# SYNTHESIS AND BIOLOGICAL STUDIES OF A TRIPODAL SCHIFF BASE DERIVED FROM 1,3,5-TRIBROMOMETHYLBENZENE AND ITS TRINUCLEAR Ce(IV) AND Nd(III) SALEN CAPPED COMPLEXES

**U. S. Oruma*, P. O. Ukoha, and N. N. Ukwueze**

Coordination Chemistry and Inorganic Pharmaceuticals Unit, Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, 410001, Nigeria.

*e-mail: susan.oruma@unn.edu.ng, Tel: +2348061222297

## ABSTRACT

*A tripodal Schiff base ligand, 1,3,5-tris(4-(4-carboxyphenyliminomethyl) phenoxy methyl) benzene (TT) was synthesized in a two-step reaction involving 1, 3, 5-tribromomethylbenzene. The ligand was used to synthesize Ce(IV) and Nd(III) salen capped complexes. These compounds were characterized using UV-Visible, IR, $^{1}H$, and $^{13}C$ NMR spectroscopies, elemental analysis, and molar conductivity measurements. The spectral studies indicate that the ligand is hexadentate and coordinates to the Ce(IV)and Nd(III) ions through the oxygen atoms of the carboxylic group. The trinuclear Ce(IV) and Nd(III) salen capped complexes were characterized as being bridged by carboxylate anions to the Ce(IV)and Nd(III) salen centers and displays a coordination number of eight by involving two hydroxyl groups in the coordination sphere. The in vitro antimicrobial activities of the ligand and complexes were investigated against Gram-negative bacteria: Escherichia coli (ATCC 6749) and Pseudomonas aeruginosa (ATCC 9027), Gram-positive bacteria: Staphylococcus aureus (ATCC 6538P) and Bacillus cereus (ATCC 14579), and fungi: Candida albicans and Aspergillus niger by the agar well diffusion technique. In vitro antimicrobial test indicate that $[\{Nd(OH)_2(salen)\}_3(TT)].3H_2O$ is more potent against the test microorganisms relative to TT and $[\{Ce(OH)_2(salen)\}_3(TT)].3H_2O$.*

**Keywords:** Tripodal Schiff base ligand; salen; Trinuclear Ce(IV) and Nd(III) Complexes; Antimicrobial activity.

## INTRODUCTION

Tripodal Schiff base ligands consist of three arms, each of which contains one or more donor atoms (N, S, P, or O) through which they can bind to one or more metals. This class of ligands has attracted so much attention due to their coordination chemistry and biological properties[1,2]. In coordination chemistry, a lot of metal complexes with novel geometries and properties have been prepared[3-6] because the extended arms of the

tripodal Schiff base ligands offer a highly protected binding pocket within the tripod unit. Moreover, the flexibility of the multi-component reaction allows for the introduction of great structural diversity, by variation of the aldehyde or carboxylic acid employed[7]. Tripodal Schiff base ligands have been synthesized and characterized [8-11]. However, there is no report on their applications in biological studies. There is also no report on the synthesis and characterization of tripodal-trinuclear [lanthanide(III) salen] capped complexes.

Our research group has reported the synthesis, characterization, antimicrobial and computational

studies of a tripodal Schiff base containing pyrimidine unit[12]. We have also reported the synthesis, characterization and biological studies of trinuclear Ce(IV) Salen Capped Complex with 5-amino-2,4,6- tris(4-carboxybenzimino)-1,3-pyrimidine [13] and with bridging 2, 4, 6-tris (4-carboxyphenylimino-41-formylphenoxy)-1, 3, 5- triazine and 2,4,6-tris(4-carboxybenzimino)-1,3,5-triazine [14].

Our interest in tripodal trinuclear lanthanide salen Schiff base complexes was aroused due to the various pharmacological properties such as antimicrobial[15], anticancer[16], cytotoxic and cytostatic activities[17-19] and antitumor activity[20] associated with lanthanide complexes.

Because of the noted physiological activities of lanthanide complexes and biological activities of tripodal Schiff base ligands, we synthesized and characterized 1,3,5-tris(4-(4 carboxyphenyliminomethyl)phenoxy methyl)benzene (TT) and its trinuclear Ce(IV) and Nd(III) salen capped complexes. The *in vitro* antimicrobial activities were also investigated.

## MATERIALS AND METHODS

### *Materials and measurements*

The chemicals used were of analytical reagent grade, purchased from Zayo–Sigma and were used as supplied without further purification. Fischer Jones melting point apparatus was used for the determination of melting points and was uncorrected. Molar conductance measurements were carried out using $10^{-4}$ mol/L solutions of the complexes in methanol at room temperature using WTW-LF 90 conductivity meter. Electronic spectra (in DMSO) were recorded on UV-Vis 1800 SHIMADZU spectrophotometer. Infrared spectra of the compounds were performed using KBr

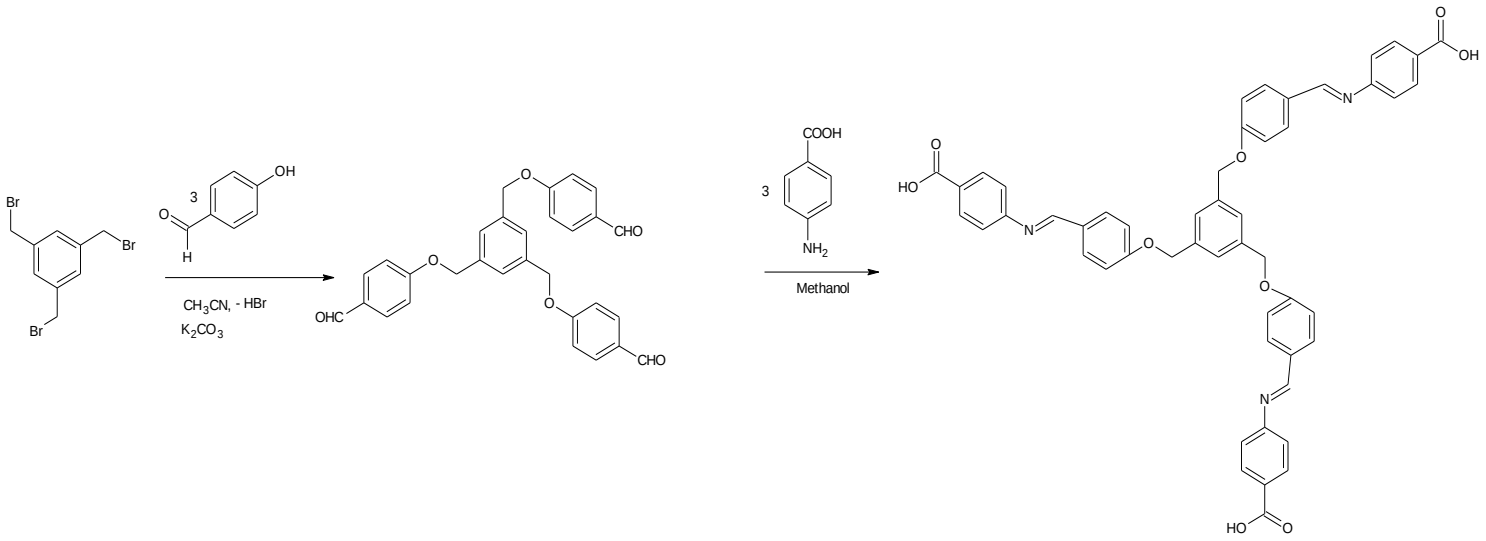
discs on a Perkin–Elmer (Waltham, Massachusetts, USA) 100 series version 10.03.08 FTIR spectrophotometer. The $^{1}H$ and $^{13}C$ NMR spectra of the compounds were recorded on a Bruker (Billerica, Massachusetts, USA) DPX 300 spectrometer in DMSO- $d_6$ at 300.13MHz and 75.47MHz respectively. Elemental analyses for C, H, and N were carried out using LECO – CHN – 932 analyzers.

***Synthesis of 1, 3, 5-tris (p-formyl phenoxy methyl) benzene (2)***

The method reported by Kocyigit and Guler (2010)[21] was adopted. 1, 3, 5-Tribromomethylbenzene (0.70 g, 2 mmol), 4-hydroxybenzaldehyde (0.76 g, 64 mmol), and 4 g of $K_2CO_3$ were stirred in 100 mL of acetonitrile at 50 °C for 4 h. It was allowed to stand and filtered. The next day, fine, orange-coloured crystals appeared as the filtrate evaporated. This was dissolved in $CH_2Cl_2$ (10 mL). Then 25 mL of 2.5 N NaOH added and shook in a separating funnel. Two layers formed. The top aqueous, white layer was discarded while the bottom yellow organic layer was collected and dried over $Na_2SO_4$. The $CH_2Cl_2$ was removed in a rotary evaporator and dried over $CaCl_2$ in desiccator. This gave 1, 3, 5-tris (formyl phenoxy methyl) benzene (**2**).

***Synthesis of 1, 3, 5- tris (4-(4-carboxyphenyliminomethyl)phenoxy methyl) benzene (TT) (3)***

$K_2CO_3$ (18 mmol, 3.0 g) was added to a solution of 4- aminobenzoic acid (1.02 g, 6 mmol) in 30 mL methanol and stirred. Then the suspension of 1, 3, 5-tris(formyl phenoxy methyl) benzene (**2**) (0.96g, 2 mmol) in 30 mL methanol was added dropwise to the above solution. The mixture was refluxed at 50 °C for 8 h and left stirring overnight. Then 1.0 N HCl solution was added and the yellow precipitate was obtained. The yellow precipitate was extracted with a 1:1 ethyl ethanoate/water mixture thrice. The organic phase was separated and dried over $Na_2SO_4$. The solvent was removed in a rotary evaporator and dried over $CaCl_2$ in a desiccator.




**1** **2** **3**

**Scheme 1: Synthesis of 1, 3, 5- tris (4-(4-carboxyphenyliminomethyl)phenoxy methyl) benzene (TT)**

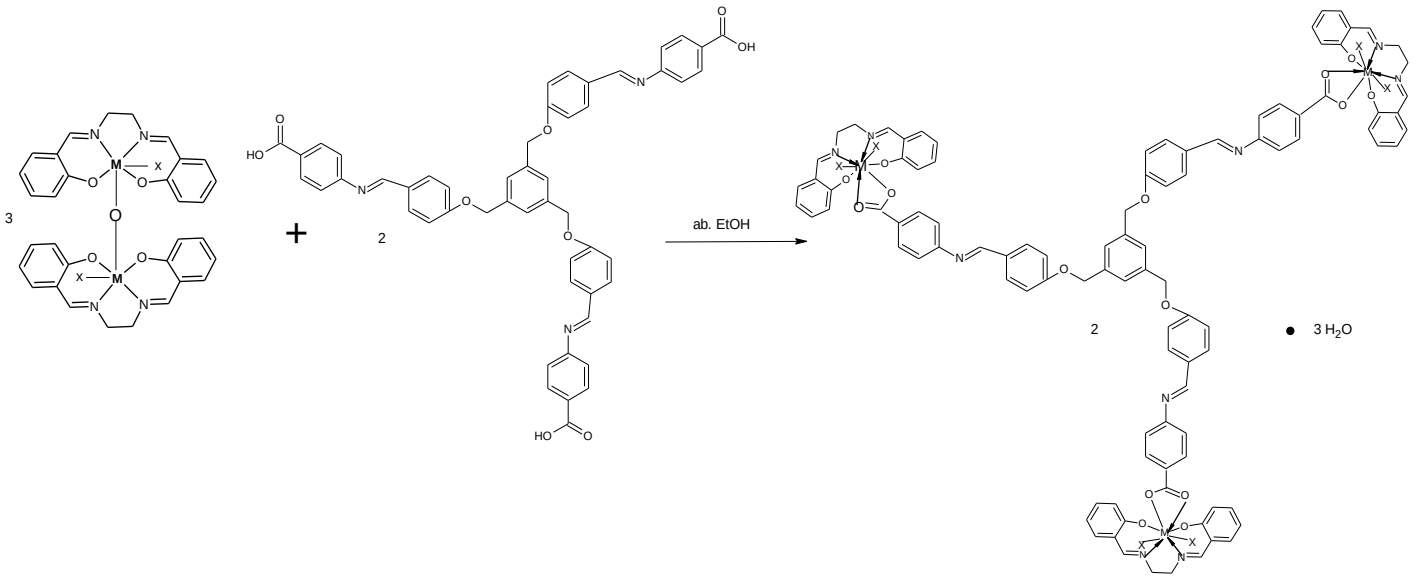
### *Synthesis of Ce(IV) ligand complex, Ce(IV)LC*

The ligand complexes were prepared by modifying the method reported by Kopel *et al.*, (1998)[22] and Uysal and Koc (2010)[23]. A solution of Ce(IV) salen complex (0.50g, 1 mmol) in absolute ethanol (20 mL) was stirred at 50 °C for 15 minutes. Excess concentrated ammonia solution (1 mL at a time) was added and stirred. The pH of the solution was monitored with the aid of a pH meter until the pH of 12. A brown precipitate was formed, filtered, and dried over $CaCl_2$. Yield = 0.43 g (63.77 %); mp of 318– 320 °C; UV (λ nm) (DMSO) (ε): 260 ($8.61\times 10^3$), 307 ($5.20 \times 10^3$); IR (KBr): 3250 (br) (O – H), 1631(s) (C═N), 1546(s) (C═C), 1199(m) (C–O), 907(s), 752(s) (C–H), 600 (s) (M-O-M), 580(m) (Ln-O), 455(m) (Ln-N) $cm^{-1}$; Anal. Calc. for $[\{Ce(OH)(salen)\}_2O]$ (862): C, 44.55; H, 3.48; N, 6.50. Found: C, 44.65; H, 3.70; N, 6.60. The UV and IR spectra are presented in supplementary materials (Figure S1 and S6).

### *Synthesis of Ce(IV) Salen Capped Complex of TT, $[\{Ce(OH)_2(salen)\}_3(TT)].3H_2O$*

Ce(IV)LC (0.53 g, 0.62 mmol) was suspended in hot absolute ethanol (25 mL) and a solution of TT (0.33 g, 0.39 mmol) in absolute ethanol was added while stirring. The reaction mixture was boiled under

reflux for 4 h. The brick- red solid formed was dried over $CaCl_2$. See Scheme 2.




**M = Ce, X = OH**

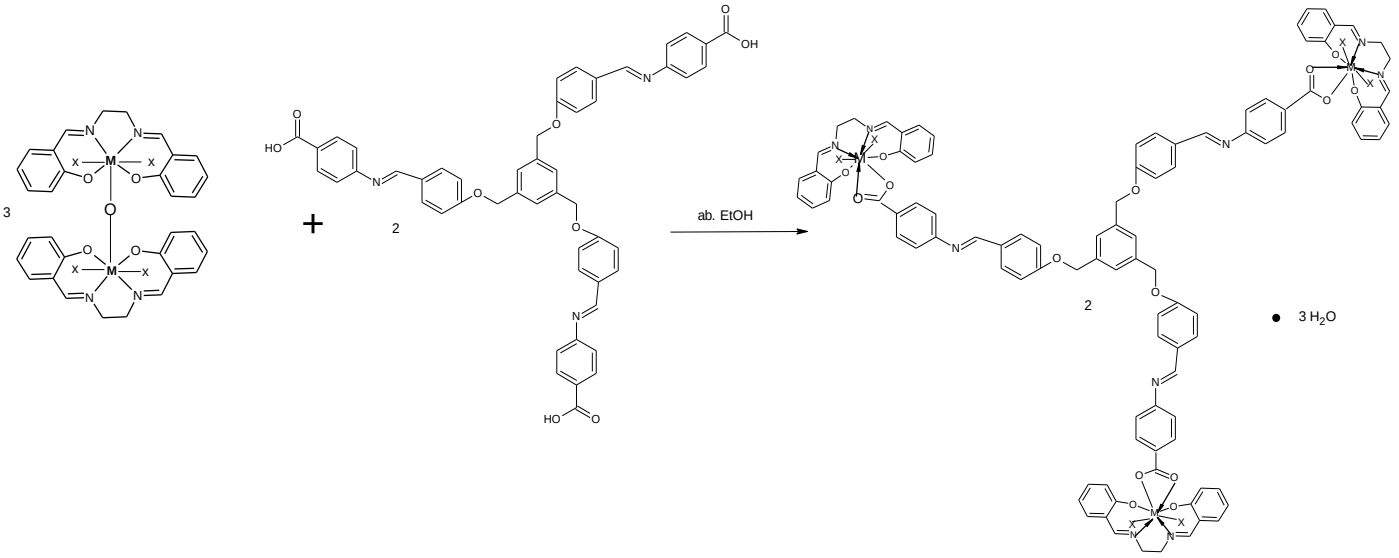
**Scheme 2: Synthesis of Ce(IV) Salen Capped Complex of TT, $[\{Ce(OH)_2(salen)\}_3(TT)].3H_2O$**

***Synthesis of Nd(III) ligand complex(Nd(III)LC)***

The ligand complexes were prepared by modifying the method reported by Kopel *et al.*, (1998)[22]. A solution of Nd(III) salen complex (0.50 g, 1 mmol) in absolute ethanol (20 mL) was stirred at 50 °C for 15 min. Excess concentrated ammonia solution (1 mL at a time) was added and stirred. The pH of the solution was monitored with the aid of a pH meter until the pH of 12. A greyish-yellow precipitate was formed, filtered, and dried over $CaCl_2$. Yield = 0.34 g (48.57 %); mp of 205 °C; UV (DMSO) $\lambda_{max}$nm (ε): 263 ($13.2 \times 10^3$), 318 ($5.54 \times 10^3$); IR (KBr): 3500 (O-H), 1623 (C=N), 1553 (C=C), 1296 (C-O), 851,753 (C-H), 597 (Ln-O-Ln), 571 (Ln-O); $^1$H NMR spectrum could not be taken due to their paramagnetic character; Anal. Calcd for $[\{Nd(OH)_2(salen)\}_2O]$ (904.48): C, 42.46; H, 3.54; N, 6.19. Found: C, 42.50; H, 3.62; N, 6.23. The UV and IR spectra are presented in supplementary materials (Figures S2 and S7).

***Synthesis of Nd(III) Salen Capped Complex of TT, $[\{Nd(OH)_2(salen)\}_3(TT)].3H_2O$***

Nd(III)LC (0.33 g, 0.37 mmol) was suspended in hot absolute ethanol (25 mL) and a solution of TT (0.13 g, 0.16 mmol) in absolute ethanol was added while stirring. The reaction mixture was boiled under reflux for 4 h. The light yellow solid formed was dried over $CaCl_2$. See Scheme 3.




**M = Nd, X = OH**

**Scheme 3: Synthesis of Nd(III) Salen Capped Complex of TT, $[\{Nd(OH)_2(salen)\}_3(TT)].3H_2O$**

***In vitro* antimicrobial activity**

The *in vitro* antimicrobial activities of TT and its trinuclear Ce(IV) and Nd(III) salen capped complexes were tested against American Type Culture Collection (ATCC) bacteria strains obtained from Rockville, MD, USA, by the Department of Microbiology, University of Nigeria, Nsukka while the fungi strains were isolated under clinical conditions. The typed bacteria culture comprises Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538P) and *Bacillus cereus* (ATCC 14579); Gram-negative bacteria: *Escherichia coli* (ATCC 6749) and *Pseudomonas aeruginosa* (ATCC 9027). The fungi strains used were *Candida albicans* and *Aspergillus niger*. The bacteria strains were tested for sterility on nutrient agar and then grown in nutrient broth at 37 °C for 24 hours while the fungal strains were tested on Sabourand Dextrose Agar (SDA) and cultured in Sabourand Dextrose Liquid

medium at 25 °C for 24 hours. The overnight cultures were subsequently diluted and suspensions were made in normal saline and adjusted to 0.5 McFarland standards[24].

***Antimicrobial assay***

Agar cup diffusion technique[25] was employed to determine the antimicrobial activities of TT and its trinuclear Ce(IV) and Nd(III) salen capped complexes. The nutrient agar and SDA plates were inoculated with 0.1 mL broth culture of the test bacteria or fungi. Using a sterile cork borer, wells (5 mm in diameter and 2.5 mm deep) were bored into the inoculated agar. Fresh stock solutions (1000 µg/mL) of the synthesized compounds were prepared in DMSO. The stock solution was further diluted with sterilized distilled water to 12.5, 25, and 50 µg/mL for antimicrobial evaluation. The wells were filled with 100 µL of the test compounds using a sterile micropipette. Standard antibiotics namely: Ciprofloxacin, Tetracycline, Gentamycin, and Fluconazole were used as positive control while sterile DMSO served as a negative control. Subsequently, 12.5, 6.25, and 3.125 µg/mL of each positive control were prepared in DMSO. The bacteria plates were incubated at 37 °C for 24 h while fungal plates were incubated at 25 °C for 24 h. Inhibition zone diameter (IZD) around each well was measured in millimeters and recorded. The graph of $IZD^2$ against the log of concentration was plotted for each plate containing a specific compound and a microorganism. The anti-log of the intercept on the *x*-axis is the MIC.

## RESULTS AND DISCUSSION

The analytical data of **TT** and its trinuclear complexes are in good agreement with the proposed molecular formula as shown in Table 1. **TT** is soluble in acetone, chloroform, ethyl acetate, DMF, and DMSO. The complexes are stable at room temperature and soluble in DMSO and DMF but insoluble in water. The reaction of the ligand complexes with **TT** gave rise to the tripodal trinuclear complexes, **[{Ce(IV)/Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** (scheme 2 and 3). These tripodal trinuclear complexes are the first examples of trisbromomethylbenzene based trinuclear complexes bridged to the Cerium (IV) and Neodymium(III) centers by COO-. Molar conductivity measurements in methanol at room temperature show that the compounds are non-electrolytes[26].

**Table 1: Elemental and physical data of 1, 3, 5- tris (4-(4 carboxyphenyliminomethyl)phenoxy methyl)benzene (TT) and its Ln complexes**

| Compound | Colour | $\Lambda_m$ ($\Omega^{-1}cm^2mol^{-1}$) | Yield g (%) | M.p. (°C) | Molar mass(g /mol) | Elemental analysis % calc. and found | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | C | | H | | N | |
| | | | | | | Calc. | Found | Calc. | Found | Calc. | Found |
| $C_{51}H_{39}N_3O_9$ | Light | - | (1.02) | 247 | 837 | 73.12 | 73.00 | 4.66 | 5.10 | 5.02 | 5.05 |
| **(TT)** | yellow | | 54.84 | | | | | | | | |
| **[{Ce(OH)$_2$(s** | Brick | 28.40 | (0.54) | 195 | 2211 | 53.73 | 53.94 | 4.21 | 4.50 | 5.70 | 5.54 |
| **alen)}$_3$(TT)].** | red | | 83.08 | | | | | | | | |
| **3H$_2$O** | | | | | | | | | | | |
| **[{Nd(OH)$_2$(s** | Light | 30.20 | (0.40) | 215 | 2223 | 53.44 | 53.26 | 4.18 | 4.20 | 5.67 | 5.50 |
| **alen)}$_3$(TT)].** | yellow | | 60.61 | | | | | | | | |
| **3H$_2$O** | | | | | | | | | | | |

***Electronic Spectra***

The UV/Vis absorption spectra of the TT and its complexes ($10^{-5}$ mol $dm^{-3}$) were carried out in DMSO at room temperature. The absorption wavelengths and the corresponding molar absorptivities (ε) are given in Table 2. The absorption spectra are displayed in supplementary materials (Figure S3, S4, and S5). The absorption spectrum of TT shows one peak at 278 nm assigned to π – π* transitions of the conjugated phenyl ring. The absorption spectra of the complexes show only one peak. A redshift was observed in the spectra of [{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O while a blue shift was observed in the spectra of [{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O. This indicates that the TT has coordinated with the lanthanide ions.

**Table 2: Electronic absorption data of 1,3,5- tris (4-(4 carboxyphenyliminomethyl)phenoxy methyl)benzene (TT) and its Ln Complexes**

| Compound | $\lambda_{max}$ | | ε x10$^3$(mol$^{-1}$dm$^3$cm$^{-1}$) | Band assignment |
|---|---|---|---|---|
| | nm | cm$^{-1}$ | | |
| **TT** | 278 | 35971 | 6.02 | π– π* |
| **[{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 267 | 37453 | 10.4 | π– π* |
| **[{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 345 | 28986 | 20.4 | n– π* |

***Infrared Spectra***

The relevant stretching frequencies of TT and its Ce(IV) and Nd(III) salen capped complex are shown in Table 3 while the spectra are presented in supplementary materials (Figures S8 – 10). The FTIR spectrum of the tripodal Schiff base ligand (TT) displayed strong vibrations of the carboxylic acid C = O and imine C = N (b) at 1692 and 1597 cm$^{-1}$ respectively. The C = O band shifted to lower frequencies in the complexes. In the complexes, the vibration due to C =N showed two bands (b) and (c). The C = N (b) band shifted to higher frequencies of about 33-34 cm$^{-1}$ in the complexes while the C =N(c) band which was absent in the tripodal Schiff base ligand was observed in the range of 1547 – 1576 cm$^{-1}$ in the complexes. A similar observation has been made in literature[27]. Furthermore, bands assignable to vibrations due to COO$^-$ groups were observed between 1301 – 1398 cm$^{-1}$ in the compounds. The shift in frequency and intensity of this band suggests the involvement of the COO$^-$ group in coordination with the Ln metal. This is further supported by the emergence of medium to weak bands around 513 – 580 and 436 – 456 cm$^{-1}$ in the complexes assigned to Ln- O and Ln –N vibrations respectively [15, 28-29]. The presence of uncoordinated water in Ce(IV)TT was made evident by broad bands observed at 3670[8, 30].

**Table 3: IR Band Assignments ($cm^{-1}$) for 1,3,5- tris (4-(4 carboxyphenyliminomethyl)phenoxy methyl)benzene (TT) and its Ln Metal Complexes**

| Compound | ν O- H | ν C – H ar | ν C =O | ν C = N | ν COO- | ν C –N | ν Ln –O | ν Ln –N |
|---|---|---|---|---|---|---|---|---|
| **TT** | - | - | 1692(s) | 1597(s)b | 1367(m)<br>1312(m)<br>1304(m) | 1159(s)<br>1122(w) | - | - |
| **[{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 3670(br) | 3044(br) | 1690(s) | 1630(s)b<br>1576(m)c<br>1547(m)c | 1392(m)<br>1368(m)<br>1302(s) | 1164(s)<br>1152(m)<br>1127(m) | 580(m) | 456(m) |
| **[{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | - | - | 1684(s) | 1634(s)b<br>1576(m)c | 1398(m)<br>1323(m)<br>1301(m) | 1162(s) | 513(m) | 436(m) |

Where C = N(b) = from azomethine linkage, C = N(c) = from salen.

**$^1$H and $^{13}$C NMR Spectra**

The $^1$H and $^{13}$C NMR spectra of TT and its Ce(IV) and Nd(III) salen capped complexes are presented in Tables 4 and 5 while the spectra are presented in supplementary materials (Figures S11 – S16). The $^1$H NMR spectra of TT and [{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O did not show the signal due to carboxylate proton, probably because the spectra were run in the range 1 – 10 ppm. In TT (Fig.1), the doublet (4H, d) at 7.82 and 7.85 ppm were due to phenyl protons (3, 4) while the singlet (3H, s) at 7.54 was due to the phenyl protons (5). The doublet (4H, d) at 7.17 and 7.19 ppm were assigned to phenyl protons (1, 2). In [{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O (Fig.1), the doublet (4H, d) at 7.85 and 7.83 were due to phenyl protons (3, 4). The singlet (3H, s) at 7.54 was assigned to protons on phenyl ring (5). The multiplet centered at 7.19 ppm was due to phenyl protons (1, 2). There also emerged a triplet (2H, t) centered at 6.43 ppm due to phenyl protons (6) on salen and a doublet (2H, d) centered at 6.03 pm due to phenyl protons (7) on salen. In [{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O the singlet at 5.25 ppm was assigned to $CH_2$ protons (8) while the singlet at 4.41 ppm was due to $CH_2$ of ethylene (9)[31]. In [{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O, the absence of signal for carboxylic proton confirms that

the trinuclear complex was formed with deprotonation of the carboxylic proton.

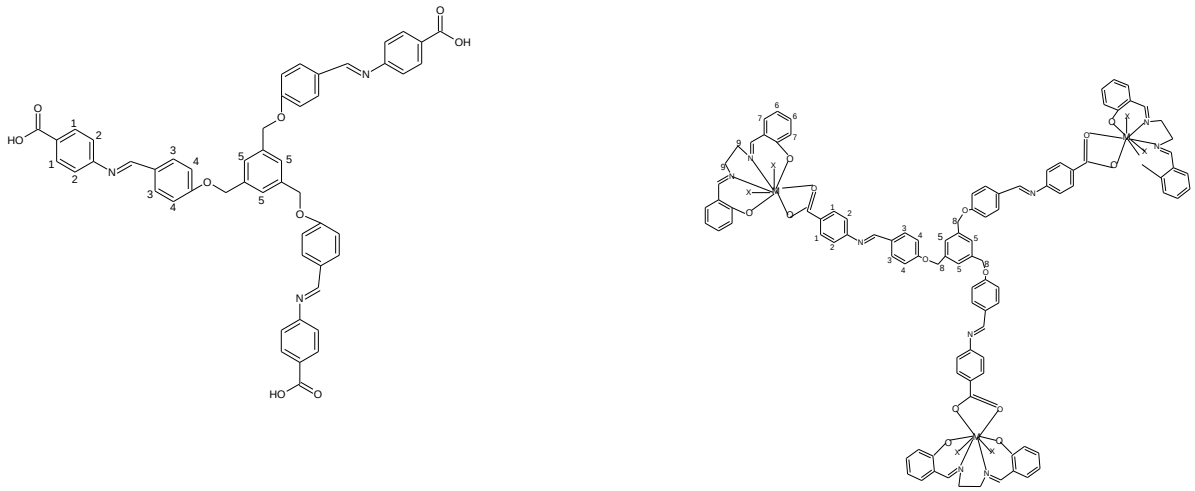


**Fig 1: Structure of TT and [{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O showing proton position**

**Table 4: $^1$H NMR Data of 1,3,5- tris (4-(4 carboxyphenyliminomethyl)phenoxy methyl)benzene (TT) and its Ln Complexes (ppm)**

| Compound | OH Carboxylate | CH = N | H$_{aromatic}$ | CH$_2$ | H$_2$O uncoordinated | DMSO |
|---|---|---|---|---|---|---|
| **TT** | - | 9.84(1H,s) | 7.17,7.19(4H,d) 7.54(3H,s), 7.82,7.85(4H,d) | 5.25(3H,s) | 3.34 | 2.5 |
| **[{Ce(OH)$_2$(salen)}$_3$ (TT)].3H$_2$O** | - | 8.29,8.67,9.84 (1H,s) | 6.03(2H,d),6.43(2H,t),7.19(4H,m), 7.54(3H,s), 7.83& 7.85(4H,d) | 4.41(4H,s), 5.25(3H,s) | 3.34 | 2.50 |
| **[{Nd(OH)$_2$(salen)} $_3$(TT)].3H$_2$O** | - | 9.86(1H,s) | 7.57(2H,t) | 5.29(4H,d) | 3.40 | 2.50 |

**Table 5: $^{13}$C NMR Data of 1,3,5 - tris (4-(4 carboxyphenyliminomethyl)phenoxy methyl)benzene (TT) and its Ln Complexes (ppm)**

| Compound | Carboxylic carbon | Azomethine carbon | Aromatic carbons | DMSO peak | CH$_2$ carbons |
|---|---|---|---|---|---|
| **TT** | 191.76 | 163.58 | 137.51, 132.24, 130.25, 127.30, 115.72. | 39.91 | 69.75 |

| | | | | | |
|---|---|---|---|---|---|
| **[{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 191.78 | 166.14, 164.10, 163.58 | 137.51, 134.40, 133.85, 132.25, 127.31, 123.66, 117.46, 115.72 | 39.89 | 69.75 |
| **[{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 191.77 | 163.61 | 132.27, 130.27, 127.32, 115.74 | 39.89 | 69.75 |

***In vitro antimicrobial activity***

The results of the *in vitro* antimicrobial screening carried out on the compounds are given in Table 6. Ciprofloxacin, Tetracycline, Gentamicin, and Fluconazole were used as positive control while sterile DMSO served as a negative control. These drugs have been chosen because they have the same mechanism of action, which is by inhibiting nucleic acid synthesis[12]. The structures of these drugs are shown in the supplementary material (Figure S17). Ciprofloxacin ($C_{17}H_{18}FN_3O_3$) belongs to fluoroquinolones and inhibits bacteria growth by preventing Deoxyribonucleic acid (DNA) synthesis before mitosis. Tetracycline ($C_{22}H_{24}N_2O_8$) inhibits the multiplication of bacteria by binding to a subunit of the ribosomes, thereby inhibiting protein and nucleic acid synthesis and consequently the death of the bacterium[32-33]. Gentamycin ($C_{21}H_{43}N_5O_7$) belongs to the class of aminoglycosides and acts by preventing protein synthesis, thereby inhibiting the synthesis of nucleic acids (DNA replication or RNA synthesis) and causes the death of the bacterium. Fluconazole is an antifungal drug ($C_{13}H_{12}F_2N_6O$) and belongs to synthetic triazoles. Fluconazole inhibits fungal cytochrome P –450, an enzyme responsible for fungal sterol synthesis, thereby causing fungal cell walls to weaken[33].

**Table 6: Inhibition zone diameter (IZD in mm) of the compounds against typed strains (ATCC CULTURES) microorganisms**

| Compound | *B.c*(ATCC 14579) | *S.a*(ATCC 6538P) | *P.a*(ATCC 9027) | *E.c*(ATCC 6749) | *C.a* | *A.n* |
|---|---|---|---|---|---|---|
| **50 µg/mL** | | | | | | |
| **TT** | 5 | 3 | 7 | 5 | - | 10 |
| **[{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 2 | 2 | 8 | 9 | - | 2 |
| **[{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 9 | 11 | 7 | 3 | 5 | 6 |
| **25 µg/mL** | | | | | | |
| **TT** | 2 | - | - | - | - | - |
| **[{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | - | - | 2 | 6 | - | - |
| **[{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 3 | 4 | - | - | - | - |
| **12.5 µg/mL** | | | | | | |
| **TT** | - | - | - | - | - | - |
| **[{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | - | - | - | - | - | - |
| **[{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | - | - | - | - | - | - |

**Key:*B.c = Bacillus cereus, S.a = Staphylococcus aureus, P.a = Pseudomonas aeruginosa, E.c = Escherichia coli, C.a = Candida albicans.A.n = Aspergillus niger*, (-) = no zone of inhibition observed.**

The results obtained (Table 6) show that the compounds inhibited the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* with inhibition zone diameter(IZD) in the range of 2 – 9, 2 – 11, 2 – 7, 3 – 9, 5, 2 –10 mm respectively. This result reflects that TT exhibits higher activity against *Aspergillus niger* while [{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O and [{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O show higher activities against, *Escherichia coli* and *Staphylococcus aureus* respectively. It was observed from the results (Table 6) that [{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O has the highest activity against *Bacillus cereus* and *Staphylococcus aureus* compared to the other compounds. Since at 25 µg/mL, it still inhibits the growth of these microorganisms.

**Table 7: Minimum Inhibitory Concentration (MIC) of the Compounds against Test Bacteria and Fungi**

| Compound | MIC (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | ***B.c*** **(ATCC 14579)** | ***S.a*** **(ATCC 6538P)** | ***P.a*** **(ATCC 9027)** | ***E.c*** **(ATCC 6749)** | ***C.a*** | ***A.n*** |
| **TT** | 25 | 50 | 50 | 50 | >50 | 50 |
| **[{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 50 | 50 | 25 | 25 | >50 | 50 |
| **[{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O** | 25 | 25 | 50 | 50 | 50 | 50 |
| **Controls** | | | | | | |
| **T** | 1.9 | 1.8 | 0.63 | 2.15 | 2.1 | 0.58 |
| **F** | 6.25 | 6.25 | 6.25 | 2.8 | 0.64 | 0.74 |
| **CP** | 1.5 | 0.70 | 0.92 | 0.65 | 2.0 | 6.25 |
| **G** | 1.4 | 2.7 | 0.71 | 2.6 | 2.5 | 0.64 |

Legend: **T** = Tetracycline, **F** = Fluconazole, **CP** = Ciprofloxacin, **G** = Gentamycin.

From Table 7, it was shown that the MIC of the controls is lower than that of the compounds. However, [{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O showed higher activity against the test organisms investigated relative to other compounds. salen centers and displays a coordination number of eight by involving two hydroxyl groups in the coordination sphere. *In vitro* antimicrobial test indicate that [{Nd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O is more potent against the test microorganisms relative to TT and [{Ce(OH)$_2$(salen)}$_3$(TT)].3H$_2$O.

## CONCLUSION

A tripodal Schiff base ligand, 1,3,5-tris(4-(4-carboxyphenyliminomethyl) phenoxy methyl) benzene (TT) and its Ce(IV) and Nd(III) salen capped complexes were synthesized and characterized. Based on analytical and spectral data, the ligand was found to be hexadentate and coordinate to Ce(IV) and Nd (III) ions through the oxygen atoms of the carboxylic group. The trinuclear Ce(IV) and Nd(III) salen capped complexes were characterized as being bridged by carboxylate anions to the Ce(IV) and Nd(III)

## ACKNOWLEDGEMENTS

The authors are grateful to Prof. Klaus Jurkschat of Technische Universität, Fakultät für Chemie und Chemische Biologie, D-

44221 Dortmund, Germany for helping with the spectral analyses. We also acknowledge the support received from the African-German Network of Excellence in Science (AGNES), the Federal Ministry of Education and Research (BMBF), and the Alexander von Humboldt Foundation (AvH).

## SUPPLEMENTARY MATERIALS

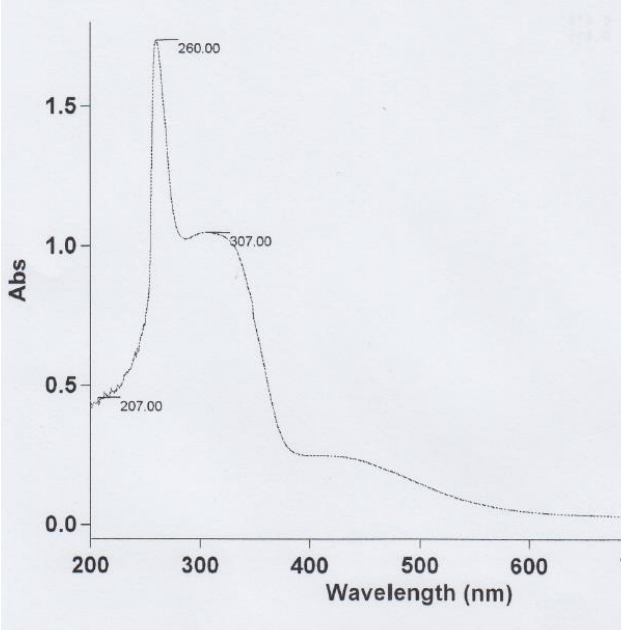



Figure S1: Electronic absorption spectrum of Ce(IV)LC

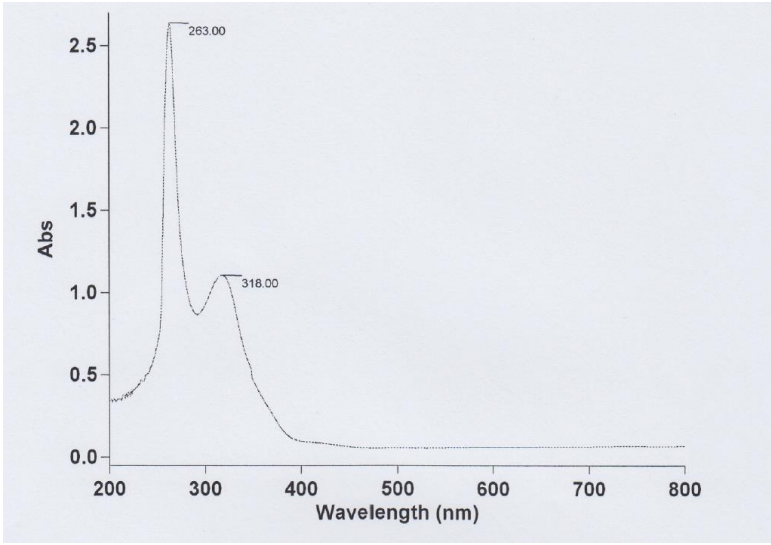



Figure S2: Electronic absorption spectrum of Nd(III) ligand complex

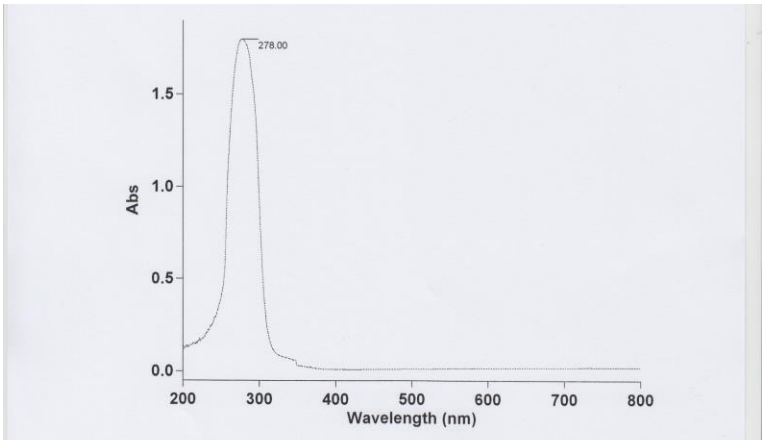



Figure S3: Electronic absorption spectrum TT

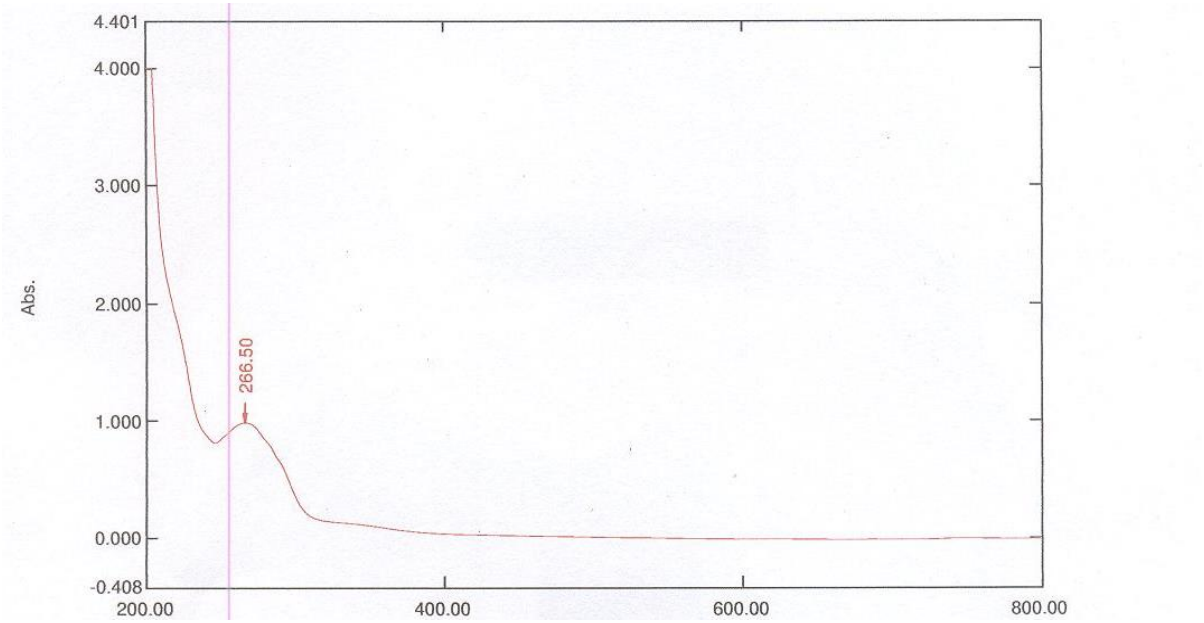



Figure S4: Electronic absorption spectrum of [{$Ce(OH)_2(salen)\}_3(TT)$].$3H_2O$

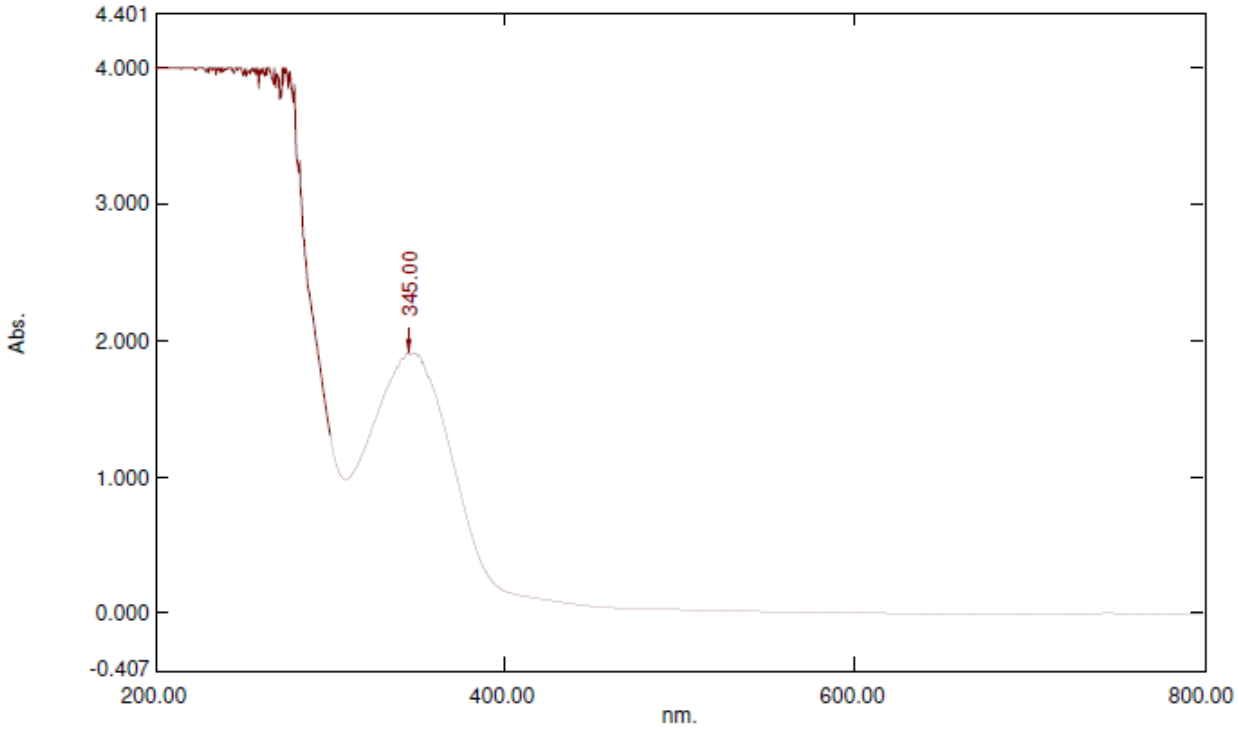



Figure S5: Electronic absorption spectrum of [{$Nd(OH)_2(salen)\}_3(TT)$].$3H_2O$

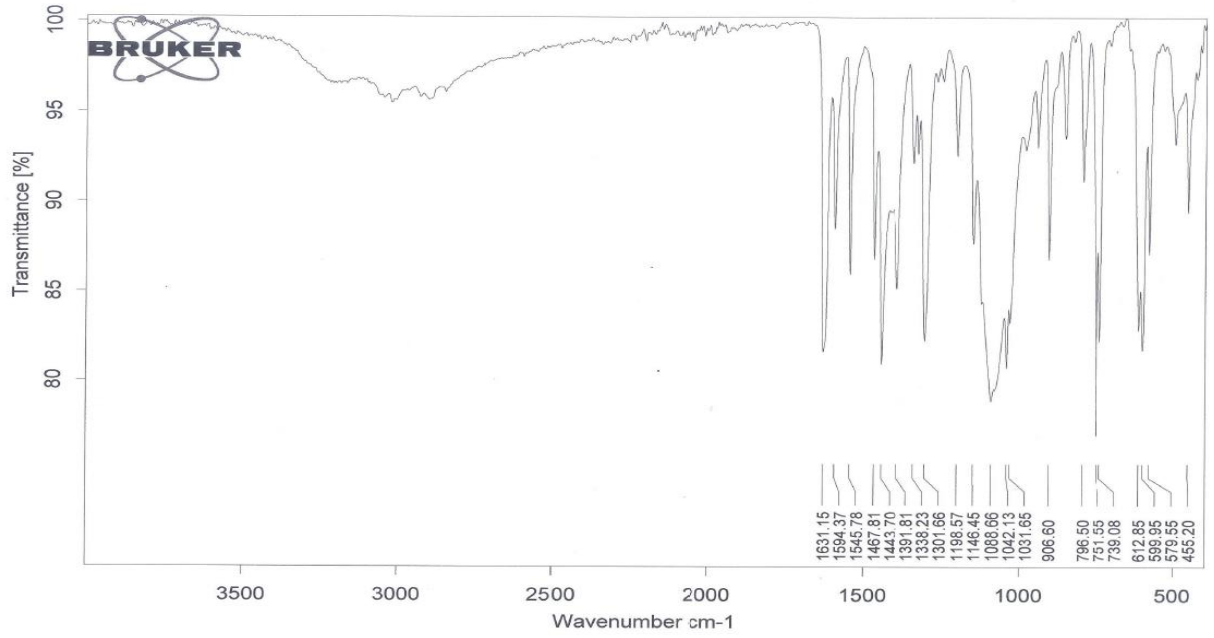



Figure S6: Infrared spectrum of Ce(IV)LC

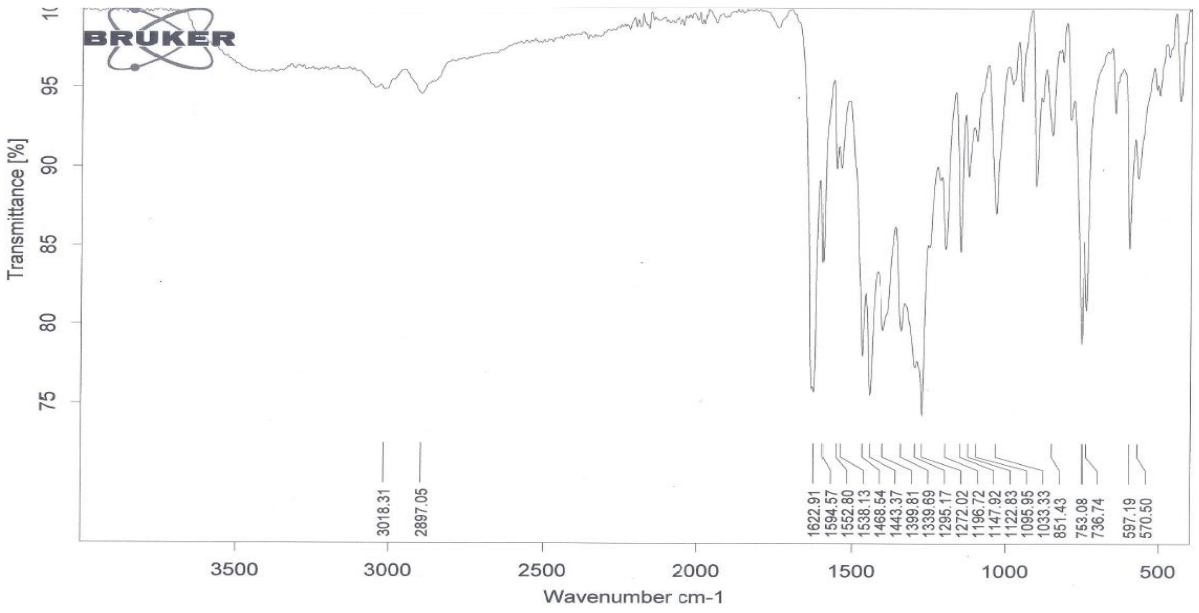



Figure S7: Infrared spectrum of Nd(III) ligand complex

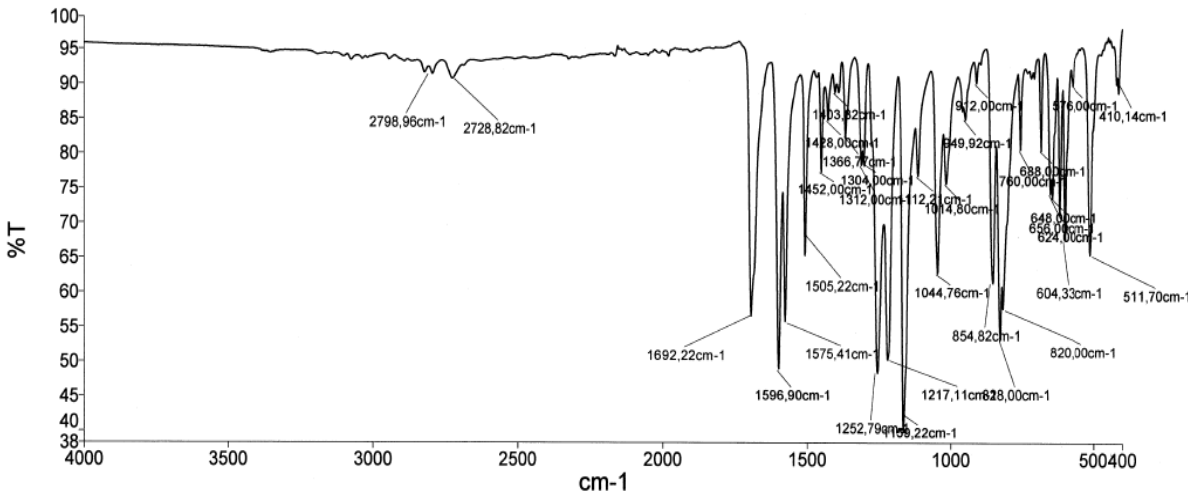



Figure S8: Infrared spectrum of TT

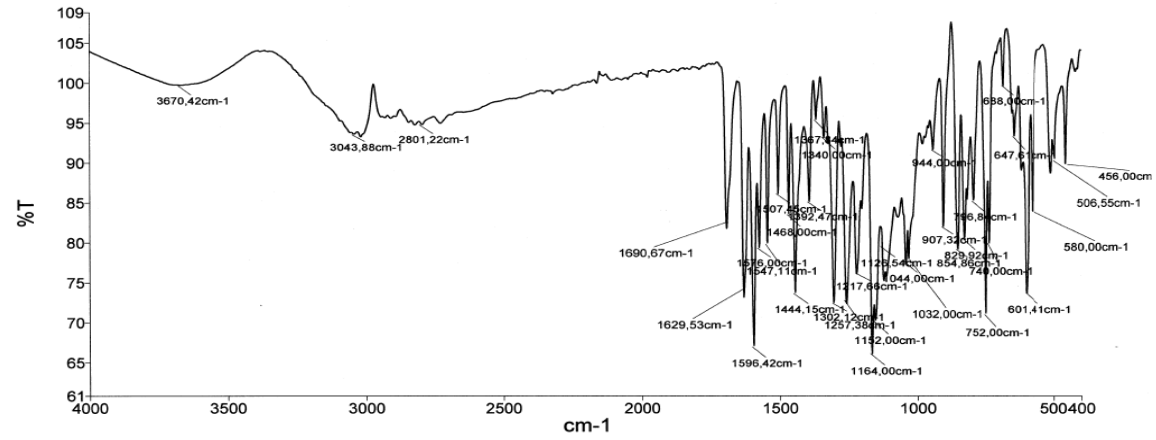



Figure S9: Infrared spectrum of [{$Ce(OH)_2(salen)\}_3(TT)$].$3H_2O$

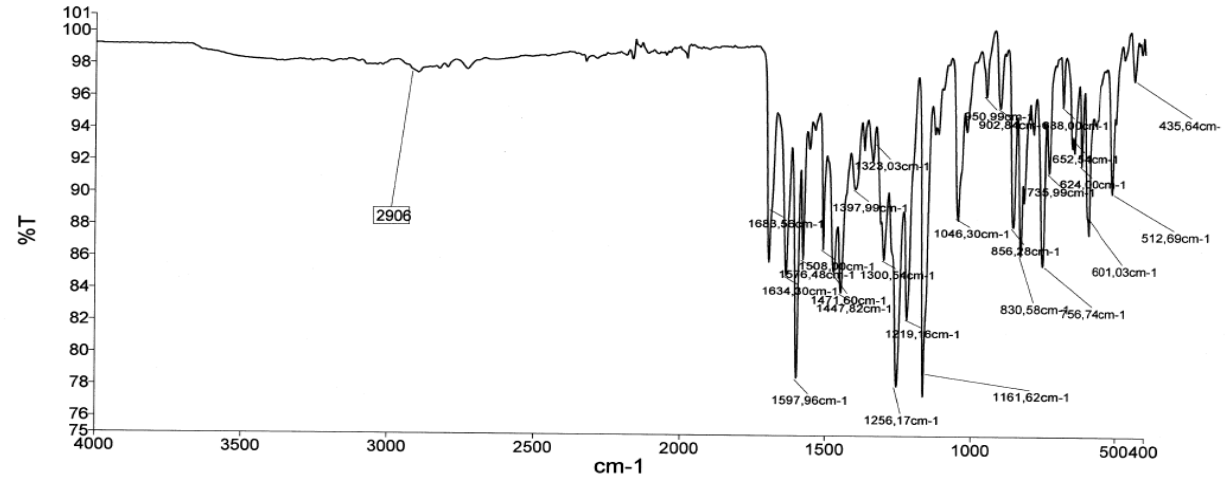



Figure S10: Infrared spectrum of {$Nd(OH)_2(salen)\}_3(TT)$].$3H_2O$

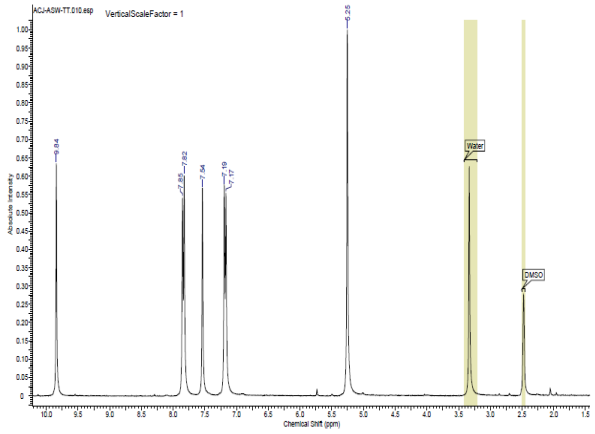



Figure S11: $^{1}$H NMR spectrum of TT

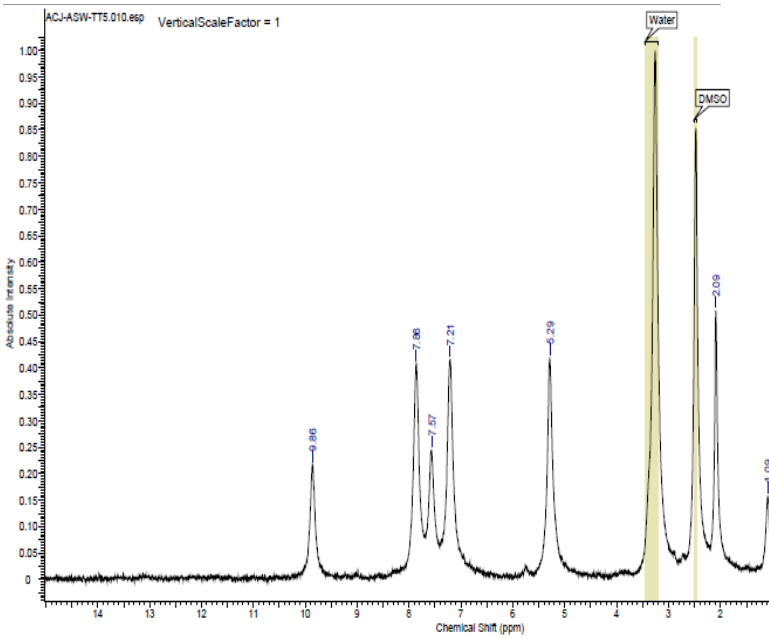



Figure S13: $^{1}$H NMR spectrum of [{Nd $(OH)_2$(salen)}$_3$(TT)].3$H_2O$

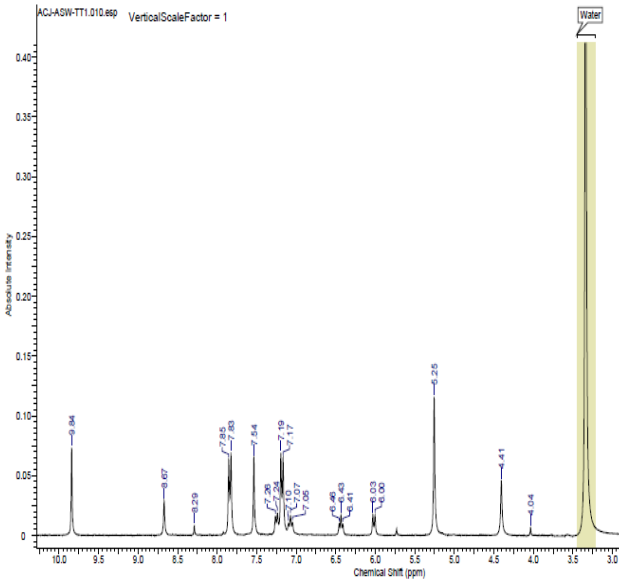



Figure S12: $^{1}$H NMR spectrum of [{Ce$(OH)_2$(salen)}$_3$(TT)].3$H_2O$

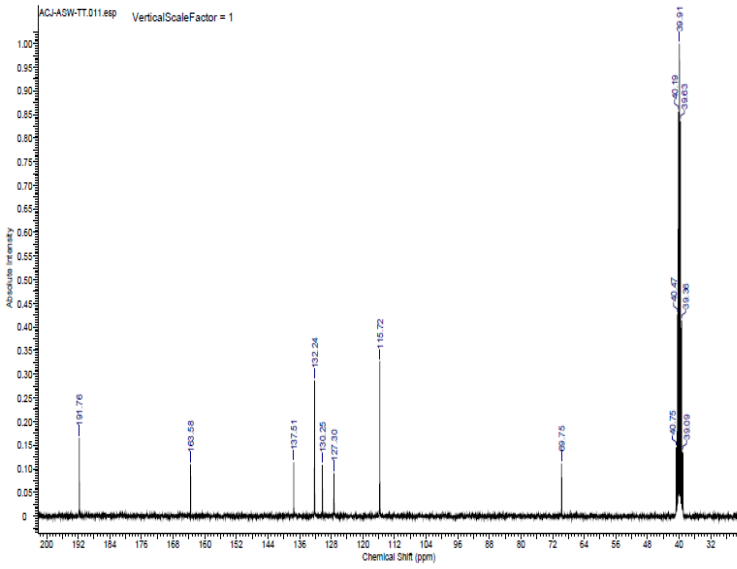



Figure S14: $^{13}$ C NMR spectrum of TT

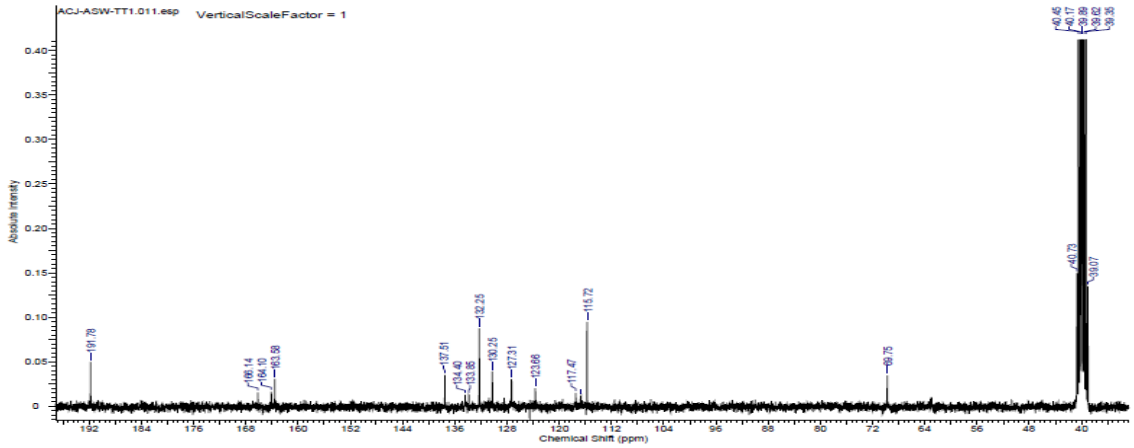



Figure S15: $^{13}$ C NMR spectrum of [{$Ce(OH)_2(salen)$}$_3$(TT)].3$H_2O$

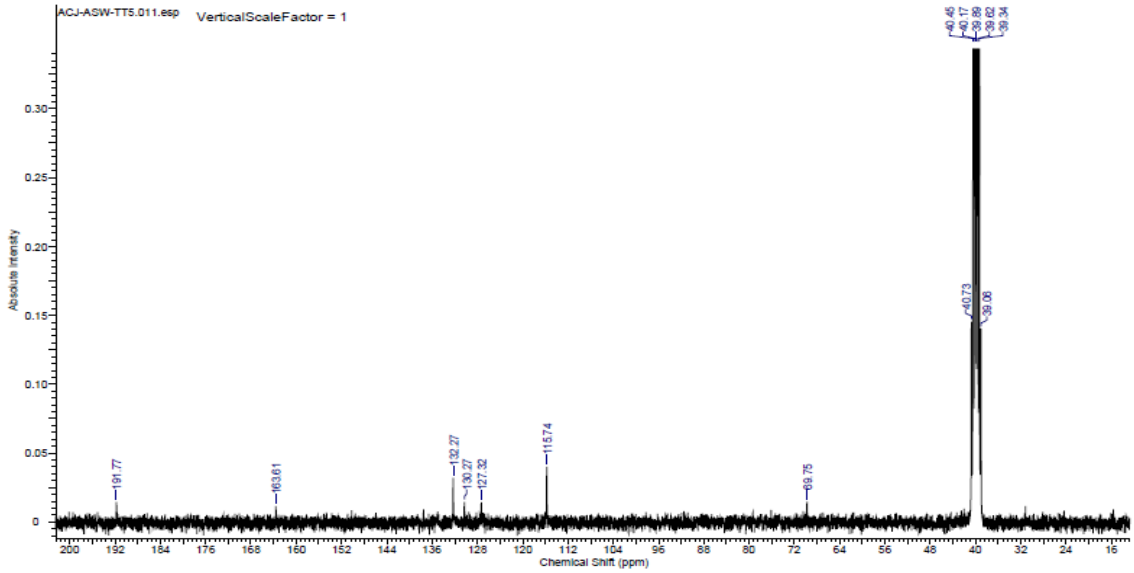
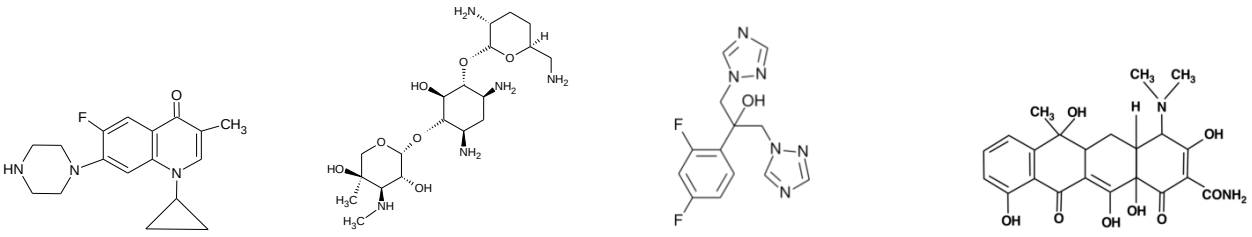



Figure S16: $^{13}$ C NMR spectrum of [{$Nd(OH)_2(salen)$}$_3$(TT)].3$H_2O$



Ciprofloxacin Gentamicin Fluconazole Tetracycline

Figure S17: Structures of the drugs used as standard.

## REFERENCES


1. Sang, Y. –L., Lin, X.-S., Li, X.-C., Liu, Y.-H. and Zhang, X.-H. (2015). Synthesis, crystal structure, and antibacterial activity of a novel phenolato- and peroxo-bridged dinuclear cerium(IV) complex with tripodal Schiff bases. *Inorg. Chem. Comm.*, 62, 115-118.

2. Wong, W.-K., Liang H. Z., Guo, J. P.,Wong, W. –Y., Lo, W. –K., Li, K. –F., Cheah, K. –W., Zhuo, Z. Y., Wong, W. –T. (2004). Template synthesis, Crystal Structure and Luminescent Properties of neutral $N_4O_3$ tripodal $Ln^{III}$ L Complexes ($Ln^{III}$ = $La^{3+}$, $Eu^{3+}$, $Gd^{3+}$, $Tb^{3+}$, $Dy^{3+}$, $Ho^{3+}$, $Er^{3+}$, $Tm^{3+}$ or $Lu^{3+}$: $H_3L$ = tris([3'-(2"-pyridyl)-5'-tert-butyl-2'-hydroxybenzylidene-2-imino] ethyl)amine). *Eur. J. Inorg. Chem.*, 829-836.

3. Casellato, U., Tamburini, S., Tomasin, P., Vigato, P. A. and Botta, M. (1996). Lanthanide(III) Complexes with a Podand Schiff Base Containing an $N_4O_3$ Coordination Site. *Inorg. Chimica Acta*, 143-145.

4. Costes, J. –P., Dupuis, A., Commenges, G., Lagrave, S. and Laurent, J. –P. (1999). Mononuclear Lanthanide Complexes of Tripodal Ligands: Synthesis and Spectroscopic Studies. *Inorg. Chimica Acta*, **285,** 49- 54.

5. Kanesato, M., Nagahara, K., Igarashi, K., Sato, K., Kikkawa, Y. and Goto, M. (2011). Synthesis, Characterization, and Emission Properties of Yttrium (III) and Europium (III) Complexes of a Tripodal Heptadentate Schiff-base Ligand, $N[CH_2CH_2N{=}CH(2\text{-}OH\text{-}3\text{-}MeC_6H_3)]_3$. *Inorg. Chimica Acta*, **367**, 225 – 229.

6. Salehzadeh, S., Nouri, S. M., Keypour, H. and Bagherzadeh, M. (2005). Synthesis of Gadolinium(III) and Samarium(III) Complexes of New Potentially Heptadentate($N_4O_3$) Tripodal Schiff Base Ligands, and a Theoretical Study. *Polyhedron*, **24,** 1478-1486.

7. Amendola, V., Boiocchi, M., Colasson, B., Fabbrizzi, L., Monzani, E., Douton-Rodriguez, M. J. and Spadini, C. (2008). Redox Active Cage for the Electrochemical Sensing of Anions. *Inorg. Chem.*, **47(11)**, 4808–4816.

8. Koc, Z. E., and Ucan, H. I. (2007). Complexes of Iron(III) Salen and Saloph Schiff Bases with Bridging 2,4,6-Tris(2,5-dicarboxyphenylmino-4-formylphenoxy)-1,3,5-Triazine and 2,4,6-Tris(4-carboxyphenylimino-4[1]-formylphenoxy)-1,3,5-Triazine.*Transition Metal Chemistry*, **32**, 597-602.

9. Uysal, S., Koc, Z. E., Celikbilek, S. and Ucan, H. I. (2012). Synthesis of Star-shaped Macromolecular Schiff Base Complexes Having Melamine Cores and Their Magnetic and Thermal Behaviours. *Synthetic Comm.*, **42**, 1033-1044.

10. Uysal, S. and Koc, Z. E. (2016). Synthesis and Characterization of Dopamine Substitute Tripodal Trinuclear [(salen/salophen/salpropen) M] (M = Cr(III), Mn(III), Fe(III) ions) Capped S-triazine Complexes: Investigation of their Thermal and Magnetic Properties. *J. of Molecular Structure*, **1109,** 119-126.

11. Kocyigit, O. (2013). A Novel Schiff Base Bearing Dopamine Groups with Tripodal Structure. Synthesis and its Salen/salophen-bridged Fe/Cr(III)

Capped Complexes. *J. of Molecular Structure*, **1034**, 69-74.

12. Oruma, U. S., Ukoha, P. O., Rhyman, L., Elzagheid, M. I., Obasi, L. N., Ramasami, P. and Jurkschat, K. (2018). Synthesis, Characterization, Antimicrobial Screening, and Computational Studies of a Tripodal Schiff Base Containing Pyrimidine Unit. *J. of Heterocyclic Chemistry*, 55, 1119 – 1129.

13. Oruma, U. S., Ukoha, P.O. and Obasi, L. N. (2020). Synthesis, Characterization and Biological studies of Trinuclear Ce(IV) salen capped complex with 5-amino-2,4,6-tris(4-carboxybenzimino) -1,3-pyrimidine. *Communication in Physical Sciences* 5(3), 403-417.

14. Oruma, U. S., Ukoha, P. O., and Ezeorah, C. J. (2020). Trinuclear Ce(Iv) Salen Capped Complexes with Bridging 2, 4, 6-Tris (4-Carboxyphenylimino-41-Formylphenoxy)-1, 3, 5-Triazine and 2,4,6-Tris(4-Carboxybenzimino)-1,3,5-Triazine: Synthesis, Characterization and Biological Studies. *Nigerian Journal of Chemical Research,* 25(2), 9-31.

15. Taha, Z. A., Ajlouni, A. M., Al-Hassan, K. A., Hajazi, A. K. and Faiq, A. B. (2011). Synthesis, Characterization, Biological Activity, and Fluorescence Properties of Bis-(salicylaldehyde)-1,3-propylene diamine Schiff base Ligand and Its Lanthanide Complexes. *Spectrochim. Acta*, Part A, 81, 317-323.

16. Dalla Cort, A., De Bernardin, P., Forte, G.and Mihan,F. Y. (2010). Metal–salophen-based receptors for anions. *Chem. Soc. Rev*., 39(10), 3863-3874.

17. Manolov, I., Kostova, I., Konstantinov, S. and Karaivanova, M. (1999). Synthesis, physicochemical characterization, and cytotoxic screening of new complexes of cerium, lanthanum, and neodymium with Niffcoumar sodium salt. *Eur. J. Med. Chem.*, 34(10), 853-858.

18. Kostova, I., Manolov, I., and Momekov, G. (2004). Cytotoxic activity of new neodymium (III) complexes of bis-coumarins. *Eur. J. Med. Chem.*, 39(9), 765-775.

19. Kostova, I., Manolov, I., Momekov, G., Tzanova, T., Konstantinov, S. and Karaivanova, M. (2005). Cytotoxic activity of new cerium (III) complexes of bis-coumarins. *Eur. J. Med. Chem.*, 40(12), 1246-1254.

20. Kostova, I., Manolov, I., Nicolova, I., Konstantinov, S. and Karaivanova, M. (2001). New lanthanide complexes of 4-methyl-7-hydroxycoumarin and their pharmacological activity. *Eur. J. Med. Chem.*, 36(4), 339–347.

21. Kocyigit, O. and Guler, E. (2010). The Investigation of Complexation Properties and Synthesis of the (salen and salophen) – Bridged Fe/Cr (III) Capped Complexes of Novel Schiff Bases. *J.Incl. Phenom Macrocyclic Chem.*, **67**, 29-37.

22. Kopel, P., Sindelar, Z. and Klicka, R. (1998). Complexes of Iron(III) Salen and Saloph Schiff Bases with Bridging Dicarboxylic and Tricarboxylic Acids. *Transition Met. Chem.*, **23**, 139-142.

23. Uysal, S. and Koc, Z. E. (2010). Synthesis and Characterization of Dendrimeric Melamine Cored [salen/salophen Fe(III)] and

[salen/salophen Cr(III)] Capped Complexes and Their Magnetic Behaviors. *J. of Hazardous Materials*, 175, 532-539.

24. Cheesbrough, M., District laboratory practice in tropical countries, P. 393 – 394, Cambridge university press, 2006.

25. Alli, A., Ehinmidu, J. and Ibrahim, Y. (2011). Preliminary phytochemical screening and antimicrobial activities of some medicinal plants used in Ebiraland. *Bayero J. of Pure and Applied Sci.*, 4(1), 10-16.

26. Ali, I., Wani, W. A. and Saleem, K. (2013). Empirical formulae to molecular structures of metal complexes by molar conductance. *Syn. React. Inorg Met.-Org and Nano-Met Chem*., 43(9), 1162-1170.

27. Uysal, S. and Ucan, H. I. (2009). The Synthesis and Characterization of Melamine Based Schiff Bases and It's Trinuclear [salen/salophen Fe(III)] and [salen/salophen Cr(III)] Capped Complexes. *J. Incl. Phenom. Macrocyclic Chem.*, 65, 299-304.

28. Lekha, L., Raja, K. K., Rajagopal, G. and Easwaramoorthy, D. (2014). Synthesis, spectroscopic characterization, and antibacterial studies of lanthanide (III) Schiff base complexes containing N, O donor atoms. *J. Mol. Struct.*, 1056, 307-313.

29. Karatas, E. and Ucan, H. I. (2014). The Synthesis and Characterization of s-Triazine- Based 8-Hydroxyquinoline Ligand and Its Salen/Salophen-Bridged Fe/Cr(III) Capped Complexes. *J. of Selcuk University Natural and Applied Science*, **3(2)**, 59-70.

30. Oruma, U. S., Ukoha, P. O. and Asegbeloyin, J. N. (2014). Synthesis, Characterization and Biological Studies of S-1,3-Benzothiazol-2-ylthiophene-2-carbothioate and its Ce(IV) and Nd(III) Complexes. *Asian J. of Chem.*, **26(22),** 7622-7626.

31. Ukoha, P. O. and Oruma, U. S. (2014). Synthesis and Antimicrobial Studies of N, $N^1$-Bis(4-Dimethylaminobezylidene)ethane-1,2-diamine (DAED) and its Nickel(II) and Platinium(IV) complexes. *J. Chem. Soc. Nigeria*, 39(2), 102-107.

32. Obasi, L. N., Oruma, U. S., Al-Swaidan, I. A., Ramasami, P., Ezeorah, C. J., A.E. Ochonogor, A. E. (2017). Synthesis, Characterization, and Antibacterial Studies of N-(Benzothiazol-2-yl)-4-chlorobenzenesulphonamide and Its Neodymium (III) and Thallium (III) Complexes. *Molecules*, 22(2), 153, 1-11.

33. Wolters, K., Clinical Pharmacology made Incredibly Easy, 3rd ed., P. 285, 286, 239, 247, 256, Lippincott, W and Wilkins, USA, 2009.