



Schiff Base, 6-Amino-2-[(4-(Dimethylamino) Benzylidene)Amino]Hexanoic Acid and its Lanthanide (III) Complexes have Antioxidant and Antimicrobial Activities

Mehwish Aftab, Tariq Mahmud*, Muhammad Asim Raza Basra, Aqsa Gulzar, Sumaira Basharat and H.M. Junaid

Institute of Chemistry, University of the Punjab, Lahore

ABSTRACT

The titled Schiff base, 6-amino-2-[(4-(dimethylamino)benzylidene)amino]hexanoic acid was synthesized by condensing *N,N*-dimethyl aminobenzaldehyde and Lysine together using ethanol as a solvent. The Schiff base was isolated and further reacted with $Ce(NO_3)_3 \cdot 6H_2O$ and $La(NO_3)_3 \cdot 6H_2O$, to afford their respective complexes. FTIR, 1H NMR, UV-Vis and ICP techniques were used for the characterization and structure elucidation of the prepared Schiff base and its La (III) and Ce (III) complexes. The proposed geometries of the complexes were established on the basis of metal ligand ratio through ICP. Schiff base showed antioxidant activity against diphenylpicrylhydrazyl (DPPH). The results showed that metal complexes are good antioxidant agents as compared to Trolox (standard) and ligand was less active as compared to Trolox. The Schiff base ligand and its metal (III) complexes showed antibacterial activity against *E. coli*, *S. aureus* by disc diffusion method and antifungal activity against *Macrophomina phaseolina*, *Fusarium oxysporum*. The metal (III) complexes are highly active under permissible limits.

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Authors' Contribution

TM conceived and designed the experimental scheme. MA executed the experimental work and wrote the article. MARB helped in performing antioxidant activities. TM and HMJ did characterization and elaboration of data. AG and SB helped in antibacterial activity studies.

Key words

Schiff base, Metal complexes, DPPH, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Schiff base was first of all prepared by Hugo Schiff in 1864 by reacting aldehyde and ketones with primary amine. These compounds have a specific functional azomethine group represented by $-C=N-$ in which the nitrogen atom is bonded to aryl or alkyl group (IUPAC, 2014). The Schiff bases are usually crystalline solids, which are slightly basic but few of them form insoluble salts by reacting with strong acids. Schiff bases prepared from aromatic amines and aromatic aldehydes show diversity of applications in many fields, e.g., analytical chemistry, biological and inorganic (Elmali *et al.*, 2003; Perry *et al.*, 1979; Singh *et al.*, 1980; Thaker *et al.*, 1996). Schiff base ligands play important role in the synthesis of Schiff base complexes, since they have ability of forming stable complexes with metal ions (Arquero *et al.*, 1985). Free amino acids in living body play an important role in many physiological activities of the human body, for example D,L-homocysteic acid (DLH) initiate cerebral activities and has been proposed as an agonist of endogenous glutamate receptors in the mammalian central nervous system (Adabiardakani *et al.*, 2012; Do *et al.*, 1986;

Haglerød *et al.*, 2009; Jia and Li, 2015). Amino acids have the ability to coordinate with the metal ions because of two donor sites *i.e.* amino group and carboxyl group (Frauscher *et al.*, 1995). It is known that the presence of metal ions bonded to ligand which is biologically active compound increases their working efficiency (Chohan *et al.*, 2005; Jayabalakrishnan *et al.*, 2003; Temperini *et al.*, 2005; Ul-Hassan *et al.*, 2004; Wang *et al.*, 2006). Novel Schiff bases have been shown to have anti-cancer and anti-inflammatory activity (Gulzar *et al.*, 2018). The Schiff base prepared here has anti-oxidant and anti-microbial activity.

MATERIALS AND METHODS

Synthesis of ligand (L)

The ligand was prepared with reacting lysine with aldehyde. The amino acid used was lysine and the aldehyde *N,N*-dimethyl aminobenzaldehyde (Fig. 1).

In a test tube 2-3 drops of dimethyl amine and 0.146 g of lysine was added in 10 ml of distilled water. In the second test tube 0.149 g of aldehyde (*N,N*-dimethyl aminobenzaldehyde) was added in 10 ml ethanol to make 0.01 M solution. This mixture was taken in round bottom flask and refluxed in round bottom flask at 80°C for 4-8 h. The orange color precipitates formed was washed recrystallized in DCM:Methanol (2:3 ratios) in a test tube. These crystals were used for formation of metal complexes.

* Corresponding author: tariqm06@yahoo.co.uk
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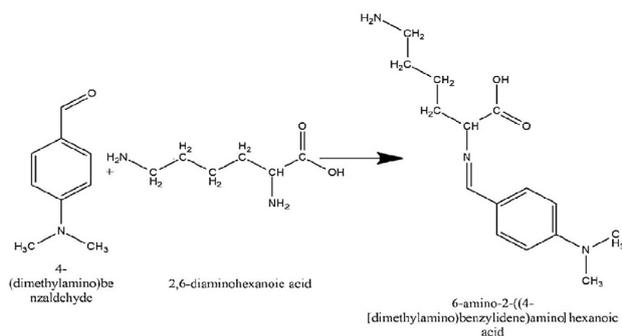


Fig. 1. Synthesis of ligand molecule.

Preparation of metal- L complexes

To make complex with La (III), two solutions 0.476 g of $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ dissolved in 10 ml ethanol in was mixed with 0.554 g of Schiff base was dissolved in 10 ml a round bottom flask and was refluxed at 90°C for 4 h precipitates of dark brown color was formed after 4 h.

For complexing with Ce (III), two solutions 0.434 g of $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ dissolved in 10 ml ethanol and 0.554 g of Schiff base dissolved in 10 ml ethanol were mixed in a round bottom flask and then refluxed at 90°C for 8 h on a hot plate/magnetic stirrer with constant stirring using water condenser. Light brown color precipitates formed after 4 h, were filtered off and washed with ethyl alcohol. These were dried at 60°C and used for characterization of the metal complexes.

Characterization of metal complexes

For estimation of La (III), sample solutions were prepared by dissolving 0.1 g of complex in 0.9 ml water to which then 2 to 3 drops of HNO_3 were added; 1ml of this solution was diluted up to 5 ml with distilled water. Metal complexes were characterized by using ICPEs, FTIR and NMR spectroscopy. Standard solutions of 1, 3, 5 ppm were prepared from the Stock solution (1000 ppm) of La^{+3} and Ce^{+3} .

Antibacterial activity

The ligand and its metal complexes were tested for their anti-bacterial activity against two bacterial species *i.e.* *E. coli* and *Staphylococcus aureus* by using disc diffusion method (Amiri-Rudbari *et al.*, 2016; Dupont and Drouhet, 1979). The ligand and metal complexes were dissolved in 30 $\mu\text{g}/\text{ml}$. DMSO was applied to a paper disc using micropipette. These discs were placed in autoclave for two hours. Transferred in the center of each Petri plate with the help of sterilized forceps and incubated for overnight at 37°C . The zone of inhibition was measured in mm around the center of the disc.

Antifungal activity

All of the ligands and their metal complexes were tested for their anti-bacterial activity against tow bacterial species *i.e.* *Macrophomina phaseolina* and *Fusarium oxysporum*. The ligand and its metal complexes were dissolved in 30 $\mu\text{g}/\text{ml}$ of DMSO, and then transferred into a test tube containing 10 ml of malt extract media. Incubation of the test tube was performed for 3-6 days at a temperature 28°C in the incubator. Then comparative studies of fungal growth in control and sample test tubes were recorded.

Antioxidant activity

0.15ml of ten different concentrations of Trolox *viz.*, 1000 mM, 500 mM, 250 mM, 125 mM, 62.5 mM, 31.25 mM, 15.62 mM, 7.81 mM, 3.9 mM and 1.95 mM was taken in 12 brown bottles of 20ml. To each bottle 1.85ml of 0.05 mM ethanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was added. The OD of mixture was taken at 517nm after 15 min, 30 min, 45 min, 1 h and then 2 h to prepare a standard curve. Similar types of dilutions of samples were prepared.

RESULTS AND DISCUSSION

The ligand (L) and its metal complexes were crystalline solid. Their colors and melting points and percentage of compounds are shown in Table I.

The ligand and its complexes are insoluble in water but soluble in organic solvents like alcohol, chloroform, acetone and DMSO. They are partially soluble in methanol and ethanol.

For culture measuring conductance of metal complexes, the ligand and its metal (III) complexes were dissolved in DMSO to prepare 10^{-3} M molar solution. The conductivities of these solutions were measured at room temperature. The conductance values are very small which showed that M (III) complexes are non-ionic in nature and non-electrolytes (Table I).

Table I.- Physical properties and conductance of ligand and its complexes.

Compound	Physical properties			Conductance ($\mu\text{S}/\text{cm}$)
	Color	Melting point	Yield (%)	
Ligand (L)	Orange Red	49°C	75	12.67
La-Ligand	Shiny Yellow	103°C	60	32.4
Ce-Ligand	Dark Brown	98°C	72	26.78

FTIR spectra of Schiff base and its metal complexes

A comparative study of the FTIR spectra was made

between the prepared ligand and its metal complexes. The confirmation of azomethine group (HC=N) was made possible by the appearance of a new band at 1595-1610 cm^{-1} . It confirmed the formation of Schiff base. FTIR data obtained for the ligand and its metal complexes depicted strong absorption bands for C-H(stretch), C-N(stretch), C-O-C (stretch) with high absorption intensities indicated the complex formation. The peaks shifted to lower wave number in case of many synthesized metal complexes (Table II).

Table II.- FTIR bands of ligand (L) and its metal complexes.

Band frequency	Ligand (L)	Ce-Ligand (L)	La-Ligand (L)
C-H	727(s)	690(s)	596(s)
C-C	2908(s)	3093(m)	3047(m)
C-N	1068(s)	1226(s)	1065(s)
C=N	1670(m)	1676(m)	1666(m)
C=C	1560(m)	1580(m)	1580(m)
N-H	3315(m)	3327(m)	3321(m)
COO ⁻	1597(m)	1560(m)	1580(m)

Estimation of metals by ICP

In order to determine the metal to ligand ratio, the concentration of metals in the synthesized metal complexes was determined by inductively coupled plasma (ICP) spectrophotometer (Table III). Then the metal complex was determined by comparing theoretical percentage with experimental percentage. This ratio has also helped in processing the structure of metal complexes.

Table III.- Emission of La-Ligand (L) and Ce-Ligand (L) complexes by ICP.

Concentration (ppm)	Emission(cps)	
	La-Ligand (L)	Ce-Ligand (L)
1	1218773.2	1275364.4
3	7790006.8	4320076.6
5	14093181.5	6744202.7
LaL and CeL ₂	49199362.9	67879659.2

Table IV shows metal to ligand ratio for La (III) and Ce (III) complexes. For La (III), the experimental percentage of 17.00% is very close to 17.0%. So metal to ligand ratio is 1: 2. For Ce (III), the experimental percentage of 31.5% is very close to 33.5%. So the metal to ligand ratio is 1:1.

¹H NMR spectra

The NMR spectra of Schiff bases were recorded in dimethylsulfoxide (DMSO) solution, using

tetramethylsilane (TMS) as standard. The signals observed in the Schiff bases under study are shown in Table V. The spectra exhibit a multiplet at 7.684, 7.655, 6.760 and 6.789 ppm for the hydrogen of the aromatic rings. The azomethine hydrogen (-CH=N-) produces a singlet to a singlet of intensity equivalent to one hydrogen at 8.32-9.71 ppm (Issa et al., 2008). The spectra of ligand showed the singlet peak at 9.650 ppm which confirmed the formation of azomethine group or Schiff base. In metal complexes this peak is shifted to 9.657 ppm in Ce(L) complex and 9.654 ppm in La(L) complex this is because the presence of lanthanide which had empty shell and hydrogen atom of azomethine group is deshielded. The spectra of showed quartet due to presence of CH₂ at 1.061ppm 1.015 ppm, 1.038 ppm and 2.487 ppm. In spectra CH peak appear at 2.938 ppm. The peak of the CH₃ group appeared at 3.547 ppm and 3.434 ppm. The peak of N-H₂ appears in the spectra of ligand at 2.493ppm. The hydrogen of the free OH-group didn't appear due to the formation of carboxylate ion during salvation process.

Table IV.- Metal to ligand ratio for La(III) and Ce(III) complexes.

M:L (Metal to ligand ratio)	Theoretical percentage	
	La(III) complex	Ce(III) complex
1:1	33.31	33.50
1:2	17	20.12
1:3	11.10	14.379

Table V.- Proton NMR spectral data of the Schiff base.

Functional groups	Chemical shift, (δ) ppm
	Ligand (L)
CH ₃ -N	3.457
Benzene H	7.648
Benzene H	6.798
HC-N	2.938
CH-	2.493
CH ₂ -	1.038
CH=N	9.650
NH ₂ -	2.493
₂ NH-CH ₂	2.487
CH ₂ -	1.051

¹³C NMR spectra

The ¹³CNMR spectra (Fig. 2B) of the ligand verify the results of ¹HNMR. In spectra the peak of azomethine group appears at 160.43. the carbon atom of the benzene ring appear at 111-124 the peak of methyl carbon appear

at 40.326 the peak of carboxylate carbon appear at 198 the peak of CH₂ appear at 26-34.

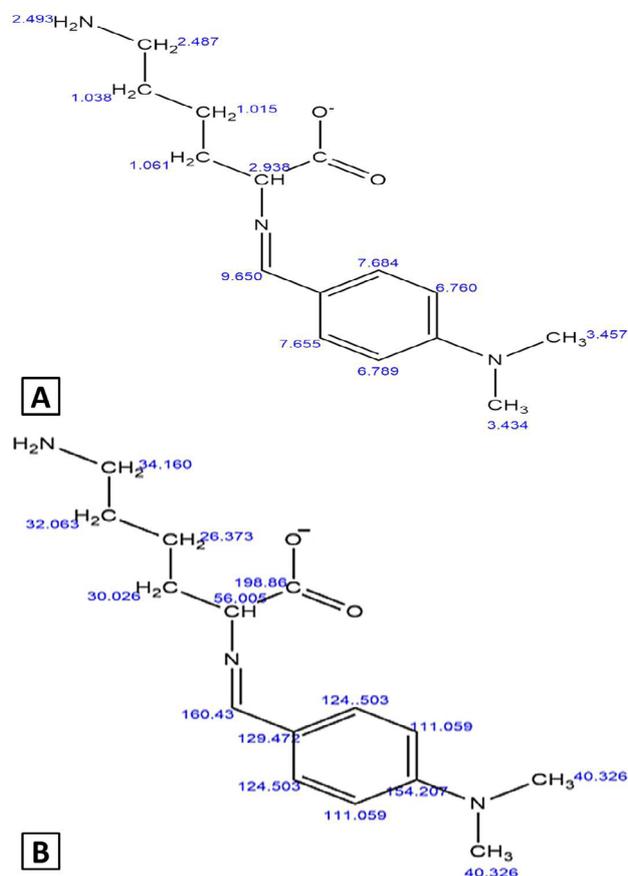


Fig. 2. Values of ¹H NMR (A) and ¹³C NMR (B) in ligand molecule.

Antioxidant activity

Figure 3 shows that complexes of lanthanum and cerium were more active as compared to their parent ligand. The increased antioxidant activity of the metal complexes due to electron withdrawing effect of metals which accelerates the discharge of hydrogen to reduce the DPPH radical. The DPPH radical scavenging capacity of the compounds could be represented in order of increasing anti-oxidant activity.

La-Ligand (L) > Ce-Ligand (L) > Trolox > Ligand (L)

Due to high inhibition showed on the DPPH radical by the ligand and its metal complexes exhibit that these compounds had ability of donating electrons and neutralize free radicals so these could be used as drugs for the treatment of pathological diseases which are produced by excess free radicals in case of stress.

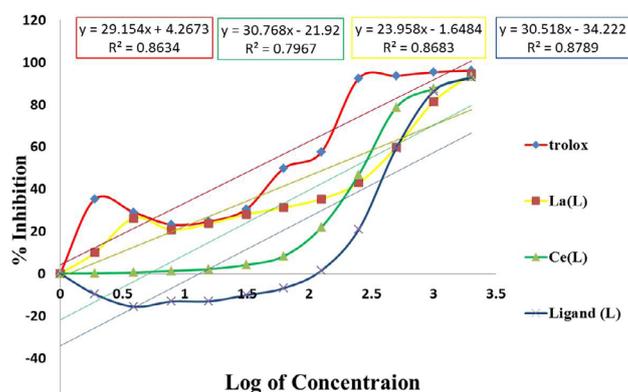


Fig. 3. DPPH scavenging activity of ligand (L) and its metal complexes.

Anti-microbial Activity

The results showed that antimicrobial activity tested for ligand as well as their metal complexes and inhibition zone diameter was noted in mm. Ligand and their complexes are biologically active. Ligand L is moderately active while its complexes are highly active against bacterial species and moderately active against fungal species. Table VI shows concentration of various compounds having IC₅₀ values at different times. Table VII shows antimicrobial activities of various ligands and their metal complexes.

Table VI.- Comparison of IC₅₀ of all compounds at different time.

Compounds	Variation of IC ₅₀ (µg) with different time				
	15 min	30 min	45 min	60 min	120 min
Trolox	225.12	216.25	223.17	209.39	252.73
Ligand (L)	526.12	502.65	496.24	451.20	425.15
La-Ligand (L)	209.36	195.2	190.08	138.177	136.73
Ce-Ligand (L)	222.50	221.36	238.30	234.44	215.55

Table VII.- Antimicrobial activity of ligand and its metal complexes.

Compounds	<i>M.</i>	<i>F.</i>	<i>E.</i>	<i>S.</i>
	<i>phaseolina</i>	<i>oxysporum</i>	<i>coli</i>	<i>aureus</i>
Ligand (L)	+	+	+	+
La-Ligand (L)	++	++	+++	+++
Ce-Ligand (L)	++	++	+++	+++

+, 1-10 mm zone of inhibition; ++, 11-20 mm zone of inhibition; +++, 21-40 mm zone of inhibition.

CONCLUSION

The present study provides an easy protocol for the production of Schiff base 6-amino-2-[(4-(dimethylamino)benzylidene)amino]hexanoic acid and its lanthanide (III) complexes. The mode of bonding and overall structures of the complexes were determined through physico-chemical and spectroscopic methods. Spectroscopic techniques well supported to our proposed structure. Complex formation study via molar ratio was investigated and results were consistent to those found in the solid complexes with a ratio of (M:L). Anti-oxidant and anti-microbial activities of the synthesized Schiff base ligand and its metal complexes were studied.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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