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Auranofin and Related Heterometallic Gold(I)-Thiolates as Potent Inhibitors of Methicillin-Resistant *Staphylococcus aureus* Bacterial Strains

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Abstract

A series of new heterometallic gold(I) thiolates containing ferrocenyl-phoshines were synthesized. Their antimicrobial properties were studied and compared to that of FDA-approved drug, auranofin (Ridaura), prescribed for the treatment of rheumatoid arthritis. MIC in the order of one digit micromolar were found for most of the compounds against Gram-positive bacteria *S. aureus* and CA MRSA strains US300 and US400. Remarkably, auranofin inhibited *S. aureus*, US300 and US400 in the order of 150–300 nM. This is the first time that the potent inhibitory effect of auranofin on MRSA strains has been described. The effects of a selected heterometallic compound and auranofin were also studied in a non-tumorigenic human embryonic kidney cell line (HEK-293).

Keywords

Antimicrobial; auranofin; heterometallic gold-thiolates; MRSA

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Appendix A. Supplementary Material

Crystallographic tables for compounds **3a** and **3d**, drawing for the crystal structure of **3a**, DFT calculations on molecules **4a** and **4d** ^{1}H, $^{31}P\{^{1}H\}$ and $^{13}C\{^{1}H\}$ NMR ($CDCl_3$) spectra for all new compounds, ^{1}H and $^{31}P\{^{1}H\}$ NMR (DMSO-d^6) spectra overtime for selected compounds (**4a, 3b, 3c, 4d** and **4e**), and MS spectra for all new compounds. DOI: …….

1. Introduction

During the past two decades there has been a renewed interest in the study of the biological activities and potential medicinal applications of gold compounds [1]. In particular, gold(I) compounds have been thoroughly explored as cytotoxic agents [2]. Many of the compounds reported contain thiolates and/or phosphines as ligands [3,4] and their mode of action seems to be based on thioredoxin reductase inhibition [5]. Some of these gold(I) thiolates (e.g. aurothiomalate and auranofin (AF) in Figure 1) are used therapeutically [6] and belong to the group of disease-modifying antirheumatic drugs (DMARDs) used to slow down or stop the progression of this rheumatic disorder. Auranofin (approved by the FDA in 1985 and commercialized under the brand name of Ridaura) has been granted orphan-drug status and its potential medicinal applications have been reviewed recently [7].

Recent reports indicate that AF is able to effectively kill the *parasit*es *Schistosoma mansoni* [8], *Trypanosoma crucei* [9], *Echinococcus granulosusm* [10], *Plasmodium falciparum* [11], *Leishmania infantinum* [12] and *Giardia lamblia* [13]. Last year, the potential of AF for the treatment of amebiasis was described [14]. In this report it was suggested that AF targets *Entamoeba histolytica* thioredoxin reductase activity [14]. In comparison, the antibacterial activities of gold(I) derivatives have been less explored [7]. Some reports on the effect of AF on *Clostridium difficile* and *Treponema denticola* in vitro have shown that disruption of the selenium metabolism is implied in the growth inhibition observed [15,16]. Both *C. difficile* and *T. denticola* utilize selenoproteins for energy. The growth inhibition effect of AF on *Staphylococcus aureus* had been reported before [17], but surprisingly this article has not been cited in any of these recent articles and reviews.

While *S. aureus* infections (staph) can be effectively treated with penicillinase-resistant β-lactam antibiotics (methicillin, *oxacillin*), resistant strains of *S. aureus*, such as the Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, are very common. Hospital associated infections caused by MRSA (HA MRSA) are a major problem in hospitals, prisons and nursing homes around the world. Patients with open wounds, invasive devices, and weakened *immune systems* are at great risk *of infection*. However, in the 1990s community associated MRSA (CA MRSA) infections emerged in persons in which the healthcare associated risk factors were generally absent. Two of the best described CA MRSA strains are US300 and US400. The first line treatment for MRSA infections is the glycopeptide antibiotic vancomycin, although vancomycin-resistant strains of S. aureus (VRSA) exist and reports of these strains are on the rise globally. Additional effective antibiotics for both HA and CA MSRA bacteria, which are currently considered a serious public health threat in the US [18], are urgently needed.

In our group we have explored the antimicrobial properties of different gold(I)-phosphine compounds, including some heterometallic gold(I)-silver(I) and gold(I)-copper(I) derivatives [19,20]. The heterometallic compounds Au_2-Ag were more active than the parent monometallic fragments against Gram-negative (*S. typhimurium* and *E. coli*) and Gram-positive bacteria (*B. cereus* and *S. aureus*) with MIC ranging from 1 to 10 μg/mL [20]. More recently, we have described the potential of gold(III) and palladium(II) heterometallic complexes ($FeAu_2$ and $FePd_2$) based on ferrocenyl iminophosphorane ligands as anticancer

agents through zinc finger protein poly-(adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP-1) inhibition. [21] Ferrocene has special properties such as low toxicity, high lipophilicity and unique electrochemical behavior which makes it an attractive motif in drug design [22].

In this context, we report here on the synthesis, characterization and antimicrobial properties of heterometallic $FeAu_2$ and FeAu thiolate gold(I) complexes containing ferrocenyl phosphines (scheme 1). Their antimicrobial properties have been studied on Gram-negative bacteria (*E. coli* and *P. aeruginosa*), Gram-positive bacteria (including *S. aureus* and MRSA US300 and US400 strains) and yeast (*S. cerevisiae*) and compared to that of AF.

2. Experimental

2.1. Materials and methods

All manipulations involving air-free syntheses were performed using standard Schlenk-line techniques under a nitrogen atmosphere or in a glove-box MBraun MOD System. Solvents were purified by use of a PureSolv purification unit from Innovative Technology, Inc. Auranofin was purchased from Enzo LifeSciences, thiophenol (a) was purchased from Alfa Aesar, 2-propanethiol (b) and 2-mercaptothiazoline (d) were purchased from Acros Organic, 1-thio-beta-D-glucose tetraacetate (c) was purchased from Sigma-Aldrich and 6-mercaptopurine monohydrate (e) was purchased from MP Biochemicals. The compound $[(Cp\text{-}PPh_2)_2Fe]$ 1,1'-Bis-diphenylphosphino-ferrocene (DPPF) was purchased from Strem chemicals and used without further purification. [AuCl(tht)] [23], $[\{AuCl\}_2(\mu\text{-DPPF})]$ (**1**) [24] and $[(Cp\text{-}PPh_2)Fe(Cp)]$ diphenylphosphino-ferrocene (MPPF) [25] were prepared by reported methods. $[\{AuSPh\}_2(\mu\text{-DPPF})]$ (**3a**) was prepared by a modification of a previously reported procedure [26], more detailed spectroscopic and crystallographic data for this published compound are included here. Purity of the compounds is based on elemental analysis and in all cases is ≥99.5%. Elemental analyses were performed on a Perkin Elmer 2400 CHNS/O Analyzer, Series II. NMR spectra were recorded in a Bruker AV400 (^{1}H NMR at 400MHz, ^{13}C NMR at 100.6 MHz, ^{31}P NMR at 161.9 MHz). Chemical shifts (δ) are given in ppm using $CDCl_3$ or DMSO-d^6 as solvent, unless otherwise stated. ^{1}H and ^{13}C chemical shifts were measured relative to solvent peaks considering TMS (tetramethylsilane) = 0 ppm; $^{31}P\{^1H\}$ was externally referenced to H3PO4 (85%). MNova was the report software package used for NMR spectra analysis. Infrared spectra (4000-250 cm^{-1}) were recorded on a Nicolet 6700 FT-IR spectrophotometer on KBr pellets. Mass spectra (ESI-MS and HR-ESI MS) were performed on an Agilent Analyzer or a Bruker Analyzer. X-ray collection was performed at room temperature on a Kappa CCD diffractometer using graphite monochromated Mo-Kα radiation (λ=0.71073 Å).

2.2. Synthesis and characterization of the new compounds

[{AuCl}(MPPF)] (2)—MPPF (0.738 g, 2 mmol) was added to a solution of [AuCl(tht)] (0.641 g, 2 mmol) in 40 mL of dichloromethane at 0°C. The mixture was stirred for 20 minutes at room temperature and then the solvent was removed to ca. 3 mL. The addition of 5 mL of cold diethyl ether afforded a yellow-brown precipitate that was isolated by filtration. Yield: 1.142 g, 95%. Anal. Calc. for $C_{22}H1_9AuClFeP$ (602.63): C, 43.85; H, 3.18.

Found: C, 44.19; H, 3.28. MS(ESI+) [m/z]: 567.0 [M − Cl]$^+$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 28.46. ^1H NMR (CDCl3): δ 7.60 (4H, m, C_6H_5-$_{orto}$), δ 7.48 (6H, m, $C_6H_{5\text{-meta/para}}$), δ 4.60 (2H, s, C_5H_4), δ 4.38 (2H, s, C_5H_4), δ 4.22 (5H, s, C_5H_5). $^{13}C\{^1H\}$ NMR (CDCl3): δ 133.5 (d, J_{PC} = 13.5 Hz, C_6H_5), δ 131.6 (s, C_6H_5), δ 130.9 (d, J_{PC} = 63.5 Hz, C_6H_5), δ 128.8 (d, J_{PC} = 11.8 Hz, C_6H_5), δ 73.4 (d, J_{PC} = 14.07 Hz, C5H4), δ 72.5 (d, J_{PC} = 9.6 Hz, C_5H_4), δ 70.2 (s, C_5H_5). IR (cm^{-1}): v335 (Au−Cl).

[{AuSR}$_2$(DPPF)] (3a-3e) and [{AuSR}(MPPF)] (4a-4e)—A mixture of the thiols (**a-e**) (0.2 mmol) and of K_2CO_3 (2.0 mmol) in 30 mL of dichloromethane was stirred for 10 minutes at room temperature. The corresponding gold complex, either [{AuCl}$_2$(DPPF)] (**1**) (0.1 mmol) or [AuCl(MPPF)] (**2**) (0.2 mmol), was added to the solution. The reaction mixture was stirred during 2 h at room temperature. The solution was subsequently filtered through celite to remove any excess reagents and undesired side-products. The solvent was removed to dryness to afford the expected gold thiolate complex. **3a (**ref 4): Yield: 0.132 g, 57%. $^{31}P\{^1H\}$ NMR (DMSO-d^6): δ 33.0. **3b**: Yield: 0.146 g, 66%. Anal. Calc. for $C_{40}H_{42}Au_2FeP_2S_2$ (1,098.62): C, 43.73; H, 3.85; S, 5.84. Found: C, 43.29; H, 3.78, S, 5.91. MS(ESI+) [m/z]: 1024.1 [M - S^{i}Pr]$^+$. $^{31}P\{^1H\}$ NMR (DMSO-d^6): δ 32.21. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 33.58. ^1H NMR (plus COSY, plus NOESY-2D, CDCl3): δ 7.48 (20H, m, C_6H_5), δ 4.78 (4H, s, C_5H_4), δ 4.25 (4H, s, C_5H_4), δ 3.73 (2H, s, C*H*$(CH_3)_2$), δ 1.51 (12H, d, $^3J_{HH}$ = 6.4 Hz, CH(C*H*$_3)_2$). $^{13}C\{^1H\}$ NMR (plus HSQC, $CDCl_3$): δ 133.5 (d, J_{PC} = 14.0 Hz, C_6H_5), δ 131.4 (d, J_{PC} = 56.4 Hz, C_6H_5), δ 131.4 (s, C_6H_5), δ 128.9 (d, J_{pc} = 11.3 Hz, C_6H_5), δ 75.1 (d, J_{PC} = 8.1 Hz, C_5H_4), 74.5 (d, J_{PC} = 13.1 Hz, C_5H_4) 72.1 (d, J_{PC} = 63.3 Hz, C_5H_4), δ 34.4 (s, *C*H$(CH_3)_2$),), δ 30.9 (s, CH(*C*H$_3)_2$).

3c: Yield: 0.206 g, 61%. Anal. Calc. for $C_{62}H_{66}Au_2FeO_{18}P_2S_2$ (1,6743.57): C, 44.46; H, 3.97; S, 3.83. Found: C, 44.93; H, 4.20, S, 3.30. MS(ESI+) [m/z]: 1311.1 [M − S(β-D-glucose tetraacetate)]+. $^{31}P\{^1H\}$ NMR (DMSO-d^6): δ 32.52. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 32.94. ^1H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 7.50 (20H, m, C_6H_5), δ 5.20 (8H, m, $H_{b,c,d,e}$-β-D-glucose tetraacetate), δ 4.93 (2H, s, C_5H_4), δ 4.87 (2H, s, C_5H_4), δ 4.36 (2H, s, C_5H_4), δ 4.30 (2H, m, H_g-β-D-glucose tetraacetate), δ 4.28 (2H, s, C_5H_4), δ 4.13 (2H, m, H_g-β-D-glucose tetraacetate), δ 3.81 (2H, m, H_f-β-D-glucose tetraacetate), δ 2.10 (6H, s, H_h-β-D-glucose tetraacetate), δ 2.04 (6H, s, H_h-β-D-glucose tetraacetate), δ 1.98 (6H, s, H_h-β-D-glucose tetraacetate), δ 1.89 (6H, s, H_h-β-D-glucose tetraacetate). $^{13}C\{^1H\}$ NMR (plus HSQC, plus HMBC, CDCl3): δ 170.7, 170.2, 169.8, 169.5 (s, C=O), δ 133.6 (m, C_6H_5), δ 131.5 (m, C_6H_5), δ 131.0 (m, C_6H_5), δ 129.0 (m, C_6H_5), δ 83.4, 77.9, 74.2, 69.0 (s, $C_{b,c,d,e}$-β-D-glucose tetraacetate), δ 75.8 (s, C_f-β-D-glucose tetraacetate), δ 75.1 (m, C_5H_4), δ 71.7 (d, J_{PC} = 65.05 Hz, C_5H_4), δ 62.9 (s, C_g-β-D-glucose tetraacetate), δ 21.2 (s, C_h-β-D-glucose tetraacetate), δ 20.8 (s, C_h-β-D-glucose tetraacetate), δ 20.7 (s, C_h-β-D-glucose tetraacetate), δ 20.6 (s, C_h-β-D-glucose tetraacetate).

3d: Yield: 0.184 g, 61%. Anal. Calc. for $C_{40}H_{36}Au_2FeN_2P_2S_4$ (1,184.71): C, 40.55; H, 3.06; N, 2.36; S, 10.82. Found: C, 40.59; H, 3.21, N, 2.45; S, 10.56. MS(ESI+) [m/z]: 751.1 [DPPF + Au]$^+$, 1066.1 [M − S(thiazoline)]$^+$. $^{31}P\{^1H\}$ NMR (DMSO-d^6): δ 32.21. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 32.15. ^1H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 7.52 (20H, m, C_6H_5), δ 4.81 (4H, s, C_5H_4), δ 4.37 (4H, s, C_5H_4), δ 4.29 (4H, m, C*H*$_2$N), δ 3.45 (4H, m,

C*H*$_2$S. ^{13}C{^{1}H} NMR (plus HSQC, CDCl3): δ 173.1 (s, CS(S)(N)). δ 133.6 (d, J_{PC} = 13.9 Hz, C_6H_5), δ 131.7 (s, C_6H_5), δ 130.7 (d, J_{PC} = 59.1 Hz, C_6H_5), δ 129.1 (d, J_{PC} = 11.6 Hz, C_6H_5), δ 75.4 (d, J_{PC} = 8.3 Hz, C_5H_4), 74.9 (d, J_{PC} = 13.3 Hz, C_5H_4) 71.6 (d, J_{PC} = 66.8 Hz, C_5H_4), δ 64.6 (s, *C*H$_2$N), δ 37.5 (s, *C*H$_2$S).

3e: Yield: 0.128 g, 52%. Anal. Calc. for $C_{44}H_{34}Au_2FeN_8P_2S_2$ (1,250.66): C, 42.26; H, 2.74; N, 8.96; S, 5.13. Found: C, 41.89; H, 3.06, N, 8.65; S, 5.37. MS(ESI+) [m/z]: 751.1 [DPPF + Au]$^+$, 1099.1 [M − S(6-mercaptopurine)]$^+$. ^{31}P{^{1}H} NMR (DMSO-d^6): δ 27.17. ^{31}P{^{1}H} NMR ($CDCl_3$): δ 27.67. ^{1}H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 8.81 (2H, s, 2-purine), δ 8.23 (2H, s, 8-purine), δ 7.52 (8H, m, C_6H_5), δ 7.46 (12H, m, C_6H_5), δ 4.68 (4H, s, C_5H_4), δ 4.35 (4H, s, C_5H_4), δ 1.62 (2H, s, 9-purine). ^{13}C{^{1}H} NMR (plus HSQC, $CDCl_3$): δ 154.1 (s, 4-purine), δ 150.8 (s, 8-purine), δ 133.4 (d, J_{PC} = 14.0 Hz, C_6H_5), δ 132.2 (d, J_{PC} = 77.6 Hz, C_6H_5), δ 131.9 (s, C_6H_5), δ 129.1 (d, J_{PC} = 11.9 Hz, C_6H_5), δ 128.4 (s, 2-purine), δ 126.3 (s, 5-purine), δ 77.2 (s, C_5H_4), 75.3 (s, C_5H_4), C6-purine not observed.

4a: Yield: 0.078 g, 58%. Anal. Calc. for $C_{28}H_{24}AuFePS$ (676.35): C, 49.72; H, 3.58; S, 4.74. Found: C, 49.62; H, 3.55, S, 4.88. MS(ESI+) [m/z]: 567.16 [M − SPh]$^+$, 677.04 [M + H],. ^{31}P{^{1}H} NMR (DMSO-d^6): δ 32.40. ^{31}P{^{1}H} NMR (CDCl3): δ 33.87. ^{1}H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 7.70 (2H, d, $^3J_{HH}$ = 7.4 Hz, P(C6*H*$_5$)), δ 7.63 (4H, dd, $^3J_{HH}$ = 7.4 Hz, $^3J_{HH}$= 12.5 Hz P(C_6H_5)), δ 7.48 (4H, m, P(C6*H*$_5$)), δ 7.26 (2H, m, S(C6*H*$_5$)), δ 7.16 (2H, m, S(C6*H*$_5$)), δ 7.03 (1H, m, S(C6*H*$_5$)), δ 4.58 (2H, s, C_5H_4), δ 4.39 (2H, s, C_5H_4), δ 4.17 (5H, s, C_5H_5). ^{13}C{^{1}H} NMR (plus HSQC, $CDCl_3$): δ 133.6 (d, J_{PC} = 13.8 Hz, P(*C*$_6$H$_5$)), δ 132.8 (s, S(*C*$_6$H$_5$)), δ 132.0 (s, S(*C*$_6$H$_5$)), δ 131.3 (m, P(*C*$_6$H$_5$)), δ 131.1 (d, J_{PC} = 68.8 Hz, P(*C*$_6$H$_5$)), δ 128.8 (d, J_{pc} = 11.3 Hz, P(*C*$_6$H$_5$)), δ 128.1 (s, S(*C*$_6$H$_5$)), δ 123.5 (s, S(*C*$_6$H$_5$)), δ 73.5 (d, J_{PC} = 13.8 Hz, C_5H_4), 72.4 (d, J_{PC} = 8.5 Hz, C_5H_4) 70.0 (s, C_5H_4).

4b: Yield: 0.130 g, 76%. Anal. Calc. for $C_{25}H_{26}AuFePS$ (642.33): C, 46.75; H, 4.08; S, 4.99. Found: C, 47.14; H, 4.05, S, 4.79. MS(ESI+) [m/z]: 567.15 [M − S^{i}Pr]$^+$, 643.05 [M + H]. ^{31}P{^{1}H} NMR (DMSO-d^6): δ 34.32. ^{31}P{^{1}H} NMR (CDCl3): δ 34.50. ^{1}H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 7.61 (4H, m, C_6H_5), δ 7.45 (6H, m, C_6H_5), δ 4.56 (2H, s, C_5H_4), δ

4.37 (2H, s, C_5H_4), δ 4.20 (5H, s, C_5H_5), δ 3.76 (1H, sept, $^3J_{HH}$ = 6.5 Hz, C*H*$(CH_3)_2$), δ 1.55 (6H, d, $^3J_{HH}$ = 6.5 Hz, CH(C*H*$_3$)$_2$). ^{13}C{^{1}H} NMR (plus HSQC, $CDCl_3$): δ 133.6 (d, J_{PC} = 13.9 Hz, C_6H_5), δ 131.4 (d, J_{PC} = 57.5 Hz, C_6H_5), δ 131.0 (s, C_6H_5), δ 128.7 (d, J_{PC} = 11.0 Hz, C_6H_5), δ 73.4 (d, J_{PC} = 13.6 Hz, C_5H_4), 72.2 (d, J_{PC} = 8.3 Hz, C_5H_4) 69.9 (s, C_5H4), δ 34.2 (s, *C*H$(CH_3)_2$),), δ 32.5 (s, CH(*C*H$_3$)$_2$).

4c: Yield: 0.106 g, 55%. Anal. Calc. for $C_{36}H_{38}AuFeO_9PS$ (930.54): C, 46.47; H, 4.12; S, 3.45. Found: C, 45.97; H, 4.23, S, 3.29. MS(ESI+) [m/z]: 567.1 [M − S(β-D-glucose tetraacetate)]$^+$, 931.1 [M + H]. ^{31}P{^{1}H} NMR (DMSO-d^6): δ 33.56. ^{31}P{^{1}H} NMR (CDCl3): δ 34.13. ^{1}H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 7.64 (4H, m, C_6H_5), δ 7.49 (6H, m, C_6H_5), δ 5.21 (4H, m, $H_{b,c,d,e}$-β-D-glucose tetraacetate), δ 4.59 (2H, s, C_5H_4), δ 4.45 (1H, m, H_g-β-D-glucose tetraacetate), δ 4.27 (7H, m, C_5H_4, C_5H_5), δ 4.16

(1H, m, H_g-β-D-glucose tetraacetate), δ 3.82 (1H, m, H_fβ-D-glucose tetraacetate), δ 2.06 (12H, m, H_h-β-D-glucose tetraacetate). $^{13}C\{^1H\}$ NMR (plus HSQC, plus HMBC, $CDCl_3$): δ 170.9, 170.8, 170.3, 169.5 (s, C=O), δ 133.7 (d, J_{PC} = 13.9 Hz, C_6H_5), 131.2 (s, C_6H_5), δ 130.7 (d, J_{PC} = 58.1 Hz, C_6H_5), δ 128.8 (d, J_{PC} = 11.5 Hz, C_6H_5), δ 83.4, 77.8, 73.6, 69.0 (s, $C_{b,c,d,e}$-β-D-glucose tetraacetate), δ 77.8 (s, C_f-β-D-glucose tetraacetate), δ 72.4 (s, C_5H_4), δ 70.1 (s, C_5H_4), δ 62.8 (s, C_g-β-D-glucose tetraacetate), δ 21.2 (s, C_h-β-D-glucose tetraacetate), δ 20.8 (s, C_h-β-D-glucose tetraacetate), δ 20.7 (s, C_h-β-D-glucose tetraacetate), δ 20.6 (s, C_h-β-D-glucose tetraacetate).

4d: Yield: 0.100 g, 72%. Anal. Calc. for $C_{25}H_{23}AuFeNPS_2$ (685.37): C, 43.81; H, 3.38; N, 2.04; S, 9.36. Found: C, 43.46; H, 3.76, N, 2.08; S, 9.87. MS(ESI+) [m/z]: 567.15 [M − S(thiazoline)]$^+$, 686.0 [M + H]. $^{31}P\{^1H\}$ NMR (DMSO-d^6): δ 32.36. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 33.1,. ^1H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 7.64 (4H, m, C_6H_5), δ 7.48 (6H, m, C_6H_5), δ 4.59 (2H, s, C_5H_4), δ 4.42 (2H, s, C5H4), δ 4.32 (2H, d, $^3J_{HH}$ = 8.0, CH_2N), δ 4. 24 (5H, s, C_5H_5), δ 3.47 (2H, d, $^3J_{HH}$ = 7.9, CH_2S). $^{13}C\{^1H\}$ NMR (plus HSQC, $CDCl_3$): δ 173.3 (s, CS(S)(N)). δ 133.7 (d, J_{PC} = 13.8 Hz, C_6H_5), δ 131.3 (s, C_6H_5), δ 130.5 (d, J_{PC} = 56.6 Hz, C_6H_5), δ 128.8 (d, J_{PC} = 11.8 Hz, C_6H_5), δ 73.6 (d, J_{PC} = 8.5 Hz, C_5H_4), 72.4 (d, J_{PC} = 13.5 Hz, C5H4) 70.17 (s, C_5H_4), δ 68.1 (s, CH_2N), δ 30.4 (s, CH_2S).

4e: Yield: 0.079 g, 55%. Anal. Calc. for $C_{27}H_{22}AuFeN_4PS$ (718.34): C, 45.14; H, 3.09; N, 7.80; S, 4.46. Found: C, 45.35; H, 3.13, N, 7.71; S, 4.59. MS(ESI+) [m/z]: 567.0 [M − S(6-mercaptopurine)]$^+$, 719.0 [M + H]. $^{31}P\{^1H\}$ NMR (DMSO-d^6): δ 29.27. $^{31}P\{^1H\}$ NMR (CDCl3): δ 28.32,. ^1H NMR (plus COSY, plus NOESY-2D, $CDCl_3$): δ 8.74 (2H, s, 2-purine), δ 8.25 (2H, s, 8-purine), δ 7.66 (4H, m, C_6H_5), δ 7.41 (6H, m, C_6H_5), δ 4.53 (2H, s, C_5H_4), δ 4.39 (2H, s, C_5H_4), δ 4.16 (5H, s, C_5H_5), δ 1.95 (1H, s, purine). $^{13}C\{^1H\}$ NMR (plus HSQC, $CDCl_3$): δ 152.1 (s, 4-purine), δ 150.7 (s, 8-purine), δ 133.7 (d, J_{PC} = 13.9 Hz, C_6H_5), δ 133.5 (s, 2-purine), δ 131.9 (d, J_{PC} = 56.8 Hz, C_6H_5), δ 131.2 (s, C_6H_5), δ 129.1 (s, 5-purine), δ 128.8 (d, J_{PC} = 11.2 Hz, C_6H_5), δ 73.51 (d, J_{PC} = 14.0 Hz, C_5H_4), δ 72.3 (d, J_{PC} = 8.55 Hz, C_5H_4), 70.1 (s, C5H4), C6-purine not observed.

2.3.Crystallographic Data for Compounds 3a and 3d

Single crystals of **3a** and **3d** (see details below) were mounted on a glass fiber in a random orientation. Data collection was performed at room temperature on a Kappa CCD diffractometer using graphite monochromated Mo-Ka radiation (l=0.71073 Å). Space group assignments were based on systematic absences, E statistics and successful refinement of the structures. The structures were solved by direct methods with the aid of successive difference Fourier maps and were refined using the SHELXTL 6.1 software package. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to ideal positions and refined using a riding model. Details of the crystallographic data are given in Table S1 (Supporting information section). These data can be obtained free of charge from The Cambridge Crystallographic Data Center via www.ccdc.cam.ac.uk/data_request/cif. (CCDC 977988 for compound **3a**, and 977989 for compound **3d**). **3a**: Crystals of **3a** (yellow prisms with approximate dimensions 0.25 × 0.23 × 0.23 mm) were obtained from a solution of **3a** in CH_2Cl_2 by slow diffusion of Et_2O at RT. **3d**: Crystals of **3d** (yellow prisms with

approximate dimensions 0.25 × 0.24 × 0.22 mm) were obtained from a solution of **3d** in CH_2Cl_2 by slow diffusion of Et_2O at RT.

2.4. DFT Studies for compounds 4a and 4d

The calculations have been performed using the hybrid density functional method B3LYP, [27,28] as implemented in Gaussian09.[29] Geometries were optimized with the 6–31G(d,p) basis set for the C, N, P, S, and H elements and the SDD pseudopotential for the iron and gold centers.[30,31] Frequency calculations have been done at the same level of theory as the geometry optimizations to confirm the nature of the stationary points.

2.5.Microbial Toxicity Assays

Bacteria and yeast were stored as glycerol stocks at −80 °C and streaked onto Meuller-Hinton or YEPD plates prior to each set of experiments. Colonies from newly prepared plates were inoculated into 5 mL of media (tryptic soy broth for *E. coli*, *P. aeruginosa* and *S. aureus* strains and YPD broth for *S. cerevisiae*) and grown overnight at 37 °C (30 °C for *S. cerevisiae*). The overnight cultures were diluted to an OD_{600} <0.01 (ThermoSpectronic, Genesys 8 spectrophotometer) in 2 mL of fresh media in sterile culture tubes. The compounds (auranofin, **3a**-**3e** and **4a**-**4e**) were brought up in DMSO to a concentration of 1 mg/mL and then diluted into the cell culture tubes at the specified concentrations. Control tubes for each cell type were inoculated with an equivalent concentration of DMSO alone. The cultures were then transferred as 200 μL volumes into a 96-well plate (typically 8 replicates were performed for each condition). The plate was incubated at the appropriate temperature and shaken in a Biotek ELx808 plate reader. Growth measurements (OD_{600}) were automatically taken every hour for 24 hours. The minimal inhibitory concentration (MIC) was determined to be the concentration at which there was negligible increase in the OD_{600} value from the initial reading after 24 hours. All samples were independently tested two or three times as described here.

2.6. Toxicity of compounds on Human Embryonic Kidney Cells (HEK-293)

The Human Embryonic Kidney 293 cells (HEK-293), a non-tumoral immortalized human cell line (obtained from American Type Culture Collection, Manassas, Virginia, USA) were cultured in DMEM (Dulbecco's Modified Eagle Medium, F-12 50/50 Mix with L-Glutamine and phenol red) (from Corning Cellgro) supplemented with 10% FBS (from Gibco Sera, Life Technology) and 1% penicillin/streptomycin, at 37°C in a humidified atmosphere of 95% of air and 5% CO2 (University of Hawaii Cancer Center, Honolulu, Hawaii, USA). For evaluation of cell viability, cells were seeded at a concentration of 5×1^3 cells/well in 90 μL DMEM complete medium without phenol red, into tissue culture grade 96-wellflat bottom microplates (Thermo Scientific BioLite Microwell Plate, Fisher Scientific, Waltham, Massachusetts, USA) and grown for 24 h. Solutions of the compounds were prepared by diluting a freshly prepared stock solution (in DMSO) of the corresponding compound in DMEM complete medium without phenol red. Afterwards, the intermediate dilutions of the compounds were added to the wells (10 μL) to obtain a final concentration ranging from 0.1 to 200 μM, and the cells were incubated for 24 h. DMSO at comparable concentrations did not show any effects on cell cytotoxicity. Following 24 h drug exposure,

50 μL of 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H–tetrazolium-5-carboxanilide (XTT) (Roche Diagnostics, Indianapolis, Indiana, USA) labeling mixture per well was added to the cells at a final concentration of 0.3 mg/mL and incubated for 4h at at 37°C in a humidified atmosphere of 95% of air and 5% CO2. The optical density of each well (96-well plates) was quantified using EnVision Multilabel Plate Readers (Perkin Elmer, Waltham Massachusetts,USA) at 450 nm wavelength to measure absorbance. The percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC_{50} value was calculated as the concentration reducing the proliferation of the cells by 50% and is presented as a mean (± SE) of at least two independent experiments each with triplicates.

3. Results and Discussion

3.1. Synthesis and characterization of the heterometallic compounds

The synthesis and partial characterization of compound **3a** had been described before [26]. The standard method employed for the synthesis of all the heterometallic complexes described here is based on the deprotonation of thiols with a base and subsequent reaction with [{AuCl}$_2$(μ DPPF)] (**1**) [24] or [AuCl(MPPF)] (**2**) to obtain compounds **3a–e** or **4a–e** respectively (Scheme 1). The synthesis of the gold(I)-chlorophosphino starting materials with DPPF (**1**) and MPPF (**2**) is by displacement of the labile ligand tetrahydrothiophene (tht) in [AuCl(tht)] [23] by the ferrocenyl phosphines. Compound **1** has been reported previously and **2** was synthesized in a similar manner (Scheme 1) by reaction of [AuCl(tht)] and MPPF [26].

All new compounds are air-stable, yellow solids that are obtained in moderate yields. The structures proposed are based on NMR and IR spectroscopy, elemental analysis, and mass spectrometry (see spectra in supporting information). The heterometallic derivatives are soluble in DMSO and in mixtures DMSO:H_2O (1:99) at micromolar concentrations. Compound **3c** is more hydrophilic and is soluble in mixtures of 50:50 at milimolar concentrations. All complexes are stable for at least 8 days at RT in DMSO solutions as determined by $^{31}P\{^1H\}$ and ^1H NMR spectroscopy (no more than 2–3% phosphine oxide generated, see selected spectra in DMSO-d^6 overtime in the SI). DMSO solutions of the compounds can be kept for several weeks or months in the freezer without noticeable decomposition. The spectroscopic data $^{31}P\{1H\}$ NMR in $CDCl_3$ and DMSO-d^6 is collected in Table 1. The main peaks observed in the MS spectra (ESI in CH_2Cl_2) are also collected in table 1. It has been reported that compounds of the type [Au(X)(PR_3)] can give rise to species [Au(PR_3)$_2$][Au(X)$_2$] (e.g. X = SR^-, SeR^-, CN^-) by ligand scrambling and dissociation [32,33]. This is relevant in order to understand the metabolism of drugs like AF [34]. AF is known to bind an SH fragment from cysteine 34 to form AlbSAuPEt_3 in vivo and subsequently displacing PEt_3 which oxidizes to PEt_3O (a process favored by increasing the affinity of the anion to bind gold(I) or retarded by the bulk and basicity of the phosphine) [34]. We have found by NMR spectroscopy and MS (HR ESI) spectrometry that the trimetallic $FeAu_2$ species [{Au(SR)}$_2$(DPPF)] (**3a**-**3e**) do not seem to undergo this type of ligand scrambling easily. No noticeable decomposition is noticed at RT in DMSO-d^6 after 8 days (figures S36–39 in SI) and sharp peaks were found in the $^{31}P\{^1H\}$ NMR spectra in

$CDCl_3$ and DMSO-d^6 solution (SI). The ESI MS spectra for **3a-3e** (Table 1 and figures S44–56 in the SI) do not show peaks that can be assigned to species $[Au(DPPF)_2]^+$.

The bimetallic FeAu species [Au(SR)(MMPF)] **4b-4e** give rise to broader signals in the $^{31}P\{^1H\}$ NMR spectra (see figures S20, S23, S26, S29, S32, S41 and S43 in SI). It is worth noting that these compounds are less soluble in DMSO than compounds **3a-3e** or **4a** (completely soluble) and the solutions prepared to get the $^{31}P\{^1H\}$ are almost saturated. As for compounds with DPPF their MS spectra (ESI+, figures S57–S65 in SI) do not show peaks which could be assigned to species $[Au(MPPF)_2]^+$ indicative of ligand scrambling. Dissociation of the SR- is evident by the peaks observed that correspond to $[Au(MPPF)]^+$ (table 1) but this is common for the ESI+ or FAB+ MS spectra of gold complexes of the type $[AuX(PR_3)]$. However with the data we have so far we cannot exclude the possibility of ligand scrambling in solution for compounds **4b-4e**.

The structures of the trimetallic $FeAu_2$ compounds **3a** and **3d** were further confirmed by X-ray single crystal diffraction (structure of **3d** shown in Figure 2). Selected bond lengths and angles for both compounds are collected in Table 2 while the drawing for the crystal structure of **3a** and crystallographic tables for **3a** and **3d** (Table S1) are in the supplementary material. A more complete table including more values for bond lengths and angles for complexes **3a** and **3d** is also included in the SI (Table S2).

Both crystal structures are very similar. The cyclopentadienyl rings are staggered by 36.09° (**3a**) and 36.88° (**3d**) around the Cp…Cp axis (Cp = center of the cyclopentadienyl ring) defined by the torsion angle C11-Cp-Cp-C13. The phosphorus atoms are located at the 1 and 3’ carbon atoms of the ferrocene rings. The molecular structures display a typical linear geometry around the gold centers with a P1-Au1-S1 angle of 171.33(5) (**3a**) and 177.75(11) (**3d**). the distances Au-S and Au-P are within the usual range of other gold(I) thiolates containing phosphine ligands [17,35]. Since we could not obtain crystals of the bimetallic complexes **4a–e** of enough quality for a single crystal X-ray determination, good estimates for the structural parameters of **4a** and **4d** were obtained from density functional calculations (see figures in SI and Table S3). The distances Au-S and Au-P are similar to those in **3d** and **3d** and the main difference is the eclipsed disposition of the two Cp rings (one containing the phosphine group attached to the gold-thiolate group) for the ferrocene moiety as well as the smaller size of the overall molecule.

3.2. Biological Activity

The antimicrobial activities of the gold(I) trimetallic Fe-Au_2 (**3a–e**) and bimetallic (**4a–e**) compounds were evaluated against Gram-negative (*Escherichia coli* and *Pseudonomas aeruginosa*), Gram-positive (*Staphyloccocus aureus*) bacteria and yeast (*Saccharomyces cerevisiae*) (Table 2). A number of the compounds analyzed in this work exhibited minimum inhibitory concentration (MIC) values in the 1 μg/mL - 100 μg/mL range (Table 3). For selected compounds the antimicrobial activities against MRSA strains US300 and US400 and two *S. cerevisiae* yeast mutants lacking thioredoxin reductase TR1 or TR2 were also evaluated (Table 3 and data not shown). The antimicrobial activity of AF was also studied against all the above mentioned microbes (Table 3). MIC values for AF against

Gram-negative (*Escherichia coli* and *P. aeruginosa*), and Gram-positive (*Staphyloccocus aureus*) are similar to those reported before [17] (see Table 3). To the best of our knowledge the antimicrobial activity of AF against MRSA strains US300 and US400 and *S. cerevisiae* has not been reported before.

Some of the new heterometallic compounds, specially the bimetallic FeAu derivatives incorporating the MPPF phosphine (**4b–e**) and trimetallic $FeAu_2$ compounds with DDPF (**4b** and **4c**) have shown activity against the Gram-positive bacterium *S. aureus* with MIC in the low micromolar range (2.4–6.6 μM or 2–6 μg/mL). None of the new heterometallic complexes or AF[17] have a MIC below the 100 μg/mL range for *E. coli* or *P. aeruginosa*. Remarkably, AF inhibits the growth of *S. aureus* and the MRSA strains USA300 and USA400 in the nanomolar range (150–300 nM or 0.1 to 0.20 μg/mL). The most active heterometallic compound **4e** inhibits the growth of *S. aureus* and the MRSA strains USA300 and USA400 at 2.8 μM. In addition, compound **4d** containing 2-thiazoline and AF were equally effective against yeast *S. cerevisiae* with MIC of 100 μg/mL.

Recent studies have reported that the mechanism of AF toxicity against the eukaryotic microbial pathogens *Entamoeba histolytica* [14], and *Giardia lamblia* [13] is linked to the inhibition of thioredoxin reductase enzyme activity. We sought to test if there were a role for AF toxicity in yeast through a similar mechanism by testing mutants of *S. cerevisiae* lacking thioredoxin reductase genes, TRR1 or TRR2. The expectation being that these mutants lacking the target of AF would be less sensitive to the drug. However, upon testing AF we found no significant difference in activity between the wild type yeast and the thioredoxin reductase mutants. It could be that the TRR1 and TRR2 genes complement one another and that a double mutant in these two genes would need to be tested to see any decreased sensitivity to AF. Or it could be simply that the effects of AF work on yeast through a different pathway unrelated to thioredoxin reductase. Additional testing in the future will be required to characterize the mechanism of activity against yeast.

The activity is not solely correlated to the gold content. The bimetallic compounds have in general lower content of gold than the trimetallic derivatives (range 21.2–30.6 % **4a–e** versus 23.5–35.8 % **3a–e**) but display in general a better activity against the MSRA strains than three of the trimetallic compounds (**3a, 3d** and **3e**). Effects such as ligand dissociation or ligand scrambling and subsequent formation of different metabolites in vivo will play a more important role for the biological activity [34]. The most active compounds are **3b, 3c** and **4e** (3.6 μM (**3b, 3c**) and 2.8 μM (**4e**) against MSRA strains). It is well established that MRSA strains carry the MecA gene, often in combination with other resistance genes, which inactivates β-lactam antibiotics such as methicillin. In the CA MRSA strains tested, it is unlikely that the presence of the MecA gene would influence the activity of AF on these cells, so that any differences in toxicity seen with AF, or AF-related compounds, is likely to be linked to differences in the genetic background of the CA MRSA strains and the routine laboratory *S. aureus* strains.

The MIC values obtained for the new heterometallic complexes on Gram-positive *S. aureus* are similar, or below, those described for some bimetallic aminothiol gold compounds containing phosphines [16]. Monometallic aminothiolates containing the triethyl phosphine

ligand (like auranofin) and aminothiols had a MIC below 1 μg/mL (like AF) on this Gram-positive bacterium [17]. It is clear that the subtle interplay of the ligands coordinated to gold may be responsible for the biological activity observed on this type of compounds [17,19]. In this context, although the new heterometallic compounds described here are very potent they have not been as efficient as AF against *S. aureus* and the CA MRSA strains. We believe that the design of complexes with ferrocenyl-based alkyl phosphines may render new heterometallic complexes with improved antimicrobial and solubility properties.

An explanation for the behavior displayed by these new thiolate gold complexes and AF could be as follows: if these compounds interfere with respiration (a mitochondrial function), then the fungi should be the most resistant, because electron transport is shielded inside internal organelles, whereas in bacteria electron transport occurs at the plasma membrane. Gram-negative bacteria have a protective outer membrane; therefore the compounds may not have ready access to the electron-transport chain. These compounds should be more active in general against Gram-positive bacteria such as *S. aureus* which is what we have observed here. The testing of these compounds against selected mutant strains of Gram-negative bacteria that have a compromised outer membrane function may help to elucidate the mechanism of these gold-based compounds and the apparent reduced activity against Gram-negative bacteria relative to Gram-positive bacteria.

Studies of the toxicity of selected compounds (AF and compound **4e**) against a non-tumorigenic human embryonic kidney cell line (HEK-293) were performed to assess the toxicity on human “normal” or healthy cells. The IC50 values obtained after 24 hours of incubation with these compounds (XTT assay) were 0.549 ± 0.022 μM (AF) and 0.403 ± 0.004 μM **4e**. Auranofin was slightly less toxic than compound **4e** to the immortalized healthy cell lines but it was 5 times or 2.5 times less toxic to the human cell line than to *S. aureus* or the resistant strains US300 and US400 respectively.

4. Conclusion

In conclusion, we have prepared a number of new heterometallic gold-thiolate complexes highly active against Gram-positive bacteria *S. aureus* and CA MRSA strains US300 and US400 (MIC in the order of one digit micromolar). Remarkably, auranofin inhibited *S. aureus*, US300 and US400 in the order of 150–300 nM or 0.1–0.2 μg/mL.

Therapeutic treatment rheumatoid arthritis with AF results in stable serum concentrations of 0.5–0.7 μg/mL [36,37]. The fact that an oral, FDA approved, drug is able to inhibit CA MRSA strains in nanomolar concentrations that are less (3.5 to 5 times) than those known to be well tolerated by patients suggests that treatment of MRSA with AF, and related gold-based compounds, could represent an important and novel option for patients.

The in vitro MIC for AF on *S. aureus* and methicillin resistant strains are in the order or below of those for recently reported oxazolidinone molecules (modifications of antibiotic linexolid) [37].This study supports the potential of the FDA approved drug AF on the treatment of diseases other than rheumatoid arthritis [14]. AF and related gold thiolates may offer an opportunity to increase the palette of inexpensive methicillin -resistant antibiotics.

In this context, further modification of ferrocenyl-phosphine ligands by substitution of aryl groups by alkyl groups may allow for the preparation of compounds with some improved biological properties in terms of antimicrobial activity and solubility.

Studies on the mechanism of the growth inhibition of AF and some closely related gold(I) thiolates in *S. aureus* and several MRSA strains are ongoing at our laboratories.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AF	auranofin
COSY	correlation spectroscopy
DPPF	1,1'-Bis-diphenylphosphino-ferrocene
DFT	Density functional theory
ESI-MS	electrospray ionization- mass spectrometry
FDA	Food and Drug Administration or USFDA, an agency of the United States Department of Health and Human Services
HEK-293	inmortalized human embryonic kidney cells
HMBC	heteronuclear multiple-bond correlation spectroscopy
HSQC	heteronuclear single-quantum correlation spectroscopy
MIC	minimum inhibitory concentration
MPPF	diphenylphosphino-ferrocene
MRSA	Methicillin-resistant *Staphylococcus aureus*
NOESY	nuclear Overhauser effect spectroscopy
SDD	Stuttgart–Dresden pseudopotential
tht	tetrahydrothiophene

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Highlights

- Synthesis of new heterometallic gold(I) compounds containing ferrocenyl-phosphines
- High antibacterial activity (one digit μM) against *S. aureus* and CA MRSA bacterial strains
- First report on the activity of auranofin (AF) against CA MSRA bacterial strains (nM levels)
- Activity of AF well below those tolerated by patients in the treatment of arthritis with Ridaura

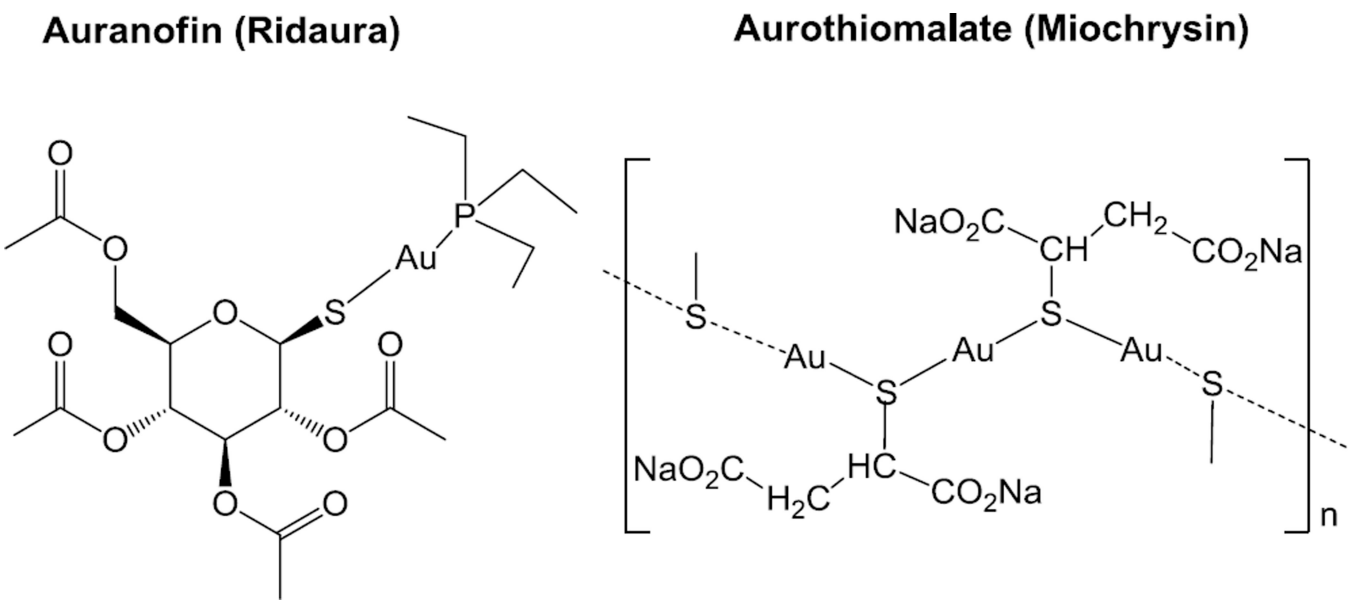


Figure 1.
Auranofin and aurothiomalate used therapeutically in the treatment of rheumatoid arthritis.

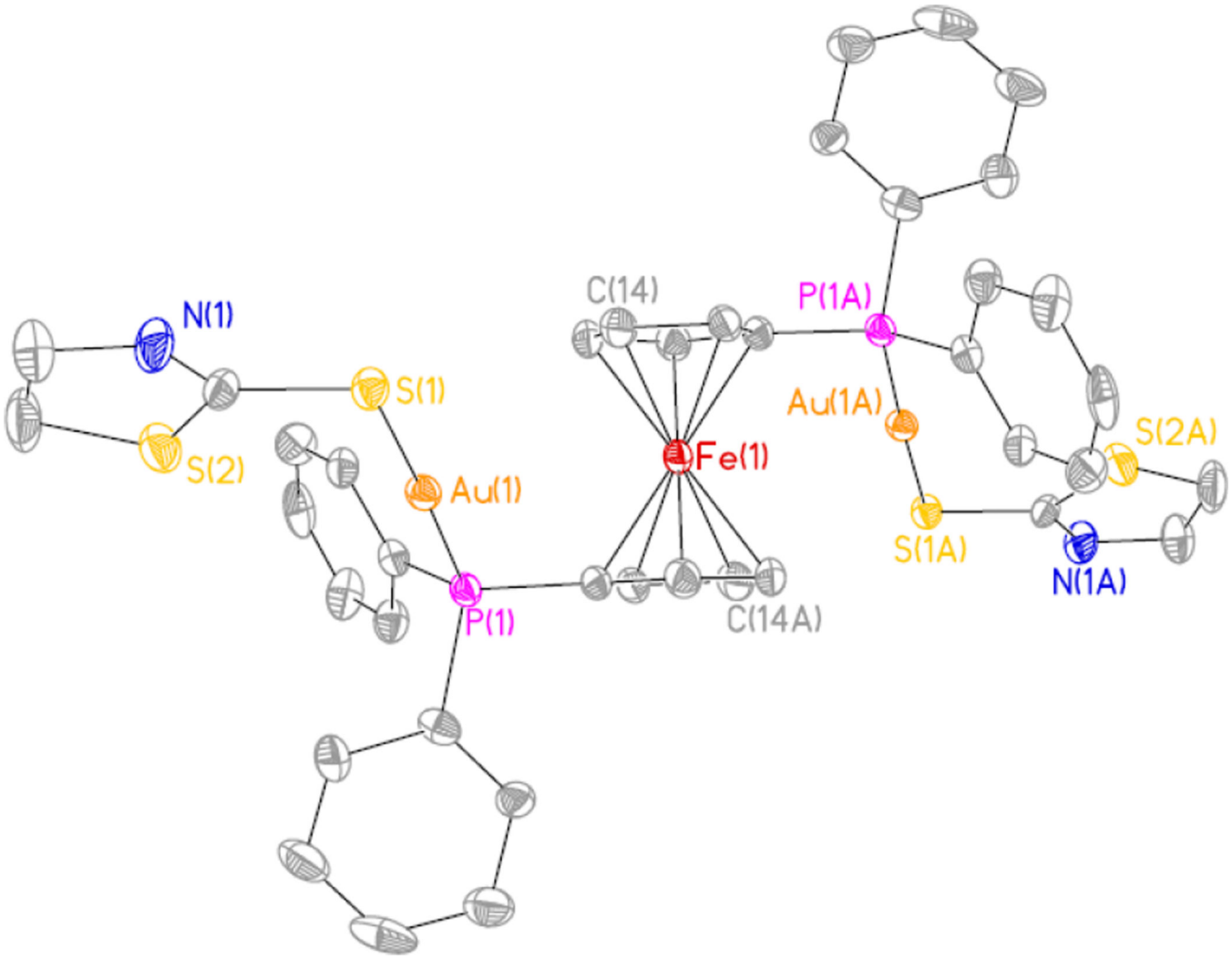
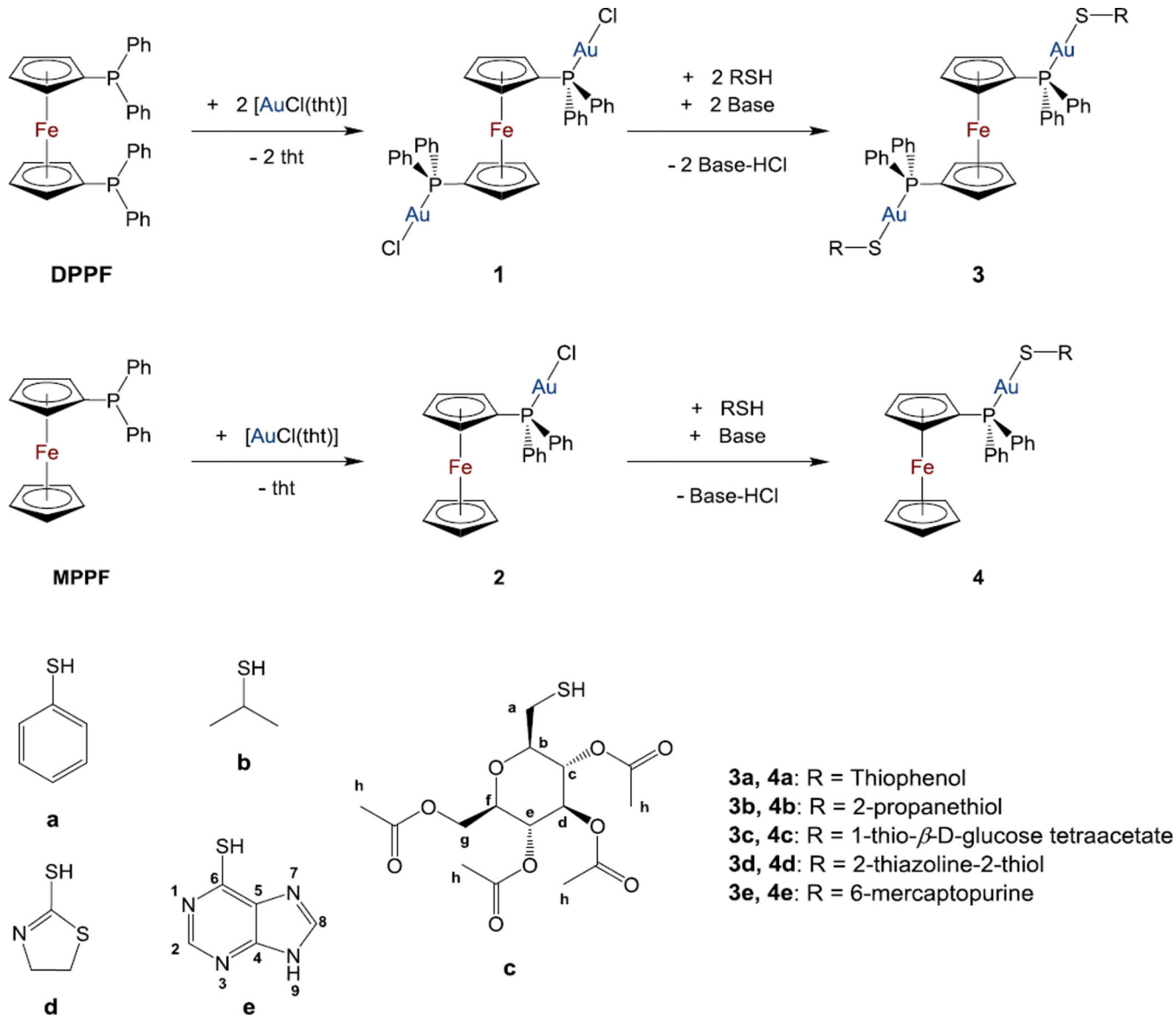


Figure 2.
Ortep view of the crystal structure of **3d**. Structure of **3a** in supplementary material.



Scheme 1.

Synthesis of heterometallic gold(I) thiolate complexes based on DPPF (**3**) or MPPF (**4**) ferrocenyl phosphines. Base = K_2CO_3 in CH_2Cl_2 or KOH/MeOH.

Table 1

$^{31}P\{^{1}H\}$ NMR data ($CDCl_3$ and DMSO-d^6) and main peaks in MS spectra (HR ESI) for the new gold compounds. $^{31}P\{^{1}H\}$ δ in DMSO-d^6 remain the same after 8 days at RT. All spectra are collected in the SI section.

Complex	$^{31}P\{^{1}H\}$ NMR (δ)		HR ESI-MS
2	28.46 (b)	29.31 (b)	$[M-Cl]^+$ or $[MPPF + Au]^+$ m/z = 567.01 (100%)
3a	32.94 (s)	33.00 (s)	$[DPPF - Ph]^+$ m/z = 477.17 (80%) $[DPPF + Au]^+$ m/z = 751.24 (100%) $[M - SR]^+$ m/z = 1057.05 (1 %)
3b	33.58 (s)	32.21 (s)	$[M - SR]^+$ m/z = 1024.05 (100 %) $[M + Au]^+$ m/z = 1295.08 (10%)
3c	32.94 (s)	32.52 (s)	$[DPPF + Au]^+$ m/z =751.10 (10%) $[DPPF + 2Au]^{2+}$ m/z = 981.10 (35%) $[M - SR]^+$ m/z = 1311.10 (100%) $[M + Au]^+$ m/z = 1871.10 (5%)
3d	32.15 (s)	32.21 (s)	$[DPPF + Au]^+$ m/z = 751.10 (100%) $[M -SR]^+$ m/z = 1066.10 (57%)
3e	27.67 (s)	27.17 (s)	$[DPPF + Au]^+$ m/z = 751.10 (58%) $[M -SR]^+$ m/z = 1099.10 (100%)
4a	33.87 (s)	32.40 (s)	$[MPPF + Au]^+$ or $[M- SR]^+$ m/z = 567.01 (75%) $[M + H]^+$ m/z = 677.04 (0.1%)
4b	34.50 (b)	34.32 (b)	$[MPPF + Au]^+$ or $[M- SR]^+$ m/z = 567.01 (75%) $[M + H]^+$ m/z = 643.05 (0.5%)
4c	34.13 (b)	33.56 (b)	$[MPPF + Au]^+$ or $[M- SR]^+$ m/z = 567.01 (95%) $[M + H]^+$ m/z = 931.10 (2%)
4d	33.17 (b)	32.36 (b)	$[MPPF + Au]^+$ or $[M- SR]^+$ m/z = 567.01 (67%) $[M + H]^+$ m/z = 686.17 (50%)
4e	28.32 (b)	29.27 (b)	$[MPPF + Au]^+$ or $[M- SR]^+$ m/z = 567.01 (10%) $[M + H]^+$ m/z = 719.03 (2%)

Table 2

Selected bond lengths [Å] and angles [°] for complexes **3a** and **3d**.

3a		**3b**	
Au(1)-P(1)	2.2546(14)	Au(1)-P(1)	2.259(3)
Au(1)- S(1)	2.3000(16)	Au(1)- S(1)	2.307(3)
Fe-C	2.032(4)-2.063(5)	Fe-C	2.025(15) -2.050(15)
S-C(41)	1.783(6)	S-C(1)	1.761(14)
P(1)-Au(1)-S(1)	171.33(5)	P(1)-Au(1)-S(1)	177.75(11)
C(14)-Fe(1)-C(14A)	180.0(3)	C(14)-Fe(1)-(C14A)	179.999(2)
C(41)-S(1)-Au(1)	100.93(19)	C(1)-S(1)-Au(1)	104.0(5)

Table 3

Minimum inhibitory concentration MIC (in μg/mL, +/−0.1) of compounds **3a–e, 4a–e** and auranofin against microbial organisms.[a]

Compound	Gram-negative		Gram-Positive			Yeast
	P. aeruginosa	*E. coli*	*S. aureus*	*US300*	*US400*	*S. cerevisiae*
3a	>100	>100	>10 (>9)[b]	----	---	>100
3b	>100	>100	4 (3.6) [b]	4 (3.6) [b]	4 (3.6) [b]	>100
3c	>100	>100	4 (2.4) [b]	6 (3.6) [b]	6 (3.6) [b]	>100
3d	>100	>100	>10 (>8) [b]	----	---	>100
3e	>100	>100	>10 (>8) [b]	----	---	>100
4a	>100	>100	2 (3) [b]	4 (6) [b]	4 (6) [b]	>100
4b	>100	>100	2 (3.2) [b]	4 (6.4) [b]	2 (3.2) [b]	>100
4c	>100	>100	2 (2.2) [b]	6 (6.6) [b]	6 (6.6) [b]	>100
4d	>100	>100	2 (3) [b]	4 (6) [b]	4 (6) [b]	100
4e	>100	>100	2 (2.8) [b]	2 (2.8) [b]	2 (2.8) [b]	>100
Auranofin	>100	>100	0.1 (0.15) [b]	0.2 (0.3) [b]	0.2 (0.3) [b]	100

[a] Auranofin and the heterometallic compounds were dissolved in DMSO. When compounds were not active in S. *aureus* below 10 μM they were not tested on US300 or US400.

[b] MIC (micromolar units) in parentheses.