Gadolinium (III) trinuclear salen capped Complexes of a series of N_2O_2 Donor Ligands: Synthesis, Characterization and Antimicrobial Studies

U. S. Oruma[1*], P. O. Ukoha[1], N. N. Ukwueze[1] and C. J. Ezeorah[2]

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

*Corresponding author E-mail: susan.oruma@unn.edu.ng, Tel: +2348061222297

ABSTRACT

Novel Gadolinium (III) trinuclear salen capped complexes of N_2O_2 donor ligands, $[\{Gd(OH)_2(salen)\}_3(TT/TTPS)].3H_2O$ were synthesized. Characterization of the compounds were done using UV-Visible, IR, ^1H, and ^{13}C NMR spectroscopies, elemental analysis, and molar conductivity measurements. The spectral studies indicate that the ligands are hexadentate and coordinate to the Gd(III) ions through the oxygen atoms of the carboxylic group. The trinuclear Gd(III)salen capped complexes were characterized as being bridged by carboxylate anions to the Gd(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 ligands 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 TTPS and $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ showed higher activity against the test organisms investigated relative to TT and $[\{Gd(OH)_2(salen)\}_3(TT)].3H_2O$. The in vivo antimalarial assay carried out on Plasmodium berghei shows a general dose-dependent significant parasitemia inhibition compared with the negative control with $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ having highest inhibition of 72.20% at 50 mg/kg and 70.28% at 25 mg/kg close to the value (87.22%) of the standard drug artesunate 5 mg/kg. Hence, $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ can serve as an antimalarial drug at doses less than 2154 mg/kg.

Keywords*:*Schiff base, Ligand complexes; Trinuclear Gd(III) Complexes; acute toxicity, antimalarial, antimicrobial

INTRODUCTION

Schiff bases have received a great deal of attention due to their ease of synthesis, availability and ability to form stable complexes with different d- and f- block

metal ions in various oxidation states[1,2]. They have been applied in developing a wide range of applications in organic[3], inorganic[4], coordination[5], bioinorganic[6] and environmental chemistry[7]. Schiff bases show biological activities like nematicidal[8], insecticidal[9], antibacterial[10], antifungal[11], antileukaemia[12], anti-inflammatory[13], anti–HIVactivity[14],antimycobacterial activity[15],antioxidant[16],anticancer [17]and plant growth regulatory activity[18]among others.

Tetradentate salen-type Schiff base ligands derived from salicylaldehyde and diamine derivatives and their complexes with lanthanide metal ions have been synthesized and characterized[19]. Salen ligand was first synthesized by Jacobsen and Katsuki in 1990, when they discovered the enantioselective epoxidation of unfunctionalized alkenes using chiral Mn(salen) complexes[20]. Salen-type ligands have been employed in the synthesis of numerous metal complexes, both with transition and non-transition metals[21] and their complexes are of great interest in chemistry because of their catalytic activities in a wide range of useful organic transformations[22]. Their ability to form ligand complexes have been used to obtain capped complexes[23, 24]**.** However, there is no report on lanthanide(III) trinuclear salen capped complexes.

Lanthanides have high (8 – 10) coordination numbers, which allows them coordinate along all directions. Hence, they can accommodate easily more than one metalloligands around themselves[25].

Our research group has synthesized tripodal trinuclear Ce(IV), Dy(III), Er(III), Gd(III) and Nd(III) salen capped complexes[26, 27].

In these works, lanthanide(III) ions have been used to synthesize 'ligand complexes', which act as receptors for tripodal ligands. This gives rise to tripodal-trinuclear [lanthanide(III) salen] capped complexes. The search for synthetic receptors able to recognise and bind anions has been a real challenge[21]. This is because the larger size and higher free energies of solvation of anions compared to cations make it more difficult for anions to bind relative to cations. Our interest in tripodal trinuclear lanthanide salen capped complexes was aroused due to the various pharmacological properties such as antimicrobial[2], anticancer[21], cytotoxic and cytostatic activitiesand antitumor activity[28] associated with lanthanide complexes. Gadolinium belongs to lanthanides with $[Xe]4f^7$ in the M^{3+} state. Gadolinium (III) complexes have been used as magnetic resonance imaging contrast agents and are widely used in clinical applications[29].

In this present work, we synthesized and characterized Gd(III) ligand complexes, which reacted with the tripodal ligands namely 1,3,5-tris(4-(4-carboxyphenyliminomethyl)phenoxymethyl) benzene (TT) and 5-amino-2,4,6 - tris(4-carboxybenzimino) -1,3- pyrimidine (TTPS) to form tripodal-trinuclear [Gadolinium(III) salen] capped complexes([{$Gd(OH)_2$(salen)}$_3$(TT/TTPS)]. $3H_2O$).The *in vitro* antimicrobial activities were also investigated.

MATERIALS AND METHODS

Materials and measurements

Analytical reagent grade chemicals, 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 – 932analyzers.

Synthesis of the ligands

The 1,3,5-tris(4-(4-carboxyphenyliminomethyl)phenoxymethyl) benzene (TT) was prepared as reported[26]and 5-amino-2,4,6 - tris(4-carboxybenzimino) -1,3- pyrimidine (TTPS) was also prepared as reported [30].

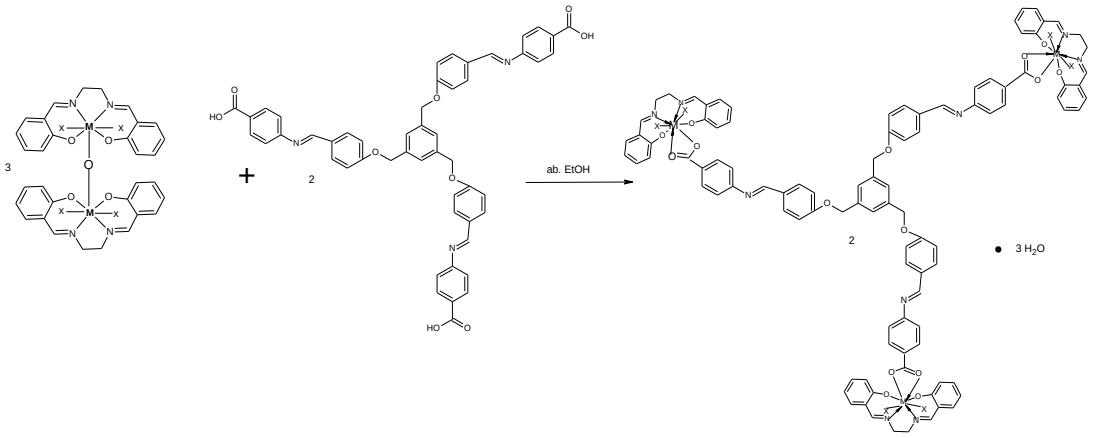
Synthesis of Gd(III) ligand complex, [{$Gd(OH)_2$(salen)}$_2$O]

The ligand complexes were prepared by modifying the method reported by Kopel *et al.*[31],and Uysal *et al.*[23,24].A solution of Gd(III) salen complex (0.50g, 10^{-3} mol) 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 pale yellow precipitate was formed, filtered and dried over $CaCl_2$. Yield = 0.40 g (59.70 %); mp of 265a °C;

UV (λ nm) (DMSO) (ε): 266 (10.6× 10^3) ; IR (KBr): 3500 (br) (O – H), 1625(s) (C═N),1543(m) (C═C), 1284(s) (C–O), 753(s) (C–H), 598(m) (M-O-M), 572(m) (Ln-O), 437(m) (Ln-N) cm^{-1}; Anal. Calc. for [{$Gd(OH)_2$(salen)}$_2$O] (931): C, 41.25; H, 1.93; N, 6.02. Found: C, 41.15; H, 2.10; N, 6.06. The UV and IR spectra are presented in supplementary materials (Figure S3 and S7).

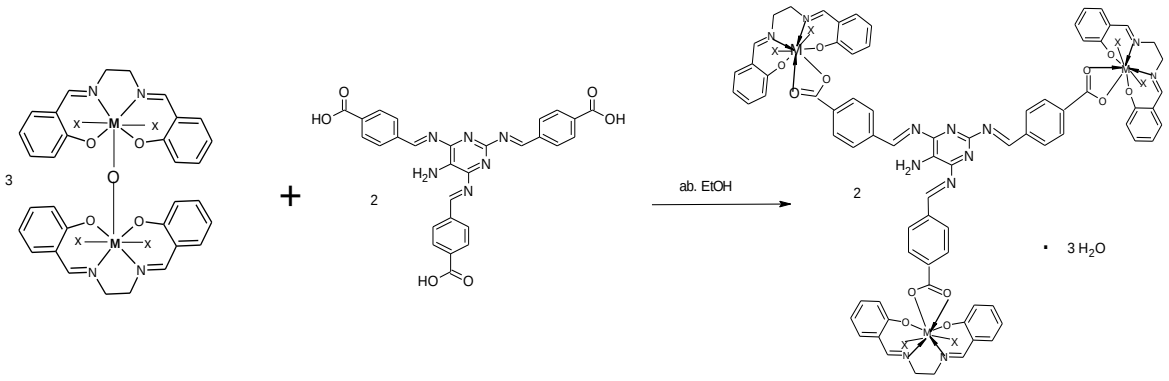
Synthesis of Gd(III) Salen Capped Complex of TT and TTPS, [{$Gd(OH)_2$(salen)}$_3$(TT/TTPS)].3H_2O

[{$Gd(OH)_2$(salen)}$_2$O] (0.00075 mol) was suspended in hot absolute ethanol (25 mL) and a solution of the ligands (0.00050 mol) in absolute ethanol was added while stirring. The reaction mixture was boiled under reflux for 4 hours. The product formed was dried over $CaCl_2$. See Scheme 1 and 2.



M = Gd, X = OH

Scheme 1: Synthesis of Gd(III) Salen Capped Complex of TT, [{$Gd(OH)_2$(salen)}$_3$(TT)].3H_2O



M = Gd, X = OH

Scheme 2: Synthesis of Gd(III) Salen Capped Complex of TTPS, $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$

In vitro antimicrobial activity

The *in vitro* antimicrobial activities of the ligand and 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[32].

Antimicrobial assay

Agar cup diffusion technique[33] was employed to determine the antimicrobial activities of the ligand and 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 ^oC for 24 h while fungal plates were incubated at 25 ^oC 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 onthe *x*-axis is the MIC.

Acute toxicity (LD_{50}) and *in vivo* antimalarial studies were determined as described in Oruma *et al*, 2018 [30].

RESULTS AND DISCUSSION

The analytical data of the complexes are in good agreement with the proposed molecular formula as shown in Table 1.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 and TTPS gave rise to the tripodal trinuclear complexes, $[\{Gd(OH)_2(salen)\}_3(TT/TTPS)].3H_2O$ (scheme 1). These tripodal trinuclear complexes are the first examples of 5-amino-2,4,6 - tris(4-carboxybenzimino) -1,3-pyrimidine and trisbromomethylbenzene based trinuclear complexes bridged to the Gadolinium(III) centers by COO-. Molar conductivity measurements in methanol at room temperature show that the compounds are non-electrolytes[34].

Table 1: Elemental and physical data of TT, TTPS and $[\{Gd(OH)_2(salen)\}_3(TT/TTPS)].3H_2O$

Compound	Colour	Λ_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
$C_{51}H_{39}N_3O_9$ (TT)	Light yellow	-	(1.02) 54.84	247	837	73.12	73.00	4.66	5.10	5.02	5.05
$C_{28}H_{20}O_6N_6$ (TTPS)	Yellow	-	(1.66) 74	348-350a	536	62.69	62.25	3.73	4.10	15.67	15.30
$[\{Gd(OH)_2(salen)\}_3(TT)].3H_2O$	Light yellow	24.60	(0.33) 49.25	205	2260	52.57	52.35	3.98	4.05	5.58	5.46
$[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$	Yellow	32.00	(0.58) 62.37	330[a]	1905	47.87	47.66	3.73	4.01	8.82	8.73

a = decomposition temperature.
Standard 1:1 electrolyte in Methanol = 80 – 122 $ohm^{-1}cm^2mol^{-1}$

Electronic Spectra

The UV/Vis absorption spectra of the ligands and its Gd(III) complexes ($10^{-5}moldm^{-3}$) 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 S1, S2, 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 spectrum of TTPS shows two peaks at 263 and 404 nm assigned to π – π* and n – π* transitions of the conjugated phenyl ring and azomethine group respectively. The absorption spectra of the complexes show only one peak. This peak is assigned to charge transfer from the ligand, TTPS, to the lanthanide ions. The changes in the values of ε also indicate the formation of the Gd(III) complexes.

Table 2: Electronic absorption data of TT, TTPS and [{Gd(OH)$_2$(salen)}$_3$(TT/TTPS)].3H$_2$O

Compound	λ_{max}		$\varepsilon x10^3(mol^{-1}dm^3cm^{-1})$	Band assignment
	nm	cm^{-1}		
TT	278	35971	6.02	π– π*
TTPS	231	43290.04	0.79	π– π*
	263	38022.81	4.94	π– π*
	404	24752.48	0.73	n– π*
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	264	37879	10.8	π– π*
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O	355	28169	14.7	LMCT

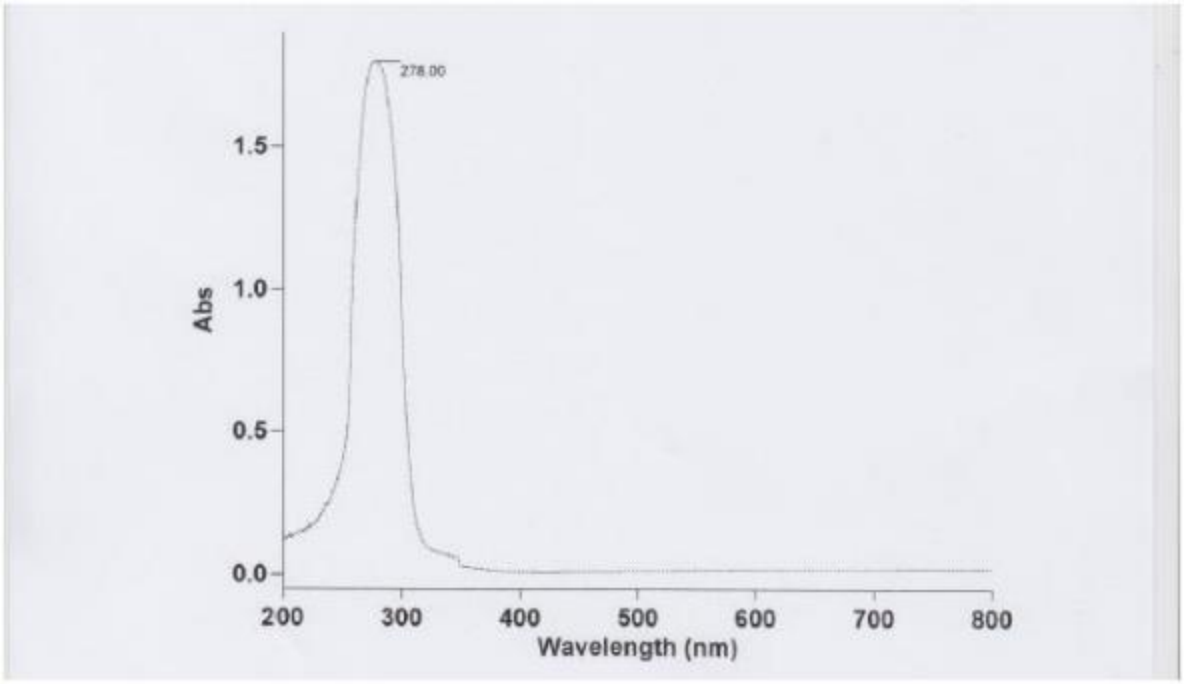


Figure S1: Electronic absorption spectrum TT

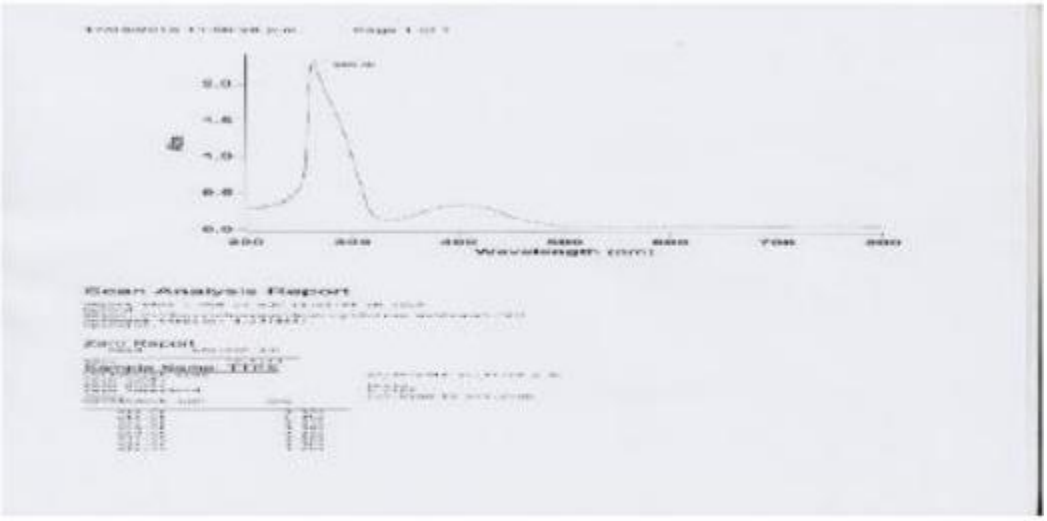


Figure S2: Electronic absorption spectrum TTPS

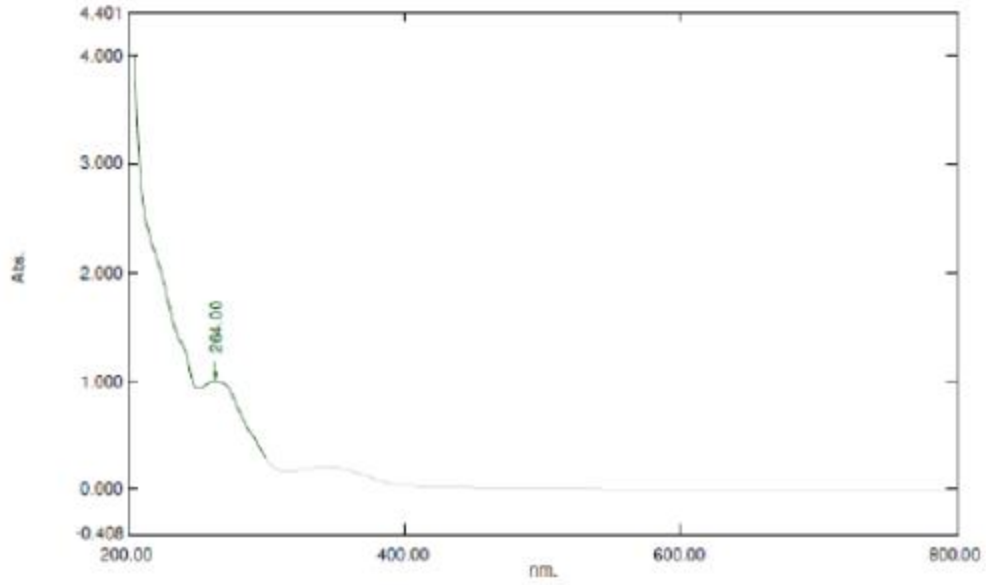


Figure S4: Electronic absorption spectrum of [{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O

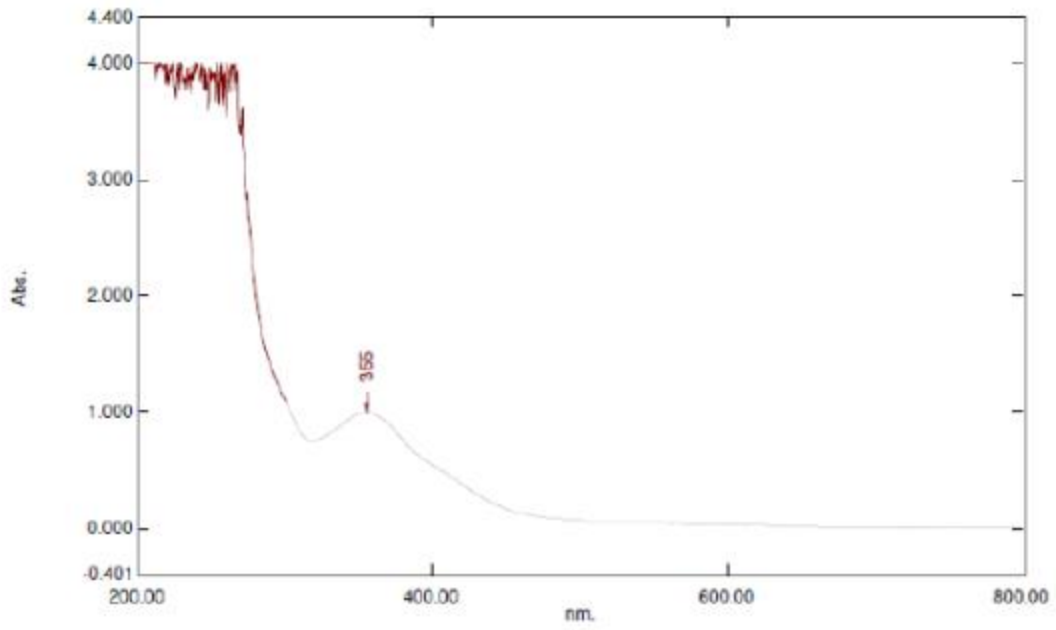


Figure S5: Electronic absorption spectrum of [{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O

Infrared Spectra

The relevant stretching frequencies of the ligands and complexes are shown in Table 3 while the spectra are presented in Fig. 1 and 2 and in supplementary materials (Figures S6, S8 – S10). The FTIR spectrum of [{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O displayed a broad band at 3400 cm^{-1} assigned to vibrations of O-H. The FTIR spectrum of the tripodalSchiff 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 complex. In the complex, the vibration due to C =N showed two bands (b) and (c). The C = N (b) band shifted to higher frequencies of about 37 cm^{-1} in the complex while the C =N(c) band which was absent in the tripodal Schiff base ligand was observed at 1576 and 1530 cm^{-1} in the complex. A similar observation has been made in literature[23]. Furthermore, bands assignable to vibrations due to COO$^-$ groups were observed between 1300 – 1399 cm^{-1} in the complex. The shift in frequency of this band suggests the involvement of the COO$^-$ group in coordination with the Gd metal. This is further supported by the emergence ofmedium to weak bands around 599, 573 and 431 cm^{-1} in the complexes assigned to Ln- O and Ln –N vibrations respectively[2, 35].

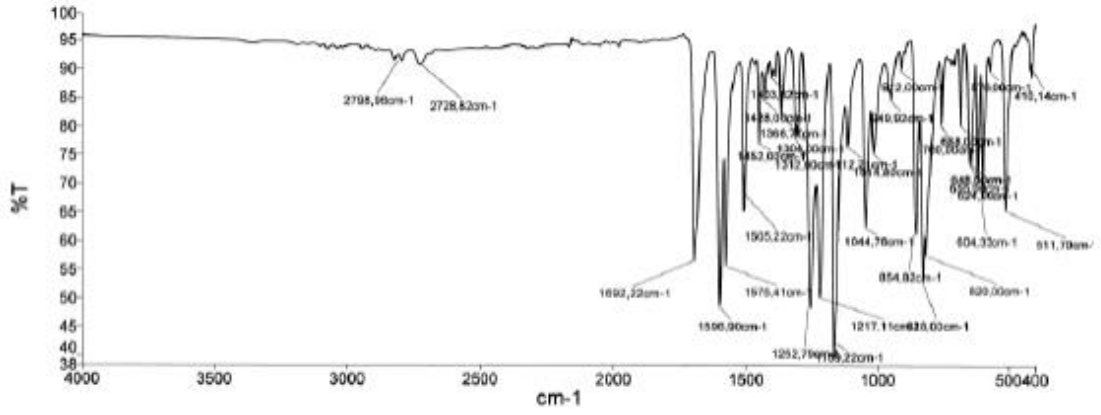


Figure S6: Infrared spectrum of TT

In TTPS, the absorption band observed at 1719 cm^{-1} was assigned to ν (C=O) of carboxylic acid. In the spectrum of the Gd(III)complex, this band shifted to lower frequency suggesting that the

carboxylic acid C = O was involved in coordination to the Gd(III) ions[36].The vibration of the azomethine C=N was observed at 1686 cm^{-1}, 1660 cm^{-1} and 1632 cm^{-1} in TTPS. The spectra of the complex show two chemically different vibrations due to C = N namely: ν (C = N) b and ν (C = N)c. The ν (C = N) b band shifted to lower wavenumber (1593 cm^{-1}) in the complex. The ν (C = N) c band was absent in the ligand but present in the complexes. This confirms that the ligand complexes capped to the COO^- group of the ligand[24]. Similar observation has been made in literature[37]. The carboxylic (COO^-) band was observed at 1420 cm^{-1} in TTPS but changed in intensity and also shifted to lower wavenumber in the complex, suggesting that the COO^- is involved in coordination[23]. Bands assignable to ν (Ln – O) were observed in the region of 544 cm^-

[12, 36].

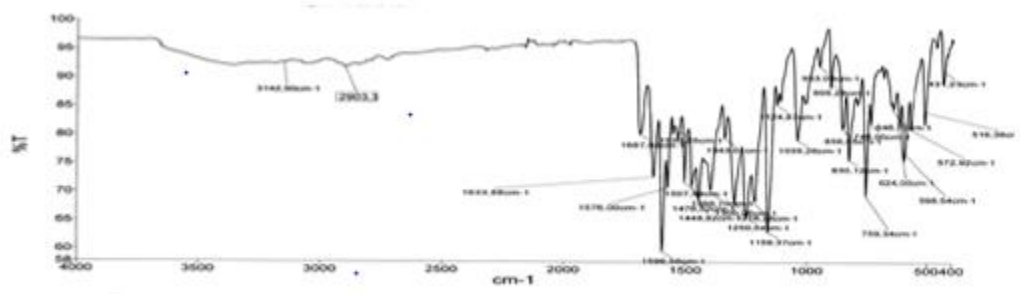


Fig. 1: FTIR Spectrum of [{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O

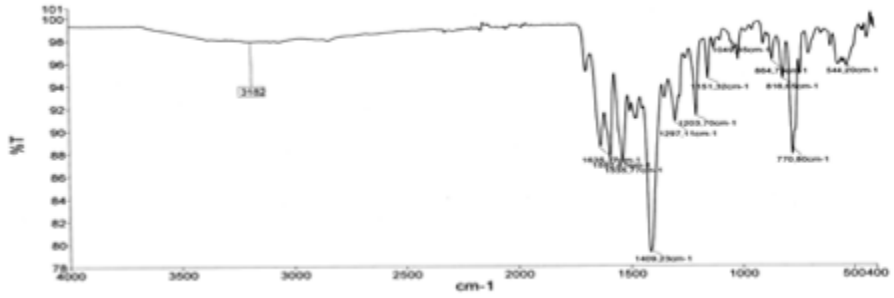


Fig. 2: FTIR spectrum of [{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O

Table 3: IR Band Assignments (cm^{-1}) for TT, TTPS and [{Gd(OH)$_2$(salen)}$_3$(TT/TTPS)].3H$_2$O

Compound	ν O- H	ν C-H ar	ν C =O	ν C =N	ν C – C	ν COO$^-$	ν C –N	ν Ln –O	ν Ln- N
TT			1692(s)	1597(s)b		1367(m)	1159(s)		
						1312(m)	1122(w)		
						1304(m)			
TTPS	-	3140(m)	1719(s)	1686(m)b		1420(m)	1200(s)		
				1660(s)b					
				1632(m)b					
				1526(m)a					

[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	3400(br)	3143(w)	1687(s)	1634(s)b 1576(m)c 1530(m)c	1399(m) 1343(m) 1300(m)	1159(s) 1125(w)	599(m) 573(m)	431(m)
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O	-	3182(w)	1635(m)	1593(m)b 1536(m)c	1409(s)	1151(m)	544(m)	

Where C = N(a)= from triazine ring, 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 the ligands are presented in Tables 4 and 5 while the spectra are presented in supplementary materials (Figures S11 – S114).The ^{1}H NMR spectra of TT and TTPS displayed the signal for azomethine proton and aromatic protons while the complexes showed no signal. This was attributed to paramagnetic nature[38] of the Gd(III) ions. A similar observation was made for ^{13}C NMR spectra of the ligands and complexes.

Table 4: ^{1}H NMR Data of TT, TTPS and [{Gd(OH)$_2$(salen)}$_3$(TT/TTPS)].3H$_2$O

Compound	OH Carboxylic	CH = N	H$_{aromatic}$	CH$_2$ = CH$_2$	H$_2$O$_{coordinated}$	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(2H,s)	2.50
TTPS	13.23(1H,s)	10.07(1H,s), 8.65(1H,s)	8.12 -7.97(H,m) 7.41(H,d), 7.38(H,d)	-	-	-
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	-	-	-	-	-	-
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O						

Table 5: ^{13}C NMR Data of TT, TTPS and [{Gd(OH)$_2$(salen)}$_3$(TT/TTPS)].3H$_2$O

Compound	Carboxylic carbon	Azomethine carbon	Carbons on triazine ring	Aromatic carbons	DMSO peak
TT	191.76	163.58	-	137.51, 132.24, 130.25, 127.30, 115.72	39.91
TTPS	193.44	166.99	-	139.29, 136.05, 130.35, 129.66, 128.73	39.90
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	-	-	-	-	-
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O	-	-	-	-	39.67

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[50]. 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[39]. 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[39].

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

	50 µg/Ml

Compound	*B.c*(ATCC 14579)	*S.a*(ATCC 6538P)	*P.a*(ATCC 9027)	*E.c*(ATCC 6749)	*C.a*	*A.n*
TT	5	3	7	5	-	10
TTPS	21	23	13	13	27	22
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	6	3	2	2	-	6
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O	4	20	8	9	29	23
		25 μg/mL				
TT	2	-	-	-	-	-
TTPS	-	9	8	5	19	-
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	-	-	-	-	-	-
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O	-	10	2	5	22	7
		12 .5 μg/mL				
TT	-	-	-	-	-	-
TTPS	-	5	3	-	5	-
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	-	-	-	-	-	-
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O	-	5	2	4	2	-

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 – 21, 3 – 23, 2 – 13, 2 – 13, 2 - 29, 6 –23 mm respectively. This result reflects that among all the compounds, [{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O exhibits higher activity against *Candida albicans,*and *Aspergillus niger*

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

	MIC (μg/mL)					
Compound	*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
TTPS	50	3.6	6.4	25	2.9	50
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O	50	50	50	50	>50	50
[{Gd(OH)$_2$(salen)}$_3$(TTPS)].3H$_2$O	50	3.8	7.3	7.5	4.8	25
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, TTPS and $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ showed higher activity against the test organisms investigated relative to TT and $[\{Gd(OH)_2(salen)\}_3(TT)].3H_2O$. In view of this, the *in vivo* acute toxicity and anti-malarial studies were conducted on TTPS and its Gd(III) complex. This observation could be linked to the number of hetero atoms in the compounds. The higher the number of hetero atoms in the compound, the more potent antimicrobial the compound. Reports have shown that compounds that have more than three hetero atoms per molecule such as polycyclic fused 1,3,5-triazines possess higher antiproliferative activity[40].

Acute toxicity (LD50).

In the acute toxicity test, death was recorded for some of the mice administered withTTPS and $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ and at concentrations of 2900 and 5000 mg/kg. TheLD50 was calculated. The result obtained was2154 mg/kg. This implies that samples of TTPS and $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ are toxicat doses above 2154 mg/kg.

In vivo antimalarial studies.

Artesunate is anantimalarial drug with molecular formular of $C_{19}H_{28}O$and was used as standard because of the presence of –COOH group in both artesunate and TTPS. The structure is shown in Figure 1. It was observed from Table 8 that the effect of the sample/drugs on packedcell volume and hemoglobin concentration of the infectedmice treated do not show an orderly pattern of dosedependenteffect; however, the effect is significantcomparing the negative control. The effect of sample onhemoglobin concentration shows same effect as in packedcell volume. The result of percentage parasitemia inhibition (Table 9) shows a general dose-dependent significant parasitemia inhibition compared with the negative control with TTPS having inhibition of 72.20% at50 mg/kg and 65.81% at 25 mg/kg and $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ 72.20 %and 70.28 % close to the value(87.22%) of the standard drug, artesunate 5 mg/kg.

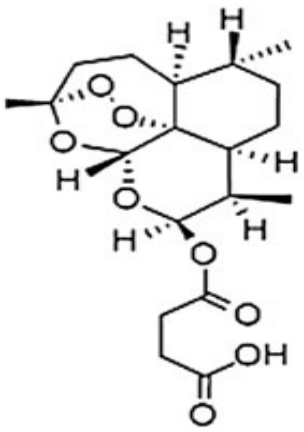


Figure 1: Structure of artesunate.

Table 8: The effect of samples on PCV and Hb on mice infected with Plasmodium berghei.

Drug/dose	PCV1 (%)	PCV2 (%)	%PCVΔ	Hb1 (g/dL)	Hb1 (g/dL)	%HΔ
TTPS 25 mg/kg	57.33 ± 3.48	46.67 ± 4.67	18.67	16.83 ± 0.32	14.70 ± 0.95	12.5
TTPS 50 mg/kg	55.00 ± 4.36	49.00 ± 4.93	10.91	16.40 ± 0.55	15.23 ± 0.90	7.32
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O 25mg/kg	46.67±1.45	39.00±3.21	16.31	15.67±0.203	13.67±0.37	12.82
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O 50 mg/kg	50.67±4.33	42.00±3.21	10.91	15.47±0.95	13.83±0.38	10.34
Artesunate 5 mg/kg	50.33 ± 4.91	44.33 ± 4.48	12.00	15.10 ± 0.71	14.43 ± 0.95	4.64
Distilled water 5 mL/kg	64.67 ± 1.20	51.00 ± 4.62	21.05	17.37 ± .088	15.20 ± 0.72	12.64

Hb, hemoglobin concentration; PCV, packed cell volume.

Table 9: Percentage parasitemia inhibition.

Drug/dose	% Parasitemia	% Inhibition
TTPS 25 mg/kg	10.67 ± 2.60	65.81
TTPS 50 mg/kg	8.67 ± 1.76	72.20
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O 25 mg/kg	9.33±3.33	70.28
[{Gd(OH)$_2$(salen)}$_3$(TT)].3H$_2$O 50 mg/kg	8.67±2.19	72.20
Artesunate 5 mg/kg	4.00 ± 0.58	87..22
Distilled water 5 mL/kg	31.33 ± 3.38	0.00

CONCLUSION

New Gd(III) salen capped complexes bearing N_2O_2 group were synthesized and characterized. Based on analytical and spectral data, the trinuclear Gd(III) salen capped complexes were characterized as being bridged by carboxylate anions to the Gd(III) salen centers and displays a coordination number of eight by involving two hydroxyl groups in the coordination sphere. *In vitro* antimicrobial test indicate thatTTPS and $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ showed higher activity against the test organisms investigated relative to TT and $[\{Gd(OH)_2(salen)\}_3(TT)].3H_2O$. The MIC of these compounds against *Candida albicans* were found to be close to that of Gentamycin(2.5 µg/mL). The *in vivo* antimalarial assay carried out on *Plasmodium berghei* shows a general dose-dependent significantparasitemia inhibition compared with the negative control with $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ having highest inhibition of 72.20% at 50 mg/kg and 70.28% at 25 mg/kg close to the value (87.22%) of the standard drug artesunate 5 mg/kg. Hence, $[\{Gd(OH)_2(salen)\}_3(TTPS)].3H_2O$ can serve as an antimalarial drug at doses less than 2154 mg/kg.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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