Conference paper

Hanna K. Maliszewska, Mahmoud A. S. Abdelhamid, María J. Marín, Zoë A. E. Waller and María Paz Muñoz*

Anticancer activity and DNA interaction of bis(pyridyl)allene-derived metal complexes

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Abstract: The constant need for novel drugs has prompted the scientific community to explore alternative structures to natural products and small and medium size organic compounds used in classic medicinal and pharmaceutical chemistry. Since the discovery of cisplatin, organometallic compounds have revealed great potential as metallodrugs and their development has exponentially grown in recent years. In this manuscript, we describe our efforts towards the synthesis of new metallodrugs by reaction of bis(pyridyl)allenes and metal complexes. Two classes of compounds are presented: one in which the allene structure is intact and the metal (Pd(II), Pt(IV) or Au(III)) coordinates to the pyridine-nitrogens; and another, in which one of the pyridines cyclises into a gold-activated allene to form β-N-stabilised gold carbenes. Both classes of compounds are active catalysts in important organic reactions, and are also promising antimicrobial, antifungal and anticancer agents. In this work, we describe the promising anticancer activity, against breast cancer cells, of the gold carbene complexes, and preliminary studies of their interaction with DNA, including non-canonical DNA structures. Our results have revealed an unusual selective stabilisation of hTeloC i-motif by one of the Au(III) carbene complexes, that opens up exciting opportunities for further development of novel DNA-binding metallodrugs.

Keywords: anticancer; DNA interaction; gold carbenes; ICPOC-25; metallodrugs.

Introduction

The medicinal properties of metal-based compounds have been explored for centuries [1]. The origins of the use of transition metal complexes in modern medicine is usually associated with the discovery of the anticancer activity of cisplatin, which was approved for clinical use in 1978 [2]. Since then, medicinal chemistry of small, inorganic transition metal complexes has developed into one of the dynamic and important areas of drug development research with more and more metal-based drugs entering pre-clinical and clinical trials [3–13]. Yet, compared to purely organic substances, the number of examples of metal-based drugs under development or available on the market is still lacking.

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***Corresponding author: María Paz Muñoz**, School of Chemistry, University of East Anglia, Earlham Road, Norwich NR4 7TJ, UK; and Chemistry Department, Lancaster University, Bailrigg, Lancaster LA1 4YB, UK, E-mail: m.munoz-herranz@lancaster.ac.uk. https://orcid.org/0000-0001-9037-349X

Hanna K. Maliszewska and María J. Marín, School of Chemistry, University of East Anglia, Earlham Road, Norwich NR4 7TJ, UK. https://orcid.org/0000-0002-5452-5457 (H.K. Maliszewska). https://orcid.org/0000-0001-8021-5498 (M.J. Marín)

Mahmoud A. S. Abdelhamid, School of Pharmacy, University of East Anglia, Earlham Road, Norwich NR4 7TJ, UK; and Department of Chemistry, University of Sheffield, Sheffield S3 7HF, UK

Zoë A. E. Waller, School of Pharmacy, University of East Anglia, Earlham Road, Norwich NR4 7TJ, UK; and UCL School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK. https://orcid.org/0000-0001-8538-0484

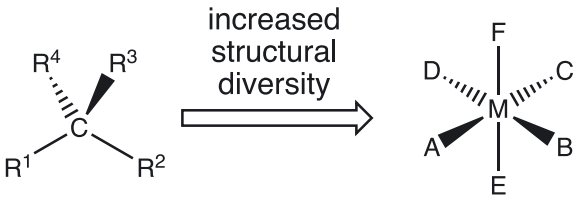


Fig. 1: Stereochemical diversity of metal coordination compounds.

However, the use of organometallic and coordination metal complexes as drug candidates has some key advantages over classic organic compounds. For example, the use of metal complexes allows an easy access to structures with varied geometries, extensive stereochemistry and three-dimensional shapes going beyond tetravalent tetrahedral carbon (Fig. 1) [14].

The wide structural diversity can be achieved by ligand exchange reactions (varying coordination number) or redox reactions at the metal centre (change in oxidation states). Additionally, coordination to the metal can beneficially modify the pharmacological properties of existing organic drugs. Other unique properties of metal-containing species, such as Lewis acidity, radioactivity, pharmacokinetics or magnetic properties are also available for fine-tuning in the rational drug design process.

Currently, the majority of transition metals [5–7, 15–19] and almost all classic ligand classes (*e.g.* metallocenes, arene-, carbonyl-, carbene-, pincer-, polynuclear- or macrocycles) [20–22] have already been investigated in some aspects of bioinorganic chemistry. Although most metal-based compounds are evaluated for their anticancer activity [5, 20, 23], many examples also show promising broader antimicrobial cytotoxicity [24]. Recently, it has been shown that metal-containing compounds outperform strictly organic molecules in terms of antibacterial and antifungal activity [25, 26].

The research into medicinal properties of Pt- and Au-based compounds is particularly extensive and well documented [27]. Pt(II) chemotherapeutics are one of the most widely employed metallodrugs and are commonly used in the treatment of various types of cancer, such as testicular, ovarian, bladder, cervical or small lung cancer [2, 28]. The research of Pt(II) drugs began with the serendipitous discovery of the antiproliferative properties of cisplatin by Rosenberg [29]. Cisplatin (**a**, Fig. 2), and second- and third-generation carboplatin (**b**, Fig. 2) and oxaliplatin (**c**, Fig. 2) are the most effective Pt anticancer therapeutics currently available on the market [30]. These complexes feature a d^8 square planar Pt centre.

DNA is the major molecular target for cisplatin [30–32]; however, quantification studies have shown that only 5–10% of its intracellular concentration can be found in the DNA fraction [33, 34]. The remaining cisplatin is believed to bind less specifically to other targets such as thiolated peptides, replication enzymes or RNA. This low level of selectivity results in serious side effects associated with Pt(II) chemotherapeutics. The other serious issue of the treatment is progressive resistance to the Pt(II) drugs. Some identified resistance mechanisms involve reduced transport of the drugs across cell membranes or enzymatic repair of platinated lesions on DNA, for example by base excision mechanism [35]. These concerns motivated renewed search for more active and safer metallodrugs over the last few decades [36].

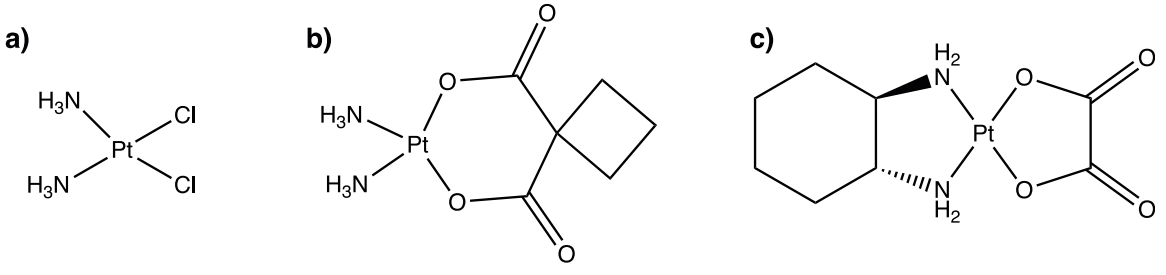


Fig. 2: Marketed Pt chemotherapeutics: (a) cisplatin; (b) carboplatin; (c) oxaliplatin.

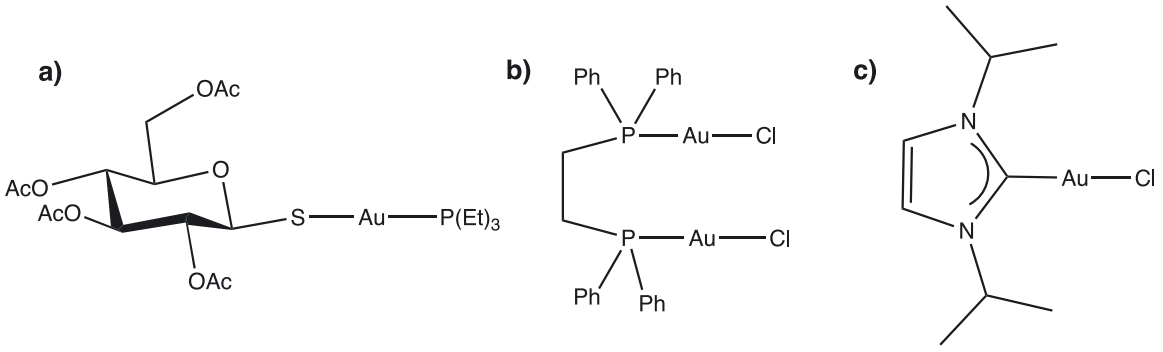


Fig. 3: Examples of Au(I) complexes with biological activity.

The use of Au-based drugs, or chrysotherapy, in modern medicine began with the observation that the Au(I) complex $K[Au(CN)_2]$ had a bacteriostatic effect towards the bacteria that cause tuberculosis [37]. Later, Au-based medicines were proven to be effective in the symptomatic treatment of rheumatoid arthritis [38]. Early use of Au(I) thiolate complexes led to the development of the highly successful, orally administered drug auranofin for this disease (**a**, Fig. 3) [1, 39].

Au(I) metallodrugs are often considered as pro-drugs, due to facile ligand substitution reactions on the metal centre. For example, "soft" Au centres have a strong binding affinity for sulphur-based ligands, and in vivo exchange reactions with thiol groups are thought to be important for the activity of Au drugs [1, 39, 40]. The anticancer activity of Au(I) complexes, including auranofin, has been demonstrated [40, 41]. Auranofin was believed to thwart DNA replication in cancer cells in a similar mechanism of action to Pt chemotherapeutics, although it was found to be inactive in the solid tumour models. Auranofin is an example of a classic two-coordinate, linear Au(I) complex with stabilising tetraacetylthioglucose and phosphine ligands. Other examples of related thiolated Au(I) complexes have been tested for antitumour activity, and tertiary phosphine complexes (**b**, Fig. 3) have shown promising toxicity towards several types of cancer [42, 43]. These complexes were believed to act by inhibition of mitochondrial function rather than DNA binding due to a weak affinity of Au(I) to coordinate O- and N-containing ligands, such as DNA nucleobases. Other Au(I) compounds with promising anticancer activity include N-heterocyclic (NHC)- or alkynyl-complexes (**c**, Fig. 3) [21, 44–48].

Historically, Au(III) complexes have been regarded as too reactive and unstable for medicinal applications. However, the fact that d^8 Au(III) is isoelectronic to Pt(II) [49] and also forms square planar complexes prompted the investigation into the anticancer properties of Au(III) compounds [41, 50–52]. Typically, Au(III) compounds have strong oxidant character and are readily reduced to Au(I) or even Au(0). The use of ligands that influence the reduction potential of the metal centre and the hydrolysis rates contribute to substantial stabilisation of Au(III) complexes in aqueous physiological conditions. Although there is some evidence that Au(III) complexes can interact with DNA, usually it is a weak interaction. It has been proposed that proteins, such as thiol-enzymes, might be major biomolecular targets for Au(III) metallodrugs [53–55], offering interesting alternatives for

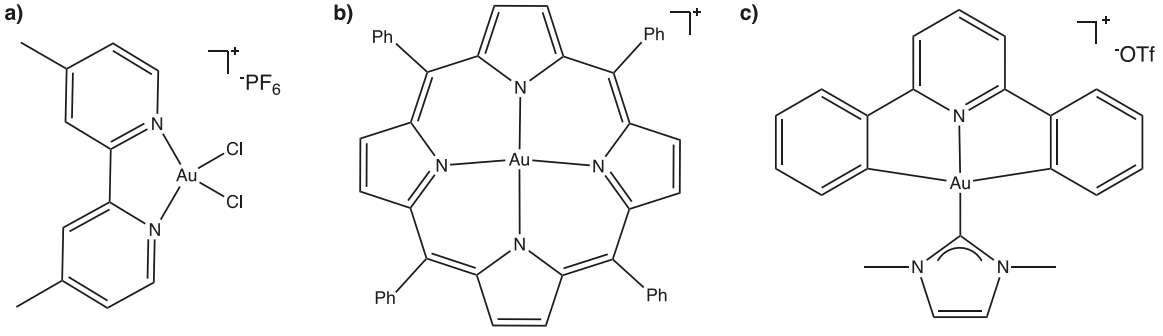


Fig. 4: Structures of Au(III) complexes investigated for anticancer applications.

treatment of cisplatin-resistant tumours. A common trend in the preparation of stable Au(III) drug candidates is the use of multidentate ligands, including the well performing bipyridines (**a**, Fig. 4), porphyrins (**b**, Fig. 4) and pincer ligands (**c**, Fig. 4) [56–63].

Existing limitations on the treatments involving use of metal-based drugs are still significant and are fuelling rapid developments in the area. The major drawbacks include severe side effects and acquired drug resistance. The control of the selectivity towards disease-related targets is especially challenging in the case of DNA-targeting drugs. DNA is ubiquitous and present in every cell in the organism rendering it a difficult target. However, its uncontrolled replication in the cancer process is the reason why more and more DNA-binding molecules are designed and synthesised. The unrealised medicinal potential and documented clinical success of some metal complexes merits further attention to metallodrugs in the context of modern drug development.

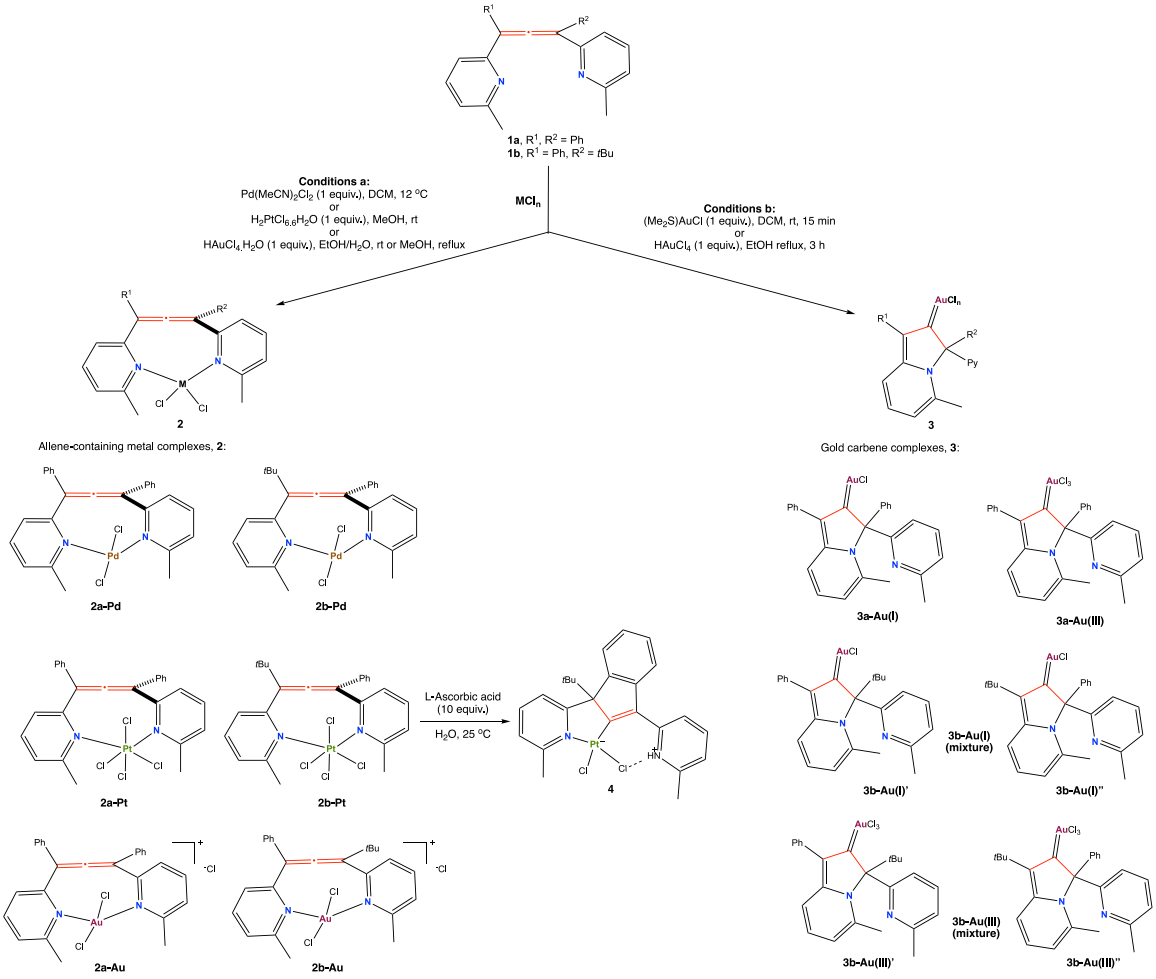
Here we present a summary of our recent attempts to develop new metallodrugs using allenes as novel scaffolds for ligand design, and their use in catalysis and as anticancer, antimicrobial and antifungal agents. We also include unreported promising results on the anticancer activity against breast cancer cells of several β-N-stabilised Au(I) and (III) carbenes recently developed in our group. DNA binding studies have revealed a selective stabilising interaction of one of the Au(III) carbene complexes with a non-canonical i-motif DNA structure. This type of DNA structure has been recently associated with biological functions [64], and stabilisation of i-motifs has been shown to affect telomere function [65]. However, a stabilising interaction of organometallic molecules with i-motif is unusual, making the examples reported here a unique opportunity for further design of DNA-binding metallodrugs.

Results and discussion

From being regarded as a chemical curiosity, the allene functional moiety has now emerged as a very useful synthetic tool of modern organic and organometallic chemistry [66]. The allene functional group is known to act as a ligand in transition metal complexes through its double bonds [67–70], but when decorated with two donor units on each of the terminal substituents, it becomes a new attractive ligand scaffold with increased coordination sites [71–73]. In our quest for novel ligand architectures with 3D arrangements that can be used in catalysis and in new metallodrug design, we have recently reported two classes of allene-derived metal complexes made by interaction of bis(pyridyl)allenes with different transition metals. Depending on the nature of the metal used and the reaction conditions (see Scheme 1 and original references [74, 75] for full details), the ligands retained their cumulene system in the new organometallic compounds (**2-Pd(II)**, **2-Pt(IV)**, and **2-Au(III)**, conditions **a**, Scheme 1) [74]. While in the presence of others, cyclisation from nucleophilic attack of one of the pyridines into the metal-activated allene afforded carbene-type complexes (**3-Au(I)** and **3-Au(III)**, conditions **b**, Scheme 1) [75, 76]. We also isolated vinyl-Pt(II)metallacycle **4**, from the attack of one of the phenyl ring substituents into the Pt-activated allene, when reacting complex **2-Pt(IV)** with L-ascorbic acid in an attempt to obtain the Pt(II) analogue by chemical reduction of the Pt(IV) complex (Scheme 1). Both allene-containing (**2**) and carbene type (**3**) complexes are characterised by non-flat, 3D geometry and feature N-heterocyclic groups, which is usually very important for the success of drug-like small molecules [77, 78]. The observed robustness of the new complexes, that could be attributed to the stabilising chelate effect or heteroatom conjugation, was an incentive to test them as catalysts and also as potential metallodrugs in three settings: as antimicrobial agents (in collaboration with the Community for Open Antimicrobial Drug Discovery – CO-ADD), as anticancer drugs, and for their specific interaction with different structures of DNA (double helical, i-motif and G-quadruplex).

All the compounds showed catalytic activities in benchmark reactions for each metal (*e.g.* Suzuki-Miyaura cross-coupling for Pd, nucleophilic addition to allenes for Au and Pt, and cycloisomerisation of enynes for Au and Pt), and the results have been published elsewhere [74, 75].

We have also reported the antimicrobial activity of all the compounds elsewhere [26]. As a summary, the free ligands (**1a** and **1b**), as well as the corresponding Pt(IV)complexes (**2-Pt**), did not show any antimicrobial activity. Both Pd(II) complexes **2-Pd**, the two Au(III) analogues **2-Au**, the vinyl-Pt(II) metallacycle **4**, and the carbenes **3-Au(I)** and **3-Au(III)**, showed moderate to good activity against MRSA (methicillin-resistant



Scheme 1: Summary of allene-derived metal complexes evaluated in this work [74, 75].

staphylococcus aureus) and the two fungal (*Candida* and *Cryptococcus*) strains tested, but all showed some level of cytotoxicity in haemolysis assays. The **3a-Au** complexes also showed moderate activity against tested Gram-negative strains (*Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii,* and *Pseudomona aeruginosa*). The best results were obtained with the Au(I) complexes made from the non-symmetrical allene: both isomers of **3b-Au(I)** were active and low-toxicity candidates, and the Au(III) analogues, **3b-Au(III)**, showed good levels of activity across the antifungal panel and no cytotoxicity or haemolysis up to 200 μM, resulting in a therapeutic index of 33,333 (virtually identical MIC – minimum inhibitory concentration – values were obtained with several samples containing different ratios of non-separable but well-characterised isomers (**3b-Au(III)′** and **3b-Au(III)″**), indicating that both isomers are responsible for the observed activity).

As well as the antimicrobial and antifungal activity, we tested all the complexes as novel anticancer agents and in their interaction with DNA, including non-canonical DNA form sequences that are widespread throughout the human genome and can be found in promoter regions of their genes (for example the gene encoding death associated protein, DAP) [79] and in the telomeres [80]. In particular, the telomeric region of DNA and associated proteins have been linked to important functions such as DNA replication and protection against DNA damage [81], and therefore the link between dysfunctional telomeric DNA and disease, *e.g.* cancer, is of much interest [82].

In this manuscript, we report unprecedented studies on the anticancer activity of the Au(I) and Au(IIII) carbenes (**3**) and Pt(II)-vinyl complex (**4**) and their interaction with DNA. Although the anticancer activity of the

allene-containing derivatives (**2**) has been reported elsewhere [74], we include here key results obtained with these complexes for comparison with the carbene analogues.

Anticancer activity

We investigated the in vitro anticancer activity of the new library of compounds against the human breast adenocarcinoma cell line MDA-MB-231, a triple-negative breast cancer cell line difficult to treat with current therapies and causing poorer outcomes for patients in comparison with other subtypes [83].

The initial experiments were carried out with a concentration range of 0.19–100 μM over an incubation period of 24 h, using the CellTiter-Blue® (CTB) fluorescent cell viability assay (see ESI, Figs. S1–S7) [84]. Although not all compounds gave a clear trend of diminished cell survival with the increased drug concentration (*e.g.* ligands **1** and **2-Pt** allene complexes did not display the characteristic sigmoidal shape of the dose response plots in the concentrations tested, see Figs. S6 and S7 in ESI, and reference [74]), we could extract the IC_{50} values in most cases [85], summarised in Table 1 (see ESI for full details). The most active compounds from the allene-type group were the symmetric Pd(II) and Au(III) compounds **2a-Pd** and **2a-Au** (entries 1 and 5, Table 1) [74]. The non-symmetric congeners (entries 2 and 6, Table 1) were substantially less active and both Pt(IV) complexes gave inconclusive results (entries 3, and 4, Table 1. We observed some precipitation of the complexes in the plates after incubation) [74]. Similarly, in the carbene-type class, the complexes derived from the symmetric bis(phenyl) allene ligand **1a** were more active than the *t*-butyl analogues (entry 7 vs. 8 and 9 vs. 10, Table 1). The free allene ligands did not show any antiproliferative properties (entries 12 and 13, Table 1), also in line with the lack of activity as antimicrobial agents [26].

We compared and validated the results obtained with the four bis(phenyl) compounds with IC_{50} values below 20 μM (**2a-Pd**, **2a-Au**, **3a-Au(I)** and **3a-Au(III)**, highlighted in Table 1), using the well established MTT proliferation assay that is most commonly used due to its low cost and high reproducibility in measuring cytotoxicity [86]. This assay measures absorbance instead of fluorescence, which allowed more accurate measurements, as some of the compounds displayed slight fluorescence, causing interference. The use of transparent plates in this assay also allowed to visually monitor the experiments to identify any precipitation of the complexes from the cell media that could lead to inaccurate results. The experiments were repeated at 24 h incubation times in the wider concentration range (see Fig. 5 for Au carbenes **3a**, and ref [74] for complexes **2a**). We obtained good viability vs. concentration responses for all four compounds with reduced experimental errors when compared to the CTB assay experiments (ESI, Fig. S8 for Au carbenes **3a**, and ref [74] for complexes **2a**).

Table 1: IC_{50} values for MDA-MB-231 cell line (24 h incubation) of allene-derived metal complexes **2**, **3** and **4**, and bis(pyridyl)allenes **1**, using CellTiter-Blue® assay.

	Entry	Compound	IC_{50} [μM]
Allene-type [74]	1	**2a-Pd**	0.3
	2	**2b-Pd**	>100
	3	**2a-Pt**	N/A[a]
	4	**2b-Pt**	>100
	5	**2a-Au**	5.9
	6	**2b-Au**	66.1
Carbene-type	7	**3a-Au(I)**	7.0
	8	**3b-Au(I)**[b]	23.6
	9	**3a-Au(III)**	10.2
	10	**3b-Au(III)**[c]	>100
	11	**4**	40.5
Ligands	12	**1a**	>100
	13	**1b**	>100

[a]Lack of clear viability profile. [b]Single isomer – **3b-Au(I)″**. [c]1:0.5, **3b-Au(III)′**:**3b-Au(III)″**.

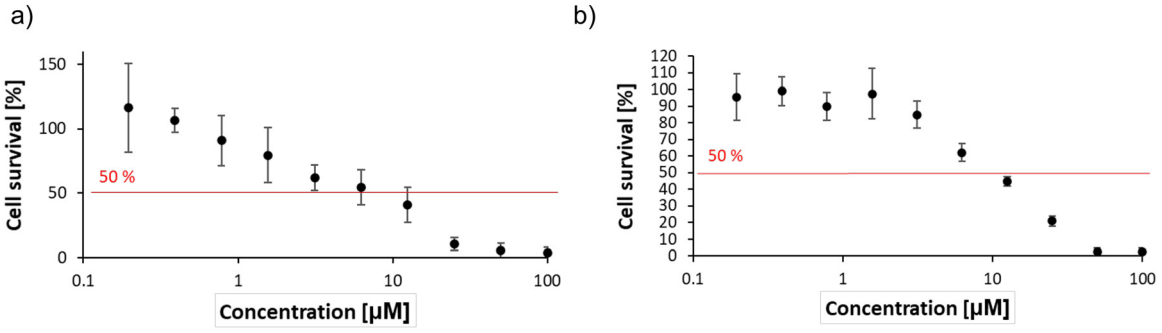


Fig. 5: MTT viability assay of MDA-MB-231 cells treated with: (a) **3a-Au(I)** and (b) **3a-Au(III)**, after 24 h of incubation.

Table 2: IC_{50} values for MDA-MB-231 cell line (24 h incubation) of selected compounds, using the MTT assays (± standard deviation).

Entry	Compound	IC_{50} [μM]
1	**2a-Pd**	3.3 ± 1.2
2	**2a-Au**	2.5 ± 1.6
3	**3a-Au(I)**	1.8 ± 0.4
4	**3a-Au(III)**	14.7 ± 2.8

The MTT assay was then carried out with these selected complexes in restricted concentration ranges in experimental and technical triplicates. The IC_{50} values were calculated as the mean of the three separate experimental runs (ESI, Figs. S9 and S10) and are presented in Table 2. In general, IC_{50} values obtained with both assays were found to be in good agreement in a similar low micromolar range.

These preliminary results confirm significant toxicity of the tested complexes against the human breast cancer cell line. Particularly, the two allene-containing compounds **2a-Pd** and **2a-Au** and the Au(I) carbene **3a-Au(I)** showed activity in the low micromolar range, which is within the range obtained for reported Pd(II) (IC_{50} *ca.* 0.5–22.9 μM) [87–91], Au(III) (IC_{50} *ca.* 0.3–11.2 μM) [92, 93], and Au(I) (IC_{50} *ca.* 2.5–>20 μM) complexes [94, 95], and are lower than reported values for cisplatin (IC_{50} *ca.* 25–50 μM depending on the study) against the MDA-MB-231 cell line [96]. In particular, results for complexes **2a-Pd** and **3a-Au(I)**, which exemplify the two classes of allene-derived complexes, are very promising and highlight the potential of 3D metal complexes based on Pd(II)- and Au(I)-centres as new scaffolds, not extensively explored to date in the context of cancer treatment.

Interaction with DNA

The mechanism of action of many metal-based drugs is often, at least partially, ascribed to their interactions with DNA as an intracellular target [15, 30–32, 97–99]. From our previous studies with the allene-derived complexes [74], we observed that although **2-Au** complexes did not interact much with any DNA type tested, in accordance with the mechanism of action of other Au(III) complexes (**2-Pt** were inactive to the level of the free ligands **1**), the **2-Pd** complexes showed a very interesting specific destabilising interaction with the hTeloG G-quadruplex.

In this work, we wanted to probe the possible source of the biological activity of the carbene type complexes by studying their interactions with different DNA types, not only with the standard double stranded helical structure, but also with non-canonical DNA secondary structures – such as G-quadruplexes and i-motifs [100]. To assess carbene complex-DNA interactions, we utilised FRET (fluorescence resonance energy transfer)-based DNA melting experiments [101] using different i-motif forming sequences from the human genome (hif-1-α [79, 102],

DAP [64, 79], and the C-rich sequence from the human telomere [101], hTeloC) as well as G-quadruplex from the human telomere and double helical DNA.

The carbene-type allene-derived metal complexes **3a-Au(I)**, **3b-Au(I)**, **3a-Au(III)**, **3b-Au(III)**, and complex **4**, were tested at a range of concentrations up to 1.0 μM (5.0 equivalents excess in respect to the DNA used at 200 nM). Higher concentrations of the complexes were not used in order to avoid non-specific interactions resulting from the sheer excess in respect to the DNA. The compounds were measured once after the pipetting of the set was completed and they were not incubated for different amounts of time. Fig. 6 (left) shows the FRET-melting curves of the fluorophore-tagged hTeloC sequence in the presence of the complexes **3a-Au(I)** and **3a-Au(III)**, as well as a control curve (marked with black squares). The graphs on the right (Fig. 6) show the first derivative of the fluorescence intensity in respect to temperature, from which the melting temperatures (T_m) can be obtained. The plots corresponding to the remaining oligonucleotide sequences and complexes can be found in the ESI (Figs. S11–S35).

Although there was no significant change in melting temperature with addition of the complex in the tested concentration range for **3a-Au(I)** ($\Delta T_m \approx 0$ °C, Fig. 6a and S11 in ESI) or compounds **3b-Au** and **4** (see ESI, Figs. S12, S15 and S13), the Au(III)-complex **3a-Au(III)** induced stabilising effect measured as a positive change in melting temperature of $\Delta T_m \approx +2$ °C at 1.0 μM concentration only for hTeloC (Fig. 6b and S14 in ESI).

Interestingly, all the compounds, including **3a-Au(III)**, showed no significant interaction with the remaining DNA sequences (ESI, Figs. S16–S35). The lack of evidence for the binding to any of the tested oligonucleotide sequences with other carbene-type complexes might suggest that any bioactivity observed in other assays for these compounds might not arise from a direct action of the metal complex and the DNA in the cells. This is in

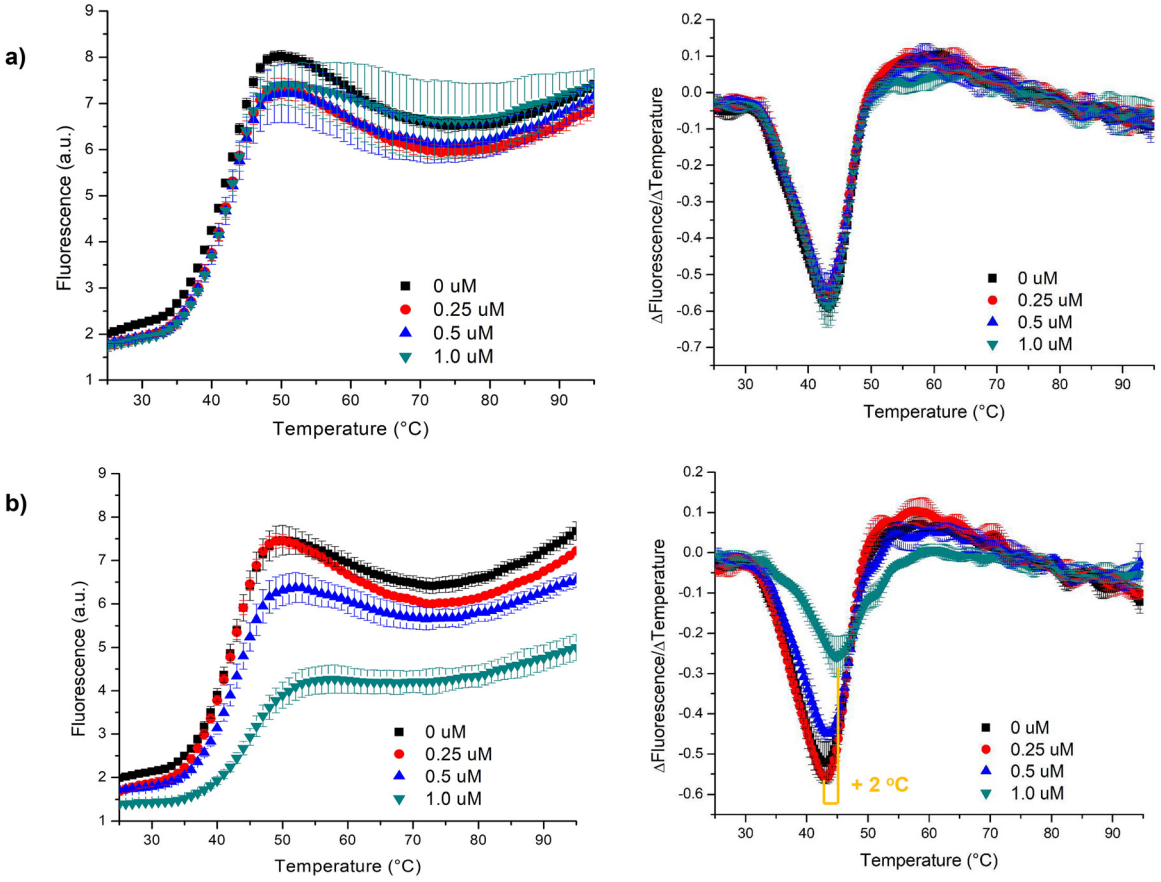


Fig. 6: FRET-melting experiments for hTeloC and complexes: (a) **3a-Au(I)**; (b) **3a-Au(III)**; FRET melting curves (left), first derivatives of fluorescence intensity in respect to temperature (right).

agreement with the mode of action of other Au complexes, that are reported to be more prone to interact with protein targets [53, 103–107].

However, the specific interactions of **3a-Au(III)** with the i-motif DNA over the G-quadruplex and double stranded DNAs is quite unusual and opens the possibility of further derivatisation of **3a-Au(III)** towards the rational design of i-motif binders for use a metallodrugs and other applications (*e.g.* catalysis).

Conclusions

We have presented here a summary of our work to date on the use of allenes as novel scaffolds for the synthesis of metallodrugs with potential DNA binding properties, including the unreported anticancer activity of β-N-stabilised-gold carbenes recently developed in our group.

The gold carbene complexes **3b-Au(I)**″ and **3b-Au(III)** derived from the non-symmetric allene **1b** have shown remarkable antimicrobial activity and low toxicity for human tissue with **3b-Au(III)** entering in vivo stage of testing. Complementary results of good anticancer activity of Pd and Au complexes **2a-Pd**, **2a-Au**, **3a-Au(I)**, and **3a-Au(III)** of symmetric ligand **1a** are presented here. These complexes showed antiproliferative activity against a human breast cancer cell line in the low micromolar range. Additionally, the source of activity of the complexes was briefly investigated in DNA binding studies. Some of the compounds exhibiting anticancer activity were among those showing the most significant binding to non-canonical DNA structures, specifically **3a-Au(III)** from the carbene group and **2a-Pd** from the allene group.

In particular, initial FRET experiments showed that **3a-Au(III)** induces a change in melting temperature of +2 °C for the hTeloC i-motif. Complex **3a-Au(III)** was not found to interact with any of the other DNA structures at low concentration, indicating there is some specific binding, likely with the loops within the structure of the i-motif. These results provide promising evidence for the utility of these complexes in specifically targeting i-motif DNA structures with therapeutic applications. Further studies of structure-DNA interaction activity of analogues of **3a-Au(III)**, as well as DNA docking modelling experiments, are currently ongoing in our group.

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