RJC

RASĀYAN ***J. Chem.***
Vol. 12 | No. 3 |1455 - 1462| July - September | 2019
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES ON 4-BROMO-2-{(Z)-[(FURAN-2-YLMETHYL) IMINO]METHYL}PHENOL PRASEODYMIUM COMPLEX

B. Preethi[1, 2], Sarojkumar Sha[4], R. Jayaprakash[3], S. Kutti Rani[1,*] and S. Hemalatha[4],

[1]Department of Chemistry, B.S.Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai-600048, (Tamil Nadu), India.
[2]Department of Chemistry, Anand Institute Of Higher Technology, Chennai-603103, (Tamil Nadu), India
[3]Department of Chemistry, School of Arts and Science, Vinayaka Mission's Research Foundation, (AVIT campus), Paiyanoor, Chennai-603105, (Tamil Nadu), India
[4]School of Life Science, B.S.Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai-600048, (Tamil Nadu), India
* E-mail: skrani@crescent.education

ABSTRACT

In our efforts to propose, Praseodymium (III) metal complex derived from 5-Bromo-2-hydroxybenzyl-2-furyl methyl for the biological efficacy. The structure of the synthesized imine and complex has been characterized by UV, IR, Mass, ^{1}H and ^{13}C NMR. The ligand and the metal complex carried for their anti-microbial tendency to control the disease-causing pathogens such as *Escherichia coli, Klebsiella pneumonia, Salmonella typhimurium, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The derived metal complex exhibited better result and is additional effective bactericides than the ligand.

Keywords: Schiff Base, Praseodymium Complex, Characterization, Antimicrobial Activity

INTRODUCTION

Rare earth metal complexes of Schiff base ligands have been examined owing to their structural diversities, remarkable coordination behavior and broad biological applications[1-4]. Lanthanides showed remarkable differences in coordination numbers and more flexible coordination geometry when compared to transition metals. These behaviors lead to the establishment of unusual multidimensional architectures. From the survey, this work described the characterization and biological values of the synthesized ligand and Praseodymium (III) metal complex. Biological studies such as antimicrobial activity by broth dilution method, biofilm analysis, DNA cleavage, were tested for the ligand and complex. Antibiogram resistance is a rising public health issue that poses a serious threat and creates different issues that limit therapeutic options[5]. Both gram-negative (GNB) and positive bacteria (GPB) are responsible for several diseases such as Urinary tract infection, pyogenic infection, post-burn infection, Pneumoniae, traveler diarrhea, Bacteremia, Septicemia and Ventilator-associated Pneumoniae. In this study the antimicrobial effects of a new LPR compound on GNB, GPB isolates are tested and evaluated.

EXPERIMENTAL

Material and Methods

Praseodymium (III) nitrate hexahydrate, 5-bromosalicylaldehyde, furfuryl amine, were purchased from Sigma Aldrich. Acetonitrile, ethanol, methanol, diethyl ether, chloroform, dimethyl sulfoxide (DMSO),

http://dx.doi.org/10.31788/RJC.2019.1235296

dimethyl formamide (DMF) were purchased from Merck. Infrared spectra recorded using KBR palate between 4000 cm-1 and 400 cm^{-1} in Jasco FTIR-6300 spectrophotometer. The electronic spectra in the 200-900 nm range were recorded on a Perkin Elmer LS25 spectrophotometer using ethanol as solvent. Mass spectra of the compounds were recorded on a GCMS-QP2010 Shimadzu mass spectrometer with Direct Inlet (DI) and Chemical ionization (CI). Bruker Advance DPZ-300 spectrometer used to record the ^{1}H-NMR and ^{13}C-NMR using DMSO-d_6 as solvent and tetra methyl silane as an internal standard at 500 MHz and 125MHz. Bacterial strains such as *E.coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 35657, *Acitobacter baumannii* ATCC 19606, *Staphylococcus aureus, Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028 were purchased from the American type culture collection.

General Procedure

Synthesis of Ligand and Metal Complex

The preparation of the ligand (LH) and it's metal complex (LPR) used in this work is shown in Scheme-1. A methanolic solution of 10 mmol 2-furfuryl amine (0.97g) was slowly added to the equal mole of 5-Bromo salicylaldehyde (1.9 g) methanolic solution. The mixture was stirred and refluxed for 1hr and the solvent was removed by rotary evaporation to obtain yellow solid. The solid crystallized from 1:1 acetonitrile-methanol by volume. The filtered pure solid crystals dried under vacuum for 2h. The metal complex was prepared as per the reported method in methanol instead of ethanol[6]. 0.56 g (2 mmol) of the dried ligand dissolved in 20mL of methanol and clear solution was added to a 15mL of methanolic solution of $Pr(NO_3)_3.6H_2O$ (0.451 g, 1 mmol) in a 2:1 molar ratio and refluxed for 2-3 h with constant stirring up to the reaction completion and it was monitored by T.L.C using 50% ethyl acetate and hexane mixture. After reaching the room temperature, the yellow solid was filtered and washed with the chilled ethanol-water mixture and kept in vacuum desiccators for 24h.

Biological Study

The microbial resistivity of the free ligand and Praseodymium complex was studied by the zone of inhibition technique using both gram –ve and gram +ve bacteria, such as *E.coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 35657, *Acitobacter baumannii* ATCC 19606, *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028[7]. As per the guidance of the National Committee for Clinical Laboratories Standard (1993b) and based on the references, broth dilution method conducted for the test samples with modification of the dilution medium and the lower concentration to inhibit the growth (MIC) of pathogens[8-10]. LH and LPR carried for MIC study using serially diluted compounds and complex showed no observable bacterial growth**.** The Minimum Inhibitory Concentration (MIC) of LH and LPR complex was noted and measured in mm scale. MIC was calculated as the minimum concentration of LPR that inhibited the bacterial growth on agar medium[11-13]. Minimum Bactericidal Concentration (MBC) was calculated by plating all MIC dilutions lacking observable turbidity. 10 µl aliquots were placed on NA plates and incubated at 37°C for 24 h. The lowest concentration of LPR compound that completely reduces the growth of bacteria in 18-24h is called MBC.

Well Diffusion Zone of LPR

This work investigated the antibacterial effect of LPR on *Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The antibacterial nature of the complex on solid agar was determined by the good diffusion test through this zone of inhibition was measured based on the serially diluted concentrations of LPR applied. All Inhibition zones, MIC and MBC results are concluded in Fig.-1 and Table–1.

Static Biofilm Formation

In addition to the antimicrobial activity, this research studied the damage or impact of LPR on biofilm of *E. coli* ATCC strain and the test was perceived through the modified method[14]. Overnight *E. coli* cultures were diluted in fresh Brain heart infusion broth medium (1:100) in borosilicate glass tubes which contained the twofold reducing concentrations of LPR compound (0.010 to 0.033 mg/ml). Tubes without compound served as positive (with bacteria) and negative controls (only broth) and were incubated for

24h. After proper incubation, the medium was removed and the tubes were cleaned with double distilled water, followed by the addition of 0.1% crystal violet in all tubes. After 20 min, the dye was washed away by double distilled water and the emergence of biofilm was confirmed by the continuation of a pinkish ring inside the tubes. The biofilm test tubes are shown in Fig.-2.

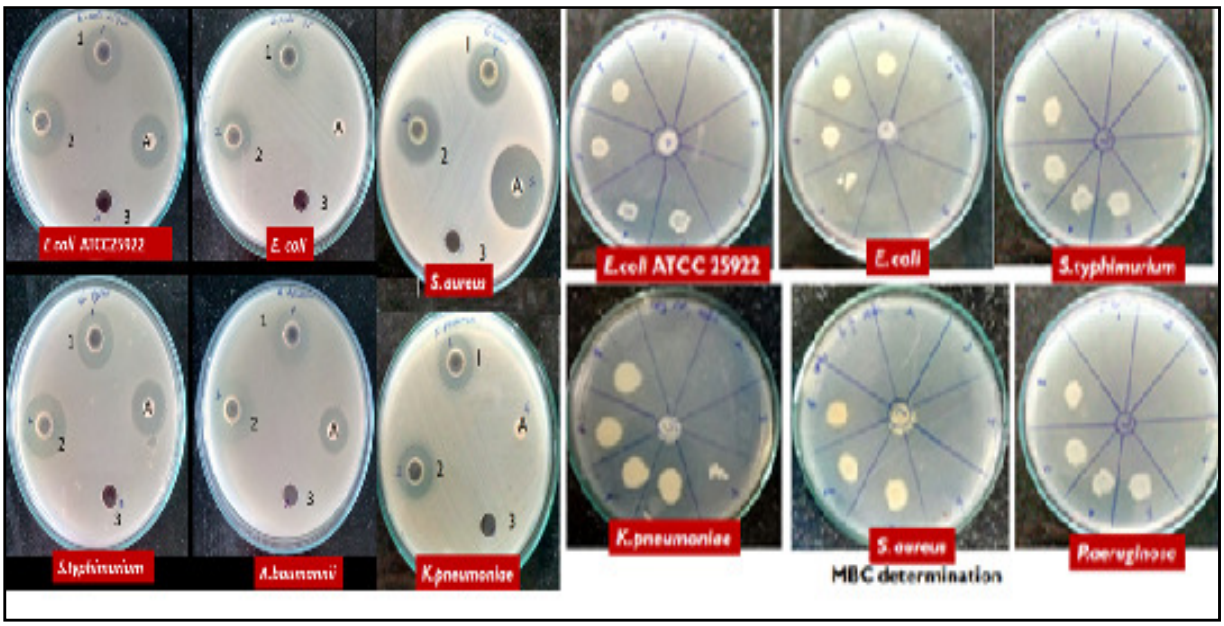


Fig.-1: Antibacterial Activity and MBC Determination using LPR

Table-1: Zone Of Inhibition, MIC and MBC of LPR

Pathogens	Zone of Inhibition in mm				MIC (mg/ml)	MBC (mg/ml)
	50 µl	75 µl	Amp 50 µl	DMSO		
E.coli ATCC25922	21	24	21	0	0.0039	0.0156
E.coli	16	18	R	0	0.0078	0.031
S.typhimurium	20	23	18	0	0.0156	0.031
A.Baumannii	20	21	16	0	0.0039	0.0156
K.pneumoniae	18	20	R	0	0.0310	0.0625
P.aeruginosa	10	15	0	0	0.0310	0.0625
S.aureus(+ve)	15	18	25	0	0.0310	0.0625

*A-Ampicillin R- Resistance 0- No inhibition

From the test, this study observed that 0.01mg/ml is the active biofilm concentration of the test LPR and ligand was not showing any film in a test tube. The biofilm formation was fully inhibited at LPR concentrations that are two and four-fold lesser than the MIC as imagined in Fig.-2.

Growth Curve of *E.coli* treated with LPR

After the biofilm study, this investigation continued until the confirm resistivity of the LPR complex against *E.coli*. Isolated *E. coli* was developed overnight at 37°C on nutrient agar dishes and they were shifted into a liquid broth medium at an initial OD of 0.1 at 600 nm. Cultures were permitted to mature further with the agitation of 180 rpm at 37°C. In certain trials, treatment with LPR was begun when the cultures reached an OD of 0.3 at 600 nm (log phase). The growth of bacteria was determined at 600 nm using Bio-Spectrophotometer and shown in Fig.3. Growth curves were measured in the "rich" liquid medium with LPR added at the beginning of the culture's growth (lag phase). Investigation of the growth curves for the *E. coli* (Ampicillin Resistant) using LPR at the lag phase reveals an abrupt control of growth and shown in Fig.-3a.

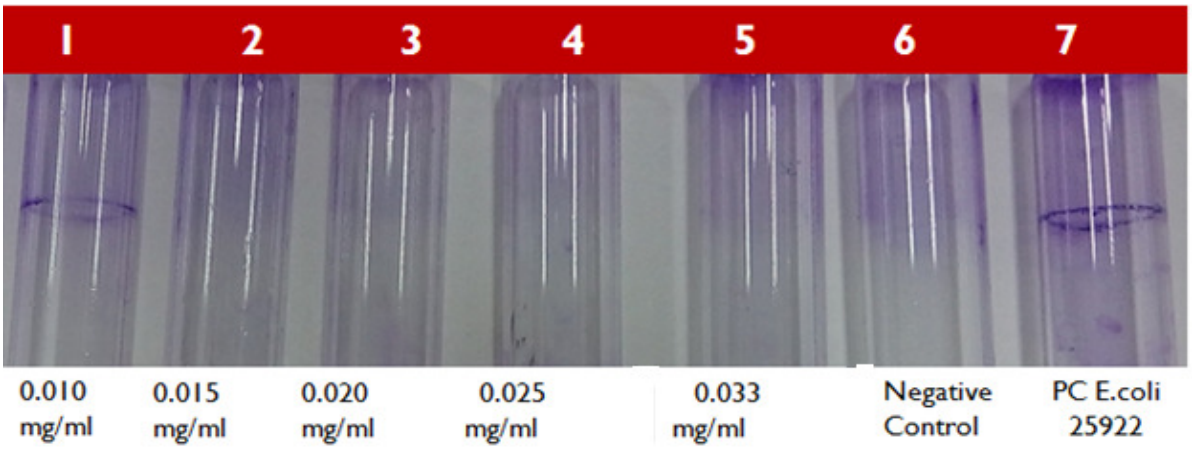


Fig.-2: Static Biofilm Formation treated with LPR; 7 +ve Control *E.coli* (ATCC 25922), 6: Negative Control, 3, 4, 5, 6, 7 treated with LPR Compound Different Concentration

Effect of LPR on Gene Expression

At last, this work extended the LPR resistivity against *E.coli* using DNA ladder assay. Genomic DNA of a single ESBL positive isolates of *E. coli* (Ampicillin resistant) was subjected to gene expression analysis and the effect of LPR was studied[15]. This study was carried out to study the effect of LPR on the expression of AmpC gene. Briefly, an ESBL producing strain was kept as a control (no treatment), the same ESBL strain was treated with LPR as well as with the antibiotic to study the gene expression. An ATCC strain of *E. coli* was kept as a negative control. Polymerase chain reaction (PCR) was used as a molecular test for the detection of AmpC plasmid-mediated (cmy) gene. The forward primer was 5-ATTCCGGGTATGGCCT-3 and the reverse was 3-GGGTTTACCTCAACGGC-5. The PCR amplification was performed in following conditions such as denaturation process at 94°C for 5 min and 30 cycles of 94°C for the 60s, annealing at 58°C for 60s, extension at 72°C for 60s and a final extension at 72°C for 5 min. After amplification of the genomic DNA, the samples were run on an agarose gel to observe the band intensities which corresponded to check whether the gene has been down-regulated and the experimental outcome presented in and shown in Fig.-3. The expression was studied as band intensities after PCR amplification. Low band intensity was observed in the treated strain as compared to the control (without treatment) and the strain treated with antibiotics (Ampicillin), showing the down regulation of AmpC gene because of the effect of LPR. The size of the amplified product was 835 bp in Fig.-3b.

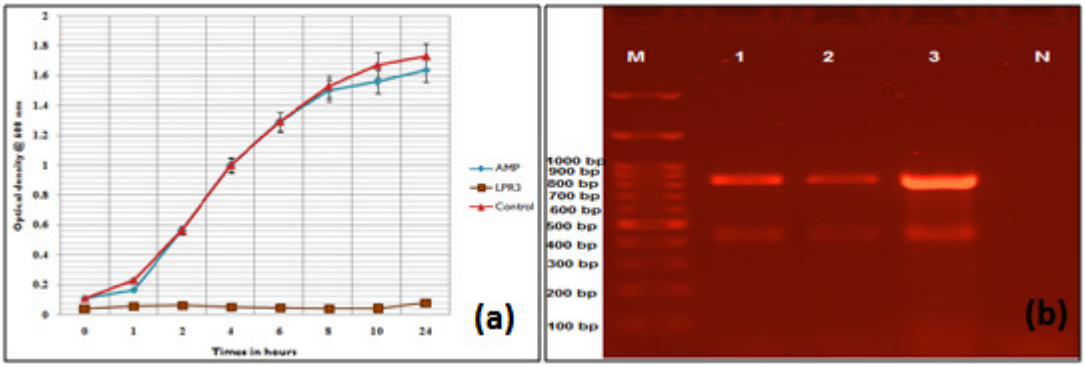


Fig.-3: (a). Growth Curves and AmpC Gene Expression Analyses of LPR (b).AmpC Gene Expression Analyses (M= 100 Base Pair Marker, Lane 3: Untreated, Lane 1: Treated with ampicillin, and Lane 2: Treated with LPR, N: negative control).

Analytical Discussion

Electronic Spectra

The UV-Vis electronic spectra are useful in the structural investigation of metal complexes. Absorption spectra of the ligand and LPR complex have been recorded in ethanol solution between 200 nm and 900

nm. LH exposed two main absorption bands at 222 and 328 for the different excitations. The band at lower energy is recognized the n →π* transition due to the conjugation between the p orbital lone pair of electrons and the N atom in imine group. Two different bands existing at higher energy region are recognized to π →π* of the benzene ring and π →π* shift for the imine group[16]. Due to the complexation, the absorption bands of the LPR are marginally stimulated to higher wavelength (red shift) region when compared to the LH. It is also attributed to the changes in inter electronic repulsion parameter of the LPR complex. The extended shift of the spectral lines signifies 'nephelauxetic' effect, which could be used as a measure of the covalent bond between the metal and ligand. From the results, absorption bands of the f-f transition could not be identified in the visible region for the LPR complex which is due to the very weak f-f transitions. The corresponding transition absorption bands are observed by the strong charge-transfer transition of the ligand. The UV spectrum of the complex, almost satisfied the reported rare earth metal complex absorption spectrum[17].

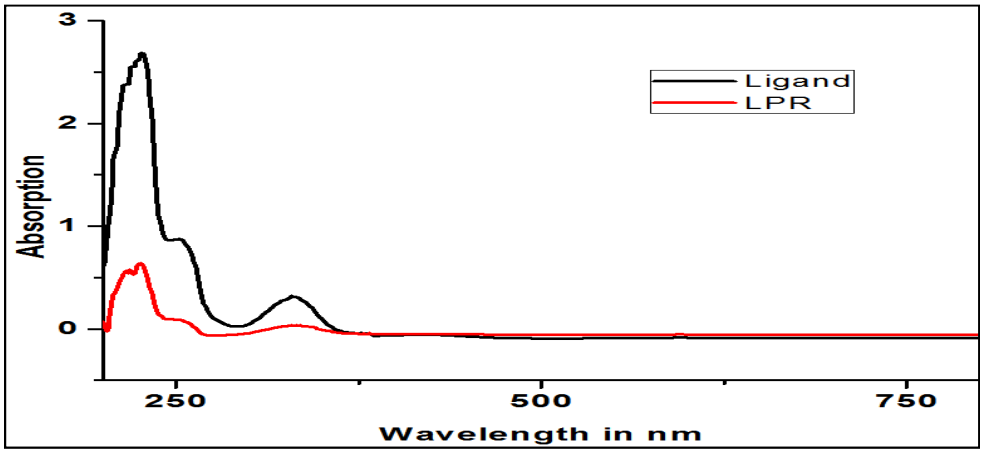


Fig.-4: Electronic Spectra of Ligand and LPR Complex

IR Spectra

The functional group of the ligand arrived from IR spectra information and the coordination behavior of the ligand to the central metal atom Praseodymium[18]. From the recorded IR data (Fig.-2) of the ligand and LPR between 400 $cm^{-1}$and 4000 cm^{-1} using KBr pellet. The peak exists at 1626 cm^{-1} assigned to the (C=N) and the frequency is shifted to 1590 cm^{-1} in the complex. This indicates that there is a covalent bond between the nitrogen atom of the imine group to the metal ion. The peak appeared at 1366 cm^{-1} in the ligand is assigned to the νCO stretching frequency, further there is a shift in the frequency was observed towards the lower value in complex indicating coordination to the metal ion through the oxygen atom. The frequency observed at 3450 cm^{-1} in a metal complex supports co-ordination without deprotonation of the hydroxyl group. The spectrum of the metal complex shows new bands at 464 cm^{-1} for M-N and 560 cm^{-1} for M-O and 1709 cm^{-1} region for M-O bond respectively. Hence the IR spectra of the LH and LPR are found to be in good agreement with their respective structural features.

^{1}H and ^{13}C NMR Spectra of Ligand

Anal. Calc for Molecular formula $C_{12}H_{10}NO_2$, ^{1}H NMR (500 MHz, DMSO-d6) δ 13.23 (s, 1H), 8.65 (s, 1H), 7.73 (d, J = 5 Hz, 1H), 7.65 (d, J = 5 Hz, 1H), 7.49 (dd, 1H), 6.88 (d, J = 10 Hz, 1H), 6.46 (T, J = 5 Hz, 1H), 6.40 (d, J = 5 Hz, 1H), 4.81 (s, 2H).^{13}C NMR(125 MHz, DMSO-d_6) 166, 160, 151, 138, 133, 130, 120, 119, 111, 109, 108, 54 (except low intensity peaks). The recorded ^{1}H NMR spectra and ^{13}C NMR of the ligand in DMSO-d_6 are shown in Fig.-6 and Fig.-7. The characteristic signals of NMR confirmed the ligand LH. The peak appearing at 13.23 ppm in the free ligand can be assigned to the hydroxy proton. Similarly, 8.65ppm singlet peak confirmed the azomethine group and the structure of ligand confirmed by the online www.nmr.db.org. This result is confirmed by the conclusion drawn on the basis of IR spectral data. The interaction between the –OH and the Praseodymium (III) ion does not increase the acidity sufficiently for ionization of the phenolic proton of the ligand. The complex formation confirmed by the physical appearance, melting point and IR, UV spectral techniques. ^{13}C-NMR

spectrum confirmed the ligand C=N functional group at 166ppm and also confirmed the CH_2-N group of the molecule and the ligand carbon number coincidence with spectrum. *Anal. Calc for* LPR complex ^{13}C NMR (125 MHz, DMSO-d_6) 166.01, 160.17, 151.17, 147.63, 143.52, 142.78, 135.09, 133.89, 120.52, 119.11, 111.13, 110.74, 110.54, 109.82, 108.16, 79.19, 78.87, 78.54, 54.88 ppm when compared to the ligand carbon NMR, complex carbon NMR showed the shifted peaks and similar peaks observed for non-bonded carbon atoms which are shown Fig.-8.

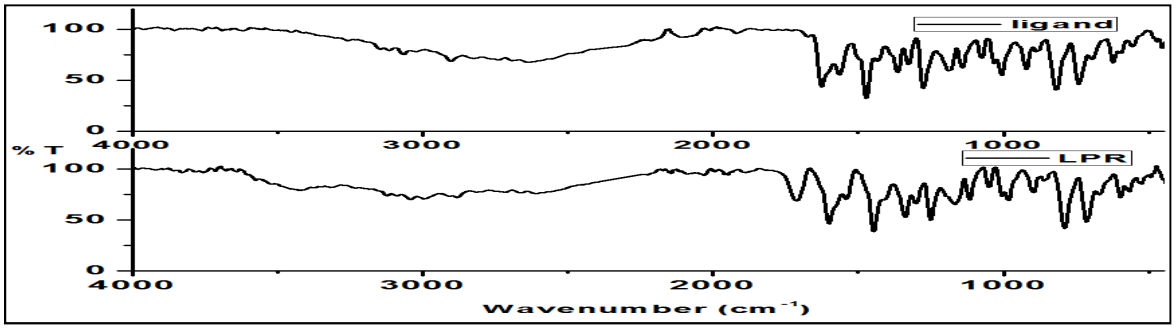


Fig.-5: IR Spectra of Ligand and LPR Complex

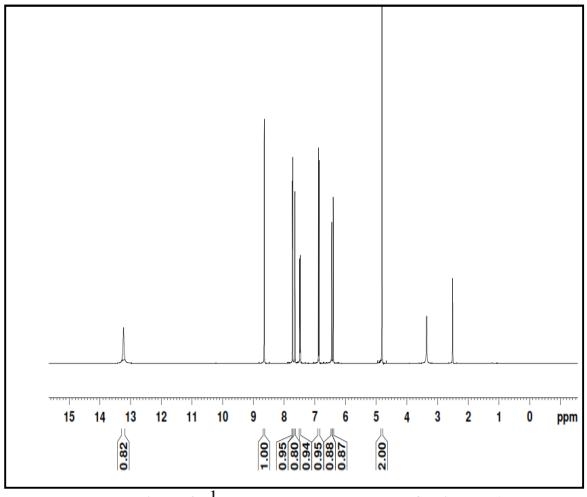


Fig.-6: ^{1}H NMR Spectra of Ligand

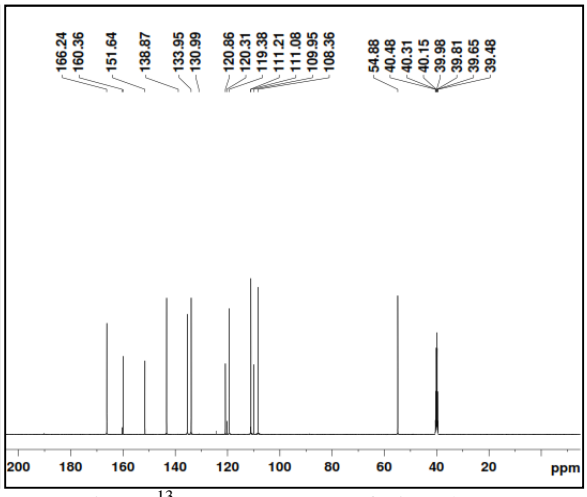


Fig.-7: ^{13}C NMR Spectra of Ligand

Mass Spectra

After the ligand conformation through the NMR studies, the complex was confirmed by the mass spectrum. From the mass, it was observed that the difference between the ligand and the complex is 571. This difference is shown because of the metal, two nitrate group, two ligands, and water molecule. The covalent bond between the ligand and metal salt is confirmed by mass spectrum base peak. $[M+H^+]$ = 844. The mass spectrum of ligand and the complex are shown in Fig.-9. From the mass and ^{13}C spectrum, this research has confirmed the complex formation and coincidence with the reported values.

RESULTS AND DISCUSSION

Ligand synthesized by condensation method and praseodymium complex derived from the ligand using nitrate salt. Both ligand and confirmed by UV (Fig.-4), IR (Fig.-5), ^{1}H-NMR (Fig.6) and ^{13}C-NMR (Fig.-7 and Fig.-8) and discussed above. The complex LPR ^{13}C-NMR and mass spectra showed the difference from the ligand confirmed the complex structure. Both NMR chemical shift of the Schiff base is exposed similarity with the reference[15]. After the compounds confirmation this research analyzed the antimicrobial activity by biofilm analysis, well diffusion and DNA ladder test of the ligand and complex. When compared to ligand, complex exhibited good inhibition zone in mm as in the order of 24, 18, 23, 21, 20, 15, and 18. This inhibition zone of the complex exhibited almost equal and greater than the standard Amoxicillin. Similarly, the MIC and MBC of the complex exist in between 0.06 and 0.01 mg/ml which is most active at low concentrations.

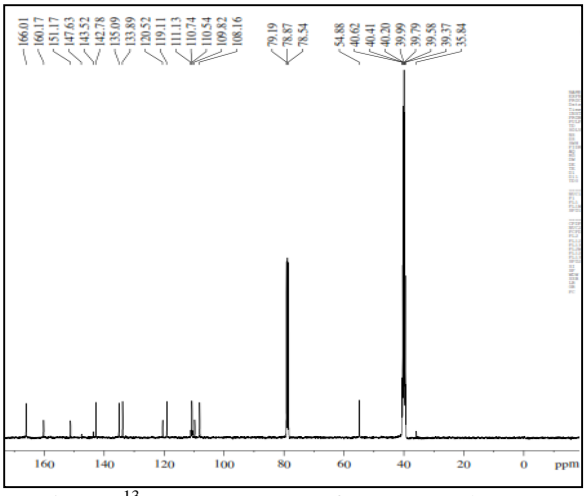


Fig.-8: ^{13}C NMR Spectra of LPR Complex

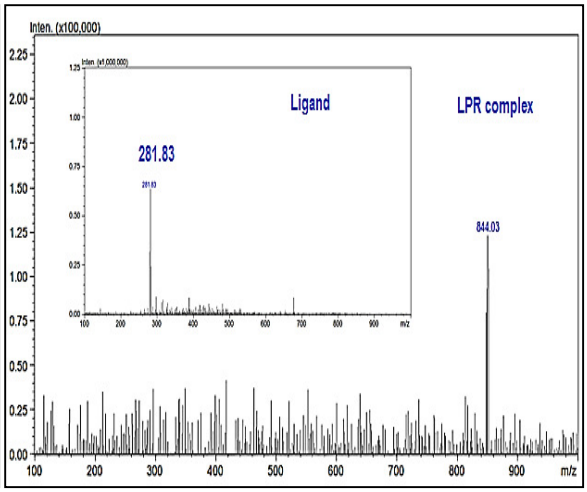
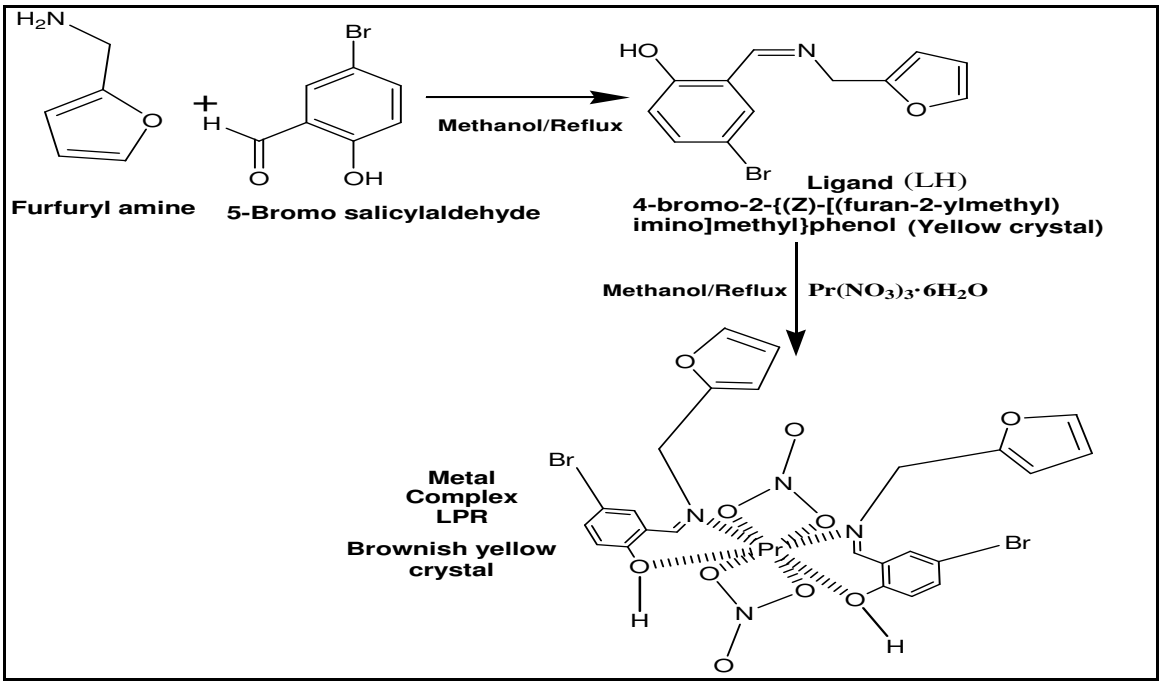


Fig.-9: Mass Spectra of Ligand and LPR Complex

The activity of the complex is confirmed by the DNA ladder test and biofilm methods. Microbial activity of the complex is supported by the DNA ladder test and control of the molecule shown through the growth control curve (Fig.-3a). From the results, this research revealed that the complex showed good results against all pathogens compared with standard Amoxicillin (Blue line). In addition to that this research will continue to enhance the activity of the complex in the future with different functional groups. This research successfully investigated the enhanced *E.coli* resistivity of our compound LPR using broth dilution method, biofilm assay, and DNA ladder assay.



Scheme-1: Scheme for the Synthesis of Ligand (LH) and its Metal Complexes

CONCLUSION

From the above results, this research concluded that the ligand with active functional groups such as –OH, -Br, and –CH=N- is bound with rare earth metal praseodymium exhibited enhanced activity against the disease-causing pathogens and have the control tendency of cell growth at lower concentrations. In addition to that the complex denatured and breaking the DNA structure of the *E.coli* pathogens when compare to other pathogens. Our work also revealed that the enhanced activity of the complex than the

standard Ampicillin against *P.aeruginosa, A.Baumannii* and *S.typhimurium*. At the same time, LPR complex showed good result against *S.aureus* (+ve) at low concentration. In future, the complex activity against *S.aureus* (+ve) will be improved by introducing or changing the functional group of LPR. LPR exhibits lower MIC and MBC against the pathogens.

ACKNOWLEDGMENT

Through this work, we wish to thank the management of Anand Institute Of Higher Technology, Chennai, India for providing lab facilities for the research. I express my deep sense of gratitude to Mr. Sathya Narayanan and Dr. R. Jayaprakash for their continuous guidance for this research.

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[RJC-5296/2019]