INTERACTION STUDIES OF SOME POTENTIAL ANTICANCER GOLD(III) COMPLEXES OF 1,2-DIAMINE CONTAINING LIGANDS WITH SOME BIOMOLECULES

BY

Khalid Hamid Omer Mohamed

A Thesis Presented to the
DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

CHEMISTRY

May 2015

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN- 31261, SAUDI ARABIA

DEANSHIP OF GRADUATE STUDIES

This thesis, written by **Khalid Hamid Omer Mohamed** under the direction of his thesis advisor and approved by his thesis committee, has been presented and accepted by the Dean of Graduate Studies, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN CHEMISTRY.**

Dr. Anvarhusein Isab
(Advisor)

Dr. Abdul-Aziz Al-Saadi
Department Chairman

Dr. Mohammed Wazeer
(Member)

Dr. Salam A. Zummo
Dean of Graduate Studies

Dr. Musa M. Musa
(Member)

12/5/15
Date

Dedication

This Thesis is dedicated to my beloved Parents, Brothers and Sisters whose love and patience were essential to its completion, as well as to my Fiancée for her prayers and support…

ACKNOWLEDGMENTS

I would like to thank my great creator almighty Allah for giving me strength, health, patience, science and power to complete this work and my MS degree. Also, I would like to express my sincere appreciations to my thesis Advisor Dr. Anvarhusein Isab for innovating research idea and for his guides, valuable comments, encouragements and continuous support during all work periods.

I would like to extend my gratitude to my Thesis committee members Dr. Mohammed Wazeer for his cooperation and continuous guidance throughout this work and to Dr. Musa Mohammed Musa for his unlimited help, and his important comments which helped me to accomplish my work smoothly. I will never forget to thank Dr. Abdel Nasser Kawde for his efforts and valuable time and Dr. Mohammed Fettouhi for his unconditional support and advices.

Special thank goes to King Fahd University of Petroleum and Minerals for giving me this chance to presume my MS degree. My appreciation is also due to Chemistry Department represented in our chairman Dr. Abd ulaziz Alsaadi and former Dr. Abd allah Al-hamdan, and to Dr. Bassam El-Ali (Graduate advisor) and to all Faculty members and staff.

Mr. Mohammed Arab and Mr. Mansour Alzaki are gratefully acknowledged for their helps and supports during conducting NMR and other spectroscopic experiments, I am also grateful to my friend Mr. Munzir Hamed Elneel for his effort with me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DACH	:	1,2-Diaminocyclohexane
en	:	Ethylenediamine
Met	:	L-Methionine
SeMet	:	DL-Selenomethionine
GSH	:	Glutathione
PSH	:	DL-Penicillamine
TmSH	:	Thiomalic acid
Imi	:	Imidazole
MAA	:	Mercaptoacetic acid
MPG	:	N-(2-Mercaptoprpinyl)Glycine
Hpm	:	2-pyridylmethanol
Terpy	:	Tripyridine
Dien	:	Diethylenetriamine
Phen	:	Phenanthrene
Cyclam	:	1,4,8,11-tetraazacyclotetradecane
TexR	:	Thioredoxin reductases

TACN	:	1,4,7-triazacyclononane
GMP	:	Guanosine monophosphate
Damp	:	2-(dimethylaminomethyl) phenol
Ala	:	L-Alanine
Gly	:	L-Glycine
His	:	L-Histidine
HEPES	:	N-(2-hydroxyethyl) piperazine – N′-(2-ethanesulfonic acid)
Hp	:	Hematoporphyrin

ABSTRACT

Full Name : Khalid Hamid Omer Mohamed

Thesis Title : Interaction studies of some potential anticancer Gold(III) complexes of 1,2-diamine containing ligands with some Biomolecules

Major Field : Chemistry

Date of Degree : May 2015

The interaction of gold(III) diammine complexes $[Au(en)Cl_2]Cl$, $[Au(en)_2]Cl_3$, $[Au(cis\text{-}DACH)Cl_2]Cl$ and $[Au(cis\text{-}DACH)_2]Cl_3$ with biologically important molecules like Glutathione (GSH), DL-Penicillamine (PSH), Mercaptoacetic acid (MAA) and N-(2-mercaptopropionyl)glycine (MPG), L-methionine (Met), L-selenomethionine (SeMet), imidazole (Imi), Thiomalic acid (TmSH) have been studied spectroscopically and electrochemically in aqueous solutions. The NMR data show oxidation of thiols to its disulfide (RSSR) along with reduction of gold(III) to gold(I). UV-Vis Kinetic scan shows formation of intermediates with formulas $[Au(diammine)(SR)X]^+$ (X: Cl in mono-complexes, N in bis-complexes) at about 231 nm and $[Au(diammine)(SR)_2]^+$ at 297 nm which are not observable in NMR. The *pseudo*-first order rate constants and activation parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ *and Ea*) were determined using UV-Vis spectrophotometeric data. The kinetics data for reaction with GSH indicates that the reactivity of ethylenediammine gold(III) complexes is higher than that for 1,2-diamminocyclohexane gold(III) complexes. The reactivity towards substitution reaction is found to be $[Au(en)Cl_2]^+ > [Au(en)_2]^{3+} > [Au(cis\text{-}DACH)Cl_2]^+ > [Au(cis\text{-}DACH)_2]^{3+}$ while the reactivity of investigated thiols varied with thiol size; *viz* MAA >> MPG > PSH > GSH. The $[Au(en)Cl_2]Cl$ gave a well defined (SWSV) signal at + 0.875V (vs Ag/AgCl sat KCl). Subsequent addition of 50 μM GSH to gold complex reduced the obtained complex signal with appearance of a new signal around +1.4V.

ملخص الرسالة

الاسم الكامل : خالد حامد عمر محمد

عنوان الرسالة : دراسة تفاعلات بعض معقدات الذهب +3 المشتقة من ليكاندات 2,1- ثنائى الامين المحتملة كمضادات للسرطان مع بعض الجزيئات الحيوية

التخصص : علوم الكيمياء

تاريخ الدرجة العلمية: مايو 2015

تفاعلات معقدات الذهب 3+ المحضرة احادى وثنائى ايثيلين داى امين وسيس داى امين سيكلو هكسان مع الجلوتاثيون , المثيونين , السلينومثيونين , الاميدازول , البنسيليامين , الثيومماليك اسيد , الميركابتو استيك اسيد والميركابتو بروبينيل جليسين قد درست فى محاليلها المائية باستخدام الاجهزة الطيفية الرنين النووى المغناطيسى والاشعة فوق البنفسجية والمرئية . الازاحة فى مقدار الازاحة الكيميائية دلت على اكسدة الثيولات والثيو ايثر بواسطة الذهب3+ الى داى سلفايدات وسلفوكسيدات بينما تم اختزال الذهب3+ الى الذهب 1+ . الطيف الالكترونى لمحلول التفاعل دلل على تكون مركب وسيط متوقع ان يكون للذهب 3+ مع المرتبطة داى امين سايكلو هكسان وجزيئين من الثيول عند طول موجى 297 نانوميتر فى حين لم يتم ملاحظة هذا المركب الوسيط باستخدام الرنين النووى المغناطيسى . عوامل التنشيط لهذة التفاعلات(ثابت المعدل, انثالبى التنشيط ,انتروبى التنشيط و طاقة التنشيط) تم حسابها تحت ظروف الرتبة الاولى غير الحقيقة باستخدام اجهزة الاشعة فوق البنفسجية والمرئية نتائج التفاعل مع الجلوتاثيون دلت على ان الفاعلية تترتب وفقا للاتى: معقد احادى اثيلين ثنائى الامين> معقد ثنائى اثيلين ثنائى الامين > معقد احادى داي امين سيكلو هكسين > معقد ثنائى داي امين سيكلو هكسين بينما فعالية الليكاندات وجدت تتبع الترتيب: الميركابتو اسيتك اسيد >> الميركابتو بروبينيل جليسين > البنسيليامين > الجلوتاثيون. المركب احادى ايثيلين داى امين اعطى قمة واضحة على باستخدام تقنية الاسكوير ويف عند قيمة +0.875 فولت لمحلول بتركيز 1 ملى مولار ضد الكترود الفضة/ كلوريد الفضة فى محلول كلوريد البوتاسيوم المشبع , الاضافة المتتابعة ل 50 ميكرو مولر من محلول الجلوتاثيون ادت الى اختزال القمة المعطاه للمعقد عند +0.875 فولت مع ظهور قمة جديدة عند حوالى 1.4+ فولت .

CHAPTER 1

INTRODUCTION

1.1 Metallodrugs in cancer treatment:

The emergence of *cisplatin* during the 1960s as a powerful anticancer agent excited a considerable deal of solicitude in the field of anti-tumour metallodrugs [1]. Although *cisplatin* has been used for decades in human cancer treatment, some resistance and toxic side effects were reported [2]. After a great success of *cisplatin* in cancer chemotherapy especial attention had been paid to gold(III) complexes due to the similarity between gold(III) complexes and cisplatin, both are isoelectric d^8 electronic system and isostructural and their complexes are tetracoordinate in centre of square planar [3]. One of the most important concerns of recent bio-metallic and bio-inorganic chemistry is to develop and introduce new metal based drugs [4–13]. The use of gold in anti-rheumatic treatment supports its pharmaceutical importance [14–17]. However gold(III) complexes are not stable enough under physiological conditions due to their high reduction potentials and fast rate of hydrolysis recently many gold(III) complexes were found to be unstable under physiological conditions and that was achieved by using property of interaction of gold(III) with biological ligands such as amino acids and peptides which are well known good chelating ligands that are able to bind with various metal ions [18], so the ligand selection is a very significant factor to improve the stability of gold(III)

complexes against hydrolysis and reduction and thus provide gold(III) complexes that are appropriate for biomedical applications [19]. Moreover the design of an efficient anticancer agents is very sophisticated game that encompass beside its inherent inhibitory action the resident time *in vivo*, dosage and delivery [20]. Because of high reduction potential of gold(III) complexes, it can be easily reduced to gold(I) or gold(0), and this is a conceivable problem in the body due to the existence of reducing thiol moieties, e.g amino acid cysteine ($HSCH_2CH(NH_2)CO_2H$). However, in recent years, an enormous number of gold(III) complexes with low reduction potentials for central metal ion were introduced; that success was achieved by utilizing polydentate ligands and it showed tangible stability under physiological conditions, this manifest very promising antitumor activity against different human tumor cell lines, both *in vitro* and *in vivo* [21]. To overcome the instability against hydrolysis, reduction reactions and to introduce gold(III) complexes suitable for biomedical applications, suitable ligands must be selected [19]. The possible involvement of gold(III) complexes in cancer treatment initiated an interest in the interactions of Au(III) with different bioligands, such as peptides and proteins. The most important amino acid in the study of interactions of metal ions with peptides and proteins is L-histidine, since it is found in the active sites of several metalloenzymes and of metal transport proteins [3].

1.2 Aim of study:

Recently, a great attention has been focused on the analysis of the interactions of cytotoxic gold (III) porphyrins with DNA. The first mechanistic studies on gold(III) porphyrins (using gold(III) mesotetraarylporphyrins) shows that they interact strongly

and directly with DNA, implying that DNA may actually constitute a preferential biomolecular target. However, in a successive study, the same authors reveal that gold(III) porphyrin acts differently from cisplatin *in vivo* as the gold compound causes DNA fragmentation rather than cross-linking; moreover, its interactions with DNA are reported to be noncovalent and reversible in nature [22]. Therefore the stability of gold(III) complexes toward the substitution reaction with some important biological target ligands such as protein, DNA, RNA and amino acids (especially sulfur containing amino acid) is consider an interesting area for research.

1.3 Technique and Methodology:

Preparation of gold(III) complexes is performed by a general method described in literature [23] for similar compounds by mixing gold(III) salt solution with each ligand solution. These complexes were characterized using elemental analysis, thermal analysis, mid- and far-IR spectroscopy, and NMR (^{1}H and ^{13}C) spectroscopy. Interactions of prepared gold(III) complexes with targeted bioligands studied using NMR by monitoring the change in chemical shifts for ^{1}H, ^{13}C and ^{15}N in the reaction mixture. The Kinetic studies of these interactions will be studied by employing UV-Vis spectrometry after investigating working wavelength and pH under *pseudo* first order condition as a function in ligand concentration. Rate of reaction and activation parameters will be calculated.

1.4 Research Objectives:

1. Interactions of the following complexes, [*cis*-1,2-(DACH)$AuCl_2$]Cl, [Au(en)Cl_2]Cl with Glutathione, Thiomalic acid, DL-Penicillamine will be studied by ^{13}C NMR.

2. Interactions of the following complexes [*cis*-1,2-$(DACH)_2Au]Cl_3$ and $[Au(en)_2]Cl_3$ with Glutathione, Thiomalic acid, DL-Penicillamine will be studied by ^{13}C NMR.

3. Interactions of the following complexes, [*cis*-1,2-(DACH)$AuCl_2$]Cl, [Au(en)Cl_2]Cl with R–S-CH_3-L-Methionine, R–Se-CH_3-(DL-Seleno-Methionine) will be studied by ^{13}C NMR.

4. Interactions of the following complexes [*cis*-1,2-$(DACH)_2Au]Cl_3$, and $[Au(en)_2]Cl_3$ with L-Methionine, DL-Seleno-Methionine) will be studied by ^{13}C NMR.

5. Interactions of the following complexes, [*cis*-1,2-(DACH)$AuCl_2$]Cl, [Au(en)Cl_2]Cl with Imidazole, Guanine and Cytosine will be studied using ^{13}C and ^{15}N NMR in solution.

6. Interactions of the following complexes [*cis*-1,2-$(DACH)_2Au]Cl_3$, and $[Au(en)_2]Cl_3$ with Imidazole, Guanine and Cytosine will be studied using ^{13}C and ^{15}N NMR in solution

7. The kinetics of these interactions will be studied using UV-Vis spectrometry and activation parameters for these interactions will also be calculated.

CHAPTER 2

LITERATURE REVIEW

2.1 Cisplatin and its anticancer activity:

Cisplatin is a powerful anticancer agent that was discovered by Rosenberg and his coworkers during an experiment using platinum electrodes to study the effect of electric fields on the growth of bacteria (*E. coli*); later this active compound was investigated and this chance observation led to such a powerful antitumor drug which is described as a tetra coordinate platinum(II) in the centre of square planar coordinated to two chloride ligands and two ammonia ligands with *cis* conformation and it is known by *cisplatin,* its chemical structure is illustrated in Figure 2.1 [6].

The possible mechanism of cytotoxicity of *cisplatin* might be through drug's interaction with DNA. Since the cisplatin is injected into the bloodstream and is believed to exist in its neutral state until after it crosses the cell membrane where one or both chlorides are displaced by aqua ligands (the chloride concentration being lower inside than outside the cell) affording cationic compounds. These cationic aqua derivatives react with the bases on DNA, most commonly with the N7 of purine bases (with guanine favored over adenine) which displace the aqua/chlorido ligands. A bifunctional adduct is formed between the {*cis*-$Pt(NH_3)_2$} unit and two adjacent bases on the same strand (the 1,2 intrastrand GG adduct accounts for >70 % of all adducts formed: 1,2 intrastrand AG adducts are the next most common at around 20 % of all lesions: 1,3 adducts and

monoadducts are much less common). The platinum center is located in the DNA major groove, and the effect of the platinum coordination to two adjacent bases is to bend (kink) the DNA by around 45°, toward the site of platination this bent DNA structure is then recognized by nuclear high-mobility group (HMG) proteins which bind and are believed to protect the lesion from DNA repair [24].

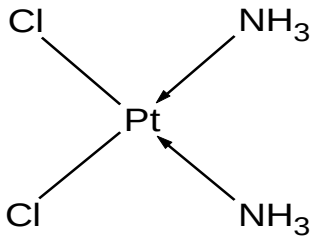


Figure2.1: Cis-diamminedichloridoPlatinum(II)

2.2 Gold complexes as anticancer agents:

Although *cisplatin* has been used for decades to treat human cancer, some toxic side effects and resistance are observed [8]. neurotoxicity, ototoxicity, anemia, nephrotoxicity and nausea (vomiting) are some of *cisplatin* side effects observed [9]. After success of *cisplatin* great interest had been paid to Au(III) complexes as new candidates for cancer treatment because Au(III) complexes are isoelectrical and isostructural with platinum(II) complexes; both are d^8 electronic systems and coordinate to four ligands in center of square planar geometry [2].

The emergence of Au(I) and Au(III) complexes overcome some of these challenges by forming strong covalent attachments to targets and added advantage of decreased toxicity [2].

Au(I) is d^{10} electronic system and complexes containing Au(I) found linear with two coordinate, the most important Au(I) complexes that has been used for medicinal purposes are thiolate and phosphene complexes, like auranofin, aurothiomalate, aurothioglucose, and auro-bis(thiosulfate), this preference of thiols and phosphines can referred to Au(I) is soft cation prefers binding with soft ligands [22].

Due to high potential of reduction and fast rate of hydrolysis Au(III) complexes were found to be unstable enough under physiological conditions like a Pt(II) complexes [25] but recently, a large number of gold(III) complexes were developed and the reduction potential of gold center was lowered by using polydentate ligands, that development was initiated by interactions properties of Au(III) with important biological molecules such as amino acids and peptides which are capable to coordinate many metal ions and form chelate of stable five member ring based on amino nitrogen and carboxylate oxygen. In peptides, this type of binding involves the nitrogen atom of amide bond(s) because the terminal amino nitrogen and carboxylate are too far from each other to coordinate to same metal ions [25].

2.3 Gold(I) complexes:

Gold(I) complexes are considered very interesting from applied and basic concern [26–28]. These complexes exhibits unusual liking between Au….Au characteristic of this sort of interaction. These interactions are stronger than van der waals but weaker than ordinary covalent bonds, and they were first found in gold complexes because of that these phenomena known by aurophilic. Gold complexes have been gaining significant role in medical field since they were used by ancient before more than 2000 years [29,30]. Today a number of gold based drugs are used to treat rheumatoid arthritis

symptoms, like aurothiomalate, aurothioglucose, aurothiosulfate, triethylphosphinegold(I)-tetraacetyl-thioglucose and aurothiopropanol sulfonate and they are also used mainly to prevent destructive progress of disease and this type of drugs which are used as inflammatory agents called Disease Modifying Antirheumatic Drugs (DMARDs), and the Antirheumatic gold compounds classified as DMARDs [8,31].

In vitro studies on auranofin showed that it also exhibit promising cell growth inhibition effects and some effectiveness was reported with *in vivo* models and that is in relationship with administrated dose in inoculated mice [32]. Moreover limited efficiency obtained in others *in vivo* studies and the reason till now is unclear. It might be that auranofin is a competent antitumor candidate, while among all tested mouse tumour models it was reported active only against i.p. P388 [33]. These observation have directly initiated immense attention on gold complexes as anticancer agents.

2.4 Gold(II) complexes:

Three stable platinum complexes were synthesized and characterized in unusual oxidation state as Pt^{+3} with octahedral geometry based on hematoporphyrin ligands. These three complexes were obtained in different kind of coordinate to HP ligand since it is controlled by M:L ratio and Pt(II) precursor; and the interaction of Pt(II) with HP leads to formation of Pt(III) accompanied with organic radical and appreciable decrease in pH. Their anticancer activity was evaluated against human cancer cell lines, $[Pt(III)HP_{-2H}.(H_2O)_2]$ shows promising cytotoxicity towards tested cancer cell lines [34]. Stable monomeric gold(II) complexes with HP in alkaline solution was obtained and formula $[Au(II)HP_{-2H}.(H_2O)_2]$ was suggested for octahedral complex (Figure 2.2); furthermore

+2 oxidation state for gold had been proven by EPR [35]. Prepared Au(II)-hematoporphyrin was studied in a panel of cancer cell lines and it displays significant cytotoxicity; moreover high sensitivity noticed with lymphoma and leukemia cells with corresponding IC_{50} values even comparable to *cisplatin* [36].

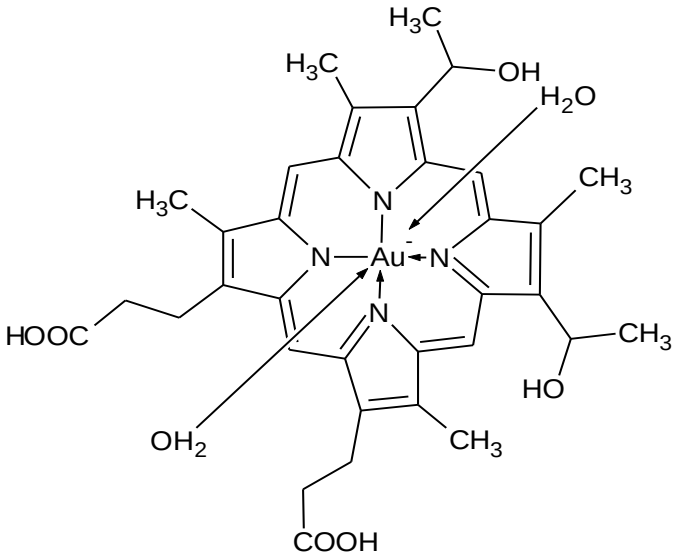


Figure2. 1: [Au(II)-HP_{-2H}.2H_2O]

2.5 Gold(III) complexes:

Depending on their similarity to *cisplatin* in structure and electronic, gold(III) complexes can be considered as strong candidates to achieve promising anticancer activity. However, involvement of gold(III) complexes as therapeutic agents has been limited by their poor stability under physiological conditions.

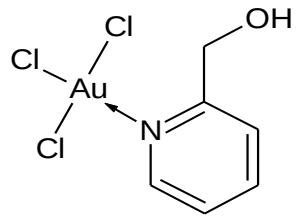
A number of gold(III) complexes were reported and their anticancer activity was investigated under physiological conditions, majority of them are gold(III) complexes containing Au-N and Au-Cl or Au-O bonds and some containing Au-C or Au-S bonds.

2.5.1 Gold(III) complexes containing Au-N bonds:

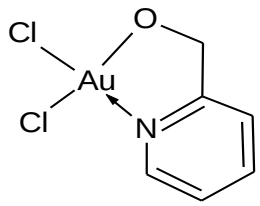
Two gold(III) complexes with 2-subsituted pyridine having the following formulas $[AuCl_3(Hpm)]$ and $[AuCl_2(pm)]$ were obtained and their solutions disposal was studied in order to investigate their ability to act as anticancer agents. Fast release of bounded chlorides was observed in water with preserve gold(III) centre and heterocyclic five member chelating site moreover this hydrolysis is faster in a physiological buffer. Both gold(III) complexes react with protein leading to conversion of gold(III) to gold(I) that was observed with albumin and transferin, Moreover rapid and strong binding of gold(III) complexes to calf thymus DNA or polynucleotide was shown, and cytotoxicity studies prove good antitumor activity for both gold(III) complexes even comparable to *cisplatin* [37]. Some bipyridine (bipy) gold(III) complexes $[Au(bipy^c\text{-}H)(OH)][PF_6]$ and $[Au(bipy)(OH)_2][PF_6]$ were found to be stable at temperature 37ºC with physiological buffers, whereas, the cytotoxicity studied on calf thymus DNA showed that interaction of tested complexes with DNA is reversible in nature and weak [38]. The use of polydentate ligands led to distinguish stabilization of gold metal centre in oxidation state of +3 under physiological-like conditions, and that can be clearly seen from their reduction potential measurements. $[Au(en)Cl_2]$, $[Au(phen)Cl_2]Cl$, $[AuCl(dien)]Cl_2$ and $[Au(terpy)Cl]Cl_2$ exhibited good cytotoxicity in A2780 ovarian cancer cells. But $[Au(cyclam)](ClO_4)_2Cl$ did not, moreover, the obtained results from cisplatin-resistance A2780 reflect the ability of these complexes to overcome drug resistance. The potency of free ligands was investigated and pointed out that the potency of free tripyridine and phenanthrene was similar to that for correspondening gold(III) complexes whereas free ethylenediamine driven ligands does not display any activity proving the activity of their

gold(III) complexes is due to presence of gold(III) centre. The results from [Au(cyclam)]$(ClO_4)_2Cl$ indicates that increasing the stability of gold(III) is in reverse relationship with activity [39].

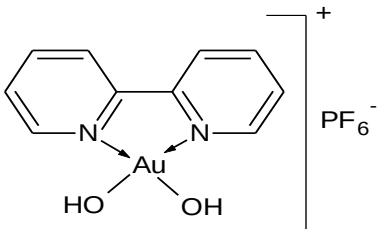
Compounds with similar structures were obtained for gold(III) using triazacyclononane ligand $[Au(TACN)Cl_2][AuCl_4]$ and $[Au(TACN)Cl_2]Cl$ The structure of first complex was determined by XRD, circular dichroism spectroscopy, UV-Vis and fluorescence were employed to study the interaction of $[Au(TACN)Cl_2]Cl$ with CT-DNA and the results shows that this complex can induce DNA double helix distortion that by replacing Ethidium bromide from DNA-EB system. Biological tests using A-549 and HCT-116 tumor cell lines proved better activity than anticancer drug *cisplatin* [40]. Zhu *et al* synthesized gold(III)-ethylenediamine complexes and studied their interactions with GMP, the electrochemistry showed the formal negative potential of gold(III)/gold(0) increased in order $[AuCl_4]^-$, $[Au(en)Cl_2]^+$ then $[Au(en)_2]^{3+}$ [41]. Furthermore, number of gold(III) complexes with alkylenediammine and N-alkyl-ethylenediamine were prepared and tested against gastric, prostate and ovarian cancer cells [42]. $[Au(en)Cl_2]Cl$ produced minimum changes in liver and kidney, showing that it is safer than other therapeutic drugs [43]. [(*sec*-butyl)(phen)$AuCl_3$] a penta-coordinate gold(III) complex, exhibits better cytotoxic properties compared with *cisplatin* itself [44]. The structure of some gold(III) containing Au-N bond is shown in figure 2.3 .



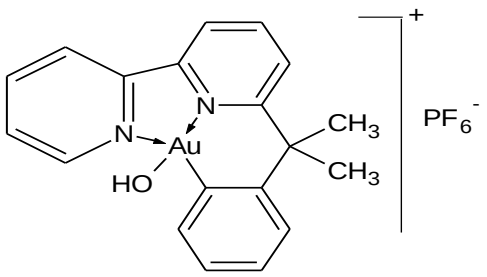
$AuCl_3(Hpm)$



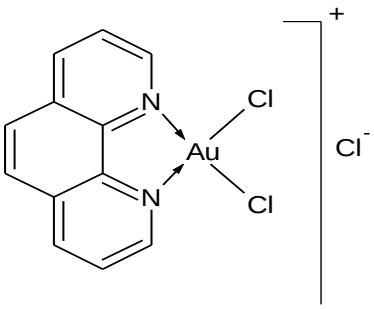
$AuCl_2(pm)$



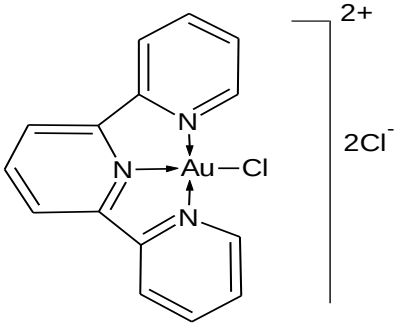
$[Au(bipy)(OH)_2][PF_6]$



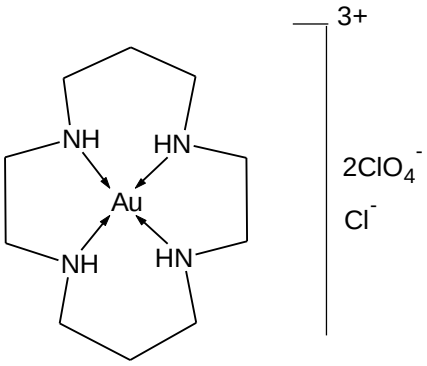
$[Au(bipy^c\text{-}H)(OH)][PF_6]$



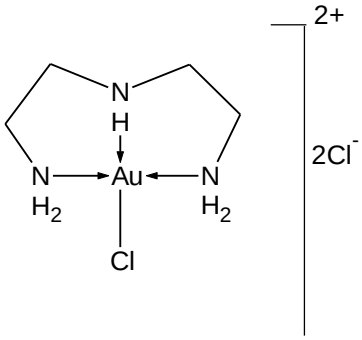
$[Au(phen)Cl_2]Cl$



$[Au(terpy)Cl]Cl_2$



$[Au(acylam)](ClO_4)_2Cl$



[Au(dien)Cl]Cl

Figure2. 2: Some of gold (III) complexes containing Au-N bonds

2.5.2 Gold (III) complexes containing Au-S bonds:

Gold(III) complexes with their Palladium(II) and Platinum(II) analogs with methylsarcosinedithiocarbamate (MSDT) were synthesized and characterized in order to study their ability to act as potential antitumor agents, [Au(MSDT)X_2] gave significant activity compared with *cisplatin* as a reference antitumor drug; and that in case of X is Cl or Br, [Au(MSDT)X_2] induced apoptosis when it was exposed to HeLa cells and HL60 cells [45]. A superior gold(III) anticancer was obtained by coordinate to N,N-dimethyldithiocarbmate (DMDT) and its *in vitro* results displayed cytotoxic behavior with value of IC_{50} value less than cisplatin by 1 to 4 times; also this activity extended to cisplatin sensitive cells that reinforce various mechanisms of action and exclude cross-resistance occurrence [46]. Gold(III) complexes with dithiocarbmate ligands gave full hydrolysis under physiological conditions during first hour with preserving oxidation state for central metal as +3 in solution. An *in vitro* study showed ability of examined complexes to bind to DNA leading to inhibition of formation of both DNA and RNA, and this can be rationalized by the high reactivity of gold(III) complexes towards some isolated biologically important macromolecules [47]. These compounds were found cause cancer cell death through apoptotic and non-apoptotic ways [48]. Recently, Isab and his coworkers reported a new type mixed ligands gold(III) complexes with general formula [(thione)$_2$Au(diamine)]Cl_3 using different diamine and thione ligands [49]. Spectroscopic characterization of these complexes indicates that all thione ligands were bounded to gold(III) centre through their thiocarbonyl sites with square planear geometry, An *in vitro* study shows cytotoxicity comparable to cisplatin against C6 glioma cells. Structural

difference like ring size of diamine ligands and thione ligands might have affected *in vitro* activity of presented compounds.

2.5.3 Gold (III) complexes containing Au-C bonds:

Different gold(III) compounds containing one or more gold(III)-carbon bonds were evaluated against TrxR and MCF-7 cancer cells. As gold(III)-carbon bonds were increased to two gold(III)-carbon bonds act as powerful inhibitors for reduction of Trx with IC_{50} value less than 2 nM. This inhibitory concentration does not correlate to ability of killing cells, and only one complex with two gold(III)-carbon bonds (Figure 2.4) was found to inhibit the formation of colony by MCF-7 breast cancer cells at micromolar concentrations [50]. A group of gold(III) based on 2-phenylpyridine and some thiolate ligands shows better *in vitro* cytotoxicity than clinical *cisplatin* against both MOLT-4 human leukemia and C2Cl2 mouse tumour cell lines [51]. Four gold(III) complexes with 2-[(dimethylamino)methyl]phenyl (damp) ligands were tested *in vitro* and *in vivo* and results showed good activity. Detailed studies on [Au(acetato)$_2$(damp)] showed that only minor cell cycle alterations occur and the compound did not cause DNA interstrand cross-linking [52]. The behavior of some gold(III)-carbon complexes was studied under physiological-like conditions to indicate that all compounds underwent hydrolysis for labile ligands were the gold(III)-carbon bonds and metal oxidation states were preserved [53,21].

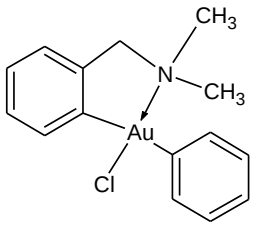


Figure2. 4: Gold(III) complex with strong TrxR inhibitory properties

2.6 Interactions of gold(III) with Amino acids:

The mechanism of interaction for gold(III) with amino acids, peptides and proteins as well as structural characterization of intermediates formed in these reactions have been investigated and reported in detail. Examples of interaction with amino acids are discussed in this section.

2.6.1 Interaction of gold(III) complexes with Glycine and Alanine:

The reactions between gold(III) complexes with amino acids Alanine and Glycine were successfully studied in acidic solutions at pH 2.90 and 2.44 respectively by using isotopically labeled amino acids and employing NMR techniques [54,55]. These reactions were carried out at room temperature using tetrachloridoaurate(III) $[AuCl_4]^-$ and ^{15}N Gly in different molar ratios, and the reaction mixture was monitored by ^1H NMR and 2D [^1H, ^{15}N] NMR in order to get more information about the reaction intermediate. The collected data showed that Glycine coordinates initially to gold(III) centre through its nitrogen atom forming intermediate product with formula [Cl_3AuN-gly] this intermediate can form chelation of five member ring by intramolecular substitution of second chlorido ligand to give [Cl_2AuNO-gly]; On the other hand, two electrons transferred to gold(III) centre from amino acid lead to formation of gold(I)-imine which is well known to be unstable and it hydrolyzes to release NH_4^+ with its corresponding aldehyde. Further reaction with additional gold(III) complexes induced decarboxylation of glyoxylic acid to obtain formaldehyde concomitant with reduction of gold(I) to elemental gold(0) and formation of CO_2 and this at 1:2 reactant ratios. All intermediates identified in this reaction according to its analogies platinum compounds chemical shifts values [54]. Similar behavior was observed with Alanine to give pyruvic

acid. Proposed mechanism for gold(III) reaction with both Glycine and Alanine in acidic solutions can be driven as first step coordinate of amino acides to gold(III) centre through their Nitrogen atoms followed by reduction of gold(III) to form gold(I)-imine complex then fast hydrolysis led to formation of glyoxylic acid and purovic acid with concomitant formation of NH_4^+ and Au(0), proposed mechanism is illustrated in (Figure 2.5). Moreover both Glyoxylic and pyruvic acid can react with additional gold(III) complex giving formic and acetic acid, respectively, carbon dioxide and Au(0) [34,35].

2.6.2 Interaction of gold(III) complexes with L-Histidine:

L-Histidine is classified as the most important amino acid because it is part of the active site in many biological systems, such as enzymes [56]. The interaction of gold(III) with L-His have been studied utilizing a number of instrumental techniques in order to determine the structure of product. The obtained results indicate that the binding of L-His to gold(III) centre and the coordination of L-His to gold(III) occur through amino group and N3 from imidazole ring, whereas, two molecules of L-His were coordinated to one gold(III) ion, $[Au(L\text{-}His)_2]Cl_3$ complex was slowly obtained after 3 hours as precipitate. This final product is concomitant with an increase in acidity of reaction solution owing to carboxyl group deprotonation [57]. Another study was for interaction of $[AuCl_4]^-$ with L-His amino acid in presence of perchloric acid. β-Imidazolyl-pyruvic acid was obtained as final product in this reaction, and kinetic studies using UV-Vis spectrophotometry [58]. The interaction of $[Au(en)Cl_2]Cl$ with L-Histidine was found to be pH dependent and the oxidation of L-His by gold(III) complex was studied by monitoring ^1H NMR chemical shifts. These studies showed fast gold(III) reduction to elemental gold(0) and L-His oxidation was observed in solution indicating preference of gold(III) binding to L-His,

hence the binding here is thought to be through amine group and N3 from imidazole ring and carboxyl group acts as counter ion. Under mentioned experimental conditions this reaction was reported as first order reaction [59].

2.3.3. Interaction of gold(III) complexes with sulfur containing amino acids:

Although gold complexes have been used as drugs based in Chrysotherapy this, use was limited to gold(I) which were administrated in the form of thiolates e.g. gold(I) thioglucose which is used in rheumatoid arthritis. The interaction of gold complexes with sulfer plays very important role in gold biochemistry as gold(III) ability to oxidize thiols to disulfides and Methionine to Methionine sulfoxide are reported as a source of gold(III) toxicity [60].

An increasing attention in the gold(III) complexes interaction with sulfur-containing amino acids was observed after discovering antiarthritic properties of gold(I) thiolates, L-Cysteine (L-Cys) is an important sulfur-containing amino acid and its interaction with gold(III) complexes was classified to be one of the reasons behind toxicity of gold(III) complexes for medical applications since it acts as a reducing agent reducing gold(III) to gold(I). L-Cys is also oxidized to generate disulfide products and cystic acid; further reduction of gold(III) complexes to elemental gold(0) occurs by cystic acid. These transformations are reported as toxic side effects in Chrysotherapy [60] such as nephrotoxicity. It was found that L-Cys is able to reduce gold(III) in aqueous solution. Cystine was found to be the major oxidation product in this reaction, with Au(III) being reduced to Au(I), which can be stabilized with excess L-cysteine. Disulfide cystine was capable of reducing Au(III) to elemental Au(0) while being itself oxidized to cysteic acid [25].

The mechanistic study of the redox reaction between $[AuCl_4]^-$ and the amino acid L-methionine was studied by UV-Vis spectrophotometry, thermal analysis, NMR, IR and FT-IR spectroscopy in acidic solution [61,62]. It was found that this reaction occurred in two stages. Initially, the very fast substitution of one chloride ion by L-Methionine molecule with formation of the short lived Au(III)–methionine complex was observed. Additionally, it was confirmed that the amino group of methionine was involved in this process even at pH below 2.00. The rate constant for the first stage of this reaction determined by applying a stopped-flow technique was found to be $983 \pm 2\ M^{-1}\ s^{-1}$ at 21°C. The second stage of this reaction was reduction of the intermediate Au (III)–methionine complex with formation of methionine sulfoxide and a Au(I) complex as the final products [62].

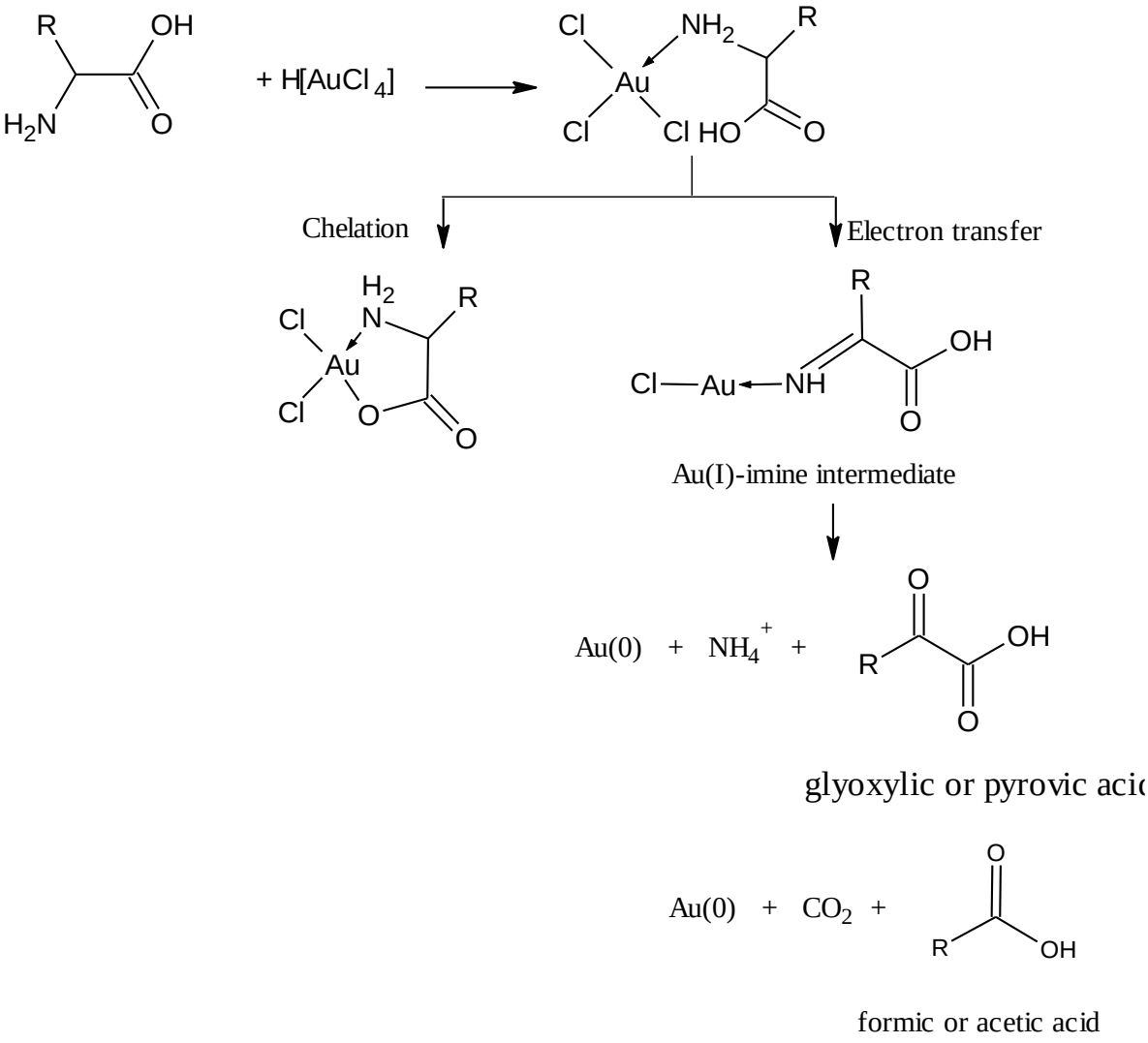


Figure2. 3: Proposed mechanism of interaction of gold(III) complexes with amino acids.

2.7 Interactions of Au(III) with Peptides:

The reactions between two dipeptides, glycyl–glycine (Gly–Gly) and glycyl–L-alanine (Gly–L-Ala) and $H[AuCl_4]$ were studied at pH 2.00 and 3.00 at 40°C by ^{1}H NMR spectroscopy. The final products in these reactions, [Au(Gly–Gly–N,N′,O)Cl] and [Au(Gly–L-Ala–N,N′,O)Cl] complexes, were identified and characterized by ^{1}H and ^{13}C NMR spectroscopy. From NMR data it was concluded that both dipeptides are tridentate

coordinated to Au(III) ion through the nitrogen atom of the terminal amino group, the deprotonated peptide nitrogen and the oxygen atom of the carboxyl group. The fourth coordination place in these square-planar complexes was occupied by the chloride ion. It was also stated that coordination of Gly–Gly and Gly–L-Ala dipeptides to Au(III) was a slow process strongly dependent on pH [3].

The redox reaction between glutathione (GSH, γ-L-Glu–L-Cys–Gly) and tetracyanidoaurate(III), $[Au(CN)_4]^-$ was investigated in aqueous solution at pH 7.4 by UV-Vis, ^{13}C NMR and electrospray ionization mass spectroscopy (ESI-MS). It was found that the reduction of $[Au(CN)_4]^-$ by GSH proceeded through two intermediates, $[Au(CN)_3(GS)]^{2-}$ and $[Au(CN)_2(GS)_2]^{3-}$. These intermediate species further react with an additional molecule of GSH to generate $[Au(CN)_2]^-$ and glutathione disulfide ($GSSG^{2-}$) as the final products of the reaction [63,64].

CHAPTER 3

Experimental work

3.1 Synthesis of gold(III) complexes:

3.1.1 Synthesis of [*cis*-1,2-(DACH)$AuCl_2$]Cl:

It was prepared by direct mixing of one equivalent $NaAuCl_4.2H_2O$ with one equivalent of diammine ligand in alcoholic media as per literature method [23]. ^{1}H NMR: δ ppm (density, multiplicity): 3.56(2, *m*), 2.14(2, *m*), 1.75(2, *m*), 1.50(2, *m*) and 1.32(2, *m*). ^{13}C NMR as δ ppm: 63.35, 26.86 and 21.44ppm.

3.1.2 Synthesis of [*cis*-1,2-$(DACH)_2$Au]Cl_3:

It was prepared by direct mixing of one equivalent $NaAuCl_4.2H_2O$ with two equivalent diammine ligand in alcoholic media as per literature[65], ^{1}H NMR as δ ppm (density, multiplicity): 3.52(2, *m*), 1.87(2, *m*), 1.65(2, *m*), 1.50(2, *m*), 1.33(2, *m*). ^{13}C NMR as δ ppm: 63.19, 62.92, 26.55, 26.10 and 20.82, 20.60 ppm.

3.2 Solution NMR Measurements:

All NMR measurements were made using a Jeol JNM-LA 500 NMR spectrophotometer. The ^{13}C NMR resonance spectra were obtained with ^{1}H broadband decoupling at a frequency of 125.65 MHz and they were referenced relative to TMS, the spectral conditions were 32 k data points, 0.963 s acquisition time, 1.00 s pulse delay and 45º pulse angle, The proton NMR spectra were obtained at a frequency of 500.00 MHz.

3.3 Electrochemical Measurements:

All square wave voltammetric measurements were carried out using A CHI660 potentiostat. The electrochemical cell contained a glassy carbon electrode (GCE; 3.0 mm diameter, Model CHI104, CH Instruments, Austin, TX) as a working electrode, Ag/AgCl (sat. KCl) reference electrode (Model CHI111, CH Instruments, Austin,TX), and a platinum wire counter electrode were inserted into a 2 mL glass cell through holes in its Teflon cover. Before each Squarewave stripping voltammetry (SWSV) or cyclic voltammetry (CV) measurement, the GCE was polished with 3.0 and 0.05 μm alumina slurries and washed with doubly distilled water Each SWSV measurement was performed at room temperature in a quiescent 0.2 M KCl aqueous solution, and with an initial potential of 0.0 V and final potential of +1.6 V.

3.4 UV-Vis and Kinetics Measurements:

All kinetic measurements were made using a Carry UV-100 UV-Vis spectrophotometer. The Scanning Kinetic mode was used to investigate the change in electronic spectra for gold(III) complexes upon interaction with GSH and to determine the effective wave length for the reactions, then the kinetic mode was used to monitor the reaction progress at selected wavelength. A solution of 0.2 mM gold(III) complexes were prepared in water containing sodium chloride added to increase stability of mono- gold(III) complexes by prevent hydrolysis of chlorides [66], series of thiols solutions were also prepared, and equal volumes of gold(III) complex solution and thiol solution were mixed and spectral changes were recorded using Scanning Kinetic mode in range from 250 nm to 350 nm.

Kinetic mode was used then to follow the reaction at three different temperatures. The collected data were plotted according to *pseudo*-first order kinetic equation (**3.1**)

$\ln A = kobs.t + \ln A_{\circ}$ Eqn (**3.1**)

Where:

$A_{\circ}$: Initial Absorption

A: Absorption at time t

t: time in minute

Pseudo-first order rate constant obtained from the slope value of the straight line correlation between *kobs* versus thiol concentration.

kobs = k[Thiol] Eqn (**3.2**)

Using Microsoft -Excel, rate constants and activation parameters have been calculated from Eyring plots

$$ln\frac{\mathrm{k}}{T} = \frac{-\Delta H^{\ddagger}}{R}\frac{1}{T} + ln\frac{kB}{h} + \frac{\Delta S^{\ddagger}}{R}$$ Eqn (**3.3**)

Where:

k: *pseudo*-first order rate constant

R: universal gas constant

T: absolute temperature

$k_{B:}$ Poltzman constant

$H^{\ddagger}$: enthalpy of activation

$S^{\ddagger}$: entropy of activation

The dependence of rate constant on Thiol concentration can be expressed as:

$k = k_{obs}[\text{Thiol}]^{n}$ Eqn (**3.4**)

It can also be written as:

$$\ln k = \log k_{obs} + n \ln[\text{Thiol}] \qquad \text{Eqn } (\mathbf{3.5})$$

The order of reaction (*n*) can be calculated using eqn(**3.5**) from plot of ln *k versus* ln [Thiol].

CHAPTER 4

Results and Discussion

4.1 Interaction of 1,2-diamminocyclohexane gold(III) complexes with L-Methionine:

4.1.1 Interaction of [Au(*cis*-DACH)Cl_2]Cl with L-Methionine:

The interaction of gold(III) with biological ligands initiate an interest in these types of interactions with different important biological ligands such as amino acids and peptides, especially the interaction with sulphur containing amino acids, namely L-cysteine (L-Cys) and L-Methionine (L-Met). These interactions are thought to be responsible for the toxic-side effects encountered in chrysotherapy such as nephrotoxicity. The interaction of L-Met with *cis*-diamminocyclohexane gold(III) chloride was studied by using NMR in acidic solution of pD 2.5 (correct according to isotope effect). The L-Met to reduce gold(III) to elemental gold at this pD of solution and the reaction involves oxidation of L-Met by gold(III) to L-Methionine sulfoxide which is recognized as final product, the disappearance of methyl resonance in ^{13}C NMR spectrum and at the same time observed resonance at about 37 ppm, assigned to methylsulfoxide resonance, is consider a strong indicator for formation of L-Methionine sulfoxide. In contrast appearance of free diamminecyclohexane as protonated ligands indicate redox of gold(III) to elemental gold(0) and loss of central metal ion. The reaction was monitored as function of L-Met ratio. The ration of gold(III) complex to L-Met was 1:2. The resonance of C1and C2 for

complex completely disappeared and the color of the solution was changed from yellow to colorless, and within this ratio the resonance of methyl at 14.5 ppm was obtained again. The ^{1}H NMR showed the same behavior and the resonance of methyl protons 1.97 ppm (3H, s) shifted downfield to 2.56 (3H, s). This is in agreement with ^{13}C results. For more investigation L-Met was oxidized using H_2O_2 (30%) at pD of reaction then both ^{1}H and ^{13}C resonance were recorded and the data gathered from this reactions was listed in (Tables 4.1 and 4.2)

Table 4. 1: ^{13}C NMR Chemical shifts in ppm for L-Methionine and oxidized L-Methionine in D_2O pD 2.5.

Position	^{13}C δ in ppm for L-Met. , pD 2.5	^{13}C δ in ppm for L-Met.+ H_2O_2 30% , pD 2.5
5	14.56	37.11
4	29.93	48.67
3	29.34	24.21
2	53.51	53.27
1	173.68	172.70

Table 4. 2: ^{1}H NMR δ in ppm for L-Methionine and oxidized L-Methionine in D_2O pD 2.5.

Position	^{1}H δ in ppm for L-Met. pD 2.5	Intensity	Multiplicity	^{1}H δ in ppm for L-Met.+ H_2O_2 30% , pD 2.5	Intensity	Multiplicity
5	1.97	3	s	2.56	3	s
4	2.09	2	m	2.85	2	m
3	2.51	2	t	2.18	2	t
2	3.89	1	t	3.88	1	t

s: singlet, m: multiplet, t: triplet

The suggested mechanism of reaction between [Au(*cis*-DACH)Cl_2]Cl and L-Met can be driven as first of all L-Met coordinate to gold(III) through sulphur atom by substitution on one chlorido ligands forming [(Met-SAu(*cis*-DACH)Cl]$^{2+}$ and this intermediate is subjected to further chlorido ligand substitution and formation of five membered ring

using nitrogen atom of amino group [Met-S,NAu(*cis*-DACH)]$^{3+}$ this intermediate could produce Met-SO-CH_3, which is identified by carbon resonance at 37 ppm along with resonance of protonated $(DACH.H_2)^{2+}$ which identified by its corresponding resonance at 50.55, 26.44 and 20.89 ppm. The resonance at 30.88 and 49.51 ppm were obtained at low concentration of L-Met (1:0.25 gold(III complex to L-Met) and completely disappeared by increasing L-Met concentration and were not observed in oxidized L-Met solution that indicate these resonances are for L-Met-gold(III), the complete removal of DACH ligands from gold(III) complex was observed at 1:2 gold(III) complex to L-Met ligand similar study was conducted using $[Au(en)Cl_2]Cl$ [67], where the interaction with $[AuCl_4]^-$ was studied in details [62]. See Figure 4.1

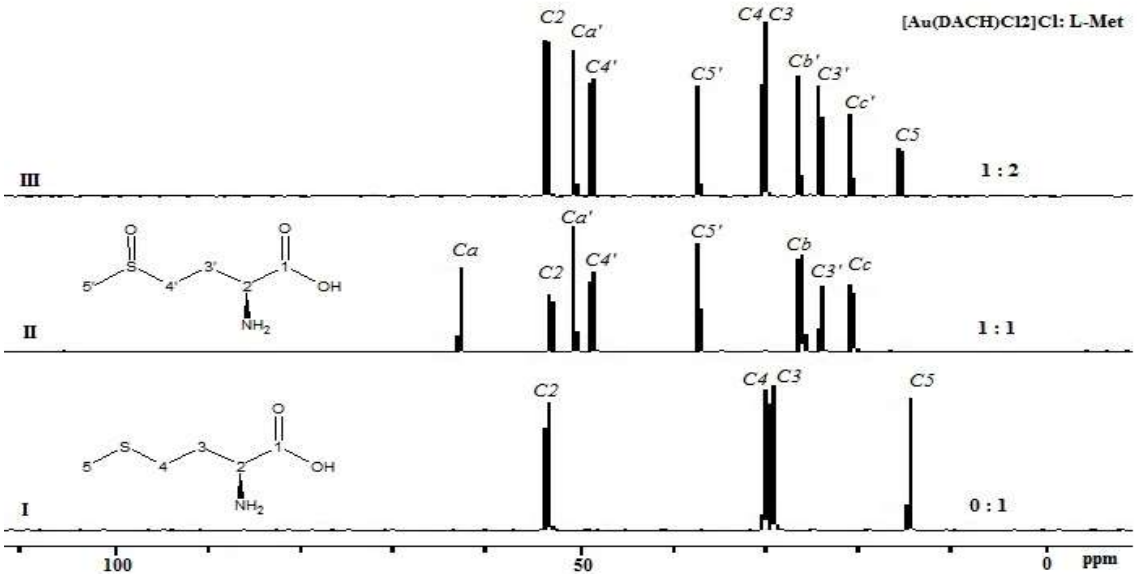


Figure 4. 1: ^{13}C NMR for L-Met I) 10 mM L-Met in D_2O II) after reaction with 10 mM $[(cis\text{-}DACH)AuCl_2]^+$ (1:1) III) after reaction with 20 mM $[(cis\text{-}DACH)AuCl_2]^+$ (1:2)

4.1.2 Interaction of [Au(*cis*-DACH)$_2$]Cl$_3$ with L-Methionine:

The interaction of L-Met with bis (*cis*-diamminocyclohexane)gold(III) was carried out using NMR as function of L-Met ratio and it shows less reactivity towards redox reaction compared with interaction with monodiamminocyclohexane complex, methysulfoxide resonance was also observed at about 37 ppm and complete removal of diamminocyclohexane ligand from bis complex occur when the ratio was raised to at molar ratio 1:2 in mono diammine complex that indicate relative stability of nitrogen tetracoordinted gold(III) complexes and their ability to protect gold center from redox and also reflect the role of *cis*-di-chlorido ligands in mono diammine complex; which is suggested to be responsible by easy hydrolyzed and formation of di aqua complexes and the last one can easily by attacked by nucleophiles to exhibit reduction or substitution depending on ligand type and donar atom nature. All resonances were shown in (Figure 4.2**).**

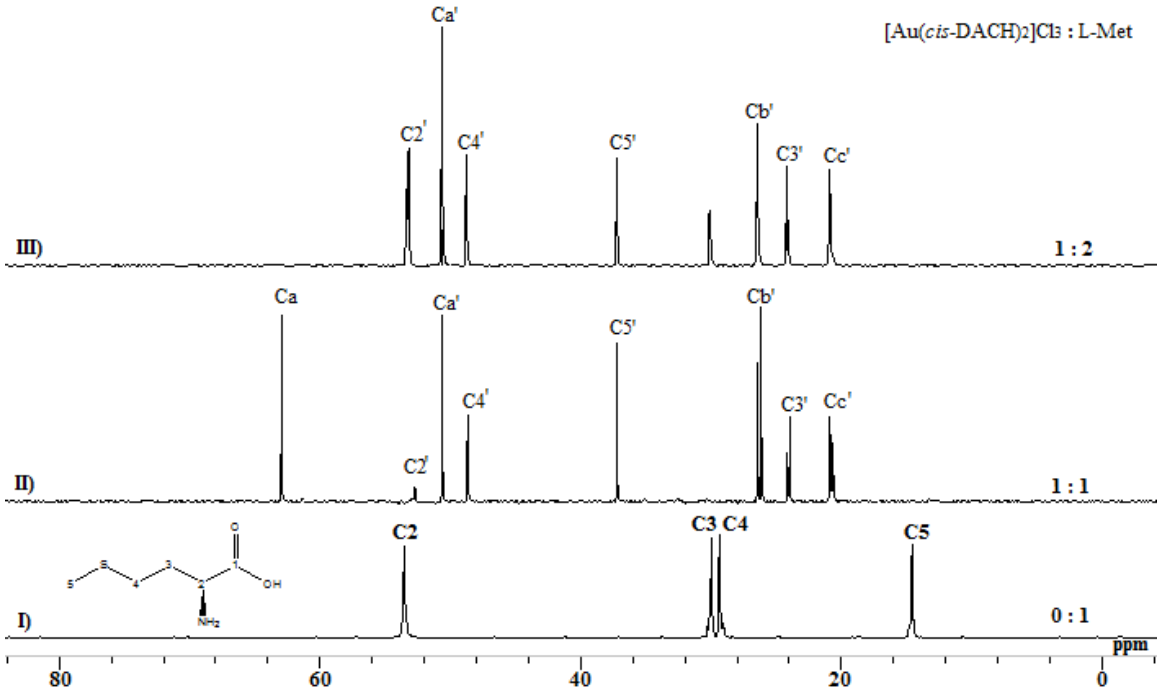


Figure 4.2:^{13}C NMR for reaction of I) L-Met with II) one equivalent [Au(*cis*-DACH)$_2$]Cl$_3$ III) 1:2 complex to L-Met at pD 2.

4.2 Interaction of [Au(*cis*-DACH)Cl_2]Cl with $_{DL}$-Selenomethionine:

Because of their essential presence in mammals Selenium compounds have gained recently more attention [68]. The interaction between Se-Met and [Au(*cis*-DACH)Cl_2]Cl was carried out by mixing both compounds directly in D_2O solution at different pD values, No change in spectram was obtained at pD 2 and 7 but when pD was raised to 12 using NaOD in D_2O clear down-field shift for C5 from δ = 3.88 ppm to C5" 35.6 ppm and C5' 37.1 ppm corresponds to [(*cis*-DACH)Au(SeMet)]$^{3+}$ and to oxidized CH_3-SeO-Met respectively [69–71]. Signals were assigned by comparison with Se–Met oxidation experiment with hydrogen peroxide [72–74] this concomitant with reduction of gold(III) which indicated by free *cis*-DACH resonance at 56.7 ppm while 59.7 ppm could be assigned to [(*cis*-DACH)Au(SeMet)]$^{3+}$ which is shifted from 63 ppm in [Au(cis-DACH)Cl_2]$^+$. ^{13}C NMR spectra are shown in (Figure 4.3).

The binding between gold(III) centre and Se-Met is in prospect to occur through the amine group or Selenium as indicated by Shoeib *et al* [75] in his computational study using methyl-seleno-cysteine.

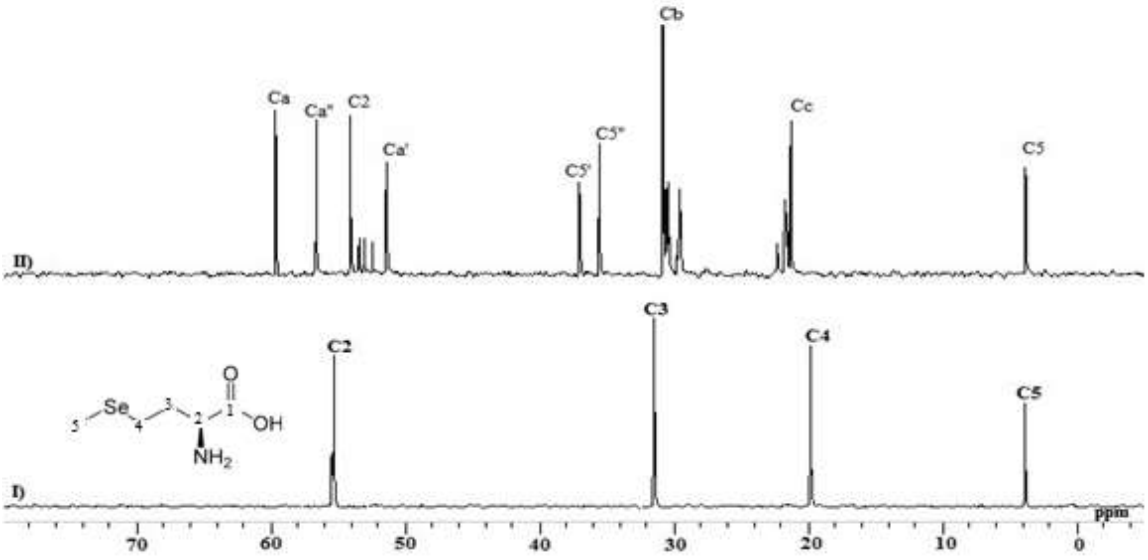
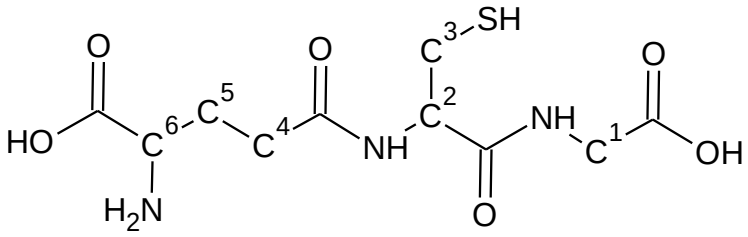


Figure 4. 3: ^{13}C NMR for reaction of SeMet, I) before reaction, II) with [Au(*cis*-DACH)Cl_2]$^+$ at pD 12.

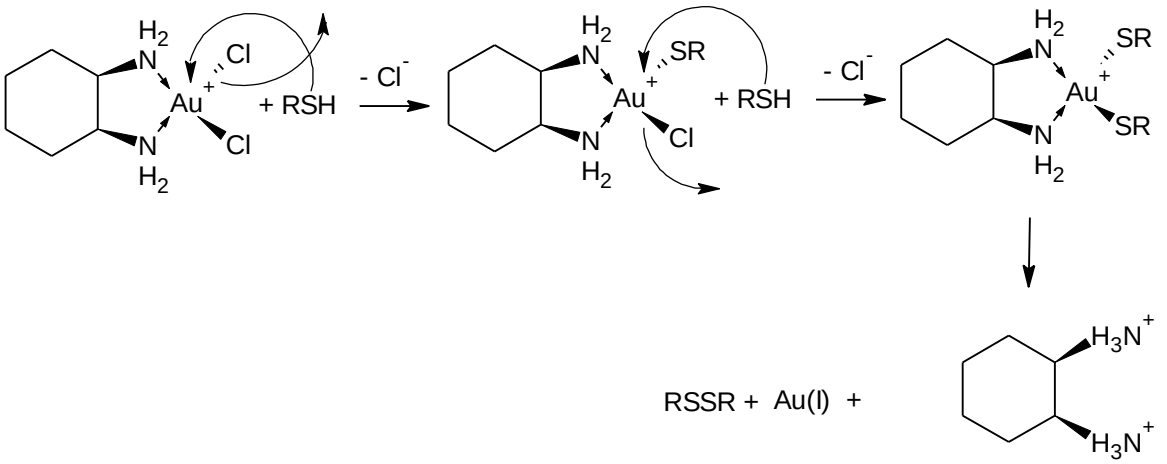
4.3 Interaction of diamminocyclohexane gold(III) with glutathione:

4.3.1 Interaction of [Au(*cis*-DACH)$_2$]Cl$_3$ with Glutathione:

In the NMR spectra (**Fig 4.4**) of 1:1 ratio of GSH with [Au(*cis*-DACH)Cl$_2$]Cl, the C3 resonance (numbering according to (scheme **4.1)** below) was observed at 39 ppm instead of 27 ppm, while the C2 resonance showed an up-field shift that indicates GSH was oxidized to its corresponding disulfide GSSG [76]. When the ratio reached 1:2, the resonance, C1 and C2 signals in [Au(*cis*-DACH)Cl$_2$]Cl were shifted up-field toward the protonated (DACH.H$_2$)$^{+2}$, implying loss of Au(III) centre and precipitation of polymeric gold(I) with GSSG from reaction mixture. Similar amount of GSH was oxidized in D$_2$O at pD 2 using 30% H$_2$O$_2$ and ^{13}C NMR spectra for oxidized GSH shows similar shifts for GSSG obtained in reaction mixture, suggesting reaction mechanism for interaction of [Au(*cis*-DACH)Cl$_2$]Cl with GSH as illustrated in (scheme **4.2**)



Scheme 4. 1: Glutathione



Scheme 4. 2: Proposed mechanism for interaction of [Au(*cis*-DACH)Cl_2]Cl with GSH in acidic solution pD 2.5

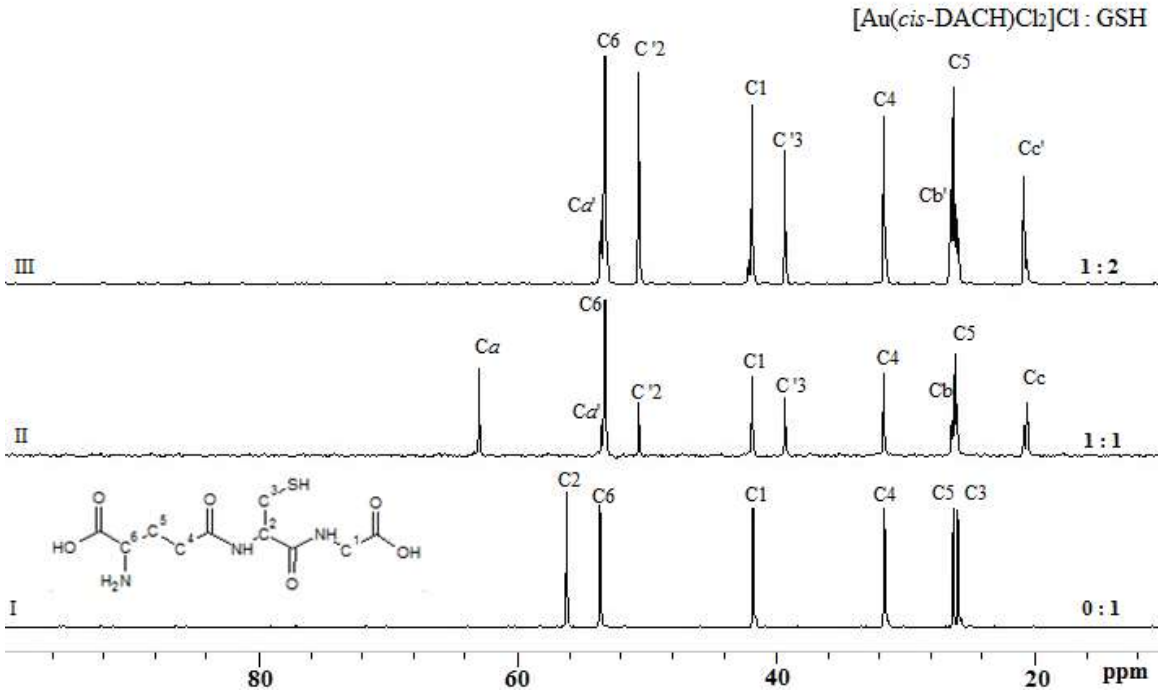


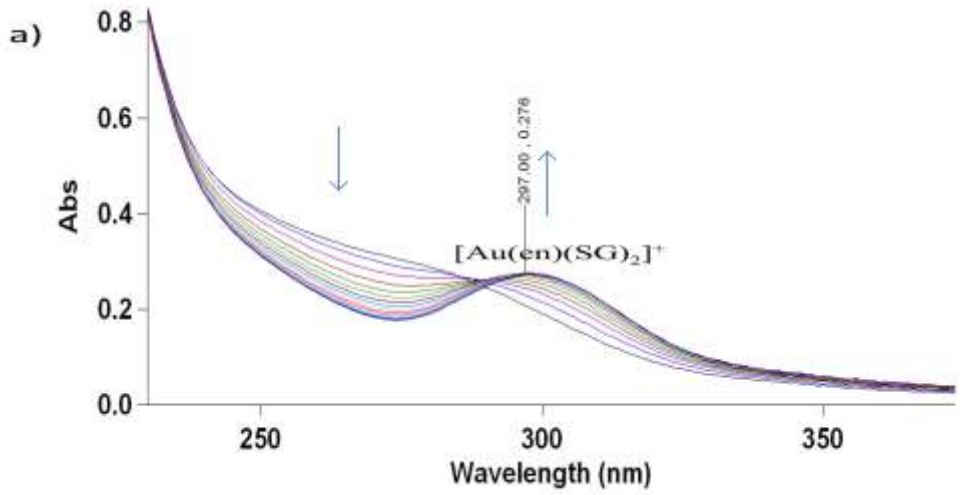
Figure 4. 4:^{13}C NMR for I) 10 mM GSH in D_2O II) after reaction with 10mM [(*cis*-DACH)$AuCl_2]^+$ (1:1) III) 20 mM GSH with 10 mM [(*cis*-DACH)$AuCl_2]^+$ (1:2).

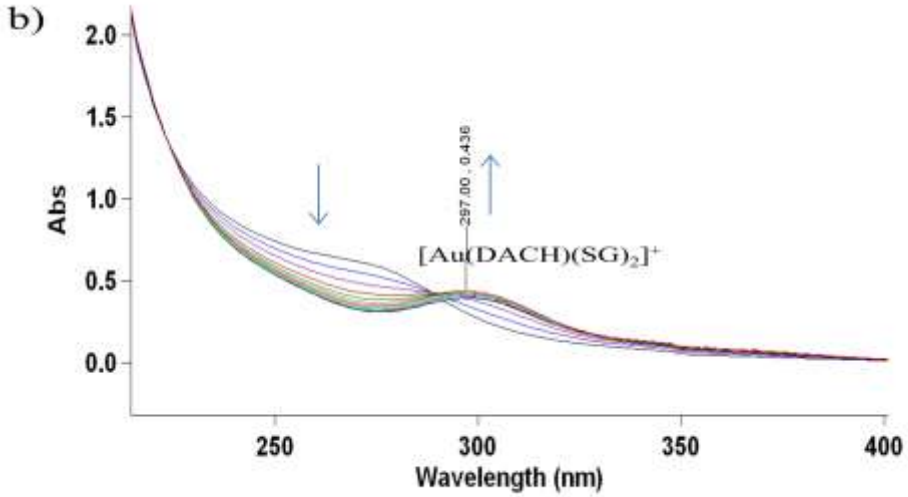
4.3.2 UV-Vis and Kinetic measurements for interaction of glutathione with [Au(*cis*-DACH)Cl_2]Cl, [Au(en)Cl_2]Cl and [Au(en)$_2$]Cl_3:

Scanning kinetics mode was used to record the electronic spectral change for reaction between glutathione with [Au(*cis*-DACH)Cl_2]Cl, [Au(en)Cl_2]Cl and [Au(en)$_2$]Cl_3, 0.2 mM gold(III) diamine complexes were prepared in aqueous solution contain 40 mM sodium chloride to avoid hydrolysis of mono-gold(III) diammines chlorides. This was found not effective in case of bis-gold(III) complexes. Glutathione was prepared in water by mixing equal volumes of gold(III) complexes solutions and GSH solution inside measuring cell and electronic scanning kinetics spectra for all complexes showed appearance of an increasing absorption band at 297 nm at 298 K, where the solution of 0.2 mM [Au(*cis*-DACH)$_2$]Cl_3 with 1mM GSH solution shows another absorption band at 240 nm. These absorption bands are assigned to formation of intermediates with general formulas [Au(diamine)$(SG)_2]^+$ and [Au(diamine)(SG)X]$^+$ respectively. At higher concentration of GSH only absorption band at 297 nm corresponds to [Au(diamine)$(SG)_2]^+$ was observed, which indicates that this intermediate is more stable than [Au(diamine)(SG)X]$^+$. Scanning kinetics spectra for 0.2 mM gold(III) complexes with 2 mM GSH solution at 298 K are shown in (Figure 4.5).

The kinetics of substitution reaction between potential anticancer gold(III) complexes [Au(*cis*-DACH)Cl_2]Cl, [Au(en)$_2Cl_2$]Cl and [Au(en)$_2$]Cl_3 with glutathione was investigated spectrophotometrically by monitoring the spectral change of gold(III) complexes solution with excess of GSH. Equal volumes from gold(III) complexes and GSH were kept till it thermally equilibrate and then mixed directly inside a UV cell and the change in absorption at selected wavelength (297 nm) under *pseudo*-first order condition as function of GSH concentration and temperature was collected. Each kinetic

measurement was repeated three times, k_{obs} under this experiment conditions was determined using the mentioned Eqn (**3.1**) and the *pseudo*-first order rate constant was obtained from linear dependence of k_{obs} *vs* total GSH concentration Eqn (**3.2**). NaCl was added in excess concentration.





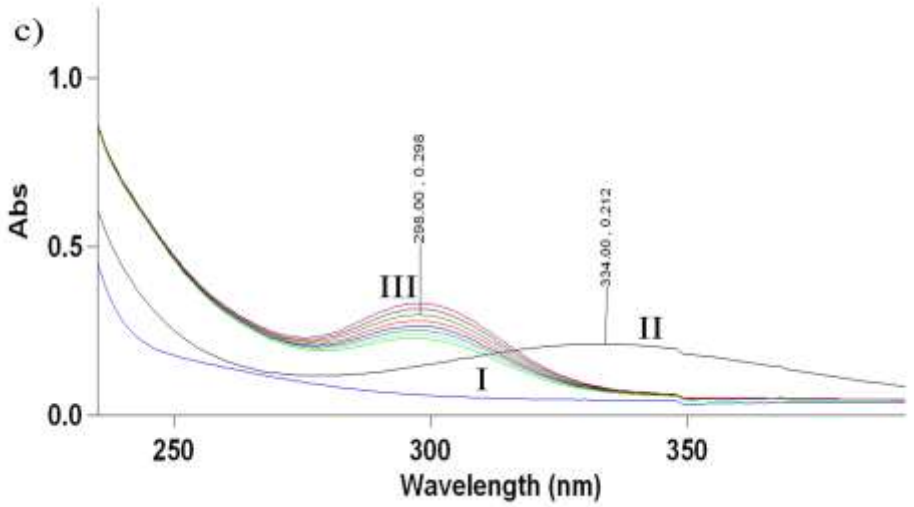


Figure 4. 5: Scanning kinetics spectra for reaction of GSH with **a)** $[Au(en)Cl_2]Cl$ **b)** $[Au(cis\text{-}DACH)Cl_2]Cl$ **c)** I) GSH II) $[Au(cis\text{-}DACH)_2]Cl$ III) 0.2 mM complex to 2 mM GSH.

Table 4. 3: Kinetic data for reaction of gold(III) diammines complexes with Glutathione.

GSH	^{a}k $M^{-1}.\ s^{-1}$	$\Delta H^{\ddagger}$ kJ. mol^{-1}	$\Delta S^{\ddagger}$ $JK^{-1}mol^{-1}$	*Ea* kJ.mol^{-1}
$[Au(en)Cl_2]^{+}$	$(217 \pm 3)\ x10^{-2}$	18 ± 0.9	-170 ± 3	21 ± 0.6
$[Au(en)_2]^{3+}$	$(103 \pm 8)\ x\ 10^{-2}$	11 ± 1	-168 ± 4	13 ± 1.7
$[Au(cis\text{-}DACH)Cl_2]^{+}$	$(46 \pm 2)\ x10^{-2}$	37 ± 0.8	-120 ± 3	40 ± 0.8
$[Au(cis\text{-}DACH)_2]^{3+}$	$(19 \pm 1)\ x\ 10^{-2}$	-	-	-

Reaction carried out in aqueous solution in presence of 20mM NaCl for $[Au(NN)Cl_2]^{+}$ complexes. ^{a} *pseudo*-first order rate constant at 298K.

From the data given in Table (**4.3**), the values of rate constants and calculated activation parameters ($\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and *Ea*) indicate that gold(III) complexes react differently from each other with GSH due to several factors. It can be seen that $[Au(NN)Cl_2]^{+}$ reacts faster than $[Au(NNNN)]^{3+}$ and this difference in reactivity can be attributed to ease of Chloride substitution by Sulphur ligands in $[Au(NN)Cl_2]^{+}$ to form short life time intermediate

$[Au(NN)(SG)_2]^+$ at 297 nm, however formation of this intermediate was not observed in NMR. DACH geometry also plays a very important role in term of reactivity of gold(III) centre as the chair conformation for DACH in its gold(III) complexes increase the stability by hindering GSH attack. Such an effect is not offered by *en* ligand, due to its small size, and the rate constant increases by a factor of 8. There is also a small contribution in DACH gold(III) complexes due to the decrease in the electrophilicity of metal centre due to cyclohexane donation. The negative entropy of activation indicate that the entropy of transition state is too low compared with reactant entropy and that can be rationalized decrease in number of molecules by of the formation of the intermediate, where two bulky GSH molecules are tied to the complexes, thereby reducing the entropy.

The order of substitution reaction between prepared square planar gold(III) complexes and GSH was determined by value of slope(n) from plot of ln *k* vs ln[GSH] (Eqn(**3.5**)). At all measured temperatures and the reaction obeys *pseudo*-first order kinetic.

4.3.3 Electrochemical study of $[Au(en)Cl_2]Cl$ interaction with glutathione:

Cyclic voltammograms were obtained for 1mM $[Au(en)Cl_2]Cl$ in HEPES buffer (25 mM, pH 7.4) in presence of 40mM NaCl at a GCE, Figure 4.6 shows well defined (CV) signal for 1 mM solution of $[Au(en)Cl_2]Cl$ in presence of HEPES buffer at about 0.940 V (Vs Ag/AgCl sat KCl) and this signal has subsequently shifted to 0.975 V and 1.04 V upon addition of 50 μM GSH solution, that indicate some interaction between GSH and gold(III) complex takes place, same fact was presented by SWSV for the mentioned gold(III) complex solution under the same experiment condition. 1mM $[Au(en)Cl_2]Cl$ in HEPES buffer is used, SWSV signal at around +0.875V (Vs Ag/AgCl sat KCl) was observed as shown in Figure 4.7. Subsequent addition of 50 μM GSH to gold(III)

complex reduced the obtained complex signal towards the blank signal, control experiment was conducted by subsequent addition of 50 µM water to gold complex and collecting obtained SWSV signal. There was no reduction in complex signal that can be taken as a strong evidence to correlate the reduction of complex signal with glutathione interaction.

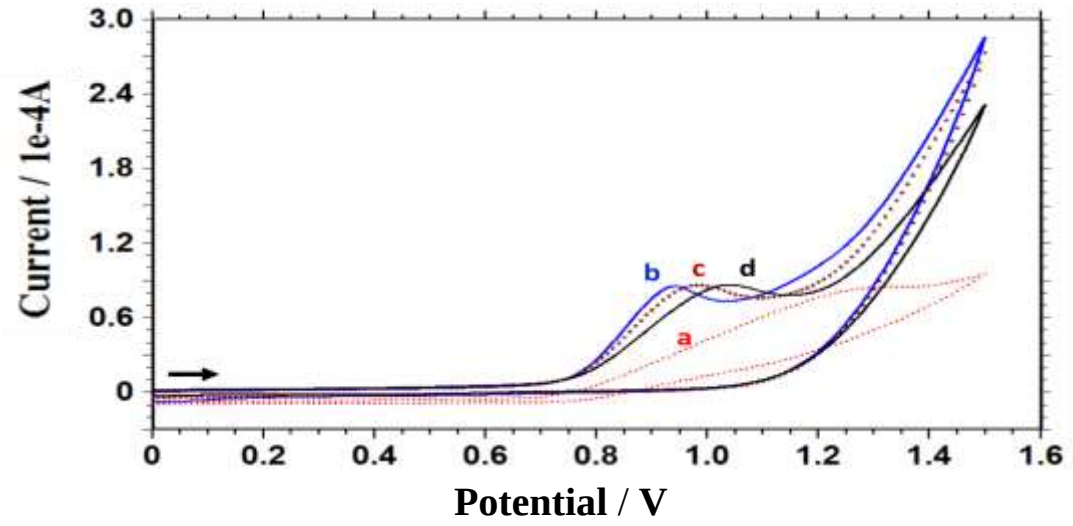


Figure 4. 6: Cyclic voltammograms in HEPES buffer (25 mM, pH 7.4) at a GCE in absence (a) and presence (b) of 1.0 mM $[Au(en)Cl_2]Cl$, and subsequent additions of 50 µM GSH aqueous solution (c, d). Electrochemistry working conditions: scan rate, 100 mVs^{-1}; sample intervals 1

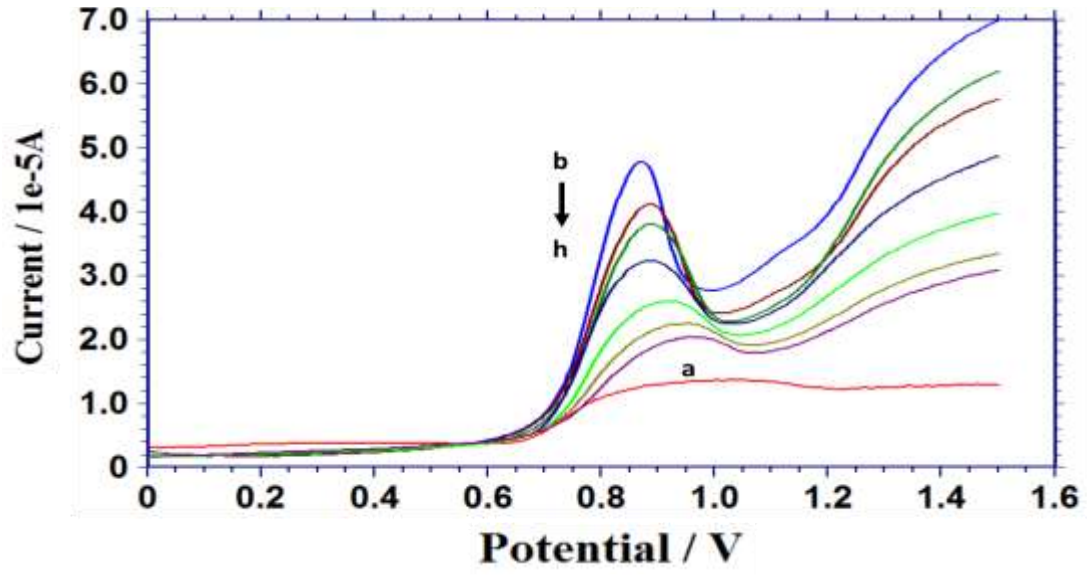


Figure 4. 7: Square wave voltammograms in HEPES buffer (25 mM, pH 7.4) at a GCE in absence (a) and presence (b) of 1.0 mM $[Au(en)Cl_2]Cl$, and subsequent additions of 50 µM GSH aqueous solution (c-h). Electrochemistry working conditions: pulse width (increment), 4 mV;

Table 4. 4: Observed rate constant for reaction of $[Au(en)Cl_2]^+$ with GSH as a function of ligand concentration and temperature in presence of 20 mM NaCl at 297 nm.

T/K	$10^{-3}C_L$/M	k_{obs}/min
288.0	1	0.0717
	2	0.1430
	3	0.2546
	4	0.3760
	5	0.4806
	6	0.5620
298.0	1	0.0917
	2	0.2469
	3	0.4169
	4	0.5047
	5	0.6475
	6	0.7496
307.5	1	0.2902
	2	0.4760
	3	0.6275
	4	0.8275
	5	0.9976
	6	1.181

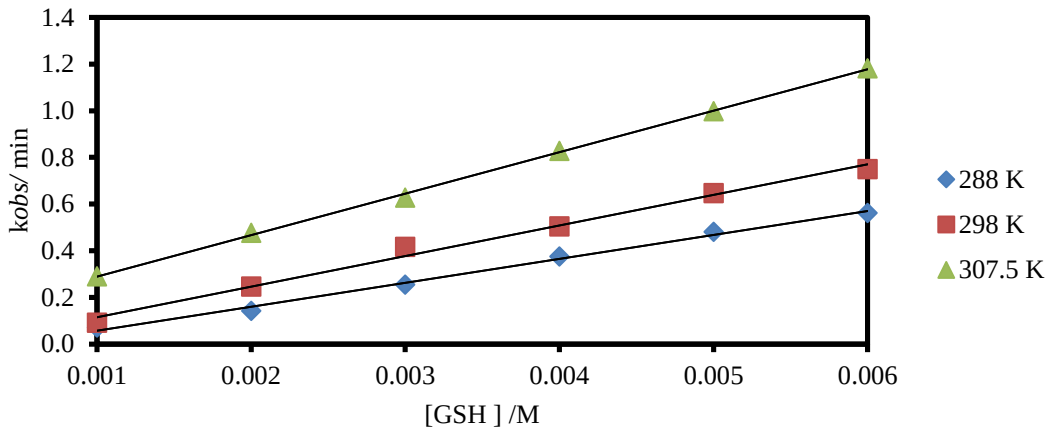


Figure 4. 8: *Pseudo*-first order rate constant for reaction of $[Au(en)Cl_2]^+$ with GSH as a function in ligand concentration and temperature in presence of 20 mM NaCl.

Table 4. 5: Observed rate constant for reaction of $[Au(en)_2]^{3+}$ with GSH as function of ligand concentration and temperature at 297 nm.

T/K	$10^{-3}C_L$/M	k_{obs}/min
288	1	0.0267
	2	0.0670
	3	0.1430
	4	0.1417
	5	0.2232
	6	0.2823
298	1	0.0338
	2	0.1015
	3	0.1941
	4	0.2405
	5	0.2699
	6	0.3578
310	1	0.0523
	2	0.1768
	3	0.2516
	4	0.3153
	5	0.3708
	6	0.4463

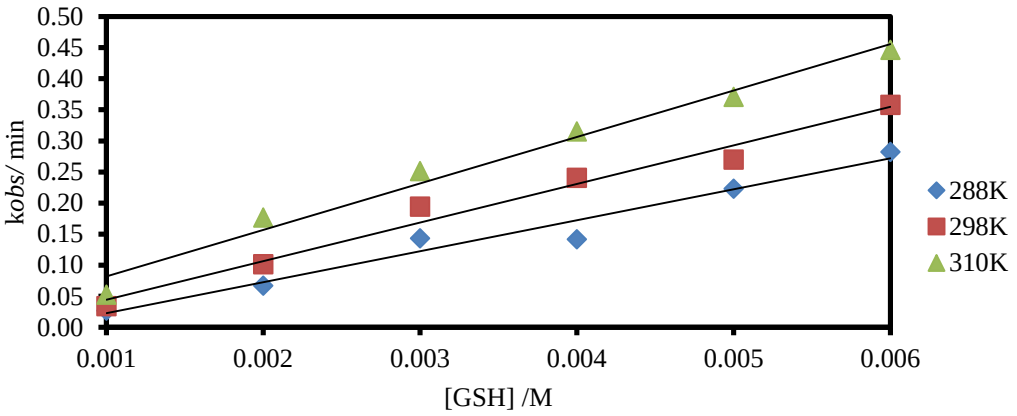


Figure 4. 9: *Pseudo*-first order rate constant for reaction of $[Au(en)_2]^{3+}$ with GSH as a function in ligand concentration and temperature.

Table 4. 6: Observed rate constant for reaction of $[Au(cis\text{-}DACH)Cl_2]^{+}$ with GSH as a function of ligand concentration and temperature in presence of 20 mM sodium chloride at 297 nm.

T/K	$10^{-3}C_L$/M	k_{obs} /min
288	1	0.0152
	2	0.0440
	3	0.0625
	4	0.0722
	5	0.0868
298	1	0.0209
	2	0.0497
	3	0.0864
	4	0.1097
	5	0.1339
303	1	0.0389
	2	0.0851
	3	0.1246
	4	0.1408
	5	0.2059

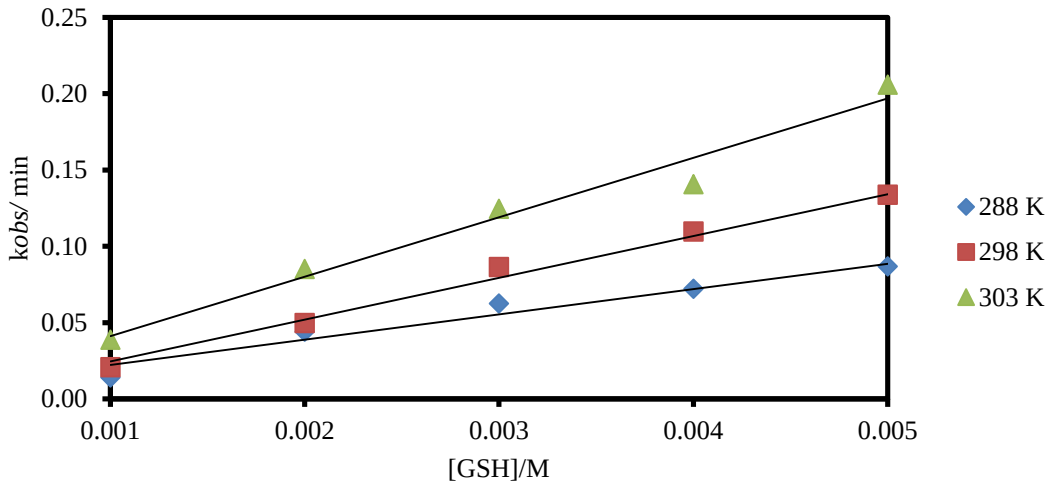


Figure 4. 10: *Pseudo*-first order rate constant for reaction of $[Au(cis\text{-}DACH)Cl_2]^+$ with GSH as a function of ligand concentration and temperature in presence of 20 mM NaCl.

The order of substitution reaction between prepared square planar gold(III) complexes and GSH was determined by value of slope(n) from plot of ln k vs ln[GSH] (Eqn(**3.5**) **Figs 4.11**) at all measured temperatures, and the reaction obeys *pseudo*-first order kinetic.

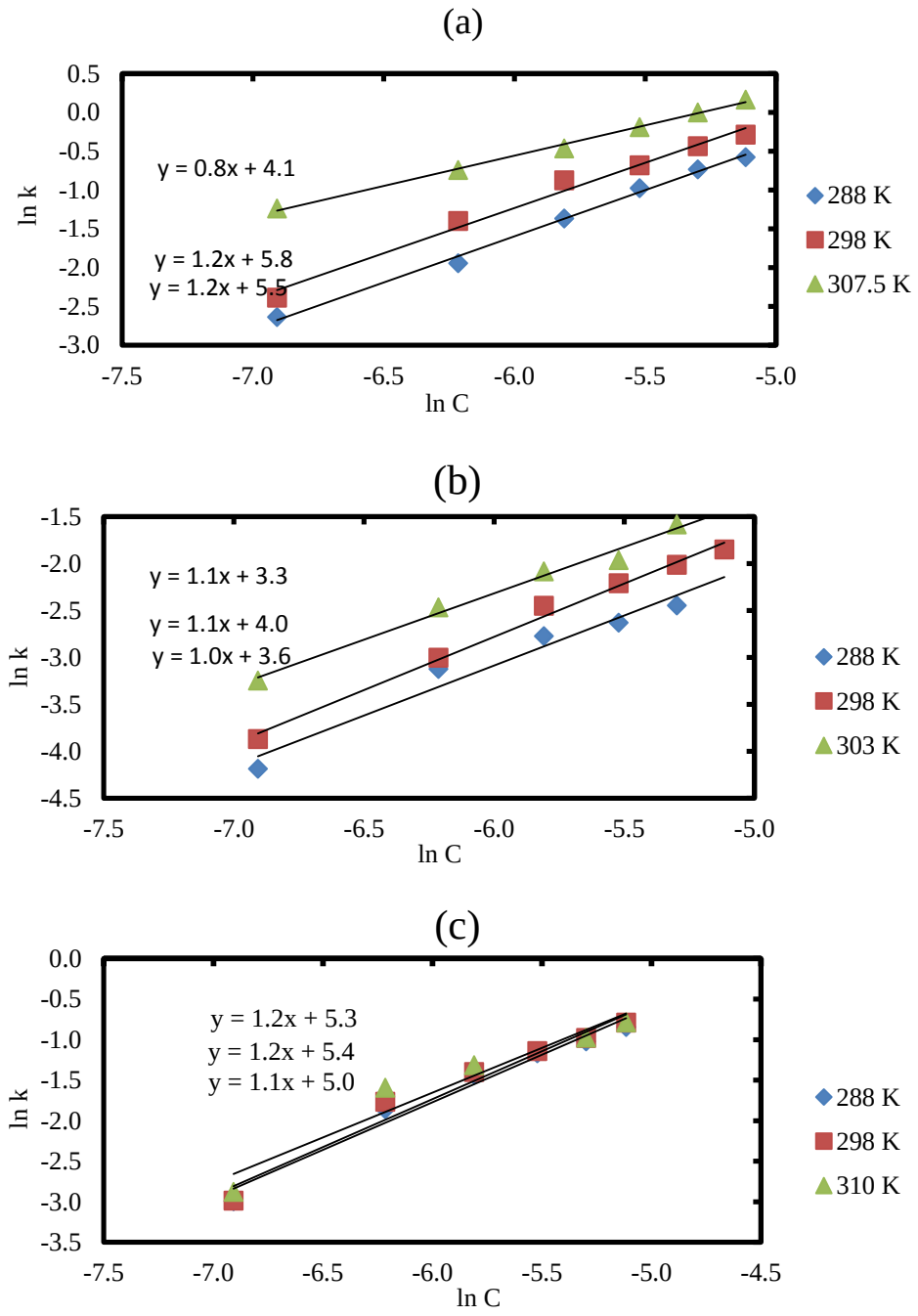


Figure 4. 11: Plots of ln*k* vs ln[GSH] for GSH for (a) $[Au(en)Cl_2]Cl$ (b) [Au(*cis*-DACH)Cl_2]Cl (c) $[Au(en)_2]Cl_3$

The reaction of GSH with potential anticancer gold(III) complexes based on diamines ligands was found to follow *pseudo*-first order kinetics under the experiment condition,

and *pseudo*-first order rate constant explains substantial fact that the selection of ligand is very important issue not only in order to improve cytotoxicity of gold(III) complexes but also to increase stability of gold(III) complexes towards biological molecules and limits toxic side effects which results from loss of gold(III) centre and formation of elemental gold(0). The reactivity of examined gold(III) complexes towards GSH can be ranked as follow $[Au(en)Cl_2]Cl > [Au(en)_2]Cl_3 > [Au(DACH)Cl_2]Cl > [Au(DACH)_2]Cl_3$.

NMR data shows formation of GSSG as final product beside free ligand at molar ratio 1:2 that means reduction of gold(III) by GSH require 2 equivalents and there is no intermediate observed. The UV-VIS spectroscopy showed the presence of intermediate with formula $[(NN)_2Au(SG)_2]^+$ and *pseudo*-first rate constant for formation of this intermediate from $[Au(en)Cl_2]Cl$, $[Au(en)_2]Cl_3$, $[Au(DACH)Cl_2]Cl$ and $[Au(DACH)_2]Cl_3$ is listed in **Table 4.3.**

4.4 Interaction of diamminocyclohexane gold(III) with imidazole:

Imidazole (1,3-diaza-2,4-cyclopentadiene) is planar hetrocyclic compound consists of three carbon atoms and two nitrogen atoms (five membered ring) in order 1,3 that is the source for systematic name 1,3-diazole, the resonance structure shows one of nitrogen atoms is forming sp^2 and its lone pair is responsible for basic characteristics of imidazole [77]. This nitrogen represents an active site for many important biomolecules like amino acid L-His. Imidazole and its derivatives have broad domain in pharmacological applications as cardiovascular activity [78], analgesic and anti-inflammatory [79], anti-neoplastic agents [80], anti- fungal agents [80] and enzyme inhibitor [81].

4.4.1 Interaction of [Au(*cis*-DACH)Cl_2]Cl with imidazole:

The interaction between potential anticancer gold(III) complex of 1,2-diaminocyclohexane [Au(*cis*-DACH)Cl_2]Cl was carried out in 10 mM gold(III)complexes in D_2O at pD 7 by observing the shifts in ^1H and ^{13}C NMR resonance. NMR spectra for reaction of [Au(*cis*-DACH)Cl_2]Cl and Imidazole (1:1) showed up field shift in carbon resonance of C1and C2 of gold(III) complex from 63 ppm to 61 ppm along with increase in intensity of peak upon addition of another equivalent from Imidazole to reaction mixture and decrease in complex peak intensity concomitant with up-field shift of C1 for Imidazole from 136.2 ppm to 126.5 ppm which indicates binding through nitrogen atom with similar up-field shift observed in Imidazole Carbons (Figure 4.13) . ^1H resonance showed an increase in resonances 7.0 -8.3 ppm due to loss of molecule symmetry upon coordination to gold(III) and the final compound thought to have the formula [(*cis*-DACH)Au$(Imi)_2]^{3+}$ (Figure 4.12)

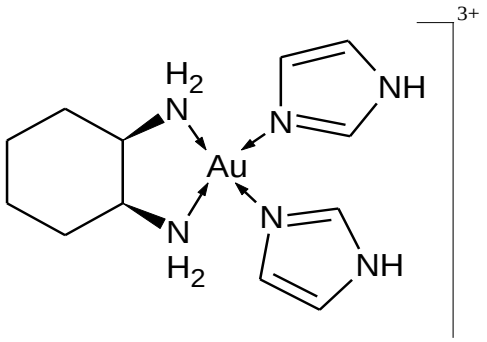


Figure 4. 12: [(*cis*-DACH)Au(Imi)$_2$]$^{3+}$

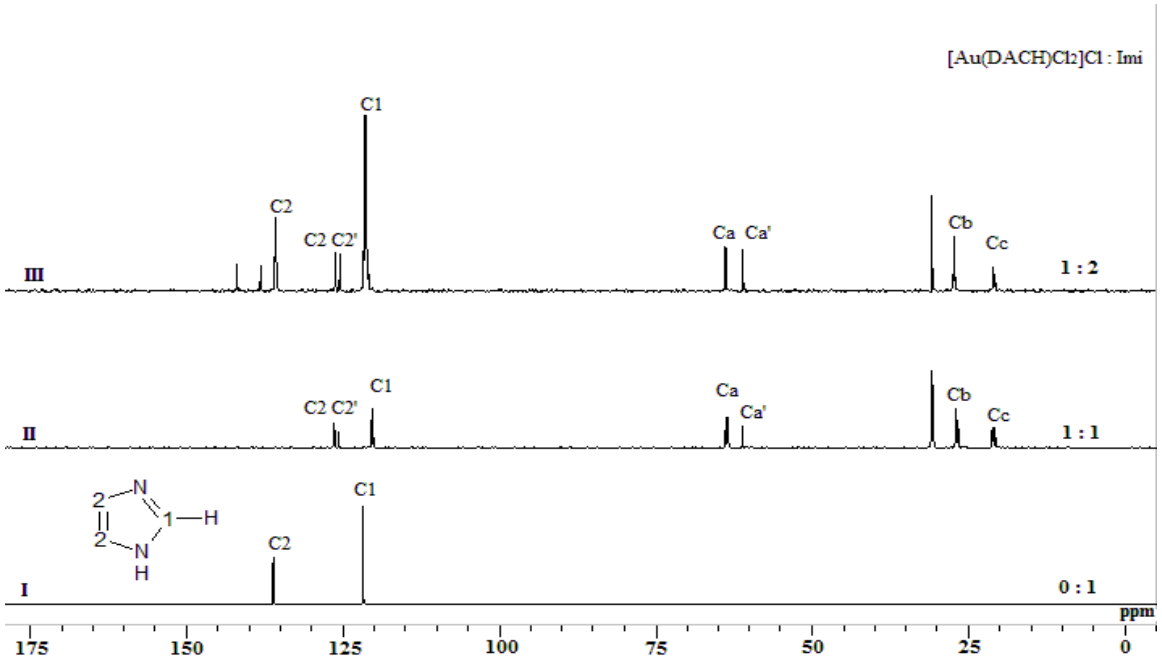


Figure 4. 13: ^{13}CNMR for reaction of Imidazole with [Au(*cis*-DACH)Cl$_2$]Cl in D$_2$O at pD 7.4 I) 10 mM Imidazole before reaction II) with 10 mM [Au(*cis*-DACH)Cl$_2$]Cl III) at 20 mM Imidazole.

4.4.2 Interaction of [Au(*cis*-DACH)$_2$]Cl$_3$ with imidazole:

Potential anticancer gold(III) complex of *cis*-1,2-diaminoyclohexane [Au(*cis*-DACH)$_2$]$^{3+}$ was prepared in 10 mM solution using D$_2$O as solvent and amount of Imidazole equivalent to 10 mM was added. The pD of solution was adjusted at 7.3 then ^{1}H and ^{13}C NMR spectra were collected at prescribed reaction conditions and the results showed

there is no interaction between gold(III) complex and imidazole takes place, since all ^{13}C resonance for both gold(III) complex and Imidazole were obtained without any shifts (Figure 4.14).

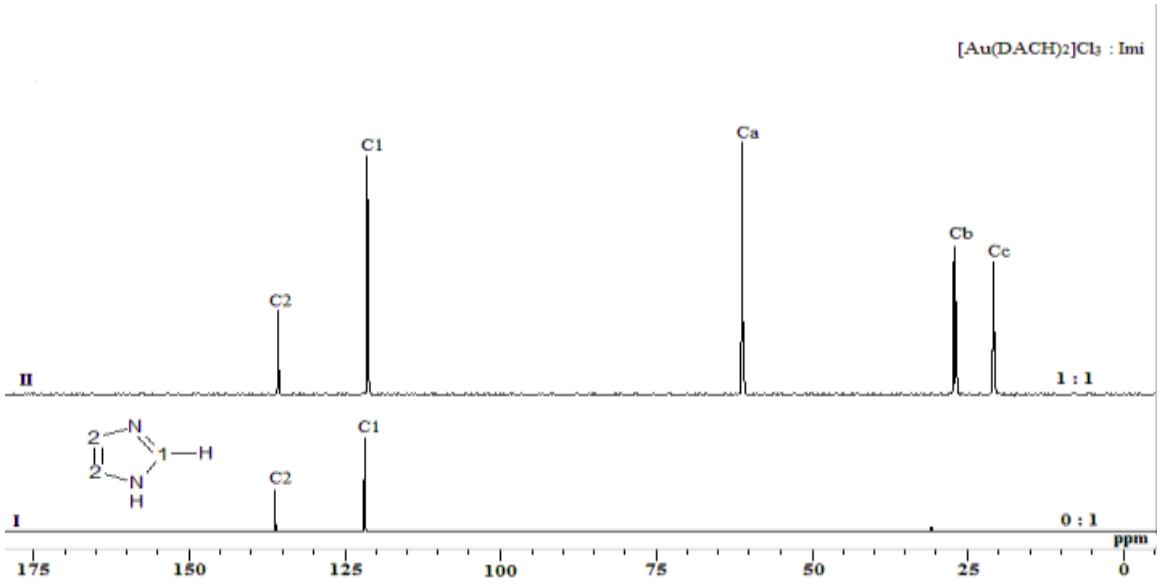


Figure 4. 14:^{13}CNMR for reaction of Imidazole with [Au(*cis*-DACH)$_2$]Cl$_3$ in D_2O at pD 7.4 I) 10 mM Imidazole before reaction II) with 10 mM [Au(*cis*-DACH)$_2$]Cl$_3$.

4.4.3 UV-Vis study of [Au(*cis*-DACH)Cl$_2$]Cl interaction with imidazole:

An aqueous solution of [Au(*cis*-DACH)Cl$_2$]Cl was prepared in presence of 40 mM sodium chloride, electronic spectra was recorded for prepared solution absorption band was obtained at 305 nm with extension coefficient 2500 $M^{-1}cm^{-1}$ assigned to MLCT from gold centre to Chloride ligands as compared with Auric acid then equal volumes from gold(III) complex solution and imidazole were mixed inside UV cell and electronic spectrum was collected again, the absorption band for gold(III) complexes completely disappeared (Figure 4.15) this can be easily explained as removal of chlorides ligands from gold(III) and replaced by Imidazole ligands and that is in agreement with NMR data.

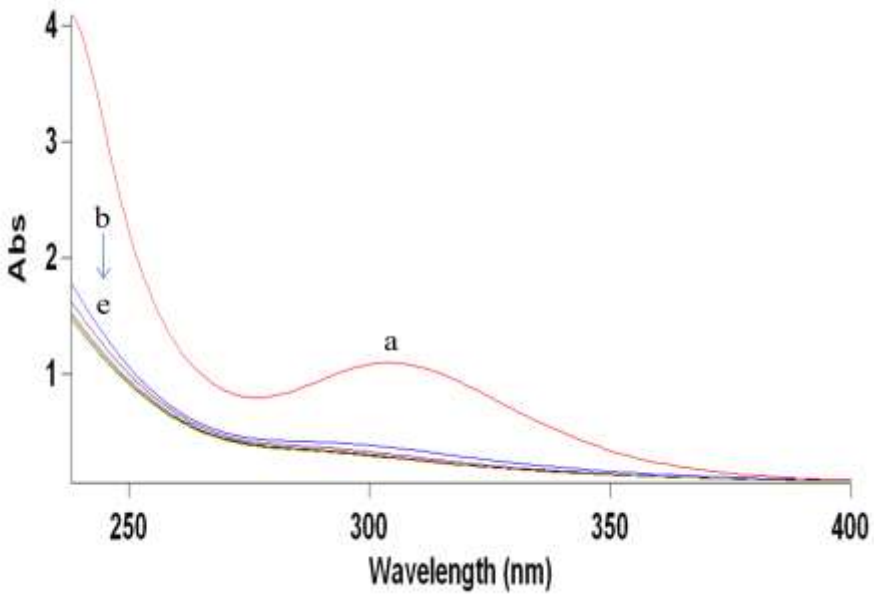


Figure 4. 15: Electronic spectra for reaction of $[Au(cis\text{-}DACH)Cl_2]^+$ with Imidazole: a) before reaction b-e): after addition of Imidazole solution.

4.5 Interaction of 1, 2-diammino-gold(III) with DL-Penicillamine:

Penicillamine is one of the chelator agents that have been used in pharmacology to remove heavy metals from body [82], It was used to treat Wilson's disease [83]. It is Penicillin metabolites although it has no antibiotic activity, Pritchard *et al.* showed the importance of Penicillamine interaction with gold in treatment of rheumatoid disease [84].

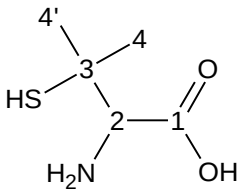


Figure 4. 16: Structure of Penicillamine

4.5.1 Interaction of [Au(*cis*-DACH)Cl_2]Cl with DL-Penicillamine:

The interaction of DL-Penicillamine with gold(III) diammine ligand complexes was studied in details using multi-nuclear NMR and UV-Vis spectrophotometer , ^{1}H and ^{13}C NMR spectra for DL-Penicillamine were obtained prior to mixing with gold(III) complex and listed in (Table 4.7), the shifts in resonance values were then monitored.

Table 4. 7: ^{13}C NMR for PSH and oxidized PSH.

	C1	C2	C3	C4, C4'
PSH	170.5	63.7	44.2	30.5, 28.4
PSH + H_2O_2 (30%)	172.0, 171.6	65.3,62.6	50.7,50.6	30.9, 28.4, 27.4, 23.3
[(*cis*-DACH)Au(SNP)]$^{2+}$	181.6	75.8	57.8	27.8, 26.5

A solution of 10 mM [Au(*cis*-DACH)Cl_2]Cl was prepared by dissolve 8.35mg gold(III) complex in 2mL D_2O and amount of 3.00 mg DL-Penicillamine was added to prepared

solution (1:1) ratio, solution pD was adjusted to 3.8, then NMR spectra was collected. DL-Penicillamine was oxidized in D_2O solution using H_2O_2 (30%) in order to investigate its disulfide resonances (**Table 4.7** above), The appearance of the resonances at ~50.5, 27.19 and 23.4 ppm in ^{13}C NMR spectrum for reaction mixture could be taken as an evidence for oxidation of DL-PSH to its corresponds disulfide which indicates that it where $[Au(cis\text{-}DACH)Cl_2]^+$ have been reduced to gold(I) and after few days white precipitate for gold(I)-PSSP was observed. When gold(III) complex and PSH were mixed (1:1) the carbonyl resonance was obtained with down-field shift at 181.62 ppm followed by resonances at 75.76 ppm and 57.76 ppm which corresponds to DL-Penicillamine-C3and C3 in chelation intermediate $[(cis\text{-}DACH)Au(SNP)_2]^+$.

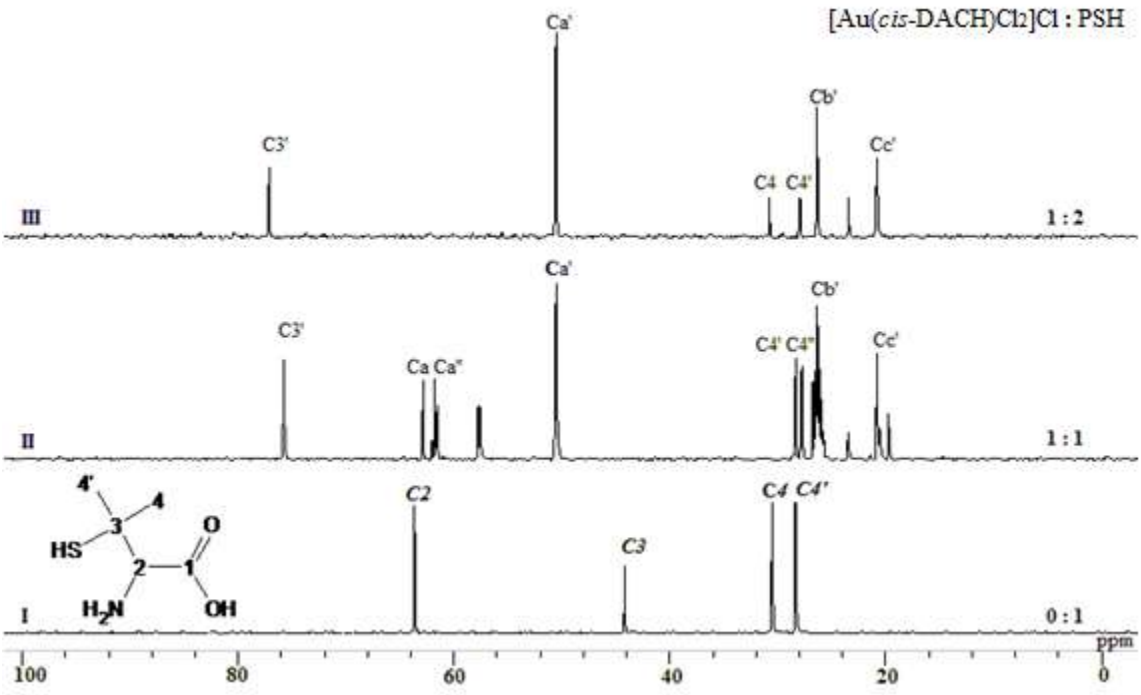


Figure 4. 17: ^{13}C NMR for reaction of I) 10 mM $[Au(cis\text{-}DACH)Cl_2]^+$ with II) 10 mM PSH III) 20 mM PSH.

4.5.2 UV-Vis and Kinetics Measurements of $[Au(en)Cl_2]Cl$ and $[Au(en)_2]Cl_3$ with DL-Penicillamine:

The Kinetics scan spectra obtained from monitoring mixture of solution of 0.5 mM gold(III)ethylenediamine complex with 0.5 mM DL-Penicillamine solution as time dependent (Figure 4.18) showed absorption band at about 233 nm, while solution of 0.5 mM gold(III) ethylenediamine complex to 1 mM DL-Penicillamine solution as time dependent (Figure 4.19) showed an increased absorption band with time at 297 nm with rapid disappearance of band at 233 nm here based on this observation the reaction between gold(III) diamine complexs can be summarized as DL-Penicillamine exchange one chloride ligand and form intermediate $[Au(en)(SP)Cl]^+$ followed by further ligand exchange to form $[Au(en)(SP)_2]^+$ this intermediate dissociate to give Penicillamine disulfide with reduction of gold(III) to gold(I) as observed in NMR .

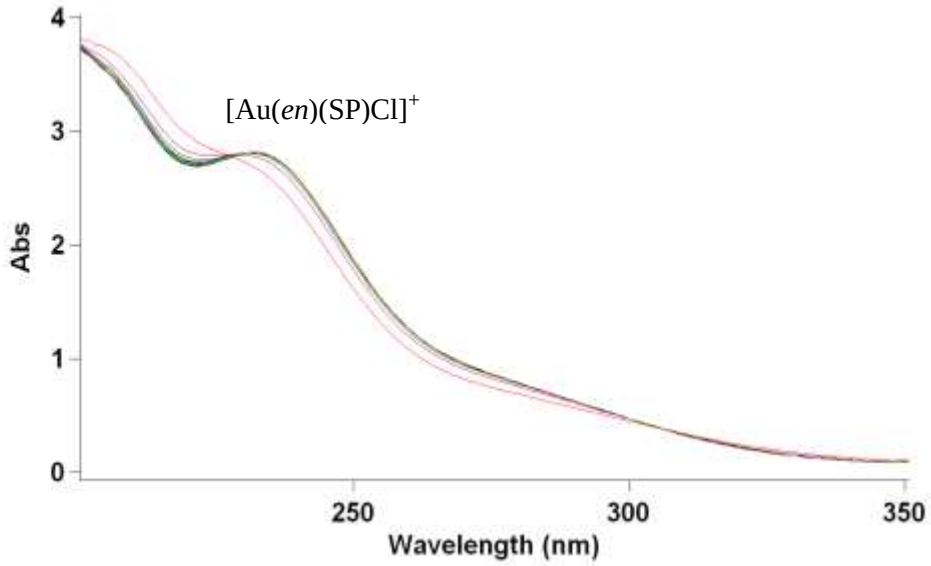


Figure 4. 18: Kinetics scan spectra for reaction of 0.5 mM $[Au(en)Cl_2]^+$ with 0.5 mM DL-Penicillamine in presence of 20 mM NaCl.

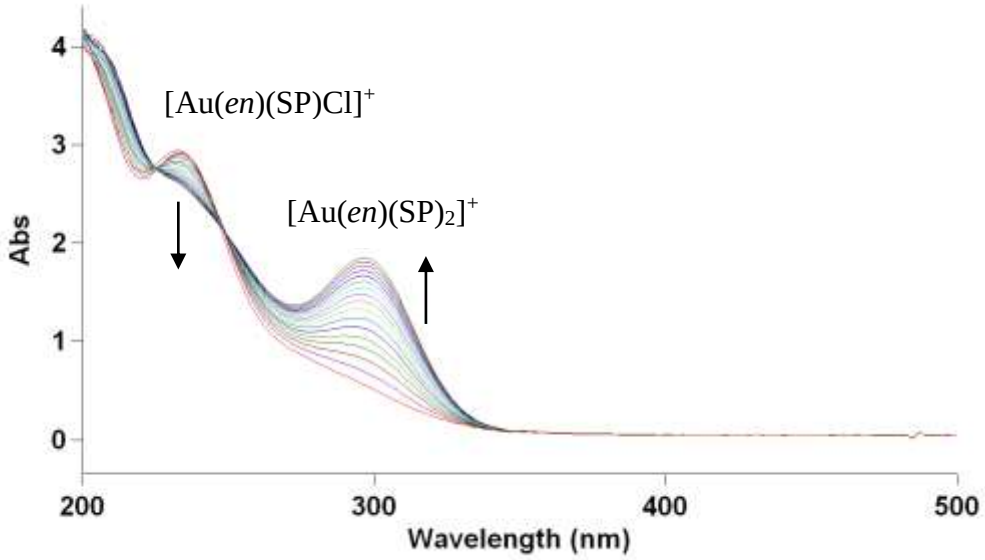


Figure 4. 19: Kinetics scan spectra for reaction of 0.5 mM $[Au(en)Cl_2]^+$ with 2 mM PSH in presence of 20 mM NaCl.

These intermediates were found to be stable under this low concentration over 48 hours where mixtures solution were kept for 48 hours and their electronic spectra were collected, absorption band at 234 nm was obtained from 1:1 mixture while absorption band at 297 nm was obtained from 1:2 mixture as shown in (Figure 4.20)

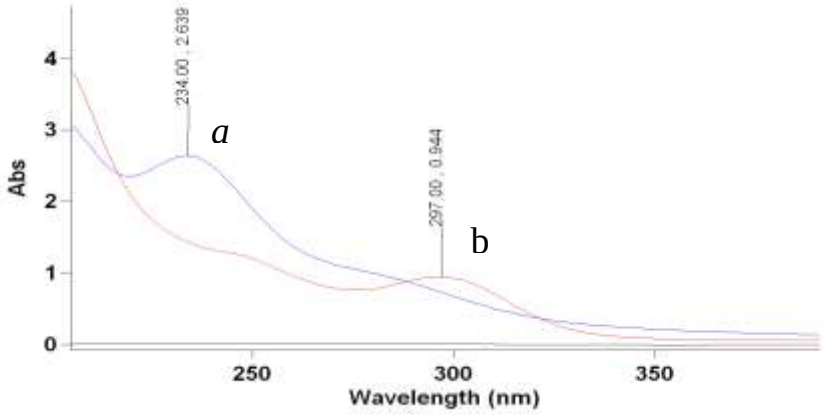


Figure 4. 20: Reaction mixtures after 48 hours a) 0.5 mM : 0.5mM b) 0.5 mM: 2mM

Kinetic measurements were performed at *pseudo*-first order condition as function of ligand concentration at 297 nm as working wavelength. Each kinetic measurement was repeated three times; then *pseudo*-first order rate constant was calculated using Eqn (3.1). Each reaction was evaluated at three different temperatures 288K, 298K and 310K, and obtained date were plotted using eyring and Arrhenius activation parameters were calculated and listed in (**Table 4.8**)

Table 4. 8: Kinetic data for reaction of PSH with **1)** $[Au(en)Cl_2]^+$ **2)** $[Au(en)_2]^{3+}$

PSH	k_{obs} / $M^{-1}.s^{-1}$			$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	*Ea*
	288K	298K	310K	kJ . mol^{-1}	$JK^{-1}mol^{-1}$	kJ . mol^{-1}
1	$(95\pm2)x10^{-2}$	$(109\pm2)x10^{-2}$	$(103\pm4)x10^{-2}$	8.0 ± 0.3	-210 ± 1	11 ± 0.3
2	$(135\pm5)x10^{-2}$	$(217\pm6)x10^{-2}$	$(260\pm5)x10^{-2}$	17 ± 3	-175 ± 12	22 ± 1

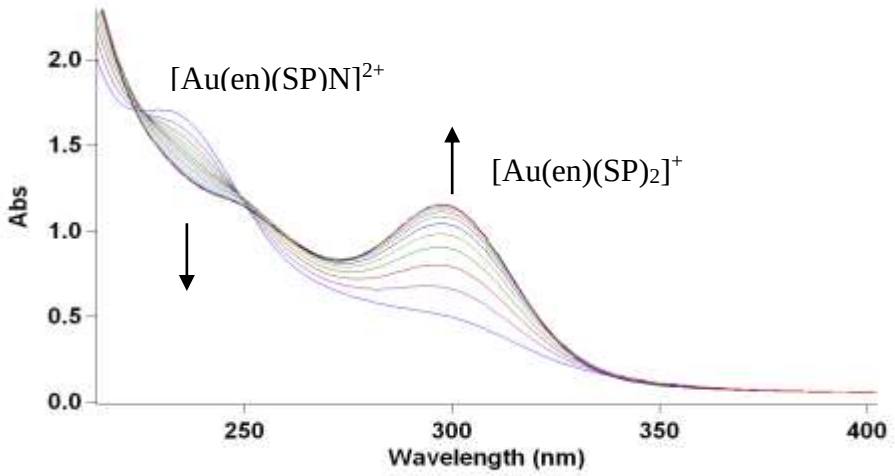


Figure 4. 21: Scanning kinetics spectra for reaction of PSH with $[Au(en)_2]^{3+}$ at 298 K.

Table 4. 9: Observed rate constant for reaction of $[Au(en)Cl_2]^+$ with PSH as a function of ligand concentration in presence of 20 mM sodium chloride at 297 nm.

T/K	$10^{-3}C_L$/M	k_{obs}/min
288	1	0.1488
	2	0.2251
	3	0.2889
	4	0.3371
	5	0.3953
	6	0.4344
298	1	0.2313
	2	0.3029
	3	0.3638
	4	0.4217
	5	0.4914
	6	0.5591
310	1	0.3379
	2	0.4464
	3	0.5105
	4	0.5887
	5	0.6681
	6	0.7352

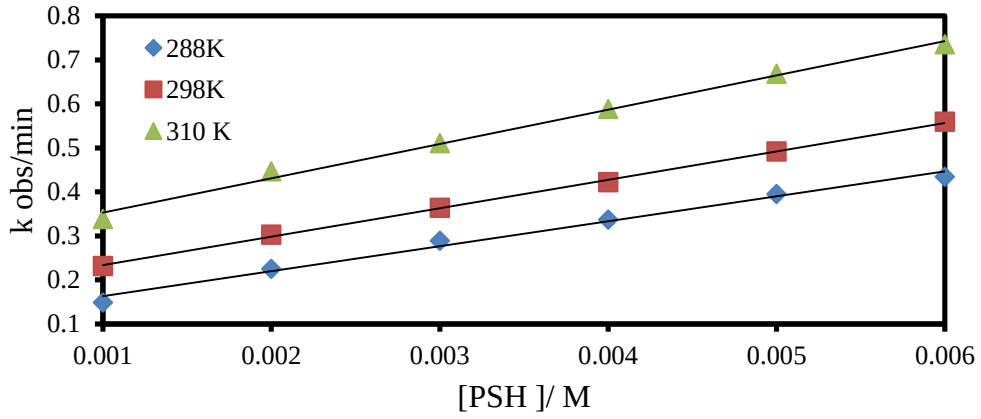


Figure 4. 22: Observed rate constant for reaction of $[Au(en)Cl_2]^+$ with PSH as a function of ligand concentration in presence of 20 mM sodium chloride

Table 4. 10: Observed rate constant for reaction of $[Au(en)_2]^{3+}$ with PSH as function of ligand concentration in presence of 20mM sodium chloride at 297 nm.

T/K	$10^{-3}C_L$/M	k_{obs}/min
288	1	0.2360
	2	0.3233
	3	0.4446
	4	0.4961
	5	0.5574
298	1	0.3434
	2	0.5244
	3	0.6290
	4	0.7569
	5	0.8727
310	1	0.6042
	2	0.7418
	3	0.8883
	4	1.0668
	5	1.2219

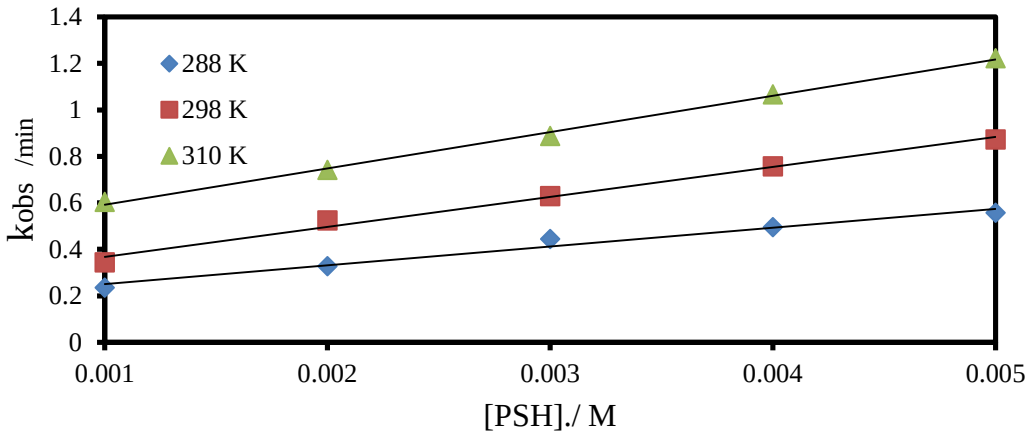


Figure 4. 23: Observed rate constant for reaction of $[Au(en)_2]^{3+}$ with PSH as a function of ligand concentration in presence of 20 mM sodium chloride.

4.5.3 UV-Vis and Kinetics Measurements of [Au(*cis*-DACH)Cl_2]Cl and [Au(*cis*-DACH)$_2$]Cl_3 with DL-Penicillamine:

Electronic spectra for reaction mixture of [Au(*cis*-DACH)Cl_2]Cl and [Au(*cis*-DACH)$_2$]Cl_3 0.2 mM with 2 mM DL-Penicillamine in their aqueous solutions containing 20 mM sodium chloride gave the same absorption bands which are at 233 nm and 297 nm, and the absorption band at 233 nm rapidly disappeared with time and absorption band at 297 nm. These absorptions thought to be sulfur to gold(III) CT (Figure 4.24) and this is consider as strong evidence for formation of intermediates with formulas [Au(*cis*-DACH)(SP)X]$^+$ and [Au(*cis*-DACH)(SP)$_2$]$^+$ respectively. That explains the reduction of [Au(*cis*-DACH)Cl_2]Cl which occurs through two consecutive steps. First step is substitution of one chloride ligand by Penicillamine ligand followed by further ligand substitution then reductive elimination to produce gold(I) with Penicillamine oxidized to Penicillamine disulfide.

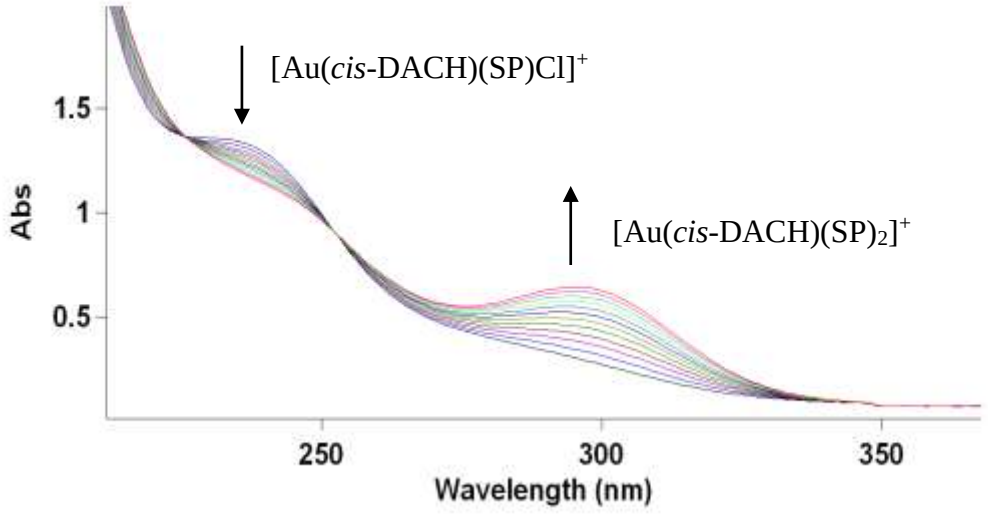


Figure 4. 24:Scanning kinetics spectra for reaction of PSH with [Au(*cis*-DACH)Cl_2]$^+$ in presence of 20 mM NaCl.

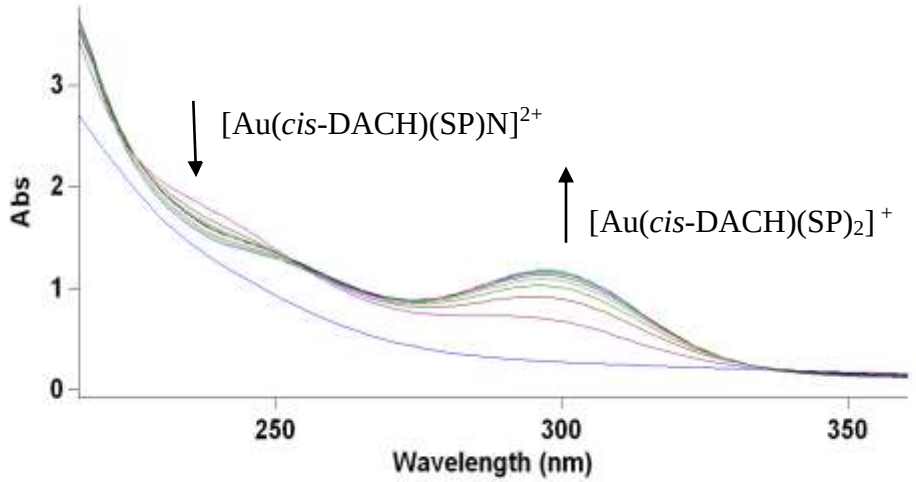


Figure 4. 25: Scanning kinetics spectra for reaction of PSH with $[Au(cis\text{-}DACH)_2]^{3+}$ in presence of 20 mM NaCl.

Scanning kinetics mode was employed to monitor the reaction progress, equal volumes from gold(III) *cis*-diamonocyclohexane complexes and DL-Penicillamine were kept till it were thermally stable then mixed directly inside UV cell at 297 nm. The reaction progress obtained as time dependant, each concentration was repeated three times and reaction was conducted at three different temperatures 288K, 298K and 310K. Data was treated using mentioned Eqns (**3.1-5**) *pseudo*-first order rate constants (*k*), entropy of activation ($\Delta S^{\ddagger}$) enthalpy of activation ($\Delta H^{\ddagger}$) and activation energy E were calculated and all kinetics data were listed in Table (**4.11**) below.

Table 4. 11: Kinetics data for reaction of PSH with 1) $[Au(cis\text{-}DACH)Cl_2]^+$ 2) $[Au(cis\text{-}DACH)_2]^{3+}$

PSH	k $M^{-1}.s^{-1}$			$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	Ea
	288K	298K	310K	kJ . mol^{-1}	$JK^{-1}mol^{-1}$	kJ. mol^{-1}
1	$(43\pm2)x10^{-2}$	$(68\pm0.8)x10^{-2}$	$(136\pm2)x10^{-2}$	36 ± 1	-120 ± 3	38 ± 1
2	$(101\pm3)x10^{-2}$	$(172\pm3)\ x10^{-2}$	$(221\pm0)x10^{-2}$	24 ± 1	-155 ± 3	26 ± 1

Table 4. 12: Observed rate constant for reaction of $[Au(cis\text{-}DACH)Cl_2]^+$ with PSH as a function of ligand concentration in presence of 20 mM sodium chloride at 297 nm.

T/K	$10^{-3}C_L$/M	k_{obs}/min
288	1	0.0402
	2	0.0665
	3	0.1013
	4	0.1211
	5	0.1404
	6	0.1734
298	1	0.0710
	2	0.1222
	3	0.1647
	4	0.2007
	5	0.2417
	6	0.2807
310	1	0.1251
	2	0.1924
	3	0.2873
	4	0.3543
	5	0.4657
	6	0.5202

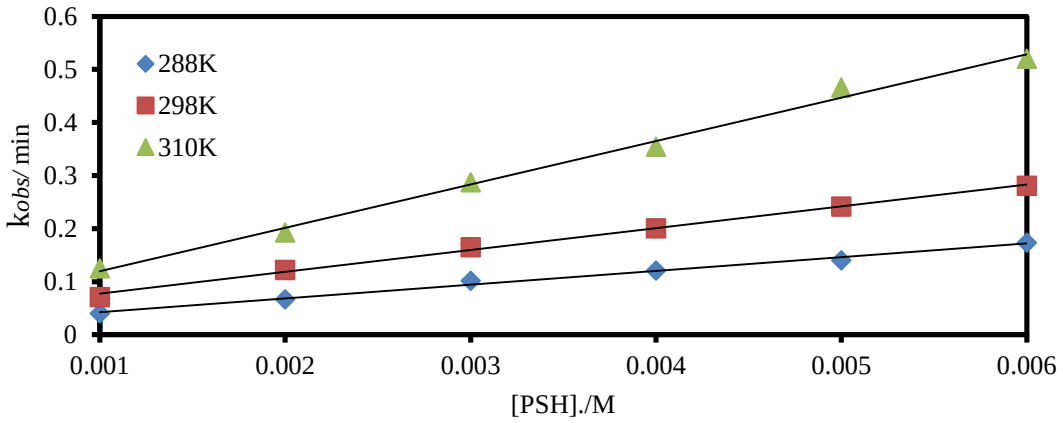


Figure 4. 26: Observed rate constant for reaction of $[Au(cis\text{-}DACH)Cl_2]^+$ with PSH as a function of PSH concentration and temperature in presence of 20 mM sodium chloride.

Table 4. 13: Observed rate constant for reaction of $[Au(cis\text{-}DACH)_2]^{3+}$ with PSH as a function in ligand concentration in presence of 20 mM sodium chloride at 297 nm.

T/K	$10^{-3}C_L$/M	k_{obs}/min
288	1	0.2240
	2	0.2882
	3	0.3634
	4	0.4050
	5	0.4686
298	1	0.4571
	2	0.5401
	3	0.6501
	4	0.7430
	5	0.8705
310	1	0.5849
	2	0.7323
	3	0.8334
	4	0.9876
	5	-

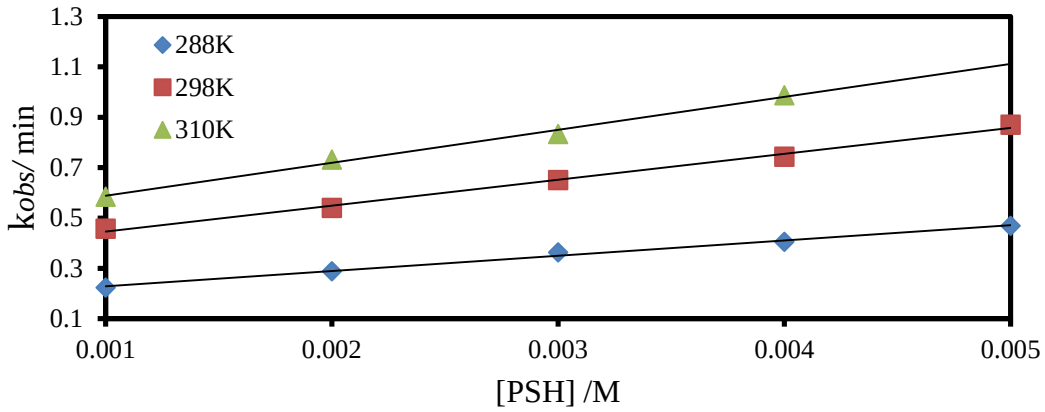


Figure 4. 27: Observed rate constant for reaction of PSH with $[Au(cis\text{-}DACH)_2]^{3+}$ as a function of PSH concentration and temperature.

4.6 Interaction of diamminegold(III) complexes with Thiomalic acid:

4.6.1 Interaction of [Au(*cis*-DACH)Cl_2]Cl complexes with Thiomalic acid:

Thiomalic acid (TmSH) complexes with gold are used fundamentally in treatment of rheumatoid arthritis because of their anti-inflammatory action [85]. Interaction of thiomalic acid with $[Au(cis\text{-}DACH)Cl_2]^+$ was studied using NMR technique in D_2O solution in acidic conditions.

^{13}C spectra for TmSH was recorded in D_2O solution prior to mixing with $[Au(cis\text{-}DACH)Cl_2]^+$ and then it has been oxidized by 30% H_2O_2. C2 resonance shifted down-field to C2' at 47 ppm, when C3 was shifted up-field to C3' at ~ 36 ppm. ^{13}C NMR spectra for reaction of TmSH with $[Au(cis\text{-}DACH)Cl_2]^+$ is shown in (Figure 4.28**)**. similar down-field was observed for C2 while C3 also showed slight up-field shift that confirm oxidation of TmSH to disulfide along with gold(III) complexes reduction as indicated by resonance at ~ 50 ppm which is assigned to C1 and C2 (indicated by Ca') in

protonated DACH ligands. Complete disappearance for Ca resonance was obtained at reaction mixture 1:2 gold(III) complex to TmSH.

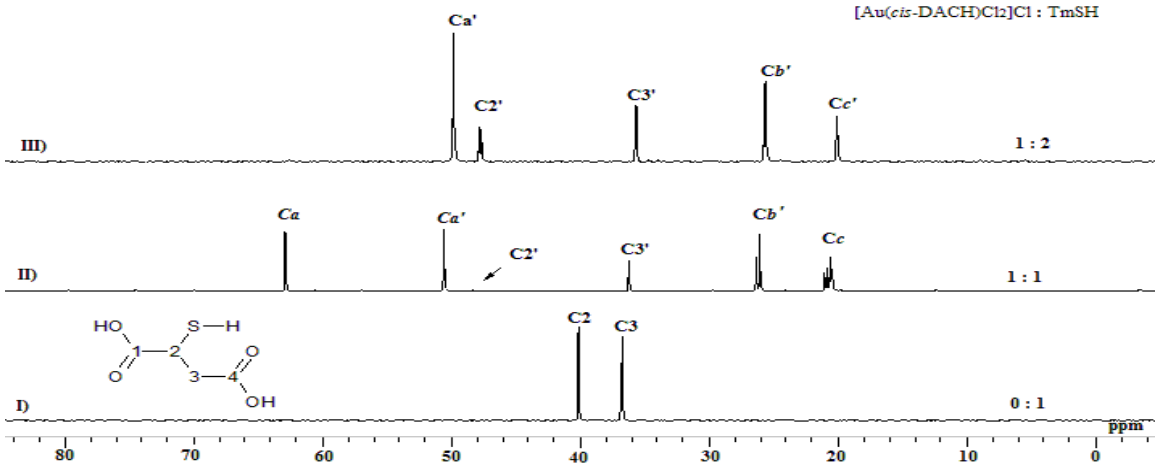


Figure 4. 28: ^{13}C NMR spectra for reaction of TmSH with [Au(*cis*-DACH)Cl_2]Cl in acidic condition, I) TmSH before reaction II) after addition of one equivalent [Au(*cis*-DACH)Cl_2]Cl III) with two equivalents [Au(*cis*-DACH)Cl_2]Cl.

4.6.2 UV-Vis for gold (III) diamminocyclohexane complex with Thiomalic acid:

Scanning kinetics mode was employed to investigate the spectral for reaction mixture between gold(III) complex and thiomalic acid, no change in electronic spectra for gold(III) complex solution was observed in low concentration with TmSH but when the ratio was raised to 1:10 (0.2 mM [Au(*cis*-DACH)Cl_2]$^+$ in 40 mM NaCl solution to 2mM TmSH solution) and monitored to 20 min intense absorption band appeared in 297-300 nm and went to disappear, this absorption band could be assigned to formation of Substitution intermediate for gold(III) complexes prior to reduction with two TmSH molecules [Au(*cis*-DACH)$(Tm)_2$]$^+$ as shown in Figure 4.29.

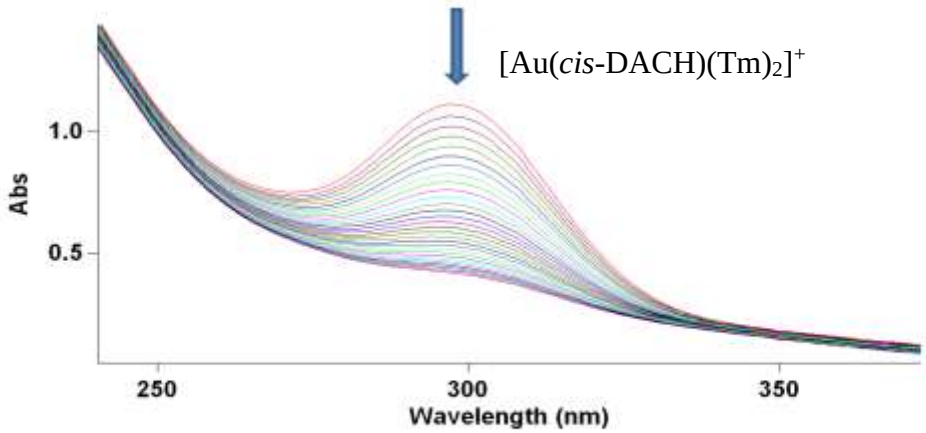


Figure 4. 29: Scanning kinetics spectra for reaction of 0.2mM [Au(*cis*-DACH)Cl_2]Cl with 2mM TmSH in presence of 20 mM NaCl.

4.7 Kinetics study of N-(2-Mercaptoprpinyl)gGlycine interaction with diamminocyclohexane gold(III) complex:

N-(2-Mercaptoprpinyl)glycine (MPG) interaction with potential anticancer gold(III) complex of [Au(*cis*-DACH)Cl_2]Cl was investigated spectrophotometerically in terms of kinetics, pseudo-first order conditions were applied to study the reaction in aquoes solution in presence of 20 mM NaCl, scanning kinetics gave well defined absorption band at 297 nm assigned by comparison to [Au(*cis*-DACH)$(MPG)_2]^+$ (Figure 4.30) bellow.

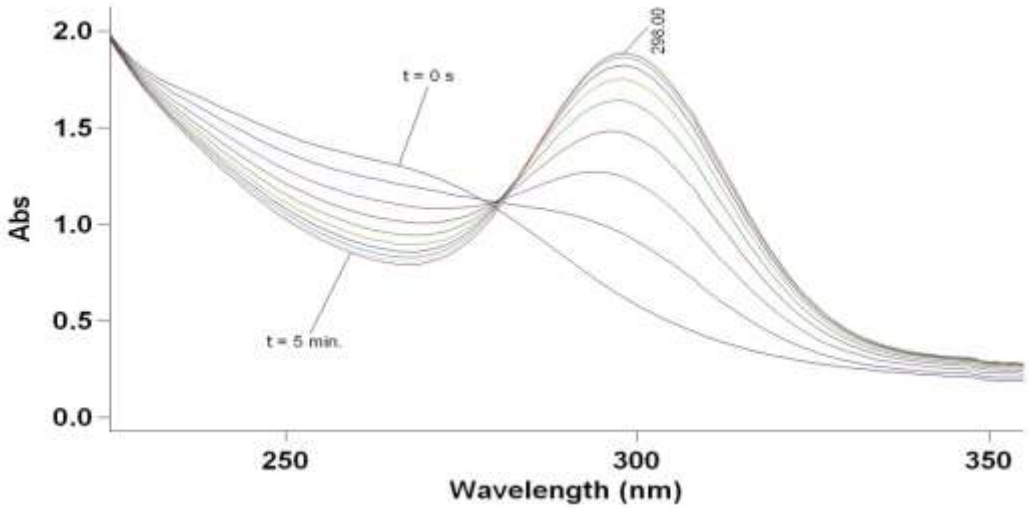


Figure 4. 30: Scanning kinetic spectra for reaction of MPG with [Au(*cis*-DACH)$Cl_2]^+$

It is clearly seen that the difference between each time dependant run is relatively large compared with previously investigated thiols (GSH and PSH) and after about 5 minutes there is no change in absorption. Furthermore rate of formation of this intermediate was calculated under ascribed condition with activation parameters (**Table 4.14**)

Table 4. 14: Kinetics data for reaction of MPG with [Au(*cis*-DACH)Cl_2]$^+$

MPG	*k* M^{-1}. s^{-1}			$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	*Ea*
	288 K	298 K	310 K	kJ . mol^{-1}	$JK^{-1}mol^{-1}$	kJ . mol^{-1}
[Au(DACH)Cl_2]$^+$	2.5	3.6	4.9	20	-163	22

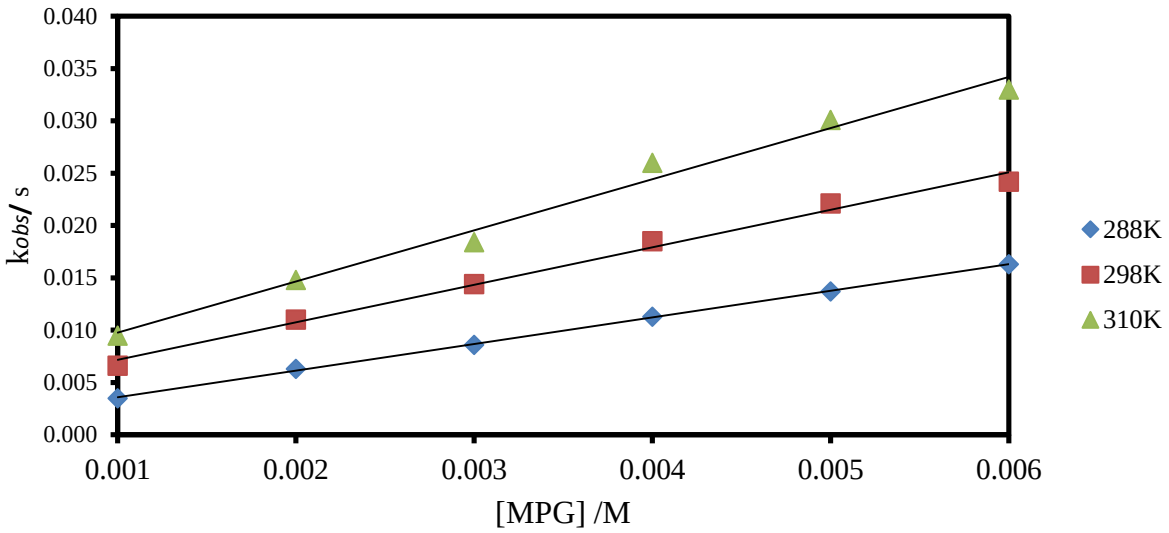


Figure 4. 31: Observed rate constant for reaction of MPG with [Au(*cis*-DACH)Cl_2]$^+$ as a function of MPG concentration and temperature in presence of 20 mM sodium chloride.

4.8 Kinetics of [Au(*cis*-DACH)Cl_2]Cl interaction with Mercaptoacetic acid :

The interaction of [Au(*cis*-DACH)Cl_2]Cl with Mercaptoacetic acid (MAA)was carried out using UV/Vis spectrophotometer, a solution of 0.2 mM of [Au(*cis*-DACH)Cl_2]Cl was prepared in presence of 40 mM NaCl. Series of MAA solution were also prepared then effective wavelength was determined as 298 nm using scanning kinetics mode at 298K.

Pseudo-first order rate constant was determined for the reaction of [Au(*cis*-DACH)Cl_2]Cl with MAA under *pseudo*-first order conditions at three different temperatures 288K, 298K and 310K, Arrhenius and Eyring equations were applied to calculate activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and Ea and calculated values showed that MAA has the highest reactivity towards substitution reaction with [Au(*cis*-DACH)Cl_2]Cl with correspondence *pseudo*-first order rate constant as 57 $\pm$ 3 $M^{-1}s^{-1}$ at 298K. All values were listed in (**Table 4.15**) bellow

Table 4. 15: Kinetics data for reaction of MAA with [Au(cis-DACH)Cl_2]$^+$

MAA	k M^{-1}. s^{-1}			$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	*Ea*
	288K	298K	310K	kJ . mol^{-1}	$JK^{-1}mol^{-1}$	kJ . mol^{-1}
[Au(*cis*-DACH)Cl_2]$^+$	43 $\pm$ 0.6	57 $\pm$ 3	99 $\pm$ 0.02	25 $\pm$ 0.4	-117 $\pm$ 1	28 $\pm$ 0.4

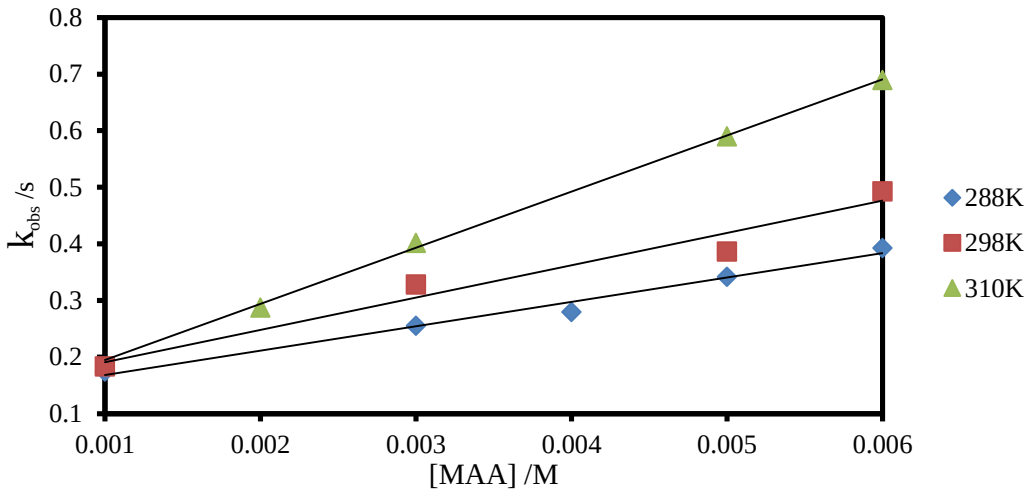


Figure 4. 32: Observed rate constant for reaction of MAA with [Au(*cis*-DACH)Cl_2]$^+$ as a function of MAA concentration and temperature in presence of 20 mM sodium chloride.

The kinetic data for all studied reactions were listed in Table (4.16) bellow in comparison with some reaction for same gold(III)-DACH complex with biologically relevant molecules.

Table 4. 16: Kinetic data for reaction of gold(III) diammines complexes with different biological molecules in aqueous solution in presence of 20mM NaCl for $[Au(NN)Cl_2]^+$ complexes.

	^{a}k $M^{-1}.\ s^{-1}$	$\Delta H^{\ddagger}$ kJ . mol^{-1}	$\Delta S^{\ddagger}$ $JK^{-1}mol^{-1}$	*Ea* kJ . mol^{-1}	Reference
$[Au(DACH)Cl_2]^+$					
GSH	(46 ± 2) $x10^{-2}$	37.3 ± 0.8	-120 ± 3	40 ± 0.8	This work
PSH	(68 ± 1) $x10^{-2}$	36 ± 1	-120 ± 3	38 ± 1	This work
Ino	9.9 ± 0.9	-	-	-	[66]
5'-IMP	6.0 ± 0.3	-	-	-	[66]
5'-GMP	66 ± 2	10 ± 3	-180 ± 10	-	[66]
L-His	75 ± 2	12 ± 3	-160 ± 10	-	[66]
KCN	148	39	-80	42	[86]
MAA	57 + 3	25 + 0.4	-117 + 1	28 + 0.4	This work
MPG	2.5	19.5	-162.6	22.0	This work
$[Au(en)Cl_2]^+$					
GSH	(217 ± 3) $x10^{-2}$	18 ± 0.9	-170 ± 3	21 ± 0.6	This work
PSH	(109 ± 2) $x10^{-2}$	8.0 ± 0.3	-210 ± 1	11 ± 0.3	This work
L-His	39 ± 3	-	-	-	[59]
$[Au(en)_2]^{3+}$					
GSH	(103 ± 8) x 10^{-2}	11 ± 1	-168 ± 4	13 ± 1.7	This work
PSH	(217 ± 6) x 10^{-2}	17 ± 3	-175 ± 12	22 ± 1	This work
$[Au(DACH)_2]^{3+}$					
GSH	(19 ± 1) x 10^{-2}	-	-	-	This work
PSH	(172 ± 3) x 10^{-2}	24 ± 1.0	-155 ± 3	26 ± 1	This work
KCN	18	11	-185	13	[86]

[a] *Pseudo*-first order rate constant at 298K.

Chapter 5

Conclusions and Recommendations

In this work we have studied the interaction of some potential anticancer gold(III) complexes of 1,2-diammine containing ligands with biologically important molecules like L-methionine, DL-selenomethionine, glutathione, imidazole, DL-penicillamine, thiomalic acid, mercaptoacetic acid and N-(2-mercaptopropinyl)glycine. Our results showed the reduction of gold(III) to gold(I) and gold(0) that occurs upon reaction with sulphur containing ligands, while nitrogen donar ligands have been substituted and coordinated to gold(III).

Final product identification was based on comparison with oxidized ligands using H_2O_2 under the same reaction conditions. thioether and selenoether were oxidized to their corresponding sulfoxide and selenoxide; where thiols oxidation products were obtained as disulfides. The reactivity of studied gold(III) complexes to form diamminodithiolato gold(III) intermediate can be racked as: ethylenediamine gold(III) complexes are faster than diaminocyclohexane gold(III) complexes where mono diamino gold(III) complexes react faster than bis diamino gold(III) complexes.

For future recommendations, more studies for gold(III) complexes interaction with biologically importance molecules will lead to better understanding for its cytotoxicity and it will help in developing and designing stable compounds with low toxicity. Interaction with nucleobases should be carried out using stopped-flow technique.

References:

[1] A. Casini, C. Hartinger, C. Gabbiani, E. Mini, P. J. Dyson, B. K. Keppler, and L. Messori, "Gold(III) compounds as anticancer agents: relevance of gold-protein interactions for their mechanism of action.," *J. Inorg. Biochem.*, vol. 102, pp. 564–575,. 2008.

[2] V. Milacic, D. Fregona, and Q. P. Dou, "Gold complexes as prospective metal-based anticancer drugs.," *Histol. Histopathol.*, vol. 23, pp. 101–108, 2008.

[3] B. Đ. Glišić, S. Rajković, and M. I. Djuran, "The reactions of $[Au(dien)Cl]^{2+}$ with L-histidine-containing dipeptides. Dependence of complex formation on the dipeptide structure," *J. Coord. Chem.*, vol. 66, pp. 424–434, 2013.

[4] S. L. Best and P. J. Sadler, "Gold drugs: Mechanism of action and toxicity," *Gold Bull.*, vol. 29, pp. 87–93, 1996.

[5] A. Casini, G. Kelter, C. Gabbiani, M. A. Cinellu, G. Minghetti, D. Fregona, H.-H. Fiebig, and L. Messori, "Chemistry, antiproliferative properties, tumor selectivity, and molecular mechanisms of novel gold(III) compounds for cancer treatment: a systematic study.," *J. bio Inorg Chem*, vol. 14, pp. 1139–1149, 2009.

[6] S. H. van Rijt and P. J. Sadler, "Current applications and future potential for bioinorganic chemistry in the development of anticancer drugs.," *Drug Discov. Today*, vol. 14, pp. 1089–1097, 2009.

[7] X. Wang and Z. Guo, "Towards the rational design of platinum(II) and gold(III) complexes as antitumour agents", *Dalton Trans.*, pp. 1521–1532, 2008.

[8] C. Frank Shaw III, "Gold-Based Therapeutic Agents," *Chem. Rev.*, vol. 99, pp. 2589–2600, 1999.

[9] P. J. Sadler and R. E. Sue, "The chemistry of gold drugs," *Met. Based Drugs*, vol. 1, pp. 107–44, 1994.

[10] I. Ott, "On the medicinal chemistry of gold complexes as anticancer drugs," *Coord. Chem. Rev.*, vol. 25, pp. 1670–1681, 2009.

[11] C. Gabbani, A. Casini, and L. Messori, "Gold(III) compounds as anticancer rugs", *Gold Bull*, vol. 40, pp. 73–81, 2007.

[12] M. A Cinellu, L. Maiore, M. Manassero, A. Casini, M. Arca, H.-H. Fiebig, G. Kelter, E. Michelucci, G. Pieraccini, C. Gabbiani, and L. Messori,

"$[Au_2(phen(2Me))_2(\mu\text{-}O)_2](PF_6)_2$, A Novel Dinuclear Gold(III) Complex Showing Excellent Antiproliferative Properties.," *ACS Med. Chem. Lett.*, vol. 1, pp. 336–339, 2010.

[13] A. Bindoli, M. P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, and L. Messori, "Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs," *Coord Chem Rev*, vol. 253, pp. 1692–1707, 2009.

[14] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettı, R. Graziani, G. Pilloni, and D. Fregona, "Gold Dithiocarbamate Derivatives as Potential Antineoplastic Agents : Design , Spectroscopic Properties , and in Vitro Antitumor Activity," *Inorg. Chem.*, vol. 44, pp. 1867–1881, 2005.

[15] A. Casini, M. A. Cinellu, G. Minghetti, C. Gabbiani, M. Coronnello, E. Mini, and L. Messori, "Structural and solution chemistry, antiproliferative effects, and DNA and protein binding properties of a series of dinuclear gold(III) compounds with bipyridyl ligands.," *J. Med. Chem.*, vol. 49, pp. 5524–5531, 2006.

[16] G. G. Graham, M. W. Whitehouse, and G. R. Bushell, "Aurocyanide, dicyano-aurate (I), a pharmacologically active metabolite of medicinal gold complexes", *Inflammopharmacology*, vol. 16, pp. 126–132, 2008.

[17] M. W. Whitehouse, "Therapeutic gold. Is it due for a come-back", *Inflammopharmacology*, vol. 16, pp. 107–109, 2008.

[18] S. Carotti, A. Guerri, T. Mazzei, L. Messori, E. Mini, and P. Orioli, "Gold(III) compounds as potential antitumor agents: Cytotoxicity and DNA binding properties of some selected polyamine-gold(III) complexes," *Inorg. Chim. Acta*, vol. 281, pp. 90–94, 1998.

[19] S. Carotti, G. Marcon, M. Marussich, T. Mazzei, L. Messori, E. Mini, and P. Orioli, "Cytotoxicity and DNA binding properties of a chloro glycylhistidinate gold(III) complex (GHAu).," *Chem. Biol. Interact.*, vol. 125, pp. 29–38, 2000.

[20] I. Kostova and B. S. P. Bentham Science Publishers, "Gold Coordination Complexes as Anticancer Agents," *Anticancer. Agents Med. Chem.*, vol. 6, pp. 19–32, 2006.

[21] M. Coronnello, E. Mini, B. Caciagli, M. A. Cinellu, A. Bindoli, C. Gabbiani, and L. Messori, "Mechanisms of cytotoxicity of selected organogold(III) compounds.," *J. Med. Chem.*, vol. 48, pp. 6761–6765, 2005.

[22] S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, and L. Messori, "Gold Compounds as AnticancerAgents : Chemistry , Cellular Pharmacology , and Preclinical Studies," *Med. Res Rev*, vol. 30, pp. 550–580, 2009.

[23] S. S. Al-Jaroudi, M. Fettouhi, M. I. M. Wazeer, A. A. Isab, and S. Altuwaijri, "Synthesis, characterization and cytotoxicity of new gold(III) complexes with 1,2-diaminocyclohexane: Influence of stereochemistry on antitumor activity," *Polyhedron*, vol. 50, pp. 434–442, 2013.

[24] M. J. Hannon, "Metal-based anticancer drugs: From a past anchored in platinum chemistry to a post-genomic future of diverse chemistry and biology," *Pure Appl. Chem.*, vol. 79, pp. 2243–2261, 2007.

[25] B. Đ. Glišić, U. Rychlewska, and M. I. Djuran, "Reactions and structural characterization of gold(III) complexes with amino acids, peptides and proteins", *Dalton Trans.*, vol. 41, pp. 6887–6901, 2012.

[26] R. V Parish, "Organogold chemistry applications," *Gold Bull.*, vol. 31, pp. 14–21, 1998.

[27] J. Vicente, M. T. Chicote, M. D. Abrisqueta, P. González-Herrero, and R. Guerrero, "Recent advances in the chemistry of gold(I) complexes with C-, N- and S-donor ligands part I: alkynyl, amino, imino and nitrido derivatives," *Gold Bull.*, vol. 31, pp. 83–87, 1998.

[28] G. B. Kauffman, "The role of gold in alchemy. Part III," *Gold Bull.*, vol. 18, pp. 109–119, 1985.

[29] G. B. Kauffman, "The role of gold in alchemy. Part II," *Gold Bull.*, vol. 18, pp. 69–78, 1985.

[30] G. B. Kauffman, "The Role of Gold In Alchemy . Part I," *GoldBull.*, vol. 18, pp. 31–44, 1985.

[31] R. Eisler, "Chrysotherapy: a synoptic review.," *Inflamm. Res.*, vol. 52, pp. 487–501, 2003.

[32] T. M. Simon, D. H. Kunishima, G. J. Vibert, M. Lymphocytic, P. Leukemia, T. M. Simon, D. H. Kunishima, G. J. Vibert, and A. Lorber, "Screening Trial with the Coordinated Gold Compound Auranofin Using Mouse Lymphocytic Leukemia P388 Screening Trial with the Coordinated Gold Compound Auranofin Using Mouse Lymphocytic Leukemia P388," *cancer Res.*, vol. 41, pp. 94–97, 1981.

[33] C. K. Mirabelli, R. K. Johnson, C. M. Sung, C. K. Mirabelli, R. K. Johnson, C. Sung, L. Faucette, K. Muirhead, and S. T. Crooke, "Evaluation of the in Vivo Antitumor Activity and in Vitro Cytotoxic Properties of Auranofin , a Coordinated Gold Compound , in Murine Tumor Models Evaluation of the in Vivo Antitumor Activity and in Vitro Cytotoxic Properties of Auranofin , a Coordinated," *Cancer Res*, vol. 45, pp. 32–39, 1985.

[34] G. Gencheva, D. Tsekova, G. Gochev, G. Momekov, G. Tyuliev, V. Skumryev, M. Karaivanova, and P. R. Bontchev, "Synthesis, Structural Characterization, and Cytotoxic Activity of Novel Paramagnetic Platinum Hematoporphyrin IX Complexes: Potent Antitumor Agents.," *Met. Based. Drugs*, vol. 2007, pp. 67376, 2007.

[35] G. Gencheva, D. Tsekova, G. Gochev, D. Mehandjiev, and P. R. Bontchev, "Monomeric Au(II) complex with hematoporphyrin IX," *Inorg. Chem. Commun.*, vol. 6, pp. 325–328, 2003.

[36] G. Momekov, D. Ferdinandov, S. Konstantinov, S. Arpadjan, D. Tsekova, G. Gencheva, P. R. Bontchev, and M. Karaivanova, "In vitro evaluation of a stable monomeric gold(II) complex with hematoporphyrin IX: cytotoxicity against tumor and kidney cells, cellular accumulation, and induction of apoptosis.," *Bioinorg. Chem. Appl.*, vol. 2008, p.p 367471, 2008.

[37] P. Calamai, S. Carotti, A. Guerri, L. Messori, E. Mini, P. Orioli, and G. P. Speroni, "Biological Properties of Two Gold(III) Complexes: $AuC1_3$(Upm) and $AuC1_2$(pm)," *J. Inorg Biochem*, vol. 13, p.p 103, 1997.

[38] G. Marcon, S. Carotti, M. Coronnello, L. Messori, E. Mini, P. Orioli, T. Mazzei, M. A. Cinellu, and G. Minghetti, "Gold(III) complexes with bipyridyl ligands: solution chemistry, cytotoxicity, and DNA binding properties.," *J. Med. Chem.*, vol. 45, pp. 1672–1677, 2002.

[39] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell, and P. Zanello, "Gold(III) complexes as potential antitumor agents: solution chemistry and cytotoxic properties of some selected gold(III) compounds.," *J. Med. Chem.*, vol. 43, pp. 3541–3548, 2000.

[40] P. Shi, Q. Jiang, J. Lin, Y. Zhao, L. Lin, and Z. Guo, "Gold(III) compounds of 1,4,7-triazacyclononane showing high cytotoxicity against A-549 and HCT-116 tumor cell lines.," *J. Inorg. Biochem.*, vol. 100, pp. 939–945, 2006.

[41] S. Zhu, W. Gorski, D. R. Powell, and J. A. Walmsley "Synthesis, Structures, and Electrochemistry of Au(III)- ethylenediamine Complexes and Interactions with Guanosine 5'- Monophosphate," *Inorg. Chem.*, vol. 45, pp. 2688–2694, 2008.

[42] M. Monim-ul-Mehboob, M. Altaf, M. Fettouhi, A. A. Isab, M. I. M. Wazeer, M. N. Shaikh, and S. Altuwaijri, "Synthesis, spectroscopic characterization and anti-cancer properties of new gold(III)–alkanediamine complexes against gastric, prostate and ovarian cancer cells; crystal structure of $[Au_2(pn)_2Cl_2]Cl_2{\cdot}H_2O$," *Polyhedron*, vol. 61, pp. 225–234, 2013.

[43] A. Ahmed, D. M. Al Tamimi, A. A. Isab, A. M. M. Alkhawajah, and M. A Shawarby, "Histological changes in kidney and liver of rats due to gold(III) compound [Au(en)Cl_2]Cl.," *PLoS One*, vol. 7, pp. e51889, 2012.

[44] C. D. Sanghvi, P. M. Olsen, C. Elix, S. B. Peng, D. Wang, Z. G. Chen, D. M. Shin, K. I. Hardcastle, C. E. MacBeth, and J. F. Eichler, "Antitumor properties of five-coordinate gold(III) complexes bearing substituted polypyridyl ligands.," *J. Inorg. Biochem.*, vol. 128, pp. 68–76, 2013.

[45] L. Giovagnini, L. Ronconi, D. Aldinucci, D. Lorenzon, S. Sitran, and D. Fregona, "Synthesis, characterization, and comparative in vitro cytotoxicity studies of platinum(II), palladium(II), and gold(III) methylsarcosinedithiocarbamate complexes.," *J. Med. Chem.*, vol. 48, pp. 1588–1595, 2005.

[46] L. Ronconi, L. Giovagnini, C. Marzano, F. Bett, R. Graziani, G. Pilloni, and D. Fregona, "Gold Dithiocarbamate Derivatives as Potential Antineoplastic Agents : Design , Spectroscopic Properties , and in Vitro Antitumor Activity," *Inorg.Chem.*, vol. 44, pp. 87–93, 2005.

[47] L. Ronconi, C. Marzano, P. Zanello, M. Corsini, G. Miolo, C. Maccà, A. Trevisan, and D. Fregona, "Gold(III) dithiocarbamate derivatives for the treatment of cancer: solution chemistry, DNA binding, and hemolytic properties.," *J. Med. Chem.*, vol. 49, pp. 1648–1657, 2006.

[48] D. Saggioro, M. P. Rigobello, L. Paloschi, A. Folda, S. A Moggach, S. Parsons, L. Ronconi, D. Fregona, and A. Bindoli, "Gold(III)-dithiocarbamato complexes induce cancer cell death triggered by thioredoxin redox system inhibition and activation of ERK pathway.," *Chem. Biol.*, vol. 14, pp. 1128–1139, 2007.

[49] B. A Al-Maythalony, M. Monim-ul-Mehboob, M. Altaf, M. I. M. Wazeer, A. A Isab, S. Altuwaijri, A. Ahmed, V. Dhuna, G. Bhatia, K. Dhuna, and S. S. Kamboj, "Some new [(thione)$_2$Au(diamine)]Cl_3 complexes: synthesis, spectroscopic characterization, computational and in vitro cytotoxic studies.," *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.*, vol. 115, pp. 641–647, 2013.

[50] P. G. Engman L, McNaughton M, Gajewska M, Kumar S, Birmingham A, "Thioredoxin reductase and cancer cell growth inhibition by organogold(III) compounds," *Anticancer Drugs*, vol. 17, pp. 539–544, 2006.

[51] D. Fan, C. Yang, J. D. Ranford, J. J. Vittal, and P. F. Lee, "Synthesis , characterization , and biological activities of 2-phenylpyridine gold (III) complexes with thiolate ligands ," *Dalt. trans.*, pp. 3376–3381, 2003.

[52] R. G. Buckley, A M. Elsome, S. P. Fricker, G. R. Henderson, B. R. Theobald, R. V Parish, B. P. Howe, and L. R. Kelland, "Antitumor properties of some 2-

[(dimethylamino)methyl]phenylgold(III) complexes.," *J. Med. Chem.*, vol. 39, pp. 5208–5214, 1996.

[53] L. Messori, G. Marcon, M. A. Cinellu, M. Coronnello, E. Mini, C. Gabbiani, and P. Orioli, "Solution chemistry and cytotoxic properties of novel organogold(III) compounds.," *Bioorg. Med. Chem.*, vol. 12, pp. 6039–6043, 2004.

[54] J. Zou, Z. Guo, J. A. Parkinson, Y. Chen, and P. J. Sadler, "Gold (III) -induced oxidation of glycine ", *Chem. Commun.*, vol. 8, pp. 1359–1360, 1999.

[55] J. Zou, J. A. Par, and P. J. Sadler, "Gold(III)-In duced Oxidation of Amino Acids and Malonic Acid: Re ac tion Path ways Studied by NMR Spec tros copy with Iso tope Labelling," *J. Chin. Chem. Soc*, vol. 49, pp. 499–504, 2002.

[56] S. Timári, C. Kállay, K. Osz, I. Sóvágó, and K. Várnagy, "Transition metal complexes of short multihistidine peptides.," *Dalton Trans.*, no. 11, pp. 1962–1971, 2009.

[57] J. A. Cuadrado, W. Zhang, W. Hang, and V. Majidi, "Speciation of gold(III)–L-histidine complex: a multi-instrumental approach," *J. Env. Monit*, vol. 2, pp. 355–359, 2000.

[58] V. Soni, R. S. Sindal, and R. N. Mehrotra, "Kinetics of oxidation of l-histidine by tetrachloroaurate(III) ion in perchloric acid solution," *Polyhedron*, vol. 24, pp. 1167–1174, 2005.

[59] B. A. Al-Maythalony, A. A. Isab, M. I. M. Wazeer, and Wazeer and Abdellatif Ibdah, "Investigation of the interaction of gold(III)–alkyldiamine complexes with l-histidine and imidazole ligands by ^{1}H and ^{13}C NMR, and UV spectrophotometry," *Inorga. Chim. Acta*, vol. 363, pp. 3200–3207, 2010.

[60] C. Frank Shaw III, M. P. Cancro, P. L. Witkiewicz, and J. E. Eldridge, "Gold(III) Oxidation of Disulfides in Aqueous Solution", *Inorg. Chem.*, vol. 19, pp. 3198–3201, 1980.

[61] B. Emillio, and C. Lucio, "Stereospecific Oxidation of Methionine to Methionine Sulphoxide," *J. Chem. Soc., Chem. Commun.*, pp. 878- 879, 1973.

[62] A. V. Vujačić, J. Z. Savić, S. P. Sovilj, K. M. Szécsényi, N. Todorović, M. Ž. Petković, and V. M. Vasić, "Mechanism of complex formation between $[AuCl_4]^-$ and L-methionine," *Polyhedron*, vol. 28, pp. 593–599, 2009.

[63] A J. Canumalla, N. Al-Zamil, M. Phillips, A.A. Isab, and C. F. Shaw, "Redox and ligand exchange reactions of potential gold(I) and gold(III)-cyanide metabolites under biomimetic conditions.," *J. Inorg. Biochem.*, vol. 85, pp. 67–76, 2001.

[64] P. M. Yangyuoru, J. W. Webb, and C. F. Shaw, "Glutathionato-S-Gold(III) complexes formed as intermediates in the reduction of auricyanide by glutathione.," *J. Inorg. Biochem.*, vol. 102, pp. 584–593, 2008.

[65] S. S. Al-Jaroudi, M. Monim-ul-Mehboob, M. Altaf, M. Fettouhi, M. I. M. Wazeer, S. Altuwaijri, and A. A. Isab, "Synthesis, spectroscopic characterization, X-ray structure and electrochemistry of new bis(1,2-diaminocyclohexane)gold(III) chloride compounds and their anticancer activities against PC3 and SGC7901 cancer cell lines," *New J. Chem.*, vol. 38, pp. 3199, 2014.

[66] A. Djeković, B. Petrović, Z. D. Bugarčić, R. Puchta, and R. van Eldik, "Kinetics and mechanism of the reactions of Au(III) complexes with some biologically relevant molecules.," *Dalton Trans.*, vol. 41, pp. 3633–3641, 2012.

[67] B. A. Al-maythalony, M. I. M. Wazeer, and A. A. Isab, "A study of [Au(ethylenediamine)Cl_2]Cl interaction with L-methionine and DL-seleno-methionine by ^{1}H and ^{13}C NMR spectroscopy," *J. Spectrosc.*, vol. 24, pp. 567–575, 2010.

[68] M. Salzmann, E. M. Stocking, L. A. Silks, and H. Senn, "NMR characterization of l , l -selenocystine with 92 % ^{77}Se enrichment," *Magn. Reson. Chem.*, vol. 37, pp. 672–675, 1999.

[69] L. R. Njaa, "Utilization of methionine sulphoxide and methionine sulphone by the young rat," *Br. J. Nutr.*, vol. 16, pp. 571–577, 1962.

[70] T. Wieland, C. Gotzendorfer, J. Dabrowski, W. N. Lipscomb, and G. Shoham "Unexpected Similarity of the Structures of the Weakly Toxic Amanitin (S)-Sulfoxide and the Highly Toxic (R)-Sulfoxide and Sulfone As Revealed by Proton Nuclear Magnetic Resonance and X-ray Analysis," *Biochemistry*, vol. 22, pp. 1264–1271, 1983.

[71] Y. H. Kim, A. H. Berry, D. S. Spencer, and W. E. Stites, "Comparing the effect on protein stability of methionine oxidation versus mutagenesis: steps toward engineering oxidative resistance in proteins," *Protein Eng. Des. Sel.*, vol. 14, pp. 343–347, 2001.

[72] and J.-L. R. Michel Seigneuret, Jean-Michel Neumann, Daniel Levy, "High-Resolution ^{13}C NMR Study of the Topography and Dynamics of Methionine Residues in Detergent-Solubilized Bacteriorhodopsin," *Biochemistry*, vol. 30, pp. 3885–3892, 1991.

[73] A. A. Isab, "^{13}C NMR studies of the interaction of Hg(II) with methionine and selenamethionine in the acidic aqueous solution," *Transit. Met. Chem*, vol. 14, pp. 235–236, 1989.

[74] J. D. Bell, R. E. Norman, and P. J. Sadler, "Coordination Chemistry in Biological Media : Reactions of Antitumor Pt (II) and Au (III) Complexes with Cell Culture Media," *J Inorg. Biochem*, vol. 31, pp. 241–246, 1987.

[75] T. Shoeib, D. W. Atkinson, and B. L. Sharp, "Structural analysis of the anti-arthritic drug Auranofin: Its complexes with cysteine, selenocysteine and their fragmentation products," *Inorg. Chim. Acta*, vol. 363, pp. 184–192, 2010.

[76] B. A. Al-Maythalony, M. I. M. Wazeer, and A. A. Isab, "^{1}H, ^{13}C NMR and UV spectroscopy studies of gold(III)-tetracyanide complex with L-cysteine, glutathione, captopril, L-methionine and DL-seleno-methionine in aqueous solution," *Inorganica Chim. Acta*, vol. 363, pp. 3244–3253, 2010.

[77] A. Bhatnagar, P. K. Sharma, and N. Kumar, "A Review on ' Imidazoles ': Their Chemistry and Pharmacological Potentials," *J. PharmTech Res.*, vol. 3, pp. 268–282, 2011.

[78] D. W. Robertson, E. E. Beedle, J. H. Krushinski, G.D. Pollock, H. Wilson, V. L. Wyss, and J.S.Hayes"Structure-Activity Relationships of Arylimidazopyridine Cardiotonics: Discovery and Inotropic Activity of 2-[2-Methoxy-4-(met hylsulfinyl)phenyll-1H-imidazo[4,5-c]pyridine," *J. Med. Chem.*, vol. 28, pp. 717–727, 1985.

[79] J. Mathiasen, F. Suzuki, T. Kuroda, T. Tamura, S. Sato, K. Ohmori, and S. Ichikawa, "New Antiinflammatory Agents.5-Phenyl-3H-imidazo[4,5-c][1,8]naphthyridin-4(5H)-ones: A New Class of Nonsteroidal Antiinflammatory Agents with Potent Activity Like Glucocorticoids," *J. Med. Chem.*, vol. 35, pp. 2863–2870, 1992.

[80] R. A. Johnson, S. Huong, and E. Huang, "Inhibitory effect of 4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)1H-imidazole on HCMV DNA replication and permissive infection," *Antivir. Res. 41*, pp. 101–111, 1999.

[81] M. D. Brewer, R. J. J. Dorgan, B. R. Manger, P. Mamalis, and R. A. B.Webster "Isothiourea Derivatives of 6-Phenyl-2,3,5,6-tetrahydroimidazo[2,l-b]thiazole with Broad-spectrum Anthelmintic Activity," *J. Med. Chem.*, vol. 30, pp. 1848–1853, 1987.

[82] I. A Jaffe, K. Altman, and P. Merryman, "The Antipyridoxine Effect of Penicillamine in Man," *J. Clin. Invest.*, vol. 43, pp. 1869–1873, 1964.

[83] J. Peisach and W.E.Blumberg, "A Mechanism for the Action of Penicillamine in the Treatment of Wilson's Disease," *Mol. Pharmacol.*, vol. 5, pp. 200–209, 1969.

[84] M. H. Pretchard and G. Niki, “Gold and penicillamine : A proposed mode of action in rheumatoid arthritis , based on synovial fluid analysis,” *Ann. Rheum. Dis.*, vol. 37, pp. 493–503, 1978.

[85] T. Murata, “Effects of bismuth contamination on the growth and activity of soil microorganisms using thiols as model compounds.,” *J. Environ. Sci. Heal.*, vol. 41, pp. 161–72, 2006.

[86] A. A.A. Seliman, M. Altaf, A.-N. Kawde, M. I. M. Wazeer, and A. A. Isab, “NMR and kinetic studies of the interactions of [Au(cis-DACH)Cl_2]Cl and [Au(cis-DACH)$_2$]Cl_3 with potassium cyanide in aqueous solution,” *J Coord Chem*, vol. 67, pp. 3431–3443, 2014.

Vitae

Name : Khalid Hamid Omer Mohamed

Nationality : Sudanese

Date of Birth : 1/21/1987

Email : Khalidhamid1440@yahoo.com

Address : King Fahd University of Petroleum & Minerals

Academic Background : BSc Honors degree in Chemistry (First Class) Sudan University of Science and Technology, Sudan

Conference Papers:

- **Khalid H. Omer**, Anvarhusein A. Isab, Abdel-Nasser Kawde, and Mohammed I. M. Wazeer "Study of glutathione interactions with anticancer Gold(III) diammines complexes using NMR, UV-VIS and electrochemistry" 249th ACS National Meeting, Colorado, US, March 2015.

Journal Papers:

1. **Khalid H. Omer**, Hassan A. Al-Mohsin, Abdel-Nasser Kawde, Mohamed Altaf, Mohammed I. M. Wazeer and Anvarhusein A. Isab "Study of the interaction of some potential anticancer gold(III) complexes with biologically important thiols using NMR, UV-Vis and Electrochemistry" (**Submitted**).
2. **Khalid H. Omer**, Mohamed Altaf, Mohammed I. M. Wazeer and Anvarhusein A. Isab. "Synthesis, Characterization and in vitro evolution of gold(III) complexes of (1)*R*,(2)*R* - diaminocyclohexane and their interactions with Glutathione"**(In Progress)**.