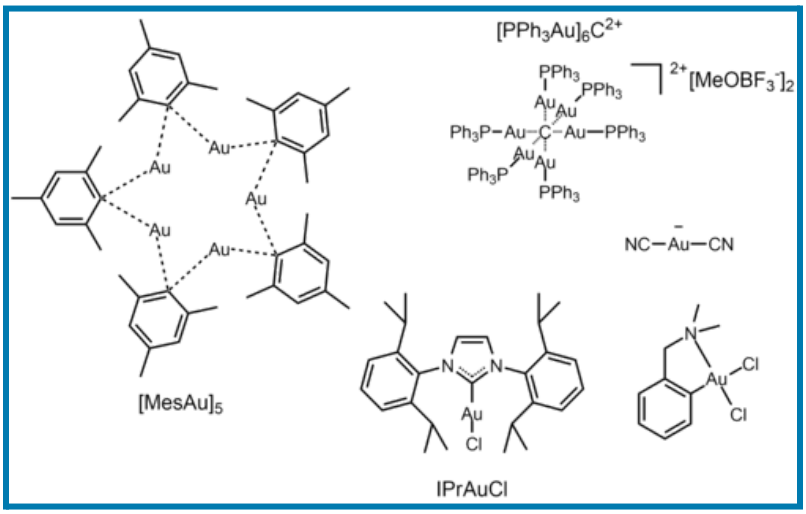
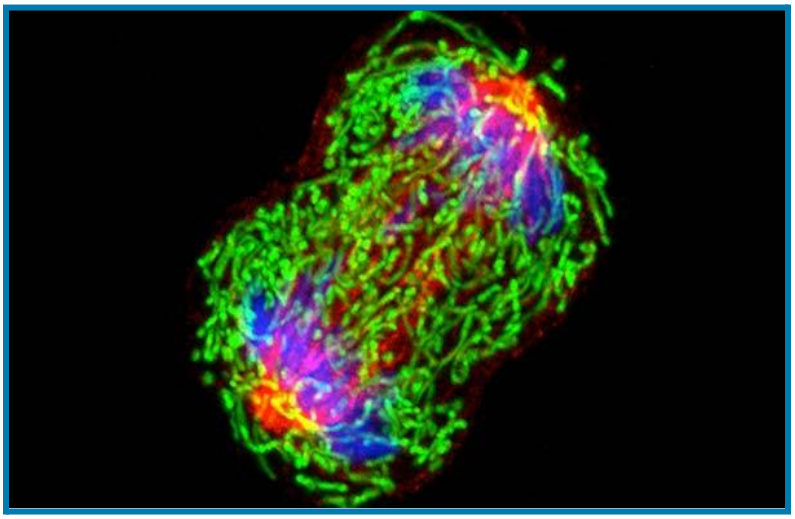
The Role of Gold Heterocyclic Thione and Selone Complexes in Cancer Research





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Abstract

Gold coordination complexes with heterocyclic thione (C=S) and selone (C=Se) ligands are investigated for their potential (or reported) use in antimicrobial and antitumor applications. More specifically, this literature survey focuses on 5- and 6-membered heterocyclic systems derived from imidazole and pyrimidine, respectively. In turn, gold, a transition metal, has been widely used in the medical field to treat a variety of diseases and conditions like rheumatoid arthritis, smallpox, and measles; gold complexes are even presently being evaluated for their potential in treating acute forms of asthma and pemphigus. Gold(I) and gold (III) complexes, specifically, have been shown to exhibit anticancer properties. Gold(I) complexes have been shown to be effective in treating cancers from non-solid tumors, like melanoma and leukemia, but were shown to be ineffective against cancers with solid tumors and harness cardiotoxic properties. Gold(III) complexes, on the other hand, have demonstrated anticancer properties alternative to cisplatin, the most common platinum-based compound used for chemotherapy, due to its cytotoxic properties with degrees of selectivity to prevent surrounding cell death. Gold complexes were synthesized to produce a 50-86% yield depending on the corresponding ligands, and analyzed through ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, mass spectrometry, and other techniques. Due to the occurrence of the COVID-19 pandemic, data was not able to be obtained from laboratory tests and analyses; therefore, this report comprises a literature review of peer-reviewed articles about gold heterocyclic thione and selone complexes and its applications contributing to anticancer drugs in an effort to provide a nuanced understanding of and an alternative to existing chemotherapy drugs.

Introduction

A Brief Overview of Metal Complex Use

Metal complexes form according to coordination chemistry, involving the use of a metal center connected to surrounding ligands; the metal acts as a lewis acid, while the surrounding ligands act as lewis bases ("Coordination Chemistry", 2019). They can be charged or neutral compounds. Metal complexes have grown increasingly popular in the biochemical industry throughout the years. This is due to the notion that "metals exhibit unique characteristics, such as redox activity, variable coordination modes and reactivity toward the organic substrate" (Frezza, et al., 2010; obtained from Ndagi, et. al, 2017). Because metals exhibit these properties, they are often found in various biological enzymes and catalytic processes; this suggests metal complex biochemical suitability for a majority of medicinal purposes. One of these medicinal purposes includes treatment of cancer.

History of Gold Complexes in Medicine

Apart from its lustrous properties in jewelry, gold has historically been used to treat various medical conditions and illnesses. According to S.P Fricker (1996, p. 53) obtained from V. Milacic & Q.P. Dou, "the earliest therapeutic application of gold can be traced back to 2500 b.c. in China" for the treatment of smallpox, skin ulcers, and measles. These benefits led people to associate it with "longevity" and the quality of being "immortal" (Milacic & Dou, 2009). Despite the medicinal benefits of gold, toxic side effects were observed; to combat this, alchemists started to mix gold with many plants. Alchemists in medieval Europe called the resulting elixir *"aurum potabile"* (Fricker, 1996). This drinkable elixir made gold biochemically suitable due to its "interactions with cyanide in the plants", and made it possible for gold to be used medically (as cited in Kostova, 2006; obtained from Milacic & Dou, 2009). Metals are generally needed for the body and serve a great purpose in helping perform cellular activities, however excess amounts can lead to cytotoxic effects.

Antibiotic Properties

The first ever "rational use" of the gold complex in the medical world was in 1890, when the famous microbiologist Robert Koch found that "$K[Au(Cn)_2]$ exhibits bacteriostatic activity

towards the tubercle bacillus [tuberculosis bacteria]" (Navarro, M, 2009; Shaw III, C.F, 1999). Koch used this compound "to kill the mycobacterium" found in tuberculosis and conducted clinical trials during the 19020s; according to the C.F Shaw III, this introduction led to the emergence of several new gold complexes used in treating the disease, about all for which "they did not provide an effective therapy" (Milacic & Dou 2009; Shaw III, 1999). Due to observed cytotoxic effects, less toxic gold (I) thiolate complexes were brought in. Although these new gold compounds were not seen to be effective in treating tuberculosis, it sparked a large push in the creation of medical gold complex use; more effective gold complexes were developed later on to treat *Mycobacterium Tuberculosis*, such as in the medical drug Auranofin (Milacic, & Dou, 2009; Thangamani, et al., 2016). Newer research on the efficacy of gold complexes in treating other medical conditions have noted their potential usefulness in medicine.

Anti-Arthritic Drugs and Auranofin

Ten years after clinical use for treatment of pulmonary tuberculosis, Jacques Forestier in the early 1930s started to experiment with some of the gold (I) thiolate complexes administered in tuberculosis treatment to treat rheumatoid arthritis--a disease which was believed to be related to tuberculosis at the time (Milacic, & Dou, 2009). The first two drugs used for this contained "aurothiomalate (ATM) and aurothioglucose (ATG), administered by intramuscular injection" (as cited in Champion, et al., 1990; obtained from Milacic & Dou 2009).

These compounds, however effective, were soon fazed out with the introduction of auranofin, [2,3,4,6-tetra-o-acetyl-L-thio-b-D-glycopyranp-sato-S-(triethyl-phosphine)-gold]. This gold-containing drug, composed of phosphine and thiol ligands, has been used due to its "well-known toxicity profile" and biocompatibility and suitability for use (Roder & Thomson, 2015).

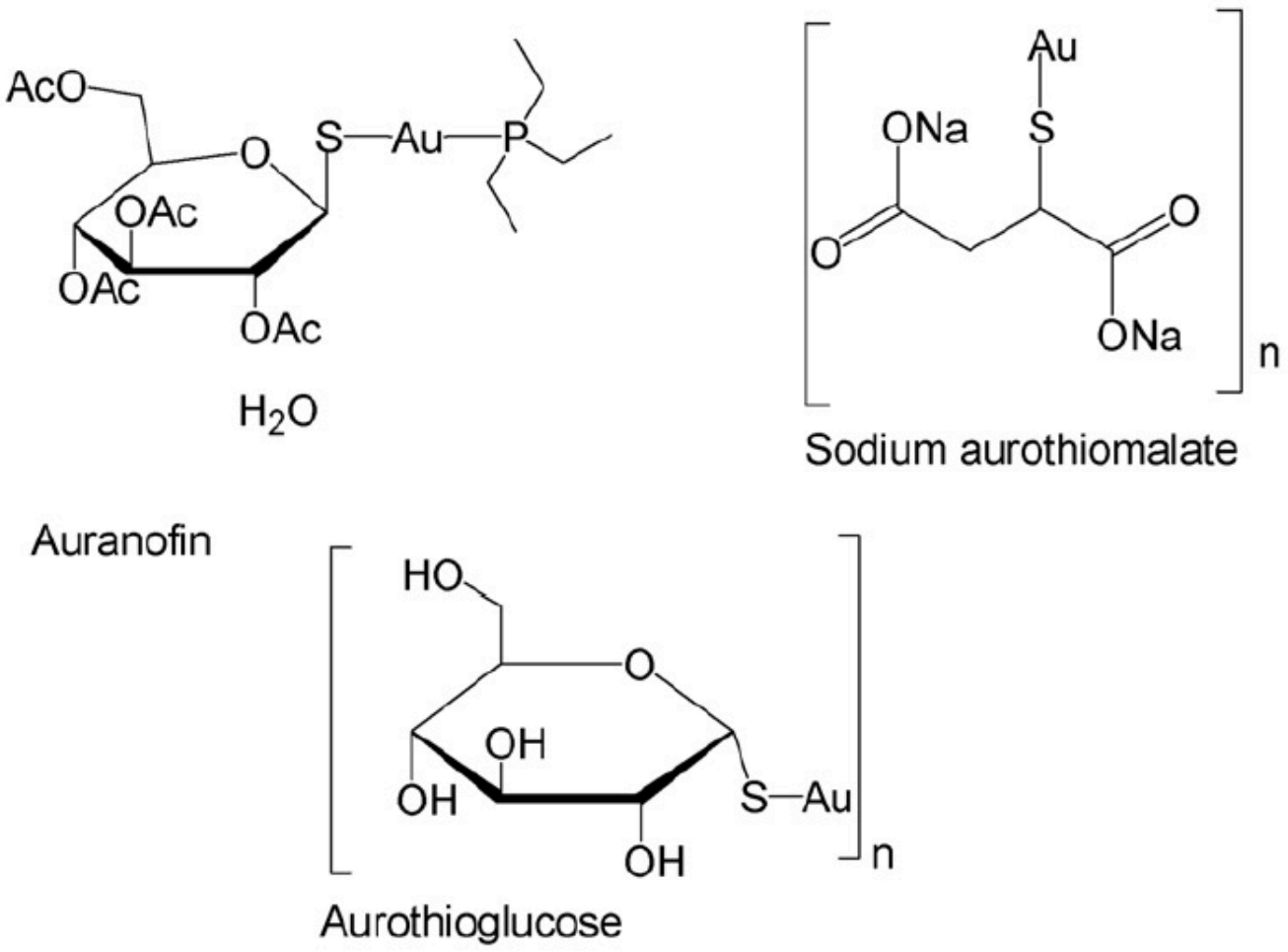


Figure 1

Anti-Parasitic Applications

There have been even claims of gold-based treatments being effective in serving as "potential antiparasitic agents" (Navarro, 2008). Gold complexes have been shown to be effective *in vitro* in combating parasites that cause malaria, trypanosomiasis, and leishmaniasis; however, further research is currently being conducted to study more of its effects on the pathogens (Navarro, 2008).

Current Anticancer Medications

Although more methods for cancer treatment are emerging into the scientific realm of medicine, there presently exists three main methods for cancer and tumor removal: Radiation Therapy, Chemotherapy, and Surgery. Radiation therapy involves the use of radio waves "to kill cancer cells and shrink tumors" by damaging the genetic material of the cells (National Cancer Institute, 2019). Chemotherapy "stops or slows the growth of cancer cells" with the use of potent, toxic drugs (National Cancer Institute, 2019). Surgery physically removes cancerous tumors from a patient's body. All of these treatments, however effective, come with disastrous side effects. Radiotherapy and chemotherapy both are not specific, and end up killing surrounding healthy cells in the process; thus, often causing extreme fatigue and weakness in patients. Surgery is the relatively safer option, however it also comes with side effects. It can lead to an increased risk for "bleeding, damage to nearby tissues, possibility for anesthetic reactions" in some people, and an increased risk for infection post surgery; it can also cause pain on the "part of the body that was operated on" if not managed properly (National Cancer Institute, 2019). Due to an increasing need for targeted cell therapies and effective treatments, metal complexes were sought out for their cytotoxic effects.

Cisplatin, a platinum-based drug discovered "in 1960 by Barnett Rosenburg", marked the beginning of research and use for metal complexes in anticancer applications (Ndagi, et al., 2017). Platinum-based complexes, generally, are particularly effective for the treatment of "cervical, ovarian, testicular, head and neck, breast, bladder, stomach, prostate and lung cancers" (Ndagi, et al., 2017). Cisplatin works by attaching to the guanine base in the deoxyribonucleic acid (DNA) molecule and forming a 'crosslink' within the molecule, effectively prohibiting formation of new DNA and the subsequent replication of cells (O'Brien, et al., 2009). Because of its known pathways, cisplatin is one of the major drugs used in chemotherapy and has been heavily relied upon in previous years in cancer treatment. Frequent use, however, has caused a surge in cisplatin-resistant cancerous tumors; therefore, calling for the creation of alternative cancer-fighting compounds. "Carboplatin, oxaliplatin, satraplatin, ormaplatin, aroplatin, enloplatin, zeniplatin, sebriplatin, miboplatin, picoplatin, satraplatin, and iproplatin" are some examples of these compounds (Ndagi, et al., 2017).

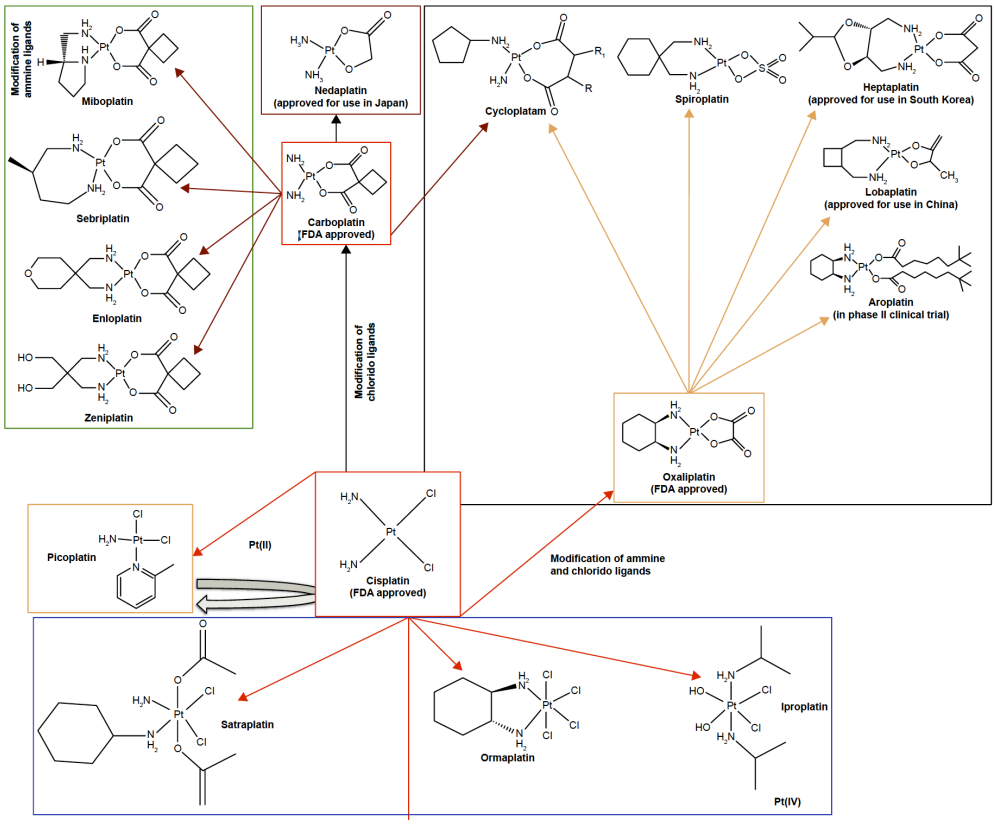


Figure 2

However effective these complexes may be in treating specific types of cancers, toxic side effects spread onto surrounding cells relating to their usage have been noted. To offer an alternative to the platinum-based treatments, scientists started to investigate gold complexes *in vivo* and *in vitro* to combat cancer. Due to its reputation as a well-known antirheumatic drug and its known avenues of functioning, scientists first started with auranofin (Ndagi, et al., 2017).

Literature Review

Antimicrobial Properties

Antimicrobial agents "kill or slow the spread of microorganisms", including bacteria and viruses (Oregon State University). As previously noted, gold complexes have traditionally been used in medicine for its disease fighting abilities and antimicrobial properties. This can be seen not only when the ancient Chinese used gold to treat smallpox, skin ulcers, furuncles, but also with tuberculosis (Huaizhi and Yuantao, 2000; Abultalem and Seleem, 2020). Auranofin, a gold-based drug developed to treat rheumatoid arthritis, has been shown to prove effective in combating nosocomial infections caused by *Clostridioides difficile* and multidrug resistant bacteria. Presently, auranofin is even undergoing tests to prove efficacy in fighting the novel coronavirus, SARS-COV-2 (Abutalem and Seleem, 2020).

Tuberculosis

Mycobacterial pathogens, however widespread, and infections are on the rise in the United States. Among these pathogens are the "pulmonary pathogens" *Mycobacterium tuberculosis* and *Mycobacterium abscessus* (Gupta, et al., 2018). *M. tuberculosis*, as the name suggests, causes tuberculosis and is proving to be increasingly resistant to current antibiotics used to treat the disease; it is "a slow-growing mycobacterium estimated to latently infect almost one-third of the human population, causing 8 million new cases of active tuberculosis cases every year" (as cited in Centers for Disease Control; obtained from Gupta et al., 2018). Tuberculosis is a disease of the lungs, in which the mycobacterium contaminates the tubercles of the lungs impairing the individual's breathing abilities. *M. abscessus*, unlike its tuberculosis counterpart, "is a rapidly growing nontuberculous mycobacterium (NTM) which causes TB-like pulmonary infections as well as soft tissue and wound infections" (as cited in Howard & Boyd, 2000; obtained from Gupta et al., 2018). Although there presently exists treatment of infections by these pathogens with antibiotics, these mycobacteria have been seen to be increasingly resistant to the medicines. As stated in Gupta and colleagues' report *Evidence for Inhibition of Topoisomerase 1A by Gold(III) Macrocycles and Chelates Targeting Mycobacterium tuberculosis and Mycobacterium abscessus*, infections caused by both pathogens "require a long multidrug regimen of over 6 months, which can lead to poor patient compliance and rising rates

of drug-resistant strains" (Gupta, et al., 2018). To search for a new treatment, Gupta and colleagues tested "bis (pyrrolide-imine)-based gold(III) compounds with potent activity against both mycobacterial pathogens and low toxicity to mammalian cells"; these compounds were demonstrated to "target topoisomerase 1A (Topo1), a recently validated target" (Gupta, et al., 2018).

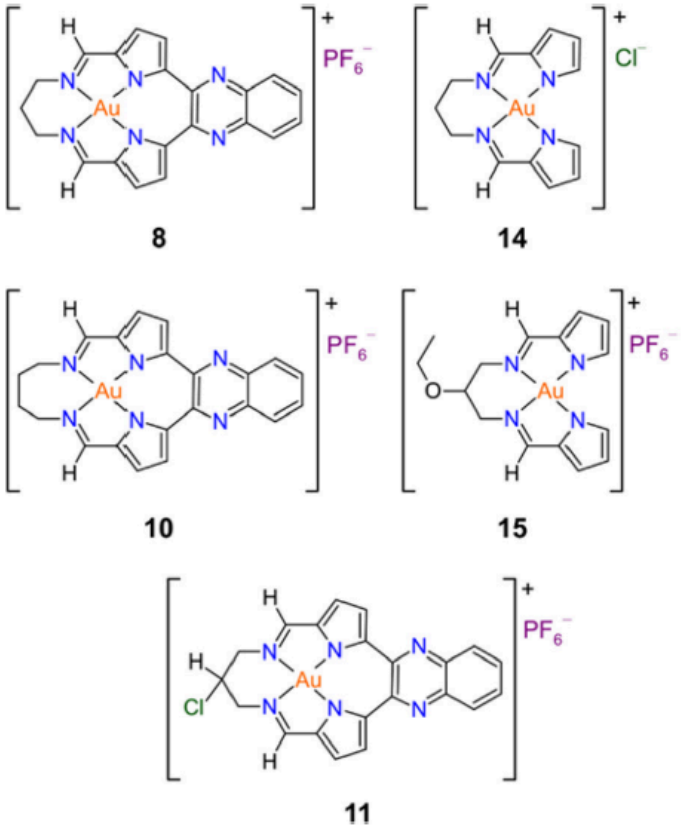


FIG 1 Structures of the bis(pyrrolide-imine) gold(III) macrocycles (compounds 8, 10, and 11) and chelates (compounds 14 and 15) active against mycobacteria. In the study by Akerman and collaborators (33), compounds 1, 3, and 5 correspond to compounds 8, 10, and 11 in the present paper, respectively.

Figure 3

These gold compounds were shown to "effectively inhibit replicating *M. tuberculosis* and *M. abscessus*". Of the nineteen gold (III) macrocycles and simple chelates tested, five inhibited specifically *M. tuberculosis* and four inhibiting *M. abscessus*; "compounds 10, 11, 14, and 15 inhibited both mycobacterial species", while compound 8 "inhibited only Mtb-RG [*M. tuberculosis* bacterial enzyme]" (Gupta, et al., 2018).

TABLE 1 Activity profiles of 5 active gold(III) complexes

Compound	MIC (μM [95% CI[a]])		IC_{50} (μM) for J774	SI[b] (IC_{50}/MIC)	
	M. tuberculosis	*M. abscessus*		*M. tuberculosis*	*M. abscessus*
8	2.72 (2.1–3.6)	849.41 (12.8–56,175)	48.48	17.82	0.06
10	0.12 (0.09–.14)	5.44 (3.0–9.8)	85.82	715.16	15.77
11	4.06 (2.7–6.0)	91.72 (53.2–158.2)	108.50	26.72	1.18
14	0.98 (0.8–1.2)	11.98 (8.3–17.3)	42.30	43.16	3.53
15	4.61 (3.4–6.2)	13.05 (9.2–18.5)	48.89	10.60	3.74

[a]CI, confidence interval.
[b]SI, selectivity index.

Figure 4

Nosocomial Infections

Nosocomial infections arise from the spread of bacteria within the environment, and can cause "mild to sever symptoms". These symptoms can include "mild to severe watery diarrhea...pseudomembranous colitis, toxic megacolon, colon perforation, sepsis, and systemic inflammatory response syndrome and shock" (). These infections have been treated with antibiotics in the past and currently with antibiotics, such as vancomycin and fidaxomicin, but have grown resistant to these medications due to overuse. In a study conducted by Nader S. Abutaleb and Mohamed N. Seleem (2020), auranofin, previously mentioned for its usefulness in treating rheumatoid arthritis, has been shown to be effective in treating nosocomial infections caused by *Clostridioides difficile* when tested for antibacterial activity *in vivo* against infected mice; the drug was found to "possess strong antibacterial and antifungal activities (write in the sources of 17, 22-25). It has also been tested for antibacterial activity *in vitro* when "in the presence of high bacterial inoculum size compared to vancomycin and fidaxomicin" (Abultalem and Seleem, 2020). Auranofin, as well as vancomycin and the control antibiotic (fidaxomicin), were first incubated with "simulated gastric fluid (SGF) and simulated intestinal fluid (SGI) for 2, 4, and 24 hours" to mimic the environment the medicines will be in; "minimum inhibitory concentrations (MICs) against two clinical *C. difficile* strains were then determined" (Abultalem and Seleem, 2020). Findings suggested that auranofin proved to be stable and unaffected "by the enzymes of gastric fluid" and intestinal fluids (Abultalem and Seleem, 2020).

(A) Simulated gastric fluid (SGF)												
	Auranofin				Vancomycin				Fidaxomicin			
C. difficile strains	0 h	2 h	4 h	24 h	0 h	2 h	4 h	24 h	0 h	2 h	4 h	24 h
ATCC BAA 1870	1	1	1	1	1	1	1	2	0.03	0.03	0.03	0.03
ATCC 43255	0.5	0.5	0.5	1	1	1	1	1	0.015	0.015	0.015	0.015
(B) Simulated intestinal fluid (SIF)												
C. difficile strains	Auranofin				Vancomycin				Fidaxomicin			
	0 h	2 h	4 h	24 h	0 h	2 h	4 h	24 h	0 h	2 h	4 h	24 h
ATCC BAA 1870	1	1	1	2	1	1	1	2	0.03	0.03	0.03	0.06
ATCC 43255	0.5	1	1	1	1	1	1	2	0.015	0.015	0.015	0.03

Table 2. MICs (μg/mL) of auranofin and control antibiotics against *C. difficile* clinical isolates after incubation with: (**A**) simulated gastric fluid (SGF), (**B**) simulated intestinal fluid (SIF), for the corresponding times (hours).

Table 1

To determine the drug's efficacy, researchers treated three groups of *C. Difficile* infected (CDI) mice with either 0.125 mg./kg., 0.25 mg./kg., and 0.5 mg./kg of auranofin for 5 days and closely monitored (Abultalem and Seleem, 2020). At clinically low achievable concentrations (0.125 mg./kg.), "auranofin...was able to protect 100% of the mice against *C. difficile* during the 5-days treatment period" and "the higher doses (0.25 mg./kg. and 0.5 mg./kg) protected only 80% and 40% of mice" (Abultalem and Seleem, 2020). Moreover, at optimal dosages auranofin protected against weight loss "till day 5".

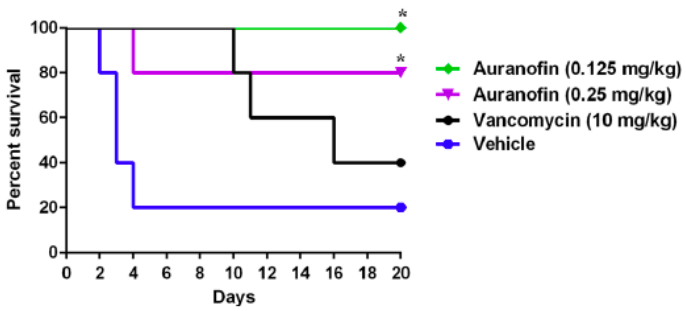


Figure 3. Efficacy of auranofin against CDI recurrence. Mice were treated with auranofin (0.125 mg/kg, and 0.25 mg/kg), vancomycin (10 mg/kg), or the vehicle for 5 days after infection with *C. difficile* spores and the treatments were stopped afterwards. Mice were monitored for survival. Kaplan–Meier survival curves were analyzed using a log-rank (Mantel–Cox) test. Asterisks (*) denote statistical significant difference between mice treated with either auranofin, or vancomycin in comparison with vehicle-treated mice.

Figure 5

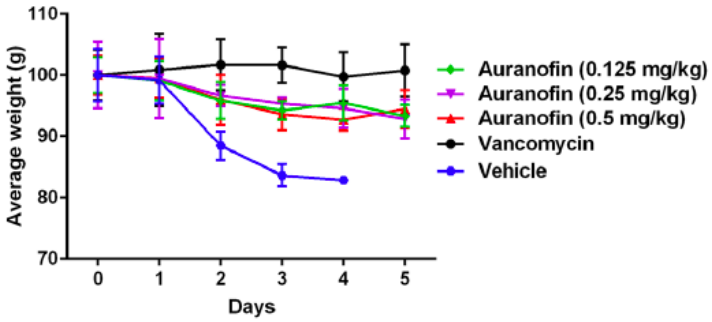


Figure 2. Average relative weight of all surviving mice. Infected mice were treated with auranofin (0.125 mg/kg, 0.25 mg/kg, and 0.5 mg/kg), vancomycin (10 mg/kg), or the vehicle for 5 days and weighed daily till the end of the experiment. The data are presented as percent relative weight (mean ± standard deviation) for each group.

Figure 6

The rise of antibiotic resistant bacteria, also known as "superbugs", has been a growing problem in the 21st century as more bacteria have increasingly shown signs of resistance to powerful drugs. This growing problem prompted a search for nuanced medications that can be used to defeat these resistant microbes.

Multi-drug Resistant Bacteria

As previously mentioned, gold-based compounds possess antimicrobial properties. Currently, the drug Auranofin seems to be increasingly scrutinized in these properties due to the assumption that it "possesses potent antibacterial activities in a clinically achievable range". This is achieved through inhibiting "multiple biosynthetic pathways including the cell wall, DNA, and bacterial protein synthesis" (Thangamani, et. al, 2016). Because bacterial protein synthesis is halted, bacteria are not able to secrete as much bacterial toxins; therefore, leading to a decrease in severity of disease symptoms or even a reduction in disease experience.

Staphylococcus aureus (*S. aureus)*, the bacteria that causes a number of "invasive diseases including pneumonia and sepsis", is currently proving difficult to control. It has become resistant to many antibiotics, such as methicillin, previously used for treatment; because *S. aureus* has shown to be resistant to methicillin, the bacteria has been called methicillin-resistant *Staphylococcus aureus* (MRSA). Because of this resistance, researchers started to look towards use of auranofin. A study conducted by Thangamani and associates published in 2016 found that

it is “a potent inhibitor of multidrug-resistant Gram-positive bacteria”, through testing multiple “multidrug-resistant Gram-positive pathogens using the broth microdilution method” (Thangamani, et al., 2016). These tests included comparing auranofin dosage to some other well-known antibiotics: vancomycin, erythromycin, fusidic acid, linezolid, and daptomycin.

	Minimum Inhibitory Concentration (MIC) (μg/ml)										
		Auranofin		Erythromycin		Fusidic acid		Linezolid		Daptomycin	
		PMBN		PMBN		PMBN		PMBN		PMBN	
Bacteria	PMBN	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)
Actnetobacter baumannti ATCC BAA19606	>256	16	0.25	64	0.5	64	0.5	256	64	>256	>256
Actnetobacter baumannti ATCC BAA1605	>256	16	0.5	64	0.5	128	1	>256	128	>256	>256
Actnetobacter baumannti ATCC BAA747	>256	16	0.25	64	1	128	0.5	>256	64	>256	>256
Escherichta colt O157:H7 ATCC 700728	256	64	0.5	128	1	>256	16	256	16	>256	>256
Escherichta colt O157:H7 ATCC 35150	256	32	0.5	128	1	>256	16	>256	16	>256	>256
Salmonella Typhtmurtum ATCC 700720	>256	128	1	256	2	>256	16	256	16	>256	>256
Klebstella pneumontae ATCC BAA 2146	>256	256	0.5	>256	128	>256	32	>256	64	>256	>256
Klebstella pneumontae ATCC BAA 1705	>256	256	1	>256	64	>256	64	>256	128	>256	>256
Pseudomonas aerugtnosa ATCC 9721	>256	>256	0.25	>256	1	>256	1	>256	4	>256	>256
Pseudomonas aerugtnosa ATCC 27853	>256	256	0.125	256	1	>256	1	>256	16	>256	>256
Pseudomonas aerugtnosa ATCC BAA-1744	>256	>256	0.25	>256	1	>256	1	>256	16	>256	>256
Pseudomonas aerugtnosa ATCC 25619	>256	256	0.25	256	1	>256	1	>256	8	>256	>256
Pseudomonas aerugtnosa ATCC 35032	>256	>256	0.5	>256	1	>256	1	>256	8	>256	>256
Pseudomonas aerugtnosa ATCC 10145	>256	256	0.25	256	1	>256	2	>256	8	>256	>256
Pseudomonas aerugtnosa ATCC 15442	>256	>256	0.25	>256	2	>256	1	>256	16	>256	>256
Escherichta colt 1411	>256	32	0.5	32	4	>256	4	>256	16	>256	>256
Escherichta colt SM1411Δ *acrAB*	>256	8	0.5	0.03	<0.03	8	<0.03	32	2	>256	>256
Escherichta colt (Novablue (DE3)-K12)	256	16	0.5	16	0.5	>256	0.5	>256	16	>256	>256
Escherichta colt (Origami-2) (*trxB/gor* mutant)	256	16	0.5	32	0.5	256	0.06	128	16	>256	>256

Table 2. MICs of auranofin and control antibiotics against Gram-negative bacteria. PMBN polymyxin B nonapeptide: (−) No PMBN was added to the media; (+) (10 μg/ml) of PMBN was added to the media.

Table 2

Auranofin proved superior to all the other antibiotics as it required the least dosage amounts in combating various gram-positive bacteria including different MRSA and vancomycin-resistant strains. The minimum inhibitory concentrations (MICs) “required to inhibit the different MRSA strains were found to be in the range of 0.0625 to 0.125 μg/ml”. This works due to inhibiting “5 major biosynthetic pathways *S. aureus*”, including cell wall, DNA, protein, lipid, and RNA synthesis. This drug has been shown to affect a vital bacterial enzyme known as Thioredoxin-Reductase, which regulates “thiol-reductase homeostasis” in the bacterial

specimens. Auranofin was also proven to be effective against “vancomycin-sensitive enterococcus and vancomycin-resistant enterococcus (VRE), *Streptococcus pneumoniae* and *Streptococcus agalactiae* with MIC values ranging from 0.0015 to 0.25 µg/ml” (Thangamani, et al., 2018). However effective it has been in gram-positive bacteria, researchers have discovered that the drug does not work very well in gram-negative bacteria due to the “thick outer membrane negating auranofin’s antibacterial activity”.

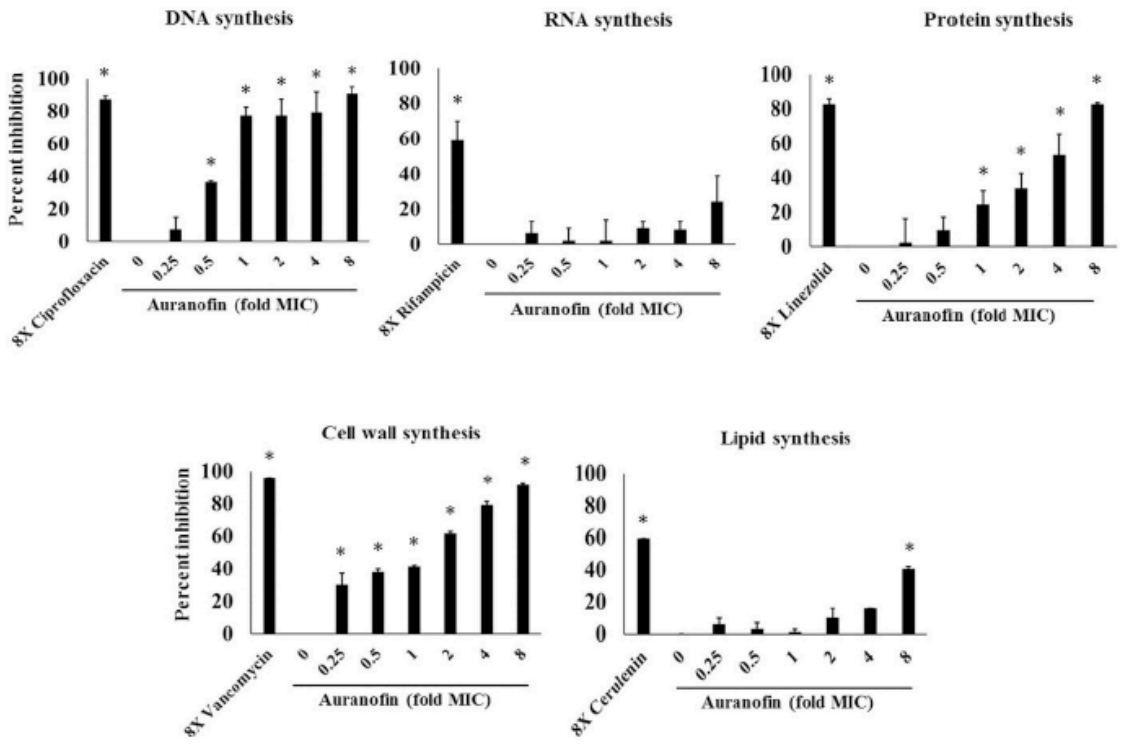


Figure 7

COVID-19

The following are preliminary results of this new study, and are subject to change with new forthcoming research on the novel SARS-COV-2 virus. SARS-COV-2 works similar to every coronavirus; the Endoplasmic Reticulum (ER) and Unfolded Protein Response (UPR) pathway is usually stressed in the cells within infection, “contributing significantly to viral replication and pathogenesis during a coronavirus infection” (as cited in Fung & Liu, 2014; obtained from Rothan et al., 2020). It has also been found that the novel coronavirus “causes acute inflammation and neutrophilia...leading to a cytokine storm with over-expression of IL-6,

TNF-alpha, monocyte chemoattractant protein (MCP-1) and reactive oxygen species" (ROS) (as cited in Mehta et al, 2020; obtained from Rothan et al., 2020). Because auranofin is known to target ""redox enzymes such as thioredoxin reductase" and inhibit "5 major pathways of biosynthesis", researchers decided to explore its effect *in vitro* on cells infected with the novel virus (Thangamani, et al., 2018; Rothan, et al., 2020). To determine its effects on cells, Rothan and a team of researchers from Georgia State University infected Huh7 cells, cells that are "highly permissive for SARS-COV-2 replication", with the novel coronavirus *in vitro*; cells were infected with a "multiplicity of infection (MOI) of 1 for 2 hours, followed by the addition of 4 μM of auranofin" (Rothan, et al., 2020). They used 1% dimethyl sulfoxide (DMSO) as a control. After 24 and 48 hours, the researchers would check on the cell cultures supernatants and cell lysates (Rothan, et al., 2020). They would then proceed to copy viral RNA structures via "RT-PCR using two separate primers specific for the viral N1 region and N2 region" (as cited in Rothan et al., 2019 and Kumar et al., 2017; obtained from Rothan et al., 2020).

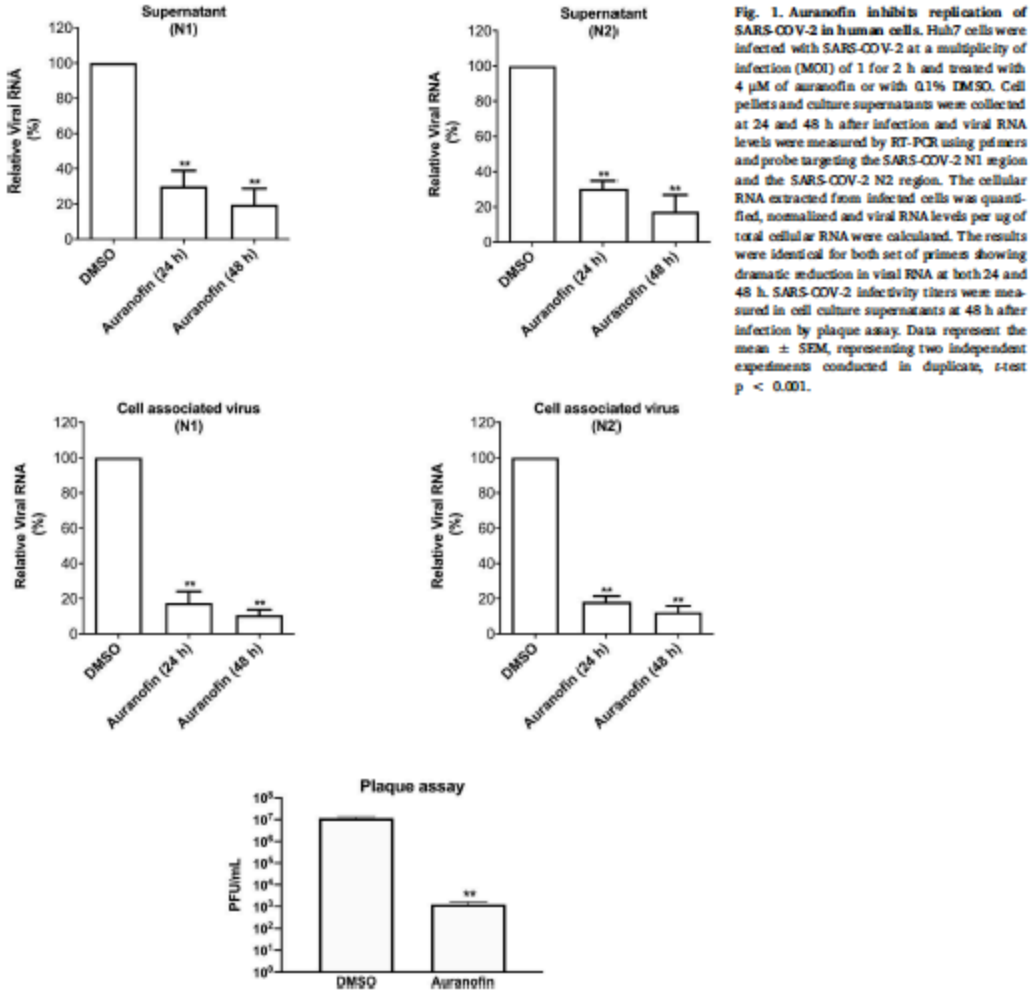


Fig. 1. Auranofin inhibits replication of SARS-COV-2 in human cells. Huh7 cells were infected with SARS-COV-2 at a multiplicity of infection (MOI) of 1 for 2 h and treated with 4 μM of auranofin or with 0.1% DMSO. Cell pellets and culture supernatants were collected at 24 and 48 h after infection and viral RNA levels were measured by RT-PCR using primers and probe targeting the SARS-COV-2 N1 region and the SARS-COV-2 N2 region. The cellular RNA extracted from infected cells was quantified, normalized and viral RNA levels per ug of total cellular RNA were calculated. The results were identical for both set of primers showing dramatic reduction in viral RNA at both 24 and 48 h. SARS-COV-2 infectivity titers were measured in cell culture supernatants at 48 h after infection by plaque assay. Data represent the mean ± SEM, representing two independent experiments conducted in duplicate, *t*-test $p < 0.001$.

Figure 8

Researchers discovered that auranofin effectively inhibited “replication of SARS-COV-2 in human cells at low micro molar concentration” and “resulted in a significant reduction in the expression of cytokines induced by virus infection”(Rothan, et al., 2020). Treatment of Huh7 cells with the antiarthritic drug led to “a 70% reduction in the viral RNA in the supernatants compared to the DMSO [control group] at 24 hours after infection”; 48 hours after infection led to an “85% reduction...compared to the DMSO”. There was an 85% reduction and 95% reduction in intracellular viral RNA levels after 24 and 48 hours (Rothan, et al., 2020).

IMPORTANT NOTE: These preliminary results, however conclusive, do not entirely prove auranofin’s effectiveness in treating COVID-19; as the researchers stated, “further animal studies are warranted to evaluate the safety and efficacy of auranofin” (Rothan, et al., 2020).

Various Medical Conditions

Use of gold complexes have been shown to prove effective in treating medical conditions, such as asthma and pemphigus.

Pemphigus

Pemphigus is a rare autoimmune disorder that affects the skin and is treated with steroids. According to a study conducted by Dr. Pandya and Dr. Dyke, gold compounds were known to be effective in treating pemphigus vulgaris; because of toxicity levels and concern over the efficacy of this treatment, however, treatment is halted in some patients. To determine the exact efficacy of the treatment, as well as toxicity levels, the two doctors "reviewed 26 patients with pemphigus who were treated with intramuscular gold over a 10-year period". They discovered that gold-based treatment was effective in 62% of the treated patients when it was used as either "a primary treatment or as a steroid-sparing agent" (Pandya and Dyke, 1996). However effective treatment was, "toxic effects were observed in 42% of patients"; these effects seemed to be resolved with the halting of treatment, leading the two doctors to believe that it may only "be effective in treating a large percentage of patients who otherwise are unable to reduce their steroid treatment" . Gold therapy in treatment of pemphigus has to, thus, "require systemic steroids when therapy is initiated". Although these results offer a grim look into gold-based therapy for this condition, more research is needed to determine if it could be potentially utilized in the future (Pandya and Dyke, 1996).

Asthma

Corticosteroids are typically used for the treatment of asthma. While treatment usually results in significant improvement for asthma patients, for others it could come with a caveat. These corticosteroids could cause a dependence in some asthmatic patients. In a study by Dr. I. Leonard Bernstein et al. (1996), auranofin was tested to determine if it could cause a reduction in "oral corticosteroid requirements" and if it would be safe "in the treatment of chronic corticosteroid-dependent asthma". To determine this, "two hundred seventy-nine patients with chronic corticosteroid-dependent asthma, requiring at least 10 mg. of steroid use a day", were randomly split into two groups: one required to take 3 mg. of auranofin twice daily for eight months and a placebo group Phase (I) of the clinical trial included a "4-week baseline period", phase (II) consisted of "a 6-month double-blinded treatment and steroid reduction period" and phase (III) included a "4-week posttreatment observation period during which steroid and auranofin doses were maintained at levels achieved by the end of phase II" (Bernstein, et al., 1996).

From this study, researchers discovered that "auranofin demonstrated a steroid-sparing effect...and appeared to be more effective in patients dependent on 10 to 19 mg. of prednisone per day". Treatment of patients with auranofin achieved "significantly higher therapeutic success (41%) than in the placebo group (27%) (p=0.01)" (Bernstein, et al., 1996). This effect was more pronounced in patients "requiring 10 to 19 mg. of prednisone per day at baseline (p<0.001)"; while a "significant reduction (≥50% of baseline) in oral corticosteroid dosage was achieved in the auranofin group (60%) compared with the placebo group (32%) (p<0.001).

Anti-arthritic drugs and auranofin

Rheumatoid arthritis is an auto-immune disease that progressively attacks the joints of individuals, "resulting in deformities, immobility, and a great deal of pain" (Fricker, 1996). After Jacques Forestier's reports on gold-based treatments of rheumatoid arthritis in the early 1930s, more gold-based drugs were produced to combat this chronic condition. According to a study published Sigler and colleagues (1974), 27 rheumatoid arthritis patients, who underwent a two year double-blind study to determine the effect of gold salts on rheumatoid arthritis, "reported clinical improvement". A follow-up study concerning radiological techniques confirmed clinical improvement in the patients by showing that there was a slowed "mean progression rate of [bone and cartilage] destruction" in patients who underwent the gold treatment (Finkelstein, et al., 1975). "Gold (I) thiolates, such as sodium aurothiomalate (Mycrosin™) and aurothioglucose (Solganol™)" were among the first gold-based drugs introduced for the treatment of arthritis; they have since been phased out with the introduction of Auranofin in 1985, due to its ability to "distribute to various tissues including the kidneys...and gives rise to nephrotoxicity" (Fricker, 1996). Auranofin, generically known as "Ridaura™, was introduced as an orally bioavailable drug for arthritis" (T.G Davis, obtained from Fricker, 1996). It has the general formula R_3PAuSR and is a "monomeric thiolate" with lipophilic properties (Fricker, 1996). An analysis of Auranofin showed that the central gold atom was "divalent and linear" and bound to "the phosphine and sulphur atoms of the triethylphosphine and thioglucose ligands", creating a ligand exchange attributable to its metabolism "most likely associated with Au-S bond cleavage" (Baran, Et al., 1569, 1996; obtained from Shoeib, et al., 2009). Through ^{13}C and ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy "under hydrophobic conditions" demonstrating decreased lability in the phosphine group, it was determined that this cleavage causes the gold center to be released and "coordinate to a biologically relevant ligand such as thiol" (Mckeage, et al., 2002; Shoeib, Atkinson and Sharp, 2009).

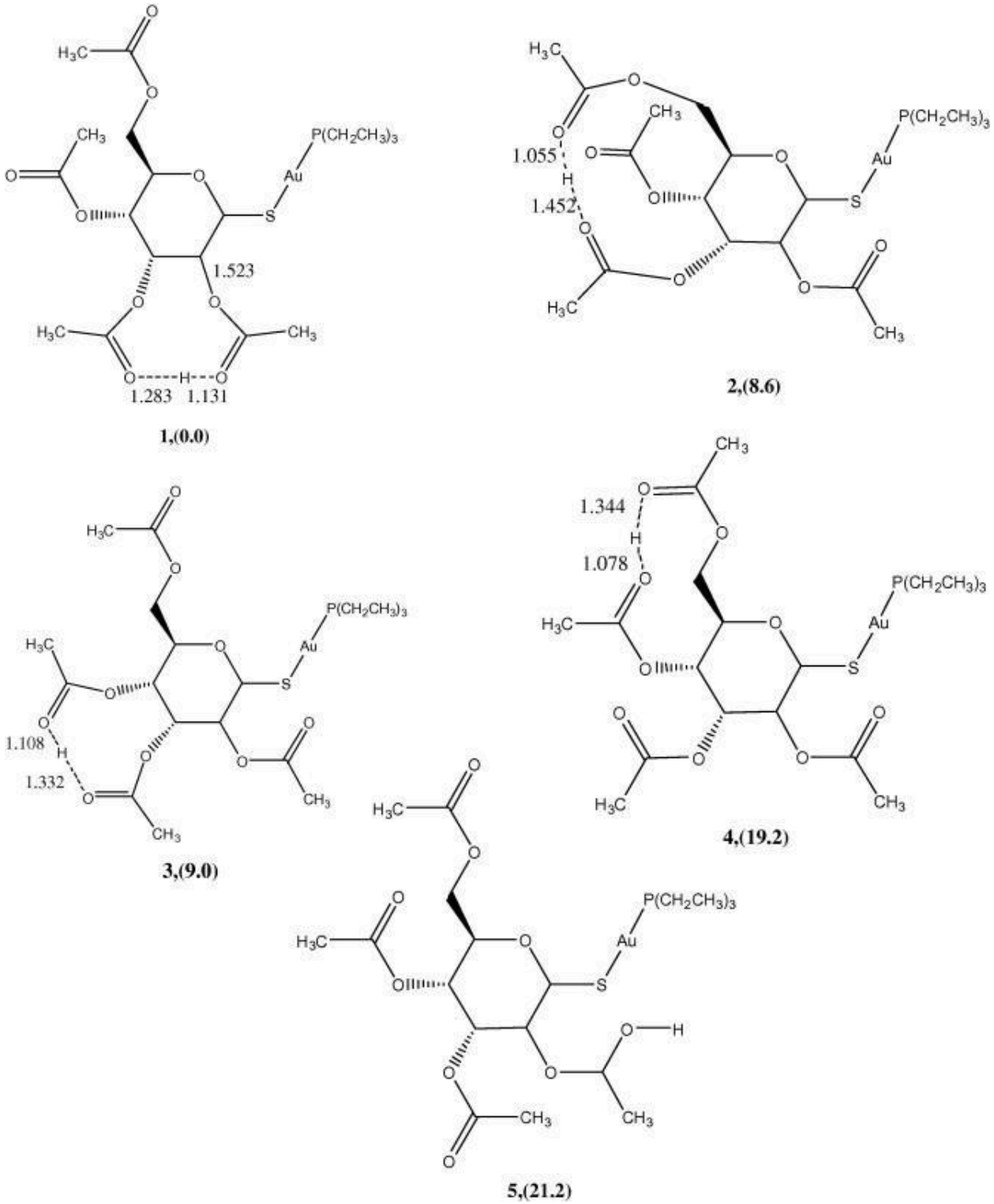


Figure 9: "Five structures were calculated for the parent [Auranofin + H]+ as shown. In all these structures the external proton resides on one of the carbonyl oxygen atoms of the substituted heterocyclic ring of the thioglucose moiety. This external proton makes

a hydrogen bond with another carbonyl oxygen atom in all but the highest energy structure found. The lowest energy species, 1, found on this surface contains the shortest (1.283 Å) and thus strongest hydrogen bond with a nearly linear HOH bond angle at 177°. Structures 2 and 3 which contain slightly longer hydrogen bonds are about 9 kcal mol–1 higher in energy than 1, while structures 4 and 5 are both calculated to be about 20 kcal mol–1 removed from the lowest energy species" (Shoeib, Atkinson and Sharp, 2009).

A study suggested that "the binding force between the gold center and the cysteine residue in the glutathione enzyme and Cathepsin B" may be responsible for the gold-based drugs anti-arthritic properties (Shoeib, Atkinson and Sharp, 2010). Auranofin is favored by medical professionals to treat rheumatoid arthritis not only due to its ability to be taken orally, but also due to "less retention of gold in the tissues...reducing renal toxicity" levels (Fricker, 1996). Renal toxicity and nephrotoxicity both refer to toxic levels within the kidneys, the organs which filter the blood, and are commonly one of the major problems when it comes to drugs and medicines; leaving the kidneys in healthy conditions is one of the major goals to designing the ideal drug.

Anti-Parasitic Applications

Auranofin is just one of the gold complexes that have been recently drawing the attention of researchers. It, as well as a growing number of gold complexes, have also been evaluated for their antiparasitic applications. Neglected tropical diseases, a term used by the World Health Organization (WHO) to describe parasitic infections that are often overlooked, remain a very big problem for poorer, developing nations. Reliance on antibiotics have led to an increasing number of drug-resistant parasites, leading to antibiotic resistant infections; furthermore, the ability to obtain antibiotics pose a problem for most members of developing nations. The use of gold complexes may provide a nuanced, cost-effective way to treat these diseases. Some of these parasitic diseases include giardiasis, malaria, trypanosomiasis, and leishmaniasis.

Giardiasis

Giardiasis, "one of the most common causes of diarrheal disease worldwide", is known as a neglected tropical disease--a parasitic disease that is all too often overlooked as they mainly affect people in developing nations. The disease is caused by the *Giardia lamblia* parasite. In previous years, people have been treated with "primarily...5-nitro antimicrobials, particularly metronidazole"; recently, the parasite has been shown to have been developing resistance to the treatment, with treatment failures occurring "in up to 20% of cases" (as cited in Tejman-Yarden et al., 2011; Escobedo and Cimerman, 2007, Upcroft and Upcroft, 2001, and Wright et al., 2003; obtained from Tejman-Yarden, et al., 2013). Because of this, researchers ran a search of "screening libraries of FDA-approved compounds"; they searched through 750 approved novel compounds "with promising *in vitro* and *in vivo* activities". One of the compounds they found, auranofin, displayed desirable characteristics (Tejman-Yarden, et al., 2013).

When tested in a 48-hour assay with *Giardia lamblia*, the drug was found to have "inhibited growth and survival of several different *G. lamblia* isolates, belonging to both assemblages A and B with half-maximal effective concentrations (EC_{50}s) of 4 to 6 μM". This dosage level suggests that "this drug may be therapeutic against giardiasis *in vivo*, particularly since the drug is given orally and giardial infection is limited to the small intestinal lumen". The drug was also seen to "completely override resistance to...metronidazole" (Tejman-Yarden, et al., 2013). The drug, as noted in some previous studies, inhibited the thioredoxin reductase (TrxR) enzyme in the *Giardia* "with an IC_{50} [inhibitory concentration 50%) of 152 $\pm$ 12 nM". When

tested against diphenyleneiodonium (DPI), "a broad spectrum-flavoenzyme inhibitor" which "marked antigiardial activity with IC_{50}s similar to those of auranofin and metronidazole", and metronidazole, the drug that the parasite is showing resistance to, auranofin displayed displayed similarities to DPI suggesting that the drug may establish effective "morphological forms of cell death" through the inhibition of TrxR (Tejman-Yarden, et al., 2013).

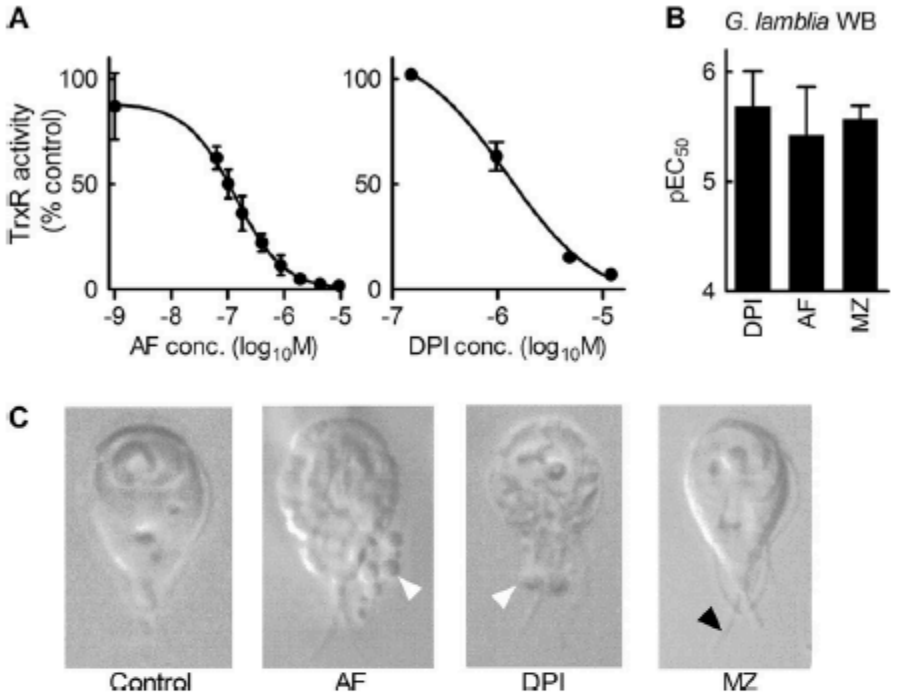


FIG 3 Inhibition of *G. lamblia* TrxR by auranofin. (A) *In vitro* activity of purified, recombinant TrxR from *G. lamblia* was determined with the substrate DTNB in the presence of increasing concentrations of auranofin (AF) or DPI. Data are expressed relative to the control activity without drugs and are shown as mean ± SD ($n = 3$). (B) The antigiardial activities of DPI, AF, and metronidazole (MZ) were determined by 48-h growth assay and are shown as pEC_{50} (mean + SD). (C) The effects of the indicated drugs on the morphology of *G. lamblia* WB were determined with the aid of a spinning-disk confocal microscope. MZ-treated cells exhibited marked slowing of flagellar beating, since flagella could be easily captured by the camera (black arrowhead), whereas the flagella in untreated cells could barely be detected under these conditions due to their rapid movement. By comparison, AF and DPI treatment caused only modest flagellar slowing but instead led to marked cellular blebbing (white arrowheads).

Figure 10

To test for its efficacy *in vivo*, researchers tested the drug against *G. lamblia* infected newborn (5 to 7 days old) mice. They were infected with "10^7 trophozites of two assemblage A isolates, 106 and WB, or the assemblage B isolate 1279". Treatment started after 2 days of

established infection, “and continued once daily for 5 days” (Tejman-Yarden, et al., 2013). Researchers discovered that auranofin proved “highly effective against *G. lamblia* WB and 106 and slightly less so against *G. lamblia* 1279 at a dose of 5 mg/kg”; “at a lower dose of 1 mg/kg, auranofin treatment resulted in only a modest 2-fold decrease in trophozoite numbers for the 106 isolate”. More rodents were tested in this study, and further analysis of liver enzymes and “liver, kidneys, spleen, and intestinal tract...suggested that auranofin was safe at an effective dose” (Tejman-Yarden, et al., 2013).

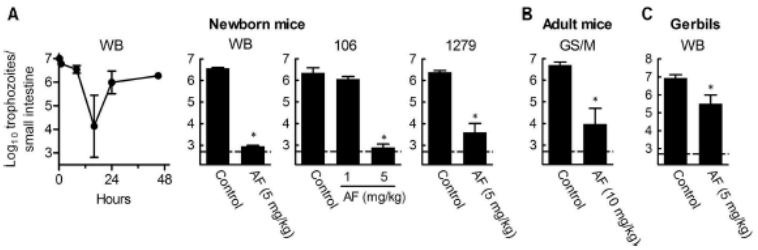


FIG 4 *In vivo* efficacy of auranofin in rodent models of giardiasis. Suckling mice (5 to 7 days old), adult mice, or adult gerbils were infected with the indicated *G. lamblia* isolates. After 2 days (mice) or 7 days (gerbils), animals were given one daily dose of auranofin (AF) for 5 days or were given solvent alone (2.5 to 25% ethanol in PBS) as a control. Afterwards, live trophozoites in the small intestine were enumerated. Data are mean + SD for 3 to 6 mice or 4 gerbils per group; $P < 0.05$ by rank sum test versus PBS-treated controls. The dashed horizontal line represents the detection limit of the assay.

Figure 11

Malaria

Malaria is a “disease caused by protozoan parasites of the genus *Plasmodium*”, with infection usually attributable to infected female *Anopheles* mosquitoes; these mosquitoes act as carriers. This disease can be caused by either *Plasmodium falciparum, vivax, ovale,* or *malariae,* also known as the “malaria parasites”, with the most series being caused by *Plasmodium falcarum* and *Plasmodium vivax* (Navarro, 2008). Presently, malaria remains a very important public health problem especially for poorer, developing nations. To combat malaria with medications, healthcare workers have employed the use of a number of organic compounds for chemotherapy--some of which includes chloroquine, hydroxychloroquine, primaquine, and amodiaquine. Chloroquine, once touted for its efficacy in treating the disease, has lost traction in combating this disease as more more “chloroquine resistant parasites emerged and spread from Asia to Africa and South America” (Navarro, 2008). Because of this, there has been a growing

need for a new re-purposed set of compounds.

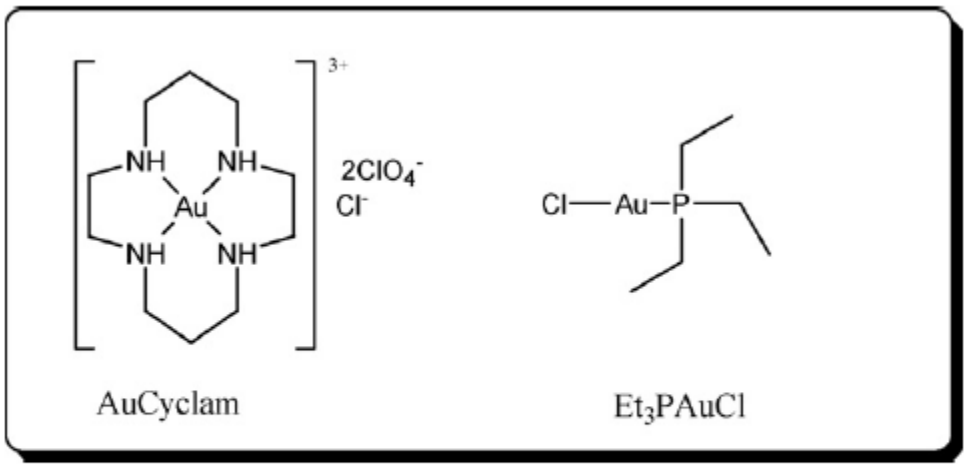


Fig. 6. Structures of Au (III) cyclam and chloro(triethylphosphine) gold (I) tested against 3D7 *P. falciparum* strain.

Figure 12

Researchers have demonstrated that metals, working synergistically with preexisting antimalarial drugs, can be used to produce "antimalarial metal agents". In 1987, scientists used "primaquine and amodiaquine as ligands in the synthesis of 32 gold compounds" all of which demonstrated activity against *P. falciparum* "to the same extent as the parental drugs" (as cited in Wash et al, 1987; obtained from Navarro, 2008).

Table 1
IC_{50} of Au (I) and Au (III) complexes against several strains of *P. falciparum*.

Compounds	IC_{50} (nM)	Strain *P.falciparum*	Reference
CQDP	50	FcB1	[43]
	110	FcB2	[42]
	70	W2	[43]
	91	K1	[43]
	10	F32	[43]
	23	D10	[53]
	352	K1	[53]
FQ	16	D10	[53]
	5	K1	[53]
Amodiaquine	100	FAN-5	[41]
Primaquine	1000	FAN-5	[41]
Au (I) compounds			
Auranofin	142	3D7	[55]
$Au(PEt_3)Cl$	21×10^2	3D7	[55]
Aurothiomalate	168×10^3	3D7	[55]
$[Au(PPh_3)(CQ)]PF_6$	5	FcB1	[42]
	40	FcB1	[43]
	23	FcB2	[42]
	70	W2	[43]
	99	K1	[43]
	18	F32	[43]
$[Au(PPh_3)(CQ)]NO_3$	40	FcB1	[43]
	40	W2	[43]
	58	K1	[43]
	10	F32	[43]
	21	D10	[53]
	62	K1	[53]
$[Au(PEt_3)(CQ)]PF_6$	10	FcB1	[43]
	50	W2	[43]
	99	K1	[43]
	13	F32	[43]
$[Au(PMe_3)(CQ)]PF_6$	40	FcB1	[43]
	50	W2	[43]
	93	K1	[43]
	17	F32	[43]
$[Au(FQ)(PPh_3)]NO_3$	11	D10	[53]
	6	K1	[53]
$[Au(L1)(PPh_3)]NO_3$	33	D10	[53]
	34	K1	[53]
$[Au(L2)(PPh_3)]NO_3$	30	D10	[53]
	31	K1	[53]
$[Au(C_6F_5)(CQ)]$	18	D10	[53]
	61	K1	[53]
$[Au(C_6F_5)(FQ)]$	10	D10	[53]
	4	K1	[53]
$[Au(C_6F_5)(L1)$	14	D10	[53]
	15	K1	[53]
$[Au(C_6F_5)(L2)]$	23	D10	[53]
	13	K1	[53]
Au (III) compounds			
$[Au(CQ)_2(Cl)_2]Cl$	43	K1	[43]
	13	F32	[43]
$[Au(CQ)(SR)(Cl)(Solv)]Cl$	54	K1	[43]
	18	F32	[43]
$KAuCl_4$	100	FAN-5	[41]
$Au(Primaquine)Cl_3$	1000	FAN-5	[41]
$Au(amodiaquine)Cl_3$	100	FAN-5	[41]
AuCyclam	439×10^3	3D7	[55]

IC_{50}: 50% inhibitory concentration; CQ: Chloroquine; SR: -thio-β-D-glucose-2,3,4,6-tetraacetate; L1: N-(7-chloro-quinolin-4-yl)-N'-[2-(N',N'-dimethylaminomethyl) ferrocenylmethyl]-ethane-1,2-diamine; L2: 3-benzyl-1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-1-[2-(N',N' dimethylaminomethyl)-ferrocenylmethyl]urea). FcB1 and FcB2: CQ-resistant Strains of *P.falciparum* [42]; FcB1, W2, and K1 strains are considered of medium CQ resistance, while F32 is CQ sensitive [43]. D10 is CQ-sensitive and K1 CQ-resistance [53].

Figure 13

When scientists combined gold compounds with chloroquine (CQ) as a ligand, there was a "strong modification of the electronic properties"; gold complex synergism with CQ or chloroquine diphosphate (CQDP) as a base created an "almost unlimited variety of possible gold-ligand fragments". The gold "complex $[Au(PPh_3)(CQ)]PF_6$ caused marked inhibition of the *in vitro* growth of *P. berghei*" and displayed effectiveness "against two chloroquine-resistant FcB1 and FcB2 strains of *P. falciparum*". Further results can be seen in the figure above.

Trypanosomiasis

Trypanosomiasis describes diseases caused by "protozoan parasites of the genus *Trypanosoma*". American trypanosomiasis, caused by *Trypanosoma cruzi*, and African trypanosomiasis, known as "sleeping sickness...caused by *Trypansoma brucei*, are two forms of diseases caused by protozoans of the genus *Trypanosoma*. "Benznidazole (nitroaromatic compounds) and nifurtimox (discontinued to the public)...have [both] proven efficient only through the acute phase", limiting usefulness of these two drugs; side effects have limited the use of these drugs even more (Navarro, 2008). African "sleeping sickness" has seen better prospects, as it can be treated by either one of four drugs, pentamide, melarsoprol, eflornithine, and suramin, "depending on the type and stage of African trypanosomiasis" (as cited in Guedes et al., 2006; obtained from Navarro, 2008). The "coordination of gold (III) to clotrimazole, producing $AuCl_3$ (CTZ)...at 10^{-5} M inhibited 60% of the proliferation of the epimastigotes of *T. cruzi* slightly higher than the free CTZ (58%) (as cited in Sánchez-Delgado et al., 1998; Navarro, 2008).

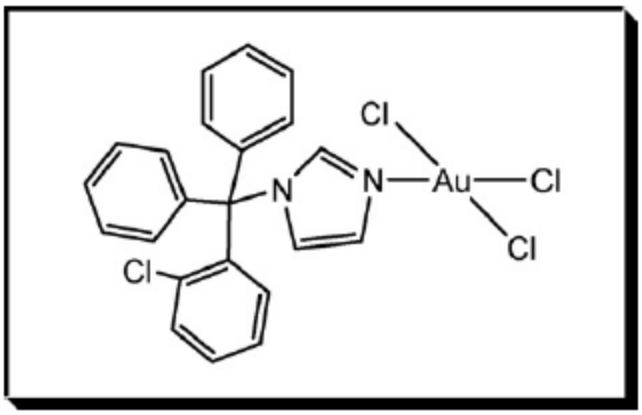


Fig. 8. The molecular structure of trichloro(clotrimazole) gold (III), active against *T. cruzi* parasite.

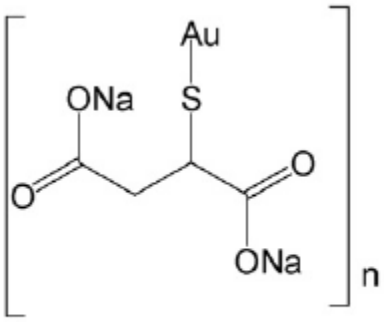
Figure 14

Leishmaniasis

Leishmaniasis is an emerging public health problem and an "uncontrolled category I disease...causing significant morbidity and mortality in Africa, Asia, and the Americas". "It is caused by flagellated protozoans of the genus *Leishmania* (Sarcomastigophora, Kinetoplastida)", and treatments, as reported by Navarro in 2008, "are far from ideal". Treatments of "prevalent antimonials (Sb(V))" as "the classic first line treatment", while effective, often "induce severe side effects"; two of the drugs used to treat this disease, sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®). These drugs "only exist in their parental forms". Other compounds have been developed for "second-line" use, when there are "unresponsive cases" (Navarro, 2008).

To find more safe and active drugs, scientists have resorted to study organometallic compounds due to findings suggesting that "metabolic pathways of kinetoplastid parasites are similar to those present in tumor cells" (as cited in Kinnamon, Steck, Rane, 1997 and Farrell, Williamson, McLaren, 1984; obtained from Navarro, 2008). To do this, the researchers used DNA metallointerclating organic compounds dppz [dipyrido[3,2-a:2',3'-c]phenazine) and dpq (dipyrido[3,2-a:2',3'-h]quinoxoline) as a ligand for metal complexes. One of the gold complexes tested, $[Au(dppz)_2]Cl_3$, was found to be "the most effective complex in the series"; it "induced a dose dependent antiproliferative effect with minimal inhibitory concentration (MIC) of 3.4 nM

and lethal doses LD_{26} of 17 nM at 48 hours". One of the forms of African leishmaniasis, kala-azar, has been seen to be effectively combated by sodium aurothiomalate when treated in 10 patients in 1987 (Navarro, 2008).



Sodium aurothiomalate

Figure 15

Use in Cancer

Gold(I) Complexes

Gold (I) atoms act as a "soft lewis acid with great affinity for soft ligands (S-thiolates and P-phosphines)...assuming a tetrahedral four-coordination, trigonal three-coordination, and linear two-coordination, the latter being the most important for medical purposes". This is possible because gold (I) "exhibits a d shell configuration" (Porchia et al., 2018). It is believed that their anticancer properties comes from "their ability to undergo facile ligand exchange with protein thiol groups, particularly those with low pK_a values" (as cited in Razi et al., 1983, Gunatillekeand & Barrios, 2006, and Krishnamurthy, et al., 2008; obtained from Yan et al., 2010).

In a study conducted by Yan et al. (2010), they found that "homoleptic d^{10} thiourea complexes represent a new paradigm in developing metal-based inhibitors of enzymes, such as [thioredoxin reductase] TrxR" and that varying ligand of metal-thiourea complexes have the potential to serve as a "new class of metal-based drug leads" (Yan et al., 2010). Gold (I) complexes have also shown great potential *in vitro* and *in vivo*. "*In vitro* cytotoxicity studies showed that the gold (I) thiourea complex $[Au^{I}(Imidazolidine)\text{-}2\text{-}thione)_2]^+$ displays potent anti-cancer activities towards a panel of cancer cell lines with IC_{50} values spanned between 3.7 and 17.4 μM" (Che & Sun, 2011). The *in vivo* study on mice conducted by Che and Sun "revealed that intraperitoneal injection of this complex at 100 mg/kg twice a week resulted in significant size reduction of the tumor of non-small lung cancer cells by 38%" (as cited in Yan et al., 2010; obtained from Che & Sun, 2011).

Gold(III) Complexes

Gold (III) acts as "a lewis acid and presents a d^8 shell configuration with a classical square planar tetra coordination", and bears isoelectronic properties similar to [platinum (II)] Pt(II) (as cited in Nobili et al., 2010; obtained from Porchia et al., 2018). Some bioactive gold (III) complexes that act as active antiproliferative agents "include tetradentate porphyrins, bidentate phenantrolines, and monodentate halides or nitrogen bonded derivatives" (as cited in Tiekink, 2008 and Casini et al., 2006; obtained from Porchia et al., 2018). From analysis of biological data, researchers found a "strong alteration of the mitochondrial membrane potential,

formation of mitochondrial membrane pores, reactive oxygen species stimulation, interaction with DNA, and apoptosis induction" (as cited in Coronnello et al., 2005; obtained from Porchia et al., 2018).

Auranofin and Cancer

Auranofin, a drug known for exhibiting anti-rheumatic activities, has been also explored for its potential as an anticancer medication; it is a "unique, linear mixed thiolato/phosphine gold (I) drug" (Porchia et al., 2018). Scientists reason that the drug may be the key to halting the progression of cancer, mainly due to the reason that it targets Thioredoxin Reductase--the "enzyme of the Thioredoxin (TRX) system that is important for maintaining the intracellular redox state" (Onodera, Momose, and Kawada, 2019). Two types of the TRX system exist within human cells: "TRX1 (encoded by the *TXN* gene) and TRX2 (encoded by the *TXN2* gene)". TRX1 transmits "oxidative signals as a molecular switch in kinase signaling pathways", while TRX2 controls the "the homeostasis of mitochondrial ROS [reactive oxygen species]"; auranofin blocks both of these systems (Onodera, Momose, and Kawada, 2019). Especially within cancer cells, researchers have noted that "inhibition [of the general TrXR enzyme] leads to an increase in cellular oxidative stress and induces apoptosis"; some cancers, such as "breast, ovarian, and lung cancers" show levels of "TrxR overexpression" (Onodera, Momose, and Kawada, 2019). Figure 12 explains how auranofin affects the TRX pathway and how it may halt cancer progression.

As cited in Madeira et al. (2012) and Nobili et al (2010), the gold-sulfur-containing compound "exhibits powerful antitumor activity in various in vitro and in vivo tumor models and has dose-dependent inhibitory activity against DNA, RNA, and protein synthesis at cytotoxic concentrations" (obtained from Onodera, Momose, and Kawada, 2019).

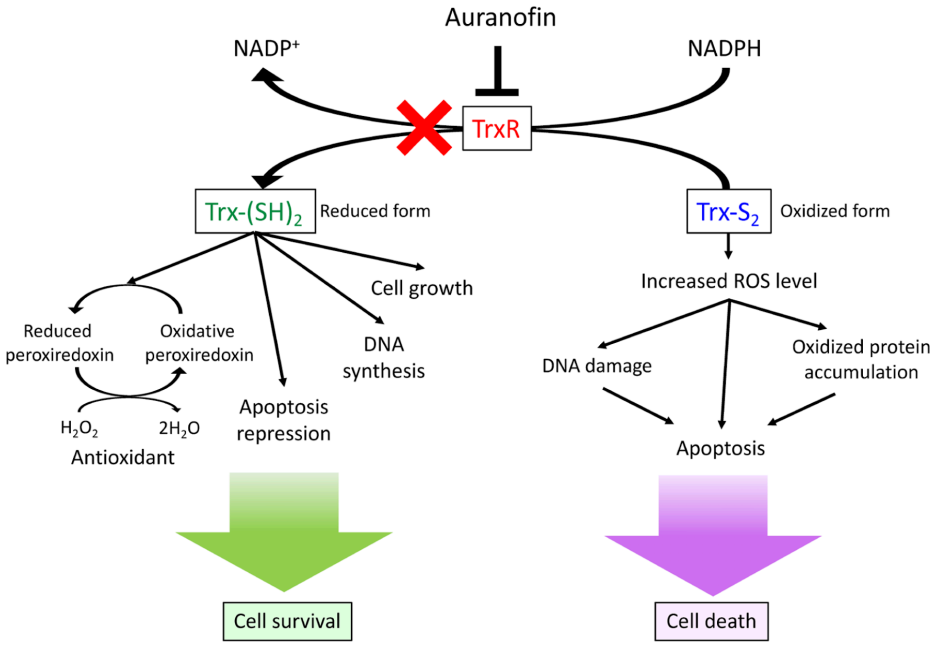


Fig. 5. Major Physiologic Functions of the Trx System in Cells

The reduced form of Trx acts as an electron donor to peroxiredoxin and ribonucleotide reductase. Peroxiredoxin scavenges H_2O_2 using Trx1 as a source of reducing equivalents. Ribonucleotide reductase reduces ribonucleotide to deoxyribonucleotide for DNA synthesis. Increasing the reduced form of Trx induces cell growth, activation of transcription factors that control gene expression, and inhibition of programmed cell death, which leads to cell survival. In contrast, auranofin-induced inhibition of TrxR leads to elevation of levels of the oxidized form of Trx, which in turn leads to an increase in intracellular oxidized substances and ROS levels, thereby inducing apoptosis. (Color figure can be accessed in the online version.)

Figure 16

Imidazole Compounds

	Carbon-Selenium Compounds	Carbon-Selenium Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds
Name	(1,3-bis[2,6-bis(propan-2-yl)phenyl]-2,3-dihydro-1H-imidazol-2-ylidene)-(1,3-dimethylimidazolidine-2-selenone)-gold hexafluorophosphate	(1,3-bis[2,6-bis(propan-2-yl)phenyl]-2,3-dihydro-1H-imidazol-2-ylidene)-(1,3-dimethylimidazolidine-2-selenone)-gold hexafluorophosphate	dichloro-(1,1'-pyridine-2,6-diylbis(3-isopropyl-1,3-dihydro-2H-imidazole-2-thione))-gold(iii) chloride methanol solvate monohydrate	(m2-Pyrrolidine-1-carbodithioato)-(m2-1,1'-methylene bis(3-n-butylimidazol-1-yl-2-ylidene))-di-gold bromide ethanol solvate	bis[1,3-bis(4-chlorophenyl)imidazolidine-2-thione]-gold trifluoromethanesulfonate n-hexane solvate
Bond Length between C and R	2.016 å	2.016 å	1st: 1.725 å 2nd: 1.678 å	1st: 4.277å 2nd: 4.286 å	1st: 1.724º å 2nd: 1.74 å (On 2nd Au connection) 3rd: 1.714 å 4th: 1.714 å
Angles Created by Bond between C and R	176.77º	176.77º	1st: 32.24º 2nd: 31.19º	1st: 167.25º 2nd: 171.17º	1st: 30.82º 2nd: 30.82º (On 2nd Au connection) 3rd: 30.65º 4th: 30.65º

	Gold-Selenium Compounds	Gold-Selenium Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds
Name	(1,3-bis[2,6-bis(propan-2-yl)phenyl]-2,3-dihydro-1H-imidazol-2-ylidene)-(1,3-dimethylimidazolidine-2-selenone)-gold hexafluorophosphate	(1,3-bis[2,6-bis(propan-2-yl)phenyl]-2,3-dihydro-1H-imidazol-2-ylidene)-(1,3-dimethylimidazolidine-2-selenone)-gold hexafluorophosphate	dichloro-(1,1'-pyridine-2,6-diylbis(3-isopropyl-1,3-dihydro-2H-imidazole-2-thione))-gold(iii) chloride methanol solvate monohydrate	(m2-Pyrrolidine-1-carbodithioato)-(m2-1,1'-methylene bis(3-n-butylimidazol-1-yl-2-ylidene))-di-gold bromide ethanol solvate	bis[1,3-bis(4-chlorophenyl)imidazolidine-2-thione]-gold trifluoromethanesulfonate n-hexane solvate
Bond Length between Au and R	2.407 å	2.407 å	1st: 2.340 å 2nd: 2.333 å	1st: 2.291 å 2nd: 2.295 å	1st: 2.289 å 2nd: 2.289 å (On 2nd Au connection) 3rd: 2.281 å 4th: 2.281 å
Angles Created by Bond between Au and R	1.76º	1.76º	1st: 46.36º 2nd: 46.06º	1st: 6.79º 2nd: 4.71º	1st: 42.86º 2nd: 42.86º (On 2nd Au connection) 3rd: 42.73º 4th: 42.73º

Pyrimidine Compounds

	Carbon-Selenium Compounds	Carbon-Selenium Compounds	Carbon-Selenium Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds	Carbon-Sulfur Compounds
Name	(pyrimidine-2-selenolato)-(triphenylphosphine)-gold	(4,6-dimethylpyrimidine-2-selenolato)-(triphenylphosphine)-gold	(4,6-Dimethylpyrimidine-2-selenolato)-triethylphosphine-gold	(6-Mercaptopurine)-triphenylphosphino-gold(i) ethanol solvate	(Purine-6-thiolato)-triphenylphosphine-gold(i) ethanol solvate	Chloro-(2-((N,N-dimethylammonium)methyl)phenyl)-(6-mercaptopurinato)-gold(iii) chloride	6-Mercaptopurinato-tricyclohexylphosphine-gold(i) ethanol solvate	tris(o-Tolyl)phosphine-(purine-6-thiolato)-gold(i) ethanol solvate
Bond Length between C and R	1.884 å	1.915 å	1.884 å	1.728 å	1.738 å	1.747 å	1.742 å	1.716 å
Angles Created by Bond between C and R	35.67º	35.95º	32.97º	31.07º	31.07º	34.53º	31.57º	31.32º

	Gold-Selenium Compounds	Gold-Selenium Compounds	Gold-Selenium Compounds	Gold-Sulfur Compounds	Gold-Sulfur Compounds	Gold-Sulfur Compounds	Gold-Sulfur Compounds	Gold-Sulfur Compounds
Name	(pyrimidine-2-selenolato)-(triphenylphosphine)-gold	(4,6-dimethylpyrimidine-2-selenolato)-(triphenylphosphine)-gold	(4,6-Dimethylpyrimidine-2-selenolato)-triethylphosphine-gold	(6-Mercaptopurine)-triphenylphosphino-gold(i) ethanol solvate	(Purine-6-thiolato)-triphenylphosphine-gold(i) ethanol solvate	Chloro-(2-((N,N-dimethylammonium)methyl)phenyl)-(6-mercaptopurinato)-gold(iii) chloride	6-Mercaptopurinato-tricyclohexylphosphine-gold(i) ethanol solvate	tris(o-Tolyl)phosphine-(purine-6-thiolato)-gold(i) ethanol solvate
Bond Length between Au and R	2.375 å	2.412 å	2.417 å	2.286 å	2.311å	2.328 å	2.316 å	2.266 å
Angles Created by Bond between Au and R	47.31º	47.69º	44.28º	43.07º	43.31º	49.06º	44.12º	43.35º

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Figures

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Figure 5-Abutaleb, N., S. & Seleem, M., N. Auranofin, at clinically achievable dose, protects mice and prevents recurrence from *Clostridioides difficile* infection. *Sci. Rep.* **2020**, *10.*

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Table:

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