Preparation and antimicrobial studies of metal complexes for schiff bases 2-(benzylidine) benzothiazole hydrazone

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الخلاصة

تضمن هذا البحث تحضير قاعدة شف 2- (benzylidine) benzothiazole hydrazone من تفاعل 2-hydrazinobenzothiazole مع p-hydroxy benzaldehyde

جرى تشخيص قاعدة شف بطريق طيف الأشعة تحت الحمراء (IR) وطيف الاشعة البنفسجية–المرئية (UV-Vis) و ثم استخدام هذه القاعدة كليكاند في تحضير المعقدات الجديدة مع ايونات عناصر البلاديوم ، النيكل، البلاتين والنحاس الثنائية و. جرى تناسق هذه الأيونات مع الليكاند من خلال ذرتي نتروجين مجموعة الأزوميثين . تم اقتراح الشكل الهندسي للمعقدات المحضرة باستخدام تقنية الامتصاص الذري أللهبي للعناصر و اطياف الأشعة تحت الحمراء و البنفسجية–المرئية (UV-Vis)إضافة لقياسات التوصيلية الكهربائية. ثم قياس النسبة المولية للفلز و الليكاند في محلول اليثانول بأتباع طريقة النسبة المولية ، وقد أعطت هذه الدراسة نتائجاً مطابقة مع تلك التي تم الحصول عليها في الحالة الصلبة ، فضلاً عن ذلك تم حساب قيمة الممتصية المولارية للمعقدات . تم تقويم الفعالية المضادة للبكتريا والفطريات للمعقدات و اختير ثلاث انواع من البكتريا (*Eschericha coli*) و (*Staphylococcus aurous*) و (*Pseudomonas aeruginosa*) ونوعين من الفطريات , *Candidas Aspergillus flavus*.

Abstract :

This work describeds the preparation of Schiff bases 2-(benzylidine) benzothiazole hydrazone from reaction 2-hydrazinobenzothiazole with p-hydroxy benzaldehyde. The product was characterized by infrared spectra (IR) and UV-Vis. Spectroscopy, this Schiff bases act as a ligand coordinating with the metal ions [Pd(II), Ni (II), Pt(II) and Cu(II)] through nitrogen of the isomethene group .The stereochemistry around the metal ion has been suggested using flam atomic absorption technique FT-IR and UV-Vis. Spectroscopy , conductivity measurement. The study of the nature of the complexes formed in (ethanolic solution) following the mole ratio method , gave results which were compared successfully with those obtained from solid state studies . As well as the molar absorptivites have been calculated. The antibacterial activity for their metal complexes were studied against three selected micro-organisms *Eschericha coli* , *Staphylococcus aurous*, *Pseudomonas aeruginosa* and *fungi Aspergillus flavus* , *Candidas* .

Introduction

A large number of Schiff base compounds are known to form an important class of biologically active compounds with a variety of pharmacologic actions[1-5] .The presence of nitrogen of azo methane group serve as very good chelating agents , this may lead to synergistic effect in biological activities as well as synthetic models in studying the effect of metal ion on some structurally related enzyme activities[4] .

Schiff bases are an important class of ligands in coordination chemistry and find extensive application in different fields[6-8]. Some of these bases exhibited antimicrobial and anticancer activities[9-11]. The biological activities were attributed mainly to azomethin group[9]. Some complexes of Schiff bases were found more active than the parent ligands against becteria and fungi[12-14] and as herbicides[13]. Complexes containing more than one metal center represent synthetic models of ferromagnetic interaction between metal centres which can

explain oxidation-reduction proceses in biological systems in addition to their catalytic and biological activities[14,15]. The aim of this work preparation new metal complexes derived from Schiff bases 2-(benzylidine) benzothiazole hydrazone and study the biological activity .

Experimental

Preparation of 2-hydrazino-benzothiazole

In a two-necked round bottomed flask connected to reflux condenser and a thermometer, 2-thio-benzothiazole (0.003 mol ,0.5 gm) was dissolved in 15 ml ethanol to this solution (N_2H_4) (0.003 mol) was added dropwise with continuous stirring at 65 C° for 3 hrs . the mixture was poured on crushed ice to give brown precipitate which was recrystalized from ethanol to give good yield (Table I) (90%), m.p 164-166 C° , 3350 cm^{-1} (NH), 3450, 3550 cm^{-1} (NH_2).

Preparation of Schiff-basses

In round bottomed flask equipped with double surface condenser fitted with calcium chloride guard tube, a mixture of hydrazine compound (0.001 mol ,0.165 gm) and para hydroxyl aldehyde (0.001 mol ,0.122 gm) in ethanol 30 ml containing a drop of acetic acid was refluxed for 12 hrs. the mixture was poured on ice water to give precipitate which was recrystalized from acetic acid (Table I)

Preparation of the metal complexes

Ethanolic solution of each of the following metal ion solts (1 mmole of $CuCl_2.2H_2O$ (0.169 gm) , Ni $(NO_3)_2.6H_2O$ (0.267 gm), $K_2Pt.Cl_6$ (0.486 gm) and $PdCl_2$ (0.176 gm)) was added to ethanolic solution (1 mmole ,0.278 gm) of schiff base (L) with stirring. The mixture was heated under reflux for 90 min. during this time a precipitate was formed . The product was filtered off , washed with hot ethanol and then dried under vacuum .

Study of complex formation in solution

Complexes of (L) with metal ions were studied in solution using mix (chloroform-ethanol)as a solvent, in order to determined [M:(L)] ratio in the complex following molar ratio method. A series of solutions were prepared having a constant concentration (10^{-3} M) of the metal ion and (L). The [M/(L)]ratio was determined from the relationship between the absorption of the absorbed light and the mole ratio of[M:(L)]. The results of complexes formation in solution were listed in Table(1).

Physical measurements and analysis :

Melting points were recorded on Gallen Kamp melting point apparatus and were uncorrected . FT-IR spectra were recorded using FT-IR 8300 Shimadzu in the rang of (4000-200) cm^{-1}, samples were measured as (CsI disc). Electronic spectra were obtained using Hitachi U-2000 and Cary 100 conc. Spectrophotometer at room temperature . The measurements were recorded using a concentration of (10^{-3} M) of the complex in DMF . The metal content was estimated Spectrophotometrically using Atomic Absorption Shimadzu AA670 Spectrophotometer . Conductivity measurements were obtained using Corning Conductivity meter 220 . These measurements were obtained in DMF solvent as (10^{-3} M) concentration at 25C^{o}.Elemental analysis (C.H.N) were carried out in College of Science / University Al - Mustansiriya , Baghdad .

Stability constant of Schiff base complexes

The apparent stability constant of the (1:1) and (1:2) (Metal:Ligand) complex were evaluated as follows :

Two sets of solutions were prepared , the first one were formulated to contain stoichiometric amount (1ml) of (10^{-3} M) ligand and (1 ml) of (10^{-3} M) of metal ion by placing in to a three series of (10 ml) volumetric flasks . The solutions of the colored complexes were diluted to the mark with ethanol . The second set were formulated to contain five fold excess (5ml) of (10^{-3} M) ligand , by placing in to a three series of (10ml) volumetric flasks followed by addition of (1ml) of (10^{-3} M) of metal ion solution , the volumes were

then completed to the mark with ethanol The absorbance (As and Am) of the solutions were measured at (λ_{max}) of maximum absorption . The stability constant (Kf), and the molar absorptivity (ε_{max}) have been calculated, were listed in , Table(2) .

Study of biological activity for complexes

The biological activity of the complexes were studied against selected types of bacteria which include *Eschericha coli* , *Staphylococcus aurous, Pseudomonas aeruginosa* and *fungi Aspergillus flavus* , *Candidas* were cultivated in nutrient agar medium .

Two ***in vitro*** techniques were proceeded for studying antibacterial activity against the two strains , DMSO was used as a solvent and as a control , for both techniques the constructions of the compounds in this solvent were (10^{-3} M) . The first technique was the Disc Sensitivity Test[10], this method involves the exposure of the zone of inhibition towered the diffusion of micro-organism on agar plate. The plates were incubated for 24hr. at 37 C^{o}, the zone of inhibition of bacterial growth around the disc was observed.

Results and discussion

The starting compound 2-hydrazinobenzothiazole (1) required for the preparation of Schiff base was obtained by the hydrazinolysis of 2-mercapto-benzothiazole, the I.R spectrum showed (NH_2) stretching band at (3450-3550 cm^{-1}) and disappearance of the (SH) stretching band at (2600-2550 cm^{-1}), the hydrazine compound converted to Schiff base by condensation with para hydroxyl aldehyde to give 2-(benzylidine) benzothiazole hydrazone. The i.r spectrum showed disappearance of the (NH_2) group stretching bands (symmetrical & unsymmetrical) at (3450-3550 cm^{-1}) and appearance of (C=N) absorption band at (1610- 1650 cm^{-1}) and U.V. spectrum showed absorption band of (342 nm) for $n \rightarrow \pi^*$ transitions for (C=N) group and absorption at (250 nm) for $\pi \rightarrow \pi^*$ transitions of the aromatic ring

Table (1) : Physical properties of ligand and their complexes

Symp.	Colour	M.P. C°	Yield %	Elemental analysis Calc. / (Foud)			M :L	Suggested formula
				C%	H%	N%		
L	White	230-232	75	62.45 (62.10)	4.08 (3.90)	15.61 (15.05)	-	$C_{14}H_{11}N_3OS$
LCu	Green	256-276	50	38.26 (38.00)	3.41 (3.12)	9.56 (9.33)	1:1	$[Cu(C_{14}H_{11}N_3OS)_2Cl_2]$
LNi	Pale green	253-255	83	29.47 (29.25)	4.03 (3.85)	12.28 (11.99)	1:1	$[Ni(C_{14}H_{11}N_3OS)_2]NO_2$
LPt	Red-brown	315-317	84	22.42 (22.20)	1.46 (1.26)	5.60 (5.47)	1:1	$[Pt(C_{14}H_{11}N_3OS)_2Cl]$
LPd	orange	288-290	90	37.75 (37.58)	2.47 (2.33)	9.43 (9.27)	1:1	$[Pd(C_{14}H_{11}N_3OS)_2]Cl_2$

Table (2) : Physical properties of ligand and their complexes

Symp.	Molar conductivity $oh^{-1}.cm^2.mol^{-1}$	Stability constant Kf (L^{-1} . mol)
LCu	88.5	3.5×10^3
LNi	20	6.1×10^4
LPt	95	2.9×10^4
LPd	35	5.3×10^5

Electronic spectra of complexes

The UV-Visible of the free ligand in 10^{-3} M (EtOH) shows two absorption at 250 and 315 nm which are attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ respectively .

a- NiL : the pale green solution of nil complex shows three peaks at 331, 319 and 450 which is assigned to LMCT ($\varepsilon \approx 50000$ L.ml^{-1} cm^{-1}) $A_2g^4 \rightarrow T_2g^4$ and $A_2g^4 \rightarrow T_2g^4$ (F), this is supporting of octahedral configuration of Ni ($3d^8$) .

b- PdL : the orange solution of PdL in 10 M solution of ethanol gives two peak at 223, 309 nm which are attributed to $\pi \rightarrow \pi^*$ and C.T of L

$\rightarrow$ MCT (L $\rightarrow$ Pd^{+2}) which is in agreement with electronic transitions of square planer (d^8) .

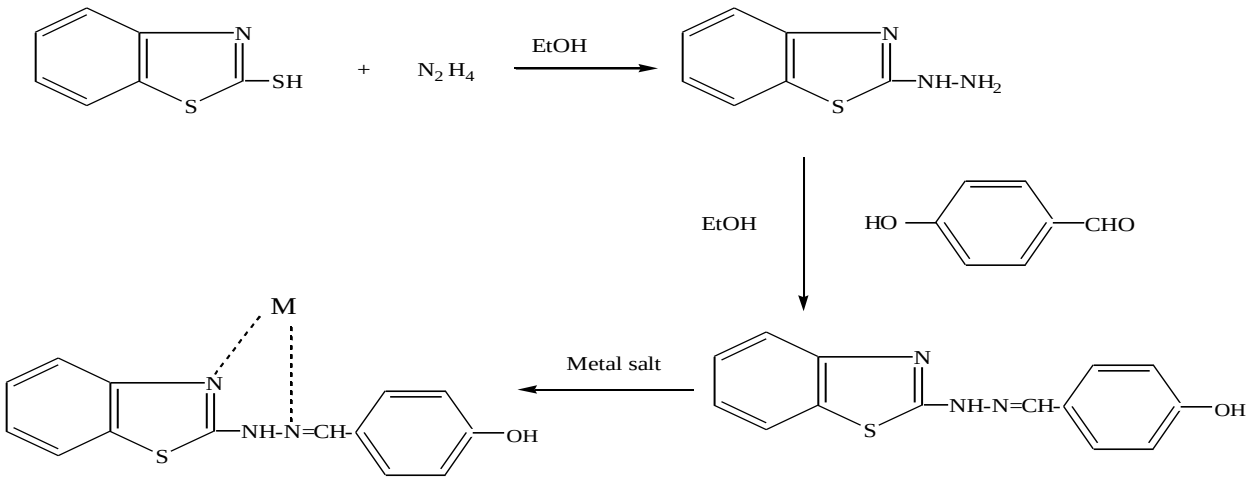
c- CuL : The pale green solution of complex in ethanol are assigned to $\pi \rightarrow \pi^*$ n $\rightarrow \pi^*$and Eg $\rightarrow$ T_2g^2 . the last peaks in the visible of d^9 – complex and this confirms the octahedral symmetry around Cu^{+2} ion

d- PtL : The electronic spectrum of PtL complex in ethanol was very complicated due to high intensits of MLCT and LMCT due to orbital contribution .

I.R. Spectra

From the data obtained the free ligand in KBr disc shows distinct vibrational modes at 1610 , 3450 and 1450 cm^{-1} which are assigned to C=N, O-H (phenalic) and N-H bonds respectively, which undergoes to red or blue shift in the formed complexes .

The isomethine of benzathiazole ring (N_3) and C=N terminal group participates in the coordination with the central ion, this is investigated from the shift of C=N to (1590-1580 cm^{-1}), and increasing (N-N) bond due to the reasonace to 1490-1470 cm^{-1}. moreover the appearance of low intensity absorptions in the range 400-480 cm^{-1} referred to (M-N) and (200-315 cm^{-1}) for (Cu-Cl) and (Pt-Cl). as well as the nitrato group NO_3^- for NiL complex showed strong absorption at 1496 cm^{-1} $(N=O)^{(g)}$, scheme (1) shows the preparation of ligand and their complexes .



Scheme (1)

Solution study :

(1)- Molar ratio method

The molar ratio method were followed to determine the [M:(L)] ratio. The results of complexes in ethanol (solvents) , suggest that the metal to ligand ratio was (1:1) which were comparable to those obtained from solid state study, Table (1) .

(2)- Stability constant of the Schiff base complexes

The apparent stability constant of the (1:1) [Metal:Ligand] (eq.1) or (1:2) [Metal:Ligand] (eq.2) complex,were evaluated using the following equations[6]:

$$(1)K = \frac{(1-\alpha)}{\alpha^2 C} \qquad (2)K = \frac{(1-\alpha)}{4\alpha^3 C^2}$$

(As and Am) of the solutions, were measured at (λ_{max}) of maximum absorption, furthermore the molar absorptivity (ε_{max}) for three complexes were calculated from the (eq.3) :

$$(3)A_m = \varepsilon_{\max}.bc$$

Biological activity

Biological Screening: Antimicrobial Activity Tests.

The biological activity of some of the prepared compounds was tested against one strain of Gram +ve bacteria (*Staphylococcus aurous*), Gram –ve bacteria (*Eschericha coli*, *Pseudomonas aeruginosa*), yeast (*Candidas*) and fungi (*Aspergillus flavus*).

Disc sensitivity test[16]was employed for the *in vitro* study for anti bacterial and anti fungal studies. This method involves the exposure of the zone of inhibition toward the diffusion of microorganism on agar plate. The plates were incubated for 24 hrs. at 37 °C, the zone of inhibition of bacterial growth around the disc was measured.

In order to complete this study, some of these new compounds were tested for their *in vitro* growth inhibitory activity against yeast (*Candidas*) and a pathogenic fungi i.e. *Aspergillus flavus*, on potato dextrose agar medium, then incubated at 30 °C for 72 hrs. The resulted are presented in table (3), Compounds (1,3,4) were nearly as active as the antibiotics against the E. Coli with (1) being the most active , also compound (1) only has activity against the *Staph.Aurous* and no effect against *Pseudomonas aeruginosa*. Moreover compounds (1-3) show similar activity against the yeast (Candidas) with (4) being the most active and all compounds shows no effect against *Aspergillus flavus*.

Table (3): Results of antimicrobial activities of the compounds (10^{-3} mg. mL^{-1})

Compound	*Staph. Aurous*	*E. Coli*	*Pseudomonas aeruginosa*	*Candida*	*Aspergillus flavus*
Control (DMSO)	0	0	0	0	0
1- CuL	7	10	0	10	0
2- NiL	0	0	0	13	0
3- PtL	0	6	0	13	0
4- PdL	0	8	0	17	0

Where:

6-8 mm: (+) 10-20 mm: (+++) (0) : no effect

8-10 mm: (++) 20-30 mm: (++++)

As a result from the study of antimicrobial for complexes, we are concluded :

1- It was of interest to investigate the effect of introducing the pharmacologically important Schiff base moiety in the structure of the some of prepared complex and their antibacterial with antifungl activities. For this purpose four complexes were prepared and the results in table (3) show large increase in the activity for some complexes, which indicate the importance of the Schiff base moiety (imin groups) in enhancement of biological activity of the studied complexes.

2- Results of the anti fungal activity of the new compounds , Table (3) showed that the metal ion chelates were more toxic toward the same micro-organism and under the identical experimental conditions. The increase in the antifungal activity of metal chelates may be due to the effect of metal ion on the normal cell process .These activities may be explained by Tweedy's Chelation Theory[17] ,according to which chelation reduces the polarity of the metal atom mainly ,because of the partial sharing of its positive charge with the donor groups of the ligand which favours permeation of the complexes through the lipid layer of cell membrane[18].

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