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1,2,3-Triazole-gold(I)-triethylposphine derivatives active against resistant Gram-positive pathogens☆

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ABSTRACT

Innovative organogold(I) antibacterial compounds were synthesized by click chemistry with triethylphosphine-gold(I) azides and an alkyne derivative. The resulting organo-gold(I) compounds exhibit high levels of antibacterial activity against Gram-positive pathogens, with particularly low MICs against *Clostridium difficile*.

Resistance to antibiotics will be a major threat for mankind in the forthcoming decades. The development of bacterial resistance to antibiotics is a natural Darwinian process which has been greatly amplified by the massive use of antibiotics against human infections, and the misuse of antibiotics to promote animal growth in farming.[1] The raise of increasingly antibiotic-resistant strains is also the result of a lack of innovation in anti-infective discovery over the four last decades. Novel antibacterial strategies are therefore urgently needed to regain control on resistant pathogens.[2]

Drugs used in the current antibiotic arsenal are exclusively constituted by elements from the first to the third periods of the Mendeleev table (carbone, hydrogen, sulfur, oxygen, nitrogen, phosphorus). With only the notable exception of boron[3] and bismuth[4] no metals were included in approved drugs dedicated to antibacterial strategies. Compounds containing other elements from the periodic table have been developed mainly against cancer, and applications of organometallic compounds in the treatment of bacterial infections have been put on standby so far for toxicity issues. However, the diffusion of ever more resistant bacterial strains and therefore the use of fully organic last resort antibiotics with significant side effects make now organometallic species competitive in the benefit *vs* risk balance for hard-to-treat patients.[4,5] Hybrid drugs made of two conjugated antibacterial molecules were shown to improve greatly the biological and/or pharmacological properties of the combined molecules.[6] Even so hybrids between antibacterial metal complexes and approved antibiotics have been only scarcely reported in the literature. Based on a β-lactam substructure ferrocene and ruthenocene hybrids were described and shown to have promising antibiotic activities.[7,8] We recently added a new string to the bow by reporting a new family of metallo-antibiotics namely chryso-lactams **2** to **5**, based on the penam scaffold of ampicillin (Amp) **1** and containing a gold(I) ion (Fig. 1).[9]

In the present article, we describe the synthesis and biological properties of a second generation of chryso-lactams and a set of gold(I)-containing fragments with inhibitory activities on Gram-positive pathogenic bacteria, including *Clostridium difficile*.

Amongst the five chryso-lactams described previously[9] compounds **2** and **3** where shown to be those having promising *in vitro* antibacterial activities. Triethylphosphane derivative **3** was much more easily synthesized compared to the trimethylphosphane **2** analog, for only a moderate difference in antimicrobial effects. Of note gold(I)-triethylphosphine complexes are already approved for a use in human health,[10] and known to inhibit bacterial growth.[11–13] We therefore built

☆ This article is dedicated to the late Pr. Ermanno Candolfi in memory of its outstanding contributions in microbiology

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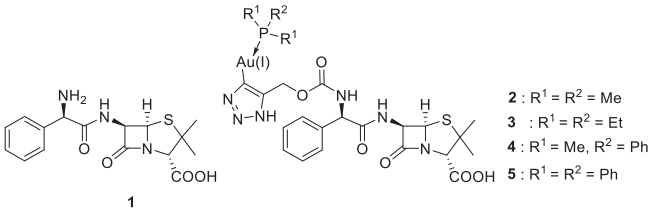
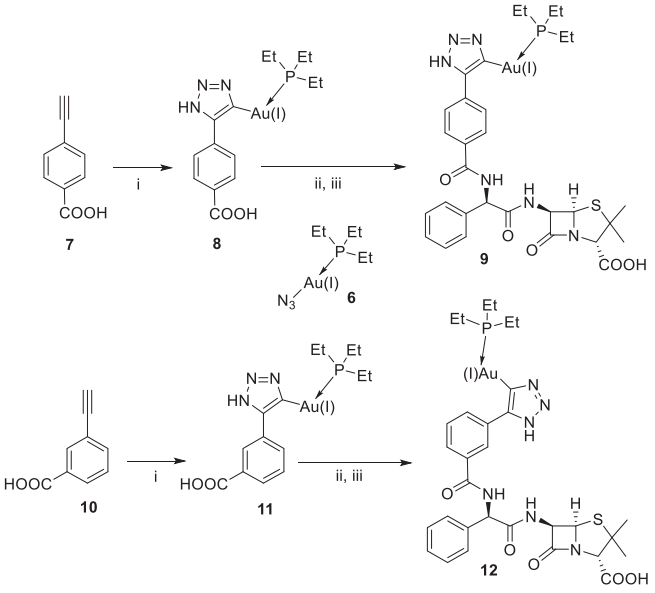


Fig. 1. Structure of ampicillin **1** and chryso-lactams **2** to **5**.

the next generation of organogold(I) compounds using solely the triethylphosphine as co-ligand for the gold(I) center. Phosphane gold(I) was introduced on the organic moiety by a Huisgen 1,3-dipolar cycloaddition, between (triethylphosphine)Au(I) azide **6**[9,14] and the terminal alkyne of an organic scaffold. In a second step, a migration of the phosphane gold(I) moiety occurs, resulting in the formation of a 1,2,3-triazole ring with, in the final conjugate, the gold(I) ion connected in position 5 of the triazole through a σ-gold-carbon bond.[14,15]

In this second generation of organogold(I) compounds we aimed more specifically to investigate the influence of the size of the penam moiety involved in the hybrid conjugate on the antibacterial properties. For this purpose, 4-ethynyl-benzoic acid **7** and 3-ethynyl-benzoic acid **10** were treated with (triethylphosphine)Au(I) azide **6**. The carboxylate functions of resulting organogold(I) compounds **8** and **11** were further activated under the form of pentafluorophenyl-esters. These activated esters were not purified and reacted with ampicillin **1** trihydrate to generate penam derivatives **9** and **12** (Scheme 1).

In the frame of a SAR study, we aimed also to assess the contribution of the β-lactam ring to the antibacterial activity of organogold(I) compounds, by synthesizing gold(I) compounds including organic fragments of the penam structure. Compounds **13** and **14** were obtained by the reaction of D-phenylglycine with the activated ester of gold(I) compounds **8** and **11** respectively. Smaller fragments **19** and **20** bearing only the phenylglycine moiety were also synthesized. For this purpose, D-phenylglycine **15** and L-phenylglycine **16** were treated with propargyl-chloroformate under basic conditions. The resulting alkynes **17** and **18** were further combined with the phosphine-gold(I) azide **6** to generate fragments **19** and **20**. We previously reported that antimicrobial properties of chryso-lactams are mostly related to the presence of the gold(I) ion.[9] To confirm this observation, the fully organic

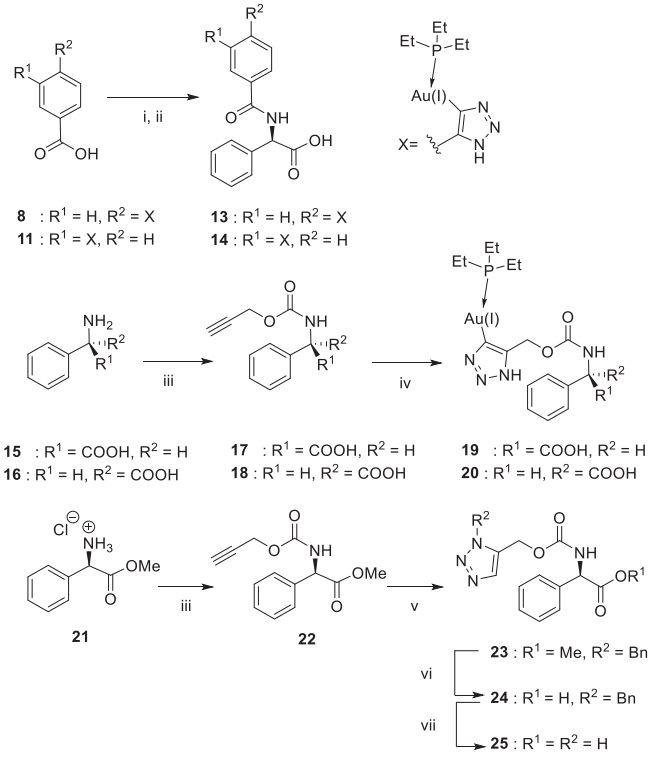


Scheme 1. Synthesis of ampicillin-Au(I) conjugates **9** and **12**. i. **6**, THF, 20 °C. ii. C_6F_5OH, EDCI.HCl, CH_2Cl_2, 20 °C. iii. **1**, NEt_3, THF/H_2O, 0 °C to 20 °C.

compound **25** was synthesized starting from D-phenylglycine methyl ester hydrochloride **21**. The reaction of compound **21** with propargyl-chloroformate led to the alkyne **22**. The later compound was then treated with benzyl azide in the presence of Cp*RuCl(cod),[16,17] and the resulting *N*-benzyl-triazole **23** was converted into the carboxylate **24** by hydrolysis with lithium hydroxide and finally converted in the expected compound **25** by catalytic hydrogenation under 6 bars (Scheme 2).

The antibacterial properties of the chryso-lactams **9** and **12** and fragments **13**, **14**, **19**, **20** and **25** were then assessed with characterized microorganisms, and compared with those of ampicillin (Amp) **1** and the chryso-lactam **3** reported previously.[9] Minimal inhibitory concentrations (MICs) were determined for *Escherichia coli*, a Gram-negative bacterium, and a set of Gram-positive pathogenic bacteria, namely *Staphylococcus aureus*, *S. epidermidis, Enterococcus faecalis*, *E. faecium* and *Clostridium difficile* (Table 1). This latter microorganism is a common cause of diarrhea in healthcare facilities and, for the most aggressive strains of hard-to-treat pseudomembranous colitis.[18]

E. coli was resistant to chryso-lactams as well as to all fragments, in the range of concentrations tested (from 0.06 to 8 $\mu g.mL^{-1}$) (Table 1). Such a resistance of Gram-negative bacteria to organometallics is well described,[4,5,19] and is attributed to the permeability barrier exerted by the bacterial outer membrane toward relatively hydrophobic molecules.[13,19] Interestingly, chryso-lactams **3**, **9** and **12** and fragments **13**, **14**, **19** and **20** proved to be much more effective that compound **25**, a fully organic molecule, on the whole set of strains tested, with *C. difficile* exhibiting a particularly high susceptibility to organometallics. These results confirmed the crucial role played by gold(I) ion in the antibacterial activity of such compounds.[12,13] Chryso-lactam **3** was previously found to be very active against both *S. aureus* and *S. epidermidis*, and to a lower extent against *E. faecalis* and *E. faecium*.[9] Chryso-lactams **9** and **12**, as well as the fragments **13**, **14**, **19** and **20** exhibited anti-staphylococcal and anti-enterococcal activities very similar to that of compound **3**, that



Scheme 2. Synthesis of chryso-lactam fragments **13**, **14**, **19**, **20** and fully organic fragment **25**. i. C_6F_5OH, EDCI.HCl, CH_2Cl_2, 20 °C. ii. D-phenylglycine, NEt_3, $_{THF}$, 20 °C. iii. Propargyl-chloroformate, NaOH, THF/H_2O, 0° to 20 °C. iv. **6**, THF, 20 °C. v. benzyl azide, Cp*RuCl(cod), toluene, 80 °C, sealed tube. vi. $LiOH{\cdot}H_2O$, THF/H_2O, 20 °C. vii. H_2 (6 bars), Pd/C, MeOH, 50 °C.

Table 1
Minimal inhibitory concentrations (MICs) in μg.mL^{-1} of ampicillin (Amp) **1**, chrysolactams **3**, **9**, and **12**, gold(I) conjugates **13**, **14**, **19** and **20,** and of gold-free fragment **25** for a panel of wild-type and resistant bacterial strains.[a]

Bacteria tested	Amp (1)	3	9	12	13	14	19	20	25
S. aureus									
ATCC 25923[b]	0.5	0.125	0.125	0.125	≤0.06	0.125	0.125	0.125	>8
ATCC 700699[c,d]	>8	0.25	0.25	0.25	0.125	0.125	0.25	0.25	>8
ATCC 29213[b]	0.5	0.125	≤0.06	0.125	0.125	0.125	0.125	0.25	>8
ST20131365[c,d]	4	0.25	0.25	0.5	0.125	0.125	0.125	0.25	>8
S. epidermidisATCC 14990[b]	1	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	>8
ATCC 35984[c]	>8	≤0.06	0.125	≤0.06	0.125	≤0.06	≤0.06	≤0.06	>8
ST20140436[c,d]	4	≤0.06	0.125	≤0.06	0.125	≤0.06	≤0.06	≤0.06	>8
ST20150446[c,d]	8	0.125	0.125	≤0.06	≤0.06	0.125	0.125	≤0.06	>8
E. faecalis									
JH2-2[b]	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.5	>8
UCN4[d]	0.5	1	0.25	1	0.125	0.25	0.25	0.25	>8
V583[d]	0.5	1	0.5	1	0.25	0.5	0.5	0.5	>8
E. faecium									
ATCC 19434 T[b]	0.5	1	0.5	0.5	0.25	0.25	0.25	0.25	>8
BM 4147[c,d]	8	2	0.5	1	0.5	0.5	0.5	0.25	>8
AUS0004[c,d]	>8	1	0.5	0.5	0.25	0.25	0.5	0.5	>8
E. coliATCC 25922[b]	2	>8	>8	>8	>8	>8	>8	>8	>8
C. difficileATCC70057[b]	0.5	0.125	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	0.125	>8

[a] The MIC values displayed are the highest values obtained in two independent experiments.
[b] wild-type reference strain.
[c] β-lactam-resistant strain;
[d] glycopeptide-resistant strain.

were not impaired by expression of resistance mechanisms to β-lactams and/or glycopeptides in the strains. Of note, as exemplified by compounds **19** and **20**, the chirality of the phenyl-glycine core, common to all our gold(I) derivatives, appeared to have no notable impact on the antimicrobial properties of the series. The same applies to the penam scaffold which appears as dispensable. Since antibacterial activities of the chryso-lactams and fragments synthetized relies on gold(I) ion, emergence of cross-resistance with currently used antibiotic families under treatment with organometallics seems very unlikely.

Heavy and noble metals derivatives are known to be more toxic for eukaryotic cells than their fully organic homologs. We thus compared the toxicity of the fragments **13** and **19** with that of chryso-lactams **3** and **9** on human healthy hepatocytes. As previously reported, ampicillin remained non-cytotoxic over the entire range of concentrations tested.[9] Penam derivatives **3** and **9** exhibited EC50s of 23.14 ± 0.18 μM (18.12 ± 0.14 μg.mL^{-1}) and 17.57 ± 1.29 μM (14.91 ± 1.08 μg.mL^{-1}) respectively, while the EC50 of fragment **13** was 11.71 ± 0.56 μM (7.61 ± 0.36 μg.mL^{-1}). EC50 of the smallest fragment **19** could not be determined due to solubility issues in the concentration range used for the other compounds. However, with 8% viable cells at 7 μM and 0% at 12 μM **19** proved to have a much higher cytotoxicity compared to **3**, **9** or **13** (See ESI). As already mentioned for chryso-lactams and other gold(I) derivatives with potential anti-infective applications, the metal center is essential for the expected activity.[9,11,12,19] Our results prove that the smaller is the organic part of gold(I) compound, the higher is the eukaryotic cytotoxicity with, in parallel, only modest changes in antibacterial activity. The organic counterpart is therefore of great importance to finely tune up the biological properties of organo-gold(I) antibacterial compounds.

In conclusion, this study describes the synthesis and the biological activities of unprecedented gold(I) derivatives including a new generation of chryso-lactams. All these organometallics exhibit high antibacterial activities *in vitro* against major Gram-positive pathogens, including multidrug resistant strains. While ineffective against Gram-negative bacteria like *E. coli*, they were able to inhibit *C. difficile* at very low concentrations (≤0.125 μg/mL), confirming that chryso-antibiotics are a promising and untapped source of therapeutic innovation against this drug recalcitrant Gram-positive pathogen.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Protocols and analytical data concerning the synthesis of compounds **9**, **12**, **13**, **14**, **19**, **20** and **25**. Experimental conditions for Minimum Inhibitory Concentrations assessments and for the *in vitro* cytotoxicity assays of compounds **3**, **9**, **13** and **19** on hepatocytes can be found online DOI

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127879.

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