Bacheloroppgave

NTNU
Norges teknisk-naturvitenskapelige universitet
Fakultet for naturvitenskap
Institutt for kjemi

Andreas Hegland Simensen

Development of Gold Auranofin Analogues against Multidrug Resistant Bacteria Including *Neisseria Gonorrhoeae*

Bacheloroppgave i Bachelor i Kjemi
Veileder: Odd Reidar Gautun
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Development of Gold Auranofin Analogues against Multidrug Resistant Bacteria Including *Neisseria Gonorrhoeae*

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Abstract

This thesis will present a review of recent literature on Auranofin, a United States Food and Drug Administration (FDA) approved gold based anti arthritic drug. Auranofin drug might be of interest for repurposing as an antibacterial agent. This is as it has been proven to have potent inhibition of multiple multi drug-resistant (MDR) gram positive bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is the reason is the reason for half of all deaths from multidrug resistance (MDR) bacteria in the United States. Auranofin lacks activity against most Gram negative bacteria, but has been found to be effective against *Neisseria gonorrhoeae* (NG). NG is the causes of the sexually transmitted disease (STD) gonorrhea. NG has been prone to development of resistance against many classes of antibiotics, and there is an urgent need for new novel treatments of the disease. In addition, recent research on analogues of Auranofin, that are more active towards Gram negative bacteria, and also lowering the cytotoxity will be presented.

Sammendrag

Denne oppgaven vil presentere en gjennomgang av nyere forskning på medikamentet Auranofin,som ble godkjent av det amerikanske legemiddelverket (FDA) som et antiaritmetisk legemiddel. Auranofin er ett gull basert medikament som har er fordi det har vist seg å være effektivt i behandlig av flere multi-legemiddelresistente (MDR) Gram-positive bakterier, inkludert Meticillin-resistente *Staphylococcus aureus* (MRSA). MRSA er årlig grunn i over halvparten av alle dødsfall på grunn av multiresistente (MDR) bakterier i USA. Auranofin mangler aktivitet mot de fleste Gram negative bakterier, men det har vist seg at det er effektiv mot *Neisseria gonorrhoeae* (NG). NG er baktereien som gir den seksuelt overførbare sykdommen gonoré. NG har vært utsatt for utvikling av resistens mot antibiotika, og det er et et prekært behov for nye nye behandlinger av sykdommen. I tillegg vil det blir sett næreme på forsjellige analoger av Auranofin, som er både mer aktive mot Gram negative bakterier, dreper bakterer bedre, og har lavere cytotoksisitet.

Contents

Symbols and abbreviation

AMR	Antimicrobial resistance
Cys	Cysteine
DMARD	Disease-modifying antirheumatic drugs
FDA	U.S. Food and Drug Administration
HAT	Human African trypanosomiasis
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NG	*Neisseria gonorrhoeae*
NTDs	Neglected tropical diseases
PMBN	Polymyxin B nonapeptide
SAR	Structure-Activity Relationship
STD	Sexually transmitted disease
TrxR	Thioredoxin reductase
Trx	Thioredoxin
USD	United States dollar
WHO	World Health Organization

1 Introduction

Alexander Fleming's coincidental discovery of the β-lactam *penicillin* in 1928 is considered to be the first antibiotic[1, 2]. His discovery revolutionized the entire medicinal industry[1], and kick started what is often referred to as the golden age in antibiotic research[2] as there was a rapid discovery of new novel classes of antimicrobial agents. It peaked in the mid 1950s[3] before a sudden halt at the beginning of the 1960s[2], with the last new class of broad spectrum antibiotics, quinolones[2], first introduced all the way back in 1962[4]. As is visualized in Figure 1.1

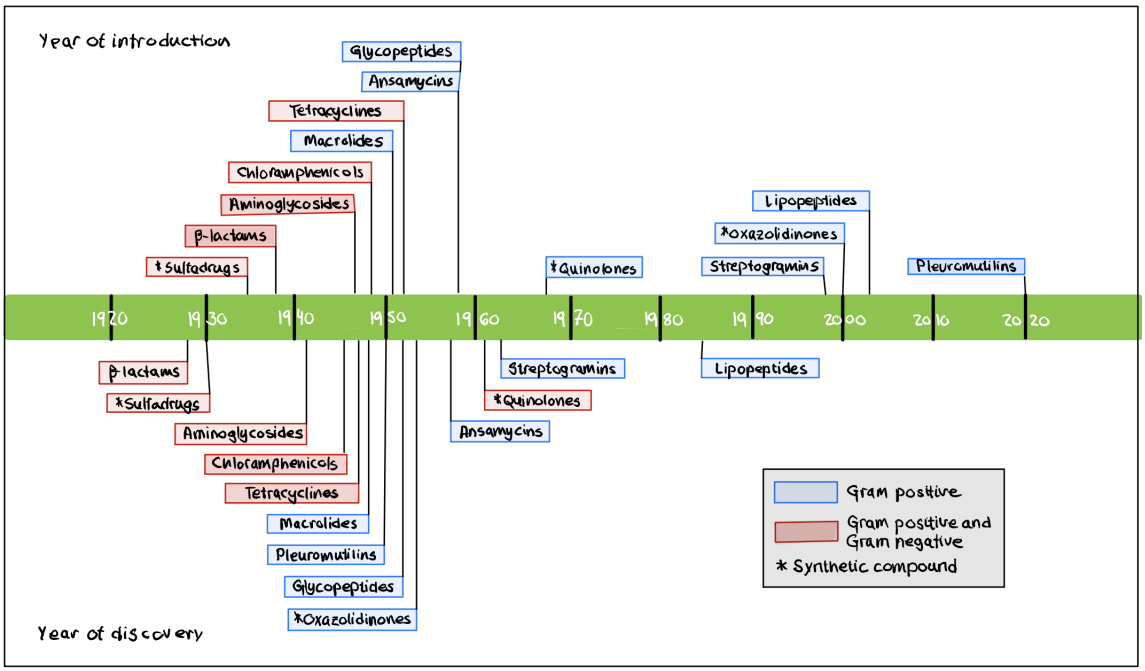

Figure 1.1: Timeline of discovery and introduction of novel classes of antibiotics. Adapted from Lewis[2].

Today there is a grave need for novel classes of antibiotics as abuse and overuse of antibiotics, especially in agriculture, has resulted in surging numbers of antimicrobial resistance (AMR)[5]. The situation for Gram negative pathogens is especially dire [6]. In fact, the emergence of multi drug resistant superbugs and microbes is characterized by World Health Organization (WHO) as one of the gravest threats towards the humanity in current day[7]. But the economic incentives lack, as development of new drugs is both incredibly time consuming and costly. Any new antibiotic would only be reserved for last case scenario where

no other treatment works, giving little profit. The result is a steady decline in development pipeline of new antibiotics[8, 9].

This has led to the screening of already approved drugs for potential repurposing. This process could significantly reduce both the cost and time needed for development of new antimicrobials[8, 9]. In such a screening process for agents against the intestinal parasite *Entamoeba histolytica*, a protozoan leading to approximately 70 000 deaths each year, it was found that Auranofin **(1)** (see Figure 1.2) was ten times more effective than metronidazole, the current treatment[10]. Auranofin is a drug approved by The United States Food and Drug Administration (FDA) in 1985 for treatment of rheumatoid arthritis[10]. Further investigations have shown that auranofin is active against several gram positive bacteria, and with modification can also become active towards gram negative bacteria[11].

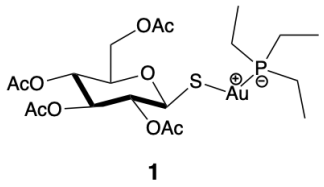

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Figure 1.2: Structure of Auranofin **(1)**

1.1 Objective

The objective of this thesis it to precent recent published research on Auranofin and analogues as an antibacterial agent. Focus will lay on how modifications of their structures can boost activity towards both Gram positive and Gram negative bacteria, but also the cytotoxicity towards different pathogens. The treatment of gonorrhea with Auranofin will be presented as an example.

2 Theory

2.1 Antibiotics

At the turn of the 20th century microbiology was in a golden age with many breakthroughs, leading to a greater interest for this new emerging field.

At the same time there was a great need for handling the tropical diseases in the European colonies, like malaria and Human African trypanosomiasis (HAT) commonly referred to as sleeping sickness[12]. Both diseases are a result of Vector-borne protozoans[13], transferred by malaria mosquitos and tsetse flies[12]. Especially HAT got a of lot attention after a huge endemic that started in 1901 around Lake Victoria in current day Uganda[12]. By 1905 the disease had killed approximately one third of the population in the area.

This resulted in a lot of research into ways of treating the diseases. German Paul Ehrlich (1854 - 1915) and Kiyoshi Shiga (1871-1957) started screening synthetic dyes for bio-activity against protozoans[14] in search of what Ehrlich called a "Zauberkugel" or "Magic bullet"[15], an agent that would be highly parasitotropic, but not organotropic. Meaning that it would have affinity towards just the wanted target, and not the rest of the tissue in the host[14, 15].

In 1905 it was proven that the arsenical drug Atoxyl® was active against *trypanosoma*[15], and it was used in treatment of HAT. The drug needed to be distributed in such high dosages that there was significant risk of damage to the nerve system, resulting in blindness[14]. Following this discovery they started looking into other arcenicals, and in 1907 discovered arsphenamine. In 1909 was proven to be active against *Treponema pallidum* spirochete, which causes the sexually transmitted disease (STD) syphilis. At this time syphilis resembled somewhat how HIV/AIDS impact the society today[14]. By this discovery Ehrlich had indeed found his "Zauberkugel". Arsphenamine was marketed under brand name Salvarsan®, when it came to market in 1910 and was until the 1940s the most sold drug in the world[12, 15].

The history about Alexander Fleming and penicillin is already presented in the introduction (Section 1), and will not be presented in more detail here.

2.1.1 Gram-stain

Bacteria is commonly categorized into one of two categories after the Gram stain test. The bacteria is categorized either as a Gram positive(+), or Gram negative(-)[16]. The difference between the two lies in the structure of cell wall. Gram positive cells have a inner membrane, keeping the cytoplasm in, and a peptidoglycan layer outside the membrane(see Figure 2.1). Gram negative cells does also have a inner membrane, and a peptidoglycan layer, but the peptidoglycanlayer is much thinner, and in additional to the piptidoglycan layer there is an extra outer membrane surrounding the molecule. The outer membrane often also contain lipopolysaccharides. (Figure 2.1).

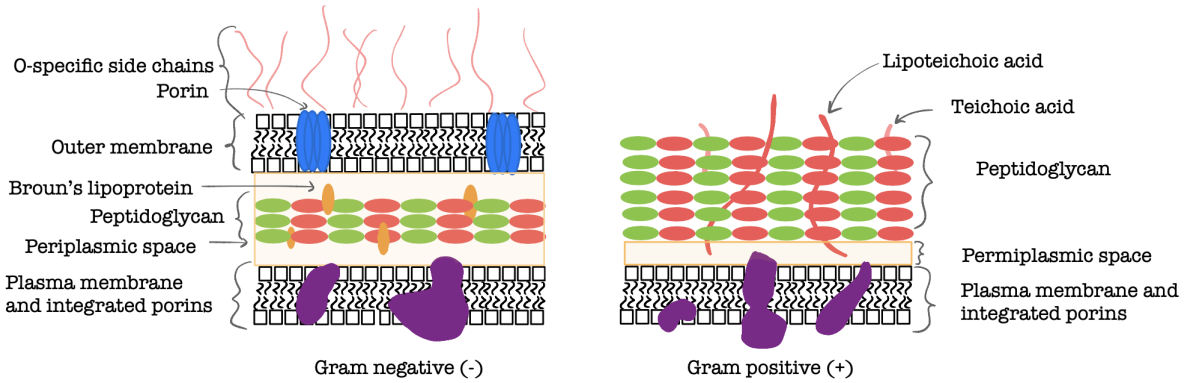

Figure 2.1: A simple illustration of a Gram negative cell wall to the left, and a Gram positive cell wall to the right. Adapted from Atanasova[17].

This outer membrane of Gram negative bacteria makes it harder for antibiotics to permit through the cell wall in to the cytoplasm[18]. The outer membrane does contain porins allowing molecules to pass by, but the structure makes it hard[19]. It is generally harder for agents to permit through the double membrane of Gram negative bacteria, resulting in less agents that are effective. The drugs that do work on both Gram positive and Gram negative is often referred to as broad spectrum antibiotics[20].

2.1.2 Antibiotic resistance, and the current deadlock

The challenges concerning AMR is escalating[5]. Bacterial cultures has the ability not only to transfer its DNA to the next generation[21], but also to other bacterial colonies, of whom

it is not related. The common transport method for DNA is thru what commonly is called horizontal transmission of genomes and DNA material. This is made possible by conjugative plasmids. This ability puts all bacteria essentially into what can be considered as one large gene pool, with the ability of transferring methods of inhibition, when certain species mutate to become resistant, towards different medicines and chemicals.

In addition there is only a limited number of natural compounds[8, 9], and a majority of the natural products have been explored, making it harder to come over new good candidates. In this way it can be said that there is a deadlock.

A 2020 study[22] of the cost of developing new drugs, based on publicly available data of FDA approved drugs in between the years of 2009 and 2018, concluded with a median price of USD 1.1 billion. Including the expenditures of expected failed drugs[22]. The expected development time is 15 years until launch[9].

One possible way of lowering the cost and time needed for development of new drugs could be to repurpose drugs already approved for human use. This is especially relevant for phase I and phase II of clinical trails, as the safety and pharmacokinetics of the drug is already known[9]. Against neglected tropical deceases (NTDs) this method might be especially important. Phase IV clinical trails, with an estimated cost of USD 100 million in 2007, would be impossible in the undeveloped world without the proper health infrastructure and financials to undertake such studies[9].

It was reported all the way back in 1999 about the inhibiting effect of Auranofin against gram positive *Novelli* et al.[23] against Gram positive *Pseudomonas aeruginosa*. But but the article did not get a lot of citations[24].

It was not until multiple reports of Auranofins activity towards multiple parasites, like Debnath et al.'s discovery of auranofin to be effective against the protozoan *Entamoeba histolytica* sparked the interest of further investigations into the drug[10]. It was than found to also have strong inhibiting powers against many multiresistent Gram positive bacteria.

2.2 Auranofin

Auranofin **(1)** is a disease-modifying antirheumatic drug (DMARD) that was approved by the (FDA) in 1985[25] for use in treatment against rheumatoid arthritis. It has been off patent since 1992[26]. The drug is orally administered[6], and helps to suppress the symptoms and therefore also helps slowing down the progression of the disease[27]. **1** was first discovered by Sutton et al. in 1972[28], and is sold under the brand name Ridaura®[24]. At first **1** looked like a greate new treatment as it was simple to administer, but it lacked the effectiveness of other gold contaning compounds like Aurothiomalate **(2)** and Aurothioglucose **(3)** that is injected intramuscularly[27]. See Figure 2.2. The current day first-line treatment/therapy is the drug Methotrexate[29]. **1** is today still in use, but only in a few rare cases[30].

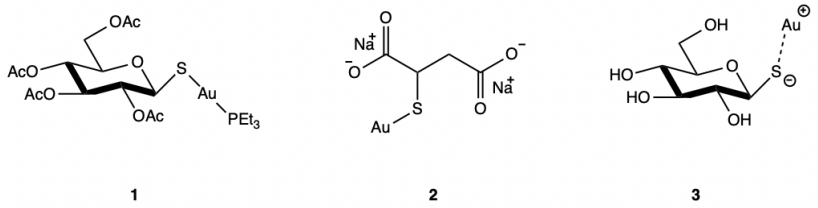

Figure 2.2: Structure of Auranofin **(1)**, Aurothiomalate **(2)** and Aurothioglucose **(3)**

Auranofin (**1**) is a monomeric metall-organic aurous complex[6] consisting of Au(I) coordinated to an peracylated β-D-glucopyranosato thiole and a soft phospine thriethyle ligand witch stabilizes the complex[6, 31]. The systematic name of **1** is (2,3,4,6-tetra-*O*-acetyl-1-thio-β-d-glucopyranosato-*S*) (triethylphosphine) gold (I)[27]. A synthetic pathway of **1** can be found in Figure 2.3.

Auranofin (**1**) is not only of interest for reposition as an antibacterial agent, but also for treatment against neurodegenerative disorders like Parkinson's and Alzheimer's, HIV/AIDS and multiple types of cancer including leukemia and lymphoma[25]. In fact **1** has and is undergoing multiple phase I and II clinical trails for use against multiple types of parasites[32, 33] and cancer types[34, 35]. There has also been completed a clinical trail against HIV[36].

2.2.1 Synthesis of Auranofin

A totalsyntesis of **1** from D-glucose is presented in Figure 2.3.

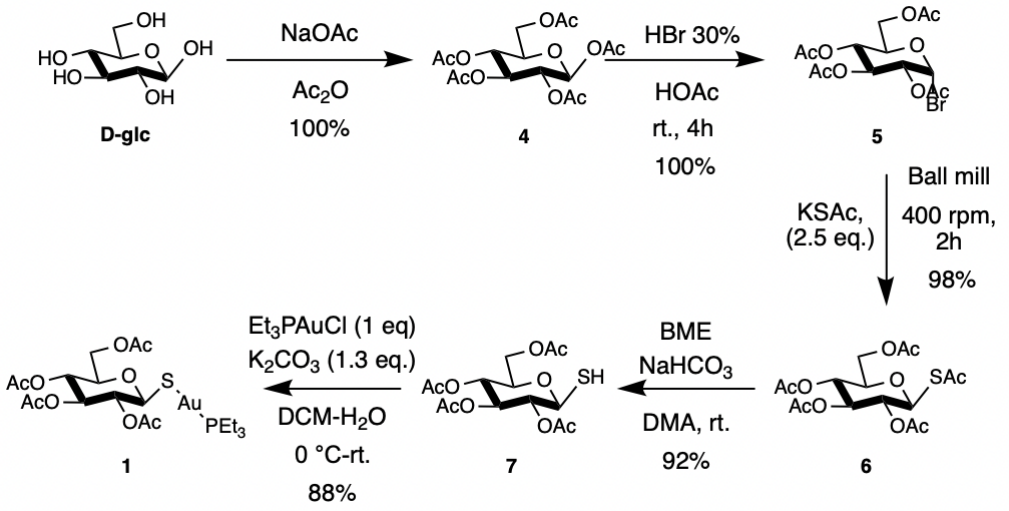

Figure 2.3: An example total syntesis of auranofin (**1**) from β - D - glucose. Adapted from Michihata et al.[37], Ferrari et al.[38], Patil et al.[39] and Shu et al.[40]

Compound **4** can be produced by adding powder β-D-glucose slowly to a mixture of acetic anhydride (Ac_2O) and sodium acetate (NaOAc) in refluxing 100% yield[37]. **5** can then be produced by reacting **4** with a 30% HBr for four hours at room temperature in glacial acetic acid in 100 % yield[38]. After that **6** can be synthesized without the use of any solvent. This was done by adding **5** and 2.5 mole equivalent of KSAc to a ball mill, and mixed for 2 hours at 400 rpm. After two hours, the product was recrystallized in Et_2O-*n*-Hex yielding **6** in 98% yield[39]. Thiol **7** was then produced by mixing **6** with 2-mercaptoethanol (BME) and 0.1 equivalent of $NaHCO_3$ at room temperature in 92% yield. The final step to product **1** was done by mixing **7** and Et_3PAuCl in dichloromethane (DCM). The solution was stirred at 0 - 5 °C and K_2CO_3 was added. This afforded 1 in 88% yield[40]. This gives **1** in an overall yield of 79% over 5 steps.

2.3 Biological activety of Auranofin

The exact way Auranofin (**1**) kills bacteria is not fully understood[6], but it is believed that **1** have multiple modes of action. **1** has high affinity towards sulfur and selenium[26].

When administered, **1** crosses into the undergo a ligands exchange, where the thiosugar of **1** is excanged for the sulphure of a the cysteine residue Cys34 of serum albumin in the bloodplasma[26, 41], and than transported by the blood stream through the body to the target[26]. It is approximately 25 % of the gold administered that is absorbed, with a peak blood concentration after 60 - 120 minutes[31]. The gold complexes has a half-life of 11 to 31.3 days in the plasma[26].

1 main mode of action is by interfering with the enzyme thioredoxin reductase (TrxR)[42], and thereby inhibiting the thioredoxin system (TrxR/Trx). The thioredoxin system is found in most types of cells[43], form simple prokaryotes cells like bacteria to mammal cells. The system contains thioredoxin reductase (TrxR), thioredoxin (Trx) and NADPH[44], and is essential for the cells ability to protect against oxidative stress and. In smaller cells like prokaryotes[43], the TrxR protein is an disulfide/dithole that is redox active.

One of the important roles of TrxR/Trx system is to maintain proteins in the cells at a reduced state[43], which is done by the TrxR enzyme with electrons from the oxidation of NADPH to $NADPH^{+}$. This is important for the cells ability to defend against oxidizing conditions. The other main function of the TrxR/Trx system is helping keeping the redox potential inside the cell low, by protonating sulphur containing proteins. A figure of the TrxR/Trx system is presented in Figure 2.4. These mechanisms gives it a central role in the cells ability to defend itself against reactive oxygen species, DNA synthesis and protein folding[42]. Some, more advanced cells do also inherit the glutathione pathway containing glutathione reductase, glutathione and NADPH, and is a pathway that is overlapping with the TrxR/Trx, and thereby makes the cells less susceptible to **1** as it acts as a backup backup[42, 45].

Some bacteria depends on the stickland fermentation for metabolism[46]. The stickland fermentation reaction[47], is a redox reaction, in which two amino acids react. This reaction is catalyzed by seleonoenzymes[46], to which auranofin has high affinity. When **1** is administered, and enters the cell, a stable seleno-auranofin complex is formed, inhibiting the

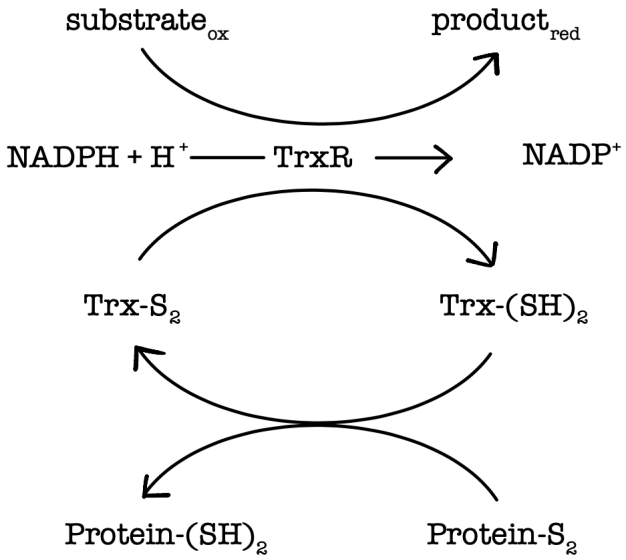

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Figure 2.4: The thioredoxin reductase pathway. Adapted from Arnér & Holmgren[43]

metabolism, resulting in the cell dying. A putative structure of the complex formed between Auranofin and the selenium is presented in Figure 2.5.

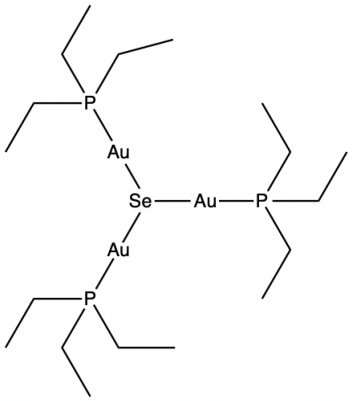

Figure 2.5: A putative structure of the stabile seleno-auranofin complex formed when Auranofin interacts with selenium containing selenoproteins in cells. Adapted from Jackson-Rosario Et al.[46]

3 Discussion

3.1 Antibacterial

Hokai et al. found in 2014[24] that **1** was quite effective against the Gram positive bacteria methicillin-resistant *Staphylococcus aureus* (MRSA)[24]. MRSA is responsible for almost half of all deaths in the United States from antibiotic resistant bacteria[11],

Thangamani et al. concluded in a 2015 study[11] that **1** was inhibiting many Gram positive bacteria[11], but lacked effectiveness against Gram negative bacteria, as the drug had trouble passing through the outer membrane of the bacteria. Thangamani et al. were able to significantly improve the inhibiting power of *1* by adding Polymyxin B nonapeptide (PMBN). PMBN is a cationic peptide that increase the hydrophobic antibiotic permeability of the cell wall of gram negative bacteria[48].

3.2 Modifications of Auranofin

In 2018 Marzo et al. publish a Structure-Activity Relationship (SAR) study[30], in which Auranofin (**1**) and different gold halide salt analogues of **1** on the form $[Au(PEt_3)X]$, X = Cl, Br or I (**8** - **10**) was tested. It was also tested with gold coordinated to the stronger ligand CN^- (**12**). These structures can be seen in Figure 3.1. In addition to this it was also tested with swapping the gold (I) metal core ion with silver (I), on the halides giving the form $[Ag(PEt_3)X]$ (**13** - **15**) and a negatively charged $[Ag(PEt_3)_2]NO_3$ (**16**) analogue. As can be seen in Figure 3.2. All analogues was also tested with the addition of PMBN against the Gram negative strains. The analogues were tested against 14 Gram positive strains, and five Gram negative strains, many of them being multi drug resistant.

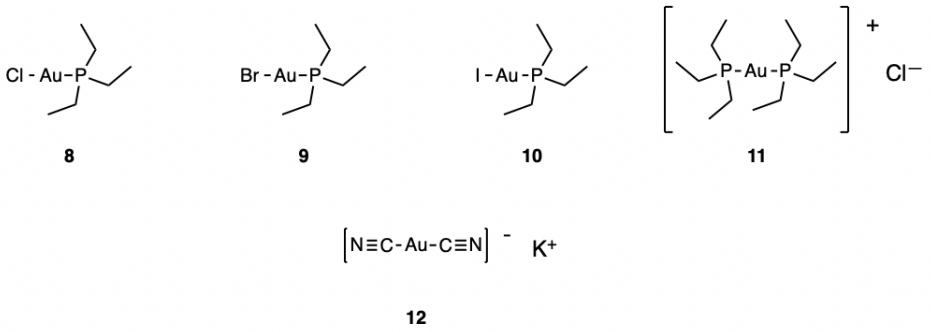

Figure 3.1: Structure analogues **8** - **10** containing Auranofin analogues with the thiole glucose changed with differet halides. **11** is a analogue with Au(I) coordinated to two triethylphosphine groups. **12** contain a center Au(I) ion coordinated to two cyanogroups.

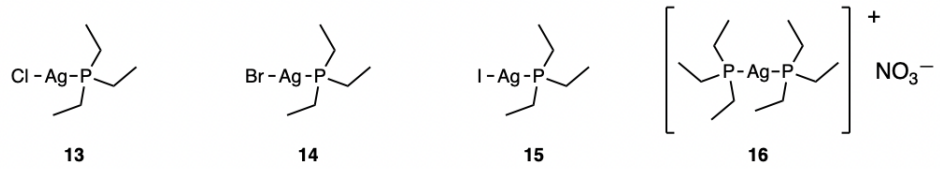

Figure 3.2: Analogues **13** - **16** containing a silver (I) center which in **13** - **15** is coordinated to a triethylphosphine and a halide. In **16**, the silver (I) center ion coordinated to two triethylphosphine, and a has a chloride counterion.

Marzo et al.[30] found **1** to work excellent against all Gram positive strains with MIC values below 1 μM. The only exception was the fungus *C. albicans*, where the effect was lower, with MIC value >11.80 μM. Against the Gram negative, **1** was lacking effect, but with addition of PMBN, **1** became active. This is all in content with what is previous reported by Thangamani et al. al[11]. The halide salts (**8** - **10**) were found to be active both towards the Gram negative and the Gram positive strains[30]. Against the Gram positive strains the results were a little improved, but quite similar compared to **1**. Towards the Gram negative strains on the other hand, the halides outperformed **1** without PMBN. The difference between the halides were minimal. Reasoned in this Marzo et al. concluded that the true pharmacophore of **1** is $[Au(PEt_3)]^+$, with the thioglucose playing a minimal role in the active inhibition, from the ease of dissociation of the thio/halide ligand. This was further indicated by the dicyano analogue **12** which got substantially higher MICs than **1** and the

halides against most pathogens tested. It was proposed that this was either because the cyano groups binds stronger to the gold (I) center ion, or that it was because of the charge of the molecule. When PMBN was added, there was an approximate twofold improvement of the MIC's against most of the pathogens. This is not a greater improvement compared to **1** and **8** - **10** which had for many of the strains an upwards of a tenfold increase in activity. This might also indicate that the cyano groups in fact is too tightly bound to the gold to be able to dissociate, and enabling the gold to interact.

It is not mentioned by Marzo et al.[30], but it can be seen from Table 3.1 that even though the halides (**8** - **10**) performance were basically equal, there was a minimal improvement. Compared to the bromo ligand, the larger and less tightly bound iodo ligand had a minimal increase in activety, while the smaller and more tightly bound chloro ligand had a small decrease in activety. This further supports Marzo et al.'s claim that $[Au(PEt_3)]^+$ is indeed the pharmacophore, and that the dissociation of the ligand is essential for the activity of the compound.

Analogue **11** containing two triethylphosphine ligands performed similarly to the halides containing a single triethylphosphine against the gram positive, and against the gram negatives when PMBN was added, as can be seen in table 3.1. This reasons for

Elements in the same group in the periodic table often contain many of the same characteristics. Because of this it might be interesting to test silver or copper as a substitute for gold to see if this might give any improvement in activity. Marzo et al. tested this, with substituting gold (I) in analogues **8** - **10**, with silver (I) giving analogues **13** - **15** (see Figure 3.2)[30]. When MIC values were compared it was found that swapping Au(I) with Ag(I) significantly decreased the activity, as can be seen when comparing MIC values of Table 3.1 with Table 3.2. Gold (I) complexes (**8** - **10**) showed MIC values below 1 μM against all Gram positive bacterias, except against *C. albicans* as previously discussed. Silver (I) complexes (**13** - **15**) were much more inconsistent, with MIC values ranging from 0.41 μM for **14** against *Staphylococcus epidermidis* (FI-1), to >30.60 μM for **13** against *Candida albicans* (FI-12). **16** did perform considerably worse compared to the halides. It was proposed by Marzo et al.[30] that the cause the decrease of in activity for the silver analogues might come from the stability of the complex. NMR analysis done after incubation in **Mueller Hinton Broth**

showed that there is a rapid reaction between the silver analogues, amino acids and peptides, where mainly the halides, but also phosphine to some extent are exchanged mainly with sulfur containing peptide chains and proteins[30]. For the Gram negative species the results were pretty much the same, but the addition of PMBN did not result in any significant improvements. It was proposed that this was a result of the rapid reaction with the amino acids and peptides present in the test.

Table 3.1: MIC [μM] values for analogues **1** and **8** - **12** with and without addition of PMBN. Gram positive are marked with (+), and Gram neagtive are marked with (-). Strains: a, *S. aureus* (ATCC 25923); b, *S. epidermidis* (FI-1); c, *E. faecalis* (ATCC 29212); d, *E. faecalis* (FI- 2); e, *E. faecalis* (FI-3); f, *E. faecalis* (FI-4); g, *E. faecium* (FI-5); h, *E. faecium* (FI-6); i, *S. salivarius* (FI-7); j, *S. parasanguinis* (FI-8); k, *S. pyogenes* (FI-9); l, *C. striatum* (FI-10); m, *C. striatum* (FI-11); n, *C. albicans* (FI-12); o, *E. coli* (CV287); p, *K. pneumoniae* (KKBO4); q, *P. aeruginosa* (Vr143/97); r, *A. baumannii* (FI-13); s, *S. maltophilia* (FI-14). Adapted from Table 1 and Table 2 in Marzo et al.[30]

Strain	**1**	**1** + PMBN	**8**	**8** + PMBN	**9**	**9** + PMBN	**10**	**10** + PMBN	**11**	**11** + PMBN	**12**	**12** + PMBN
a (+)	0.09	-	≤0.18	-	≤0.16	-	≤0.14	-	≤0.13	-	27.77	-
b (+)	0.09	-	≤0.18	-	≤0.16	-	≤0.14	-	≤0.13	-	1.73	-
c (+)	≤0.09	-	≤0.18	-	≤0.16	-	0.14	-	≤0.13	-	0.87	-
d (+)	0.09	-	≤0.18	-	≤0.16	-	≤0.14	-	≤0.13	-	1.73	-
e (+)	0.37	-	≤0.18	-	≤0.16	-	0.14	-	≤0.13	-	1.73	-
f (+)	0.18	-	≤0.18	-	≤0.16	-	0.14	-	≤0.13	-	0.87	-
g (+)	0.09	-	≤0.18	-	0.16	-	0.14	-	≤0.13	-	6.94	-
h (+)	0.02	-	≤0.18	-	0.16	-	0.14	-	≤0.13	-	6.94	-
i (+)	0.09	-	≤0.18	-	0.16	-	0.14	-	≤0.13	-	3.47	-
j (+)	0.09	-	≤0.18	-	0.32	-	0.14	-	≤0.13	-	1.73	-
k (+)	0.18	-	≤0.18	-	≤0.16	-	0.28	-	0.27	-	0.21	-
l (+)	≤0.09	-	≤0.18	-	≤0.16	-	≤0.14	-	≤0.13	-	6.94	-
m (+)	≤0.09	-	≤0.18	-	≤0.16	-	≤0.14	-	≤0.13	-	27.77	-
n	>11.80	-	5.70	-	10.12	-	18.10	-	>17.07	-	>27.77	-
o (-)	>47.09	1.47	11.41	2.85	10.12	1.26	9.05	1.13	34.13	1.07	55.54	27.77
p (-)	>47.09	11.77	11.41	5.70	10.12	5.06	9.05	4.52	34.13	2.13	55.54	27.77
q (-)	>47.09	≤0.37	>22.82	0.36	>20.25	0.32	>18.10	0.56	>64.27	≤0.53	55.54	3.74
r (-)	>47.09	2.94	11.41	1.43	10.12	1.26	9.05	1.13	17.07	1.07	55.54	27.77
s (-)	>23.55	1.47	11.41	1.43	10.12	1.26	9.05	1.13	17.07	0.53	27.77	13.88

Wu et al. published in 2019 a SAR study where they tested over 40 different gold analogues of **1** against a control specie of gram negative *Escherichia coli* in addition to the ESKAPE

Table 3.2: MIC [μM] values for analogues **13** - **16** with and without addition of PMBN. Gram positive are marked with (+), and Gram neagtive are marked with (-). Strains: a, *S. aureus* (ATCC 25923); b, *S. epidermidis* (FI-1); c, *E. faecalis* (ATCC 29212); d, *E. faecalis* (FI- 2); e, *E. faecalis* (FI-3); f, *E. faecalis* (FI-4); g, *E. faecium* (FI-5); h, *E. faecium* (FI-6); i, *S. salivarius* (FI-7); j, *S. parasanguinis* (FI-8); k, *S. pyogenes* (FI-9); l, *C. striatum* (FI-10); m, *C. striatum* (FI-11); n, *C. albicans* (FI-12); o, *E. coli* (CV287); p, *K. pneumoniae* (KKBO4); q, *P. aeruginosa* (Vr143/97); r, *A. baumannii* (FI-13); s, *S. maltophilia* (FI-14). Adapted from Table 3 and Table 4 in Marzo et al.[30]

Strain	**13**	**13** + PMBN	**14**	**14** + PMBN	**15**	**15** + PMBN	**16**	**16** + PMBN
a (+)	0.96	-	0.82	-	1.42	-	16.72	-
b (+)	0.48	-	0.41	-	1.42	-	8.36	-
c (+)	1.91	-	1.63	-	2.83	-	16.72	-
d (+)	0.48	-	0.41	-	1.42	-	16.72	-
e (+)	0.48	-	0.82	-	0.71	-	16.72	-
f (+)	0.96	-	1.63	-	0.71	-	16.72	-
g (+)	1.91	-	1.63	-	2.83	-	16.72	-
h (+)	1.91	-	1.63	-	2.83	-	16.72	-
i (+)	30.60	-	13.07	-	11.33	-	16.72	-
j (+)	30.60	-	26.15	-	22.68	-	>16.72	-
k (+)	30.60	-	26.15	-	22.68	-	>16.72	-
l (+)	0.48	-	0.41	-	0.71	-	0.26	-
m (+)	0.48	-	0.41	-	0.71	-	0.26	-
n	>30.60	-	>26.15	-	>22.68	-	>16.72	-
o (-)	7.56	3.82	6.54	3.27	11.33	5.67	19.70	19.70
p (-)	7.56	7.56	6.56	6.54	11.33	11.33	19.70	19.70
q (-)	3.82	1.91	3.27	1.63	5.67	1.42	19.70	2.46
r (-)	1.91	1.91	1.63	1.63	5.67	2.83	4.92	4.92
s (-)	1.91	0.96	1.63	0.82	2.83	1.42	2.46	2.46

pathogens[6]. The ESKAPE pathogens is a group of bacterias (***E**nterococcus faecium*, ***S**taphylococcus aureus*, ***K**lebsiella pneumoniae*, ***A**cinetobacter baumannii*, ***P**seudomonas aeruginosa*, ***E**nterobacter species*[49]), were WHO deemed the situation and need for new antimicrobial agents especially dire in a 2017 report[49]. In addition to this Wu et al. tested the cytotoxicity of the analogues against A549 human cells and found most analogues to have lower cytotoxicity than **1**[6].

Wu et al. found that **1** effectively inhibited the two Gram positive bacterias *S. aureus* and *E. faecium*, with MICs of 0.03μM and 0.12μM[6]. The inhibition could be changed by modification of the molecule. By swapping out the ligands coordinated to the the gold (I) they were able to increase the activity. This can be seen in Table 3.3 and Table 3.4. They also concluded that especially the thiol ligand plays an essential role for both the activity and the cytotoxicity of the molecule. This is not in accordance with what was reported by Marzo et al. that stated that the thiosugar ligand does not play a significant role for the activity[30]. Wu et al.[6] found that triethylphosphine performed better that the two other tested, trimethylphosphine and tri-*n*-butylphosphine ligands also tested. See table **??**.

Against *E. faecium* the most effective thio ligands tested were found to be the aromatics, with electron donating substituent of NH_2 (**17**, **18**)) and MeO (**19**) in addition to electron withdrawing the F_3C (**21**)) (see Figure 3.3)[6]. The MIC values as low as 0.02 μM, one eight of that of **1**. Mic values for all different aromatic thioligands tested can be found in Table 3.3.

Against *S. aureus* the best results were obtained for **17**, **20**, **22**, **23**, and **25**, with **25** (see Figure 3.3 and Figure 3.4) having MIC levels as low as 0.003/0.006 μM[6]. See Table 3.3 for full data set.

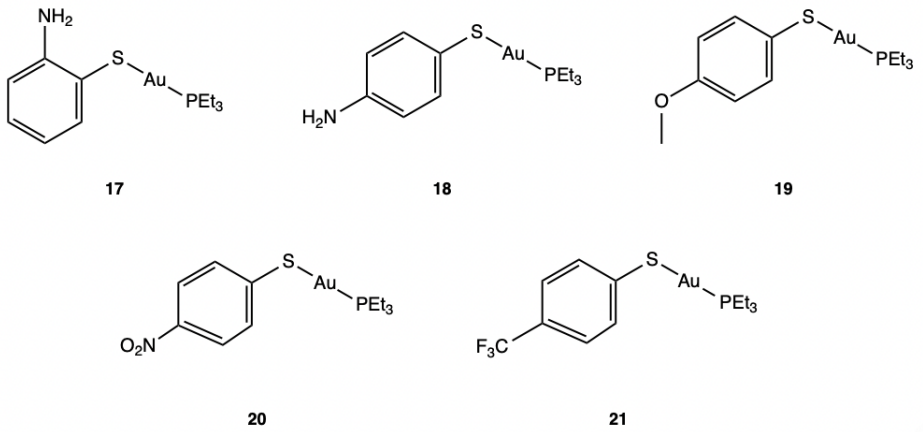

Figure 3.3: Auranofin analogues containing aryl phosphines ligands. Adapted from Wu et al.[6]

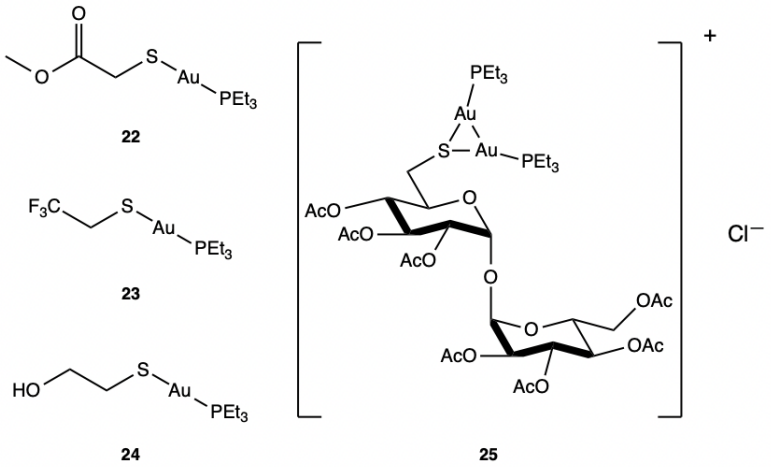

Figure 3.4: Selection of auranofin analogues containing aliphatic phosphine ligands with high inhibition against *S. aureus*. Adapted from Wu et al.[6]

Table 3.3: MIC values [μM] for a selection of auranofin analogues that were prepared and tested by Wu et al.[6], and found to perform well. Structures are presented in Figure 3.3 and Figure 3.4. Gram positive strains are marked with (+), and Gram negative strains are marked with (-). Adapted from figure 1 and 2 of Wu et al[6].

	E. faecium (+) **ATCC 700221**	*S. aureus* (+) **JE2 (USA300)**	*K. pneumoniae* (-) **ATCC 700603**	*A. baumannii* (-) **NCTC 13420**	*P. aeruginosa* (-) **NCTC 13437**	*E. cloacae* (-) **NCTC 13405**	*E. coli* (-) **ATCC 25922**	log p
1	0.2/0.09	0.04	377	47	377	189	24	0.56
17	0.02	0.009	146	18/36	291	18/37	36	0.61
18	0.02	0.07/0.02	36	18	146	36	9	1.36
19	0.02/0.1	0.02	141	35	282	141	35	2.01
20	0.06/0.1	0.0004/0.02	>546	>546	>546	>546	>546	>3.28
21	0.02	0.02/0.1	>520	520	>520	>520	>520	>3.18
22	0.1/0.3	0.005/0.02	76	19	305	38	76	1.75
23	0.6	0.009	149	37/74	149	47	74	2.08
24	0.2/0.3	0.03	20	10	41	5/10	10	1.19
25	0.05	0.003/0.006	>194	12	>194	24	24	-0.28

Against the Gram negative bacteria Wu et al. found that the phosphine ligand play an important role in the activity of the molecule[6]. The aliphatic phosphines performed much better than the aromatic phosfines. It was tested with aryls containing both electron donating and electron withdrawing substituents with minimal change in activity. It was also test with triarylphosphines coordinated to an Au(I) center ione, coordinated to both β-D-thioglucose

and chloride, but they were all lacking inhibiting powers. For the aliphatic compounds, trimethylphosphine, triethylphosphine and tri-*n*-butylphosphine ligands were tested, also with Au(I) coordinated to both β-D-peracylatedthioglucose (**26**, **1**, **27**) and chloride (**28**, **8**, **29**) (See Figure 3.5). Wu et al. found a general correlation between the activity and the size of the alkyl group, where methyl (**26** and **28**) performed better than triethyl (**1** and **8**), which again performed better than the worst of three tested, the tri-*n*-butylphosphine ligand (**27**, **29**). MIC values of the different compound can be found in Table 3.3, and Table 3.4.

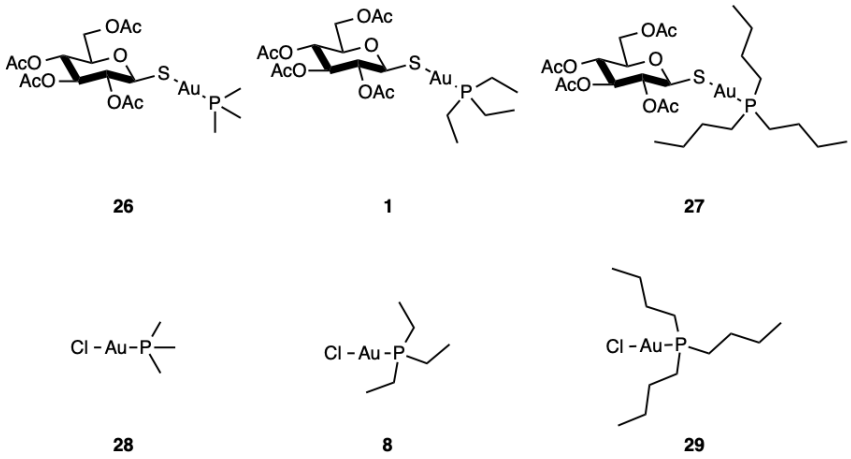

Figure 3.5: Auranofin analogues with aliphatic phosphine substituents of different length coordinated to thiosugar gold(I) (**26**, **1** and **27**), and cloro gold(I) (**28**, **8** and **29**). Adapted from Wu et al.[6]

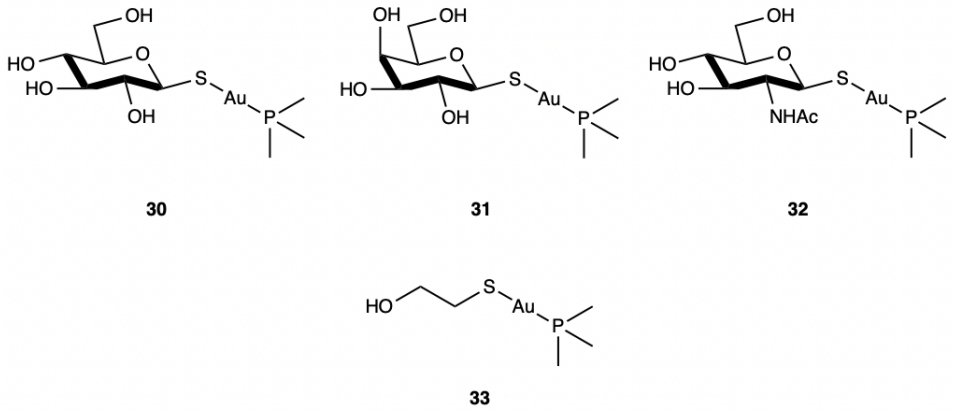

Figure 3.6: Auranofin analogues **30** - **32** containing deacylated sugars, and **33** with a mercaptoethanol ligand. All are coordiantede to a trimethylphosphine group. Adapted from Wu et al.[6]

Wu et al. found that some of the tested thio groups containing different aromatics and aliphatics performed well against *K. pneumoniae*(see Table 3.3[6], while the different sugar structures that were tested did not give any improvement. **33** performed the best, with a MIC of 11 μM. That gives a 40 fold improvement over **1** which was rather ineffective with a MIC of 377 μM.

Against *A. baumannii* Wu et al. found the best performing Auranofin analogues were the ones containing a trimetylphosfine (see table 3.4 and 3.3)[6], while modifications to sugar, or addition of aromatic or aliphatic groups to the phenol had little effect. The best performing analogue was **26** and **33**, with respective MIC values of 6/13 μM for and 6/3 μM, compared to 47 μM for **1**. Wu et al. tested two parallels, and got different vales. Thereby the double value.

P. aeruginosa was the odd one out, compared to the other Gram negative bacterias tested by Wu et al.[6] They concluded that the most important ligand for the activity against *P. aeruginosa* was the thiole. The major contribution for its activity was expected to be the lipophilicity of the the analogue. The deacylated sugar analogue **30** - **32**, with Log P values of <0, was all inactive against *P. aeruginosa* (see Table 3.4), but had quite improved MICs for all the other Gram negative bactera compared to that of **1**. As table 3.4 shows, the best performing auranofin analogue against *P. aeruginosa* were the mercapoethanole **24**, with a MIC of 41 μM, an approximate nine-fold improvement over **1** with a MIC of 377 μM.

The activity of the analogues tested against the *E. cloacae* strain by Wu et al.[6] were found to be dependent on the structure of the phosphine ligand, but also to be dependent on the thio group. More so than for the other gram negative bacteria tested. For the phosphine ligand, short alkyl chain of methyl was the most efficient. For the thiole ligand, aromatic groups with deactivating substituents like NO_2 (**20**) and CF_3 (**21**), was found to be less inhibiting than **1**. Other than that, all other thioles tested was found to improve the activity, with the best performing analogue being **33**, with a MIC of 3 μM, an 65 fold improvement over **1** with MIC of 189 μM.

Table 3.4: MIC values [μM] for a selection of analogues that was found by Wu et al.[6] to perform well. The Structures are presented in Figure3.5 and Figure 3.6. Gram positive strains are marked with (+), and Gram negative strains are marked with (-). Adapted from Table 3 and Table 4 of Wu et al[6].

	E. faecium (+) **ATCC 700221**	*S. aureus* (+) **JE2 (USA300)**	*K. pneumoniae* (-) **ATCC 700603**	*A. baumannii* (-) **NCTC 13420**	*P. aeruginosa* (-) **NCTC 13437**	*E. cloacae* (-) **NCTC 13405**	*E. coli* (-) **ATCC 25922**	Log P
26	0.2/0.4	0.09	13	6/13	101	3	6	0.35
1	0.2/0.09	0.04	377	47	377	189	24	0.56
27	3/5	3	>336	84	>336	>366	>366	>4.32
28	0.4	0.1/0.2	7/13	7/13	52	3	7	0.16
8	0.09/0.2	0.001/0.02	46	11/23	92	23	23	1.74
29	5	2	147	74/147	74	>589	>589	>3.99
30	0.3/0.5	0.3/0.5	34	17/4	>547	4/9	9(9)	-1.63
31	0.3/0.5	0.3	34	17/9	>547	4	9	-1.88
32	0.2/0.5	0.5	31	16/8	>503	8/16	8/31	-2.03
33	0.3	0.3	11	6/3	23/91	3	1/6	-0.15

3.3 *Neisseria gonorrhoeae*

Since the first classes of antibiotics came on the market[50], there have been struggles with the gram negative bacteria *Neisseria gonorrhea* (NG) responsible for the sexually transmitted disease (STD) Gonorrhoea developing resistance. There is currently species of NG with resistance towards all major classes of antibiotics[51], including Cefriaxone, the current drug of choice. And with the emergence of multi drug resistant strains, the situation and need for new anti gonnoccol agents is especially dire.

Foerster et al. conducted in 2019 a screening of a library of 1200 FDA approved agents (Prestwick Chemical Library)[52]. The agents were tested against the three reference strains of NG *N. gonorrhoeae* WHO F, *N. gonorrhoeae* O and *N. gonorrhoeae* P. They found 68 compounds with inhibiting powers, and one of them was Auranofin (**1**). **1** was found to have a MIC of 1 μg/mL for all tested strains, and with a rapid inhibition of the bacteria with concentrations above that of the MIC in a time-kill assay. While they got the same MIC values for all strains, this was not the result of the time-kill assay. Here they found more varying results with the highest effect against the WHO F strain.

Yang et al. published a paper in 2022[53] where they tested **1** against 575 different strains,

including multi resistant strains. While Foerster et al.[52] got MIC values of 1 μg/mL for **1** against all tested strains, Yang et al. found only nine of the tested strains to have a MIC value of 1 μg/mL. Here 94 % of the tested strains were found to have MIC values between 0.25 μg/mL and 0.5 μg/mL.

Elkashif et al. posted in 2020[50] a paper, where three gold containing compounds Auranofin (**1**), sodium Aurothiomalate (**2**) and Aurothioglucose (**3**) (see Figure 2.2) where tested against 48 different strains of NG, including 5 WHO reference strains. Auranofin (**1**) was also tested in combination with Azithromycin, Tetracycline, Cefixime and Ciprofloxacin. In addition to testing the the different compounds against NG, they also tested different isolates of *Lactobacillus*[50]. Lactobacillus are Gram positive bacteria, that forms a protective biofilm in the female genital tract and vagina, and is in a symbiotic relationship with the body, and helps to protect against infections, including NG[50, 54].

Of the analogues tested by Elkashif et al.[50], **1** was found to the most effective, with MICs in the range of 0.03 μg/mL to 0.25 μg/mL. The second most effective was **2** with MICs between 0.06 μg/mL to 32 μg/mL, and the least effective being **3** with MIC values from 0.125 μg/mL to 16 μg/mL.

As development of ressistance is a huge problem with NG, it is normal to use a dual treatment of two antibiotics. This is normally a mix of Azithromycin and Ciprofloxacin. This is a method of slowing down the progression of the mutations, as there is a higher success rate of treatment. Elkashif et al.[50] performed tests with **1** in combination with the antibiotics Azithromycin, Tetracycline, Cefixime and Ciprofloxacin. They found that the combination worked will, with increased inhibition for all four tested antibiotics in combination with **1**.

Another study that also concluded that **1** is well suited for combination treatment was published by Yu et al. in 2022[55]. In this a combination of **1** and the antiprotozoal drug pentamidine was tested against multiple Gram negative bacteria. While NG was not included in this study, it was found that the combination of the two drugs significantly increased the activity, especially against *K. pneumoniae*. It was found that the drug combination enhanced the permeability of **1**, and thereby improving its effectiveness against Gram negative pathogens.

Anyhow, the testing of **1**, **2** and **3** against different *lactobacillus* by Elkashif et al.[50], found that while **1** inhibited growth, **2** and **3** did not, with MICs of >128 μg/mL against all strains tested.

4 Conclusion

The research assessed in this thesis show promising potential for Auranofin (**1**) as a new antibiotic, with high potency against many Gram positive, bacteria including MRSA. In addition to this, (**1**) has proven to be potent against many strains of *Neisseria gonorrhoeae*, a bacteria where the need for novel treatment methods is especially dire. As (**1**) already have been used in the treatment of rheumatoid arthritis in almost 40 years, the drug is well tested and the profile and safety of the drug is well known. This makes potential reprising faster and cheaper.

In addition to the promising outlooks for **1**, SAR studies have found that gold containing analogues of **1** in some cases increase the inhibiting powers, while also lowering the cytotoxicity. It has been found that the pharmacophore of **1** is most probably the phosphine ligand in addition to the gold center ion.

For activity towards Gram positive bacteria, a trietylphosphine ligand is best suited. Towards Gram negative bacteria, a trimetylphosphine ligand is preferred. In addition to this, the thiol group is important for the susceptibility, with aromatic thio ligands performing best against Gram positive bacteria.

Against Gram positive bacteria gold (I) should be coordinated to a trietylphosphine ligand and an aromatic thio ligand as this gave best results. For treatment of Gram negative bacteria, gold (I) should be coordinated with trimetylphosphine and an aliphatic thiole.

The overall best performing Auranofin analogue investigated in this thesis is **33**, tested by Wu et al., found to be the best inhibitor of *K. pneumoniae A. baumannii* and *E. cloacae*, with the biggest improvement against *E. cloacae* with an 65 fold improvement over (**1**) lowering the MIC from 189 μM to 3μM.

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