

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/103453/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Casini, Angela, Estrada-Ortiz, Natalia, Gabbiani, Chiara, Guarra, Federica, Groothuis, Geny, de Graaf, Inge, de Jager, Marina and Marchetti, Lorella 2017. Anticancer gold N-heterocyclic carbene complexes: a comparative in vitro and ex vivo study. *ChemMedchem* 12 (17) , pp. 1429-1435. 10.1002/cmdc.201700316 file

Publishers page: <http://dx.doi.org/10.1002/cmdc.201700316>
<<http://dx.doi.org/10.1002/cmdc.201700316>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Anticancer gold N-heterocyclic carbene complexes: a comparative *in vitro* and *ex vivo* study

Natalia Estrada-Ortiz,^[a] Federica Guarra,^[b] Inge A. M. de Graaf,^[a] Lorella Marchetti,^[b] Marina H. de Jager,^[a] Geny M. M. Groothuis,^[a] Chiara Gabbiani^[b] and Angela Casini^{*[a,c]}

[a] N. Estrada-Ortiz, Dr. I. A. M. de Graaf, M. H. de Jager, Prof. G. M. M Groothuis
Dept. Pharmacokinetics, Toxicology and Targeting, Groningen Research Institute of Pharmacy
University of Groningen
Antonius Deusinglaan 1, 9713AV Groningen, The Netherlands.
*E-mail: casinia@cardiff.ac.uk

b. F. Guarra, Prof. C. Gabbiani.
Department of Chemistry and Industrial Chemistry
University of Pisa
Via Moruzzi, 3, 56124 Pisa, Italy.

c. Prof. A. Casini*
School of Chemistry
Cardiff University
Main Building, Park Place, CF103AT Cardiff, United Kingdom.

Supporting information for this article is given via a link at the end of the document

Abstract: A series of organometallic Au(I) N-heterocyclic carbene (NHC) complexes was synthesized and characterized on anticancer activity in four human cancer cell lines. The compounds' toxicity in healthy tissue was determined using precision cut kidney slices (PCKS) as a tool to determine the potential selectivity of the Au complexes *ex vivo*. All evaluated compounds presented cytotoxic activity towards the cancer cells in the nano- or low micromolar range. The mixed Au(I) NHC complex - (ter-butylethynyl)-1,3-bis-(2,6-diisopropylphenyl)-imidazol-2-ylidene gold(I), bearing an alkynyl moiety as ancillary ligand, showed high cytotoxicity in cancer cells *in vitro*, while being barely toxic in healthy rat kidney tissues. The obtained results open new perspectives towards the design of mixed NHC-alkynyl gold complexes for cancer therapy.

Metals and metal complexes have been used for medicinal applications since ancient times. For example, copper was the first metal described as sterilizing agent between 2600 and 2200 B.C. (Smith Papyrus). Furthermore, the Ebers Papyrus from 1500 B.C. describes the use of copper to reduce inflammation and the use of iron to treat anaemia, while approximately at the same time, zinc was being used to heal wounds.^[1,2] In recent years, medicinal inorganic chemistry has become a rapidly growing field with a broad range of medical applications for inorganic and metal-based compounds, including mineral supplements (Fe, Zn, Cu, Se), diagnostics (Gd, Mn, Ba, I), antimicrobial (Ag), anticancer (Pt), antiulcer (Bi) and antiarthritic (Au) agents, among others.^[3,4]

The interest in developing new metal-containing therapeutic compounds increased largely after the success of cisplatin in the treatment of solid malignancies.^[5] Unfortunately, platinum(II)-containing compounds present several limitations such as toxicity in healthy tissues, restricted spectrum of activity and development of resistance.^[5,6]

Subsequently, an extensive number of metal-containing compounds were described with interesting cytotoxic activities, displaying diverse mechanisms of action and pharmacological profiles.^[7-10] Within this framework, gold-based complexes are particularly interesting due to their different possible oxidation

states (e.g. Au(I) and Au(III)), stability and ligand exchange reactions, which confer them different mechanisms of activity compared to cisplatin.^[4,11,12]

Early studies on the anticancer activity of the Au(I) complex auranofin ([Au(I)(2,3,4,6-tetra-O-acetyl-1-(thio-κS)-β-D-glucopyranosato)(triethylphosphine)]), presently used in the clinic to treat severe rheumatoid arthritis, revealed activity levels similar to cisplatin *in vitro*,^[13] which subsequently led to a large number of Au(I) complexes being evaluated for antiproliferative effects.

Interestingly, most of the anticancer gold complexes reported so far, appear to exert their activity via the interaction with protein targets,¹²⁻¹⁴ and only in a few cases DNA binding has emerged as a likely route to cancer cell death.^{15,16}

Among the various families of gold compounds tested for their anticancer effects in the last decade, a variety of organometallic gold(I/III) N-heterocyclic carbene (NHC) complexes were designed, featuring anticancer activity in the micromolar or sub-micromolar range *in vitro*.^[17-21] Specifically, gold(I) NHC derivatives exert their effects via different pathways, including: (i) mitochondrial damage (common for cationic gold(I) biscarbene complexes, which behave as delocalized lipophilic cations (DLC) being able to accumulate selectively inside the mitochondria of cancer cells due to their higher mitochondrial membrane potential),²¹⁻²⁴ (ii) inhibition of the seleno-enzyme thioredoxin reductase,^[26-28] (iii) inhibition of protein tyrosine phosphatases (PTP)^[29] and (iv) stabilization of DNA G-quadruplexes.^[30-32]

Moreover, gold(I) NHC complexes are appealing from a synthetic point of view due to their high stability with respect to ligand exchange reactions, relatively easy synthetic procedures, possibility of functionalization leading to increased structural diversity, as well as tuneable lipophilic-hydrophilic properties to enhance biological activity.^[11,19,33,34]

Therefore, herein we report the synthesis of four Au(I) NHC complexes (**1-4**) (Figure 1) including: mono- (**1**) and bis-NHC (**2**) compounds featuring a 1-butyl-3-methyl-imidazol-2-ylidene ligand. This ligand was chosen to obtain gold complexes possessing favourable lipophilicity, in line with previous

studies.^[33] Based on the same NHC scaffold, an auranofin-type complex (**3**) was obtained, where the NHC moiety replaces the phosphane ligand. Moreover, a mixed NHC-alkynyl complex (**4**) was synthesized, with a 1,3-bis-(2,6-diisopropylphenyl)-imidazol-2-ylidene ligand. The latter was chosen since it was impossible to stabilize the mixed NHC-alkynyl metal complexes using the 1-butyl-3-methyl-imidazol-2-ylidene ligand.

It should be noted that, recently, Au(I) complexes of the type (alkynyl)Au(I)(phosphane), containing an anionic alkynyl group as well as a neutral phosphane ligand, have been shown to possess interesting anticancer effects *in vitro* and *in vivo*.^[35,36] However, to the best of our knowledge the anticancer properties of mixed NHC-alkynyl Au(I) complexes have not been reported so far.

Finally, for comparison purposes and to evaluate the effect of the metal ion on the biological activity, the Pt(II) (**5**) analogue of **2** was also synthesized.

The biological activity of our series of Au(I) NHC complexes was evaluated both *in vitro* and *ex vivo*. Specifically, the cytotoxicity of the compounds was tested in four cancer cell lines, namely a p53 wild-type and a p53 null variant of HCT 116 (colorectal carcinoma), MCF-7 (breast adenocarcinoma) and A375 (malignant melanoma). Additionally, the new compounds were tested for their toxicity on healthy rat kidney tissue *ex vivo* using precision cut kidney slices (PCKS).^[37] In PCKS, all cells remain in their natural environment maintaining the original cell-cell and cell-matrix contacts, which are absent in classical 2D cell cultures *in vitro*.^[37,38] This technique is an FDA-approved model for drug toxicity and metabolism studies.^[37,38] Recently, we have successfully used the precision cut tissue slices technique to study the toxic effects of experimental anticancer organometallic compounds,^[31,39,40] aminoferrocene-containing pro-drugs,^[41] ruthenium-based kinase inhibitors,^[42] as well as supramolecular metallacages as possible drug delivery systems.^[43]

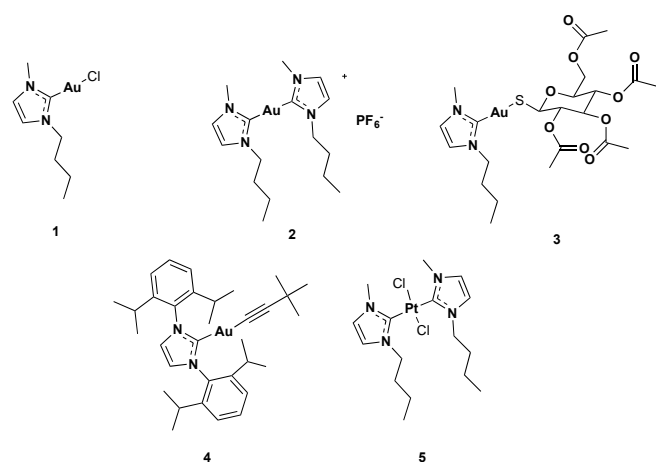


Figure 1. Structure of the reported metal NHC complexes.

Synthesis and characterization

The carbene (1-butyl-3-methyl-imidazol-2-ylidene)gold(I)chlorido Au(BMIm)Cl (**1**) and the bis-carbene bis(1-butyl-3-methyl-imidazole-2-ylidene)gold(I) hexafluorophosphate [Au(BMIm)₂]PF₆ (**2**) were synthesized according to previously reported procedures,^[34] and adapted established protocols.^[33,44] Thus, synthesis of compound **1** was achieved by a transmetalating route from the correspondent Ag(I) carbene.

Compound **2** was synthesized by reaction of Au(SMe₂)Cl with 2 equiv. of 1-methyl-3-butyl-2-ylidene, prepared *in situ* by deprotonation of the correspondent imidazolium salt with LiHMDS.

Complex **3** is a new analogue of auranofin bearing thio-β-glucose-tetraacetate as ancillary ligand while replacing the phosphine with the NHC carbene ligand. The compound was obtained in high yields by adapting a procedure previously published by Baker et al.^[45] that allows the substitution of a chlorido ligand using K₂CO₃ in CH₂Cl₂. The compound was found to be soluble in chlorinated solvents (dichloromethane and chloroform), in acetone and in DMSO. This new gold(I) complex was characterized in solution by ¹H and ¹³C NMR in CDCl₃ (Figures S1 and S2 in the Supplementary material). The signals are consistent with the ones reported for similar compounds, confirming the proposed structure.^[33,44]

The most diagnostic feature in the ¹³C NMR spectrum of compound **3** (Figure S2) is the carbene carbon signal at 183.7 ppm, which shows a downfield shift compared with the signal at 171.9 ppm of the corresponding precursor **1** with chlorido as ancillary ligand, most likely due to the better donating ability of the thiolate ligand.^[44] The signal of the thiol carbon (C1') shows a downfield shift from 78.9 ppm to 83.0 ppm as a consequence of the coordination to the gold(I) centre. In the ¹H NMR spectrum (Figure S1) the coordination of the thiolate is confirmed by the absence of the S-H resonance. In FT-IR spectrum the most diagnostic feature is the band of the carbonyls at $\tilde{\nu} = 1744 \text{ cm}^{-1}$. (Figure S3).

Preparation of the gold(I) alkynyl compound **4** was performed according to already established procedures,^[46,47] by reacting 3,3-dimethyl-1-butyne with the precursor gold(I) NHC carbene (Au(IPr)Cl) in presence of a strong base (ButOK) in MeOH. The compound was characterized in solution by ¹H and ¹³C NMR in CDCl₃ (Figure S4 and S5). The ¹³C NMR spectrum is featured by the signal of the carbene carbon at low field ($\delta = 191.9 \text{ ppm}$). Furthermore, the quaternary carbons of the alkynyl moiety are downfield shifted ($\delta = 114.1$ and 112.5 ppm) compared to the signals in the precursor ($\delta = 92.7$ and 66.5 ppm) owing to the coordination to gold(I). In the ¹H NMR the signal of the four isopropyl protons is a diagnostic feature, featuring a septet at 2.60 ppm as well as the corresponding doublets of the methyl groups at 1.34 and 1.18 ppm. Another characteristic feature of the spectrum is the singlet of the three methyl groups of the alkynyl moiety at 1.10 ppm. In the FT-IR spectrum the weak band of the triple bond at $\tilde{\nu} = 2116 \text{ cm}^{-1}$ can be recognized (Figure S6).

Compound **5** is a platinum(II) derivative bearing the same NHC ligand of compounds **1**, **2**, **3**. It was synthesized by transmetalating routes from the correspondent Ag(I) carbene by adapting previously reported procedures.^[48] Thus, **5** was obtained by refluxing Ag(BMIm)Cl with 0,5 eq of K₂PtCl₄ in CH₂Cl₂ for 4 days.

The ¹H NMR spectrum of **5** in CDCl₃ (Figure S7) shows the superposition of two sets of signals (shifted of $\delta \sim 0.03 \text{ ppm}$) due to the presence of two isomers. ¹⁹⁵Pt NMR spectrum confirms the presence of two complexes in which the metal nucleus resonates at close chemical shifts ($\delta = -3177.8$; -3179.0 ppm) (Figure S8). The nature of the isomers can be better elucidated

through ^{13}C NMR spectrum (Figure S9). It suggests the presence of the two rotamers *trans-syn* and *trans-anti* as frequently observed for nickel(II) and palladium(II) complexes of two unsymmetrical NHC ligands.^[49–51] As a matter of fact, only one carbenic carbon signal at chemical shift typical of *trans*-bis(NHCs)PtX₂ complexes is present ($\delta = 167.3$ ppm).^[48] The formation of the *cis* isomer can therefore be excluded, since it would have given a carbenic carbon signal at significantly upfield shifted chemical shift as reported for similar compounds ($\delta \sim 132$ – 150 ppm).^[52,53]

The stability of gold compounds **3** and **4** was monitored by ^1H NMR spectroscopy of mixtures of water and DMSO-*d*₆ during 7 days at room temperature. The complexes were found to be stable in solution in these conditions (Figures S10–S11).

Moreover, the two complexes were reacted with 1.5 equivalents of DL-homocysteine, as an intracellular model nucleophile, in CD₃OD. ^1H NMR spectra revealed that no reaction with **3** occurred after 24 h. Indeed, neither the signals of the complex nor of homocysteine showed any variation (Figures S12–14).

On the contrary, **4** was found to react with homocysteine with the formation of a new species that could be identified as the product of the substitution of the alkyne with the thiol. Indeed, a series of ^1H NMR spectra registered during 24 h shows the progressive conversion of **4** into a new complex (Figure S15), most likely involving coordination of the amino acid to the gold centre with substitution of the alkynyl moiety. In fact, after 24 h, new signals in the region of aromatic protons are visible (from $\delta = 7.55$, 7.50 , 7.36 ppm of the precursor to $\delta = 7.62$ ppm, $\delta = 7.54$, 7.37 ppm), the signal of one of the doublets of the methyl groups in the isopropyl moiety shifted from $\delta = 1.23$ to 1.26 ppm. Another relevant feature is the simultaneous progressive disappearance of the signal of the methyl groups of the alkyne at $\delta = 1.03$ with the formation of a new signal at $\delta = 1.22$ ppm, consistent with the presence of a dissociated 3,3-dimethylbutyne moiety (Figure S16–S18). Furthermore, the integration of the signals of coordinated homocysteine with the ones of the NHC moiety let us suppose that only the alkyne was substituted by the amino acid nucleophile in these conditions (Figure S19). This hypothesis was further confirmed by ^{13}C NMR spectrum of the mixture after 24 h, where the carbenic carbon signal is still present ($\delta = 173.7$ ppm) (Figure S20).

In vitro cell viability assays

The antiproliferative properties of the Au(I) NHC complexes **1–4** and of cisplatin and auranofin as comparison, were assessed using the MTT assay in the human cancer cell lines HCT116 p53 wt, HCT116 p53 null, MCF-7 and A375.

All the tested Au(I) complexes presented antiproliferative effects in the evaluated cell lines in the low μM range (Table 1). Instead, the Pt(II) complex **5** was scarcely active ($\text{EC}_{50} > 50$ μM). The latter result is in accordance with other studies on Pt(II) bis-NHC complexes with chlorido ligands (see ref 20 and citations therein) featuring moderate cytotoxic effects *in vitro*. Notably, the compounds **1**, **2** and **3** showed superior activity, in the nM or low μM range, compared with cisplatin and auranofin in three of the four cell lines, A375 being the exception. Overall, no significant differences could be observed between the EC_{50} values for the mono-carbene (**1**), bis-carbene (**2**), and auranofin-analogue (**3**) in the different cancer cell lines. Interestingly, the alkynyl derivative (**4**) was effective although ca. 4–10 fold less potent compared to the other gold NHC complexes. However, in

general **4** was markedly more active or as active as cisplatin, except in the A375 cell line. Additionally, the lack of differences observed between the HCT116 p53 wt and p53 null cells treated with the gold complexes indicates that their toxicity mechanism is independent of p53 activity, in contrast to cisplatin. The latter is known to induce rapid p53-dependent apoptosis and, as a secondary effect, p53-independent cell cycle arrest.^[54,55]

Table 1. EC_{50} values of Au(I) NHC complexes in various human cancer cell lines, in comparison to auranofin and cisplatin, after 72 h incubation.

| Compound | EC_{50}^a (μM) | | | |
|-----------|--------------------------------------|----------------|-----------|-----------|
| | HCT116 p53wt | HCT116 p53null | MCF-7 | A375 |
| 1 | 0,5 ± 0,3 | 0,6 ± 0,2 | 0,8 ± 0,4 | 2 ± 1 |
| 2 | 0,6 ± 0,4 | 0,24 ± 0,09 | 1,1 ± 0,3 | 1,0 ± 0,6 |
| 3 | 0,8 ± 0,2 | 1 ± 0,4 | 2 ± 0,8 | 1,2 ± 0,2 |
| 4 | 9 ± 3 | 8,8 ± 0,9 | 6 ± 2 | 10 ± 1 |
| 5 | > 50 | > 50 | > 50 | ND |
| auranofin | 5,1 ± 0,7 | 7,10 ± 0,01 | 7 ± 2 | 1,3 ± 0,8 |
| cisplatin | 11 ± 3 | 20,6 ± 0,9 | 12 ± 2 | 3,7 ± 0,9 |

^a The reported EC_{50} values were calculated using a nonlinear fitting of log[concentration] vs response and are presented as a mean (\pm SD) of at least three independent experiments. ND: not determined.

Ex vivo toxicity evaluation

Due to their potent cytotoxic effects in cancer cells, complexes **1–4** were tested for their possible toxicity in an *ex vivo* model in healthy rat kidney tissue using the PCKS technology.^[37,38]

Kidney was selected since cisplatin is well known to induce severe nephrotoxicity in patients.^[56]

Hence, kidney slices were incubated with various concentrations of each gold complex, and after 24 h the viability of the tissues was determined measuring the ATP content (Table 2 and Figure 2). Auranofin and cisplatin were also tested for comparison.

The gold complexes, including auranofin, displayed a concentration dependent toxicity profile, with complexes **1** and **2** as the most toxic, with TC_{50} below 1 μM . Interestingly, compounds **1–3** were more toxic than cisplatin (ca. 6–12-fold). Remarkably, complex **4** showed no toxicity up to 50 μM . For compounds **1–3** the safety margin for toxicity was small to absent with a ratio of TC_{50} PCKS/ EC_{50} cells between 0,4 and 3,3. However, for compound **4** the TC_{50} PCKS/ EC_{50} cells ratio was higher than 5,0 to 8,3 indicating selective toxicity towards cancer cells compared to healthy kidney tissue.

The differential effects of complexes **1** and **4** (5 μM concentration) on kidney slices were further assessed by histomorphology using Periodic acid-Schiff staining (PAS) to evaluate slice integrity and particularly to visualize the basement membranes and epithelial brush border in the proximal tubule cells, as reported in the experimental section. After 24 h incubation, the untreated kidney slices show minor morphological changes, indicated by pyknosis and swelling of some of the tubular cells (Figure 3A). Marked effects were observed upon treatment with complex **1**, which induced dilatation of Bowman's space in the glomerulus and necrosis of the distal tubule cells, as well as discontinuation of the brush border in the proximal tubule cells at some sites (Figure 3B). In contrast, exposure of slices to complex **4** (Figure 3C) did not

induce significant morphological changes compared to controls. Auranofin treatment led to damage of the distal tubule cells and loss of nuclei from the proximal tubule cells. Moreover, cisplatin treatment (25 μM corresponding to 2-fold the TC_{50} , Figure 3E) showed damage to the proximal tubular cells with loss of nuclei and discontinuation of the brush border; additionally, damage of the distal tubule is evident as previously reported in the literature.^[57]

Table 2. Toxicity of Au(I) NHC complexes (TC_{50} values) in PCKS in comparison to auranofin and cisplatin.

| Compound | TC_{50}^a (μM) | TC_{50} (PCKS) / EC_{50} (cells) |
|-----------|--------------------------------------|--|
| 1 | $0,8 \pm 0,3$ | 0,4 - 1,6 |
| 2 | $0,8 \pm 0,7$ | 1,3 - 3,3 |
| 3 | $2,1 \pm 0,4$ | 1,1 - 2,6 |
| 4 | > 50 | >5,0 - 8,3 |
| auranofin | $2,9 \pm 1,4$ | 0,4 - 2,2 |
| cisplatin | 12 ± 6 | 0,6 - 3,2 |

^a The reported TC_{50} values were calculated using a nonlinear fitting of $\log[\text{concentration}]$ vs response and are presented as a mean (\pm SD) of at least three independent experiments.

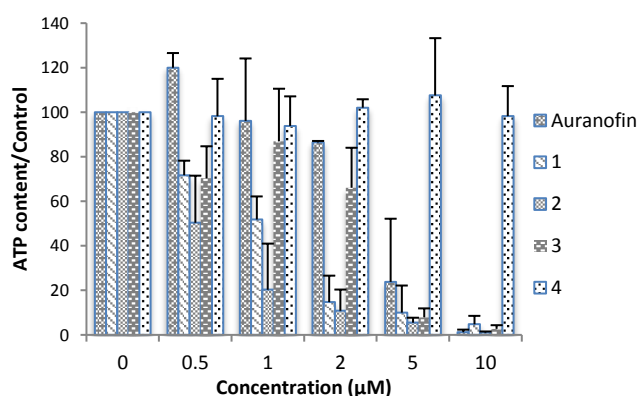


Figure 2. Viability of rat (PCKS) relative to the controls (untreated slices) after treatment with complexes 1-4 and auranofin for 24 h. The error bars show the standard deviation of at least three independent experiments. We performed an ANOVA analysis with each individual experiment as random effect and a Tukey HSD post-hoc test for pairwise comparisons.

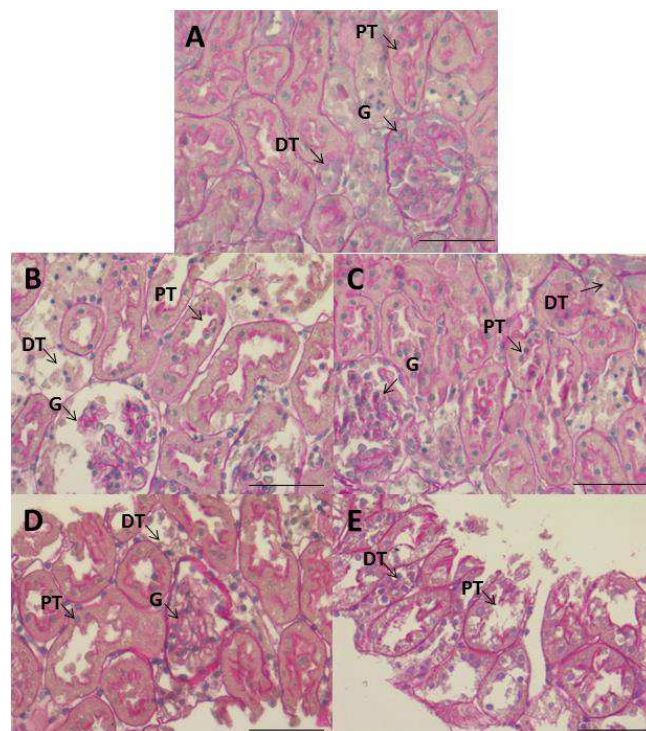


Figure 3. Morphology of rat kidney slices. A: 24 h control incubation; B: complex 1; C: complex 4; D: auranofin; E: cisplatin. The Au(I) complexes were incubated at 5 μM concentration for 24 h, while cisplatin at 25 μM for 24 h. PT: proximal tubule, DT: distal tubule, G: glomerulus. Scale bar indicates 50 μm .

Conclusions

The broad spectrum and synthetic possibilities in organometallic chemistry allowed us to develop and study different NHC ligands “fine-tuning” the physico-chemical properties of the resulting Au(I) complexes, and, possibly, achieving selectivity towards cancer tissue.

Herein, we reported the synthesis of complexes 3 and 4, as a continuation of our previous work where we described the synthesis and very preliminary biological evaluation of complexes 1 and 2.^[34] In this study, complexes 1-4 showed interesting cytotoxic activity against HCT116 p53 wt and p53 null, MCF-7 and A357, in the low μM range. Instead, the Pt(II) complex 5 was poorly cytotoxic, indicating the essential role of Au(I) ions in the biological activity. However, complexes 1-3 and auranofin displayed severe toxicity in healthy kidney tissue (PCKS), even higher than cisplatin, indicating that these compounds, when administered *in vivo*, may also induce severe nephrotoxicity. Conversely, the mixed NHC and alkynyl complex 4 appears to be at least 5-fold less toxic in healthy tissue, while maintaining antiproliferative effects at the low micromolar concentration. The reduced bioactivity of this compound may be partly due to its lower stability in the presence of nucleophiles, such as sulfur donor ligands. However, the selectivity of 4 for cancer cells with respect to healthy tissues is still promising in comparison to the other tested Au(I) NHC complexes. This initial result prompts us to develop new mixed organometallics with enhanced selectivity against cancer cells. Moreover, ongoing studies in our labs are aimed at further investigating the mechanisms of action of toxicity in cancer cells and kidney slices. So far, pilot studies using mass spectrometry (MS) analysis of complexes 1-4 showed no reactivity towards model protein

targets (cytochrome c and lysozyme), while **1** and **2** could bind to the copper chaperon protein Atox-1 upon complete ligand loss.^[34]

Overall, we believe that it is important that toxicology studies, as those presented here using our *ex vivo* model, should be conducted as early as possible on new experimental metallodrugs to select the optimal chemical scaffolds and to orient the drug design at its early stages

Experimental section

General

Unless stated otherwise the reactions were performed under inert atmosphere of nitrogen in anhydrous conditions. Solvents and reagents were used without prior treatments. NMR spectra were recorded on a Varian Gemini 200 BB instrument (¹H, 200 MHz; ¹³C, 50.3 MHz, ¹⁹⁵Pt, 42.8 MH) at room temperature; frequencies are referenced to the residual resonances of the deuterated solvent. UV-visible spectra were recorded on an Agilent Cary 60 spectrophotometer. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Spectrum One Perkin Elmer instrument equipped with an UATR unit.

Synthetic procedures

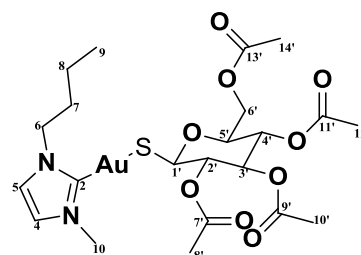
The carbenes (1-butyl-3-methyl-imidazol-2-ylidene)silver(I)chlorido Ag(BMI)Cl, (1-butyl-3-methyl-imidazol-2-ylidene)gold(I)chlorido Au(BMI)Cl (**1**) and the bis-carbene bis(1-butyl-3-methyl-imidazole-2-ylidene)gold(I) hexafluorophosphate [Au(BMI)₂]PF₆ (**2**) were synthesized according to procedures previously reported by us^[34] and their purity was confirmed by elemental analysis, and resulted to be > 98 %.

Synthesis of thio-β-D-glucose-tetraacetate-(1-butyl-3-methyl)-imidazol-2-ylidene-gold(I) (**3**)

Precursor **1** was reacted with thio-β-D-glucose-tetraacetate to prepare compound **3**. 0.1 mmol of **1** was dissolved in 15 ml of CH₂Cl₂, 1 mmol of K₂CO₃ and 0.1 mmol of thio-β-glucose-tetraacetate (tgt) were added to the solution. The suspension was stirred at room temperature for 10 min in the dark. The mixture was filtered and the solution was concentrated by evaporation to about 2 ml. The product was precipitated with hexane and washed to obtain a light brown solid (yield > 99%).

¹H NMR (CDCl₃, 293K) δ (ppm) = 6.90 (m, 2H, H₄ and H₅); 5.12-5.03 (m, 3H, tgt); 4.25-4.12 (m, 2H, H₆, 3H tgt); 3.83 (s, 3H, H₁₀); 3.76-3.68 (m, 1H, H₅); 2.08 (s, 3H, OAc); 2.02 (s, 3H, OAc); 1.99 (s, 3H, OAc); 1.96 (s, 3H, OAc); 1.83 (apparent quintet J= 7.4 Hz, 2H, H_{7a-b}); 1.68 (s, H₂O); 1.37 (apparent sextet J= 7.4 Hz, 2H, H_{8a-b}); 1.26 (hexane); 0.96 (t J= 7.4 Hz, 3H, H₉); 0.86 (hexane).

¹³C NMR (CDCl₃, 293K) δ (ppm) = 183.2 (C₂); 170.8 (C=O); 170.3 (C=O); 169.9 (C=O); 169.7 (C=O); 121.4 (C₄ or C₅); 120.3 (C₄ or C₅); 83.3 (C_{1'}); 77.9 (C_{2'}); 75.8 (C_{5'}); 74.5 (C_{3'}); 69.2 (C_{4'}); 63.2 (C_{6'}); 50.9 (C₆); 36.1 (C₁₀); 33.4 (C₇); 31.4 (hexane); 22.8 (hexane) 21.4 (OAc); 21.0 (OAc); 20.9 (2OAc); 19.8 (C₈); 14.1 (hexane); 13.9 (C₉).



IR (solid state): $\tilde{\nu}$ (cm⁻¹) = 2962 (w-m); 2916 (w); 2870 (vw); 1744 (m, CO); 1567 (vw); 1469 (w); 1412(w); 1258 (s); 1220 (m); 1086 (s); 1016 (vs); 911 (w-m); 865 (w-m); 795 (vs); 734 (w-m); 680 (w-m). Anal. Calc. (%) for C₂₂H₃₃AuN₂O₉S: C 37.70, H 4.75, N 4.00; found: C 37.64, H 4.65, N 3.98.

Synthesis of (ter-butylethynyl)-1,3-bis-(2,6-diisopropylphenyl)imidazol-2-ylidene-gold(I) (**4**)

The compound was prepared according to previously reported procedures.^[46,47] The colourless solid was obtained with 80% yield.

¹H NMR (CDCl₃, 293K) δ (ppm)= 7.48 (t, J= 7.4 Hz, 2H, CH-aromatic); 7.28 (d, J=7.4 Hz, 4H, CH-aromatic); 7.06 (s, 2H, CH-imidazole); 2.61 (sept, J= 6.9 Hz, 4H, CH(CH₃)₂ isopropyl); 1.35 (d, J= 6.8 Hz, 12H, CH(CH₃)₂ isopropyl); 1.18 (d, J= 6.8 Hz, 12H, CH(CH₃)₂ isopropyl); 1.10 (s, 9H, C(CH₃)₃ ter-butylethynyl).

¹³C NMR (CDCl₃, 293K) δ (ppm) = 191.9 (NCN imidazol); 145.7 (CH aromatic); 134.7 (C aromatic); 130.4 (CH aromatic); 124.2 (CH aromatic); 123.2 (CH imidazole); 114.05 (Au-CC); 112.51 (Au-CC); 32.6 (C(CH₃)₃ ter-butylethynyl); 28.9 (CH(CH₃)₂ isopropyl); 28.3 (C(CH₃)₃ ter-butylethynyl); 24.5 (CH(CH₃)₂ isopropyl); 24.3 (CH(CH₃)₂ isopropyl).

IR (solid state): $\tilde{\nu}$ (cm-1) = 3150 (w); 2966 (m/s); 2923 (m, sh); 2864 (w/m); 2116 (vw, CC); 1956 (vw); 1890 (vw); 1825 (vw); 1593 (w); 1720 (vw); 1677 (vw); 1650 (vw); 1558 (w); 1458 (s); 1410 (m); 1387 (w/m); 1364 (m); 1354 (w/m); 1343 (w/m); 1329 (w/m); 1275 (w); 1249 (m); 1208 (w/m); 1208 (w/m); 1181 (w/m); 1104 (w/m); 1082 (w/m); 1062 (w/m); 1032 (w); 981 (w); 944 (m); 805 (s); 764 (s); 731 (s); 697 (s); 561 (w). ESI-MS (DMSO-MeOH), positive mode exact mass for [C₃₃H₄₅AuN₂]⁺ (667.3321): measured m/z 667.3322 [M]⁺. Anal. Calc. (%) for C₃₃H₄₅AuN₂: C 59.45, H 6.80, N 4.20; found: C 59.39, H 6.58, N 4.18.

Synthesis of *trans*-dichlorido-bis(1-butyl-3-methyl-imidazole-2-ylidene)platinum(II) (**5**)

161 mg of K₂PtCl₄ (0,388 mmol) were suspended in 15ml of CH₂Cl₂, afterwards 218 mg of Ag(BMI)Cl (0,775 mmol) were added. The mixture was refluxed for 4 days and then filtered on celite. Solvent was removed under reduced pressure and the product was obtained with 86% yield.

¹H NMR (CDCl₃, 293K), *syn* and *anti* δ (ppm)= 6.80 (m, 2H, imidazole); 4.54-4.46 (m, 8H, NCH₂CH₂CH₂CH₃); 4.11, 4.08 (s+s, 12H, NCH₃); 2.14-1.96 (m, 8H, NCH₂CH₂CH₂CH₃); 1.63 (H₂O); 1.46-1.43 (m, 8H, NCH₂CH₂CH₂CH₃); 1.00 (t J² = 7.3Hz; 6H, NCH₂CH₂CH₂CH₃); 0.99 (t J²= 7.3 Hz, 6H, NCH₂CH₂CH₂CH₃).

¹³C NMR (CDCl₃, 293K), *syn* and *anti* δ (ppm)= 167.3 (NCN); 121.3 (s+d, J_{Pt-C}= 23.3 Hz, CH imidazole); 120.1 (s+d, J_{Pt-C}= 24.5 Hz, CH imidazole); 50.0 (s+d, J_{Pt-C}= 18.9 Hz, NCH₂CH₂CH₂CH₃); 37.1 (NCH₃); 37.0 (NCH₃); 33.2 (NCH₂CH₂CH₂CH₃); 20.2 (NCH₂CH₂CH₂CH₃); 14.0 (NCH₂CH₂CH₂CH₃); 13.9 (NCH₂CH₂CH₂CH₃).

¹⁹⁵Pt NMR (CDCl₃, 293K), *syn* and *anti* δ (ppm)= -3177.8; -3179.0.

NMR stability studies in aqueous media and reactivity with homocysteine.

$4 \cdot 10^{-3}$ mmol of **3** were dissolved in 0.4 ml of DMSO- d_6 /H₂O 60:40, while $6 \cdot 10^{-3}$ mmol of **4** were dissolved in 0.4 ml of DMSO- d_6 /H₂O 80:20. ¹H NMR spectra were registered at various time intervals (0h, 24h, 1 week). Afterwards, $5.0 \cdot 10^{-3}$ mmol of **3** or $4.2 \cdot 10^{-3}$ mmol of **4** were reacted with 1.5 equivalents of DL-homocysteine in 0.4 ml of CD₃OD. Reactions were monitored through ¹H NMR (0h, 6h, 24 h) and ¹³C NMR.

Cell lines

The human colorectal carcinoma HCT 116 p53 null and HCT 116 wt p53 variants (kindly provided by Dr. Götz Hartleben, University of Groningen), the human breast adenocarcinoma MCF-7 (Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH), and human malignant melanoma A375 (kindly provided by Prof. Sylvestre Bonnet, Leiden University) were cultured in DMEM (Dulbecco's Modified Eagle Medium) containing glutamax supplemented with 10% FBS and 1% penicillin/streptomycin (all from Invitrogen), at 37 °C in an incubator (Thermo Fisher Scientific, US) with humidified atmosphere of 95% of air and 5% CO₂.

In vitro cell viability assays

Cells in an exponential growth rate were seeded (8000 cells per well) in 96-wells plates (Costar 3595) grown for 24 h in complete medium. Solutions of the gold compounds were prepared by diluting a stock solution (10^{-2} M in DMSO, ethanol in the case of auranofin) in culture medium (DMSO or ethanol in the culture medium never exceeded 1%). Subsequently, different dilutions of the compounds were added to the wells to obtain a final concentration from 0.5 to 100 μM. Following 72h of exposure, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the cells at a final concentration of 0.50 mg/ml in PBS (phosphate buffered saline solution, pH 7.4) and incubated for 2.5 h. The incubation medium was removed and the violet formazan crystals in the cells were dissolved in DMSO, and the optical density of each well was quantified at 550 nm, using a multi-well plate reader (ThermoMax microplate reader, Molecular devices, US). The percentage of surviving cells was calculated from the ratio of absorbance between treated and untreated cells. Each treatment was performed in quadruplicate. The EC₅₀ value was calculated as the concentration causing 50% decrease in cell number and is presented as a mean (± SD) of at least three independent experiments.

Preparation of rat Precision-Cut Kidney Slices (PCKS) and toxicity studies ex vivo

Male Wistar rats (Charles River, France) of 250-300 g were housed under a 12 h dark/light cycle at constant humidity and temperature. Animals were permitted *ad libitum* access to tap water and standard lab chow. All experiments were approved by the committee for care and use of laboratory animals of the University of Groningen and were performed according to strict governmental and international guidelines. Kidneys were harvested (from rats anesthetized with isoflurane) and immediately placed in University of Wisconsin solution (UW, ViaSpan, 4°C) until further use. After removing fat, kidneys were cut in half lengthwise using a scalpel, and cortex cores of 5 mm diameter were made from each half perpendicular to the cut surface using disposable Biopsy Punches (KAI medical, Japan). PCKS were made as described by de Graaf et al.^[37,38] The cores were sliced with a Krumdieck tissue slicer (Alabama R&D, Munford, AL, USA) in ice-cold Krebs-Henseleit buffer, pH 7.4 saturated with carbogen (95% O₂ and 5% CO₂). Kidney slices weighing about 3 mg (~150 μm thickness), were incubated individually in 12-well plates (Greiner bio-one GmbH,

Frickenhausen, Austria), at 37°C in Williams' medium E (WME, Gibco by Life Technologies, UK) with glutamax-1, supplemented with 25 mM D-glucose (Gibco) and ciprofloxacin HCl (10 μg/mL, Sigma-Aldrich, Steinheim, Germany) in an incubator (Panasonic biomedical) in an atmosphere of 80% O₂ and 5% CO₂ with shaking (90 times/min). Stock solutions of compounds **1** to **4**, auranofin and cisplatin were prepared as for the studies on cell lines. The final concentration of DMSO and ethanol during the PCKS incubation was always below 0.5% to exclude solvent toxicity. For each concentration, three slices were incubated individually for one hour in WME and subsequently, different dilutions of the compounds were added to the wells, to obtain a final concentration from 0.5 to 50 μM. After this, PCKS were incubated for 24 h. After the incubation, slices were collected for ATP and protein determination, by snap freezing them in 1 ml of ethanol (70% v/v) containing 2 mM EDTA with pH=10.9. After thawing the slices were homogenized using a mini bead beater and centrifuged. The supernatant was used for the ATP assay and the pellet was dissolved in 5N NaOH for the protein assay. The viability of PCKS was determined by measuring the ATP using the ATP Bioluminescence Assay kit CLS II (Roche, Mannheim, Germany) as described previously.^[37] The ATP content was corrected by the protein amount of each slice and expressed as pmol/μg protein. The protein content of the PCKS was determined by the Bio-Rad DC Protein Assay (Bio-Rad, Munich, Germany) using bovine serum albumin (BSA, Sigma-Aldrich, Steinheim, Germany) for the calibration curve. The TC₅₀ value was calculated as the concentration reducing the viability of the slices by 50%, in terms of ATP content corrected by the protein amount of each slice and relative to the slices without any treatment, and is presented as a mean (± SD) of at least three independent experiments.

Histomorphology

Kidney slices were fixated in 4% formalin for 24 hours and stored in 70% ethanol at 4°C until processing for morphology studies. After dehydration, the slices were embedded in paraffin and 4 μm sections were made, which were mounted on glass slides and PAS staining was used for histopathological evaluation. Briefly, the glass slides were deparaffinised, washed with distilled water, followed by treatment with a 1% aqueous solution of periodic acid for 20 minutes and Schiff reagent for 20 minutes, the slides were rinsed with tap water, finally, a counterstain with Mayer's haematoxylin (5 min) was used to visualize the nuclei.

Statistics

A minimum of three independent experiments were performed with the cells, with 4 replicas for each condition. PCKS were prepared from 3 rats and in each experiment slices were exposed in triplicate. The EC₅₀ and TC₅₀ values were calculated as the concentration reducing the viability of the cells or slices by 50%, relative to the untreated samples using a nonlinear fitting of log[concentration compound] vs response and is presented as a mean (± SD) of at least three independent experiments. Statistical testing was performed with one way ANOVA with each individual experiment as random effect. We performed a Tukey HSD post-hoc test for pairwise comparisons. In all graphs and tables the mean values and standard deviation (SD) are shown.

Acknowledgements

The authors thank the Department of Sciences, Technology, and Innovation COLCIENCIAS (Colombia) for supporting N.E.O. The ERASMUS program is acknowledged for a fellowship awarded to F.G.. C.G. acknowledges Beneficentia Stiftung for generous financial support.

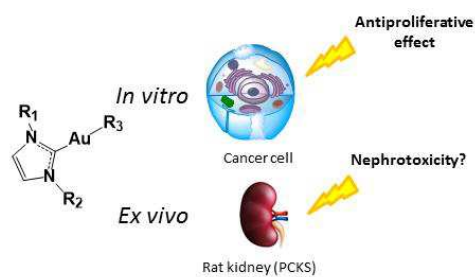
Keywords: gold NHC complexes • organometallics • alkynyl • cytotoxicity • tissue slices

References:

[1] H. H. A. Dollwet, J. R. J. Sorenson, *Trace Elem. Med.* **1985**, *2*, 80–87.
 [2] C. Orvig, M. J. Abrams, *Chem. Rev.* **1999**, *99*, 2201–2204.
 [3] R. H. Fish, G. Jaouen, *Organometallics* **2003**, *22*, 2166–2177.
 [4] D. Gaynor, D. M. Griffith, *Dalton Trans.* **2012**, *41*, 13239–13257.
 [5] L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573–584.
 [6] N. J. Wheate, S. Walker, G. E. Craig, R. Oun, *Dalton Trans. Camb. Engl.* **2003** **2010**, *39*, 8113–8127.
 [7] K. D. Mjos, C. Orvig, *Chem. Rev.* **2014**, *114*, 4540–4563.
 [8] J. B. Mangrum, B. J. Engelmann, E. J. Peterson, J. J. Ryan, S. J. Berners-Price, N. P. Farrell, *Chem. Commun. Camb. Engl.* **2014**, *50*, 4056–4058.
 [9] W. H. Ang, A. Casini, G. Sava, P. J. Dyson, *J. Organomet. Chem.* **2011**, *696*, 989–998.
 [10] A. de Almeida, B. L. Oliveira, J. D. G. Correia, G. Soveral, A. Casini, *Coord. Chem. Rev.* **2013**, *257*, 2689–2704.
 [11] B. Bertrand, A. Casini, *Dalton Trans.* **2014**, *43*, 4209–4219.
 [12] C. Nardon, G. Boscutti, D. Fregona, *Anticancer Res.* **2014**, *34*, 487–492.
 [13] S. Nobili, E. Mini, I. Landini, C. Gabbiani, A. Casini, L. Messori, *Med. Res. Rev.* **2010**, *30*, 550–580.
 [14] F. Mendes, M. Groessel, A. A. Nazarov, Y. O. Tsybin, G. Sava, I. Santos, P. J. Dyson, A. Casini, *J. Med. Chem.* **2011**, *54*, 2196–2206.
 [15] P. Shi, Q. Jiang, Y. Zhao, Y. Zhang, J. Lin, L. Lin, J. Ding, Z. Guo, *JBIC J. Biol. Inorg. Chem.* **2006**, *11*, 745–752.
 [16] M. N. Patel, B. S. Bhatt, P. A. Dosi, *Inorg. Chem. Commun.* **2013**, *29*, 190–193.
 [17] I. Ott, *Coord. Chem. Rev.* **2009**, *253*, 1670–1681.
 [18] S. J. Berners-Price, A. Filipovska, *Met. Integr. Biometal Sci.* **2011**, *3*, 863–873.
 [19] L. Oehninger, R. Rubbiani, I. Ott, *Dalton Trans. Camb. Engl.* **2003** **2013**, *42*, 3269–3284.
 [20] W. Liu, R. Gust, *Coord. Chem. Rev.* **2016**, *329*, 191–213.
 [21] M. A. Cinellu, I. Ott, A. Casini, in *Bioorganometallic Chem.* (Eds.: G. Jaouen, M. Salmann), Wiley-VCH Verlag GmbH & Co. KGaA, **2014**, pp. 117–140.
 [22] P. J. Barnard, M. V. Baker, S. J. Berners-Price, D. A. Day, *J. Inorg. Biochem.* **2004**, *98*, 1642–1647.
 [23] P. J. Barnard, S. J. Berners-Price, *Coord. Chem. Rev.* **2007**, *251*, 1889–1902.
 [24] J. L. Hickey, R. A. Ruhayel, P. J. Barnard, M. V. Baker, S. J. Berners-Price, A. Filipovska, *J. Am. Chem. Soc.* **2008**, *130*, 12570–12571.
 [25] S. Sundelacruz, M. Levin, D. L. Kaplan, *Stem Cell Rev.* **2009**, *5*, 231–246.
 [26] A. Bindoli, M. P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori, *Coord. Chem. Rev.* **2009**, *253*, 1692–1707.
 [27] R. Rubbiani, I. Kitanovic, H. Alborzina, S. Can, A. Kitanovic, L. A. Onambele, M. Stefanopoulou, Y. Geldmacher, W. S. Sheldrick, G. Wolber, et al., *J. Med. Chem.* **2010**, *53*, 8608–8618.
 [28] A. Citta, E. Schuh, F. Mohr, A. Folda, M. L. Massimino, A. Bindoli, A. Casini, M. P. Rigobello, *Metallomics* **2013**, *5*, 1006–1015.
 [29] D. Krishnamurthy, M. R. Karver, E. Fiorillo, V. Orrú, S. M. Stanford, N. Bottini, A. M. Barrios, *J. Med. Chem.* **2008**, *51*, 4790–4795.
 [30] L. Stefan, B. Bertrand, P. Richard, P. Le Gendre, F. Denat, M. Picquet, D. Monchaud, *ChemBioChem* **2012**, *13*, 1905–1912.
 [31] B. Bertrand, L. Stefan, M. Pirrotta, D. Monchaud, E. Bodio, P. Richard, P. Le Gendre, E. Warmerdam, M. H. de Jager, G. M. M. Groothuis, et al., *Inorg. Chem.* **2014**, *53*, 2296–2303.
 [32] C. Bazzicalupi, M. Ferraroni, F. Papi, L. Massai, B. Bertrand, L. Messori, P. Gratteri, A. Casini, *Angew. Chem. Int. Ed Engl.* **2016**, *55*, 4256–4259.
 [33] M. V. Baker, P. J. Barnard, S. J. Berners-Price, S. K. Brayshaw, J. L. Hickey, B. W. Skelton, A. H. White, *Dalton Trans.* **2006**, 3708–3715.
 [34] L. Messori, L. Marchetti, L. Massai, F. Scaletti, A. Guerri, I. Landini, S. Nobili, G. Perrone, E. Mini, P. Leoni, et al., *Inorg. Chem.* **2014**, *53*, 2396–2403.
 [35] A. Meyer, C. P. Bagowski, M. Kokoschka, M. Stefanopoulou, H. Alborzina, S. Can, D. H. Vlecken, W. S. Sheldrick, S. Wölfl, I. Ott, *Angew. Chem. Int. Ed.* **2012**, *51*, 8895–8899.
 [36] V. Andermark, K. Göke, M. Kokoschka, M. A. Abu el Maaty, C. T. Lum, T. Zou, R. W.-Y. Sun, E. Aguiló, L. Oehninger, L. Rodríguez, et al., *J. Inorg. Biochem.* **2016**, *160*, 140–148.
 [37] I. A. M. de Graaf, P. Olinga, M. H. de Jager, M. T. Merema, R. de Kanter, E. G. van de Kerkhof, G. M. M. Groothuis, *Nat. Protoc.* **2010**, *5*, 1540–1551.
 [38] I. A. de Graaf, G. M. Groothuis, P. Olinga, *Expert Opin. Drug Metab. Toxicol.* **2007**, *3*, 879–898.
 [39] B. Bertrand, A. Citta, I. L. Franken, M. Picquet, A. Folda, V. Scalcon, M. P. Rigobello, P. Le Gendre, A. Casini, E. Bodio, *J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem.* **2015**, *20*, 1005–1020.
 [40] J. K. Muenzner, T. Rehm, B. Biersack, A. Casini, I. A. M. de Graaf, P. Worawutputtpong, A. Noor, R. Kempe, V. Brabec, J. Kasparkova, et al., *J. Med. Chem.* **2015**, *58*, 6283–6292.
 [41] S. Daum, V. F. Chekhun, I. N. Todor, N. Y. Lukianova, Y. V. Shvets, L. Sellner, K. Putzker, J. Lewis, T. Zenz, I. A. M. de Graaf, et al., *J. Med. Chem.* **2015**, *58*, 2015–2024.
 [42] R. Rajaratnam, E. K. Martin, M. Dörr, K. Harms, A. Casini, E. Meggers, *Inorg. Chem.* **2015**, *54*, 8111–8120.
 [43] A. Schmidt, V. Molano, M. Hollering, A. Pöthig, A. Casini, F. E. Kühn, *Chem. Weinh. Bergstr. Ger.* **2016**, *22*, 2253–2256.
 [44] M. V. Baker, P. J. Barnard, S. K. Brayshaw, J. L. Hickey, B. W. Skelton, A. H. White, *Dalton Trans.* **2005**, 37–43.
 [45] M. V. Baker, P. J. Barnard, S. J. Berners-Price, S. K. Brayshaw, J. L. Hickey, B. W. Skelton, A. H. White, *J. Organomet. Chem.* **2005**, *690*, 5625–5635.
 [46] L. Gao, D. V. Partyka, J. B. Updegraff, N. Deligonul, T. G. Gray, *Eur. J. Inorg. Chem.* **2009**, *2009*, 2711–2719.
 [47] D. S. Laitar, P. Müller, T. G. Gray, J. P. Sadighi, *Organometallics* **2005**, *24*, 4503–4505.
 [48] C. P. Newman, R. J. Deeth, G. J. Clarkson, J. P. Rourke, *Organometallics* **2007**, *26*, 6225–6233.
 [49] T. Shibata, S. Ito, M. Doe, R. Tanaka, H. Hashimoto, I. Kinoshita, S. Yano, T. Nishioka, *Dalton Trans.* **2011**, *40*, 6778–6784.
 [50] J. C. Bernhammer, H. V. Huynh, *Organometallics* **2014**, *33*, 1266–1275.
 [51] K.-H. Yu, C.-C. Wang, I.-H. Chang, Y.-H. Liu, Y. Wang, C. J. Elsevier, S.-T. Liu, J.-T. Chen, *Chem. – Asian J.* **2014**, *9*, 3498–3510.
 [52] T. Rehm, M. Rothmund, J. K. Muenzner, A. Noor, R. Kempe, R. Schobert, *Dalton Trans.* **2016**, *45*, 15390–15398.
 [53] S. Ahrens, E. Herdtweck, S. Goutal, T. Strassner, *Eur. J. Inorg. Chem.* **2006**, *2006*, 1268–1274.
 [54] D. B. Zamble, T. Jacks, S. J. Lippard, *Proc. Natl. Acad. Sci.* **1998**, *95*, 6163–6168.
 [55] A. di Pietro, R. Koster, W. Boersma-van Eck, W. A. Dam, N. H. Mulder, J. A. Gietema, E. G. E. de Vries, S. de Jong, *Cell Cycle* **2012**, *11*, 4552–4562.
 [56] M. D. Hall, M. Okabe, D.-W. Shen, X.-J. Liang, M. M. Gottesman, *Annu. Rev. Pharmacol. Toxicol.* **2008**, *48*, 495–535.
 [57] A. E. M. Vickers, K. Rose, R. Fisher, M. Saulnier, P. Sahota, P. Bentley, *Toxicol. Pathol.* **2004**, *32*, 577–590.

1
2
3
4

Entry for the Table of Contents



5
6
7
8
9

A novel series of gold(I) N-heterocyclic carbene (NHC) have been prepared and characterized. The antiproliferative activities of the compounds were tested in four human cancer cell lines and their toxicity in healthy tissue was determined using precision cut kidney slices (PCKS) showing interesting activities in both *in vitro* and *ex vivo* models.