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Short communication

Antibacterial properties of water-soluble gold(I) N-heterocyclic carbene complexes

Gabriela A. Fernández [a], María S. Vela Gurovic [a,*,1], Nelda L. Olivera [b,2], Alicia B. Chopa [a,3], Gustavo F. Silbestri [a,*,1,2]

[a] INQUISUR, Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, B8000CPB Bahía Blanca, Argentina
[b] CENPAT, Bvd. Brown 2915, U9120ACD Puerto Madryn, Argentina

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ABSTRACT

The antibacterial properties of water-soluble gold(I) complexes [1-methyl-3-(3-sulfonatopropyl)imidazol-2-ylidene]gold(I) chloride (**C1**), [1-mesityl-3-(3-sulfonatopropyl)imidazol-2-ylidene]gold(I) chloride (**C2**), [1-(2,6-diisopropylphenyl)-3-(3-sulfonatopropyl)imidazol-2-ylidene]gold(I) chloride (**C3**) and [1,3-bis(2,6-diisopropyl-4-sodiumsulfonatophenyl)imidazol-2-ylidene]gold(I) chloride (**C4**) and the respective ligands were assessed by agar diffusion and broth macrodilution methods against Gram-positives *Staphylococcus aureus*, *Enterococcus faecalis* and *Micrococcus luteus* and the Gram-negative bacteria *Yersinia ruckeri*, *Pseudomonas aeruginosa* and *Escherichia coli*. Viability after treatments was determined by direct plate count. The bactericidal activity displayed by **C1** and **C3** was comparable to that of $AgNO_3$.

Au(I) phosphine auranofin and its chloro analog Et_3PAuCl, the first class of Au complexes approved for clinical use in the treatment of rheumatoid arthritis, have been considered leading structures for the development of antitumoral agents [1,2]. The need of better chemical stability led to the introduction of new types of ligands. Since 1991, a variety of NHC (N-heterocyclic carbene) complexes have been synthesized including both transition and main group metals [3–5]. Their strong σ-donating nature facilitates tighter ligand binding to the metal, thereby increasing their stability [6]. The biological properties can be tuned and modified by the nature of the respective metal as well as the coordinated NHC ligands [7–9]. Among them, silver and gold complexes have shown promising antimicrobial and antitumoral properties.

The silver ion exerts a non-specific bacterial inhibition and is likely that the cell membrane is the primary target of Ag+ [6]. Silver-based antimicrobial agents such as 1% silver sulfadiazine ointment and 0.5% (w/w) $AgNO_3$ are traditional antiseptics with a broad-spectrum of antimicrobial activities. However, the current use of silver is limited to certain medical applications due to two major toxicity concerns, namely low hemocompatibility and argyria, both of which have been attributed to the interaction between silver and blood cysteine, an amino acid with a thiol side chain [10]. Since the mechanism of action of gold and gold complexes involves the high affinity of Au for protein components such as thiols and selenols [1], a parallelism between gold and silver interaction with biological samples could be suggested. The metal–NHC bond of gold complexes is more stable than the silver–NHC bond, which would mean better kinetics and reduced unspecific binding for the former [6]. Au–NHC complexes follow different mechanisms than those of the common antibiotics [11]. Some Au(I)–NHC complexes inhibited bacterial proliferation by blocking cytokinesis [12]. More recently, it has been demonstrated that pyrazine functionalized pincer Au(I)–NHC complexes strongly bind to both Lys and Dap-Type peptidoglycan layers causing drastic damage to the bacterial cell wall and increasing the membrane permeability [13]. This may be of great value when treating persistent infections, because the antibacterial agents that target the bacterial membrane typically retain activity against metabolically inert bacteria [14].

Water solubility has been considered of main interest in the development of both Ag- and Au-NHC complexes as therapeutic agents [1,11,13]. It should be mentioned that in contrast to the variety of water-soluble phosphane ligands available [15,16] there is little bibliography related to water-soluble NHC complexes [17], and none of the antibacterial Au(I)–NHC complexes hitherto informed is water soluble. Recently, Silbestri has reported the synthesis and structural characterization of water-soluble gold(I)–NHC complexes [17]. Herein, we report the antibacterial properties of these complexes, as well as the respective ligands,

* Corresponding authors. Tel.: +54 291 4595100; fax: +54 291 4595187.
E-mail addresses: svela@uns.edu.ar (M.S. Vela Gurovic), gsilbestri@uns.edu.ar (G.F. Silbestri).
[1] These authors contributed equally to this work.
[2] Member of CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas).
[3] Member of CIC (Comisión de Investigaciones Científicas).

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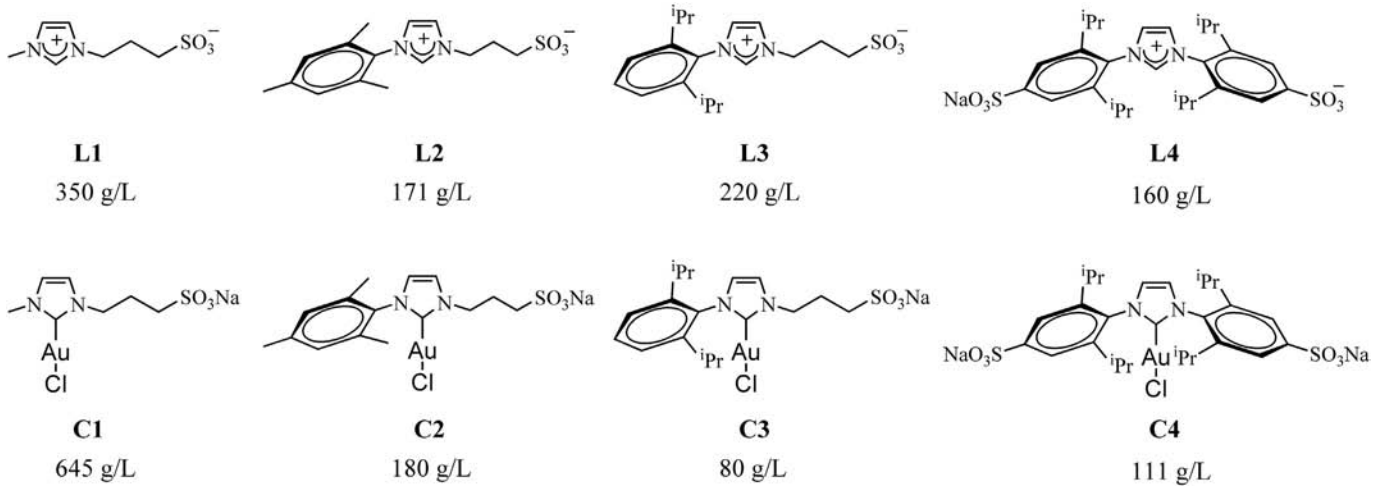

Fig. 1. Structure and water solubility of sulfonated gold(I)–NHC complexes and respective ligands.

against six different bacterial strains and compare them with reference antibiotics, and with silver nitrate at concentrations at which it is used as antiseptic. Fig. 1 shows the selected compounds (**C1**–**C4**) and the corresponding ligands (**L1**–**L4**) for the present study.

Antimicrobial activities of complexes **C1**–**C4** and the respective ligands were tested against the Gram-negative bacterial strains *Yersinia ruckeri* ATCC 29473, *Pseudomonas aeruginosa*,[4] *Escherichia coli* ATCC 25922, and the Gram-positives *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Enterococcus faecalis* CCMA-2975[5] and *Micrococcus luteus*.[6] Complexes and ligands were tested by the Kirby–Bauer method according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines [18]. Stock solutions of all compounds were dissolved in Milli-Q water. Minimum inhibitory concentrations[7] (MICs) were determined in Tripticase-soy broth (TSB) by macrodilution procedures according to the BSAC guidelines [18]. To assess the bactericidal effect, the compounds were tested at their respective MICs.[8]

All complexes were active against *M. luteus* excepting **C4**. **C1** slightly inhibited the growth of *E. faecalis* and *E. coli*, while **C2** was active against *Y. ruckeri*. Among ligands only **L3** displayed a strong inhibition against the Gram positives while **L1**, **L2** and **L4** were inactive to all strains (Table 1). **C1** and **C3** inhibited the growth of the majority of the strains at 1024 μg/mL (Table 2). **C1** inhibited the growth of *E. coli* at 512 μg/mL, being the respective ligand inactive in all treatments. Although **C3** and **C4** inhibited the growth of *M. luteus* at 256 μg/mL, it was not possible to relate the activity to the complex itself because the respective ligands were also active at this concentration.

Water solubility is of main concern in drug development. However, none of the complexes described in Table 3 are hydrophilic. Additionally, the authors claim that the Au complexes are antibacterial but many of them do not inform the activity of the ligands. In some cases the MICs of both complex and ligand are equal, so that the ligand itself is responsible of the antibacterial effects and not the complex. The MICs found for complexes **C1** and **C3** are in the order of those found for antiseptics such as polyvinylpyrrolidone–iodine [23]. This led us to compare the antimicrobial properties of these complexes with known antimicrobials which may exert their activities through more general mechanisms and not by inhibition of specific targets such as enzymes and nucleic acids. Silver nitrate has been used topically at a concentration of 0.1% in antibacterial creams and pharmaceutical products associated with sulfadiazine and other analogs to prevent infections in severe burns [24]. Based on this value, the effects produced by **C1** and **C3** at their MIC (1024 μg/mL ≅ 0.1% w/v) were compared with those of $AgNO_3$ against all the strains, excepting *M. luteus*, which was exposed to 256 μg/mL of each compound (Fig. 2). Antibacterial activities found for **C1** and **C3** were similar to antiseptic agents such as silver nitrate. **C1** was bactericidal against *P. aeruginosa* and **C3** against *S. aureus*. All the treatments reduced more than two $\log_{10}$ the viability of *E. faecalis*, and more than one $\log_{10}$ that of *M. luteus*. $AgNO_3$ caused a two $\log_{10}$ drop in the cell viability of *E. coli* while **C1** reduced only one $\log_{10}$. The remaining treatments were at least bacteriostatic at the concentrations tested. Selectivity towards Gram-positive bacteria could be suggested for **C3**, while **C1** inhibited both Gram positives and negatives. Among the tested complexes, **C1** is the smallest and simplest complex. These structural features, together with its hydrophilic nature, could have contributed to a better permeation through the outer membrane of the Gram-negative bacteria causing the subsequent antibacterial effects [24].

It has been demonstrated that MIC values of Ag + and Ag(I)–NHC complexes vary considerably depending on the medium used for the antimicrobial assays. Previous studies showed that MICs of Ag(I)–NHCs in Luria–Bertani broth (LB) are higher than those assessed in cation adjusted Mueller–Hinton broth [25–27]. This effect is attributed to the inactivation of the silver cation by non-target molecules such as NaCl and proteins [11,14], which are found at high concentrations in rich media such as LB and TSB. In that order of ideas, it is probable that the media used in this study (TSB) could have promoted a partial inactivation of the Au(I)–NHC complexes, thus causing an increase in MIC values.

In summary, the main advantage of **C1** and **C3** when compared with other Au(I)–NHC complexes previously reported, is the hydrophilic nature conferred by the sulfonic groups which improves the physicochemical properties of these compounds for their use in living tissues and pharmaceutical formulations. None of the antimicrobial Au–NHC reported up to now are water soluble, since all required DMSO for solubilizing the agent in buffers and media used for the assays (Table 3). This supports further studies on the synthesis of novel sulfonated Au(I)–NHCs in order to find ligands which improve the antimicrobial activity exerted by the complex, by increasing the affinity to the bacterial structures and reducing the binding to unspecific components of the surrounding media.

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[6] *M. luteus* belongs to the microbial collection of CENPAT (Centro Patagónico).
[7] MIC refers to the lowest concentration of the tested substance which inhibited the visible growth of the microorganism.
[8] Aliquots of the diluted sample were spread over TS agar plates and further incubated for 20 h at 37 °C. The compounds were considered bactericidal at the concentration tested when no visible CFU was observed on the plate.

Table 1
Bacterial growth inhibition of complexes and ligands by the agar diffusion test.[a]

Compound	Diameter of the zone of inhibition [mm][b]					
	Y. ruckeri	*P. aeruginosa*	*E. coli*	*S. aureus*	*E. faecalis*	*M. luteus*
C1	–	–	**11.20 ± 0.20**	9.70 ± 2.50	**11.60 ± 0.60**	**25.60 ± 0.60**
C2	**12.0 ± 3.20**	8.25 ± 1.05	8.65 ± 0.25	8.60 ± 1.40	7.95 ± 0.75	**14.75 ± 2.75**
C3	–	7.50 ± 0.30	–	8.60 ± 0.40	–	**11.73 ± 0.73**
C4	–	–	–	8.3 ± 1.1	–	8.55 ± 1.35
L1	–	–	–	–	–	–
L2	–	–	–	–	–	9.60 ± 2.40
L3	–	–	8.90 ± 1.70	**17.20 ± 1.30**	–	**27.70 ± 4.70**
L4	–	–	–	–	–	–
Pen[c]	**30.23 ± 1.13**	–	**23.73 ± 0.43**	**11.70 ± 0.60**	**30.13 ± 2.58**	**38.70 ± 1.20**
Cip[d]	**25.85 ± 1.85**	**38.45 ± 1.45**	**26.17 ± 3.22**	**34.95 ± 1.00**	**25.50 ± 2.20**	**25.17 ± 1.17**

[a] Complexes (**C**) and ligands (**L**) were tested at 500 μg.
[b] Diameters >10 mm were considered active (bold).
[c] Penicillin G 5 UI.
[d] Ciprofloxacin 5 μg.

Table 2
Inhibitory concentrations of complexes and ligands.[a]

Compound	MIC [μg/mL]					
	Y. ruckeri	*P. aeruginosa*	*E. coli*	*S. aureus*	*E. faecalis*	*M. luteus*
C1	1024	1024	512	1024	1024	256
C2	–	–	–	–	–	–
C3	1024	1024	1024	1024	1024	256
C4	–	–	–	–	–	1536
L1	–	–	–	–	–	–
L2	–	–	–	–	–	–
L3	–	–	–	–	1536	512
L4	–	–	–	–	–	1536
$AgNO_3$	1024	1024	1024	1024	1024	256
Cip[b]	0.5	0.5	0.5	<0.25	0.5	1.0
Cip/MBC[c]	4.0	4.0	2.0	0.5	16.0	8.0

[a] Each experiment was run in duplicate.
[b] Ciprofloxacin.
[c] Minimum bactericidal concentration.

Table 3
Minimum inhibitory concentrations of previously reported Au(I)–NHC complexes.

Complex type	Ligand type	MIC [μg/mL]				
		G +	G −	Ligand	Solubility[a]	Ref.
$[Au_2L]Br_2$	N,N′-olefin bis-imidazolium	1.75	0.87	n.t.[b]	DMSO	[11]
[AuL]Cl	1-Benzyl-3-tert-butylimidazol-2-ylidene	6	–[c]	n.t.	DMSO	[12]
$[Au_2L]Cl_2$	2,6-Bis(1-methyl imidazol) pyrazine	2	4	256	DMSO	[13]
$[AuL_2]Cl$	1,3-Dialkylimidazolidin-2-ylidene	3.12	3.12	3.12	DMSO	[19]
[AuL]Cl	1-Trimethoxybenzyl-3(tert-butylbenzyl) benzimidazole-2-ylidene	12.5	200	n.t.	DMSO	[20]
[AuL]Cl	Bis-iminoacenaphthene	630	630	<40	DMSO	[21]
$[AuL_2]AuCl_2$	N,N′-dialkylbenzimidazol-2-ylidene	12.5	50	n.t.	DMSO	[22]

[a] The need of DMSO (dimethyl sulfoxide) for dissolving the complexes and the chemical nature of the ligands were considered as criteria of water solubility.
[b] Not tested.
[c] Inactive. G + = Gram positive strains, G − = Gram negative strains.

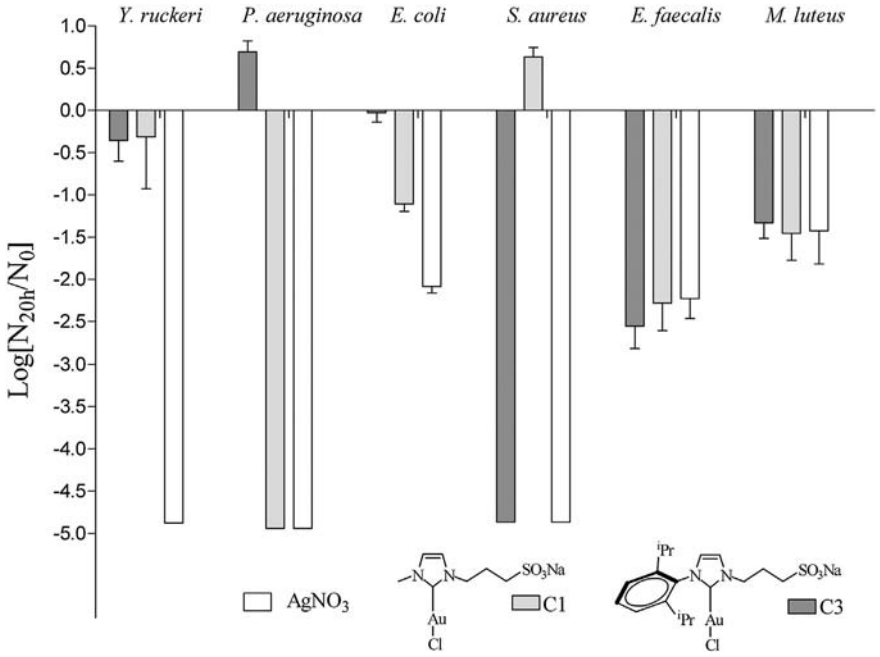

Fig. 2. Bactericidal effects of **C1**, **C3** and silver nitrate on the viability of G + and G − bacteria at 1.02 mg/mL excepting *M. luteus* (0.25 mg/mL). Values shown are the means of three replicates from three independent experiments. Error bars represent the error of the mean. N_{20h}: CFU/mL after 20 h of incubation; N_0: initial inoculum size ≅ 10^5 CFU/mL.

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References

[1] S.J. Berners-Price, A. Filipovska, Metallomics 3 (2011) 863–873.
[2] R.P. Sharma, J. Smillie, D.G. Palmer, Pharmacology 30 (1985) 115–120.
[3] S.P. Nolan, N-Heterocyclic Carbenes in Synthesis, Wiley-VCH, Weinheim, 2006.
[4] F. Glorius, N-Heterocyclic Carbenes in Transition-Metal Catalysis, Springer, Berlin, 2007.
[5] S. Diez-Gonzalez (Ed.), N-Heterocyclic Carbenes, R. Soc. Chem. (2011) 442 p.
[6] J. Alex, P. Ghosh, Dalton Trans. 39 (2010) 7183–7206.
[7] L. Oehninger, R. Rubbiani, I. Ott, Dalton Trans. 42 (2013) 3269–3284.
[8] W. Liu, R. Gust, Chem. Soc. Rev. 42 (2013) 755–773.
[9] J.L. Hickey, R.A. Ruhayel, P.J. Barnard, M.V. Baker, S.J. Berners-Price, A. Filipovska, J. Am. Chem. Soc. 130 (2008) 12570–12571.
[10] B. Zhang, Y. Luo, Q. Wang, Int. J. Antimicrob. Agents 38 (2011) 502–509.
[11] T. Samanta, G. Roymahapatra, W.F. Porto, S. Seth, S. Ghorai, S. Saha, J. Sengupta, O.L. Franco, J. Dinda, S.M.N. Mandal, PLoS ONE 8 (2013) e58346, http://dx.doi.org/10.1371/journal.pone.0058346.
[12] S. Ray, R. Mohan, J.K. Singh, M.K. Samantaray, M.M. Shaikh, D. Panda, P. Ghosh, J. Am. Chem. Soc. 129 (2007) 15042–15053.
[13] G. Roymahapatra, S.M. Mandal, W.F. Porto, T. Samanta, S. Giri, J. Dinda, O.L. Franco, P. K. Chattaraj, Curr. Med. Chem. 19 (2012) 4184–4193.
[14] C.P. Randall, L.B. Oyama, J.M. Bostock, I. Chopra, A.J. O'Neill, J. Antimicrob. Chemother. 68 (2013) 131–138.
[15] B. Cornils, W.A. Herrmann, Aqueous-Phase Organometallic Catalysis: Concepts and Applications, 2nd ed., 2004. (VCH).
[16] S. Sanz, L.A. Jones, F. Mohr, M. Laguna, Oganometallics 26 (2007) 952–957.
[17] G.A. Fernández, A.S. Picco, M.R. Ceolín, A.B. Chopa, G.F. Silbestri, Organometallics 32 (2013) 6315–6323.
[18] J.A. Andrews, J. Am. Chem. Soc. 48 (Suppl. S1) (2001) 5–16.
[19] I. Özdemir, A. Denizci, H.T. Öztürk, B. Cetinkaya, Appl. Organomet. Chem. 18 (2004) 318–322.
[20] I. Özdemir, N. Temelli, S. Günal, S. Demir, Molecules 15 (2010) 2203–2210.
[21] R.R. Butorac, S.S. Al-Deyab, A.H. Cowley, Molecules 16 (2011) 2285–2292.
[22] Ö. Dogan, N. Kaloglu, S. Demir, I. Özdemir, S. Günal, I. Özdemir, Monatsh. Chem. 144 (2013) 313–319.
[23] T. Koburger, N-O. Hübner, M. Braun, J. Siebert, A. Kramer, J. Antimicrob. Chemother. 65 (2010) 1712–1719.
[24] S. Silver, FEMS Microbiol. Rev. 27 (2003) 341–353.
[25] A. Melaiye, R.S. Simons, A. Milsted, F. Pingitore, C. Wesdemiotis, C.A. Tessier, W.J. Youngs, Agent. J. Med. Chem. 47 (2004) 973–977.
[26] K.M. Hindi, T.J. Siciliano, S. Durmus, M.J. Panzner, D.A. Medvetz, D.V. Reddy, L.A. Hogue, C.E. Hovis, J.K. Hilliard, R.J. Mallet, C.A. Tessier, C.L. Cannon, W.J. Youngs, J. Med. Chem. 51 (2008) 1577–1583.
[27] A.A. Massoud, V. Langer, Y.M. Gohar, M.A.M. Abu-Youssef, J. Jänis, G. Lindberg, K. Hansson, L. Öhrström, Inorg. Chem. 52 (2013) 4046–4060.