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Melody A.J. Rhine Date

Molecular Design of 2,9- disubstituted-1,10-phenanthroline Gold (III) complexes: Inhibiting Thioredoxin Reductase and Investigating Cytotoxicity

by

Melody A.J. Rhine

Adviser: Jack F. Eichler

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An abstract of
A thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Molecular Design of 2,9- disubstituted-1,10-phenanthroline Gold (III) complexes: Inhibiting Thioredoxin Reductase and Investigating Cytotoxicity

By Melody A.J. Rhine

Cancer is one of the leading medical concerns of modern times. Cisplatin has proven to be one of the most potent drugs available for anti-cancer chemotherapy. As a direct result of the success of the metallopharmaceutical cisplatin, this paper investigates the antitumor properties of various gold (III) complexes, which in recent years have been investigated as potential alternatives to platinum-based drugs. These compounds reported here, which are complex ions of protonated phenanthroline ligands and $AuCl_4^-$ anions [2,9-di-*n*-butyl-1,10-phenanthrolineH$^+$ $AuCl_4^-$ (**1**), 2,9-di-*sec*-butyl-1,10-phenanthrolineH$^+$ $AuCl_4^-$ (**2**), 2,9-dimethyl-1,10-phenanthrolineH$^+$ $AuCl_4^-$ (**3**)] were synthesized and then characterized with ^{1}H NMR, IR, and UV-vis spectroscopy. The biological activity of these compounds was examined by a variety of methods, including reduction by Glutathione (GSH), thioredoxin reductase (TrxR) enzyme inhibition, and cancer cell cytotoxity. Initial data indicates that complexes **1**, **2** and **3** are not easily reduced by Glutathione, a reductant that is naturally occurring in most biological systems. In addition, these compounds exhibit significant activity for TrxR inhibition and are highly cytotoxic to the studied cancer cell lines.

Molecular Design of 2,9- disubstituted-1,10-phenanthroline Gold (III) complexes: Inhibiting Thioredoxin Reductase and Investigating Cytotoxicity

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Figures and Tables

1. Introduction

According to the American Cancer Society, cancer was the second leading cause of death in 2006.[1] With cancer at the forefront of health concerns, it is vital that new and improved approaches to treatment are created to eradicate the cancerous cells; one of the most common approaches is chemotherapy, which oftentimes utilizes bio-inorganic molecules that enter cancer cells and damage the DNA. Currently, *cis*-Diamminedichloroplatinum, more commonly known as cisplatin, is a platinum-based form of chemotherapy and is one of the most widely prescribed drugs for the treatment of various lines of cancer (Figure 1.) Also, it is probably the most successful metallotherapeutic.

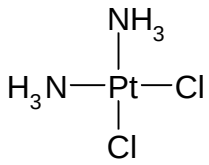

Figure 1. Structure of cisplatin.

It is widely used to treat testicular, ovarian, bladder, lung, and stomach cancers, among many others.[2] Despite its usefulness as an anticancer agent, there are two major limitations of cisplatin: its degree of toxicity to normal cells and tumors exhibiting resistance to the drug, thus eliminating its efficacy. There are many side effects that result from the use of this therapeutic agent, such as nausea and vomiting, nephrotoxicity, neurotoxicity, blood test abnormalities, and low red and white blood cell counts.[3-4] Some cancer cell lines are inherently resistant to the

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[1] *American Cancer Society.* www.cancer.org.
[2] Trzaska, S. "Cisplatin." *Chemical and Engineering News.* **2005**: *83*, 25.
[3] Wong, E. Giandomenico C.M. "Current status of platinum-based antitumor drugs." *Chem. Rev.* **1999**: 99, 2451-2466.

drug (e.g. colon cancer, non-small-cell lung cancer) whereas others develop resistance over time (e.g. ovarian cancer, small-cell lung cancer).[5] This resistance may be a result of increased glutathione levels and/or DNA repair mechanisms. Once cisplatin has entered the body, the cellular environment is rich in thiol-containing species, such as glutathione; these thiols can bind to cisplatin, thus deactivating the platinum complex.[6]

Given these limitations, the development of alternative therapeutics is warranted. Due to the success of cisplatin, much research has been conducted with metal complexes that are analogous to Platinum(II). There are similarities between the molecular structures of Pt(II) and Au(III) coordination complexes. Gold(III) was initially targeted because it is isoelectronic to Pt(II); both have a d^8 electronic configuration and typically form low-spin complexes. While some tetrahedrally- coordinated gold(I) complexes have displayed some potency against various tumors, the square-planar geometries of gold(III) complexes have recently been shown to target DNA, indicating potential for new anti-tumor agents.[7] In addition, it was found that patients treated with gold therapies for rheumatoid arthritis were found to be less likely to develop cancer.[8]

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[4] Kelland in *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug.* Lippert, B. (ed.), Helvetica Chimica Acta, **1999**: 498.
[5] *Ibid.*
[6] Kelland, L.R. "Preclinical Perspectives on Platinum Resistance." *Drugs*, **2000**: *59*, Suppl. 4, 1-8.
[7] Tiekink E.R. "Gold derivatives for the treatment of cancer." *Crit Rev Oncol Hematol*, **2002**: *42* (3), 225-48.
[8] Ho, Soo Yei and R.T. Tiekink. "Gold-Based Metallotherapeutics: Use and Potential." *Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine.* **2005**.

Hence, Au(III) therapeutics are a logical entry point into alternative anti-cancer metallopharmaceuticals.[9] Past research has shown that many metallopharmaceuticals consist of metal-ligand interactions that allow drugs to enter cancer cells and damage DNA without causing much harm to normal cells; this DNA deformation occurs through 3 modes: external binding, groove binding, and intercalation.[10] In fact, the intrinsic cytotoxicity of various cancer lines of the gold(III) compounds is comparable to that of cisplatin.[11] In addition, phenanthroline-based complexes with metals other than gold(III) exhibit cytotoxic properties,[12] and a range of phenanthroline-based complexes are significantly more active *in vitro* anti-cancer agents than cisplatin against selected cancer lines through inhibition of DNA synthesis, both intercalatively and non-intercalatively.[13-14]

It has recently been found that gold drugs can have a different mechanism leading to cytotoxity in cancer cells with DNA binding. Cisplatin typically binds to the DNA, which inhibits DNA repair, whereas DNA binding of gold(III) complexes have been found to be variable and less frequent. DNA does not represent the primary target for many gold(III) compounds; a poor affinity toward

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[9] Palanichamy, K., Ontko, A. "Synthesis, characterization, and aqueous chemistry of cytotoxic Au (III) polypyridyl complexes." *Inorganica Chemica Acta*. **2006**: *359,* 44-52.
[10] Kabanov, A. and Okano, T. Kuwler. *Polymer Drugs in the Clinical Stage: Advantages and Prospects.* Plenum Publishing: New York, **2003**.
[11] Bruni, B. *et al.* "Structure and Cytotoxic Properties of Some Selected Gold (III) Complexes." *Croatica Chemica Acta*, **1999**: *72*, 221-229.
[12] Heffeter, P. *et al.* "Anticancer activity of the lanthanum compound [tris(1,10-phenanthroline)lanthanum(III)]trithiocyanate (KP772; FFC24)." *Biochemical Pharmacology.* **2006**: *71* (4), 426-440.
[13] Devereux, M. *et al.* "Synthesis, X-Ray crystal structures and biomimetic and anticancer activities of novel copper (II) benzoate complexes incorporating 2-(4'-thiazolyl) benzimidazole (thiabendazole), 2-(2-pyridyl) benzimidazole and 1,10-phenanthroline as chelating nitrogen donor ligands." *J. Inorg. Biochem.* **2007**: *101*, 881-892.
[14] Hirohama, T., Y.Kuranuki, E. Ebina, T. Sugizaki, H. Arii, M. Chikira, P.T.Selvi, M. Palaniandavar. *J Inorg Biochem*. **2005**: *99*, 1205-1219.

calf thymus DNA was measured for a number of gold(III) compounds.[15] One of the many limitations of cisplatin is that it targets quickly dividing normal cells (hair follicles, etc.). In an effort to reduce some of these side effects, the gold(III) compounds were designed not to bind to DNA. In addition, gold (III) compounds have been shown to be extremely efficient inhibitors of mitochondrial thioredoxin reductase, an enzyme whose activity is essential to a cell's growth and survival.[16]

Given that previous gold(III) phenanthroline complexes have shown cytotoxicity, we pursued the synthesis of 2,9-disubstituted phenanthroline ligands in the hopes that it would provide an opportunity to protect the gold(III) center from glutathione reduction. An increase in alkyl chain size will protect the metal from interaction with the reductants, and therefore, it was thought that the reducing agents will cause less deactivation of the gold complexes with the increased bulkiness of alkyl substituents. In addition, the use of 2,9-disubstituted phenanthroline provided an opportunity to probe the nature of DNA binding: if the binding occurred through the gold(III) center, the 2,9-phenanthroline would inhibit the binding whereas if the binding occurred via the phenanthroline backbone (through intercalation), the 2,9-disubstituted phenanthroline should have no effect on DNA binding. (Binding via intercalation could potentially be inhibited by 5,6-disubstituted phenanthroline ligands. See **Figure 2**.) Hence, the overall goal of this research was to develop gold(III) compounds that exhibit activity against the TrxR enzyme and cytotoxicity sans DNA binding.

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[15] Casini, A., *et al.* "Structural and Solution Chemistry, Antiproliferative Effects, and DNA and Protein Binding Properties of a Series of Dinuclear Gold(III) Compounds with Bipyridyl Lignads." *J. Med. Chem.*, **2006**: 49, 5524-5531.

[16] Rigobello, M., Messori, L, Marcon, G., *et al.* "Gold complexes inhibit mitochondrial thioredoxin reductase: consequences on mitochondrial." *J Inorg Biochem*, **2004**: *98*, 1634-1641.

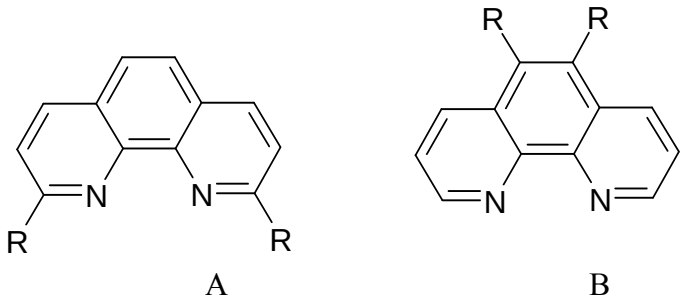


Figure 2. A) Structure of 2,9-disubstituted phenanthroline-based ligand and B) 5,6-disubstituted phenanthroline-based ligand where R = methyl, *n*-butyl, *sec*-butyl alkyl chains

The gold(III) complexes were synthesized via a two-step process: 1) Alkylation of 1,10-phenanthroline via nucleophilic aromatic substitution,[17] and 2) Formation of the gold(III) complex.[18] This procedure was conducted for all gold(III) complexes. (See **Figure 3**.) The synthesized complexes include a 1,10-phenanthroline gold (III) complex and 2,9-disubstituted alkyl-1,10-phenanthroline gold(III) complexes where alkyl = methyl, *n*-butyl, and *sec*-butyl. After synthesis and full characterization, the target compounds were then tested for biological activity, including a TrxR inhibition assay, glutathione reduction, and cytotoxicity testing.

[17] Pallenberg, A.J., Koenig, K.S., and D.M. Barnhart. "Synthesis and Characterization of Some Copper(I) Phenanthroline Complexes." *Inorg. Chem*., **1995**: *34*, 2833.
[18] Palanichamy, Ontko, *et al.* "Synthesis, characterization, and aqueous chemistry of cytotoxic Au (III) polypyridyl complexes." *Inorganica Chemica Acta,* **2006**: *359*, 44-52.

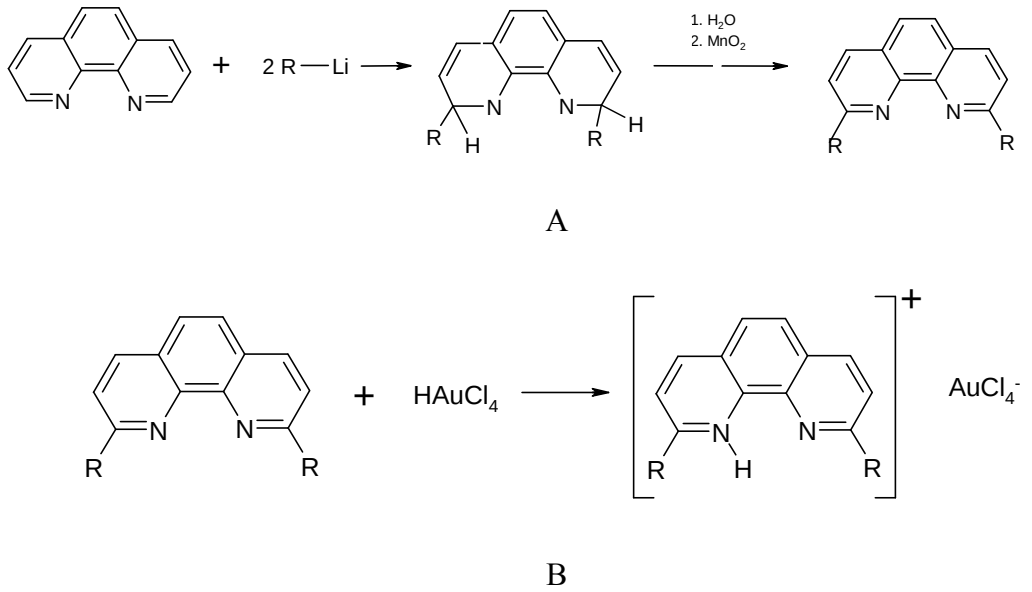


Figure 3. A) Synthesis of ligand R = $-CH_2(CH_3)CH_2CH_3$, $-(CH_2)_3CH_3$ B) Synthesis of Au(III) complex R = $-CH_3$, $-CH_2(CH_3)CH_2CH_3$, $-(CH_2)_3CH_3$

2. Experimental

2.1 General Procedures

The 2,9-dialkylphenanthroline ligands (alkyl = *sec*-butyl, *n*-butyl) were synthesized as described in the literature.[19] $HAuCl_4 \cdot 3H_20$, silver tetrafluoroborate, silver trifluoroacetate (Alfa Aesar), and all solvents were used without further purification. Neocuproine (2,9-dimethyl-1,10-phenanthroline) was purchased from Alfa Aesar and used without further purification. The gold starting materials were weighed and solvated under a nitrogen atmosphere (either in an inert atmosphere glovebox or in a nitrogen-purged glovebag) and subsequently refluxed in normal atmospheric conditions with the respective phenanthroline ligand. Aside from eliminating exposure to direct sunlight, no

[19] Pallenberg, A.J., Koenig, K.S., and D.M. Barnhart. "Synthesis and Characterization of Some Copper(I) Phenanthroline Complexes." *Inorg. Chem*., **1995**: *34*, 2833.

special handling measures were taken with the final gold(III) complexes. ^{1}H-NMR spectra were recorded on a Varian Mercury 300 MHz spectrophotometer at ambient temperature; chemical shifts were referenced to dimethyl sulfoxide. Infrared spectra were recorded as KBr pellets on a Varian Scimitar 800 Series FT-IR Spectrophotometer, UV/VIS spectra were recorded on a Cary 50 UV/VIS spectrophotometer using 1.0 cm quartz cuvettes, and elemental analyses were completed by Atlantic Microlab Inc., Norcross, GA.

2.2 Ligand Synthesis

Figure 3 shows the synthesis of the *sec*-butyl ligand. Under a nitrogenous atmosphere, *sec*-butyllithium (0.0111 mol) reacted with 1,10-phenantroline (0.00557 mol) in anhydrous toluene via lithium dispersion. The stirred 1,10-phenanthroline suspension was cooled to 0ºC; afterward, the *sec*-butyllithium was added dropwise over 0.5 hours. The resulting deep-red solution stirred overnight at room temperature. After a night of stirring, 40 mL H_2O was added, which caused the solution to turn bright yellow. The organic phase was extracted and stirred in excess MnO_2 for 5 hours at room temperature. After drying over $MgSO_4$, the mixture was gravity filtered and evaporated, thus leaving 1.07 g of the 2,9-disubstituted *sec*-butyl-1,10-phenanthroline ligand. (See **Figure 3**.) The ligand was characterized and then used for the synthesis of the respective gold complex. 2,9-di-*n*-butyl-1,10-phenanthroline was synthesized in a similar fashion.

2.3 Synthesis of RphenH$^+$ [AuCl$_4$]$^-$

Each gold (III) complex was synthesized through two overall steps: 1. Alkylation, which yielded a disubstituted phenanthroline-based ligand,[20] and 2. Formation of the gold (III) complex. The gold (III) complex was synthesized using a hydrogen salt ($HAuCl_4 \cdot 3H_20$), thus yielding a protonated gold(III) complex with a $AuCl_4^-$ counter ion.[21] (See **Figure 3B.)**

2.3.1 Synthesis of Compound **1 (*n*-butyl)**

2,9-di-*n*-butyl-1,10-phenanthroline (0.340 g, 1.2 mmol) was dissolved in 15 mL of MeOH and added dropwise to $HAuCl_4 \cdot 3H_2O$ (0.447 g, 1.2 mmol) in 20 mL of MeOH, upon which a deep purple solution was produced. After refluxing for 1 hour at 80 °C, the solution turned dark yellow. Evaporation *in vacuo* produced a red/orange solid, which was washed with 20 mL of ether (the ether wash was discarded). A yellow solid was isolated and then dried under vacuum at 35 °C (0.361g, 49%). Yellow needles suitable for X-ray diffraction studies were obtained by slow recrystallization from dichloromethane. Elemental analysis found: C, 38.27%; H, 3.98%; calculated: C, 37.98%; H 3.99%. $\lambda_{max}(MeCl_2)$/nm 232.0 (26 070), 289.0 (20 150) and 318.0sh (6 800). IR: ν_{max}/cm^{-1} 3178 (NH), 3073 (CH), 2958, 2930, 2872 (CH), 1624, 1605 (conj. CC). ^{1}H NMR (300 MHz, dmso) δ 8.85 – 8.79 (m, 2H, H-7,12), 8.17 (s, 2H, H-9,10), 8.06 – 7.99 (m, 2H,

[20] Palanichamy, Ontko "Synthesis, characterization, and aqueous chemistry of cytotoxic Au (III) polypyridyl complexes." *Inorganica Chemica Acta,* **2006**: *359*, 44-52.
[21] Abbate, F., P. Orioli, B. Bruni, G, Marcon, L. Messori. "Crystal structure and solution chemistry of the cytotoxic complex 1,2-dichloro(o-phenanthroline) gold (III) complex." *Inorganica Chemica Acta*. **2000**.

H-6,13), 3.26 (s, 4H, H-4,15), 1.81 (s, 4H, H-3,16), 1.42 (d, 4H, H-2,17), 0.94 (t, 6H, H-1,18) (See **Figure 4** for NMR numbering scheme.)

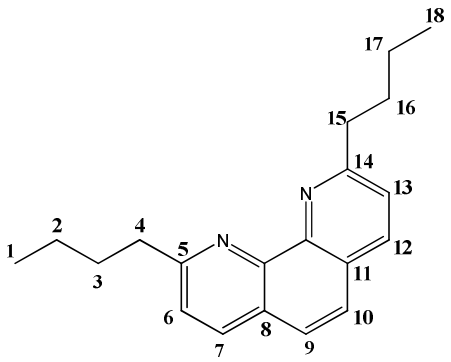


Figure 4. NMR numbering scheme for **1**. Hydrogens are labeled according to the carbon to which they are directly bonded.

2.3.2 Synthesis of Compound **2 (*sec*-butyl)**

2,9-di-*sec*-butyl-1,10-phenanthroline (0.523 g, 1.8 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.705 g, 1.8 mmol) were combined in a procedure analogous to the synthesis of **1**, yielding 0.900 g of a reddish/orange solid, **2** (79.6%). Yellow needles suitable for X-ray diffraction studies were obtained by slow-evaporation from dichloromethane. Elemental analysis found: C, 37.99%; H, 3.99%; calculated: C, 37.98%; H, 3.99%. λmax($MeCl_2$)/nm 231.0 (33 670), 287.9 (31 440) and 319.1 (11 770). IR: ν_{max}/cm^{-1} 3183 (NH), 3079 (CH), 2963, 2928, 2871 (CH), 1619, 1606 (conj. CC). ^{1}H NMR (300 MHz, cdcl3) δ 8.88 (d, 2H, H-7,12), 8.24 (s, 2H, H-9,10), 8.06 (d, 2H, H-6,13), 3.75 (m, 14.2, 2H, H-3,15), 2.09 (m, 13.7, 2H, H-2), 1.86 – 1.68 (m, 2H, H-17), 1.48 (d, 6H, H-4,16), 0.93 (t, J = 7.4, 6H, H-1,18). (See **Figure 5** for NMR numbering scheme.)

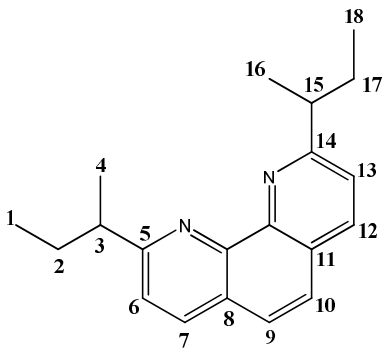


Figure 5. NMR numbering scheme for **2**. Hydrogens are labeled according to the carbon to which they are directly bonded.

2.3.3 Synthesis of Compound **3 (methyl)**

2,9-di-methyl-1,10-phenanthroline (0.375 g, 1.8 mmol) and $HAuCl_4 \cdot 3H_2O$ (0.705 g, 1.8 mmol) were combined in a procedure analogous to the synthesis of **1**, yielding 0.249 g of a yellow/orange solid (41.1%). After a slow recrystallization, a yellow solid resulted. λmax($MeCl_2$)/nm 237 (33 670), 282 (31 440) and 317.0 (11 770). IR: ν_{max}/cm^{-1} 3205 (NH), 3076 (CH), 2966, 2928, 2893 (CH), 1625, 1604 (conj. CC). ^{1}H NMR (300 MHz, dmso) δ 8.377-8.349 (d, 2H, H-4,9), 7.706 (s, 2H, H-6,7), 7.552-7.525 (d, 2H, H-3,10), 1.986-1.963 (m, 6H, H-1,12). (See **Figure 6** for NMR numbering scheme.)

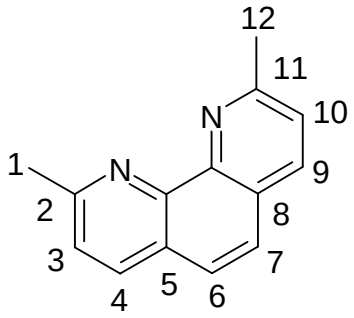


Figure 6. NMR numbering scheme for **3**. Hydrogens are labeled according to the carbon to which they are directly bonded.

*2.4 X-Ray Crystallography for Compounds **1** and **2***

X-ray crystallography was performed by mounting each crystal onto a thin glass fiber from a pool of Fluorolube™ and immediately placing it under a liquid nitrogen cooled N_2 stream, on a Bruker AXS diffractometer. The radiation used was graphite monochromatized Mo Kα radiation (λ = 0.7107 Å). The lattice parameters were optimized from a least-squares calculation on carefully centered reflections. Lattice determination, data collection, structure refinement, scaling, and data reduction were carried out using APEX2 version 1.0-27 software package.

Each structure was solved using direct methods. This procedure yielded the Au atoms, along with a number of the Cl, N, and C atoms. Subsequent Fourier synthesis yielded the remaining atom positions. The hydrogen atoms were fixed in positions of ideal geometry and refined within the XSHELL software. These idealized hydrogen atoms had their isotropic temperature factors fixed at 1.2 or 1.5 times the equivalent isotropic U of the C atoms to which they were bonded. The final refinement of each compound included anisotropic thermal parameters on all non-hydrogen atoms.

2.5 Cytotoxicity Testing: Cell lines and Culture

Dimethyl sulphoxide (DMSO) and all cell culture reagents and media were purchased from Sigma-Aldrich. To test the effects of compounds **1, 2**, and **3** on cell growth of head, neck, and lung cancer cell lines, sulforhodamine B (SRB) cytotoxicity assays were adapted from Skehan *et al*. 25 Cells maintained in medium with 5% FBS were seeded in 96-well plates at a density of 4,000

cells/well overnight prior to drug treatment. Afterwards, drugs were added in a range of concentrations as single agents in various concentrations (0–30 μM), followed by incubation at 37˚C and 5% CO_2 for 72 hours. Cells were fixed for 1 hr with 10% cold trichloroacetic acid. Plates were washed 5 times in water, airdried and then stained with 0.4% SRB for 10 min. After washing 4 times in 1% acetic acid and air-drying, bound SRB was dissolved in 10 mM unbuffered Tris base (pH 10.5). Plates were read in a microplate reader by measuring absorbance at 492 nm. Cell growth inhibition was measured by determining cell density with sulforhodamine B assay (24) at 72 hours after addition of the drugs. Percentage of inhibition was determined by comparison of cell density in the drug-treated cells with that in the untreated cell controls in the same incubation period. The percent survival was then calculated based upon the absorbance values relative to untreated samples. The experiment was repeated 3 times.[22]

2.6 Thioredoxin Reductase Assay

Compounds **1, 2**, **3** were prepared in 4 mL (10 mg/mL DMSO). $NaAuCl_4$, and $KAuCl_4$ were used as controls. For inhibition of TrxR, 50 nM recombinant rat TrxR1 (8.3 U/mg) was reduced by the addition of 250 μM NADPH to the assay buffer (50 mM Tris pH 7.5, 2 mM EDTA) and subsequently incubated 15 min at room temperature with increasing concentrations of the compounds. Two aliquotes of 195 μl were subjected to a microtiter plate and 250 μM NADPH and 2.5 mM DTNB was added. Immediately, the DTNB reduction to TNB^- was followed at 30°C for 5 min at 412 nm using the VersaMax (Molecular devices). A

[22] Zhang, X., Zhang, H., Tighiouart, M., *et al*. "Synergistic inhibition of head and neck tumor growth by green tea (—)-epigallocatechin-3-gallate and EGFR tyrosine kinase inhibitor." *Int. J. Cancer.* **2008**: *123*, 1005.

linear slope was calculated for the same 30 s interval of all samples within one run and the percentage of activity compared to enzyme incubated with assay buffer only was calculated for all treated samples.

3. Results and Discussion

3.1 Synthesis of Compounds and Spectroscopic Characterization

In comparison to the free disubstituted phenanthroline ligands, each gold(III) complex had a downfield chemical shift of over 1.0 ppm for the aromatic and alkyl hydrogens in the ^{1}H NMR spectra. There was a methanol peak in the spectra of the gold (III) complexes at 3.0 ppm; this was residual methanol from the recrystallization. Initial interpretation of this data was that the synthesized compounds had direct metal-ligand coordination, which was thought to be responsible for the downfield shift of the ligand protons.

The characterization of compounds **1**-**3** by UV/VIS still did not clearly indicate the presence of direct coordination (or lack thereof.) The synthesized salts possess absorption maxima at 289 nm (**1**), 288 nm (**2**), and 287 nm (**3**) assigned as intraligand phen $\pi \rightarrow \pi^*$ transitions; 318 nm (**1**), 319 nm (**2**), and 317 **(3**) assigned as a Ligand-to-Metal Charge Transfer (LMCT) band; and broad weak absorptions between 350 and 450 nm (**1, 2**, **3**), assigned as d-d transitions.

However, x-ray crystal structures and elemental analyses clearly show the protonated nitrogen and $[AuCl_4]^-$ counterion using the $HAuCl_4$ synthesis. This could be a direct result of the acidity of $HAuCl_4$ and the increase in nitrogen basicity by the addition of alkyl groups to the aromatic ligand backbone. The

protonation led to additional steric hindrance, which affected the reactivity of said reactions. The synthesis of gold(III) salts possessing protonated nitrogen donor ligands, as found here, is uncommon but not unprecedented. Cao, *et al.* recently reported similar protonation to the BBPMA (bis(2-pyridylmethyl)-N-benzylamine) ligand; however, using a method involved $NaAuCl_4$ instead of the hydrogen salt, their synthesis led to direct coordination between the ligand and gold(III) metal center.[23] Other results in our laboratory indicate direct coordination between the Au(III) metal and phenanthroline ligand with a slightly modified synthesis using $NaAuCl_4 \cdot 2H_2O$ and $AgBF_4$.

3.2 X-ray Crystallography

In previously reported studies, salts with a protonated nitrogen donor ligand (including protonated bipyridine and protonated bis(2-pyridylmethyl)amine)and the $AuCl_4^-$ counterion have been structurally characterized.[24,25] However, it appears that compounds **1** and **2** are the first examples of a protonated phenanthroline ligand with the $AuCl_4^-$ anion. Within these compounds, $AuCl_4^-$ has a square planar geometric structure (Figures 7 and 8), and for the first time, compounds **1** and **2** are examples of $AuCl_4^-$ salts that have a hydrogen bond between one of the chloride ligands and the nitrogen donor ligand. Complexes **1** and **2** have interatomic distances (Au-Cl$^{\cdots}$H-N) of 2.906 Å and 3.000Å

[23] L. Cao, M.C. Jennings and R.J. Puddephatt. "Amine-Amide Equilibrium in Gold(III) Complexes and a Gold(III)-Gold(I) Aurophilic Bond." *Inorg Chem.* **2007**: *46*, 1361.
[24] X.P Zhang, G. Yang and S.W. Ng. "Diaquabis(2,4-dichlorophenoxyacetato-*O*)bis(1*H*-imidazole-N^3)cobalt(II)." *Acta Cryst.*, **2006**: *E62*, m2618.
[25] M. A. Ivanov, M. V. Puzyk, and K. P. Balashev. "Spectroscopic and electrochemical properties of dichlorodiimine complexes of Au(III) and Pt(III) with 1,4-diazine derivatives of *o*-phenanthroline." *Russian Journal of General Chemistry*, **2003**: *73*, 1821.

respectively. According to a review of M-Cl···H-N interactions for the crystallographic database, the aforementioned hydrogen bonds are characterized as intermediate, a category including M-Cl···H-N bond distances from 2.52-2.95 Å, to long, a category including M-Cl···H-N bond distances from 2.95-3.15 Å.[26]

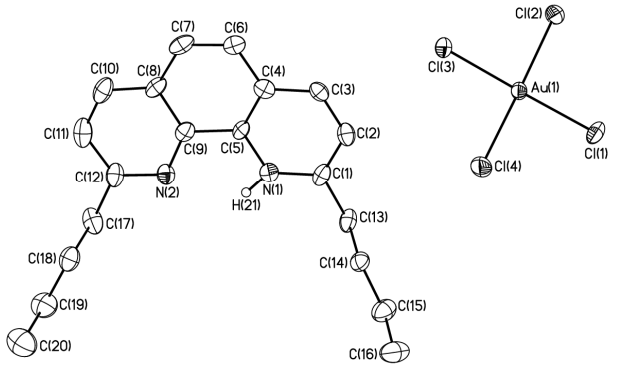


Figure 7. Molecular structure and numbering scheme of **1**. (35% probability) Hydrogen atoms have been omitted for clarity.

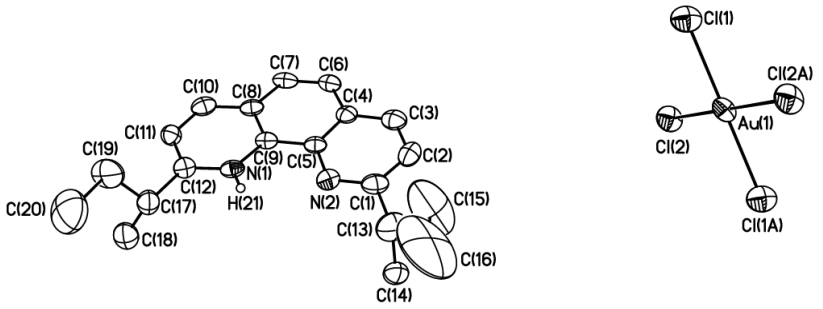


Figure 8. Molecular structure and numbering scheme of **2**. (35% probability) Hydrogen atoms have been omitted for clarity.

[26] G. Aullón, D. Bellamy, L. Brammer, E.A. Bruton, and A.G. Orpen, *Chem. Comm*., **1998:** 653.

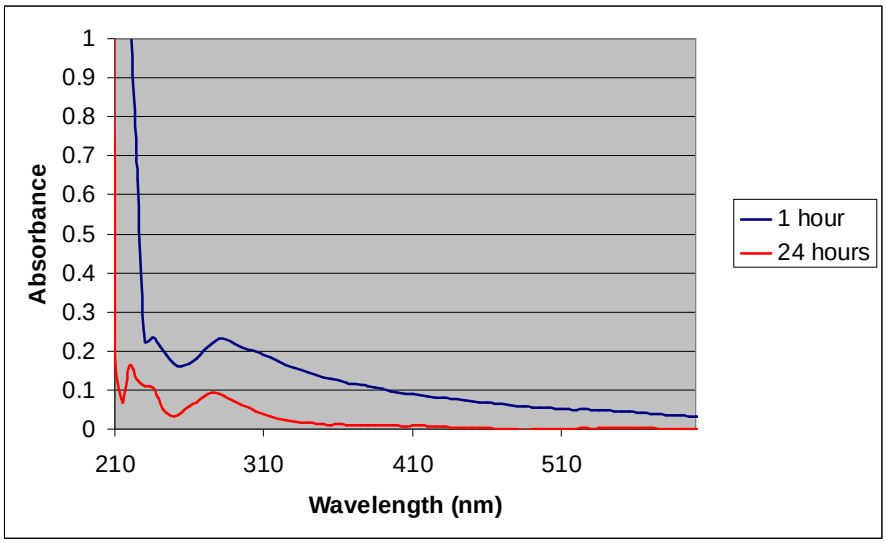
Table 1: Selected interatomic distances (Å) and angles (º) for compounds **1** and **2**.

1		**2**	
Au-Cl···H-N	2.906	Au-Cl···H-N	3.000
Au(1)-Cl(2)	2.271(3)	Au(1)-Cl(1)	2.278(2)
Au(1)-Cl(3)	2.273(3)	Au(1)-Cl(1)#1	2.278(2)
Au(1)-Cl(1)	2.275(3)	Au(1)-Cl(2)	2.2807(18)
Au(1)-Cl(4)	2.278(3)	Au(1)-Cl(2)#1	2.2807(18)
Cl(2)-Au(1)-Cl(3)	88.85(10)	Au(2)-Cl(3)#2	2.273(2)
Cl(2)-Au(1)-Cl(1)	90.30(11)	Au(2)-Cl(3)	2.273(2)
Cl(3)-Au(1)-Cl(1)	178.79(13)	Au(2)-Cl(4)	2.277(2)
Cl(2)-Au(1)-Cl(4)	178.43(12)	Au(2)-Cl(4)#2	2.277(2)
Cl(3)-Au(1)-Cl(4)	90.04(11)	Cl(1)-Au(1)-Cl(1)#1	180.00(9)
Cl(1)-Au(1)-Cl(4)	90.83(11)	Cl(1)-Au(1)-Cl(2)	90.34(7)
Cl(1)#1-Au(1)-Cl(2)	89.66(7)	Cl(3)#2-Au(2)-Cl(4)	90.06(8)
Cl(1)-Au(1)-Cl(2)#1	89.66(7)	Cl(3)-Au(2)-Cl(4)	89.94(8)
Cl(1)#1-Au(1)-Cl(2)#1	90.34(7)	Cl(3)#2-Au(2)-Cl(4)#2	89.94(8)
Cl(2)-Au(1)-Cl(2)#1	180.00(14)	Cl(3)-Au(2)-Cl(4)#2	90.06(8)
Cl(3)#2-Au(2)-Cl(3)	180.0	Cl(4)-Au(2)-Cl(4)#2	180.00(1

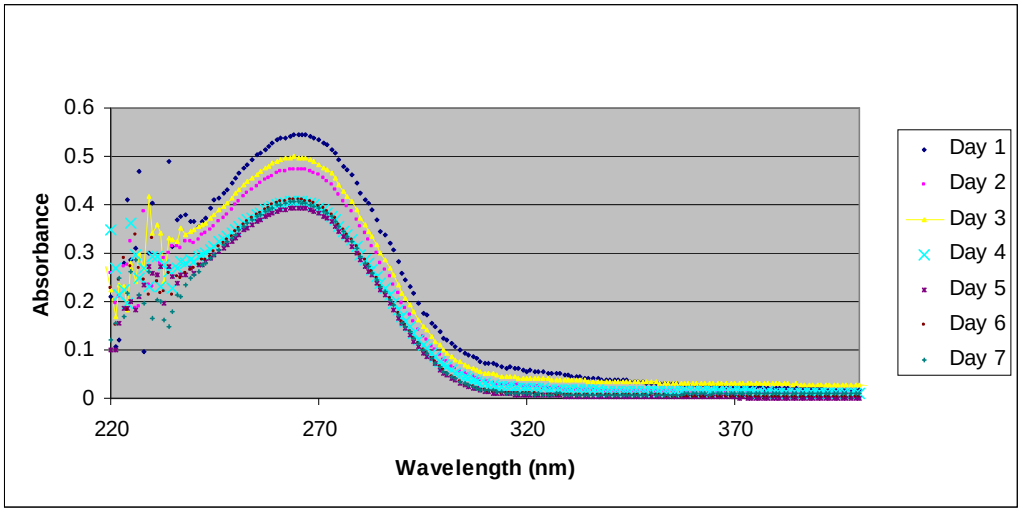
3.3 Biological Activity

3.3.1 Stability in Buffer

Stability tests were run on compound **1**, which was used as a representative compound. A minimum amount of dimethyl sulfoxide (DMSO) was used to dissolve the complex, which was then diluted in a phosphate buffer (pH 7.4), a solution of concentration $5.0*10^{-5}$ M was made and observed daily for a period of 7 days. The sample was stored in a dark environment throughout the 7-day period. Since there was little change in the absorbance, it was determined that the compounds indeed are stable in this buffer. This provides some evidence that the use of these compounds in biological conditions can be pursued despite the slight decrease in absorption intensity. There appears to be no significant shift in the absorption maxima at 320 nm, which is the absorption that arises due to the gold(III) metal ion. Therefore, a reasonable conclusion to draw is that the complex is indeed stable in the buffer.



A



B

Figure 9. A) UV-VIS Absorption spectrum of stability of Compound **1** (*n*-butyl phenH^+ gold(III) complex; $5.0*10^{-5}$ M) in phosphate buffer (pH 7.4) at room temperature over a 24-hour period B) UV-VIS Absorption spectrum of stability of Compound **1** (*n*-butyl phenH^+ gold(III) complex; $5.0*10^{-5}$ M) in phosphate buffer (pH 7.4) at room temperature over a 7-day period.

3.3.2 Glutathione reduction

Using a concentration of $5.0*10^{-5}$ M for each ligand and complex in the aforementioned phosphate buffer, glutathione, the reference reducing agent, was presented at a 2:1 molar ratio. Through observing the changes in absorbance via UV-VIS spectroscopy, any reduction of the gold(III) metal center could be detected. Since the absorbance of each complex changed a negligible amount, it can be deduced that very little reduction occurred for compounds **1**, **2**, and **3**. **Figure 10** shows the results of compound **1** over an 8-hour period, with the assumption that this *n*-butyl gold(III) complex can be used as a representative example of the data from complexes **2** and **3**.

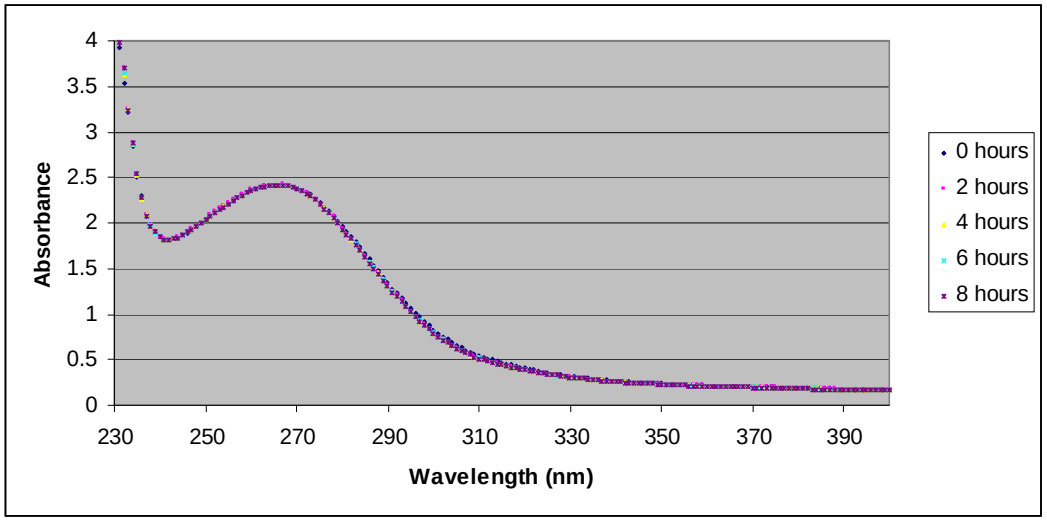


Figure 10. UV-VIS Absorption spectrum of Compound **1** (*n*-butyl phenH^+ gold(III) complex; $5.0*10^{-5}$ M) in presence of reduced glutathione ($1.0*10^{-5}$ M) in phosphate buffer (pH 7.4) at room temperature.

When these experiments were conducted with an unsubstituted protonated phenanthroline gold(III) complex, more reduction was apparent in the UV-VIS spectrum. However, since this test with the unsubstituted protonated

phenanthroline gold(III) complex was conducted under slightly different conditions, it will need to be repeated under the same conditions (phosphate buffer of pH 7.4, same concentrations, and so on) in order to confirm these results.

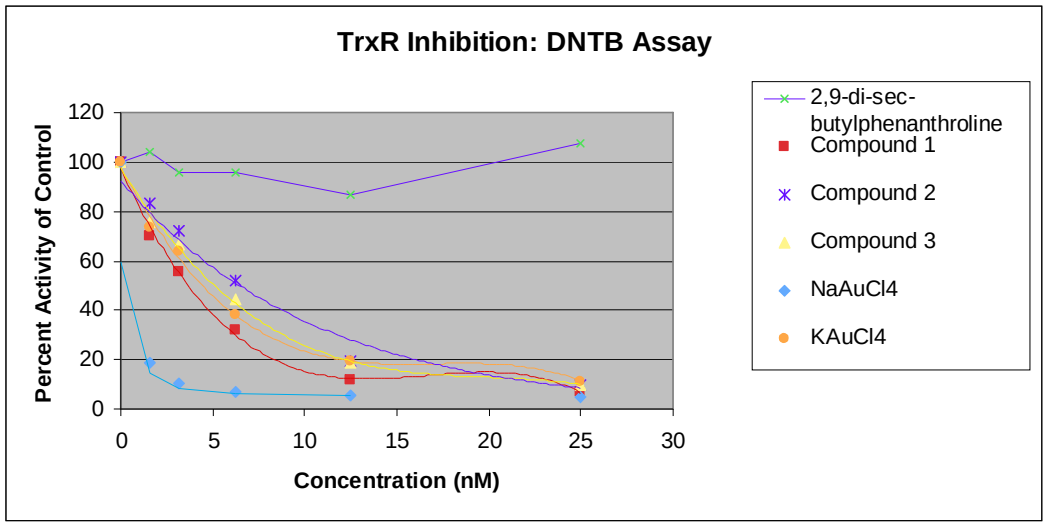
3.3.3 Thioredoxin Reductase Inhibition and Cytotoxicity

Compounds **1**, **2**, and **3** show significant activity of TrxR inhibition compared to the free ligand; their activity is comparable to that of $KAuCl_4$, which is typically quite potent against thioredoxin reductase. These results were expected since both $KAuCl_4$ and our synthesized Au(III) compounds both have $AuCl_4^-$ counter anions.

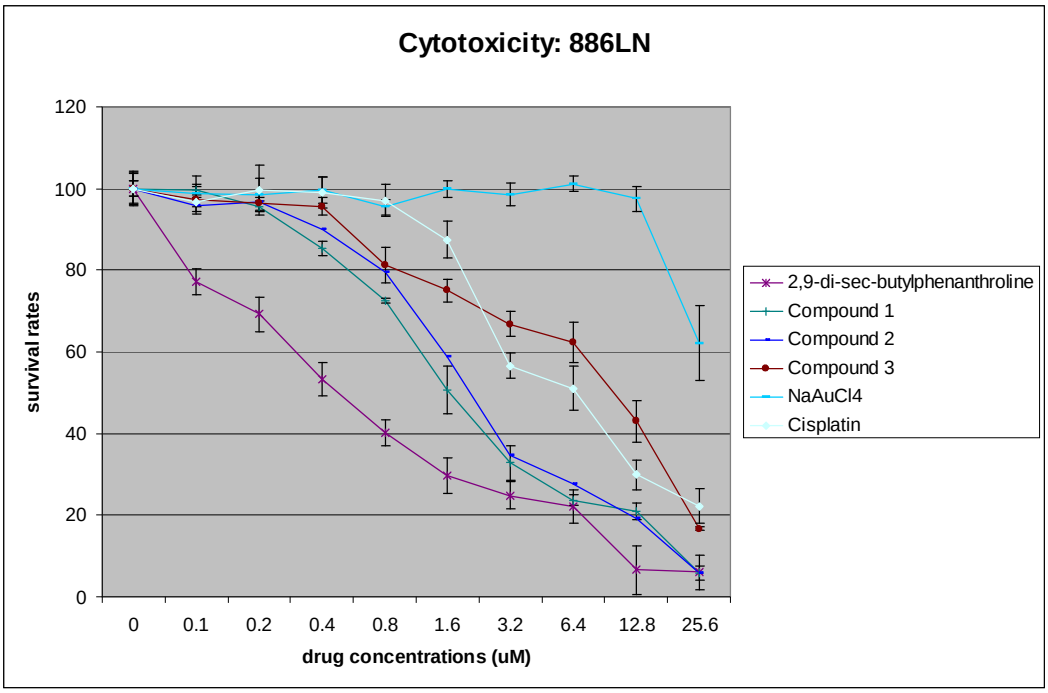
Cytotoxicity results indicate that compound **3** has a potency that is comparable to that of cisplatin against cancer cell line 886LN (a head/neck cancer cell line.) Compounds **1** and **2** appear to have much higher potencies than cisplatin, and all 3 compounds (as well as the free sec-butyl ligand) have much higher potencies against line 886LN than the $NaAuCl_4$ starting material, which exhibits no activity until extremely high concentrations are reached. The cancer cell survival rates are plotted as a percentage relative to a non-treated cell culture versus the concentration of the drug. As is shown by our compounds, the efficacy of the cytotoxicity of a compound increases as the concentration necessary to reduce the survival rate decreases. Similarly, when tested with cancer cell line Tu212 (also a head/neck cancer cell line), all four of the compounds again

exhibited higher efficacy in being toxic to the cells than the commonly utilized cisplatin.

The inactivity of $NaAuCl_4$ indicates that the $AuCl_4^-$ counterion by itself has little anticancer activity; however, the free ligand also exhibits extremely potent activity against the cell lines. This shows that the $AuCl_4^-$ anion may not contribute to the cytotoxicity of the gold(III) compounds. In addition, the free ligand has no activity against TrxR whereas the protonated phenanthroline gold(III) complexes show significant inhibition against the enzyme. Further studies are needed to determine if TrxR inhibition is indeed the mechanism of cytotoxicity.



A



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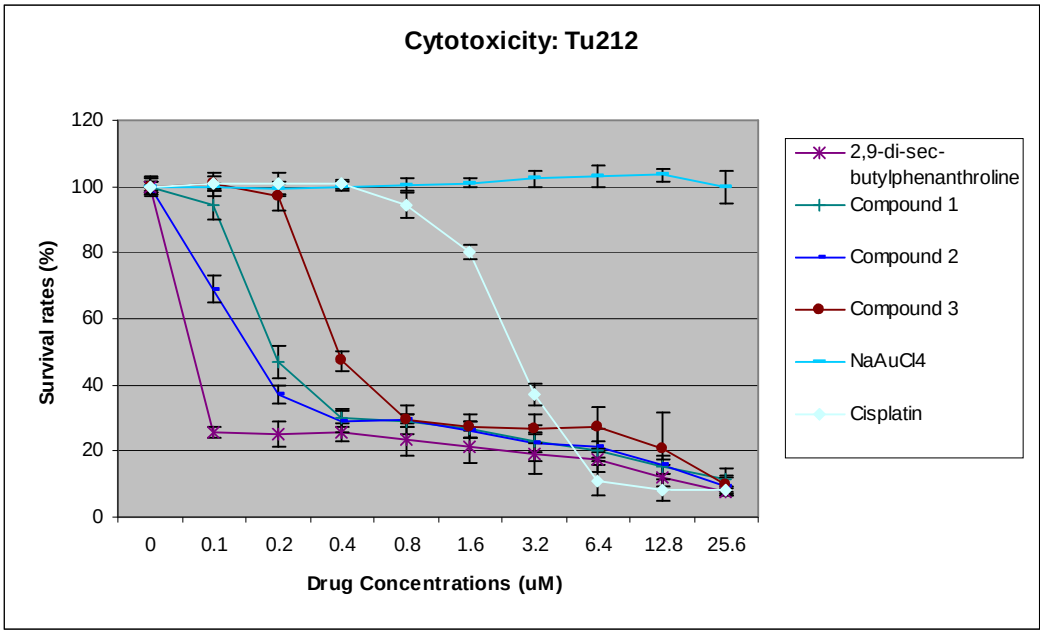

C

Figure 11. A) Thioredoxin Reductase Inhibition Activity, B) Cytotoxicity data against cancer cell line 886LN, and C) Cytotoxicity data against cancer cell line Tu212; % survival as compared to control cell culture containing no drug

4. Conclusions and Future Research

2,9-di-*n*-butyl-1,10-phenanthrolineH$^+$ $AuCl_4^-$ (**1**), 2,9-di-*sec*-butyl-1,10-phenanthrolineH$^+$ $AuCl_4^-$ (**2**), 2,9-dimethyl-1,10-phenanthrolineH$^+$ $AuCl_4^-$ (**3**) were successfully synthesized and characterized. Throughout the tests with the reducing agents, the absorbances of the complexes without alkyl groups, such as the 2,9-phenanthroline gold(III) complex, decreased at a more rapid rate than those with larger alkyl groups, such as sec-butyl and n-butyl. However, since the conditions under which these glutathione reduction reactions were run were lightly different; therefore, the glutathione experiments need to be rerun with all of the compounds under the same biological conditions. In addition, the gold(III) complexes with longer alkyl substituents have shown more biological activity and inhibition of thioredoxin reductase. As a result of the cytotoxic properties that have already been discovered, future research could unveil even more medicinal and pharmaceutical benefits of these gold(III) complexes.

Future research needs to focus on finding new experiments that will help us determine with some certainty the mechanism through which these compounds are cytotoxic. More specifically, these new tests will aid in determining whether or not TrxR enzyme inhibition is the means of cytotoxicity. This will include an examination of other synthesized 2,9-disubstituted R-1,10-phenanthroline gold (III) complexes and 5,6-disubstituted R-1,10-phenanthroline gold (III) complexes (R= methyl, ethyl, isopropyl, *t*-butyl, etc.) in order to investigate the functionality of these complexes as potential biological cytotoxic antitumor agents. DNA binding experiments could be run in order to determine whether or not these

complexes bind to DNA. Since past research indicates that gold(III) complexes have variable binding to calf thymus DNA, the value in these cytotoxic complexes would dramatically rise if they are found to function as antitumor agents through a mechanism other than DNA binding. This could eliminate some of the negative side effects that result from mutagenic activity (i.e. binding through intercalation.) Determining the stability of cisplatin in the reference phosphate buffer would be an excellent future experiment in order to compare its stability to that of our complexes. Experiments using glutathione and ascorbic acid, both naturally occurring reductants, could further be used to determine whether the ligand provides additional protection against reduction of the gold relative to the free ligand. After preliminary testing, it seems that the ligand may provide some protection to the metal center through hydrogen bonding.