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In the search for new gold metalloantibiotics: In vitro evaluation of Au(III) (C^S)-cyclometallated complexes

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ABSTRACT

A series of (C^S)-cyclometallated Au(III) cationic complexes of general formula $[Au(dppta)(dtc)]^+$, $[Au(dppta)(azmtd)]^+$ and $[Au(dppta)(azc)Cl]^+$ (dppta = N,N-diisopropyl-*P*,*P*-diphenylphosphinothioic amide-κ^2C,S; dtc = dithiocarbamate-κ^2S,S′; azc = azolium-2-dithiocarboxylate-κ^1S; azmdt = azol(*in*)ium-2-(methoxy)methanedithiol-κ^2S,S′) were synthetized and tested against a panel of bacterial strains belonging to different Gram-positive and Gram-negative species of the ESKAPE group of pathogens. Among the tested compounds, complex **4c** had the higher Therapeutic Index (TI) against multidrug resistant strains of *S. aureus*, *S. epidermidis* and *A. baumannii*, showing a more favourable cytotoxicity profile than the reference gold metalloantibiotic Auranofin.

1. Introduction

The alarming rate of antimicrobial resistance (AMR) is one of the biggest worldwide crises that public health confronts. For common bacterial infections, high rates of resistance against antibiotics frequently used in their treatment have been observed worldwide, indicating that we are running out of effective antibiotics. The World Health Organization (WHO) has identified a group of antibiotic-resistant bacteria that represent a critical danger to global health [1,2]. However, there are only 27 antibiotics in clinical development that address the priority pathogens, of which only two fulfil all the criteria's to be considered as completely innovative: no cross-resistance, new chemical class, new target and new mechanism of action [3].

Owing to the urgent need of new drugs to fight against resistant bacteria, the inclusion of metal ions in drug design have gained considerable recent attention [4]. Although yet to be advanced to the clinic, metalloantibiotics have several advantages over purely organic drug candidates [5]. The vast diversity of types of oxidation states, coordinating ligands, and geometries make metal-based complexes very useful in accessing a highly underexplored chemical space for drug development, and especially for the design of new antimicrobials [6]. In addition, the synthesis of metallodrugs requires fewer steps when compared to organic compounds. Furthermore, metal-based complexes may provide unique modes of action: exchange or release of ligands, redox activation and catalytic generation of toxic species (reactive oxygen species - ROS), and depletion of essential substrates, making them able to abolish enzyme activities, disrupt membrane function or damage DNA [7].

The interest of metal ions for the treatment of bacterial infections is not new. The antimicrobial properties of silver have been known for centuries and there are records of the use of metallic silver that date back six millennia [8]. Before the discovery of penicillin, silver nitrate was widely used as antimicrobial agent [9]. One example to illustrate the efficiency and the potential activity of silver compounds is silver sulfadiazine, known as Silvadene®, which was approved in 1968 for the treatment of infections in burn wounds. This compound slowly liberates

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silver ions, which can disrupt cell membranes of both Gram-positive and Gram-negative bacteria and inhibit their growth [10]. Taking into account these features, a wide variety of new classes of silver complexes have attracted attention for their potent antibacterial action [11,12]. Among them, N-heterocyclic carbene (NHC) complexes are particularly relevant, because of the ability of NHC ligands to protect metal ions, modulating the silver release and controlling the systemic release of silver ions [13]. Youngs and co-workers reported the first example of an antibacterial silver NHC complex [14]. After this pioneering work, the number of reported silver NHC complexes with antibacterial activity increased exponentially [15,16]. An excellent review by Isbel et al. covers the most relevant studies on antibacterial NHC silver complexes from the first report of Youngs' group in 2006 to 2023 [17].

The rapid advance of AMR led researchers to consider repurposing old drugs for different clinical diseases as antimicrobials. Considering the antibacterial properties of silver complexes, gold metallodrug auranofin (RidauraTM) was evaluated as antibacterial agent. This Au(I) phosphine complex, in clinical use since 1985 for the treatment of severe rheumatoid arthritis [18], has been found to exert potent antibacterial activity at clinically applicable concentrations [19,20]. The therapeutic potential of Auranofin as antimicrobial agent sparked the interest in gold metalloantibiotics. Hence, in the past few years a wide range of gold complexes have been assessed for their antibacterial properties.

Since the seminal review by Glišić and Djuran in 2014, [21] a wide number of reviews are available giving an overview of their antibacterial properties of gold complexes. The antibacterial properties of Au(III) complexes, gold NHC complexes and phosphine gold(I) complexes were briefly addressed in 2018, 2019 and 2020, respectively [22–24]. More recently, Ratia et al. [25], and Chew and co-workers [26] provided a detailed description of the antibacterial activity of gold complexes, highlighting their great potential for development of novel classes of antibiotics to fight bacterial resistance.

To address the global shortage of effective antibiotics, our group is devoted to the development of novel families of gold metalloantibiotics [27–30]. Here we report on the extension of our efforts in developing antimicrobial (CˆS)-cyclometallated Au(III) complexes by comparing a series of (dppta)Au(III) complexes (dppta = N,N-diisopropyl-*P*,*P*-diphenylphosphinothioic amide-κ^2C,S) with different auxiliary ligands of the type κ^2S,S′-dithiocarbamate, κ^1S-azol(*in*)ium-2-dithiocarboxylate and κ^2S,S′-azol(*in*)ium-2-(methoxy)methanedithiol. These compounds were assessed towards clinically relevant Gram-positive and Gram-negative bacteria. The biological activities of the gold complexes were studied and compared with those of auranofin, the reference metallodrug currently being explored for potential therapeutic application in bacterial infections. The effect of alterations of the ligand skeleton and the anion on the biological properties of these complexes is also discussed.

2. Materials and methods

2.1. Synthesis of Au(III) complexes

The target compounds were synthetized using established procedures with minor modifications [29,30]. For the preparation of Au(III)-dithiocarbamate hexafluorophosphate complexes **2** (Scheme 1) [29], the corresponding dithiocarbamate salt was added to a solution of the [Au(dppta)Cl_2] complex **1** [27] in methanol. Then, after addition of an aqueous solution of KPF_6, the resulting [Au(dppta)(dtc)]PF_6 **2a-h** complexes precipitated from the solution and were isolated in analytically pure form by filtration and washing with water and diethyl ether.

The preparation of Au(III)-dithiocarbamates **3** was accomplished by addition to the morpholine dithiocarbamate sodium salt to a methanolic solution of the [Au(dppta)Cl_2] complex **1** (Scheme 1). Then, complexes **3b-e** were isolated in analytically pure form after addition of aqueous $NaSbF_6$, $NaBF_4$, NaOTf or $KAuCl_4$, respectively, filtration and washing with water and diethyl ether. For the isolation of complex **3a**, water was added and, after removal of methanol under reduced pressure, the aqueous phase was extracted with CH_2Cl_2, dried over Na_2SO_4 filtered and evaporated under vacuum. The solid residue was then washed with diethyl ether to afford analytically pure **3a**. Complexes **2** and **3** were fully characterized by ^{1}H, ^{13}C and ^{31}P NMR, IR and HRMS spectroscopy.

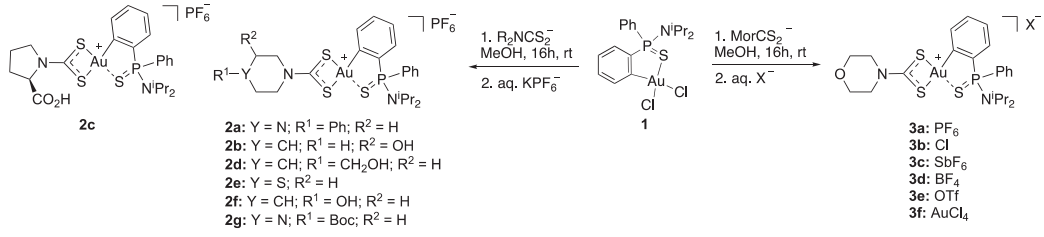
Au(III) azol(*in*)ium-2-(methoxy)methanedithiolate complexes **4** were synthetised by reaction of the corresponding azol(*in*)ium dithiocarboxylate zwitterionic ligands with a methanolic solution of [Au(dppta)Cl_2] complex **1** and subsequent addition of aqueous KPF_6 (Table 2) [30]. The desired complexes **4a-g** precipitated from the solution and were isolated in analytically pure form by filtration and washing with water and diethyl ether. For the synthesis of Au(III) azol(*in*)ium dithiocarboxylate complexes **5a-g**, a solution of [Au(dppta)Cl_2] complex **1** in acetonitrile was treated with the corresponding azol(*in*)ium dithiocarboxylate zwitterionic ligand. After addition of aqueous KPF_6, target complexes **5a-g** were precipitated from the solution and isolated in analytically pure form after filtration and washing with water and diethyl ether (Scheme 2). Spectroscopic data of complexes **4** and **5** were agree with those previously reported in the literature by our research group in a seminal paper [30].

More detailed information about the experimental procedures and full spectroscopic data for complexes **2**–**5** are included in the Supporting Information.

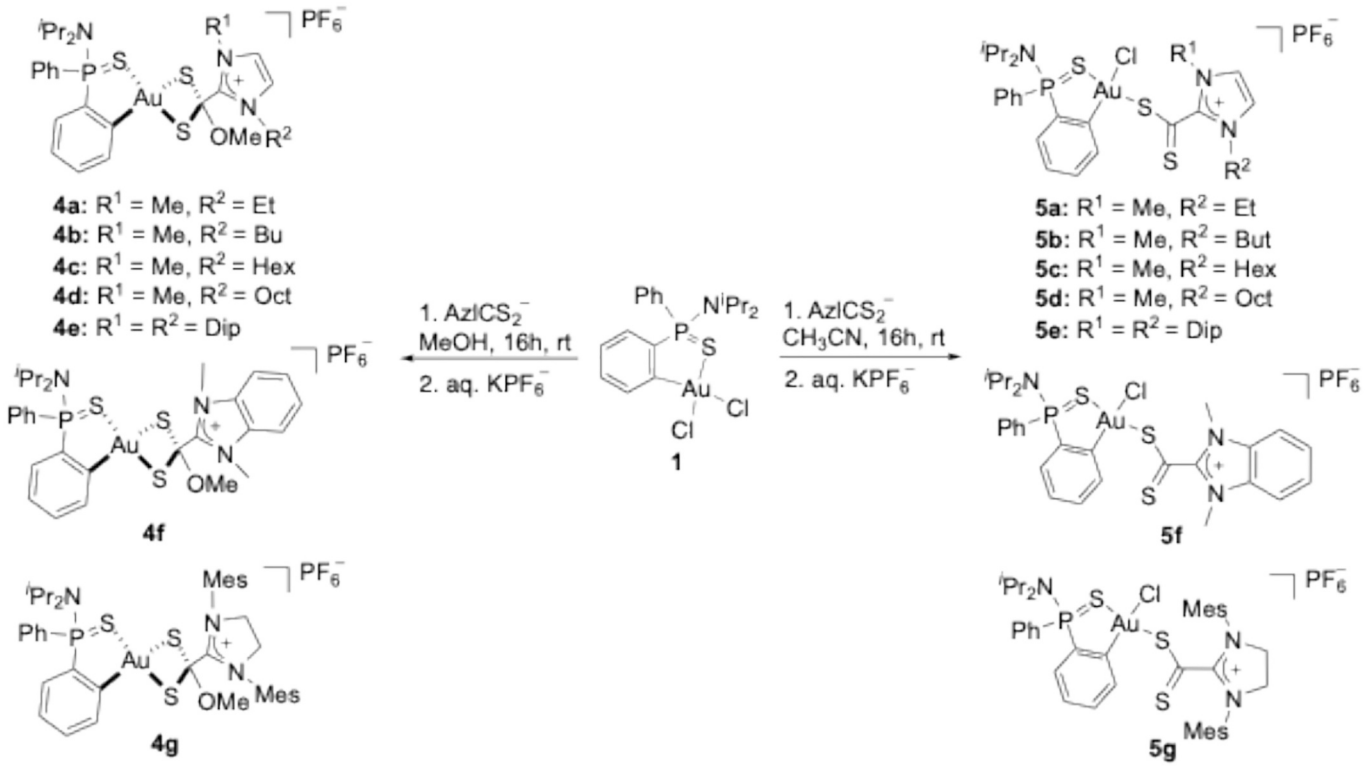
2.2. Antimicrobial activity

Antimicrobial activity was evaluated using a broth microdilution method following the Clinical & Laboratory Standards Institute (CLSI) guidelines [31]. Minimum inhibitory concentrations (MIC) determination was performed by a serial dilution method in 96-well microtiter plates. Bacteria were cultured at 37 °C in ISO-Sensitest broth (Oxoid, Madrid, Spain). After 18 h of cultivation, bacterial suspensions were made in physiological saline (0.9 %, *w/v* NaCl) and their turbidity was standardized to 0.5 McFarland. The final density of bacterial inoculum was 5×10^5 CFU/mL. The tested concentrations of gold-ranged from 0.05 mg/L to 1024 mg/L. The inoculum was added to all wells and the plates were incubated at 37 °C for 24 h. Molecules are considered active at MIC $\leq$2 mg/L, with moderately active at MICs between 2 and 32 mg/L, and inactive at MICs >32 mg/L.

Ciprofloxacin served as positive control, while one non-inoculated well, free of any antimicrobial agent, was also included to ensure medium sterility. The reference gold metalloantibiotic Auranofin was also



Scheme 1. Synthesis of Au(III) complexes **2** and **3**.



Scheme 2. Synthesis of Au(III) complexes **4** and **5**.

included for comparative purposes. MIC was defined as the lowest concentration of the test compound that inhibited visible growth (red colored pellet on the bottom of the wells after the addition of TTC). All experiments were carried out in triplicate.

2.3. Cytotoxicity

The preliminary cytotoxicity tests (Table 1) were developed on Jurkat E6.1 commercial cell line using the XTT cell proliferation assay. Study was conducted in sterile 96-well microplates and cells were spread to a density of 10^5 in 100 μL/well. Serial concentrations of the tested compounds were also included in the rows, starting with a range of 64 mg/L. After 24 h incubation with the conditions mentioned before, 50 μL of XTT (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate) were added to each well and incubated for additional 4 h. Measurement of the results were made on the Epoch™ spectrophotometer plate reader at a wavelength of 450–500 nm and 630–690 nm, and CC_{50} calculations were done using GeneData Screener Software.

Cytotoxicity on liver-derived lines were performed on HepG2 and THLE-2 using the MTT metabolic test. Cells were seeded at a density of 10.000/well in a 96-wells plate and were incubated in a humidified atmosphere at 37 °C with 5 % CO_2 for 24 h. Solutions of Au(III) complexes were prepared at 25 mM in 100 % DMSO and analyzed in an increasing dose curve from 100 to 0.2 μM. After 72 h of treatment, plates were treated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at 5 μg/mL in Minimum Essential Medium Eagle (MEM) for 2 h. Then, DMSO was added to the plates to solubilizing the formazan crystals formed in viable cells and plates were stirred for 5 min to homogenize the solution. Absorbance was measured at 570 nm by Envision Multiplate Reader (PerkinElmer), and CC_{50} calculations were done using GeneData Screener Software.

2.4. Stability and solubility

Stability was evaluated by ^1H and ^{31}P NMR. NMR spectra were recorded on Bruker Avance III HD 300 (^1H 300 MHz; ^{13}C 75 MHz; ^{31}P 121 MHz). Chemical shifts are given in ppm using tetramethylsilane (TMS) as internal standard for ^1H and 85 % H_3PO_4 for ^{31}P as external standard.

Solubility was estimated from the concentration of test compound that produced an increase in UV absorbance above the background levels (DMSO in buffer). The results were presented as an estimated precipitation range (lower bound and upper bound). It is assumed that at some point between the upper and lower bound range, the compound will precipitate. Test compound was dissolved in DMSO at 10 mM. Compounds were 6 points 1/3 serially diluted in DMSO to reach an assay final concentration range from 100 to 0.41 μM. 2 μL of DMSO compound serial dilutions were dissolved in 198 μL of phosphate buffer pH 7.4 at 100 mM. Assay plates were incubated for 2 h at 37 °C. After incubation time absorbance was determined at 612 nm.

3. Results

3.1. Preliminary screening to identify potential gold metalloantibiotics

Initially, the in vitro antimicrobial activity of complex families **2–5** was assessed using a panel of bacteria representing microorganisms of clinical importance (Table 1). The strains were selected among the most representative Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative bacteria (*Stenotrophomonas maltophilia*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*). The performance of complexes **2–5** as antimicrobial agents was compared with the behavior of clinical antibiotic ciprofloxacin and gold complex auranofin since, as it has been stated before, is the reference metallodrug which is currently being explored for potential therapeutic application in bacterial infections.

κ^2S,S'-Dithiocarbamate complexes **2** were active against all the

Table 1
Preliminary in vitro evaluation of the (C^S)-cyclometallated Au(III) complexes.

Complex	Antimicrobial activity (MIC, mg/L)						Citotoxicity[g] (CC_{50}, mg/L)[h]
	S. aureus[a]	*S. epidermidis*[b]	*S. maltophilia*[c]	*P. aeruginosa*[d]	*E. coli*[e]	A. baumanii[f]	
2a	1	1	4	>128	32	2	–
2b	0.5	0.5	8	16	16	2	–
2c	8	2	>128	>128	>128	>128	–
2d	0.25	0.25	4	32	16	2	1.46 ± 0.08
2e	0.25	0.25	16	32	32	8	1.67 ± 0.03
2f	0.25	0.25	4	8	8	2	2.48 ± 0.31
2 g	1	0.5	2	8	32	4	–
3a	0.125	0.125	2	8	8	2	1.81 ± 0.29
3b	0.125	0.25	4	16	8	4	2.16 ± 0.18
3c	0.5	0.25	2	8	8	2	–
3d	0.125	0.125	4	16	8	2	1.43 ± 0.30
3e	0.25	0.5	8	32	16	2	–
3f	0.25	0.5	4	16	8	4	–
4a	2	2	32	>128	>128	16	–
4b	2	1	4	32	16	4	2.23 ± 0.05
4c	1	1	4	64	>128	2	10.80 ± 1.65
4d	32	8	>128	>128	>128	64	–
4e	8	4	4	>128	>128	>128	–
4f	2	1	4	>128	>128	2	1.81 ± 0.29
4 g	32	32	32	>128	>128	>128	–
5a	64	64	>128	>128	>128	>128	–
5b	32	32	>128	>128	>128	>128	–
5c	16	16	>128	>128	>128	>128	–
5d	8	8	>128	>128	>128	64	–
5e	32	16	>128	>128	>128	>128	–
5f	4	2	>128	>128	>128	32	–
5 g	32	16	>128	>128	>128	>128	–
CIP[i]	>128	>128	0.5	>128	>128	>128	–
AUR[j]	0.125	0.125	16	64	>128	64	–

[a] ATTC 25923.
[b] ATCC 29887.
[c] ATCC13637.
[d] ATCC 27853.
[e] ATCC 25922.
[f] ATCC 19606.
[g] Jurkat E6.1. cell line.
[h] Concentration of test compounds required to reduce cell viability by 50 %.
[i] Ciprofloxacin.
[j] Auranofin.

Gram-positive bacteria tested. Complexes **2d-f** presented the higher activity, in the range of 0.25 mg/L. The activity of complexes **2a** and **2 g** is fourfold lower (1.0 mg/L). The activity of **2c** was just moderate against Gram-positive strains, especially for *S. aureus* (8.0 mg/L). Regarding Gram-negative strains, complexes **2b**, and **2d-g** showed moderate activity against *S. maltophilia*, *E. coli*, A. baumanii and *P. aeruginosa*. Complex **2a** resulted moderately active against *S. maltophilia*, *A. baumannii* and *E. coli* but inactive against *P. aeruginosa*. Complex **2c** did not show activity against any Gram-negative bacterial strain. Among the synthetized derivatives **2**, the most active complex against all the studied strains was **2f**, which interestingly also display the lowest toxicity (2.48 mg/L). The antibacterial activity of the free dithiocarbamate ligands was also evaluated for comparative purposes, and none of them displayed any relevant activity (Supporting Information, Table S1).

The antibacterial activity of complexes **3** followed a similar trend, showing higher activities against Gram-positive bacteria. Among them, complexes **3b** and **3d**, bearing a chloride and a trifluoroborate counter anion, respectively, were the most active against most of the studies strains. Even though the activity of **3d** against *S. epidermidis* and A. baumanii was slightly higher, complex **3b** have a more favourable toxicological profile (2.16 mg/L) when compared to complex **3d** and parent complex **3a** (1.43 and 1.81 mg/L for **3d** and **3a**, respectively). Finally, complexes **3e** and **3f** follow the activity pattern of most of the complexes studied, showing high activity against Gram-positive strains but moderate activity against Gram-negative strains.

Regarding, κ^2S,S′-azol(*in*)ium-2-(methoxy)methanedithiol complexes **4**, **4a**, **4b**, **4c** and **4f** displayed moderate against Gram-positive strains, in the range between 1 and 2 mg/L for *S. aureus* and S. epidermis, and just slightly lower to Gram-negative *S. maltophilia* (Table 1). In contrast, no activity against *P. aeruginosa* and *E. coli* strains was observed. Interestingly, complexes **4c** and **4f** were more active to multidrug-resistant *A. baumannii*, with MICs of 2 mg/L, which is better than those of the reference antibiotics. Complexes **4d**, **4e** and **4 g** were only moderately active against Gram-positive *S. aureus* and S. epidermis, showing MICs between 4 and 32 mg/L, and inactive against all Gram-negative species. Among κ^2S,S′-azol(*in*)ium-2-(methoxy)methanedithiol complexes **4**, compound **4c** was the most active against most of the studied bacterial strains and also the least toxic derivative in the preliminary cytotoxicity tests, with a CC_{50} of 10.80 mg/L.

Finally, κ^1S-azol(*in*)ium-2-dithiocarboxylate complexes **5** presented lower or null activity against all the microbial strains tested. In the same way, free azol(*in*)ium-2-dithiocarboxylate ligands display negligible antibacterial activity against all the studied bacterial strains (Supporting Information, Table S1).

3.2. Stability and solubility of au(III) complexes

To investigate the stability of the Au(III) compounds under physiological conditions, solutions of complexes **2f**, **3b**, **4c** and **5f** in DMSO-d_6 were treated with phosphate buffer saline (PBS), heated to 37 °C and monitored by ^{31}P NMR over 48 h (Fig. S37, S39 and S41, Supporting

Information). No significant deviation of chemical shift of the phosphorous signal was observed over the time for complexes **2f**, **3b** and **4c**, indicating that both the (CˆS)-cyclometallated and the (SˆS)-bidentate ligands are suitable to stabilize Au(III) under physiological conditions. The ^{1}H NMR data also confirmed that the chemical integrity of the complex is maintained (Figs. S38, S40 and S42, Supporting Information). On contrary, ^{31}P NMR data of complex **5f**, bearing a (CˆS)-cyclometallated main ligand and two monodentate ancillary ligands, display evident degradation over the time.

The stability of the Au(III) complexes under the conditions used for the biological evaluation was also assessed. Thus, solutions of complexes **2f**, **3b**, **4c** and **5f** in DMSO-d_6 were treated with ISO-Sensitest culture medium and incubated at 37 °C during 24 h and the ^{31}P NMR showed that complexes **2f**, **3b** and **4c** were perfectly stable under those conditions (Figs. S43-S45, Supporting Information).

In order to evaluate their redox stability, solutions of complexes **2f**, **3b** and **4c** in DMSO-d_6 and PBS were treated with the reducing peptide glutathione (GSH). The ^{31}P NMR spectra of the resulting samples showed that the metallocenter maintained its chemical integrity in all cases (Fig. S46, S48 and S50, Supporting Information). The stability of the aurocycle was further confirmed by ^{1}H NMR (Figs. S47, S49 and S52, Supporting Information).

As solubility in water is a relevant property for drug development, the kinetic solubility for all the studied complexes was also determined. Complexes **2a**, **3c**, **3d**, **3f**, **4a-g** and **5a-g** showed low solubility limits, with values between 3 and 11 μM; Complexes **2b**, **2d-g**, **3a** and **3e** showed intermediate solubility limits, with values between 11 and 30 μM; Complexes **2c** and **3b** showed acceptable solubility limits, with values between 30 and 100 μM and > 100 μM respectively.

3.3. Evaluation of the antibacterial activity and toxicity of selected au(III) complexes

Complexes **2f**, **3b,** and **4c** were tested against a panel of multiresistant strains of *S. aureus*, S. epidermis and *A. baumannii* of clinical origin. As depicted in Table 2, both complexes **2f** and **3b** showed a good activity against Gram-positive *S. aureus* and S. epidermis. However, a decrease of activity was observed for Gram-negative *A. baumannii*, which is much more pronounced for **3b**. Complex **4c** is less active against both Gram-positive strains, but in this case, there is no selectivity towards Gram-positive strains and the activity against Gram-negative *A. baumannii* is virtually the same.

The in vitro cytotoxicity of Au (III) complexes **3b**, **2f** and **4c** was evaluated in tumoral HepG2 and non-tumoral THLE-2 mammalian cell lines from liver tissue (Table 3). Complexes, **3b** and **2f** displayed relatively high cytotoxicity against both cell lines. On contrary, Au(III) complex **4c** showed CC_{50} values above 10 μM in both cell lines (10.7 μM and 12.4 μM in the tumoral Hep G2 cell line and in the non-tumoral THLE-2 cell line, respectively).

Table 3
Citotoxicity on liver cell lines.

Complex	Cell Line	Citotoxicity (CC_{50}, μM)[a]
2f	HepG2	1.23 ± 0.05
	THLE-2	0.98 ± 0.05
3b	HepG2	1.47 ± 0.13
	THLE-2	2.08 ± 0.13
4c	HepG2	10.75 ± 1.00
	THLE-2	12.42 ± 0.80
AUR	HepG2	3.26 ± 0.25
	THLE-2	1.21 ± 0.20

[a] Concentration of test compounds required to reduce cell viability by 50 %.

4. Discussion and conclusions

4.1. Au(III) complexes

During our investigations directed to the development of novel gold metalloantibiotics, we described a novel family of (CˆS)-cyclometallated Au(III) κ^{2}S,S′-dithiocarbamate complexes. [29] Among them, complex complex **3a** exhibit relevant antibacterial activity against multiresistant microorganisms, ranged from 0.125 to 2 mg/L and from 2 to 8 mg/L among Gram-positive and Gram-negative pathogens, respectively [28]. However, its relatively high toxicity rules it out as a candidate for the development of metalloantibiotics. With the aim of improving the therapeutic window, in the present study we performed a series of structural modifications on both the dithiocarbamate ligand and the counteranion of complex **3a**, giving rise to novel complexes **2a-g** and **3b-f**. On addition, we also evaluated the antibacterial activity of related (CˆS)-cyclometallated Au(III) κ^{1}S-azol(*in*)ium-2-dithiocarboxylate complexes **4** and (CˆS)-cyclometallated Au(III) κ^{2}S,S′-azol(*in*)ium-2-(methoxy)methanedithiol complexes **5**, previously developed in our research group [30].

4.2. Selection of bacteria

For the preliminary evaluation of the antibacterial activity of Au(III) complexes **2**–**5**, reference ATCC strains including both Gram-negative and Gram-positive species of relevance in clinical settings were chosen. All the selected strains, except the *S. maltophilia* species, were resistant to ciprofloxacin, one of the most used antibiotics from treating different types of infections caused by both Gram-positive and Gram-negative bacteria.

The selected metallodrug candidates **2f**, **3b** and **4c** were tested against a panel of MDR strains of clinical origin. The resistance phenotypes are listed in Table 2. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains 162,065–705, 163,501–000, 161,071–210 and 162,058–967 are respiratory isolates from cystic fibrosis patients at the Hospital Clinic of Barcelona (Barcelona, Spain). *Staphylococcus epidermidis* a FG22014, FG03015 and FG14013 are wound isolates from the

Table 2
Evaluation of selected Au(III) complexes against a broad panel of resistant bacterial strains.

Entry	Bacterial species	Strain ID	Resistance	MIC (mg/L)			
				3b	**2f**	**4c**	AUR
1	*S. aureus*	162,065–705	CIP, LVX, CLI, ERI, PEN	0.25	0.25	1	0.125
2	*S. aureus*	163,501–000	CIP, LVX, CLI, ERI	0.5	0.25	2	0.125
3	*S. aureus*	161,071–210	CIP, LVX, CLI, ERI	0.25	0.25	2	0.125
4	*S. aureus*	162,058–967	CIP, ERI, AMK, TET, CHL, VAN	0.25	0.25	2	0.125
5	*S. epidermidis*	FG22014	PEN, CIP, ERI, CTX, CRO, CHL	0.25	0.5	1	0.125
6	*S. epidermidis*	FG03015	PEN, CIP, ERI, OXA	0.5	0.25	1	0.125
7	*S. epidermidis*	FG14013	PEN, CIP, ERI, CTX, GEN, CHL, OXA	0.25	0.5	1	0.125
8	A. baumanii	AbCr17	PanR	2	1	2	32
9	A. baumanii	Ab177	OXA-58	4	2	4	64
10	A. baumanii	Ab210	OXA-24	8	1	2	32

Hospital Clinic of Barcelona (Barcelona, Spain). *Acinetobacter baumannii* OXA-24 and OXA-58 were originally identified in 43 hospitals in Spain during February–March 2010 during the project GEIH-REIPI-Ab 2010 [32]; they produce OXA-24 and 58-like β-lactamases and are highly resistant to several clinical antibiotics (Table 2). *Acinetobacter baumannii* Abcr17 is a cerebrospinal fluid isolate from the Virgen del Rocío Universiy Hospital (Seville, Spain). This is a pan-drug resistant strain, meaning that is nonsusceptibility to all agents in all antimicrobial categories, including carbapenems [33].

4.3. Stability of the Au(III) metallocenter

When exploring the potential of Au(III) complexes as therapeutic agents, one should also bear in mind the redox characteristics of the noble metal center. Stabilization of the oxidation state (+3) is crucial to observe any biological activity; otherwise, the metal center would eventually undergo reduction, leading to the formation of metallic gold [34]. To prevent this reduction process, a useful strategy is the inclusion of Au(III) into a cyclometallated center. Hence, most of the biologically relevant Au(III) gold complexes have the same general formula [(CˆY)Au (L)] (Y = N, S), in which the gold atom forms a Au—C σ-bond, as well as a coordinate Au—N or Au—S bond [35]. The two remaining positions at the square planar gold(III) center are usually occupied by two monodentate or one bidentate anion. In this regard, further stabilization of the Au(III) center by means of a bidentate ligand prevent ligand exchange reactions with biological nucleophiles leading to inactivation of the compound, meaning that Au(III) complexes bearing a bidentate ligands show improved stability under biological conditions compared to those bearing two monodentate ligands [36].

In the search for useful gold metalloantibiotics, it is crucial to evaluate the stability of the Au(III) complexes **2**–**5** under physiological conditions. A relevant advantage these derivatives is the presence of a phosphorous atoms in the (CˆS)-cyclometallated ligand, allowing the easy evaluation of the stability of the gold(III) metallocenter by ^{31}P NMR. NMR studies confirmed the stability of complexes **2f**, **3b** and **4c**, bearing a bidentate (SˆS) ancillary ligand in addition to the principal (CˆS) cyclometallated ligand. On contrary, complexes **5f** containing two monodentate ancillary ligands are not stable under physiological conditions and are therefore of no interest for the development of therapeutic agents.

Despite the promising biological activity of several cyclometallated Au(III) complexes, their application in pharmacology have been severely hampered by its ability to oxidize numerous biologically relevant molecules. As it is already known Au(III) complexes are redox active and can be easily reduced to Au(I)/Au(0). In this sense, Au(III) reduces to Au(I)/Au(0) causing the oxidation of sulfur containing amino acids, such as L-Cys and tripeptide glutathione (GSH, L-γ-Glu-Cys-Gly). The oxidation of thiol containing amino acids by metal ions has been studied for decades because of their important role in both the structure and function of proteins [37]. Oxidatively modified proteins accumulate during aging, oxidative stress and in age-related diseases, and some of them were found in liver, heart, skeletal muscle, kidney, and in regions of the brain. Since GSH is a thiol containing peptide ubiquitous in biological systems present at mM concentrations in mammalian cells, we thought it would be interesting to study the behavior of the selected gold complexes in the presence of GSH.

In addition to their stability under physiological conditions, complexes **2f**, **3b,** and **4c** show remarkable redox stability, making them suitable as candidates for the development of therapeutic agents.

4.4. Antibacterial activity and cytotoxicity

The κ^2S,S′-dithiocarbamate complexes **2** displayed activity against all the Gram-positive bacteria tested, which were most pronounced in case of the hydroxyl containing dithiocarbamate ligands **2b**, **2d** and **2 f**. The lower activity of complexes **2a** and **2 g** could be explained due to the presence of bulky substituents making it more difficult for the complexes to reach the molecular components of target cells. In comparison to Gram-positive bacteria, similar structure-activity-relationships were observed for Gram-negative strains, but with a considerable increase in MIC values. The preference for Gram-positive over Gram-negative bacteria is also observed for Au(I) complex Auranofin (Table 1) and has been previously reported for dithiocarbamate and NHC gold complexes. Our previous studies on the mode of antibacterial action of Au (IIII) κ^2S, S′-dithiocarbamate complexes concluded that, analogously to Auranofin [2], inhibition of bacterial TrxR is a main target of these gold complexes [28]. The strong dependence of Gram-positive bacteria on an intact Trx/TrxR system explains the enhanced activity of gold complexes over Gram-positive bacteria when compared to Gram-negative bacteria. The only exception is complex **2c** that, despite being moderately active against Gram-positive bacteria, has null activity against Gram-negative strains. A plausible hypothesis for this result could be the effect of the carboxylic acid moiety in the charge balance of the referred complex. The mechanism of action Au(IIII) κ^2S,S′-dithiocarbamate complexes is multimodal; for Gram-negative strains, that can compensate the loss of the Trx/TrxR system with their glutathione system, the main mode of antibacterial action is the disruption of the bacterial membrane via electrostatic interactions. Gram-negative bacteria are coated with negatively charged lipopolysaccharide molecules, which have a higher affinity for the positively charged complexes, leading to an increased uptake resulting in intracellular damage [28,38].

The change of counter anion has a limited effect on activity, as demonstrated by the very similar activity of complexes **3a**-**f**. However, counter anions play a major role in the cytotoxicity and solubility of Au (III) dithiocarbamate complexes. In this regard, the substitution of the hexafluorophosphate counter anion of complex **3a** for a chloride resulted in almost a one-fold decrease of cytotoxicity for complex **3b**. On addition, complex **3b** has the best solubility profile of all the studied complexes **2**–**5**.

In general terms, κ^2S,S′-azol(*in*)ium-2-(methoxy)methanedithiol complexes **4** are less active than κ^2S,S′-dithiocarbamate complexes **2** and **3**. All complexes **4**, except complexes **4e** and **4 g**, presented good activities against MDR Gram-positive microorganisms, moderate activity against Gram-negative species *S. maltophilia* and *A. baumannii* and low activity against Gram-negative species *P. aeruginosa* and *E. coli*. Although compound **4b** presented the highest activity of all the family against Gram positive bacterial strains, this is in any case modest when compared to ciprofloxacin.

Even though the data set is limited, it is possible to gather some useful information regarding the structure-activity relationships (SAR). In general terms, more sterically hindered ligands resulted in less active complexes. Thus, the bulky substituents in complexes **4d**, **4e** and **4 g** contributed to their low effectivity, probably by complicating the intermolecular interactions between the complexes and molecular components of target. For Gram-positive strains, the steric effect of the size of the ligands was less pronounced, with small and medium-sized **4a-c** and **4e** having similar activities against *S. aureus* and S. epidermis. In the case of the Gram-negative *S. maltophilia*, *P. aeruginosa* and *E. coli* strains, the activities of complexes **4c** and **4d** were lower when compared to complex **4b** bearing smaller ligands, which is in line with the hypothesized steric effect. However, the steric effect alone cannot explain the pattern observed for the activity of the Au(III) complexes against Gram-negative *A. baumannii*. In this case, the best activity in all the series is observed for complex **4f** bearing a benzazoyl ligand and complex **4c** containing a hexyl aliphatic chain in the azoyl ligand. Complexes **4a** and **4b**, with an ethyl and butyl aliphatic chains, respectively, displayed a MIC eight-fold and two-fold higher, respectively. As stated before, the mechanism previously proposed for the action of gold complexes on *A. baumannii* involves membrane permeation, caused by the intercalation of multiple molecules of the gold complex within the bilipid layer [38]. The intercalation would be more effective with complexes bearing ligands with bigger aliphatic residues,

whereas bulkier ligands complicate the union to the active center of the enzymes. This hypothesis explains why the activity towards *A. baumannii* improves when increasing the size of the ligand from **4a** to **4b** and **4c** and then decreases steeply for complex **4d** bearing an octyl aliphatic chain in the azolium ligand. Complexes **4e** and **4 g** bearing very bulky substituents in the azol(*in*)ium ligand showed poor activity.

Regarding κ^1S-azol(*in*)ium-2-dithiocarboxylate complexes **5**, the lack of activity is hypothesized to be related with their poor stability.

The therapeutic index (TI), which is typically considered as the ratio between the dose of the drug that that causes toxicity to the dose that proves to be effective, is an important parameter used to identify the drug candidates that have an appropriately balanced safety–efficacy profile [39]. The prediction of the safety–efficacy profile at an early stage of the drug discovery process it is crucial to avoid late-stage failures. From the results of the primary screening of antibacterial activity and cytotoxicity, the therapeutic indexes of complexes in Table 1 were determined. The wider therapeutic window for *S. epidermidis* corresponds to complex **2f**, for *S. aureus* to complex **3b** and for *A. baumannii* to complex **4c**, with values of 9.92, 17.28 and 5.40 respectively. On account of their potentially more favourable safety profile, complexes **2f**, **3b** and **4c** were selected as potential candidates for the development of novel metalloantibiotics.

Complexes **2f**, **3b,** and **4c** were then tested against multi-resistant microbial strains of clinical origin of the three bacterial species of interest: *S. aureus*, *S. epidermidis* and *A. baumannii*. Despite **2f** and **3b** display a good activity against Gram positive *S. aureus* and *S. epidermidis*, is slightly below the reference gold drug auranofin. Interestingly, all three complexes were more active against *A. baumannii* resistant strains than auranofin. Complexes **2f** and **4c** show a very interesting antibacterial profile against multidrug-resistant *A. baumannii* (MRAB) and carbapenem-resistant *A. baumannii* resistant (CRAB). On addition, complex **4c** is 10 times less toxic than approved gold drug Auranofin, showing CC_{50} values above 10 μM in the non-tumoral THLE-2 cell (12.42 μM). Considering that this Au(III) complex has a 16-fold lower MIC against resistant *A. baumannii* strains when compared to auranofin, complex **4c** have a much improved therapeutic window for the treatment of resistant infections caused by *A. baumannii* species.

A. baumannii constitute one of the most important multidrug-resistant (MDR) microorganisms isolated in hospitalised patients worldwide and it is currently considered one of the most important nosocomial pathogens [40]. *A. baumannii* exhibits an outstanding ability acquire resistance to antibiotics, including carbapenems and even polymyxins, representing a phenomenal challenge for achieving effective antibacterial treatment [41]. Consequently multidrug-resistant *A. baumannii* is considered the most critical pathogen in the global priority list of antibiotic-resistant bacteria of the World Health Organization [42]. Hence, it is critical to discover and develop alternative antimicrobial strategies. Taking into account that there are currently few therapeutic options to treat MRAB and CRAB infections, Au(III) complex **4c** is a potential candidate to develop potential future therapeutic strategies to fight against *A. baumannii* infections in hospital settings.

In conclusion, several (SˆC)-cyclometallated Au(III) complexes based on an ortho-substituted phosphinothioic amide and differing in ligand denticity and structure have been biologically evaluated for their antimicrobial activities against a broad range of bacterial strains belonging to different Gram-positive and Gram-negative species. Among these derivatives, complexes **2f, 3b**, and **4c** were selected for further evaluation due to their antibacterial activity and promising cytotoxicity. The wider therapeutic window was observed for complex **4c**. Given the antibacterial activity, the high resistance profile of the strains tested, and the relatively low in vitro toxicity, complex **4c** is a good candidate as metalloantibiotic.

CRediT authorship contribution statement

Paula Pérez-Ramos: Writing – review & editing, Methodology, Investigation. **Yaiza Gabasa:** Writing – review & editing, Methodology, Investigation. **Enmanuel Cornielle:** Methodology, Writing – review & editing. **Humberto Rodríguez-Solla:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Sara M. Soto:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Raquel G. Soengas:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sara M. Soto has patent A gold(III) complex, a conjugate of the gold (III) complex, a pharmaceutical composition comprising the gold(III) complex and uses and a process for preparing the gold(III) complex issued to Institute of Global Health of Barcelona. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Abbreviations

AMR	antimicrobial resistance
WHO	World Health Organization
ROS	reactive oxygen species
NHC	N-heterocyclic carbene
NMR	nuclear magnetic resonance
IR	Infrared
HRMS	High resolution mass spectrometry
CLSI	Clinical & Laboratory Standards Institute
MIC	Minimum inhibitory concentrations

(*continued on next page*)

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CFU	Colony forming unit
TTC	Triphenyl tetrazolium chloride
XTT	Sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	Dimethylsulfoxide
TMS	Tetramethylsilane
PBS	Phosphate buffer saline
GSH	Glutathione
CC_{50}	Concentration of test compounds required to reduce cell viability by 50 %
TI	Therapeutic index
MRSA	Multidrug-resistant *S. aureus*
MRAB	Multidrug-resistant *A. baumannii*
CRAB	Carbapenem-resistant *A. baumannii*
MDR	multidrug-resistant

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinorgbio.2024.112735.

References

[1] Antimicrobial Resistance Collaborators, Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, Lancet 399 (2022) 629–655, https://doi.org/10.1016/S0140-6736(21)02724-0.

[2] Antimicrobial resistance surveillance in Europe 2023–2021 data, European Centre for Disease Prevention and Control and World Health Organization, Stockholm, 2023.

[3] M. Miethke, M. Pieroni, T. Weber, M. Brönstrup, P. Hammann, L. Halby, P. B. Arimondo, P. Glaser, B. Aigle, H.B. Bode, R. Moreira, Y. Li, A. Luzhetskyy, M. H. Medema, J.L. Pernodet, M. Stadler, J.R. Tormo, O. Genilloud, A.W. Truman, K. J. Weissman, E. Takano, S. Sabatini, E. Stegmann, H. Brötz-Oesterhelt, W. Wohlleben, M. Seemann, M. Empting, A.K.H. Hirsch, B. Loretz, C.M. Lehr, A. Titz, J. Herrmann, T. Jaeger, S. Alt, T. Hesterkamp, M. Winterhalter, A. Schiefer, K. Pfarr, A. Hoerauf, H. Graz, M. Graz, M. Lindvall, S. Ramurthy, A. Karlén, M. van Dongen, H. Petkovic, A. Keller, F. Peyrane, S. Donadio, L. Fraisse, L.J.V. Piddock, I. H. Gilbert, H.E. Moser, R. Müller, Towards the sustainable discovery and development of new antibiotics, Nat. Rev. Chem. 5 (2021) 726–749, https://doi.org/10.1038/s41570-021-00313-1.

[4] H. Li, H. Sun, Metal complexes as resistance breakers or metalloantibiotics for combating antimicrobial resistance, in: R.A. Scott (Ed.), Encyclopedia of Inorganic and Bioinorganic Chemistry, 2024, pp. 1–19.

[5] C.N. Morrison, K.E. Prosser, R.W. Stokes, A. Cordes, N. Metzler-Nolte, S.M. Cohen, Expanding medicinal chemistry into 3D space: metallofragments as 3D scaffolds for fragment based drug discovery, Chem. Sci. 11 (2020) 1216–1225, https://doi.org/10.1039/C9SC05586J.

[6] A. Frei, A.D. Verderosa, A.G. Elliott, J. Zuegg, M.A.T. Blaskovich, Metals to combat antimicrobial resistance, Nat. Rev. Chem. 7 (2023) 202–224, https://doi.org/10.1038/s41570-023-00463-4.

[7] A. Frei, J. Zuegg, A.G. Eliot, M. Bakerb, S. Braese, C. Brown, F. Chen, C. Dowson, G. Dujardin, N. Jung, A.P. King, A.M. Mansour, M. Massi, J. Moat, H.A. Mohamed, A.K. Renfrew, P.J. Rutledge, P.J. Sadler, M.H. Todd, C.E. Willans, J.J. Wilson, M. A. Cooper, M.A.T. Blaskovich, Metal complexes as a promising source for new antibiotics, Chem. Sci. 11 (2020) 2627–2639, https://doi.org/10.1039/C9SC06460E.

[8] J.W. Alexander, History of the medical use of silver, Surg. Infect. 10 (2009) 289–292, https://doi.org/10.1089/sur.2008.9941.

[9] K. Mijnendonckx, N. Leys, J. Mahillon, S. Silver, R. Van Houdt, Antimicrobial silver: uses, toxicity and potential for resistance, Biometals 26 (2013) 609–621, https://doi.org/10.1007/s10534-013-9645-z.

[10] A.B. Lansdown, Metallothioneins: potential therapeutic aids for wound healing in the skin, Wound Repair Regen. 10 (2002) 130–132, https://doi.org/10.1046/j.1524-475X.2002.20101.x.

[11] S. Medici, M. Peana, G. Crisponi, V.M. Nurchi, J.I. Lachowicz, M. Remelli, M. A. Zoroddu, Silver coordination compounds: a new horizon in medicine, Coord. Chem. Rev. 327–328 (2016) 349–359, https://doi.org/10.1016/j.ccr.2016.05.015.

[12] J.S. Möhler, W. Sim, M.A.T. Blaskovich, M.A. Cooper, Z.M. Ziora, Silver bullets: a new lustre on an old antimicrobial agent, Biotechnol. Adv. 36 (2018) 1391–1411, https://doi.org/10.1016/j.biotechadv.2018.05.004.

[13] N.A. Johnson, M.R. Southerland, W.J. Youngs, Recent developments in the medicinal applications of silver-NHC complexes and imidazolium salts, Molecules 22 (2017) 1263, https://doi.org/10.3390/molecules22081263.

[14] A. Kascatan-Nebioglu, A. Melaiye, K. Hindi, S. Durmus, M.J. Panzner, L.A. Hogue, R.J. Mallett, C.E. Hovis, M. Coughenour, S.D. Crosby, A. Milsted, D.L. Ely, C. A. Tessier, C.L. Cannon, W.J. Youngs, Synthesis from caffeine of a mixed N-heterocyclic carbene-silver acetate complex active against resistant respiratory pathogens, J. Med. Chem. 49 (2006) 6811–6818, https://doi.org/10.1021/jm060711t.

[15] K.M. Hindi, M.J. Panzner, C.A. Tessier, C.L. Cannon, W.J. Youngs, The medicinal applications of imidazolium carbene- metal complexes, Chem. Rev. 109 (2009) 3859–3884, https://doi.org/10.1021/cr800500u.

[16] L. Ronga, M. Varcamonti, D. Tesauro, Structure–activity relationships in NHC–silver complexes as antimicrobial agents, Molecules 28 (2023) 4435, https://doi.org/10.3390/molecules28114435.

[17] S.R. Isbel, S.A. Patil, A. Bugarin, NHCs silver complexes as potential antimicrobial agents, Inorg. Chim. Acta 563 (2024) 121899, https://doi.org/10.1016/j.ica.2023.121899.

[18] E.T. Walz, M.J. Dimartino, D.E. Griswold, A.P. Intoccia, T.L. Flanagan, Biologic actions and pharmacokinetic studies of auranofin, Am. J. Med. 75 (1983) 90–108, https://doi.org/10.1016/0002-9343(83)90481-3.

[19] B.B. Fuchs, R. RajaMuthiah, A.C. Souza, S. Eatemadpour, R.D. Rossoni, D. A. Santos, J.C. Junqueira, L.B. Rice, E. Mylonakis, Inhibition of bacterial and fungal pathogens by the orphaned drug auranofin, future, Med. Chem. 8 (2016) 117–132, https://doi.org/10.4155/fmc.15.182.

[20] S. Thangamani, H. Mohammad, M.F. Abushahba, T.J. Sobreira, V.E. Hedrick, L. N. Paul, M.N. Seleem, Antibacterial activity and mechanism of action of auranofin against multi-drug resistant bacterial pathogens, Sci. Rep. (2016), https://doi.org/10.1038/srep22571.

[21] B.Đ. Glišić, M.I. Djuran, Gold complexes as antimicrobial agents: an overview of different biological activities in relation to the oxidation state of the gold ion and the ligand structure, Dalton Trans. 43 (2014) 5950–5969, https://doi.org/10.1039/C4DT00022F.

[22] P. Patanjali, R. Kumar, S. Bharti, A. Kumar, P. Chaudhary, R. Singh, Reviewing gold(III) complexes as effective biological operators, Main Group Chem. 17 (2018) 35–52, https://doi.org/10.3233/MGC-180247.

[23] M. Mora, M.C. Gimeno, R. Visbal, Recent advances in gold–NHC complexes with biological properties, Chem. Soc. Rev. 48 (2019) 447–462, https://doi.org/10.1039/C8CS00570B.

[24] J.R. Stenger-Smith, P.K. Mascharak, Gold drugs with $\{Au(PPh_3)\}^+$ moiety: advantages and medicinal applications, ChemMedChem 15 (2020) 2136–2145, https://doi.org/10.1002/cmdc.202000608.

[25] C. Ratia, R.G. Soengas, S.M. Soto, Gold-derived molecules as new antimicrobial agents, Front. Microbiol. 13 (2022) 846959, https://doi.org/10.3389/fmicb.2022.846959.

[26] C.I. Yeo, C.H.P. Goh, E.R.T. Tiekink, J. Chew, Antibiotics: a "GOLDen" promise? Coord. Chem. Rev. 500 (2024) 215429 https://doi.org/10.1016/j.ccr.2023.215429.

[27] C. Ratia, V. Cepas, R. Soengas, Y. Navarro, M. Velasco-de Andrés, M.J. Iglesias, F. Lozano, F. López-Ortiz, S.M. Soto, A C∧S-cyclometallated gold(III) complex as a novel antibacterial candidate against drug-resistant bacteria, Front. Microbiol. 13 (2022) 815622, https://doi.org/10.3389/fmicb.2022.815622.

[28] C. Ratia, V. Ballén, Y. Gabasa, R.G. Soengas, M. Velasco-de Andrés, M.J. Iglesias, Q. Cheng, F. Lozano, E.S.J. Arnér, F. López-Ortiz, S.M. Soto, Novel gold(III)-dithiocarbamate complex targeting bacterial thioredoxin reductase: antimicrobial activity, synergy, toxicity, and mechanistic insights, Front. Microbiol. 14 (2023) 198473, https://doi.org/10.3389/fmicb.2023.1198473.

[29] S. Sueiro, C. Ratia, R.G. Soengas, M.J. Iglesias, F. López-Ortiz, S.M. Soto, Gold(III) complexes activity against multidrug-resistant bacteria of veterinary significance, Antibiotics 12 (2022) 1728, https://doi.org/10.3390/antibiotics11121728.

[30] P. Pérez-Ramos, M.A. Mateo, D. Elorriaga, D. García-Vivó, R.G. Soengas, H. Rodríguez-Solla, Coordination of azol(in)ium dithiocarboxylate ligands to Au (III): unexpected formation of a novel family of cyclometallated Au(III) complexes, DFT calculations and catalytic studies, Dalton Trans. 53 (2024) 9433–9440, https://doi.org/10.1039/D4DT01184H.

[31] Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Third Informational Supplement M100-S23, CLSI, Wayne, PA, USA, 2013.

[32] F. Fernández-Cuenca, M. Tomás-Carmona, F. Caballero-Moyano, G. Bou, L. Martínez-Martínez, J. Vila, J. Pachón, J.M. Cisneros, J. Rodríguez-Baño, A. Pascual, In vitro activity of 18 antimicrobial agents against clinical isolates of Acinetobacter spp.: multicenter national study GEIH-REIPI-Ab 2010, Enferm. Infecc. Microbiol. Clin. 31 (2013) 4–9, https://doi.org/10.1016/j.eimc.2012.06.010.

[33] A.-P. Magiorakos, A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, S. Harbarth, J.F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D.L. Paterson, L. B. Rice, J. Stelling, M.J. Struelens, Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, Clin. Microbiol. Infect. 18 (2012) 268–281, https://doi.org/10.1111/j.1469-0691.2011.03570.x.

[34] E.A. Pacheco, E.R.T. Tiekink, M.W. Whitehouse, Gold Chemistry, Wiley-VCH Verlag GmbH & Co. KGaA, 2009, pp. 283–319.

[35] B. Bertrand, S. Spreckelmeyer, E. Bodio, F. Cocco, M. Picquet, P. Richard, P. Le Gendre, C. Orvig, M.A. Cinellu, A. Casini, Dalton Trans. 44 (2015) 11911–11918, https://doi.org/10.1039/C5DT01023C.

[36] M.D. Đurović, Ž.D. Bugarčić, R. van Eldik, Stability and reactivity of gold compounds – from fundamental aspects to applications, Coord. Chem. Rev. 338 (2017) 186–206, https://doi.org/10.1016/j.ccr.2017.02.015.

[37] L.A. Ba, T. Burkholz, T. Schneider, C. Jacob, in: A.B. Hughes (Ed.), Role of Cysteine in Amino Acids, Peptides and Proteins in Organic Chemistry vol. 5, Chapter 10, Wiley-VCH, Heidelberg, 2012, pp. 361–394.

[38] T. Samanta, G. Roymahapatra, W.F. Porto, S. Seth, S. Ghorai, S. Saha, J. Sengupta, O.L. Franco, J. Dinda, S.M. Mandal, N,N'-Olefin functionalized bis-imidazolium gold(I) salt is an efficient candidate to control keratitis-associated eye infection, PLoS One 8 (2013) e58346, https://doi.org/10.1371/journal.pone.0058346.

[39] P. Muller, M. Milton, The determination and interpretation of the therapeutic index in drug development, Nat. Rev. Drug Discov. 11 (2012) 751–761, https://doi.org/10.1038/nrd3801.

[40] I. Roca, P. Espinal, X. Vila-Farrés, J. Vila, The *Acinetobacter baumannii* oxymoron: commensal hospital dweller turned pan-drug-resistant menace, Front. Microbiol. 3 (2012) 148, https://doi.org/10.3389/fmicb.2012.00148.

[41] N. Karah, A. Sundsfjord, K. Towner, Ø. Samuelsen, Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*, Drug Resist. Updat. 15 (2012) 237–247, https://doi.org/10.1016/j.drup.2012.06.001.

[42] World Health Organization, Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics, Available at: https://www.who.int/publications/i/item/9789240093461.