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PLATINUM (II) AND PALLADIUM (II) DERIVATIVES OF HETEROCYCLIC SULPHONAMIDE IMINES: SYNTHESIS, CHARACTERIZATION AND THEIR BIOLOGICAL ASPECTS

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ABSTRACT

The present article describes the synthesis and characterization of a new series of Pt (II) and Pd(II) complexes with two N∩O donor ligands, salicylaldehyde sulphathiazole and salicylaldehyde sulphaguanidine. The 1:2 reactions of metal chlorides ($PtCl_2$ and $PdCl_2$) with monobasic bidentate ligands resulted in the formation of coloured solids. All the synthesized compounds were characterized by melting point determinations, elemental analyses and a combination of IR, and ^1H NMR spectroscopic techniques for structural elucidation. Probable square planar structures for the resulting derivatives have been proposed on the basis of above characterization. The ligands, and their complexes, have also been screened in vitro for their antimicrobial activity against a number of pathogenic fungal and bacterial strains. Besides, the DNA cleavage activity of the complexes has also been studied. The studies indicate that the metal chelates are more potent than the respective ligands.

Keyword: Palladium (II) And Platinum (II) Complexes, Elemental Analyses, Antimicrobial Activity and DNA Cleavage Activity.

I INTRODUCTION

Schiff bases and their metal complexes exhibit a number of biological activities such as antibacterial, antiviral, and antitumor, because of their specific structures. Schiff base complexes have found applications as magnetic materials, catalysts and in the biological engineering field [1-2]. Schiff bases obtained by condensation reaction usually act as bi-, tri- or poly-dentate ligands and form stable complexes with transition metals. Schiff bases show their versatile use in the synthesis of various inorganic compounds, in identification and determination of carbonyl compounds, use in the preparation of various dyes and in pharmaceutical industry.

Sulpha drugs are a group of compounds used for eliminating wide range of infections in human and other animal systems. Many chemotherapeutically important sulpha drugs, like sulphadiazine, sulphathiazole possess SO_2NH moiety which exhibit an important toxophoric function[3].

The coordination chemistry, biological effects and toxicology of platinum and palladium complexes, such as their requirements in pharmacological activities, are areas of increasing research interest [4]. It has been well established that certain platinum and palladium complexes are of biological importance due to their anticancer [5], antitumor [6], antiamoebic [7] and catalytic activity [8].

It has been reported that the activity of sulphur-containing ligand increases on complexation [9-11]. In view of the diversified chelating behaviour of sulphonamide imines as well as biological importance of palladium and platinum complexes, it has been considered worthwhile to synthesize, characterize some new palladium(II) and platinum(II) derivatives of sulphonamide imines and to investigate their physico-chemical and structural features as well as the biological activity.

II EXPERIMENTAL

2.1 Analytical Methods and Physical Measurements

The metal salt, $PtCl_2$ and $PtCl_2$ was commercial product and was used as received. All the reagents used were of AR grade and the solvents used were dried, distilled and purified by the standard methods. Nitrogen was estimated by the Kjeldahl's method [12] and sulfur was estimated by Messenger's method [13]. Palladium was estimated gravimetrically and chlorine was estimated volumetrically by Volhard's method [14]. The Rast Camphor Method was used to carry out the molecular weight determinations. Infrared spectra of the ligands and their complexes were recorded with help of Nicolet Magna FTIR-550 spectrometer on KBr pellets. ^{1}H NMR spectra were recorded in DMSO –d6 using TMS as the internal standard.

2.2 Preparation of the Ligands

The ligands, i.e. salicylaldehyde sulphathiazole and salicylaldehyde sulphaguanidine, L^1H and L^2H respectively, used during these investigations were prepared by the condensation of salicylaldehyde with the sulpha drugs. An ethanolic solution of salicylaldehyde (0.1mol) was added slowly to an ethanolic solution containing sulpha drugs (0.1 mol) in 1:1 molar ratio under stirring for 15 min, and then refluxed on a water bath for five-six hours. On cooling overnight in a refrigerator, crystals separated out which were further purified by washing with ethanol and finally recrystallized with acetone.The analytical results came in good consistence with the proposed formulas as in Table 1 (Fig. 1).

2.3 Synthesis of the Metal Complexes

2.3.1 Platinum complexes

The 1:1 water–ethanol solution of $PtCl_2$ was mixed with an ethanolic solution of the ligands in 1:2 molar ratio. To obtain the Pt $(L^n)_2$ (where n= 1&2) type of complexes aqueous ammonia was added dropwise to the reaction mixture until it was weakly alkaline (pH ca. 8.0). The reaction mixture was then heated under refluxed for about 1 h. On cooling, the complexes were separated out which were filtered and washed with ethanol and dried in vacuum. On the other hand, $[Pt(L^n)_2]Cl_2$ type of complexes have been synthesized by stirring the above reaction mixture (solution of $PtCl_2$ + L^nH in 1:2 molar ratio) on a magnetic stirrer for about 2–3 h in the presence of few

drops of concentrated HCl. The resulting product was recovered by filtration, washed with ethanol and dried in vacuum.

2.3.2 Palladium complexes

The methanolic solution of $PdCl_2$ was mixed with methanolic solution of the ligands in 1:2 molar ratio. Aqueous ammonia was added dropwise to the reaction mixture, until it was weakly alkaline (pH ca. 8.0) and this reaction mixture was then heated under reflux for about 1 h to synthesize $Pd(L^n)_2$ type of complexes. To obtain the $[Pd(L^n)_2]Cl_2$ type of complexes the methanolic solution of $PdCl_2$ and L^nH in 1:2 molar ratio was stirred on a magnetic stirrer for 2–3 h in presence of few drops of concentrated HCl. The resulting products were recovered by filtration, washed with methanol and dried in vacuum.

III BIOLOGICAL STUDIES

3.1 Anti-microbial studies

3.1.1 In- vitro Antifungal Activity

The newly prepared complexes were also screened for their antifungal activity against *Altenaria alternata*, *Aspergillus niger*, *Fusarium oxysporum* and *Macrophomina phaseolina* in DMSO by agar diffusion method. Agar media was prepared by dissolving peptone (10 g), D-glucose (40g) and agar (20 g) in distilled water (1000 mL) and adjusting pH to 5.7. Normal saline water was used to make suspension spore of fungal strain lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get suspension of corresponding species. Twenty millilitres of agar media were poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for 1 h using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37° C for 96 h. The fungal activity of each compound was compared with *Bavistin* as standard drug. The medium with DMSO as solvent was used as a negative control whereas media with *Bavistin* (standard antifungal) were used as positive control. The experiments were performed in triplicates. The cultures were incubated for 96 h at 35°C and the growth was monitored and the percentage of inhibition was calculated by equation:

$$\% \text{ inhibition} = 100(C-T)/C$$

Where, C and T are the diameters of the fungal colony in the control and the test plates, respectively.

3.1.2 In- vitro Antibacterial Activity

The newly prepared compounds were screened for their antibacterial activity against *Staphylococcus aureus*, *Klebsiella aerogenous*, *Escherichia. coli* and *Pseudomonas cepacicola* by paper disc plate method. Each compound was dissolved in DMSO and solutions of the concentrations (500 and 1000 ppm) were prepared separately. Paper discs of Whatman filter paper (No. 42) of uniform diameter (5 mm) were cut and sterilized in an autoclave. The paper discs soaked in the desired concentration of the complex solutions were placed aseptically in the petri dishes containing nutrient agar media (agar 20 g + beef extract 3 g + peptone 5 g) seeded with bacteria strains separately. The petri dishes were incubated at 37°C and the inhibition zones were recorded after 24 h of incubation. The antibacterial activity of common standard antibiotic *Tetracyclin* was also recorded using the same procedure as above at the same concentrations and solvent. The medium with DMSO as solvent

was used as a negative control whereas media with *Tetracyclin* (standard antibiotics) were used as positive control. The experiments were performed in triplicates.

3.2 DNA cleavage activity

3.2.1. Preparation of culture media

Nutrient broth (peptone, 10; yeast extract, 5; NaCl, 10 in g/L) was used for culturing of *E. coli* . The 50-mL medium was prepared and autoclaved for 15 min at 121 ° C under 15 lb pressures. The autoclaved media was inoculated with the seed culture and *E. coli* was incubated for 24 h.

3.2.2. Isolation of DNA

The fresh bacterial culture (1.5 mL) was centrifuged to obtain the pellet, which was then dissolved in 0.5 mL of lysis buffer (100 m m Tris pH 8.0, 50 m m EDTA, 10 % SDS). To this 0.5 mL of saturated phenol was added and incubated at 55 ° C for 10 min. Then, it was centrifuged at 10,000 rpm for 10 min and equal volume of chloroform:isoamyl alcohol (24:1) and 1/20 volume of 3m sodium acetate (pH 4.8) was added to this supernatant and centrifuged at 10,000 rpm for 10 min. To this supernatant three volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation. The pellet was dried and dissolved in TE buffer (10 m m Tris pH 8.0, 1 m m EDTA) and stored in cold conditions.

3.2.3 DNA cleavage analysis

Agarose gel electrophoresis cleavage products were analyzed by the agarose gel electrophoresis method. Test samples (1 mg/mL) were prepared in DMF. The samples (25 µ g) were added to the isolated DNA of *E. coli* . The samples were incubated for 2 h at 37 ° C and then 20 µ l of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 m EDTA/l) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. The gel was removed and stained with 10.0 g/mL ethidium bromide for 10 – 15 min, and the bands were observed under a UV transilluminator and photographed to determine the extent of DNA cleavage, and the results were compared with standard DNA marker.

IV RESULTS AND DISCUSSION

The metal chloride interacts with the ligands in 1:2 molar ratios in the presence of few drops of concentrated HCl to form $[M(LH)_2]Cl_2$ type of complexes as follows:

$$MCl_2 + 2LH \rightarrow [M(LH)_2]Cl_2$$

However, complexes of the type $[(Pd(L)_2)]$ were obtained when reactions were carried out in the presence of aqueous NH_4OH. The reactions may be written as:

$$MCl_2 + 2LH + 2NH_4OH \rightarrow [M(L)_2] + 2NH_4Cl + 2H_2O$$

where M= Pd(II) and Pt(II) and LH is the ligand molecule.

The reactions proceed easily and all the complexes are coloured solids. All the complexes are soluble in DMSO, DMF and $CHCl_3$ and insoluble in common organic solvents.

4.1 Spectroscopic Characterization

4.1.1. IR Spectra

The significant IR bands of the ligands and platinum (II) and palladium(II) complexes were observed that are useful for the establishment of the mode of the coordination of the ligands to the metal ion. Several significant changes with respect to the ligands bands on complexation suggest coordination through the azomethine nitrogen and oxygen of the salicylaldehyde. A comparison of the IR spectra of the complexes and the ligands L^1H and L^2H show that the stretching vibration bands of *v*(-OH) of the ligands at 3255cm^{-1} is absent in the spectra of the substitution complex. This disappeareance of –OH signals confirms the deprotonation of salicylaldehyde -OH group and its involvement in coordination. The *v*(C=N) bands in complexes appear at 1600-1598 cm^{-1}; significantly lower than the free ligands values indicating coordination by the azomethine nitrogen atoms of the Schiff bases. However, no *v*(M-Cl) bands in the region 295-345cm^{-1} is observed in the spectra of $[M(LH)_2]Cl_2$ type of complexes, suggesting the chloride is ionic in these complexes. Non-ligand bands at 415–428, 352–360, 442-448 and 410-415 cm^{-1} have been assigned to v(Pt–N,) v(Pd–N), v(Pt-O) and v(Pd-O) respectively. The overall IR spectral evidence suggests that both ligands are bidentate, coordinating through oxygen and azomethine-nitrogen forming a six-membered chelate ring.

4.1.2. ^{1}H NMR Spectra

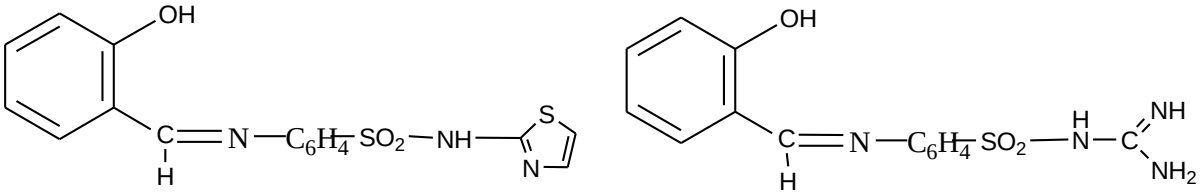
4.1.2.1. ^{1}H NMR spectra of the ligands

The ^{1}H NMR spectral data of the ligands were recorded in DMSO-d6 taking TMS as an internal standard. The free ligands exhibit OH proton resonance signals at δ12.10-12.20 ppm. The free ligands show a complex multiplet at δ6.50–8.85 for the aromatic protons. A singlet at δ10.15ppm due to the −NH proton appear in the spectra of the ligands.

4.1.2.2. ^{1}H NMR spectra of the complexes

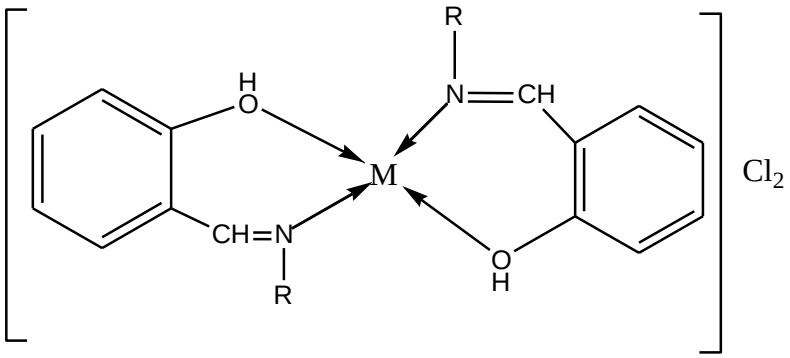
The ^{1}H NMR spectra of the complexes further support the bonding pattern as discussed above. The free ligands exhibit OH proton resonance signals at δ12.10-12.20 ppm, which completely disappear in the spectra of metal complexes. This indicates the deprotonation of OH group as a result of bonding through phenolic oxygen to the metal atom in the substitution complex. Further, in the spectra of the complexes, a downfield shift in the position of the aromatic protons also indicates the coordination of the azomethine nitrogen to the metal atom. The signal due to –NH remains unaltered in the complexes indicating that the –NH group is not taking part in the complexation.

On the basis of above discussion the following structures as shown in the Fig. 1 and 2 have been proposed for the ligands and the metal complexes.

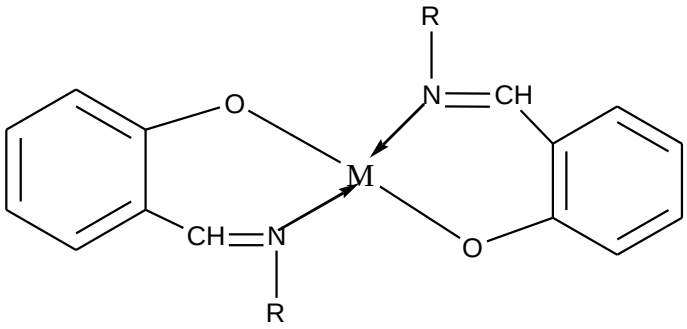


salicylaldehyde sulphathiazole(L^1H) salicylaldehyde sulphaguanidine(L^2H)

Fig.1 Structure of the Ligands



Addition Complex



Substitution Complex

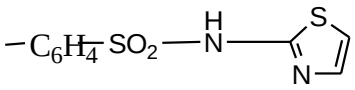
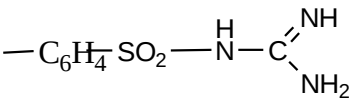
Where, M = Pt and Pd and R =  and 

Fig.2 Structure of the synthesized Complexes

4.2 Biological studies

4.2.1 In vitro antifungal and antibacterial studies

All the compounds tested against the fungi, *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum*, and *Macrophomina phaseolina* and the bacteria *Staphylococcus aureus*, *Klebsiella aerogenous*, *Escherichia Coli*, and *Pseudomonas cepacicola* and were found to be active. The results have been summarized in Tables 2 and 3 The biological activity of metal complexes exhibited markedly much promising results than the ligands against all the test bacterial/fungal strains. It was evident that overall potency of the ligands was enhanced on

coordination with metal ions. It has been suggested that the ligands with nitrogen and oxygen/sulfur donor systems inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. Moreover, coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [15] within the chelate ring system formed during coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favours its permeation more efficiently through the lipid layer of micro-organism [16], thus destroying them more aggressively.

DNA cleavage activity

The representative ligands and their complexes are studied for their DNA cleavage activity by the agarose gel electrophoresis method against DNA of *E. coli* in the presence of H_2O_2. From the gel picture it is clear that Lanes 1, 2, 3, 4, 5, 6, 7& 8 have shown cleavage activity, which was confirmed by observing the tail in the DNA band. The tail was missing in L3 & L4 indicating the non-cleavage activity. This shows that the Schiff base alone does not show any apparent cleavage, whereas its complexes do show. The results indicated the important role of metal in these isolated DNA cleavage reactions. The cleavage efficiency of the complexes compared with that of the control is due to their efficient DNA-binding ability. The metal complexes were able to convert super coiled DNA into open circular DNA. The general oxidative mechanisms proposed account for DNA cleavage by hydroxyl radicals via abstraction of a hydrogen atom from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed. The cleavage is inhibited by the free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction.

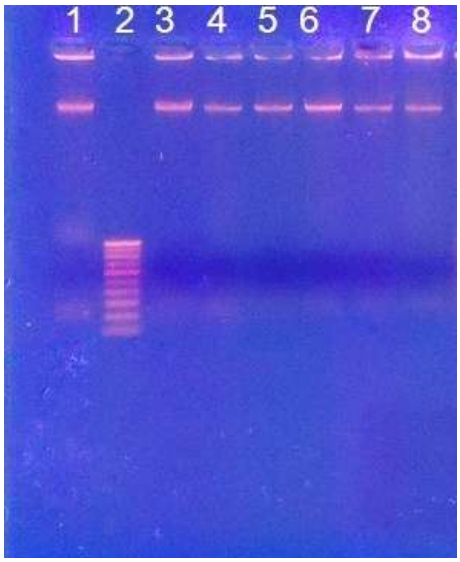
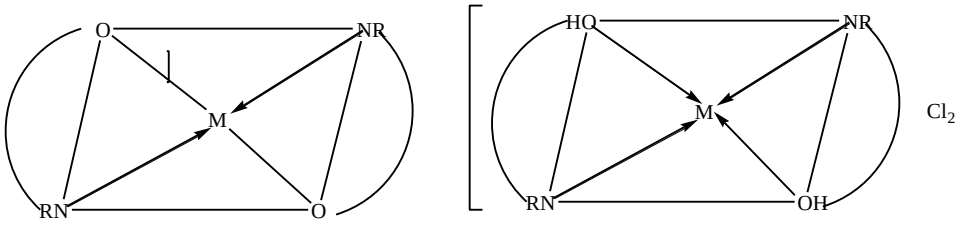


Fig.4 DNA cleavage gel diagram of ligands and synthesized compounds. , Lane 1 : (C *E. Coli)*, Lane 2: (standard molecular weight marker), Lane 3&4: (*E. Coli* DNA cleavage of L^1H & L^2H), Lanes 5 – 8, (*E. coli* DNA treated with the complexes): $[Pt(L^1H)_2]Cl_2$ $[Pt(L^2H)_2]Cl_2$ $[Pd(L^1H)_2]Cl_2$ & $[Pd(L^2H)_2]Cl_2$, respectively.

V CONCLUSIONS

We have synthesized biologically relevant ligands and their Pt(II) and Pd(II) complexes. Thus, on the basis of the above spectral features, as well as the analytical data, square planar geometries shown in Fig. 3 have been suggested for the both the Pt(II) and Pd(II) complexes. The antimicrobial results indicated that the complexes showed promising antibacterial and antifungal activities. Both the ligand and their respective metal complexes were found to be sensitive against all the fungal and bacterial strains and the metal complexes are more potent antimicrobial agents than the free ligand. The results indicated that the palladium and platinum complexes exhibited almost similar antimicrobial activity. Furthermore, DNA cleavage studies revealed that metal complexes cleave DNA more efficiently in comparison to the ligands.



Substitution Product Addition Product

Fig.3

REFERENCES

[1] H-Y.Zhang, J.Lei, Y-Y.Chen, Q-A.Wu, Y-S.Zhang, L-H.Gao, Synthesis and Reactivity in Inorganic and Metal-OrganicChemistry, 31,2001 973.

[2] E.Abele, Activation of silicon bonds by fluoride ion in the organic synthesis in the new millenium: A Review. Main Group Metal Chemistry, 28,2005, 45.

[3] H. Singh, V. K. Srivastava, S.N. Shukla, M. K. Srivastava, M. K. Upadhyay, Indian Journal of Chemistry, 33A, 1994, 350.

[4] M. Navarro, A. Betancourt, C. Hernández, E. Marchan, Braz. Chem. Soc. 19, 2008,1355.

[5] C.F. Caires, Antonio, Anti-Cancer Agents, 7,2007, 484.

[6] G. Garcia-Friaza, A. Fernandez-Botello, J.M. Perez, M.J. Prieto, V. Moreno, J. Inorg. Biochem.100, 2006 1368.

[7] N. Bharti, Shailendra, S. Sharma, F. Naqvi, A. Azam, Bioorg. Med. Chem. 11,2003,2923.

[8] W. Nazihah, W. Ibrahim, M. Shamsuddin, B.M. Yamin, Malaysian J. Anal. Sci. 11,2007, 98.

[9] M. Jain, R.V. Singh, International Journal for Chemical Science,1(1),2003,17.

[10] M. Jain, S. Gaur, S. C. Diwedi, S.C. Joshi, A.Bansal, R.V.Singh, Phosphorus, Sulfur, and Silicon,179, 2004,1517.

[11] M. Jain, S.Gaur, V.P. Singh, R.V. Singh, Applied OrganometallicChemistry. 18(2),2004, 73.

[12] J.T. Makode, A.S. Aswar. Indian J. Chem., **43**A, 2004,2120.

[13] A.I. Vogel. ‘*’A Textbook of Organic Quantitative Analysis’’*, 5th Ed., (PEL, London) 2004, 243.

[14] A.I. Vogal "*A Text Book of Quantitative Inorganic Analysis", (*Longmans Green ELBS, London), 1962.

[15] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols, J.L. Mc Laughlin, Planta Med. 45,1982,31–34.

[16] Z.H. Chohan, Synth. React. Inorg. Met-Org. Chem. 34, 2004, 833–846.

Table 2: Fungicidal screening data of the ligands and their respective metal complexes. (Average percentage inhibition after 96 hours; conc. in mg/well (w/v)

Compound	***Alternaria alternata***			***Aspergillus niger***			***Fusarium oxysporum***			***Macrophomina phaseolina***		
	0.5	**1.0**	**2.0**	**0.5**	**1.0**	**2.0**	**0.5**	**1.0**	**2.0**	**0.5**	**1.0**	**2.0**
L^1H	36	38	40	30	33	34	28	30	35	27	31	34
$[Pt(L^1H)_2]Cl_2$	45	48	50	38	39	41	30	33	39	29	33	38
$[Pt(L^1)_2]$	47	52	53	41	40	42	33	36	40	31	33	40
$[Pd(L^1H)_2]Cl_2$	42	45	48	33	36	40	27	30	35	26	30	36
$[Pd(L^1)_2]$	44	48	50	35	36	42	30	33	35	28	33	39
L^2H	35	37	40	27	30	31	25	29	35	22	25	33
$[Pt(L^2H)_2]Cl_2$	41	45	48	33	34	38	28	30	35	26	35	37
$[Pt(L^2)_2]$	43	45	51	35	39	40	31	35	39	30	36	41
$[Pd(L^2H)_2]Cl_2$	39	41	42	29	31	33	25	27	31	22	26	33
$[Pd(L^2)_2]$	42	43	45	30	33	34	25	31	34	29	31	35
Bavistin	85	100	100	84	100	100	84	100	100	82	100	100

Table 3 : Bactericidal screening of the ligands and their respective metal complexes : Inhibition after 24 hours (conc. in mg/well (w/v)

Compound	Diameter of inhibition zone (mm)							
	Staphylococcus aureus		*Klebsiella*		*Escherichia coli*		*Pseudomonas*	
	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
L_1H	8	10	5	8	6	8	4	5
$[Pt(L^1H)_2]Cl_2$	12	14	8	10	11	10	6	9
$[Pt(L^1)_2]$	12	16	10	11	13	10	8	11
$[Pd(L^1H)_2]Cl_2$	9	12	8	9	10	11	6	8
$[Pd(L^1)_2]$	10	13	10	11	7	8	7	10
L_2H	7	9	6	7	5	8	4	9
$[Pt(L^2H)_2]Cl_2$	15	16	7	8	9	13	6	7
$[Pt(L^2)_2]$	15	18	8	9	10	12	7	9
$[Pd(L^2H)_2]Cl_2$	13	15	4	5	7	9	4	5
$[Pd(L^2)_2]$	14	16	4	6	9	11	5	8
Tetracyclin	15	18	6	11	17	18	6	10

Table 1. Analytical data and physical properties of the ligands and their complexes.

Compound	Color	M.P. (°C)	Analysis (%) Found (Calcd.)						Mol.Wt. Found (Calcd.)
			C	H	N	S	Cl	M	
L^1H	Crystalline white	120	53.12 (53.33)	3.45 (3.88)	11.42 (11.66)	17.22 (17.77)	-	-	360
$[Pt(L^1H)_2]Cl_2$	Light yellow	140-144	38.54 (38.94)	2.45 (2.83)	8.14 (8.51)	12.46 (12.98)	7.06 (7.20)	19.25 (19.78)	986.07
$[Pt(L^1)_2]$	Light yellow	146-149	41.89 (42.05)	2.16 (2.84)	9.09 (9.19)	13.92 (14.01)	-	21.05 (21.36)	913.07
$[Pd(L^1H)_2]Cl_2$	Brick red	152-154	42.56 (42.78)	3.05 (3.12)	9.11 (9.36)	14.08 (14.26)	7.56 (7.91)	11.42 (11.85)	897.42
$[Pd(L^1)2]$	Dark brown	158-160	46.15 (46.57)	3.10 (3.15)	10.01 (10.18)	15.03 (15.32)	-	12.46 (12.90)	824.42
L^2H	Creme	114	52.45 (52.99)	4.02 (4.10)	17.14 (17.66)	9.65 (10.09)	-	-	374

$[Pt(L^2H)_2]Cl_2$	Light Yellow	144-146	37.01 (37.33)	2.45 (2.88)	12.08 (12.44)	7.01 (7.11)	7.49 (7.88)	20.95 (21.6)	900.07
$[Pt(L^2)_2]$	yellow	146-148	40.10 (40.62)	2.45 (2.90)	12.94 (13.54)	7.65 (7.73)	-	23.06 (23.58)	827.07
$[Pd(L^2H)_2]Cl_2$	Brown	162-164	41.02 (41.40)	3.12 (3.20)	13.49 (13.80)	7.45 (7.88)	8.45 (8.75)	12.78 (13.11)	811.42
$[Pd(L^2)_2]$	Dark brown	165-168	45.26 (45.87)	3.11 (3.25)	15.11 (15.16)	8.26 (8.66)	-	14.07 (14.52)	732.42