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Antimicrobial activity and synthesis of metal chelate of Cadmium with ligand 2-amino-1,4-naphthoquinone and 2-amino-3-chloro-1,4-naphthoquinone-1 oxime

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ABSTRACT

Metal chelates of Cadmium (Cd) with ligand 2-amino-1,4-naphthoquinone (ANQ) and 2-amino-3-chloro-1,4-naphthoquinone 1-oxime (ACNQO) were synthesized. These metal complexes i.e.,Cd $(ANQ)_2$ and Cd $(ACNQO)_2$ have been characterized by modern analytical techniques such as elementary analysis, FTIR, electronic spectra, mass spectroscopy, thermogravimetric analysis. The antimicrobial activity of the ligand and metal chelate were evaluated for bacterial and fungal pathogens such Bacillus subtilis NCIM 2063, Staphylococcus aureus NCIM 2079, Escherichia coli NCIM 2065, Proteus vulgaris NCIM 2813, Aspergillus niger NCIM 501, Candida albicans NCIM 3471. The results are compared to Cisplatin as standard. Study concluded that Cadmium metal complex with both ligands shows good antibacterial activities when compared with standard Cisplatin

Keywords; 2-amino-1,4-naphthoquinone, 2-amino-3-chloro-1,4-naphthoquinone 1-oxime, Synthesis,bacterial and fungal pathogens, antimicrobial activity.

I. INTRODUCTION

The compound like 2-amino-1,4-Naphthoquinones, 2-amino-3-chloro-1,4-Napthoquinones have an active amino group in the 2-position, which have excellent biological applications which includes antimalarial, antibacterial, antitubercular, antitumor agents larvicides, herbicides and fungicides (Prescott, 1969; Hodnettet al.,1983; Clark, 1984). So, researches always focused on these molecules, derivatives, metal complexes. Cadmium (Atomic number 48, Atomic weight 112.411, Electron configuration: [Kr] $4d^{10}5s^2$) is bluish white metal having physical properties like malleable, ductile, soft in nature. To complete the Octet rule Cd is easily donate the two electrons in outermost valence shell, so it gives two electrons and form a stable complex, on other hand ligand 2-amino-1,4-naphthoquinone & 2-amino-3-chloro-1,4-naphthoquinone 1-oxime are ability to take the electron and form the stable complex, hence synthesis and characterization of these molecules were selected for research. Synthesis of ligand 2-amino-1,4-naphthoquinone & 2-amino-3-chloro-1,4-naphthoquinone 1-oxime are reported in various methods (Camaraet al.,2008; Sharmaet al.,2013; Mandkeet al.,2017). This paper summarizes the synthesis and characterization with modern analytical tools of Cd metal chelates with ligands 2-amino-1,4-naphthoquinone & 2-amino-3-chloro-1,4-naphthoquinone 1-oxime, also microbiological activities are studied and reported.

MATERIALS USED FOR SYNTHESIS AND SYNTHESIS PROCESS

The ligand 2-amino-1,4-naphthoquinone was synthesized from 1, 4 naphthoquinone. 1,4 naphthoquinone which was supplied by Fluca chemicals and ligand 2-amino-3-chloro-1,4-naphthoquinone-1 oxime was synthesized from 2-amino-3-chloro-1,4-naphthoquinone which was supplied by Sigma-Aldrich.

Synthesis:

Synthesis of 2-amino-1,4-naphthoquinone from 1,4-naphthoquione:

About 2.0 gm 1,4-naphthoquinone was dissolved in 50 mL mixture of Tetrahydrofuran: Water (40:10). To this solution 2.5 gm of Sodium Azide (Saturated) was added. Sufficient amount of glacial acetic acid was added in the reaction mixture to acidify the reaction mixture. This reaction mixture

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was stirred for 6 hours at room temperature. Evaporate the solution to obtain the red-brown solid. Recrystallization was done with the help of Methylene chloride solvent.

Synthesis of 2-amino-3-chloro-1,4-naphthoquinone-1 oxime from 2-amino-3-chloro-1,4-naphthoquinone: About 5.0 gm Sodium hydroxide was dissolved in 40 mL of water, to which about 4.1 gm of 2-amino-3-chloro-1,4-naphthoquinone was added slowly. To this mixture a solution of 2 gm hydroxyl amine hydrochloride dissolved in 40 mL distilled water was added. This entire mixture was warmed at 50-60^{0}C for one hour on water bath. After one hour the reaction mixture was cooled to room temperature and then cooled to about 5^{0}C in an ice bath. Further to this solution cooled distilled water was added, then it was neutralized by freshly prepared dilute Hydrochloric acid. Dilute hydrochloride acid was added till precipitation is formed. Filter the precipitate and washed with the cold water. Solid was obtained; the solid was dried on hot plant.

Cd chelate with 2-amino-1,4-naphthoquinone:

2 mM of 2-amino-1,4-naphthoquinone was prepared in methanol and the solution was shake well to form a clear solution (Ligand solution), further reflux the solution for 15-20 minutes.

1 nM of Cadmium sulphate (Cd SO_4 8/3 H_2O, Merck) was prepared in water stirred well to from clear solution (Metal solution). A drop wise the metal solution was added into ligand solution under reflux condition, maintained the temperature of solution about 60^{0}C. This reaction mixture was heated for half hour under reflex condition. pH of solution was checked and adjusted to pH 6.5 with dilute Ammonia solution. Reflux the reaction mixture continue and pH of the solution was checked and if require pH of solution was adjusted to 6.5. Continued the reflux for two hours, after reflux cool the solution to room temperature and after filtration the solid was obtained. The solid was dried on hot plate and used for further characterization and microbiological studies.

Cd chelate with 2-amino-3-chloro-1,4-naphthoquinone-1oxime:

Dissolved 0.44 gm of 2-amino-3-chloro-1,4-naphthoquinone-1 oxime (2 mM) in 20 mL of methanol and reflux the solution for about 15-20 minutes to form a clear solution (Ligand solution). Dissolved 0.26 gm of Cadmium sulphate in 10 mL water, the solution was stirred and clear solution was obtained (Metal solution). Drop wise this metal solution was added in ligand solution under reflux condition, during addition temperature was maintained about 60^{0}C. Further the reaction mixture was heated for half hour under reflex condition. Cool the reaction mixture and pH of solution was checked and adjusted to 6.5-6.7 with dilute Ammonia solution. Reflux was continued and pH of solution was checked, if required the pH of solution was adjusted to 6.5. Continued the reflux for two hours, after reflux the solution was cooled and filtered to obtained the solids. The solid was dried on hot plate and used for further study.

Antibacterial activity using disc diffusion method: The modified paper disc diffusion method was employed to determine the antibacterial activity of aqueous, ethanol, methanol and acetone extracts. Turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002). Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth.Then the prepared antibacterial disc was placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37^{0}C.The antibacterial activity was evaluated and diameters of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as strong (>20mm), moderate (16-19mm) and mild (12-15mm) and less than 12mm was taken as inactive (NCCLS, 2002; Dahikar and Bhutada, 2013).

Antimicrobial activity: The agar well diffusion method was employed to determine the antibacterial activity of ligand and metal chelate. In case of bacterial suspension, the turbidity of inoculums was matched with McFarland turbidity standard (NCCLS, 2002). Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth.Ampicillin 10 mcg/disc (Hi-Media, Mumbai) were used as positive control The plates were incubated for 18h at 37^{0}C.The antibacterial activity was evaluated and diameters of inhibition zones were measured. For antifungal properties, 0.1 ml fungal suspension of 10^5 CFU ml^{-1} was uniformly spread on PDA plate to form lawn cultures. After incubation of 24 h at 37^{0}C, zone of

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inhibition of growth was measured in mm. The antifungal activity was classified according to the zone of inhibition such as strong (19-22mm), moderate (15-18mm) and mild (11-14mm). Griseofulvin 10mcg (Hi-Media disc) was used as positive control.

II. RESULTS AND DISCUSSION

Instrumental analysis:

The synthesized compound was subjected to structural elucidation by elemental analysis, FTIR, Electronic spectra, Mass spectroscopy, Thermogravimetry analysis, X-ray diffraction and metal content by ICP-MS.

Fourier-transform infrared spectroscopic study:

FTIR studied was done to evaluation functional groups and confirmation of the structure. FTIR spectra was recorded on Perkin Elmer instrument in KBr matrix with range 4000-400cm^{-1}. Typical functional groups identifications by IR spectroscopy of ligand and metal complex are summarized in Table1.

Table 1: Typical functional groups by IR spectroscopy of ligand and metal complex,

Compound → Functional group ↓	Typical IR frequencies (cm^{-1}) ↓	Experimental IR frequencies (cm^{-1})			
		ACNQO	ANQ	Cd $(ACNQO)_2$	Cd $(ANQ)_2$
M-O	700-500	--	--	690, 675, 670	703, 695
M-N	700-500	--	--	646, 623, 572, 556	648, 621
C-Cl	850-550 S	770	--	778	--
C-H	900-700 B 1465-1365 B	861, 835, 811, 797,788, 782, 770, 749, 722, 710 1470, 1434, 1421, 1396, 1391, 1379, 1369, 1360	906, 874, 860, 850, 832, 821, 803, 798, 779, 754, 726 1478, 1442, 1419, 1395, 1365	887, 881, 866, 856, 843, 838, 818, 798, 778, 744, 729, 710 1472, 1402, 1384, 1358	876, 796, 757, 725, 703, 695 1473, 1456, 1420, 1377, 1342
C=C	995-790 B 1670-1600 S	994, 967, 926, 861, 835, 811, 797 1675, 1668, 1660, 1653, 1634, 1617, 1603	986, 919, 906, 874, 860, 850, 832, 821, 803, 798 1662, 1615, 1609, 1605, 1600	941, 887, 881, 866, 856, 843, 838, 818, 798 1660, 1648, 1587	998, 988, 973, 962, 876, 796 1682, 1659, 1590,
C-N	1342-1266	1340, 1327, 1315, 1296, 1281, 1270,	1320, 1270	1342, 1331, 1325, 1316, 1310, 1301, 1280	1342, 1315, 1276
N-O	1550-1500 S	1497	--	1523, 1491	--
C=N	1690-1640 S	1675, 1668, 1660, 1653	--	1660, 1648	--
C=O	1870-1650 S	1864, 1830, 1675, 1668, 1660, 1653	1866, 1842, 1686, 1662	1832, 1660, 1648	1843, 1682, 1659
N-H	3500-2800 S	3456, 3273, 3098	3398, 3390	3175	3231
O-H	3700-3584 B	3671, 3612	--	3672, 3612	--

B=Bending, S=Stretching, M=Metal,

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For IR frequency evaluation study, Gaussian 09 software was used. Above IR frequencies are matches with literature values. Fig- 1 to 4 indicates the IR spectra for ligand and metal complexes.

Figure 1: IR spectra for 2-amino-3-chloro-1,4-naphthoquinone 1-oxime (ACNQO)

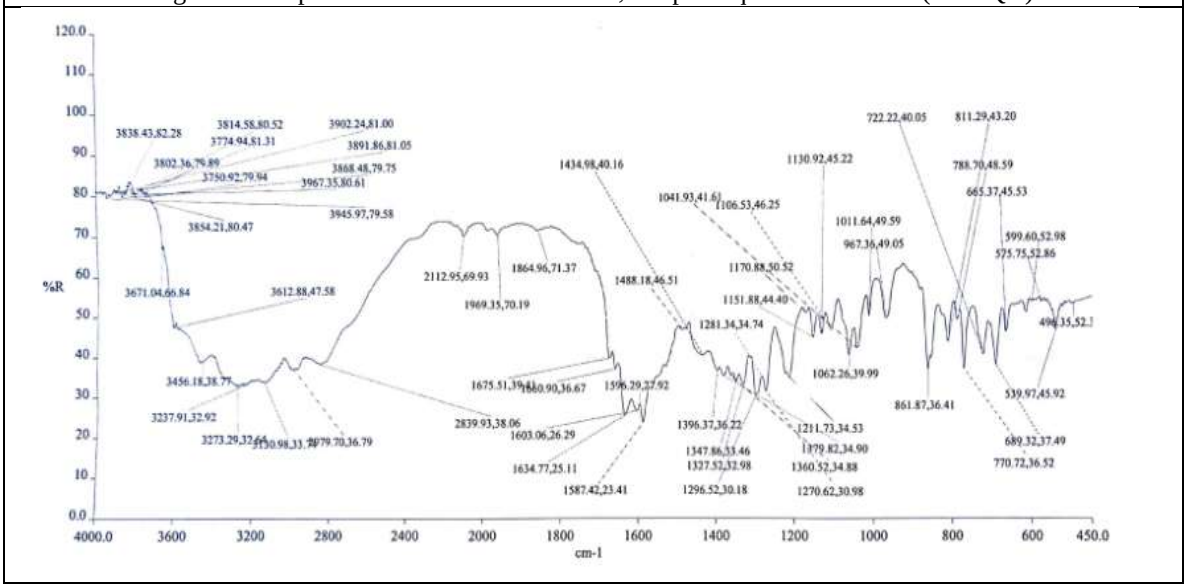

Figure 2: IR spectra for 2-amino-1,4-naphthoquinone (ANQ)

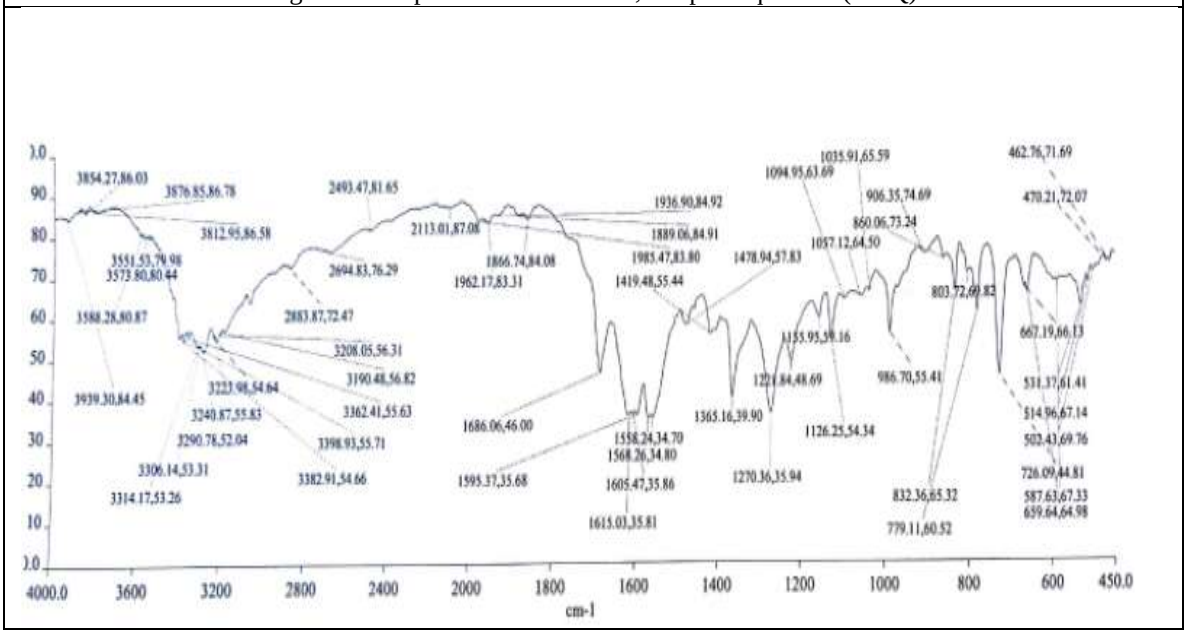

Figure 3: IR spectra for Cd metal complex with 2-amino-3-chloro-1,4-naphthoquinone 1-oxime (ACNQO)

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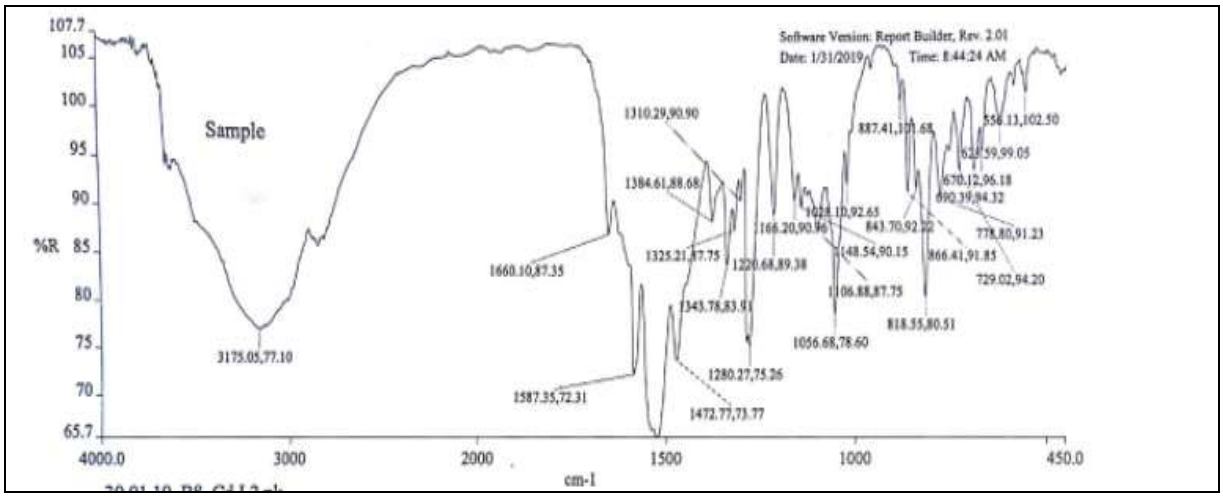
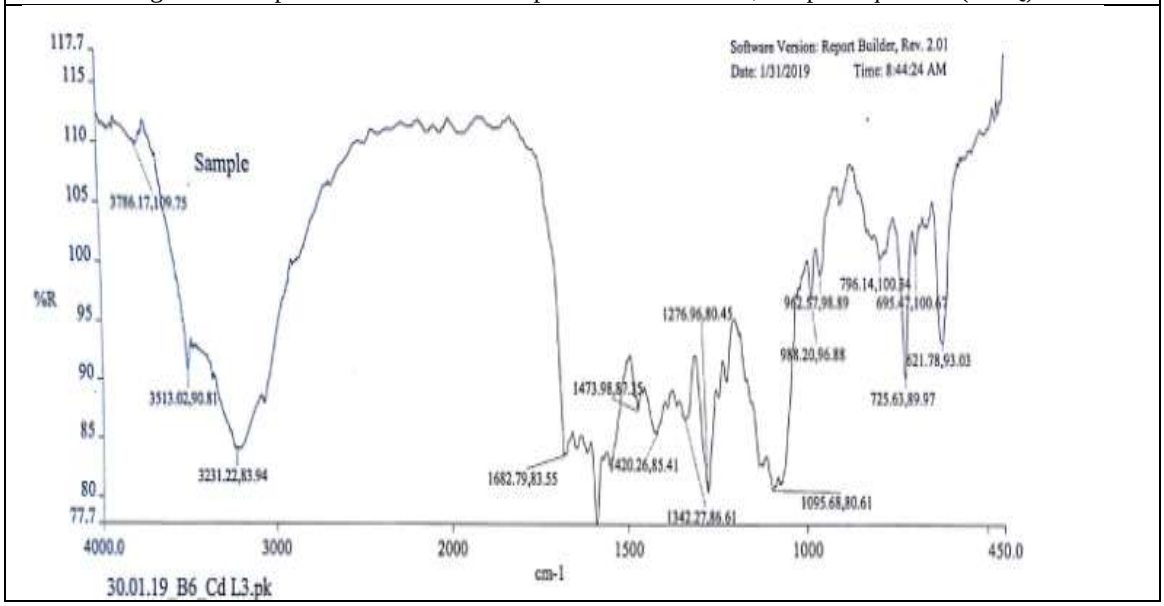


Figure 4: IR spectra for Cd metal complex with 2-amino-1,4-naphthoquinone (ANQ)



UV spectrophotometric study (Electronic spectroscopy):

Electronic spectroscopic study was conducted to evaluate the UV spectrum of metal chelate and co-relation with ligand. UV spectra had been recorded on Shimadzu instrument in solvent DMSO for metal chelates and UV scan of ligand is recorded in methanol solvents. In UV spectroscopy, a beam of UV-Visible light is passed through the sample solution; a molecule absorbs the UV or visible radiations and goes to existed. The electron moves from occupied molecular orbital to unoccupied molecular orbital. Hence this spectroscopy is also called as electronic spectroscopy.

Energy transitions are observed as $\eta \rightarrow \pi^*$, $\eta \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, $\sigma \rightarrow \pi^*$, $\sigma \rightarrow \sigma^*$.

Table 2: Experimental λ_{max} observed

λ → Compound ↓	**λ_{max1}**	**λ_{max2}**	**λ_{max3}**
$Cd(ACNQO)_2$	271 nm	339 nm	431 nm

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Cd $(ANQ)_2$	273 nm	442 nm	--

Observed λ_{max} are due to energy transitions of metal complex.

Metal analysis by ICP MS: Inductively coupled plasma mass spectrometry (ICP-MS)
% Metal content was analyzed with help of ICP MS (Inductive couple plasma mass spectroscopy) and compared with the theoretical values and summarized the data Table 5.

Table 3: Result of Metal content by ICP MS

Metal content → Compound ↓	**% Metal content**	
	Theoretical	**Experimental**
Cd L2	20.23	21.95
Cd L3	24.50	24.72

Experimental results of metal contents are matches with the theoretical contents.

Antimicrobial activity

Antimicrobial activities of ligand and metal chelates was evaluated and compared with Cisplantin drug and data was summarized. The antimicrobial activity of the ligand and metal chelate were evaluated for bacterial and fungal pathogens such Bacillus subtilis NCIM 2063, Staphylococcus aureus NCIM 2079, Escherichia coli NCIM 2065, Proteus vulgaris NCIM 2813, Aspergillus niger NCIM 501, Candida albicans NCIM 3471.A 0.2 ml of culture of each type of micro-organism was spread with sterile swabs on different plates. Four or five wells were prepared in the agar with 8.0 mm cork borer on each plate. The test material was prepared in Dimethyl Sulfoxide (DMSO) as a stock solution. A 50 µl of the stock solution was added in each well. The stock solution was added. Cisplatin was used as standard (Std). The plates were incubated at 37°C for 24 hours. After incubation the zone of inhibition was measured in millimeter (mm). Antibacterial activity against specified organisms in terms of Zone of inhibition in mm calculated and compared. Data indicated that Cadmium metal complex with both ligands shows good antibacterial activities when compared with standard Cisplatin.

Table 2: Antimicrobial Activities of Ligand and Metal Chelates

Ligand And Metal Chelate	Bacillus subtilis	Staphylococcus aureus	Escherichia coli	Proteus vulgaris	Aspergillus niger	Candida albicans
ACNQO	18	24	25	23	16	22
ANQ	19	18	23	16	18	22
Cd (ACNQO)2	19	18	20	18	20	24
Cd (ANQ)2	17	20	22	20	18	21

III. CONCLUSIONS

Ligand 2-amino-1,4-naphthoquinone (ANQ) and 2-amino-3-chloro-1,4-naphthoquinone 1-oxime (ACNQO) were synthesized and also metal chelates were synthesized. These ligands and metal complexes were characterized by elemental analysis, FTIR, Electronic spectra, Mass spectroscopy, Thermogravimetry, Differential scanning calorimetry, X-ray diffraction and metal content by ICPMS. The results are compared to Cisplatin as standard. Study concluded that Cadmium metal complex with both ligands shows good antibacterial activities when compared with standard Cisplatin

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