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(Aminophosphane)gold(I) and silver(I) complexes as antibacterial agents

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ABSTRACT

This manuscript describes the synthesis of new Au(I) and Ag(I) complexes with aminophosphane ligands and a study of their antibacterial activity against Gram-negative *Salmonella enterica* serovar *typhimurium* and *Escherichia coli* and Gram-positive *Listeria monocytogenes* and *Staphylococcus aureus*. The bactericidal assays revealed the effectiveness of these compounds on paradigm Gram-negative and Gram-positive pathogens, showing a moderate antimicrobial activity, comparable with the antibiotics of reference, for all gold(I) complexes and the silver(I) complexes without coordinated PPh_3 groups. For those complexes that were found to show inhibitory activity, serial dilutions in liquid broth method were performed for determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration).

1. Introduction

The use in medicine of penicillin in the 1940s, erythromycin in the 1950s and methicillin in the 1960s greatly contributed to control bacterial diseases. However, the extended and uncontrolled use of antibiotics soon brought the need for the development of new antimicrobial agents because of the rapid increase of bacterial resistance, decreasing antibiotic efficacy and complicating the treatment of infections. The acquired bacterial resistance to vancomycin was slower and required nearly thirty years to found antibiotic resistant strains [1]. Since then, a large number of transition metal compounds have been screened against bacteria and fungi. For gold(I), research has been principally focused on compounds with a SAuP or NAuP core, exploiting the analogy to Auranofin [2]. Studies have shown good bactericidal or bacteriostatic results [3–5], some of them including Auranofin and related complexes were shown as potent inhibitors of methicillin-resistant *Staphylococcus aureus* [6], and they also present antitumor, antimalarial, or antirheumatic activities [7]. Other important types of metal complexes presenting good antibacterial activity are those related to N-heterocyclic carbene ligand NHCs, especially silver but also gold complexes [8].

Silver(I) compounds also show a wide spectrum of effective antimicrobial activity. Silver nitrate has been used since the seventeenth century for the prevention of neonatal conjunctivitis and is still in use today [9]. The most commonly used silver compound is silver(I) sulfadiazine ([Ag((4-aminophenyl)sulfonyl)(pyrimidin-2-yl)azanide]$_n$), which is widely used in medicine to treat and prevent the development of bacterial infections in skin burns [10]. Nowadays numerous silver(I) compounds with AgN, AgP or NAgP unit have been synthesized [11–14], and more recently AgC from NHCs [15,16] and have presented cytotoxic activity against Gram-positive and Gram-negative bacteria, although the mechanisms of action of silver(I) compounds are not completely understood.

Infections caused by Gram-positive and Gram-negative bacteria are of major concern due to the increased incidence of drug resistant strains. The food-borne *Salmonella typhimurium* and *Escherichia coli* are the most prevalent Gram-negative pathogens in humans and animals. These bacteria cause enteric diseases that are commonly treated by β-lactam antibiotics. However, the prolonged administration of these antibiotics has resulted in a striking correlation between drug use and the emergence of antibiotic resistant strains. β-Lactam antibiotics interfere with bacterial cell wall biosynthesis that eventually leads to cell death. The mechanism of action is to bind covalently to their targets, the penicillin-binding proteins (PBPs), enzymes involved in cell wall synthesis in the cytoplasmic membrane of the bacteria. Antibiotics penetrate the outer membrane of Gram-negative bacteria through protein pores called porins. The acquired resistance to β-lactam antibiotics may be due to a range of diverse factors: mutations in the PBPs that reduce or prevent the binding to the antibiotic, porin mutations which preclude or reduce outer-membrane permeability [17], hyper-expression of efflux pumps that expel antibiotics outside

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the bacteria or β-lactamases produced by bacteria to cleave the antibiotic. The combination of these factors results in Gram-negative resistance to β-lactam antibiotics [18,19].

With regard to Gram-positive organisms, *Listeria monocytogenesis* is a food-borne human pathogen responsible for *listeriosis*, a disease that although not common in healthy people, can cause severe diseases including abortions and meningitis. The number of *listeriosis* cases has increased in recent years in several industrialized countries [20–23] and although treatment with ampicillin or penicillin in combination with gentamicin (or other aminoglycoside) [24] is effective, the recent increase in antibiotic resistance is a concern. For example, strains N53-1 and 4446 are resistant to gentamicin and other aminoglycosides, and some strains are able to persist within different types of food processing plants for years and consequently are repeatedly exposed to biocides, something that could potentially impact on bacterial resistance [25–28].

S. aureus is another Gram-positive bacteria causative of serious hospital-acquired infections. Nowadays strains with intermediate levels of resistance to vancomycin or even resistant appear more frequently [29]. One reason for this could be the bifunctional acetyltransferase(6′)-Ie-phosphotransferase(2″)-Ia[AAC(6′)-Ie-APH(2″)-Ia], the most important aminoglycoside-resistance enzyme in Gram-positive bacteria, that confers resistance to almost all known aminoglycoside antibiotics in clinical use [30]. However resistance is not only related to aminoglycoside antibiotics like vancomycin, there are *S. aureus* strains resistant to methicillin that are also resistant to practically all β-lactam group (penicillin, oxacillin, ampicillin, amoxicillin and augmentin), and macrolides (clindamycin and azithromycin). These multidrug-resistant strains are spreading outside hospitals and represent a serious concern [31] and sensitive to the aminoglycoside group (amikacin), macrolide antibiotics (nitrofurantoin) and the quinolone group (ciprofloxacin, ofloxacin and norfloxacin) [32–35].

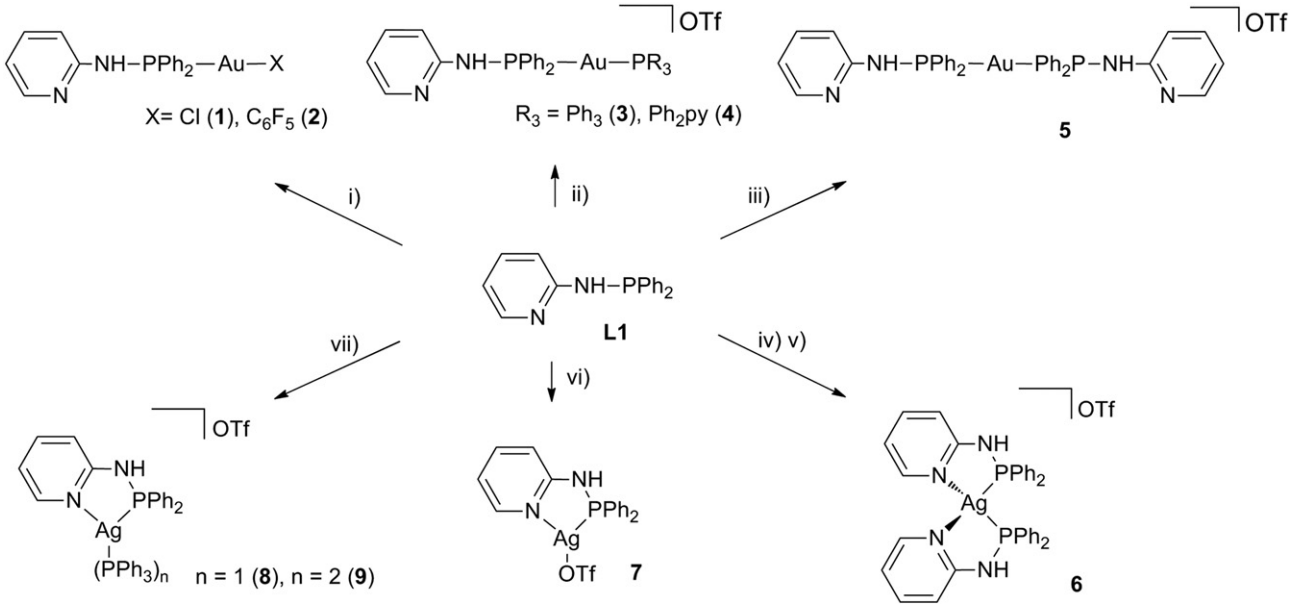
In this work we describe the synthesis and structural characterization of new gold and silver complexes with aminophosphane ligands. These ligands were chosen because other phosphane-gold derivatives are known to have antibacterial activity and because the presence of the amine group allows an easy functionalization, making possible the introduction of relevant biological moieties. These compounds were tested in vitro to study their activity against Gram-negative *S. typhimurium* SV5015 and *E. coli* ATCC 10536 strains and the Gram-positive *L. monocytogenes* EGD-e and *S. aureus* ATCC 11632 strains using the paper disk diffusion method (for the qualitative determination) and for those complexes that were found to show inhibitory activity, serial dilutions in liquid broth method were performed (for determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration)). This paper is a continuation of a previous study using the same ligand [36], in which the susceptibility of (aminophosphane)gold(I) thiolate complexes against *Enterococcus faecalis* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* TG1 was demonstrated. Some of them were found to exhibit powerful antibacterial activity, being more efficient against Gram-positive microorganisms.

2. Results and discussion

2.1. Synthesis and characterization

Herein we report the synthesis and characterization of a series of gold(I) and silver(I) derivatives with two different aminophosphane ligands: 2-(diphenylphosphanylamino)pyridine (Ph_2PNHpy) (Scheme 1) and 3-(diphenylphosphanylamino)-1,2,4-triazole [$Ph_2PNH(Htrz)$] (Scheme 2) [37]. Spectroscopic characterizations of the metal complexes were performed using IR, ESI + mass spectra, and multinuclear NMR spectroscopy, ^1H, $^{19}F\{^1H\}$, $^{31}P\{^1H\}$, and $^{13}C\{^1H\}$ NMR.

In the ESI + (positive mode of electrospray ionization) mass spectra of **3**–**6**, **8**, **9**, **12** and **14** the cation peak $[M - OTf]^+$ is observed and in **10** the protonated molecular peaks $[M + H]^+$ are seen. The IR spectra of **2** and **11** show the bands corresponding to the presence of the pentafluorophenyl groups bonded to the gold atom. Their $^{19}F\{^1H\}$ NMR spectra show three signals corresponding to *ortho*, *meta* and *para* fluorine atoms, respectively, of the pentafluorophenyl ring with small shifts compared to the starting material $[Au(C_6F_5)(tht)]$ (tht = tetrahydrothiophene). The ^1H NMR spectra of the compounds in d6-DMSO or d6-*acetone* present the expected resonances for all of the protons (see the Experimental section) and show a low or high-field shift of the aromatic protons indicatives of the successful coordination of the metal to the ligand through the phosphorus (**1**–**5**, **10**–**12**) or the phosphorus and the nitrogen atom (**6**–**9**, **13**–**14**). The ^1H NMR spectra of complexes **3**, **4**, **8** and **9** show the corresponding phosphine group signal, indicative of the coordination of one or two phosphine group. The $^{31}P\{^1H\}$ NMR spectra provide a great deal of information about



Scheme 1. Synthesis of gold(I) and silver(I) complexes with Ph_2PNHpy (**L1**). i) [AuX(tht)]; ii) [Au(OTf)(PR_3)]; iii) 0.5 [Au(tht)$_2$](OTf); iv) 0.5 Ag(OTf); v) [Ag(OTf)(tht)]; vi) Ag(OTf); vii) [Ag(OTf)(PPh_3)$_n$].