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Optimizing the interaction between a new complex of platinum and copper with immunoproteins

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Abstract---New two complexes of Platinum IV and Cupper II were synthesized and characterized in this research, Mannich base derivative L was synthesized as ligand containing 2-thion 1,3,4oxadiaol ring. A bidentate ligand was characterized by UV, FT-IR, CHNS, ^{1}HNMR and ^{13}C-NMR techniques while the complexes were characterized by UV, FTIR, CHNS, flame atomic absorption, molar conductivity and Magnetic susceptibilities. Supported by theoretical calculations which completed by MOE program for molecular docking of the complexes with ICAM-1 and IL-1 Immunoproteins, the optimizing conditions for this binding were carried out to establish the best concentration of ICAM-1 and IL-1, volume ratio for both complexes and proteins, incubation time and temperature for the binding. The pointed optimal conditions for ICAM-1 were : concentration 300 ng/mL, volume ratio 40:60, time 90 min and 35^{o}C for binding it's with platinum complex, concentration 300ng/mL, 60:40 volume ratio, time 60 min and 35 ^{o}C to bind with Cu complex. For IL-1 as: concentration 120pg/mL, volume ratio 20:80, time 60 min and 35^{o}C to bind with platinum complex and concentration 600 pg/mL, 80:20 volume ratio, time 60 min and 35 ^{o}C for its binding with Cu complex. Molecular docking carried out by MOE program confirm the success binding between Pt and Cu complexes with ICAM-1 and IL-1 by many stable conformations.

Keywords---interaction, new complex, platinum, immunoproteins.

Introduction

The interaction of metals with proteins is main goal for researchers as many endogenous proteins or enzyme using metals for their stabilization or function (El-fakharany and Redwan, 2019). Metal complexes also used for the diagnosis or treatment or various diseases, and certain metal ions are also concerned in diseases and toxicity(Paul *et al.*, 2019). Platinum complexes cisplatinum and carboplatinum interaction with protein and DNA discovered among tens of years and used as anticancer(Neelakantan *et al.*, 2018), these compounds have proven successful in treating of various types of tumor in the human body(Ii, Base and Protein, 2017).

Later, after knowing the characteristics that must be present in the chemical complex to work as a chemotherapy the treatment is still dose- -limited by side effects, narrow therapeutic window and acquired resistance(Harding, 2001) . These problems in the therapy have increased the interest in developing alternative strategies based on different metals with improved pharmacological properties, several complex of transition elements platinum and others were discovered and prepared by many researchers and test its activity as antibacterial, antiviral, antifungal and antitumor (Rodzik *et al.*, 2020) (Adam, Hassan and Hello, 2012). The most important characteristic of found in complexes rather than other types of chemical compounds is the easily interaction with molecules of proteins and nucleic acids (DNA and RNA)due to presence of several atoms capable of donating an electron pair and forming a coordination bond between empty orbitals of transition metals and amino acids in proteins or nitrogen base in nucleic acids.(Lee *et al.*, 2017)(de Sousa, Wlodarczyk and Monteiro, 2014)(Chen, Seukep and Guo, 2020).

Metal ions interact with proteins via coordinate bond formation with the side chains of amino acid, most common the imidazole heterocyclic on histidine residues, the carboxyl groups on glutamate and aspartate residues or sulfur on cysteine (Díaz *et al.*, 2020). The use of metal complexes, with one or more soft ligand that can exchange to allow formation of protein bound with complex (Lee *et al.*, 2017)(Jing *et al.*, 2017). Molecular docking is trial to find the best matching between two molecules or predict the preferred orientation of one ligand when bound in an active binding site to form stable complex(Chen, Seukep and Guo, 2020) (Neelakantan *et al.*, 2018).Considering that the binding between proteins and ligands is a complicated dynamic interaction process at the lowest energy conformations(Sciortino *et al.*, 2018). Many softwares had used for docking purpose like Molecular of Environment MOE, GOLD from university of Cambridge, AUTODOCK scripps research institute USA, GEMDOCK institute of bioinformatics national chiao TUNG university TAIWAN, …etc.

With obviously indications we focus on copper II and platinum IV complexes with thiadiazol ring derivative ligand. The present investigation concern with knowing the optimal conditions to interaction of our complexes with Intra Cellular Adhesion Molcule-1 ICAM-1 and Interlukine-1 IL-1. These protein were secreted in epithelial tissue in respiratory system(Rashad *et al.*, 2019)(Kaneko *et al.*, 2019), in high concentration case these protein caused rigidity of airways wall developing into breathing hardening, many disease will occur such as Asthma and

Bronchitis(Anbarasan, Bavanilatha and Latchumanadhas, 2015)(Dinarello, Dc and Dinarello, 2013). Dysfunction of ICAM-1 and IL-1 by our complexes is a result of interaction between them. Furthermore, our goal is supported by docking study by using computational calculations.

Materials and Methods

Materials and measurements

The reagents were obtained commercially and used without further purification.

Syntheses
General procedure for the synthesis of ligand

A 0.001 mole (0.214 g) of 6-Methoxy-2-naphthylacetic acid was mixed with 15 mL of Methanol and 10 drops of H_2SO_4 was added gradually to solution of . The mixture was refluxed at 60 ^oC for 4 h. An ester product precipitate was separated out and washed with acetone Akram *et al.*, 2018 The ester product 0.001mole(0.22 g) was refluxed with hydrazine (10.5 mL) in presence of methanol and refluxed at 60 ^oC for 1.5 h. An amide product precipitate was separated out and washed with acetone (Al-Jeboori, Abdul-Ghani and Al-Karawi, 2008). The amide product0.001mole (0.22g) was refluxed with CS_2 (7.5 mL) in presence of methanol and KOH and then refluxed at 70 ^oC for 2 h. A thiolate salt product precipitate was separated out and washed with acetone. 15drops of HCl was added slightly to salt to release the 1,3,4-Oxadiazole product (Pollak and Stanovnik, 1965). Last step and according Mannich reaction the ligand was synthesized by adding 5 mL of piprydine and 15 mL of formaldehyde to the solution of 0.001 mole 0.28 g from 1,3,4 Oxadiazole in methanol, the mixture was refluxed at 75 ^oC for 3 h. A precipitate was separated out and washed with acetone and dried at room temperature (Roman *et al.*, 2007).

General procedure for the synthesis of platinum (IV) and copper (II)-complexes

The complexes were prepared as shown in study (Zheng *et al.*, 2014)by the addition of heated solution of the metals salt , 0.001mole (0.225 g) of $CuCl_2.5H_2O$ and 0.0005mole (0.187 g) $PtCl_4.2H_2O$ in appropriate amount of Methanol [10mL]to the stirred and heated solution [65 °C] of the 1,3,4Oxadiazole 0.001 mole (0.37 g) previously dissolved in the Methanol.(Abdulameer, Abbas and Mosa, 2019) All the complex were characterized by melting point, flame atomic absorption, molar conductivity, magnetic moment, FT-IR, CHNS, and UV-visible spectroscopies .

Characterization

UV-Vis spectrum were taken for ligands and both of complexes by UV-160PC-Shimadzu spectrophotometer in chloroform. FT-IR spectrum were taken for ligands by FT-IR.8400S Shimadzu Spectrophotometer .in the range of 4000-350.cm-1, at college of science, University of Baghdad. Samples were measured as CsI disc. Elemental analyses spectrum were taken for ligand by Fison EA 1108

analyzer. ^{1}HNMR and ^{13}CNMR were taken by BRUKER AV 400 Avance-III (500 MHz and 125 MHz) instrument in d6 DMSO solution with theTMS as internal standard. Flame atomic absorption for complexes were taken by Nova350 Spectrophotometer.

Determination the Optimizing Conditions

For both complexes the value at LD50 which obtained from the cytotoxicity test was depended as constant concentration at all experiments of optimizing including choice the perfect concentration of ICAM-1 and IL-1, perfect volume that mixed with each complex, perfect time and perfect temperature.

Optimizing Conditions: Binding of ICAM-1 with cupper and platinum complexes
Determination the optimum concentration :

Five solutions had prepared by mixing of 100 µL from 875 µg/mL of Pt complex with 20 µL of [75, 150, 300, 600, 900] ng/mL of ICAM-1. Also for Cu complex five solutions had prepared by mixing of 100 µL from 1365 µg/mL of Cu complex with 20 µL of [75, 150, 300, 600, 900] ng/mL of ICAM-1. Then the absorbance taken at 282 nm for all solutions by UV-Vis spectrophotometer, the perfect concentration of ICAM-1was determined that achieves the highest absorption.

Determination the optimum volume ratio

Depending on the perfect concentration of ICAM-1 in obvious step and for choosing perfect volume for both Pt, Cu complexes and ICAM-1, five solutions were prepared by mixing a fixed concentration of ICAM-1 and complex with variable volumes according the ratios complex: ICAM-1 [100:0, 80:20, 60:40, 40:60, 20:80, 0:100] µL. Then the absorbance taken at 282 nm for all solutions by UV-Vis spectrophotometer, the solution has highest absorption has the perfect ratio of mixing.

Determination the optimum incubation time

One solution prepared by mixing perfect ratio of volumes for both complexes and ICAM-1 and then absorbance taken at 282nm by UV-Vis spectrophotometer after 30, 60, 90, 120 min. the solution has highest absorption is the perfect time of mixing.

Determination the optimum incubation temperature

Four solutions of Pt, Cu complex and ICAM-1 were prepared using perfect concentration, volumes ratio and time for mixing; each solution incubate at different temperature [20, 30, 35, 40] ^{0}C. Then the absorbance taken at 282 nm for all solutions, by UV-Vis spectrophotometer the solution has highest absorption has the perfect temperature of mixing.

Optimizing Conditions: Binding of IL-1 with cupper and platinum complexes
Determination the optimum concentration

Five solutions had prepared by mixing of 100 µL from 875 µg/mL of Pt complex with 20 µL of [15, 30, 60, 120, 180] pg/mL of IL-1. Also for Cu complex five solutions had prepared by mixing of 100 µL from 1365 µg/mL of Cu complex with 20 µL of [15, 300, 60, 120, 180] pg/mL of IL-1. Then the absorbance taken at 288 nm for all solutions by UV-Vis spectrophotometer, the perfect concentration of IL-1 the solution which have highest absorption.

Determination the optimum volume ratio

After knowing the perfect concentration in obvious step and for choosing perfect volume for both Pt, Cu complexes and IL-1, five solutions were prepared by mixing a fixed concentration of ICAM-1 and complex with variable volumes according the ratios complex : IL-1 [100:0, 80:20, 60:40, 40:60, 20:80, 0:100] µL. Then the absorbance taken at 288 nm for all solutions by UV-Vis spectrophotometer, the solution has highest absorption has the perfect ratio of mixing.

Determination the optimum incubation time

One solution prepared by mixing perfect ratio of volumes for both complexes and IL-1 and then absorbance taken at 282nm by UV-Vis spectrophotometer after 30, 60, 90, 120 min. the solution has highest absorption is the perfect time of mixing.

Determination the optimum incubation temperature

Four solutions of Pt, Cu complex and IL-1 were prepared using perfect concentration, volumes ratio and time for mixing; each solution incubate at different temperature [20, 30, 35, 40] ^{0}C. Then the absorbance taken at 288 nm for all solutions, by UV-Vis spectrophotometer the solution has highest absorption has the perfect temperature of mixing.

Docking study

MOE molecular of environment program was used for theoretical calculations. The chemical calculations for both two metal complexes were carried out to reveal the coordination and binding site between the Pt and Cu complexes with both of ICAM-1 and IL-1.

Results and Discussion

Characterization

UV chart for the ligand give two peaks at 278 nm and 315 nm belong to electronic transitions $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ respectively and one band as a shoulder at 350 nm showed in the figure 1, assigned to ($n \rightarrow \pi^*$) intra ligand transition(Xavier and Srividhya, 2014).

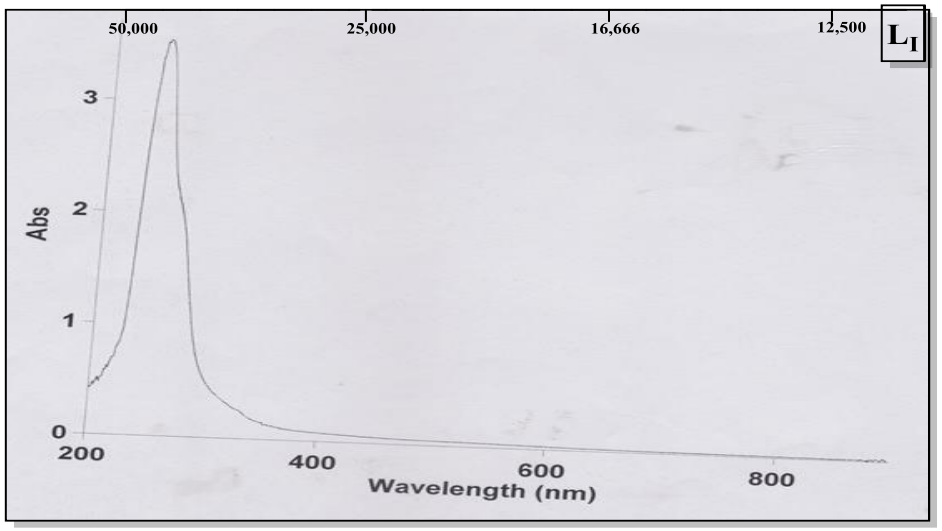


Fig 1. UV spectrum for ligand

For both complexes the peaks were shifted for a high wavelength region and appear at 508 , 460 nm and 425 nm for Pt complex belong to $^{1}A_{1}g \rightarrow {}^{3}T_{1}g$, .$^{1}A_{1}g \rightarrow {}^{3}T_{2}g$.and L→Pt respectively fig2, while for Cu complex appear at 698 nm and 450 nm due to $^{2}B_{1}g \rightarrow {}^{2}A_{1}g$ and $^{2}B_{1}g \rightarrow {}^{2}B_{2}g + {}^{2}Eg$ electronic transitions respectively fig3 (Al Zoubi *et al.*, 2018).

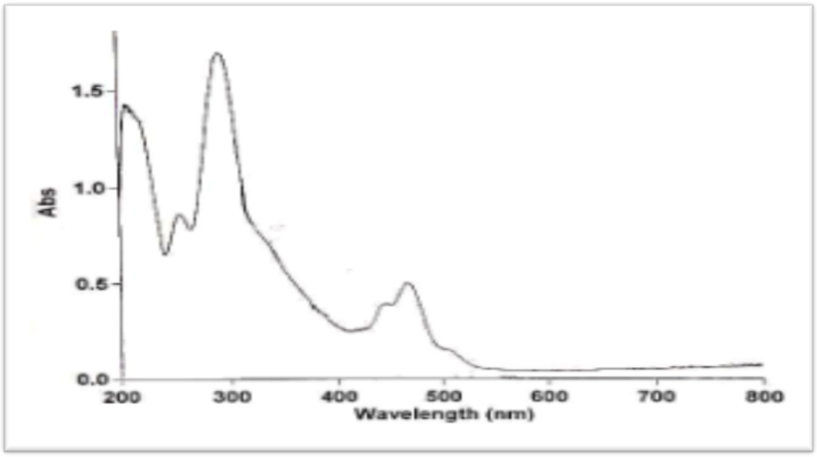


Fig 2. UV spectrum for Pt L

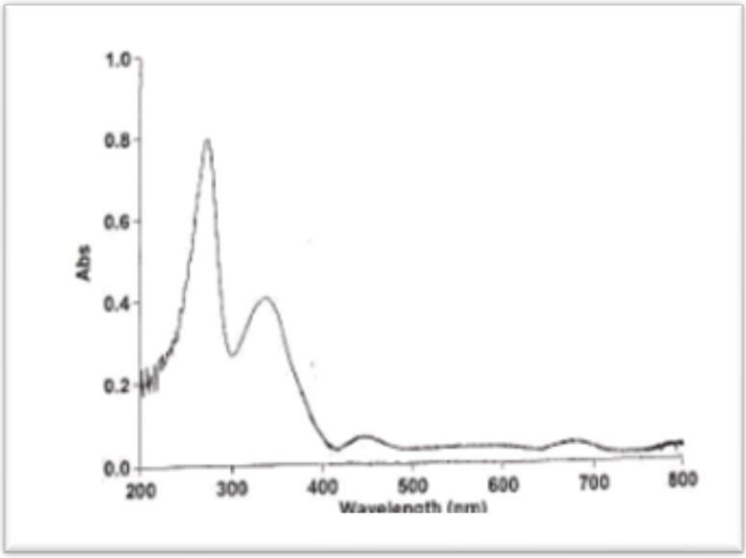


Fig 3. UV spectrum for Cu L

FT-IR spectrum for ligand give the bands at : 2995, 2837 cm^{-1} belong to vibration of N-CH_2-N , 1593 cm^{-1} due to vibration of C=N and 1062 cm^{-1} belong to stretching the bonds C=S, fig.4 (Lashanizadegan, Shayegan and Sarkheil, 2016).

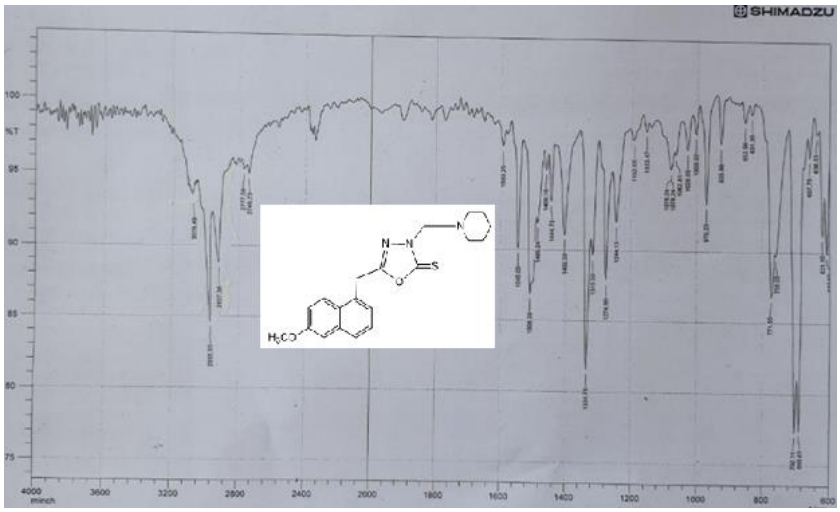


Fig 4. FT-IR spectrum for ligand

^{1}HNMR spectrum for ligand show the signals: multiple signals at 0.5-0.9, singlet 3.86, singlet 3.87, singlet 4.8-4.9 and multiplet 7.13-7.8 ppm attributed to : CH_2 in pipyridine ring (C1), two aliphatic protons CH_2 (C2), three protons at OCH_3 (C3), two protons in CH_2 (C4) and aromatic protons in phenyl ring (C5) respectively, fig5 (mahdi *et al.*, 2014). The data of ^{13}C-NMR (L) displayed good solubility in DMSO, Fig(3.00). The carbon nuclear magnetic resonance spectral data gave additional support for the composition of the ligand (L). According to

the results obtained from the chemical shifts spectra, the molecular structure of the ligand (L) can be illustrated as shown in Table1. (Al-Adilee and Hessoon, 2015)

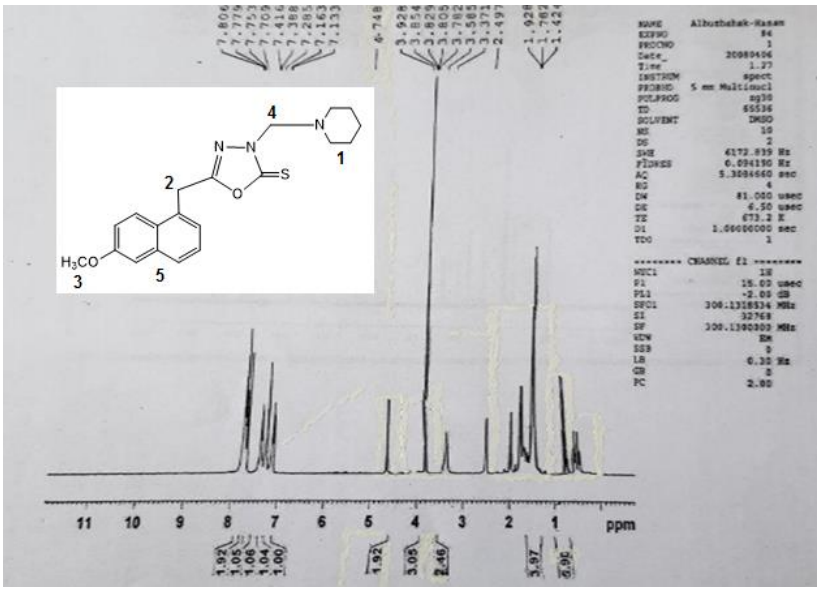
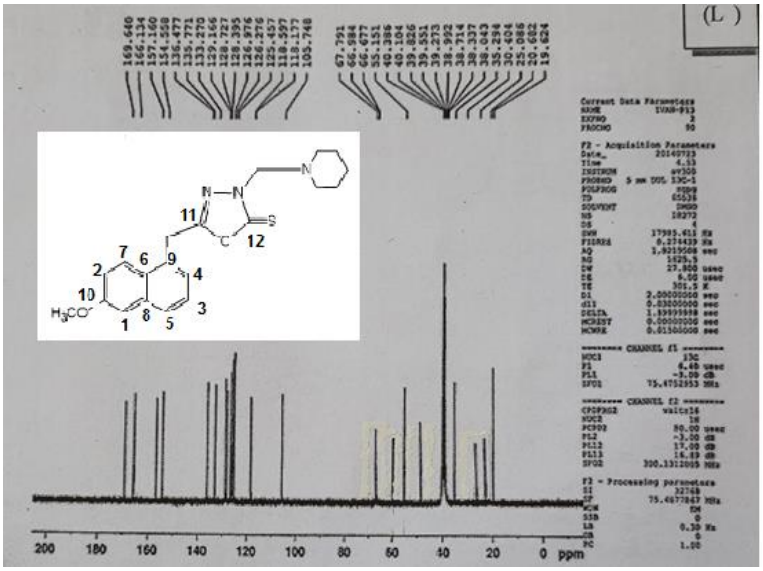
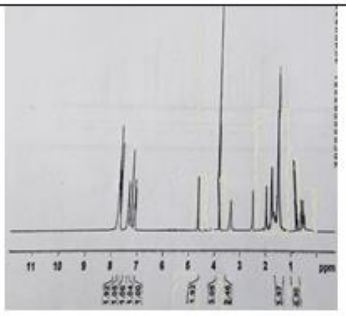
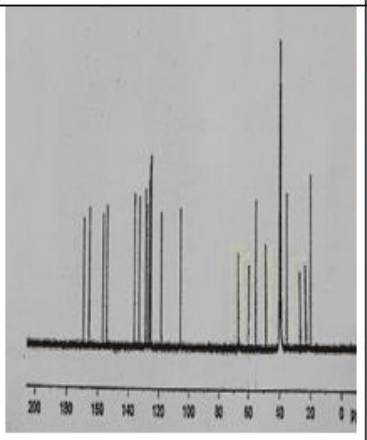


Fig 5. HNMR spectrum for ligand



^{13}C-NMR for ligand

Table 1
^{1}H NMR and ^{13}C NMR spectral data, chemical shifts and assignments of Ligand

Symbol	*Assignment ligand*	*Chemical shifts (ppm) (no. of protons and carbons)*
L		--multiple signals at 0.5-0.9 ppm, for CH_2 in pipyridine ring (C1) --singlet 3.86 ppm for two aliphatic protons CH_2 C2 -- singlet 3.87 ppm for three protons at OCH_3 -- singlet 4.8-4.9 ppm for two protons in CH_2 -- multiplet 7.13-7.8 ppm, for aromatic protons
L		C of pipyridine 20,25.5,28,51 ppm CH_2 56 ppm OCH_3 60 ppm CH_2 Mannich 67.7 ppm C1 106 ppm, C2 118.5 ppm, C3 126.3 ppm C4 127 ppm, C5 128.4 ppm, C6 129ppm, C7 129.2 ppm, C8 135.7 ppm, C9 136.5 ppm C10 157.2 ppm, C11 166.2 ppm, C12 169.6 ppm

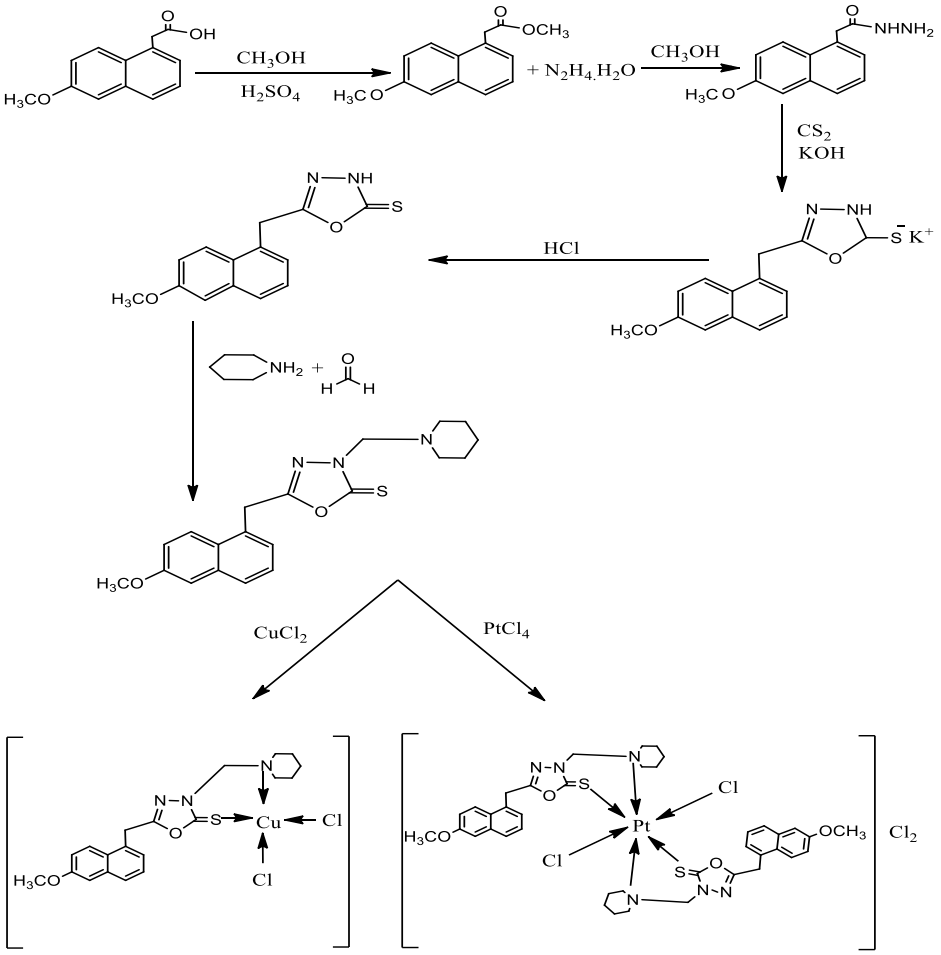
Elemental analysis for Ligand and complexes reveal approximately same ratio of C, H, N and S between the calculated and found ratios. Atomic flame absorption analysis showed proximate amount of Metals with the theoretical ratio. Table2 show the Elemental analysis and physical properties :

Table 2
Some analytical and physical data of (L) ligand and its complexes

Comp.	*Empirical formula*	*M.wt g.mol^{-1}*	*M.P C^{0}*	*Color*	Elemental analysis % Experimental (theoretical)				
					C	*H*	*N*	*S*	*M*
L	L $C_{20}N_3O_2SH_{23}$	369	150-152	White					
CuL	[Cu L Cl_2] H_2O [Cu($C_{20}H_{23}N_3SO_2$)Cl_2]	503.44	250	Dark green	48.04 (47.67)	4.79 (4.57)	8.52 (8.34)	6.68 (6.36)	13.12 (12.62)
PtL_2	[Pt $(L)_2Cl_2$] Cl_2 [Pt	1151.0	310	Dark reddish	46.12 (45.8	4.67 (4.39)	8.94 (8.02)	6.49 (6.11)	19.04 (18.62)

	$(C_{40}H_{46}N_6S_2O_4)Cl_2]\ Cl_2$			brown	1)				

The obvious analyses indicate success the synthesis of ligand and also complexation with each of Cu^{+2} and Pt^{+4}, so we suggest the flowchart of synthesis as below:



Scheme 1. Flowchart of synthesis of complexes

Binding studies between the Immunoproteins and Complexes
ICAM-1 with Pt and Cu complex
Optimum concentration of ICAM-1

After mixing of 100 µL from solution of 875 µg/mL Pt complex with five separated tube contain ICAM-1 solution in room temperature, the absorption was taken at 282 nm for each solution. The result showed high absorption belong to solution containing 300ng ofICAM-1, so the 300ng concentration chosen as optimum concentration to bind with platinum complex, fig6. After mixing of 100 µL from solution of 1365 µg/mL Cu complex with five separated tube contain ICAM-1

solution at room temperature, the absorption was taken at 282 nm for each solution. the result showed high absorption belong to solution containing 300 ng ofICAM-1, so the 300ng concentration chosen as perfect concentration to bind with Cu complex. We noticed that Pt and Cu complexes need the same concentration from ICAM-1 for the best binding results, as we expected the complexes bind with the equal number of ICAM-1 molecules also that attributed may be to the nature of ICAM-1 structure with five specific domain have a groups capable to coordinate with each coordinated metals Cu^{+2} and Pt^{+4}.

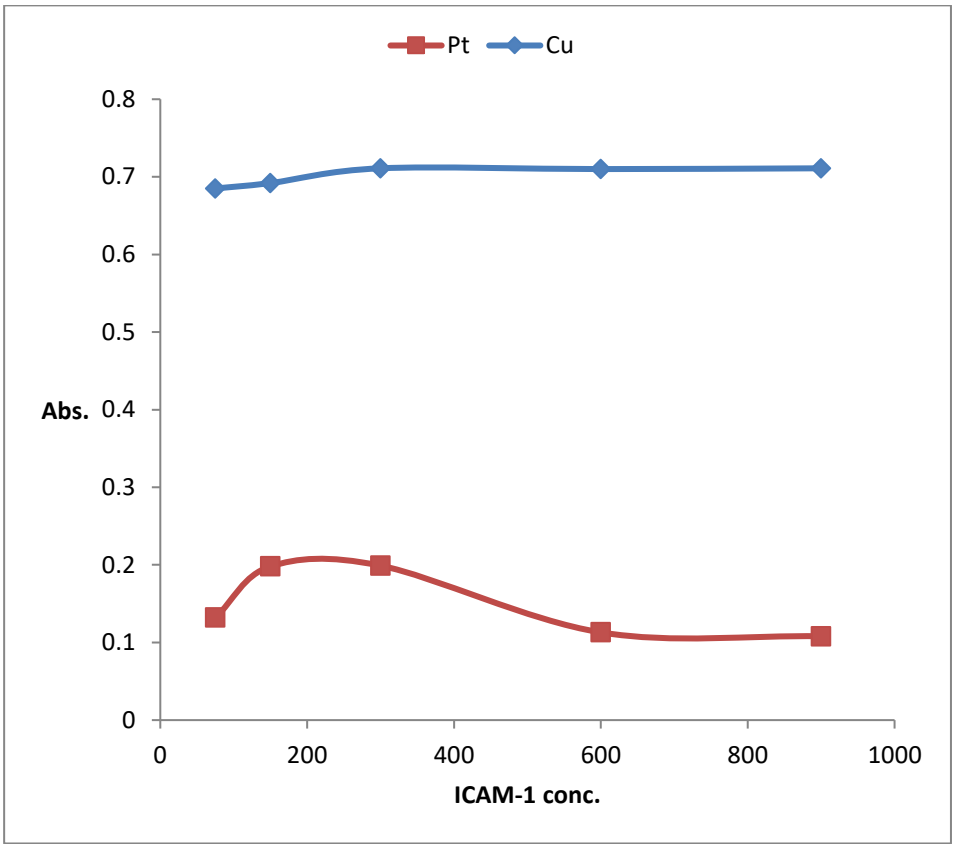


Fig 6. Absorption of different concentrations of ICAM-1 with Cu and Pt complex

Optimum volume of ICAM-1

After fixing of the ICAM-1 concentration at 300 ng, the solutions of different ratios were mixing also at room temperature and the measure the absorption at 288 nm. The fourth solution which contain [40:60µL] from Pt complex : ICAM-1 has a high absorption, so we fixed that best volumes for binding is 40 µL from complex and 60 µL from ICAM-1. After fixing of the ICAM-1 concentration at 300ng, the solutions of different ratios were mixing also at room temperature and the determine the absorption at 282nm. The third solution which contain [60:40µL] from Cu complex : ICAM-1 has a high absorption, so we fixed that best volumes for binding is 60 µL from complex and 40 µL from ICAM-1, fig7. The differences between best volume ratio of ICAM-1 with each Cu and Pt complexes is may due

to the size of the platinum complex which contains two ligands, sp^3d^2 hybridization and octahedral geometry, it needed 40:60 ratio, while it needed a ratio of 60:40 may be to the smaller size of the copper complex and its geometry where it is a square planer.

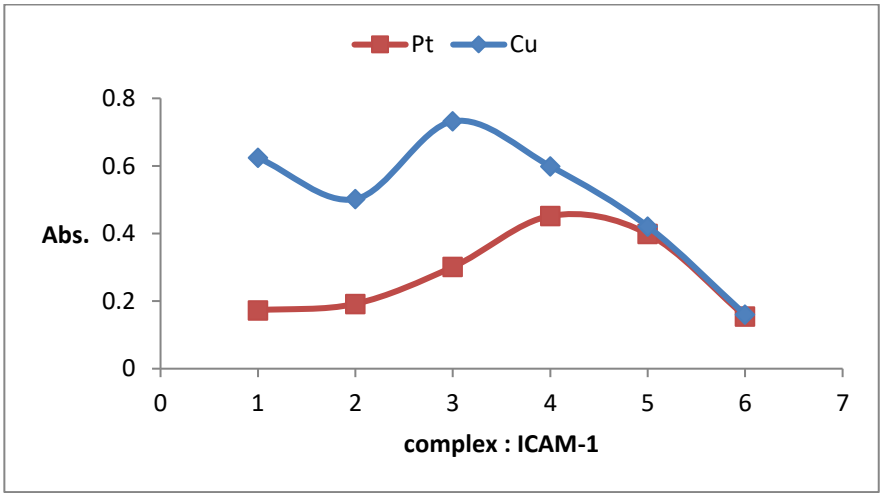


Fig 7. Absorption of of ICAM-1 with Cu and Pt complex in different volume ratios.

Optimum Time

A solution of icam-1 and Pt complex was prepared by mixing of 40 µL from 875 µg/mL complex and 60 µL /300 ng/ml of icam-1 at room temperature and then absorption was taken at 282nm after 30, 60, 90, 120 min. The solution give a best absorption after 90 minute of mixing so this time was fixed as perfect time for the binding. solution of icam-1 and Cu complex was prepared by mixing of 60 µL from 1365 µg/mL complex and 40 µL /300 ng/ml of icam-1 at room temperature and then absorption was taken after 30, 60, 90, 120 min. The solution give a best absorption after 60 minute of mixing so this time was fixed as Optimum time for the binding. Fig8. As expected the platinum complex need more time than copper complex to bind with ICAM-1 may be to the bulky size of platinum complex compared with copper complex since it needs more time to coordinate with the domains of IACM-1.

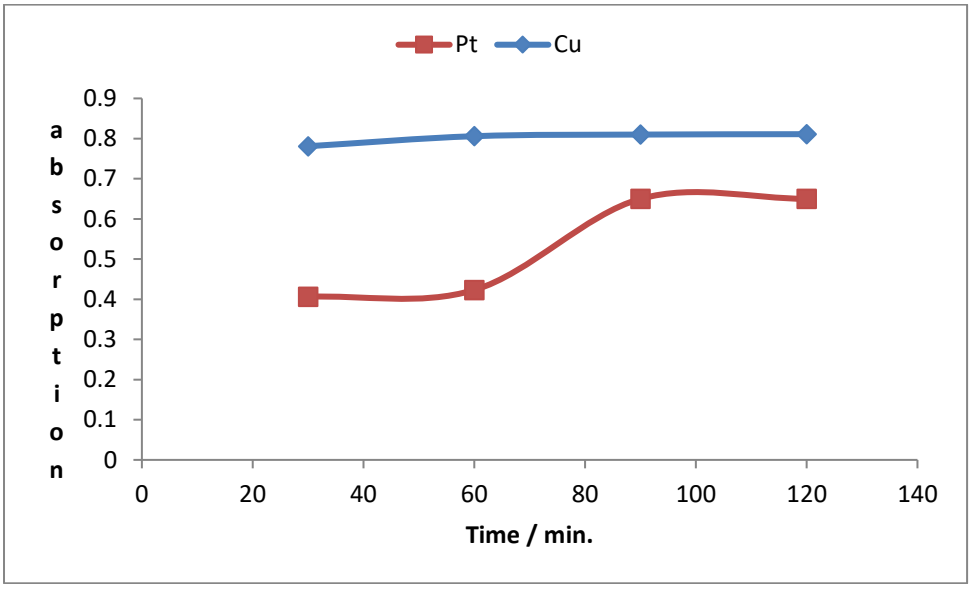


Fig 8. absorption of ICAM-1 with Cu and Pt complex at different times

Optimum temperature for mixing

four solutions from ICAM-1 and Pt complex (40 µL from 875 µg/mL complex and 60 µL /300 ng/ml of icam-1) has prepared and mixed for 90min. these solutions incubated separately at four different temperatures and then read the absorption at 282 nm. At 35°C the solution give a best absorption, so this temperature was fixed as perfect for binding study. four solutions from ICAM-1 and Cu complex (60 µL from 1365 µg/mL complex and 40 µL /300 ng/ml of icam-1) has prepared and mixed for 60min. these solutions incubated separately at four different temperatures and then read the absorption at 282 nm. At 35°C the solution give a best absorption, so this temperature was fixed as optimum for binding study fig9. At 20°C there is low binding of ICAM-1 with both complexes that's attributed to little activation energy at this temperature capable to replacement a few chloride ligands of complex with the donor groups at amino acids in the ICAM-1 structure, with increasing in temperature at 30 °C give a higher amount of bound molecules caused by high energy owned by complexes and ICAM-1, the best result of binding obtained at 35 °C and the degradation occur at 40 °C may be to the breaking the bonds between ICAM-1 and complexes and occurring the denaturation the protein molecule.

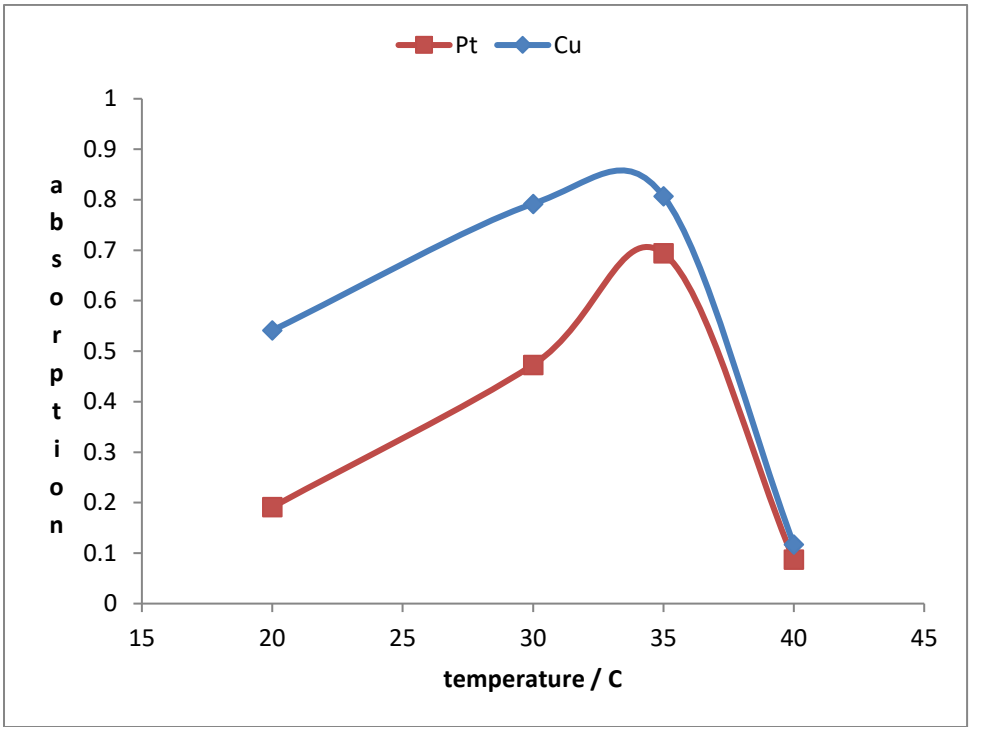


Fig 9. absorption of solutions of ICAM-1 with Cu and Pt complex at different temperatures

IL-1 with Pt and Cu complex
Optimum concentration of IL-1

After mixing of 100 µL from solution of 875 µg/mL Pt complex with five separated tube contain IL-1 solution in room temperature, the absorption was taken at 288 nm for each solution. the result showed high absorption belong to solution containing 120 pg ofIL-1, so the 120 pg concentration chosen as perfect concentration to bind with platinum complex, fig10. After mixing of 100 µL from solution of 1365 µg/mL Cu complex with five separated tube contain IL-1 solution in room temperature, the absorption was taken at 288 nm for each solution. the result showed high absorption belong to solution containing 60 pg ofIL-1, so the 60 pg concentration chosen as optimum concentration to bind with Cu complex. From these result we noticed that Pt complex need 120 pg concentration while Cu complex need 60 pg to give best result of binding, this is caused may be to the differences of charge of the metal ions since the platinum exist with +4 oxidation state which facing a hardening to coordinate with IL-1 contained a bulky network of hydrogen bonding in its structure while the copper exist in +2 oxidation state so it needs less concentration from IL-1 to binding. NOTE this character is not found in case of ICAM-1 because the ICAM-1 contain five domains to coordinate and don't effected by number of bind sites.

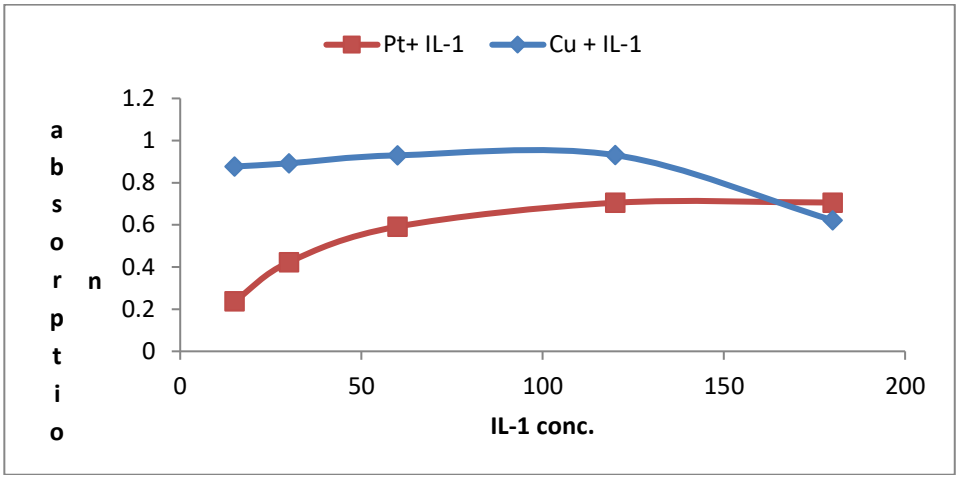


Fig 10. absorption of different concentrations of IL-1 with Pt and Cu complexes

Optimum volume of IL-1

After fixing of the IL-1 concentration at 120 pg, the solutions of different ratios were mixing also at room temperature and the measure the absorption at 288 nm. The fifth solution which contain [20:80µL] from Pt complex : IL-1 has a high absorption, so we fixed that best volumes for binding is 20 µL from complex and 80 µL from IL-1, After fixing of the IL-1 concentration at 60 pg, the solutions of different ratios were mixing also at room temperature and the measure the absorption at 288 nm. The second solution which contain [80:20µL] from Cu complex : IL-1 has a high absorption, so we fixed that best volumes for binding is 80 µL from complex and 20 µL from IL-1, fig11. The differences between best volume ratio of IL-1 with each Cu and Pt complexes is also may be to the size of the platinum complex which contains two ligands, sp^3d^2 hybridization and octahedral geometry and smaller size of the copper complex and its geometry where it is a square planer, since the number of IL-1 sites capable to bind with the metals is less than ICAM-1 sites so that IL-1 is effected by geometry of complex and steric hindrance.

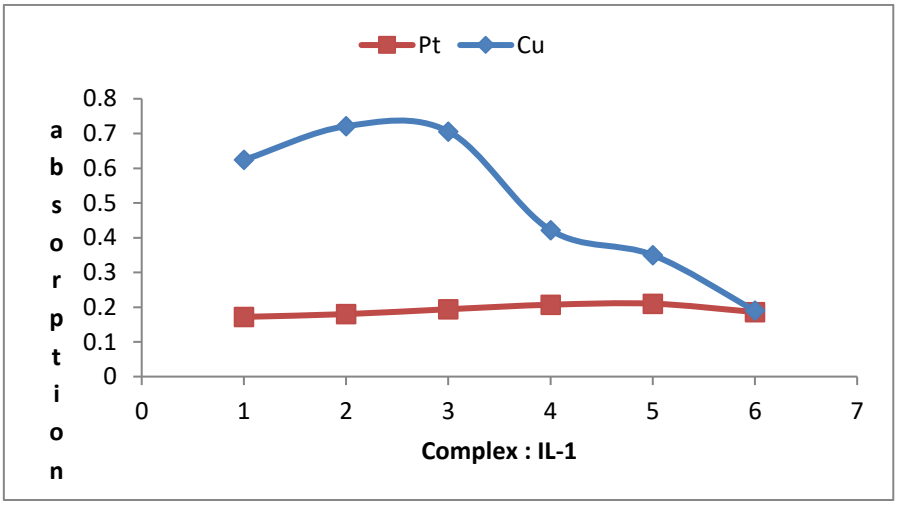


Fig 11. absorption of different volume ratios of IL-1 with Cu and Pt complex

Optimum Time

A solution of IL-1 and Pt complex was prepared by mixing of 20 µL from 875 µg/mL complex and 80 µL /120 pg/ml of iL-1 at room temperature and then absorption was taken at 288nm after 30, 60, 90, 120 min. The solution give a best absorption after 60 minute of mixing so this time was fixed as perfect time for the binding, A solution of IL-1 and Cu complex was prepared by mixing of 80 µL from 1365 µg/mL complex and 20 µL / 60 pg/ml of iL-1 at room temperature and then absorption was taken at 288nm after 30, 60, 90, 120 min. The solution give a best absorption after 60 minute of mixing so this time was fixed as optimum time for the binding, fig12. Both complexes need to same period to give the best may be because it occupy all the bind sites at same time and stay constant with any more time.

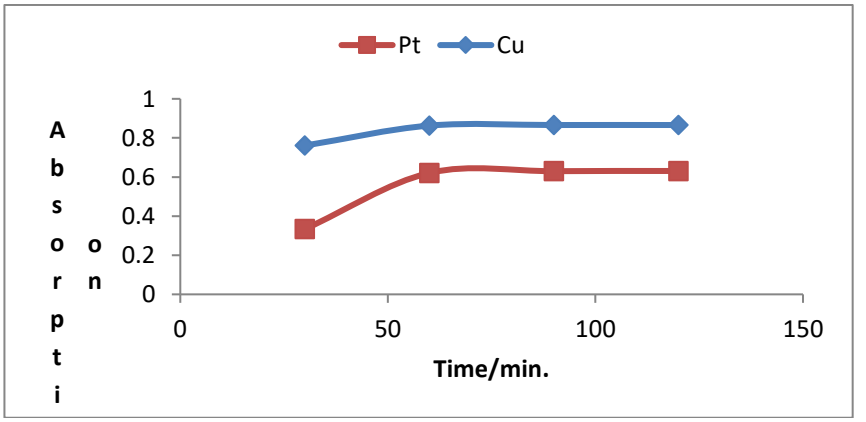


Fig 12. absorption of solution of IL-1 with Cu and Pt complex at different times.

Optimum temperature for mixing

four solutions from IL-1 and Pt complex (20 µL from 875 µg/mL complex and 80 µL /120 pg/ml of icam-1) has prepared and mixed for 60min. these solutions incubated separately at four different temperatures and then read the absorption at 288 nm. At 35°C the solution give a best absorption, so this temperature was fixed as perfect for binding study. four solutions from IL-1 and Cu complex (80 µL from 1365 µg/mL complex and 20 µL /60 pg/ml of iL-1) has prepared and mixed for 60min. these solutions incubated separately at four different temperatures and then read the absorption at 280 nm. At 35°C the solution give a best absorption, so this temperature was fixed as optimum for binding study, fig13.

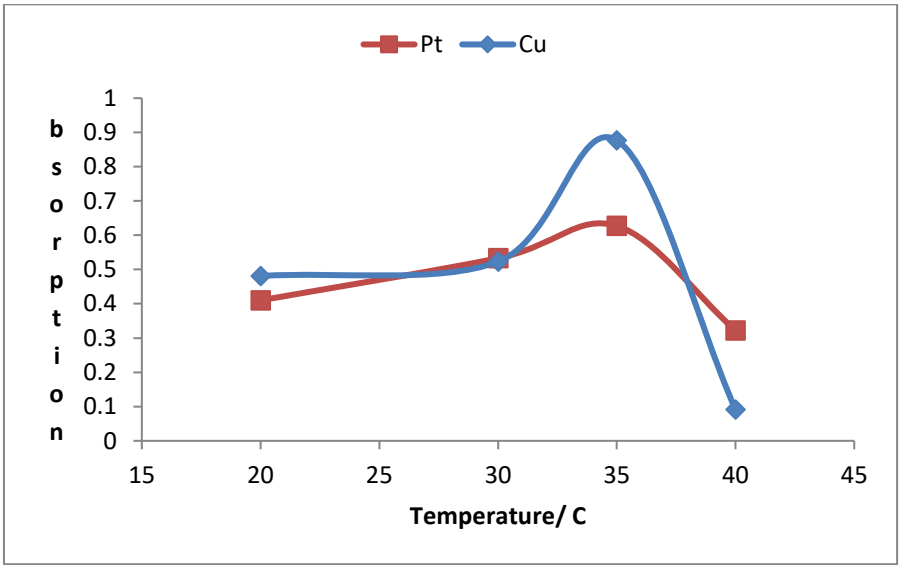


Fig 13. absorption of solution of IL-1 with Cu and Pt complex at different temperatures

As we explained in the binding of ICAM-1 with complexes, there is low binding of IL-1 At 20°C with both complexes that's may be to the little activation energy at this temperature capable to replacement a few chloride ligands of complex with the donor groups at amino acids in the IL-1 structure, with increasing in temperature at 30 °C give a higher amount of bound molecules caused by high energy owned by complexes and IL-1, the best result of binding obtained at 35 °C and the degradation occur at 40 °C may be to the breaking the bonds between IL-1 and complexes and occurring the denaturation the protein molecule.

Docking Study of complexes with ICAM-1 and IL-1

Molecular docking completed by MOE program and the data of proteins were collected from Protein Data Bank PDB coded as 2WRY for IL-1 and 1LC1 for ICAM-1. The calculations revealed that Pt complex can bind with ICAM-1 by five binding conformations, two favorable conformation as show below :

conformation1

Ligand	Receptor				Interaction	Distance	E (kcal/mol)
C 26	O	THR	28	(A)	H-donor	3.04	-1.2
6-ring	CE1	HIS	18	(A)	pi-H	4.33	-0.5
5-ring	CG2	THR	28	(A)	pi-H	4.39	-1.1
5-ring	N	ILE	81	(A)	pi-H	3.61	-1.0
6-ring	CB	PHE	82	(A)	pi-H	4.29	-0.9

conformation2

Ligand	Receptor				Interaction	Distance	E (kcal/mol)
6-ring	CG	MET	80	(A)	pi-H	4.67	-0.9
5-ring	N	ILE	81	(A)	pi-H	4.11	-2.4
6-ring	CB	PHE	82	(A)	pi-H	4.03	-0.5
6-ring	CB	PHE	82	(A)	pi-H	4.27	-0.5

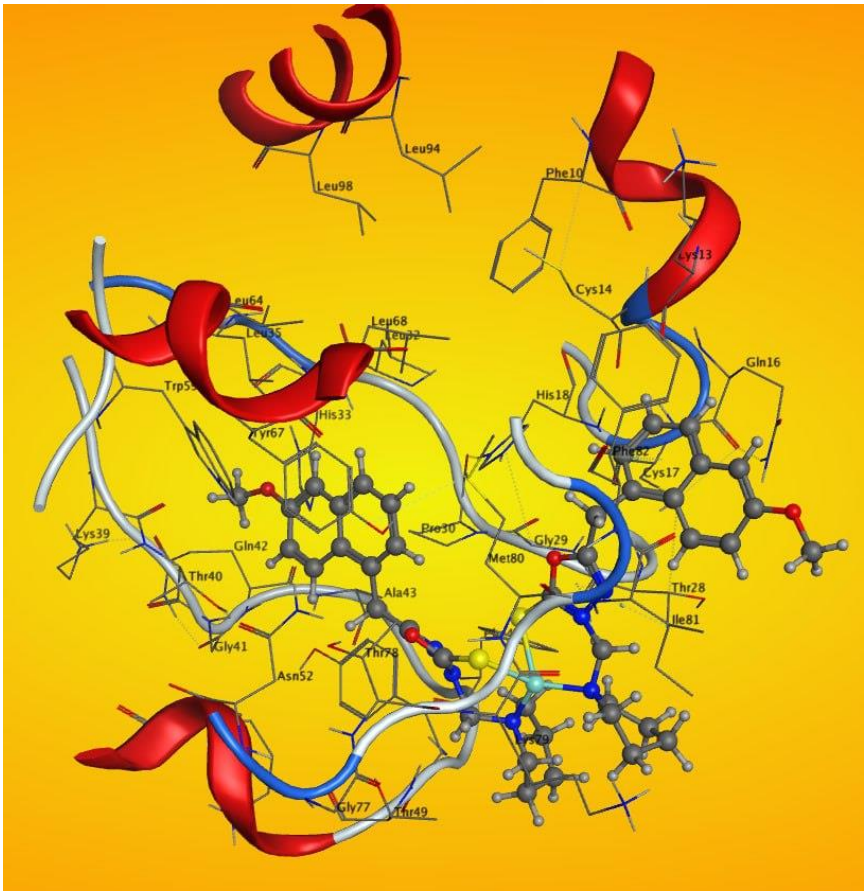


Fig 14. Three dimension image for the binding of platinum with ICAM-1

While with IL-1 results one favorable conformation with one bind by hydrogen bond with glutamine amino acid 42

Ligand	Receptor				Interaction	Distance	E (kcal/mol)
S 25	CB	GLN	42	(A)	H-acceptor	3.70	-0.5

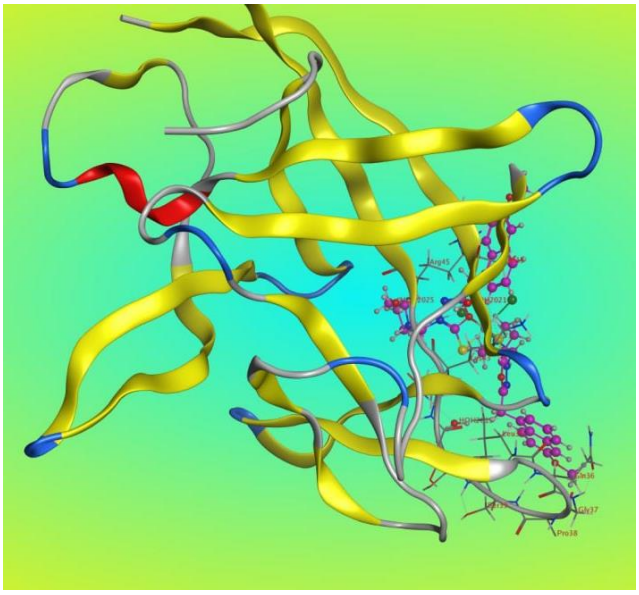


Fig 15. Three dimension image for the binding of platinum complex with IL-1

For Cu complex the result show also two conformations for binding with ICAM-1 for each of them one hydrogen bond formed between protein and Oxygen atom from ligand in the first conformation and with thiol group in other conformation as shown below :

conformation 1

Ligand		Receptor				Interaction	Distance	E (kcal/mol)
O	27	CE1	HIS	33	(A)	H-acceptor	3.21	-0.5

conformation 2

Ligand		Receptor				Interaction	Distance	E (kcal/mol)
S	1	N	THR	49	(A)	H-acceptor	3.99	-1.4

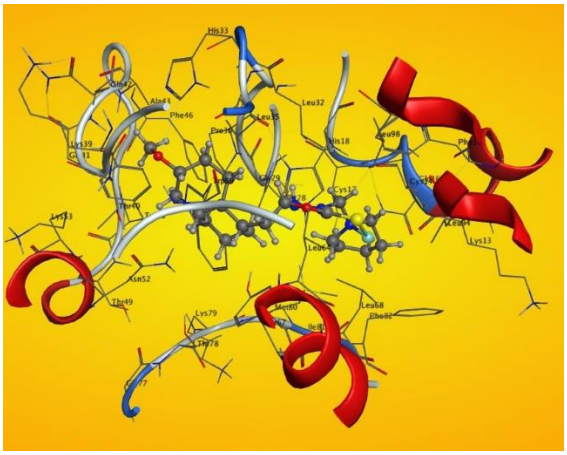


Fig 16. Three-dimension image for the binding of Cu complex with ICAM-1

While with IL-1 the complex of Cu^{+2} show the ability to binding by two conformation with some ionic bond in each of them as shown below :

conformation 1

Ligand		Receptor				Interaction	Distance	E (kcal/mol)
C	76	OE1	GLU	86	(A)	H-donor	3.21	-0.9
N	73	OE1	GLU	86	(A)	Ionic	3.52	-1.8

conformation 2

Ligand		Receptor				Interaction	Distance	E (kcal/mol)
N	40	OE2	GLU	104	(A)	H-donor	2.92	-2.0
O	27	NE2	GLN	90	(A)	H-acceptor	2.99	-1.8
N	42	OE2	GLU	104	(A)	Ionic	3.32	-2.6

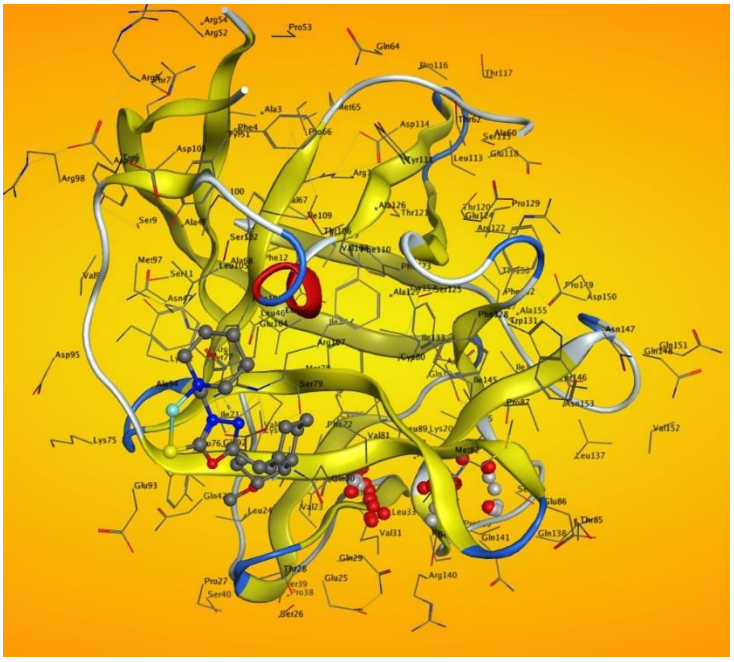


Fig 17. Three dimension image for the binding of Cu complex with IL-1

We notice that the successful of binding complexes with two protein by different ways these different came from different chemical characters of both complexes and immune protein. ICAM-1 have a five special domains capable to bind with each complex with two stable conformations. The energies and bond strength varying between Pt and Cu due to size and shape of complex, Pt complex has two ligand molecules so its capable to form more hydrogen bond with amino acid residues in protein. IL-1 has no special domains like ICAM-1 so it introduce binding conformations with little number of bonds having relatively similar energies and distances. This results are dealing with the optimum conditions we obtained since Pt complex required more concentrations of IL-1 than Cu complex to give high absorbance in experimental studies, in case of ICAM-1 the two complexes need the same concentration of ICAM-1 to give high absorbance.

Conclusion

A new complexes were synthesized and characterized with multi techniques, had applied to use it as drug by study the binding with immunproteins ICAM-1 and IL-1 that responsible on respiratory diseases. We focus to knowing the optimal conditions for the binding including the best concentration have to use, volume ratio for both complex and protein, best time and temperature for incubation. The best condition which give a higher absorption than other experiment and selected as optimum conditions for binding, also we noticed a differences between conditions for both complexes and proteins due to chemical conformation and geometries for molecules, finally we conclude the complexes can act as drugs came from its ability to bind and may blocking to ICAM-1 and IL-1 supported by computational studies.

Conditions	ICAM-1		IL-1	
	Pt complex	Cu complex	Pt complex	Cu complex
Concentration	300 ng/ml	300 ng/ml	120 pg/ml	60 ng/ml
Volume ratio (mL)	40:60	60:40	20:80	80:20
Time (min.)	90	60	60	60
Temperature (^{0}C)	35	35	35	35

These results were supported by theoretical calculations obtained from docking study. Since it confirm the binding and loading complexes on the proteins with many stable conformation.

References

Abdulameer, J. H., Abbas, S. K. and Mosa, M. J. (2019) 'Synthesis and characterization of nickel, palladium, platinum and cupper with new oxazipine ring', IOP Conference Series: Materials Science and Engineering, 571(1). doi: 10.1088/1757-899X/571/1/012099.

Adam, F., Hassan, H. E. and Hello, K. M. (2012) 'The synthesis of N-heterocyclic carbene-silica nano-particles and its catalytic activity in the cyclization of glycerol', Journal of the Taiwan Institute of Chemical Engineers. Taiwan Institute of Chemical Engineers, 43(4), pp. 619–630. doi: 10.1016/j.jtice.2012.01.013.

Akram, M. et al. (2018) 'Synthesis, biological evaluation and molecular docking studies of mannich bases derived from 1, 3, 4-oxadiazole-2-thiones as potential urease inhibitors', Tropical Journal of Pharmaceutical Research, 17(1), pp. 127–134. doi: 10.4314/tjpr.v17i1.18.

Al Zoubi, W. et al. (2018) 'Synthesis, characterization, and biological activity of Schiff bases metal complexes', Journal of Physical Organic Chemistry, 31(2), pp. 1–7. doi: 10.1002/poc.3752.

Al-Adilee, K. J. and Hessoon, H. M. (2015) 'Synthesis, identification, structural, studies and biological activity of some transition metal complexes with novel heterocyclic azo-schiff base ligand derived from benzimidazole', 7(8), pp. 89–103.

Al-Jeboori, M. J., Abdul-Ghani, A. J. and Al-Karawi, A. J. (2008) 'Synthesis and structural studies of new Mannich base ligands and their metal complexes', Transition Metal Chemistry, 33(7), pp. 925–930. doi: 10.1007/s11243-008-

9134-3.
Anbarasan, C., Bavanilatha, M. and Latchumanadhas, K. (2015) 'ScienceDirect ICAM-1 molecular mechanism and genome wide SNP ' s association studies', Indian Heart Journal. Elsevier Ltd. doi: 10.1016/j.ihj.2015.03.005.
Chen, G., Seukep, A. J. and Guo, M. (2020) 'Recent Advances in Molecular Docking for the Research and Discovery of Potential Marine Drugs', Marine drugs, 18(11). doi: 10.3390/md18110545.
de Sousa, G. F., Wlodarczyk, S. R. and Monteiro, G. (2014) 'Carboplatin: Molecular mechanisms of action associated with chemoresistance', Brazilian Journal of Pharmaceutical Sciences, 50(4), pp. 693–702. doi: 10.1590/S1984-82502014000400004.
Díaz, K. et al. (2020) 'Biological activities and molecular docking of brassinosteroids 24-norcholane type analogs', International Journal of Molecular Sciences, 21(5), pp. 8–10. doi: 10.3390/ijms21051832.
Dinarello, C. A., Dc, W. and Dinarello, C. A. (2013) 'Interleukin-1 in the pathogenesis and treatment of inflammatory diseases Interleukin-1 in the pathogenesis and treatment of inflammatory diseases', pp. 3720–3732. doi: 10.1182/blood-2010-07-273417.
El-fakharany, E. M. and Redwan, E. M. (2019) 'RSC Advances'. Royal Society of Chemistry, (November). doi: 10.1039/c9ra07127j.
et al. (2014) 'Preparation and Characterization of Some Metal Complexes with New Heterocyclic Schiff-Azo Ligand', Journal of Al-Nahrain University Science, 17(4), pp. 51–58. doi: 10.22401/jnus.17.4.08.
Harding, M. M. (2001) 'D57, 401±411 Harding Geometry of metal±ligand interactions', pp. 401–411. Available at: http://www.ebi.ac.uk/pdb/.
Ii, N., Base, S. and Protein, D. N. A. (2017) 'Ni(II) Complexes with Schiff Base Ligands: Preparation, Characterization, DNA/Protein Interaction and Cytotoxicity Studies', (Ii). doi: 10.3390/molecules22101772.
Jing, Z. et al. (2017) 'Study of interactions between metal ions and protein model compounds by energy decomposition analyses and the AMOEBA force field', Journal of Chemical Physics, 147(16). doi: 10.1063/1.4985921.
Kaneko, N. et al. (2019) 'The role of interleukin-1 in general pathology'. Inflammation and Regeneration, 5, pp. 1–16.
Lashanizadegan, M., Shayegan, S. and Sarkheil, M. (2016) 'Copper(II) complex of (±)trans-1,2-cyclohexanediamine azo-linked Schiff base ligand encapsulated in the nanocavity of zeolite-Y for the catalytic oxidation of olefins', Journal of the Serbian Chemical Society, 81(2), pp. 153–162. doi: 10.2298/JSC150708085L.
Lee, R. F. S. et al. (2017) 'Versatile Tool for the Analysis of Metal-Protein Interactions Reveals the Promiscuity of Metallodrug-Protein Interactions', Analytical Chemistry, 89(22), pp. 11985–11989. doi: 10.1021/acs.analchem.7b02211.
Neelakantan, M. A. et al. (2018) 'Interaction of Amino Acid Schiff Base Metal Complexes with DNA/BSA Protein and Antibacterial Activity: Spectral Studies, DFT Calculations and Molecular Docking Simulations', Applied Organometallic Chemistry, 32(4), pp. 1–19. doi: 10.1002/aoc.4259.
Paul, A. et al. (2019) 'DNA/protein binding and molecular docking studies of two tetranuclear Cu(II) complexes with double-open-cubane core like structure', Inorganica Chimica Acta. Elsevier, 495(July), p. 119005. doi: 10.1016/j.ica.2019.119005.
Pollak, A. and Stanovnik, B. (1965) '37 (1965) 215', 37(Iii), pp. 215–221.

Rashad, N. M. et al. (2019) 'Intercellular adhesion molecule-1 expression and serum levels as markers of pre-clinical atherosclerosis in polycystic ovary syndrome', Journal of Ovarian Research. Journal of Ovarian Research, 12(1), pp. 1–12. doi: 10.1186/s13048-019-0566-5.

Rodzik, A. et al. (2020) 'Interactions of whey proteins with metal ions', International Journal of Molecular Sciences, 21(6), pp. 1–26. doi: 10.3390/ijms21062156.

Roman, G. et al. (2007) 'Synthesis of new Mannich Bases derived from 5-phenyl-1,3,4-oxadiazole-2- thione and investigation of the conformational isomers of dimethyl 5-(5-phenyl-2-thioxo-1,3,4-oxadiazole-3-methylamino)isophthalate', Revue Roumaine de Chimie, 52(12), pp. 1103–1110. doi: 10.1002/chin.200901136.

Sciortino, G. et al. (2018) 'Prediction of the interaction of metallic moieties with proteins: An update for protein-ligand docking techniques', Journal of Computational Chemistry, 39(1), pp. 42–51. doi: 10.1002/jcc.25080.

Xavier, D. A. and Srividhya, N. (2014) 'Synthesis and Study of Schiff base Ligands', IOSR Journal of Applied Chemistry, 7(11), pp. 06–15. doi: 10.9790/5736-071110615.

Zheng, K. et al. (2014) 'Synthesis, structure and molecular docking studies of dicopper(II) complexes bridged by N-phenolato-N′-[2-(dimethylamino)ethyl]oxamide: The influence of terminal ligands on cytotoxicity and reactivity towards DNA and protein BSA', New Journal of Chemistry, 38(7), pp. 2964–2978. doi: 10.1039/c4nj00092g.