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Steroid–Au^{I}–NHC Complexes: Synthesis and Antibacterial Activity

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A series of gold(I) pioneer complexes bearing *N*-heterocyclic carbenes and steroid derivatives (ethynylestradiol and ethisterone) with the generic formula [Au(R_2-imidazol-2-ylidene)(steroid)] (where R = CH_3 or $CH_2CH_2OCH_3$) were synthesized, and the X-ray structure of a rare of gold(I)–estradiol derivative is discussed. Toxicity studies reveal notable antibacterial activity of the gold-based compounds, which is significantly increased in vivo by the presence of the estradiol unit. Toxicity profiling was estimated in vitro versus Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, and in vivo on *Galleria mellonella* larvae against *E. coli*.

The search and design of novel compounds exhibiting antibacterial or antimicrobial activity has evolved in line with the progress of chemistry, and today there is a wide variety of examples. An interesting case is that of metal complexes, already used in ancient times, which confer attributes not found in natural products or derivatives. Modern variations of such complexes are closely related to cutting-edge ligands in chemistry, as those based on *N*-heterocyclic carbenes (NHCs).[1] In fact, they have grown in importance as ligands in bioactive complexes over the last few years. The first report on antibacterial and antifungal properties of metal–NHC complexes dates from 1996,[2] and since then, their use has become widespread.[3] In particular, the incorporation of NHC ligands to the bioactive silver and gold metals has opened up new possibilities for the design of novel compounds. Thus, while Ag–NHC complexes exhibit excellent properties as antimicrobials,[4] antibacterials,[5] antibiotics,[6] or anticancer agents,[7] the antitumor activity of certain gold complexes, underpinned in many cases by the stability of their Au^{I}–NHC bonds,[8] has been the focus of numerous investigations.[7,9] Nevertheless, although less common, there are a number of NHC-based gold compounds that show antimicrobial activity.[10]

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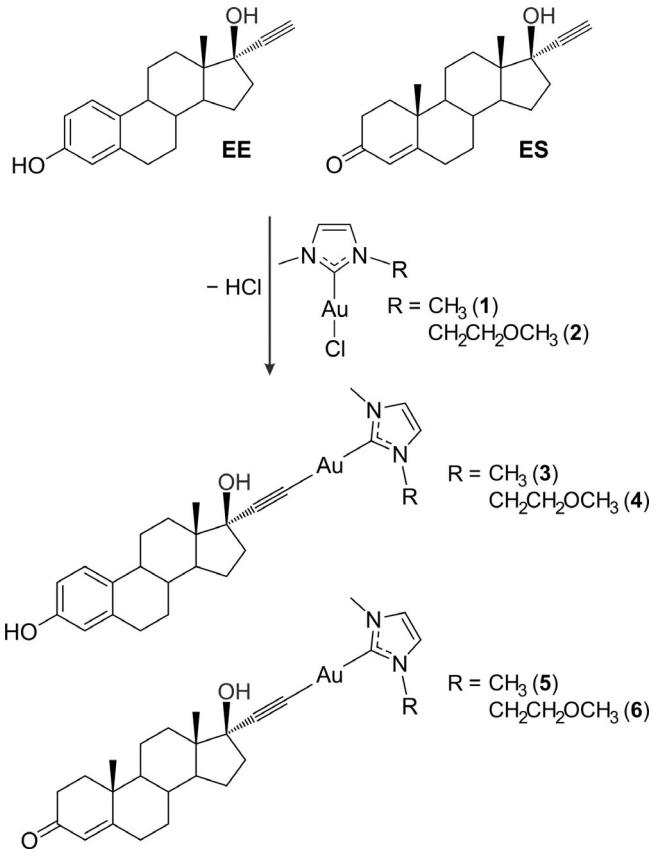
In this work, we further develop the nature of antimicrobial Au^{I}–NHC complexes. Our approach is an attempt to improve the activity of these compounds by molecular camouflage, with the incorporation of an estrogen derivative to the coordination sphere of the Au^{I} center. Estrogens have been used in the past as targeting ligands to synthesize selective metal-based anticancer drugs.[11] Steroids such as estradiol and testosterone are an important class of carrier molecules for anticancer agents; sex hormones play an important role in the growth and development of certain types of cancer cells (e.g., ovarian and prostate).[12] Estradiol and testosterone are attractive delivery vectors, as 60–75% of cancer tumors (breast, ovarian, and prostate) overexpress the estradiol and testosterone receptors. Herein we report the first complexes bearing both NHC ligands and hormone derivatives. The antimicrobial activity of this new class of Au^{I} complexes was investigated in vitro versus Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria and in vivo on *Galleria mellonella* larvae.

The complex [Au(Me_2Im)Cl] (**1**) (Me_2Im = 1,3-dimethylimidazol-2-ylidene) was prepared from the corresponding silver precursor as reported.[13] An analogous method was followed for the synthesis of [Au(MeMeOEtIm)Cl] (**2**) (MeMeOEtIm = 1-(2-methoxyethyl)-3-methylimidazol-2-ylidene). In that case, the imidazolium salt [HMeMeOEtIm]Cl was isolated from the reaction of 1-methylimidazole with 1-chloro-2-methoxyethane.[14] Hereafter, the resulting compound **2** was obtained as a white powder. The ^{1}H NMR spectrum ($CDCl_3$) of **2** shows two doublets at 7.09 and 6.89 ppm, corresponding to the H4 and H5 protons of the imidazole ring; the *N*-CH_2 and CH_2-*O* signals appear as multiplets centered at 4.32 and 3.70 ppm, respectively. The CH_3-*N* and *O*-CH_3 signals are observed as singlets at 3.83 and 3.33 ppm, respectively. Crystals of **2** (CCDC 5391621) were grown by slow diffusion of diethyl ether into a saturated dichloromethane solution. Salient geometrical features are as expected for this kind of compounds.[15] A view of the molecular structure of **2** and further details are provided in the Supporting Information.

Reactions of compounds **1** and **2** with the estrogens ethynylestradiol (**EE**) and ethisterone (**ES**) in the presence of sodium methoxide results in the corresponding alkynylgold(I) complexes [Au(Me_2Im)(EE^-)] (**3**), [Au(MeMeOEtIm)(EE^-)] (**4**), [Au(Me_2Im)(ES^-)] (**5**), and [Au(MeMeOEtIm)(ES^-)] (**6**) (Scheme 1 and Supporting Information).

NMR spectroscopy confirmed that the coupling between the starting carbene complexes **1** and **2** and the estrogens **EE** and **ES** was successful. The ^{1}H NMR spectra of **3** and **4** are very sim-



Scheme 1. Synthesis of complexes **3**–**6**.

ilar. In the case of complex **3**, there are five peaks between 7.12 and 6.56 ppm, which correspond to the imidazole H4 and H5 protons and to the three aromatic protons of the phenyl ring of the estradiol. On the other hand, ^{1}H NMR spectra of **5** and **6** are similar to those of **3** and **4**, with the only exception that there is a single aromatic proton in the testosterone moiety at 5.71 ppm for both **5** and **6**. All four complexes (**3**–**6**) show several multiplets in the region 2.50–0.90 ppm corresponding to the CH and CH_2 protons of the three rings of estradiol or testosterone. Complete NMR characterization (^{1}H NMR, ^{13}C NMR, HSQC, HMBC) for compounds **3**, **4**, **5**, and **6** is provided in the Supporting Information.

Compound **4** could be isolated as a crystalline material by slow diffusion of diethyl ether into a solution of **4** in wet dichloromethane at −20 °C. The structure of complex **4** was unequivocally determined by single-crystal X-ray diffraction (CCDC 1539163), although the quality of the crystals was poor. This was probably due to the rapid decomposition brought about by the presence of nine water molecules and four crystallographically different units of **4** in the asymmetric unit. Decomposition refers here to the loss of crystallinity, as complexes **3**–**6** are stable in solution for several days. Figure 1 shows a view of one of the molecules of **4**. In the case of complex **4**, the gold ions are coordinated to the NHC ligands and to the alkynyl fragments. C–Au–C bond angles show minimal distortions. Mutual positioning of the estradiol fragment and the imidazole ring form almost right angles. The crystal packing shows the formation pairs of molecules of **4**, which built columns along the *b* axis separated by water molecules (Supporting Information). Within these constructions, the estradiol fragments wrap the Au–NHC skeleton, which shows Au–Au separations of 3.531(2) Å (Au1–Au2) and 3.309(3) Å (Au3–Au4). Aurophilic contacts between pairs of gold atoms should not be discarded here,[15b] especially for the latter distance, which is slightly shorter than two times the van der Waals radius of gold (3.32 Å).

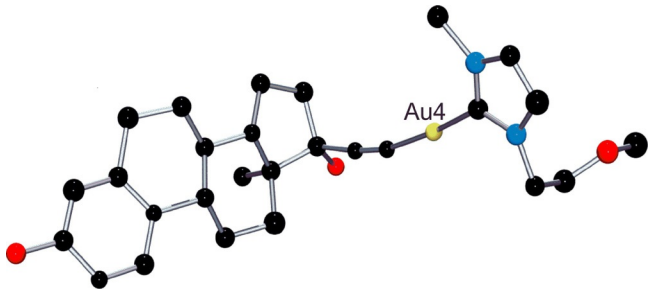


Figure 1. View of the complex [Au(MeMeOEtIm)(EE^{-})] (**4**).

To test the antimicrobial activity, the carbene Au^{I} precursors **1** and **2**, the four complexes bearing estrogens (**3**–**6**), and the two pro-ligands ethynylestradiol (**EE**) and ethisterone (**ES**) were screened in in vitro antibacterial tests against two bacterial strains: *S. aureus* (Gram positive) and *E. coli* (Gram negative). Activities as MIC_{50} values are listed in Table 1, and are similar to those of previously reported Au^{I}–NHC complexes.[16]

As expected, both compounds **EE** and **ES** are inactive, and the presence of the metal center is of fundamental importance for antimicrobial activity. The two carbene precursors **1** and **2** are the most active among the complexes tested in both Gram-positive and Gram-negative bacteria, with very low MIC_{50} values (average MIC_{50}: 4.1 and 5.85 μM for **1** and **2**, respectively). The presence of the estrogens seems to slightly decrease the in vitro antimicrobial activity with respect to the two precursors **1** and **2**. Besides, complexes **3** and **4**, bearing an estradiol moiety, were much more active than the corresponding testosterone species **5** and **6** (average MIC_{50}: 8.2 and 39.4 μM for **3** and **4**, and 95.0 and 69.2 μM for **5** and **6**, respectively). This could be correlated with the higher solubility of estradiol

Table 1. Antimicrobial activity of selected compounds.

Compd	MIC_{50} [μM][a]	
	S. aureus	*E. coli*
1	7.02 ± 0.9	1.17 ± 0.3
2	7.02 ± 0.7	4.68 ± 0.5
3	14.1 ± 1.8	2.34 ± 0.4
4	75 ± 3.8	4.68 ± 0.6
5	115 ± 6.6	75 ± 4.3
6	129 ± 7.8	9.37 ± 0.7
EE	> 150	> 150
ES	> 150	> 150

[a] Minimum compound concentration required to inhibit growth by 50% relative to positive control; values are the mean ± SD of triplicate experiments.

derivatives **3** and **4** in comparison with the testosterone derivatives (**5** and **6**). All complexes are considerably more active against the Gram-negative *E. coli* (average MIC_{50}: 16.2 μM) than against the Gram-positive *S. aureus* (average MIC_{50}: 57.8 μM).

In vivo toxicity profiling was carried out using the *G. mellonella* insect model as described in the Supporting Information. Due to the many similarities between the immune system of insects and the innate immune system of mammals,[17,18] insects have become popular choices for measuring the virulence of microbial pathogens and for assessing the in vivo activity of antimicrobial drugs.[19,20] Larvae of *G. mellonella* (the greater wax moth) can be used to assess the pathogenicity of microbial isolates[21] and the in vivo toxicity and therapeutic potential of antimicrobial drugs;[5b,22] in fact, they can yield results that are comparable to those obtained using mammalian models.[23,24] Insects have the benefit of being inexpensive to purchase and house and can give results in 24–48 hours. *G. mellonella* larvae were administered the test solutions by direct injection into the haemocoel through the last pro-leg. Survival was monitored after 15, 24, and 48 h at 37 °C, and death was assessed by the lack of movement in response to stimulus together with discoloration of the cuticle.

The toxicity of all of the test samples was assessed at three different concentrations (150, 75, and 37.5 μM). We tested the two precursors **1** and **2** together with the estradiol derivatives **3** and **4**, which resulted in the most active species on the in vitro studies. All the four tested complexes do not show any toxicity until 150 μM concentration after 24 h. Furthermore, the antimicrobial activity of complexes **1**–**4** was studied in vivo infecting the larvae with *E. coli* after treatment with nontoxic concentration of the gold(I) complexes **1**–**4**. As described in the Supporting Information, the larvae were treated with the highest nontoxic concentration of each complex (150 μM), incubated for 2 h at 37 °C, and then inoculated with *E. coli*. The larvae were incubated again at 37 °C, and the effect on viability was monitored after 15, 24, and 48 h (Figure 2). The larvae inoculated with complexes **3** and **4** (those containing the estradiol moiety) demonstrated increased survival with respect to the control. In particular, complex **4** seems to be the most active, with larval survival of > 80 %. Complexes **1** and **2** (the carbene precursors) did not show any significant increase in larval survival with respect to the control, even if in vitro studies showed the highest activity.

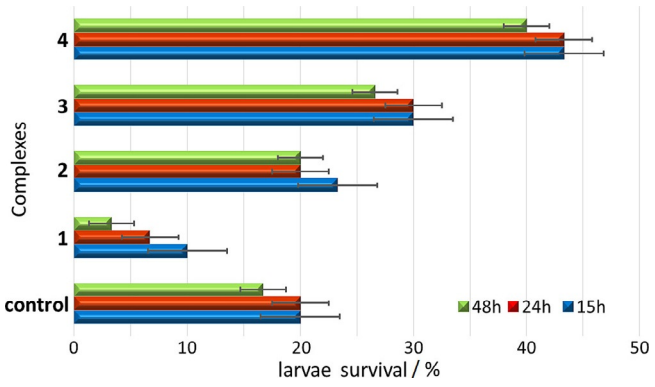


Figure 2. Percentage survival of *G. mellonella* larvae inoculated with complexes **1**–**4** and followed by inoculation of *E. coli* after 15, 24, and 48 h.

In conclusion, pioneering complexes that combine NHC ligands and hormone derivatives (ethynylestradiol and ethisterone) showed interesting in vitro antibacterial properties. The toxicity studies reveal that the presence of estradiol positively affects the in vivo antibacterial activity of the gold(I) carbene complexes. This finding may represent a significant new development in the use of metal–estrogens as effective antimicrobial agents.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: antibacterial agents · estrogens · gold(I) · NHC ligands · steroids

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