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Expanding the biological utility of bis-NHC gold(I) complexes through post synthetic carbamate conjugation†

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Abstract

We report the synthesis of a novel hydroxyl-functionalised heteroleptic bis-NHC gold(I) complex that permits conjugation to various amines *via* carbamate bond formation. The resulting derivatives were studied *in vitro* using cell proliferation assays and fluorescent microscopic imaging of human cancer cell lines.

Recently, NHC gold(I) complexes have attracted growing interest due to their catalytic,^{1–8} luminescent,^{9–14} and biological properties.^{15–19} While homoleptic bis-NHC gold(I) complexes are common, examples of heteroleptic bis-NHC gold(I) complexes that contain two different NHCs, are rare.^{20–22} Heteroleptic bis-NHC systems, owing to their asymmetric nature, can be easily exploited for incorporating (1) unique imidazolium *N*-substituents and (2) a single discrete site of functionalization. These complementary features facilitate the synthesis of chemical libraries that may, in turn, may permit structural–activity relationships (SARs) to be explored in a biological context. To date, only limited studies of the post-synthetic modification or conjugation chemistry of functionalised bis-NHC gold(I) complexes have been carried out,²³ whereas analogous studies in the context of mono-NHC gold(I) complexes are common.^{24–28} Notably, Engeser *et al.* reported a series of amino-acids and a small peptide linked to homoleptic bis-NHC gold(I) conjugates through ester linkages.²³ Ester linkages present both advantages and disadvantages due to their lability in biological milieus, including blood, serum, or plasma, characterized by high esterase activity.^{29,30} Here, we report the synthesis of heteroleptic bis-NHC gold(I) complex **1** equipped with a hydroxyl group (Scheme 1) and show that it may be used to create bis-NHC gold(I) conjugates containing carbamate linkages. Carbamates represent an easy-to-access functionality that is typically robust under cellular and biological conditions and which, as

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Conflicts of interest

There are no conflicts to declare.

such, is often exploited as a linker in medicinal chemistry.³¹ A further attractive feature of carbamates is that, in many instances, they enhance cell membrane permeability.³¹ Our design consideration was thus that by using a range of N-terminal amines and creating a series of heteroleptic-bis-NHC gold(I) carbamate analogues we would be positioned to study their basic pharmacological properties and SAR features.

The preparation of heteroleptic bis-NHC gold(I) complex **[1][Br]** is shown in Scheme 1b. Its synthesis utilized “golden synthon” methodology originally developed by Nolan *et al.* that involves combining a NHC–Au–OH and an imidazolium bromide in the presence of toluene (see ESI†).^{20,32} The isolation of **[1][Br]** as a bromide salt was facilitated by water washings, a procedure that allowed it to be obtained in an overall yield of 70%. To the best of our knowledge, **[1][Br]** is the first example of a hydroxyl functionalized heteroleptic bis-NHC gold(I) system that is positioned for further post-synthetic modification.

In an effort to improve the yields for the subsequent functionalization of the hydroxyethyl group present in **1**,²³ anion exchange was carried out by using sodium hexafluorophosphate to generate **[1][PF₆]** in near quantitative yield. ESI-negative mass spectrometry provided support for the expected anion exchange. Complex **[1][PF₆]** was then reacted with *p*-nitrophenylchloroformate to give the corresponding carbonate ester intermediate **4**.

Single crystals suitable for X-ray diffraction analysis were grown for both **[1][PF₆]** and **4** via slow diffusion of diethyl ether into a dichloromethane solution of the respective complex. The molecular structures for **[1][PF₆]** and **4** are presented in Fig. 1. Both complexes are characterized by overall linear geometries as reflected in C_{NHC}–Au–C_{NHC} bond angles of 177°. The Au–C_{NHC} bond lengths in **[1][PF₆]** and **4** are in the 2.00 Å range, values that are in accord with those noted in the literature.^{33,34}

Functionalization of complex **4** was then effected by combining it with a series of amine-containing precursors (Scheme 1). A summary of the functionalized bis-NHC gold(I) complexes produced in this way and the conditions used for their preparation is provided in Fig. 2 and Table 1, respectively. All carbamates were characterized using ¹H and ¹³C NMR spectroscopy, as well as high res ESI-MS. In all these Au(I) bis-NHC complexes, the C_{NHC}–Au–C_{NHC} subunits (see Fig. 1 for colour coding) exhibit different chemical shifts in ¹³C NMR, owing to their asymmetric nature. In the presence of 2–3 equivalents of amine, dichloromethane and triethylamine (*i.e.*, protocol 1), complete conversion to the corresponding conjugate was observed by HPLC and LCMS when the amine was (i) activated (**5**), (ii) primary in its basic form (**6**), (iii) primary in the form of the corresponding hydrochloride salt (**7**), (iv) secondary (**8**), and (v) benzylic (**9**, **10**) (Fig. 2). Purification of conjugate **7** was effected by HPLC chromatography, whereas purification of **10** was performed using column chromatography over silica gel using 20 : 1 CH₂Cl₂/methanol (v/v) as the eluent. Such column purifications are rare in the context of Au(I) carbene chemistry and are taken as support for the notion that the species generated from **4** via carbamate

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conjugation are chemically robust. Single crystals of complexes **5**, **8**, **9** and **10** suitable for X-ray diffraction analysis were obtained *via* slow diffusion of diethyl ether into the respective solutions in CH₂Cl₂. Representative structures are shown in Fig. 1 and in the ESI,† pp. 48–51.

Protocol 1 proved ineffective for preparing carbamate conjugates of aromatic amines. Aromatic amines are present in many pharmaceuticals, such as the anti-leprosy drug dapsone and some antimalarials (*e.g.*, primaquine). However, they are relatively weak nucleophiles and often require activation for coupling reactions. Hence, we modified the coupling conditions to include hydroxybenzotriazole (HOBt) as the activating agent and DMF as a solvent (protocol 2). With this new protocol, complexes **11** and **12** were successfully synthesized and fully characterized (*cf.* Fig. 2 and Table 1). Single crystals of **11** suitable for X-ray diffraction analysis were obtained, and the resulting structure is presented in Fig. 1.

To create a set of complexes that might allow SAR insights to be obtained, various amine classes were used to prepare derivatives of **4**. Specific amine motifs were chosen due to their prevalence in therapeutics or because they are recognised as having biological utility. For example, the morpholine motif present in complex **8** was chosen because it is a known pharmacophore that appears in a number of cancer therapeutics.³⁵ Likewise, complex **7** contains doxorubicin, an approved anticancer agent.³⁶

Once in hand, complexes **5**, **7–8**, and **10–11** were screened for antiproliferative and mechanistic activity. Previously, we^{33,34} and others^{17,37,38} have shown that Au(I) complexes, including various Au(I) NHC complexes, inhibit thioredoxin reductase (TrxR), an enzyme that is overexpressed in multiple cancers and thought to be a potential biological target for cancer treatment.^{39,40} Therefore, the A549 human lung cancer cell line, known to overexpress TrxR,⁴¹ was chosen for study. Auranofin was used as a validated positive control in these tests of anticancer activity due to its recognised ability to inhibit TrxR.^{42,43} The aforementioned complexes were screened for their ability to inhibit cancer cell growth using an MTT assay. It was found that all of conjugates produced in the context of the present study displayed high anticancer potency in the A549 cell line, providing inhibition constants (IC₅₀) varying from 0.11 μM (conjugate **8**) to 0.56 μM (conjugate **7**) (see ESI† Table S1).

Considered in concert, the IC₅₀ values deviated little compared to the starting hydroxyethyl complex **1** and other published systems.³⁷ Importantly, no substantial decrease was seen as the result of carbamate functionalization. On the other hand, a 5-fold reduction in the anticancer potency of **7** relative to doxorubicin alone was observed (see ESI,† Fig. S4). Nevertheless, even for this system, the overall IC₅₀ value remains relatively potent 1 μM range (Fig. 3).

To assess whether complexes **5**, **7–8**, and **10–11** could serve as TrxR activity inhibitors, standard tests involving the reduction of the cell-permeable cofactor lipoate to dihydrolipoate were carried out. Treatment of A549 cells (from ATCC) with 1.25 mM of each respective complex for 6 h followed by live cell colorimetric imaging for 3 h revealed

relative TrxR inhibition that correlated well to the IC₅₀ value of each complex (see ESI† Fig. S5). Furthermore, the inhibition of complexes [1][PF₆], **5**, and **11** were found to inhibit TrxR to the same degree as auranofin within error (*p*-value > 0.05). In contrast, **8** was found to inhibit TrxR to a greater extent than auranofin (*p*-value < 0.0001) (see ESI† Table S2). We thus propose that carbamate functionalization represents a viable strategy for functionalization of Au(I) bis-NHC complexes.

The utility of functionalization was illustrated in the case of **7** and **10**. These complexes contain inherently fluorescent doxorubicin and pyrene motifs. Known fluorescent Au(I)-bis-NHCs are relatively rare^{44,45} compared to the corresponding Au(I)-mono-NHC species.^{27,46,47} Systems **7** and **10** represent such fluorescent species. They could be analysed readily using the same excitation and emission maxima as used to monitor the constituent fluorophores (see ESI† Fig. S6–S11). These complexes were thus evaluated for their utility as fluorescent probes *in vitro*. To this end, A549 lung cancer cells were treated with each complex and subsequently subjected to fluorescent microscopic imaging (*cf.* Fig. 4 and Fig. S12–S15, ESI†). Mitochondrial localization of Au-bis-NHCs is well validated,^{38,48} and this proved true for both complexes **7** and **10** as inferred from the fluorescent overlap of the emission signal from each complex and the mitochondrial probe Mitotracker Red (Fig. 4 and Fig. S12, ESI†). In the case of **7**, fluorescent imaging of the mitochondria could be achieved at probe concentrations as low as 500 nM (see Fig. S14, ESI†). However, such low concentrations could not be used effectively in the case of complex **10** since it is not effectively excited at a wavelength (*e.g.*, 405 nm) compatible with our fluorescent microscopic imaging set up (see Fig. S11, ESI†).

In conclusion, we have described the synthesis of a hydroxyl-functionalized heteroleptic Au(I)-bis-NHC and shown that this species (**1**) may be carried on to create carbamate-lined conjugates with different amines. Using this approach, we effected the synthesis and isolation of a number of conjugates, many of which showed good antiproliferative activity in the A549 human lung cancer cell line.

Of particular interest is complex **7** containing the anthracycline doxorubicin. The accepted mechanism of action for doxorubicin is the inhibition of topoisomerase II inside the nucleus.³⁶ This nuclear localization was reconfirmed by us (see ESI† Fig. S15). On the other hand, when incorporated within conjugate **7**, doxorubicin was found to be redirected to the mitochondria (Fig. 4). This redirection might be responsible for the slight reduction of potency relative to doxorubicin alone (see discussion above and ESI† Table S1 and Fig. S4).⁴⁹ On the other hand, it has been proposed that targeting doxorubicin derivatives to the mitochondria can eliminate or reduce the nuclear effects linked with cardiotoxicity.⁵⁰ Thus, in analogy to other reported systems that effect redirection of doxorubicin to the mitochondria, reduced systemic and cardiotoxicity effects might be expected for conjugate **7**. Its biological potential and that of other conjugates produced in the present study are thus the topic of ongoing investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

(i) Toluene, 90 °C, 1 d, then NaPF₆ (6 equiv.), 8 h (ii) 4-nitro-phenylchloroformate (8 equiv.), DCM, TEA (4 equiv.), 2 d (iii) (protocol 1) amines (3 equiv.), DCM, TEA (3 equiv.), 12–36 h. (iv) (protocol 2) HOBt (1.5 equiv.), DMF, aromatic amines (3 equiv.).

Fig. 1. ORTEP representation of **1**, **4**, **10**, and **11** rendered using POV-Ray. Thermal ellipsoids are at the 50% probability level.

Fig. 2.
Au-NHC conjugates synthesized.

Fig. 3. Cell proliferation profiles of A549 lung cancer cells (ATCC) treated with representative conjugates of the present study as judged by MTT assay after 72 h of incubation. IC₅₀ values derived from these studies are given in Table S1 (*cf.* ESI†).

Fig. 4. Confocal microscopy images of A549 human lung cancer cells post-treatment with (i) vehicle only (ii) 5 μ M doxorubicin (iii) 5 μ M Au(i)-NHC-DOX conjugate **7** for 6 h.

Table 1Types of amines attached *via* the present methodology

Type of amine	Example	Protocol	Yield ^a (%)
Activated primary	5	1	79
Normal primary	6	1	53
Primary ammonium salt (an FDA approved drug doxorubicin)	7	1	15
Secondary	8	1	61
Benzyllic	9	1	40
Benzyllic ammonium salt	10	1	40
Aromatic primary	11	2	77
Aromatic secondary	12	2	36

^aYields were calculated based on isolated product.