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Synthesis, Structural Characterization and Antibacterial studies of new Schiff base 5-(Hydroxymethyl)-4-{(1Z)-[2-N(3-hydroxylphenyl]ethanimidoyl)-2-ethyl}pyridine-3-ol-hydrochloride and its Lanthanide(III) perchlorate complexes

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ABSTRACT

Schiff base, 5-(hydroxymethyl)-4-{(1Z)-[2-N-(3-hydroxylphenyl]ethanimidoyl)-2-ethyl}pyridine-3-ol-hydrochloride (**L**) was synthesized. New series of lanthanide(III) perchlorate complexes of the ligand with the general formula $[Ln(L)_2(ClO_4)](ClO_4)_2$, where Ln = La, Pr, Nd, Sm, Gd, Tb and Er were prepared and characterized based on elemental analysis, molar conductivity, magnetic susceptibility and spectroscopic studies. Conductance study indicates 1:2 ionic ratios for the complexes in solution. Electronic absorption spectra of the complex show intra-ligand and charge transfer transitions. Bonding of the metal ion through N- and O- donor atoms of the ligands is revealed by IR studies and the chemical environment of the protons is also confirmed by NMR studies. The infrared spectra show that Schiff base acts as a bidentate ligand through azomethine nitrogen atom and phenolic oxygen atom. One of the perchlorate ions was coordinated bidentately to the central metal ion while two perchlorate ion remains outside the coordination sphere. The electrolytic behavior of complexes indicates the presence of counter anions ions in side coordination sphere. Based on electronic spectral data and magnetic susceptibility measurements, geometry has been proposed for each complex. Schiff base ligand and their metal complexes have been screened for antibacterial activity by disc diffusion method against eight bacteria (*E.coli, B.cereus, B.subtilis, S. aureus, S.typhimurium, K. pneumonia, Pseudomonas aeruginosa, and Klebsiella sps*). It was shown that the complexes exhibit significantly enhanced antibacterial activity than that of the free ligand.

Keywords: Schiff base complexes, lanthanide perchlorates, biological activity, antibacterial study.

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INTRODUCTION

Schiff base complexes have occupied an interesting and important research area due to their simple synthesis and multifaceted applications. In recent years, there has been an increasing interest in the synthesis of new Schiff base and their complexes with potential and safer antibacterial agents [1]. Schiff bases are well known for their anticancer and antiviral activities and its metal complexes have been widely studied because of their anti-fungal, anti-bacterial, anticancer, anti-tubercular activities, herbicidal applications and chelating abilities [2-4]. Researchers put their efforts in this field to synthesize Schiff bases ligands and their complexes, which possesses antibacterial, antifungal, antimicrobial, anticonvulsant, anti HIV, anti-inflammatory and antitumor activity. Many reports have indicated the use of organometallic in biosciences [5]. Metallo organic compounds are effective as drugs and powerful antimicrobial agents; these are of great importance [6].

The strongly ionic nature of the bonding in solid lanthanide complexes results in coordination geometries that impart a balance between electrostatic and steric demands which determining a factor for complexation. Being buried into the atom, *f* electrons stay away from bonding. Lack of interaction with the 4*f* orbitals minimizes ligand field stabilization energies (LFSE), and promotes a greater lability of the complexes. Great interests have been paid in the chemistry of lanthanide Schiff base complexes in recent years. On account of the pharmacological applications of Schiff base complexes of rare earths; our group is interested in synthesizing new lanthanide complexes and to investigate their antimicrobial properties. This paper reports the synthesis, physico-chemical and spectral characterization of Schiff base derived from pyridoxal hydrochloride and 3-aminophenol and biological study of the ligand and its complexes.

MATERIALS AND METHODS

High purity Pyridoxal hydrochloride (Aldrich, USA), 3-Aminophenol, (E. Merck, India), Nutrient agar, Potato dextrose (Himedia) are purchased and used without further purification. Solvents used are methanol, ethanol, DMF (E. Merck, India). The conductivities of all the complexes measured at room temperature using Toshniwal Conductivity Bridge with a dip type conductance cell with platinum electrode having cell constant 0.9658cm^{-1}. The infrared spectra are recorded on a FTIR spectrophotometer in the range 400-4000cm^{-1} (KBr discs). The electronic spectral data are recorded at 300K in HPLC grade DMF solutions using a Shimadzu UV 160A spectrophotometer in the range 190-900nm at SAIF, CUSAT, Kochi. NMR spectra recorded on a Bruker DRX -500 spectrophotometer using DMSO–d$_6$ solvent. The magnetic susceptibility of the complexes was measured at room temperature using Sherwood Scientific Gouy balance. The Schiff bases and complexes are analyzed for C, H and N content on a Heracus CHN Rapid Analyzer (1104 28) at SAIF, CUSAT, Kochi.

Nutrient agar and Potato Dextrose agar (Himedia) were used as media for bacterial studies. Human pathogenic bacterial cultures were obtained from Department of Marine Biology, Microbiology and Biochemistry, School of Marine science, CUSAT, Kochi and from

Department of Biochemistry, CUSAT, Kochi. Fungal isolates were obtained from the department of Environmental Science, CUSAT, Kochi. The test was carried out by disc diffusion method using different concentrations (0.005M. 0.002M and 0.001M) in DMF extract. Sterile paper discs (5 mm diameter) were soaked each with 100µL of the extract and placed on bacteria (106 CFU/mL) seeded plate. Cultures were incubated at 37^0C for 24h. After incubation of all cultures, zone of inhibitions of bacterial growth were observed. The experiment was done in triplicates and average zone diameter was measured in mm. The fungal culture plates were inoculated and incubated at 25± 20^0C and the diameters (in mm) of the inhibition zones after 48 hrs were measured [7**].**

Synthesis of ligand

5-(Hydroxymethyl)-4-{(1Z)-[2-N-(3-hydroxylphenyl]ethanimidoyl)-2-ethyl}pyridine-3-ol-hydrochloride (**L**) was synthesized by adding methanolic solution of 0.05molar pyridoxal hydrochloride to 0.05molar solution of 3–aminophenol. The reaction mixture was refluxed on a water bath for about 5–6 h, crystalline solid product formed was washed with ether followed by acetone and finally recrystallized from absolute alcohol [8]. (yield 56%; m.p 150^{o}C, %C–57.65(57.05),H–4.85(5.13), N–10.1(9.50), UV(DMF) –(nm) 266, 340; IR(KBr/ nujol) ν_{max} – (cm^{-1}) 1602,1317,1203, 3600, 1027, 3100; ^{1}H NMR(DMSO,400 MHz)–δ = 8.2, 6.4,3.4,9.8ppm.

Synthesis of complexes

All the seven complexes (complexes **a-g**) were prepared by mixing a methanolic solution of $Ln(ClO_4)_3.nH_2O$, where Ln = La, Pr, Nd, Sm, Gd, Tb and Er with Schiff base in the same solvent in a 1:2molar ratio. The resulting mixture was refluxed on a water bath for 5h. Solid products separated were collected by filtration, washed with acetonitrile followed by ether, dried *in vacuum* over P_4O_{10} [9].

RESULTS AND DISCUSSION

All complexes were dark brown colored and hygroscopic in nature. The complexes were found to be soluble in methanol, ethanol, acetone, DMF, DMSO and insoluble in acetonitrile, benzene, carbon tetrachloride, and nitrobenzene. The electrical conductance measurements were carried out for all the complexes in methanol, ethanol, acetone and DMF. The conductance values for the perchlorate complexes were presented in Table 2. The electrical conductance values (in ohm^{-1}mol^{-1}cm^2) are in the range 160-200, 70-100, 130-170 and 160-200 for methanol, ethanol, DMF and Acetone respectively. These values suggest that these complexes are having a 1:2 electrolytic type [10]. Hence these complexes **a-g** may be formulated as $[Ln(L)_2ClO_4](ClO_4)_2.$ Where Ln = La, Pr, Nd, Sm, Gd, Tb and Er.

The metal content for all the complexes were determined by reported methods [11]. The percentage of carbon, nitrogen and hydrogen were analyzed on Analytic Functional Testing VarioEL III CHNS 11035060. The data obtained for the all complexes were summarized in Table 1. Magnetic moment values revealed a paramagnetic nature for all other complexes, except lanthanum, which lacks unpaired electron. The observed values of magnetic moments were found to be lower than those expected and were compared with the values calculated for free ions (Van-Vleck values) or free ions. The results suggest less interaction of 4*f* with the environment owing to the spatial confinement [12].

Table.1 Analytical data of complexes a-g

Complex	color	Yield (%)	µ(eff)	%M	%N	%C	%H
a	Brown	65	--	12.2(12.5)	5.1(5.0)	30.1(28.9)	3.8(3.2)
b	Brown	60	2.35	12.1(12.7)	5.6(5.1)	30.5(28.4)	3.1(3.3)
c	Brown	56	2.79	11.9(12.9)	6.1(5.0)	28.3(27.9)	3.6(3.3)
d	Brown	58	3.95	12.1(13.4)	6.5(5.1)	29.3(27.8)	3.2(3.3)
e	Brown	55	6.85	12.1(13.9)	5.6(5.0)	27.6(27.9)	3.0(3.2)
f	Brown	58	7.55	12.6(13.2)	6.7(4.9)	28.9(27.9)	3.0(3.2)
g	Brown	58	8.89	12.5(12.8)	6.8(5.2)	29.1(27.3)	3.6(3.0)

Table.2 Molar conductance of complexes a-g

No	Complex	Conductance (ohm^{-}1mol^{-1}cm^{2})			
		DMF	MeOH	EtOH	Acetone
a	$C_{38}H_{45}N_8O_{19}Cl_5La$	180	170	85	180
b	$C_{38}H_{45}N_8O_{19}Cl_5Pr$	175	185	88	190
c	$C_{38}H_{45}N_8O_{19}Cl_5Nd$	165	210	90	180
d	$C_{38}H_{45}N_8O_{19}Cl_5Sm$	160	200	82	175
e	$C_{38}H_{45}N_8O_{19}Cl_5Gd$	150	185	82	170
f	$C_{38}H_{45}N_8O_{19}Cl_5Tb$	185	188	80	180
g	$C_{38}H_{45}N_8O_{19}Cl_5Er$	180	185	90	180

Spectral Investigations

Infrared spectral analysis

The important infrared spectral bands of the ligand and its complexes are displayed in Table 3. The prominent vibrational frequency bands in the ligand were observed at 1602cm^{-1}, attributed to C=N(azomethine group), another guide peak at 1317cm^{-1} attributed to phenolic C-O (heterocyclic ring), two other medium intensity bands observed at 1245cm^{-1}, 1190cm^{-1} attributable to C-O (phenolic) and C-O (alcoholic) respectively [13].

In all the complexes, the C=N stretching band observed at 1602cm^{-1} suffer a negative shift to an extent of +15cm^{-1} indicating the involvement azomethine group in coordinate bond formation. The medium intensity frequency band of phenolic C-O observed at 1245cm^{-1} and 1190 cm^{-1} remain unaffected in position and intensity suggesting the non-participation of these groups in complexation. The band displayed at 1317cm^{-1} was shifted to ±45cm^{-1} which indicates a coordinate bond is formation at this oxygen atom. Broad bands in the range 3540-3560cm^{-1} and 3410-3480cm^{-1} indicate the presence of water coordinated to the metal ion. Appearance of δ(OH) in the region 1350-1360cm^{-1} and another band at 900cm^{-1} attributable to the out of plane bending of (OH) [14]. Medium bands in the region 1400-1600cm^{-1}are attributed to pyridyl in plane vibrations and bands displayed at 3150-3200cm^{-1} ascribed to N-H stretching vibrations practically remain unshifted suggesting the nonparticipation of the heterocyclic nitrogen atom in coordinate bond formation.

Non-ligand bands were displayed by all the complexes. The triply split band maxima at ~1140-1150cm^{-1}, ~1110-1115cm^{-1} and~ 1020-1030cm^{-1} are the ν_8, ν_6, ν_1 modes respectively of coordinated perchlorate of C_{2v} symmetry [14]. In addition, strong bands in the range 928-945cm^{-1} and at 630-635cm^{-1} assignable for ν_2 and ν_3 vibrational modes of bidentately coordinated perchlorate group of C_{2v} symmetry were also observed. In addition, very strong and unsplit band at 1078-1090 cm^{-1} region were appeared in all complexes attributable to ν_3 mode of perchlorate ion suggesting that there are perchlorate group present outside the coordination sphere. Presence of ionic perchlorate group was further supported by the appearance of strong band in the region 620-624cm^{-1} in all the complexes. Appearance of a new band in the region 370-380cm^{-1} and 420-460cm^{-1} assignable for Ln-N and Ln-O further support the coordinate bond formation to metal ion [15,16].

Table.3 IR spectral data (cm^{-1}) of complexes a-g

Complex	C=N	C-O^1	C-O^2	O-H^1	O-H^2	Ln-O
a	1636	1345	1245	3548	3279	380
b	1664	1365	1245	3546	3245	379
c	1668	1340	1245	3540	3288	375
d	1638	1363	1245	3555	3210	381
e	1609	1358	1245	3570	3269	370
f	1615	1350	1245	3560	3268	372
g	1603	1338	1245	3550	3280	369

UV–visible spectra of complexes

The solution state electronic spectra of complexes in DMF shows a red shift in the $\pi \rightarrow \pi^*$ transition in the region 270-275nm and the $n \rightarrow \pi^*$ transitions occurred in the range 345-358nm confirming the coordination of the ligand to the metal ions. Bands due to *f-f* transitions are observed in complexes **d, e** and **g**. The electronic spectral data of selected complexes and their tentative assignments were displayed in Table 5. The band near 420-450nm is assigned to a combination of $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the azomethine and other chromophores. The observed bathochromic shift gives strong support for the coordination of the azomethine nitrogen [16].

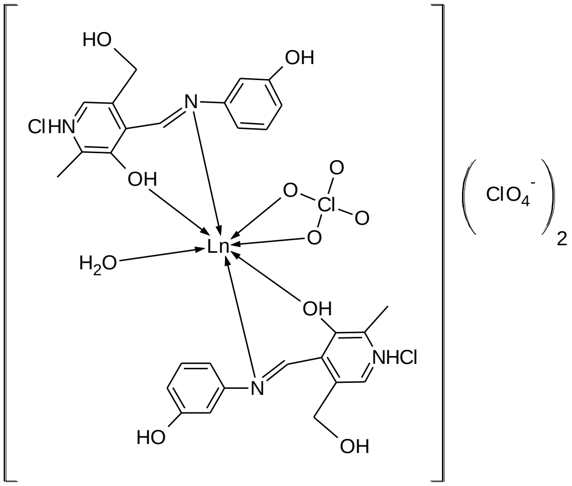
The red shift of the hypersensitive bands with respect to aqua ions was utilized to calculate the nephelauxetic effect (β). Spectral parameters like the nephelauxetic ratio β, ($\beta = \nu$ (complex)/ν (aquo), the covalency factor $b^{1/2}$, Sinha Parameter δ and covalency angular overlap parameter η, have been calculated from the hypersensitive transition of the lanthanide(III) ions. The values for Nd, Sm and Er were illustrated in Table 5. The positive value of (1-β) and δ in these complexes suggest the covalent nature of metal ligand bonding [17, 18].

Table 5. ***f-f*** **transitions of complexes d, e and g**

Complex	$E_{max(complex)}$	$E_{max(aquo)}$	Terms assigned	β	$b^{1/2}$	δ%	η
d	21276 19047 21997	21360 19100 22117	$^4I_{9/2}\rightarrow{}^2G_{9/2}$ $^4I_{9/2}\rightarrow{}^4G_{7/2}$ $^4I_{9/2}\rightarrow{}^4G_{11/2}$	0.9960 0.9972 0.9945	0.0313 0.0263 0.0368	0.3948 0.2782 0.5455	0.0628 0.0527 0.0738
e	17762 16584 15361	17972 16680 15470	$^4G_{5/2}\rightarrow{}^6H_{5/2}$ $^4G_{5/2}\rightarrow{}^6H_{7/2}$ $^4G_{5/2}\rightarrow{}^6H_{9/2}$	0.9883 0.9942 0.9929	0.0540 0.0379 0.0419	1.1823 0.5788 0.7095	0.1087 0.0760 0.0842
g	15310 19230 20530	15480 19340 20640	$^4I_{15/2}\rightarrow{}^4F_{9/2}$ $^4I_{15/2}\rightarrow{}^4F_{11/2}$ $^4I_{15/2}\rightarrow{}^4H_{7/2}$	0.9890 0.9943 0.9943	0.0523 0.0377 0.0377	1.1103 0.5720 0.5720	0.1053 0.0756 0.0756

NMR studies

The diamagnetic lanthanum complex exhibit signals at lower fields compared to ligand. The downfield shift in the resonance of these protons compared to the ligand are the consequences of coordinate bond formation with OH and HC=N. In the ligand, signals of azomethine hydrogen was observed at δ = 8.1ppm. In the complex, the signals are recorded at δ= 8.3ppm suggesting coordination through azomethine group. Signals appeared as multiplets at 6.8 to 7.3ppm attributable to aromatic protons also displayed downfield shift in the region δ = 6.9ppm to δ = 7.6ppm in the complex suggest electronic changes due to coordination. Singet observed at 3.6ppm attributed to OH (alcoholic) practically remain in complex also suggesting non-participation of this OH. Another singet observed at 6.6ppm was appeared in complex also suggesting non-deprotonation of the phenolic OH during complexation [19]. Based on the experimental evidences, tentative structure is proposed for the complexes (Fig I).



Anti bacterial study

Nutrient agar medium was prepared as per the methods described in literature [20] and autoclaved at 120°C and 15kg/cm^2 pressure. This was then allowed to cool to 60°C 20- 25 mL of this solution was introduced into petri plates. When attained room temperature, bacterial suspension of 20µL was added and swabbed and kept for 15minute for uniform spreading. Among the seven complexes synthesized, six of them were selected for antibacterial study (complexes **a-f**).

Inhibition against *E.coli*

Among the six investigated complexes, a promising activity against *E.coli* was displayed by complex **g.** It displayed an inhibition zone of 20mm diameter. Except **c**, all complexes show higher activity compared to ligand. Complex **c** exhibited an activity of 8mm. Complexes **a, b** and **d** exhibited inhibitory activity comparable to that of ligand with zone of inhibition 10mm each.

Inhibition against *Pseudomonas aeruginosa*

Among the six complexes, only two of them (complexes **d** and **f**) exhibited inhibitory activity against this bacterium. Complex **f** was found to be moderately active with inhibition zone 12mm while **d** displayed an inhibition comparable to that of ligand. The free ligand exhibited a zone with diameter 8mm.

Inhibition against *Bacillus cereus*

The results indicated the efficacy of complex **f** against this bacterial strain. This complex exhibited a marginal enhancement in the inhibitory activity compared to the free ligand. The inhibition exerted by this complex against *B. cereus* was measured to be 15mm. Complexes **a** and **d** displayed moderate activity where as **c** show a minimum activity.

Inhibition against *Klebsiella sps*

The free ligand was inactive against this organism. Only two of the six complexes (complexes **d** and **f)** were shown to have inhibitory activity against *Klebsiella sps,* with measured inhibition zone of 8mm each.

Inhibition against *Staphylococus aurous*

The ligand was found to be inactive against this organism. All of the six complexes assayed exhibited activity against this species. A promising inhibitory activity was displayed by complex **f** , the zone of the inhibition was observed to be 21mm. Medium activity were exhibited by **a** and **c** . Low activity was displayed by **b** and a moderate activity was displayed by **d**.

Inhibition against *B. subtilis*

The free ligand displayed no activity against this bacterium. All complexes exhibited inhibitory property in a medium range. Among the complexes, very low activity was displayed by complex **b**. Complex **f** was the one among the five having higher activity compared to other complexes. Complexes **a, c** and **d**- exhibited clear zone of 8mm diameter. Complex **b** show a clear zone of 5mm diameter and complex **f** -exhibited a clear zone of 10mm.

Inhibition against *Klebsiella pneumonia*

Ligand exhibited an inhibition zone of 12mm against this bacterial strain. Compared to this none of the six complexes displayed any enhancement in their activity towards this organism. Complex **a** and **f** were the two complexes which are found to be active but the inhibition zone were found to less than that of the ligand, 10mm and 12mm respectively for complexes **a** and **f**

Inhibition against *Salmonella Typhimurium*

Ligand was inactive against this organism. The results of the evaluation show that three of the complexes exhibited inhibitory activity against this species. According to the results obtained, among the three, the most active one was complex **f** with zone of inhibition18mm, the other two complexes **a** and **d** displayed moderate activity of 10mm each.

Table 6. Antibacterial study of Ligand and complexes **a, b, c, d,** and **f**

compound	*E.coli*	*Pseudomonas aeroginosa*	*B. cereus*	*Klebsiella sps*	*Staphylococcus aurous*	*B.subtilis*	*Klebsiella pneumonia*	*Salmonella typhimurium*
L	9	8	13	-	-	-	12	-
a	10	-	11	-	8	8	10	10
b	10	-	-	-	6	5	-	-
c	8	-	7	-	8	8	-	-
d	10	8	10	8	10	8	-	10
f	20	12	15	8	21	10	12	18

CONCLUSION

A new Schiff base ligand and its lanthanide complexes were synthesized and characterized by various physical and spectral methods. The Schiff base and its complexes exhibit varying antibacterial activity against human pathogenic bacteria. The variation in the effectiveness of the different compounds against different organisms depends on their impermeability of the microbial cells or on the difference in the ribosome of the microbial cells. All the complexes show better antibacterial activity when compared to the free ligands and metal precursors. Terbium complex was shown to be active against all organisms evaluated. This complex can be used as an effective toxic agent against *P.aeruginosa* and *S.aureus.*

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