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Mathieu Michaut, Alexandre Steffen, Jean-Marie Contreras, Christophe Morice, Aurélie Paulen, et al.. Chryso-lactams:Gold(I) derivatives of ampicillin with specific activity against Gram-positive pathogens. *Bioorganic and Medicinal Chemistry Letters*, 2020, 30 (9), pp.127098. 10.1016/j.bmcl.2020.127098 . hal-03006818

HAL Id: hal-03006818

<https://hal.science/hal-03006818>

Submitted on 17 Nov 2020

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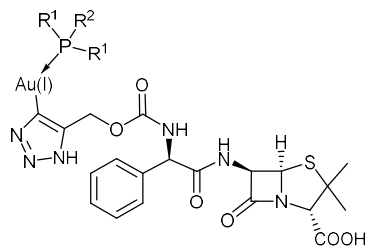
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Chryso-lactams: Gold(I) derivatives of ampicillin with specific activity against Gram-positive pathogens

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X = O, R¹ = Me, Et or Ph, R² = Me, Et or Ph

X = O, R¹ = Me, R² = Ph

X = CH₂, R¹ = R² = Et

X = NH, R¹ = R² = Et

MIC (<i>S. epidermidis</i>)	≥ 0.06 µg.mL ⁻¹
MIC (<i>S. aureus</i>)	≥ 0.125 µg.mL ⁻¹
MIC (<i>E. faecium</i>)	≥ 0.5 µg.mL ⁻¹



Chryso-lactams: Gold(I) derivatives of ampicillin with specific activity against Gram-positive pathogens

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ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

ABSTRACT

Gold(I) derivatives of a β -lactam antibiotic were synthesized by click chemistry with phosphine-gold(I) azides and an alkyne derivative of ampicillin. The resulting organogold conjugates had promising high levels of antibacterial activity against Gram-positive bacteria, including resistant strains.

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Keywords:

Antibiotic

Gold(I) complex

β -lactam

Azide-alkyne Huisgen cycloaddition

Gram-positive pathogen

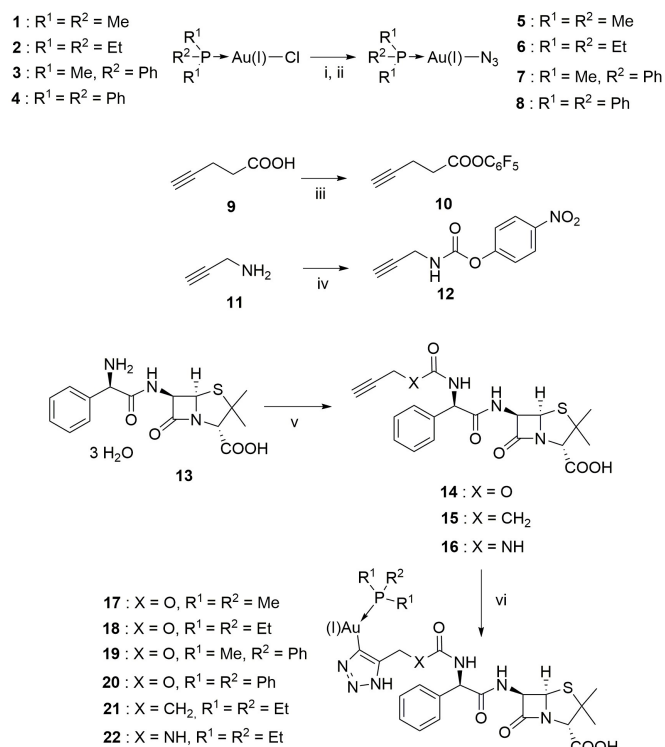
Gold is a precious metal and the symbol of continuity in time and, by extension, of health and eternal youth. For this reason, many civilizations have made use of gold in their traditional medicines. For example, Ayurvedic medicine has used the properties of metals to treat many diseases over thousands of years. Gold has been used to treat cancer, vascular disorders, arthritis and fertility problems.¹⁻⁴ In occidental medicine, chrysotherapy (therapy based on gold compounds) is already used to treat rheumatoid arthritis,⁵ asthma⁶ and skin diseases⁷ and promising therapeutic strategies for cancer are also being investigated.⁸⁻¹¹ Gold(I) derivatives have also been reported to have antibiotic activity.¹²⁻²⁰ The conjugation of approved antibiotics with metal complexes, like ferrocenyl, already led to interesting antibacterial compounds.²¹ However, the conjugation of an antibiotic with gold(I) has never before been reported. We describe here the synthesis and biological evaluation of unprecedented organo-gold(I) derivatives of β -lactam antibiotics.

Phosphane gold(I) was added to antibiotics by 1,3-dipolar cycloaddition, with a phosphane gold(I) azide complex and a penam scaffold functionalized with a terminal triple bond. During this reaction, an additional migration of the phosphane gold(I) moiety occurs, resulting in the formation of a 1,2,3-triazole ring with the gold center in position 5.^{10,22} In the final

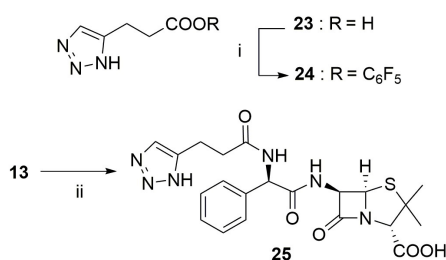
conjugate, the gold(I) center is connected to the antibiotic through a σ -gold-carbon bond. The (trimethylphosphine)Au(I) azide **5**, the (triethylphosphine)Au(I) azide **6**, the (dimethylphenyl)Au(I) azide **7** and the (triphenylphosphine)Au(I) azide **8** were prepared according to a published procedure.²⁰ Commercially available phosphine gold(I) chlorides **1**, **2**, **3** and **4** were treated with thallium(I) acetylacetonate, and the resulting phosphine Au(I) acetylacetonates were treated with trimethylsilyl azide to obtain the expected reagents **5**, **6**, **7** and **8**. Three different chemical functional groups were evaluated for connection of the phosphine-gold(I)-triazole moiety to the ampicillin molecule: carbamate (compounds **17** to **19**), amide (compound **21**) and urea (compound **22**). Ampicillin **13** was thus treated with propargyl chloroformate, the pentafluorophenyl ester **10** of pentynoic acid **9**, or the *p*-nitrophenyl chloroformate **12** prepared from propargylamine **11**. The resulting alkynes **14**, **15** and **16** were then treated with gold(I) azides **5** to **8** to obtain the expected conjugates **17** to **22**. (Scheme 1).

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The fully organic penam **25** was prepared, to make it possible to distinguish the relative importances of the gold ion and the 1,2,3-triazole moiety for antibacterial activity. For this purpose, the 3-(1*H*-1,2,3-triazol-5-yl)propanoic acid **23** was synthesized according a published procedure.²³ The carboxyl group of **23** was then activated in the form of a pentafluorophenyl-ester **24** and reacted with ampicillin **13** to generate the expected penam derivative **25** (Scheme 2).



Scheme 1. Synthesis of phosphine gold(I) azides **5**, **6**, **7** and **8**, and synthesis of ampicillin-Au(I) conjugates **17** to **22**. i. Tl(acac), toluene, 20°C. ii. TMSN₃, toluene/MeOH, 20 °C. iii. C₆F₅OH, EDCI, CH₂Cl₂, 20°C. iv. pNO₂PhOCOCl, NEt₃, THF 0°C to 20°C. v. Propargyl chloroformate, **10** or **12**, NEt₃, THF/H₂O, 0°C to 20°C. vi. **5**, **6**, **7** or **8**, THF, 20°C.



Scheme 2. Synthesis of ampicillin analog **25**. i. C₆F₅OH, EDCI.HCl, CH₂Cl₂, 20°C. ii. NEt₃, THF/H₂O, 20°C.

The antibacterial properties of the chryso-lactams **17** to **22** were then evaluated with characterized microorganisms and compared with those of ampicillin (Amp) **13**, alkyne **14** and fully organic penam **25**. Most of the pathogenic bacterial species used in the evaluation assays were Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Enterococcus faecalis* and *E. faecium*). Minimal inhibitory concentrations (MIC) were also determined for *Escherichia coli*, a Gram-negative bacterium (Table 1). However, this bacterium turned out to be relatively resistant to the chryso-lactams in the range of concentrations

tested. The resistance of Gram-negative bacteria to gold(I) derivatives has been reported to be due mostly to the low permeability of the bacterial outer membrane. Indeed, the presence of a membrane-permeabilizing agent greatly increases the antibacterial activity of gold(I) drugs against Gram-negative pathogens.¹⁹ By contrast, compounds **17** to **22** proved to be more effective than their non-metallic parental compounds against several pathogenic Gram-positive strains. The size of the phosphine ligand of the gold(I) derivative of ampicillin appeared to be crucial for biological activity. Indeed, compound **20**, with its triphenylphosphine ligand, generally had lower activity than ampicillin. A decrease in ligand size (dimethylphenylphosphine) increased antibiotic activity, but compound **19** had a level of activity similar to that of ampicillin for only a subset of bacterial strains. The optimal ligand was triethylphosphine, as compound **18** was more active against *Staphylococcus* species and several *Enterococcus* strains than ampicillin. Surprisingly, however, the use of a trimethylphosphine ligand (compound **17**) did not further increase antibacterial activity against Gram-positive bacteria but resulted in moderate activity against *E. coli*. This finding is consistent with recently reported data for antibacterial gold(I) derivatives bearing trimethylphosphine as a ligand.^{24,25} Conjugates **18**, **21** and **22** had similar antibacterial profiles, demonstrating that the nature of the linker attaching the phosphine-Au(I)-triazole moiety to the penam scaffold does not greatly influence biological activity. Finally, purely organic penam derivatives **14** and **25** were less active than ampicillin, highlighting the negligible contribution of the triazole moiety and the major importance of the gold(I) center for antibacterial activity (Table 1). Chryso-antibiotic **18** thus appeared to the most promising of the gold(I) compounds synthesized. Other triethylphosphine gold(I) derivatives, such as auranofin, have already found uses in human medicine.^{25,26} The MICs of compound **18** were low for wild-type susceptible strains of *S. aureus*, *S. epidermis* and *E. faecium*. More importantly, they were not affected by the most prevalent mechanisms of resistance to β -lactams (e.g., methicillin and oxacillin) in *Staphylococcus* sp. or known mechanisms of resistance to glycopeptides (e.g., vancomycin) in *Enterococcus* sp. These data suggest that cross-resistance between chryso-lactams and drugs classically used against Gram-positive bacteria should not emerge easily. The activity of compound **18** was similar to that of ampicillin with reference strains of *E. faecalis*.

The toxicity of gold(I) is a critical issue for the further development of molecules of therapeutic potential, such as chryso-antibiotics. The cytotoxicity of compound **18** was assessed on healthy human hepatocytes and compared with that of ampicillin **13**, used as a control. Compound **18** did not affect cell viability at concentrations of up to 10 μ M (7.9 μ g.mL⁻¹), but was toxic at higher concentrations, with only 40% viable cells at 22.5 μ M (17.7 μ g.mL⁻¹) and 10% at 25 μ M (19.7 μ g.mL⁻¹). By contrast, ampicillin remained non-cytotoxic over the entire range of concentrations tested (see ESI). These data clearly characterize an interesting therapeutic window for compound **18** even the strong antibacterial activity of this molecule is counterbalanced by a peripheral toxicity to host cells.

The current antibiotic arsenal mostly consists of purely organic compounds, with carbon, hydrogen, sulfur, oxygen and nitrogen atoms involved in the inhibitory processes. However, the emergence and rise of increasingly resistant bacterial strains, has led to the use of last-resort antibiotics known to have significant adverse effects.^{27,28}

Table 1. Minimal inhibitory concentration (MIC) in $\mu\text{g}\cdot\text{mL}^{-1}$ of ampicillin (Amp), penam **14** and gold(I) conjugates **17** to **22** and **25** for a panel of wild-type and resistant strains.^a

Bacteria tested	Amp(13)	14	17	18	19	20	21	22	25
<i>S. aureus</i>									
ATCC 25923 ^c	0.5	1	0.5	0.125	1	4	0.125	0.125	2
ATCC 700699 ^{d,e}	>8	8	0.5	0.25	2	8	0.125	0.25	>8
ATCC 29213 ^c	0.5	1	NA ^b	0.125	1	4	0.125	0.125	2
ST20131365 ^{d,e}	4	4	NA ^b	0.25	2	8	0.125	0.25	>8
<i>S. epidermidis</i>									
ATCC 14990 ^c	1	2	0.125	≤0.06	0.125	4	≤0.06	≤0.06	1
ATCC 35984 ^d	>8	>8	0.25	≤0.06	0.5	4	≤0.06	0.125	>8
ST20140436 ^{d,e}	4	>8	NA ^b	≤0.06	1	8	≤0.06	0.125	>8
ST20150446 ^{d,e}	8	4	NA ^b	0.125	1	4	0.125	0.125	>8
<i>E. faecalis</i>									
JH2-2 ^c	0.5	1	1	0.5	1	8	0.5	0.5	4
UCN41 ^c	0.5	2	0.5	1	2	8	0.5	0.25	8
V583 ^c	0.5	1	NA ^b	1	2	8	0.5	0.5	4
<i>E. faecium</i>									
ATCC 19434T ^c	0.5	2	1	1	2	4	0.5	0.5	8
BM 4147 ^{d,e}	8	>8	1	2	4	8	1	0.5	>8
AUS0004 ^{d,e}	>8	>8	NA ^b	1	2	8	0.5	0.5	>8
<i>E. coli</i>									
ATCC 25922 ^c	2	>8	8	>8	>8	>8	>8	>8	>8

^a The MIC values displayed are the highest values obtained in two independent experiments. ^b Not Assessed (NA). ^c Susceptible strain. ^d β -lactam-resistant strain. ^e Glycopeptide-resistant strain.

Compounds containing other elements from the periodic table have been developed against cancer, but other applications of these compounds in the treatment of bacterial infections have been limited by peripheral toxicity issues, particularly for molecules administered intravenously. Organometallic compounds should be more and more competitive in terms of the benefit vs risk balance for the patient. Chryso-lactams proved to have a specific activity on Gram-positive microorganisms and the low permeability of Gram-negative bacteria outer envelope seems to impair the antibacterial efficiency of many gold(I) compounds.¹⁹ Therefore, the vectorization of organometallic drugs using nutrient uptake systems, should increase their therapeutic potential (antibacterial activity, cytotoxicity).^{29,30}

This article describes the synthesis of the first conjugates of β -lactam antibiotics with phosphine-gold(I) complexes. In these conjugates, ampicillin was connected to the metal complex through a 1,2,3-triazole linker. Triethylphosphine derivative **18** proved to be an effective molecule against Gram-positive bacteria, with an activity 120 times that of ampicillin with certain strains of *S. aureus*, *S. epidermidis* and *E. faecium*. This compound escapes the most common mechanisms of resistance to β -lactams and glycopeptides. With, for certain bacterial strains, a selectivity index *ca* 130, compound **18** will serve as lead molecule in the development of the next generation of chryso-lactams. However, the mode of action of compound **18** and its ability to select resistant isolates should now be investigated. This information will be crucial for the further development of chryso-antibiotics with better antibacterial properties, manageable toxicity profile and competitive with strategies based on antibacterial gold nanoparticles.^{31,32}

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 the present article.

Acknowledgments

G.L.A.M. and I.J.S. thank the CNRS for global financial support and TTO Conectus for investment in the valorization and patenting process. A.P thanks the *Ministère de l'Éducation Nationale, de l'Enseignement Supérieur et de la Recherche (MENESR)* for a PhD fellowship. M.M. thanks Nathalie Wagner for purifying compounds by semi-preparative HPLC and the entire Prestwick Quality Control team for compound analysis. We thank Mr Loïs Andrey for his technical assistance with drug susceptibility testing. Authors finally thank Pr. Frédéric Laurent (Hospices Civils de Lyon/Université de Lyon I, Lyon, France) and Pr. Vincent Cattoir (CHU Rennes/Université de Rennes 1, Rennes, France) for providing respectively *Staphylococcus* and *Enterococcus* strains.

References and notes

- Beaudet D, Badilescu S, Kuruvinschetti K, Sohrabi Kashani A, Jaunky D., Ouellette S, Piekny A, Packirisamy M. Comparative study on cellular entry of incinerated ancient gold particles (Swama Bhasma) and chemically synthesized gold particles *Sci. Rep.*, 2017; 7: 10678.
- Shah ZA, Vohzora SB. Antioxidant/restorative effects of calcined gold preparations used in Indian systems of medicine against global and focal models of ischaemia. *Pharmacol. Toxicol.* 2002; 90: 254-259.

3. Brown CL, Bushell G, Whitehouse MW, Agrawal DS, Tupe SG, Paknikar KM, Tiekink ERT. *Nanogoldpharmaceutics. Gold Bull.* 2007; 40: 245-250.
4. Jain V, Rai A, Misra S, Singh KM. Seminal gold content in healthy fertile men in India. *Int. J. Ayurveda Res.* 2010; 1: 172-174.
5. Berglöf FF, Berglöf K, Walz DT. Auranofin: an oral chrysotherapeutic agent for the treatment of rheumatoid arthritis. *J. Rheumatol.*, 1978; 5: 74-78.
6. Klaustermeier WB, Noritake DT, Kwong FK. Chrysotherapy in the treatment of corticosteroid-dependent asthma. *J. Allergy Clin. Immunol.*, 1987; 79: 720-725.
7. Thomas I. Gold therapy and its indications in dermatology. A review. *J. Am. Acad. Dermatol.*, 1987; 16: 845-854.
8. Shaw III CF. Gold-Based Therapeutic Agents. *Chem. Rev.* 1999; 99: 2589-2600.
9. Hickey JL, Ruhayel RA, Barnard PJ, Baker MV, Berners-Price SJ, Filipovska A. Mitochondria-targeted chemotherapeutics: the rational design of gold(I) *N*-heterocyclic carbene complexes that are selectively toxic to cancer cells and target protein selenols in preference to thiols. *J. Am. Chem. Soc.* 2008; 130: 12570-12571.
10. Köster SD, Alborzinia H, Can S, Kitanovic I, Wöfl S, Rubbiani R, Ott I, Riesterer P, Prokop A, Merz K, Metzler-Nolte N. A spontaneous gold(I)-azide alkyne cycloaddition reaction yields gold-peptide bioconjugates which overcome cisplatin resistance in a p53-mutant cancer cell line. *Chem. Sci.* 2012; 3: 2062-2072.
11. Chen W-H, Chen J-X, Cheng H, Chen C-S, Yang J, Xu X-D, Wang Y, Zhuo R-X, Zhang X-Z. A new anti-cancer strategy of damaging mitochondria by pro-apoptotic peptide functionalized gold nanoparticles. *Chem. Commun.* 2013; 49: 6403-6405.
12. Glišić BĐ, Djuran MI. 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.*, 2014; 43: 5950-5969.
13. Mora M, Gimeno MC, Visbal R. Recent advances in gold-NHC complexes with biological properties. *Chem. Soc. Rev.*, 2019; 48: 447-462.
14. Stenger-Smith J, Chakraborty I, Mascharak PK. Cationic Au(I) complexes with aryl-benzothiazoles and their antibacterial activity. *J. Inorg. Biochem.* 2018; 185: 80-85.
15. Schmidt C, Karge B, Misgeld R, Prokop A, Brönstrup M, Ott I. Biscarbene gold(i) complexes: structure-activity-relationships regarding antibacterial effects, cytotoxicity, TrxR inhibition and cellular bioavailability. *Med. Chem. Comm.* 2017; 8: 1681-1689.
16. Fernández GA, Vela Gurovic MS, Olivera NL, Chopra AB, Silbestri GF. Antibacterial properties of water-soluble gold(I) *N*-heterocyclic carbene complexes. *J. Inorg. Biochem.* 2014; 135: 54-57.
17. Mizdal CR, Stefanello ST, da Costa Flores V, Agertt VA, Bonez PC, Rossi GG, da Silva TC, Antunes Soares FA, de Lourenço Marques, de Campos MMA. The antibacterial and anti-biofilm activity of gold-complexed sulfonamides against methicillin-resistant *Staphylococcus aureus*. *Microb. Pathog.* 2018; 123: 440-448.
18. Abdelkhalek A, Abutaleb NS, Mohammad H, Seleem MN. Antibacterial and antivirulence activities of auranofin against *Clostridium difficile*. *Int. J. Antimicrob. Agents.* 2019; 53: 54-62.
19. Thangamani S, Mohammad H, Abushahba MFN, Sobreira TJP, Hedrick VE, Paul LN, Saleem MN. Antibacterial activity and mechanism of action of auranofin against multi-drug resistant bacterial pathogens. *Sci. Rep.* 2016; 6: 22571.
20. Patra M, Gasser G, Metzler-Nolte N. Small organometallic compounds as antibacterial agents. *Dalton Trans.* 2012; 41: 6350-6358.
21. Edward IE, Epton R, Marr G. A new class of semi-synthetic antibiotics: ferrocenyl-penicillins and-cephalosporins. *J. Organomet. Chem.* 1976; 107: 351-357.
22. Partyka DV, Updegraff III JB, Zeller M, Hunter AD, Gray TG. Carbon-Gold Bond Formation through [3 + 2] Cycloaddition Reactions of Gold(I) Azides and Terminal Alkynes. *Organometallics*, 2007; 26: 183-186.
23. Thomson CG, Le Grand D, Dowling M, Brocklehurst CE, Chinn C, Elphick L, Faller M, Freeman M, Furninger V, Gasser C, Hamadia A, Hardaker E, Head V, Hill JC, Janus DI, Pearce D, Poulaud A-S, Stanley E, Sviridenko L. Development of autotaxin inhibitors: A series of zinc binding triazoles. *Bioorg. Med. Chem. Lett.* 2018; 28: 2279-2284.
24. Holmes I, Naylor A, Negoita-Giras G, Powell J, Charles I, Alber D. Gold (I)-phosphine compounds as anti-bacterial agents WO2015181550A1, 2015.
25. Wu B, Yang X, Yan M. Synthesis and Structure-Activity Relationship Study of Antimicrobial Auranofin against ESKAPE Pathogens. *J. Med. Chem.* 2019; 62, 17: 7751-7768.
26. Skalska J, Kiebal MM, Hilchey SP, Dai Y, Grant S, Brookes PS, Peterson DR, Maggirwar SB, Bernstein SH. The Rheumatoid Arthritis Drug Auranofin Has Significant *In Vitro* Activity in MCL and DLCL and Is Synergistic with a Glutathione Depleting Agent. *Blood*, 2012; 120: 1658.
27. Kelesidis T, Falagas ME. The safety of polymyxin antibiotics. *Expert Opin. Drug Saf.* 2015; 14:1687-1701.
28. Zavascki AP, Nation RL. Nephrotoxicity of Polymyxins: Is There Any Difference between Colistimethate and Polymyxin B? *Antimicrob. Agents Chemother.* 2017; 61: e02319-16.
29. Laurent Q, Batchelor LK, Dyson PJ. Applying a Trojan Horse Strategy to Ruthenium Complexes in the Pursuit of Novel Antibacterial Agents. *Organometallics*, 2018; 37: 915-923.
30. Sauvageot E, Elie M, Gaillard S, Daniellou R, Fechter P, Schalk JJ, Gasser V., Renaud J-L, Mislin GLA. Antipseudomonal activity enhancement of luminescent iridium(III) dipyriddyamine complexes under visible blue light. *Metallomics.* 2017; 9:1820-1827.
31. Brown AN, Smith K, Samuels TA, Lu J, Obare SO, Scott ME. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* and methicillin-resistant *Staphylococcus aureus*. *Appl. Environ. Microbiol.* 2012, 78, 2768-2774.
32. Cui Y, Zhao Y, Tian Y, Zhang W, Lü X, Jiang X. The molecular mechanism of action of bactericidal gold nanoparticles on *Escherichia coli*. *Biomaterials.* 2012, 33, 2327-2333.

Appendix A. Supplementary Data

Protocols and analytical data concerning compounds **14** to **22** and compound **25**, experimental conditions for Minimum Inhibitory Concentration assays, additional MICs on resistant *Staphylococcus* strains for compound **18** compared to ampicillin, concentrations assessments for the *in vitro* cytotoxicity of compound **18** and ampicillin can be found online DOI xxxxxxxx