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NHC*-gold(I) bioconjugated to carbohydrates and peptides as targeted anticancer drugs

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Targeted delivery of potent cytotoxic drugs to cancer cells minimizes systemic toxicity and potentially avoids side effects. NHC*-Au-Cl has already been proven to be a potent anticancer agent based on the stabilising and lipophilic properties of the 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene (NHC*) ligand. One possibility is the chemoselective thiosugar conjugation to NHC*-Au-Cl in order to have active uptake of the resulting NHC*-Au-SR primarily into tumour tissue through the Warburg effect. In addition, a strategy based on chemoselective cysteine conjugation of NHC*-Au-Cl to human serum albumin or Trastuzumab to potentiate drug-ligand ratio, pharmacokinetics, as well as drug efficacy and safety is presented. These strategies are essential steps forward towards the use of gold-based anticancer agents as targeted therapies.

INTRODUCTION

Metal complexes with *N*-heterocyclic carbene (NHC) ligands are used in catalysis [1], as materials [2] and as metal-based drugs [3,4]. NHC ligands are easily chemically modified, like 1,3-dibenzyl-4,5-diphenylimidazol-2-ylidene, in order to serve as a lipophilic part in drug-like molecules. These NHC ligands can act as excellent two electron bond donors, which are stronger σ-donors than phosphine ligands making them ideal ligands to stabilise coinage metal NHC complexes as potential antibiotic or anticancer drug candidates [5-7]. The resulting NHC-M(I) complexes may be an alternative to Auranofin (triethylphosphino gold(I) tetraacetyl β-D-thioglucoside), a drug used in the treatment of rheumatoid arthritis, and now evaluated for its chemotherapeutic potential against microorganisms and as an anti-proliferative drug [8].

RESULTS AND DISCUSSION

The anticancer drug candidate 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene gold(I) chloride (NHC*-AuCl) and its 2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl-1'-thiolate derivative (NHC*-AuSR) [9-11], which is a potential ligand for glucose transporters, were made from the corresponding NHC*-Ag-Br complex transmetallating the ligand onto dimethylsulfido gold monochloride; the reaction is shown in figure 1.

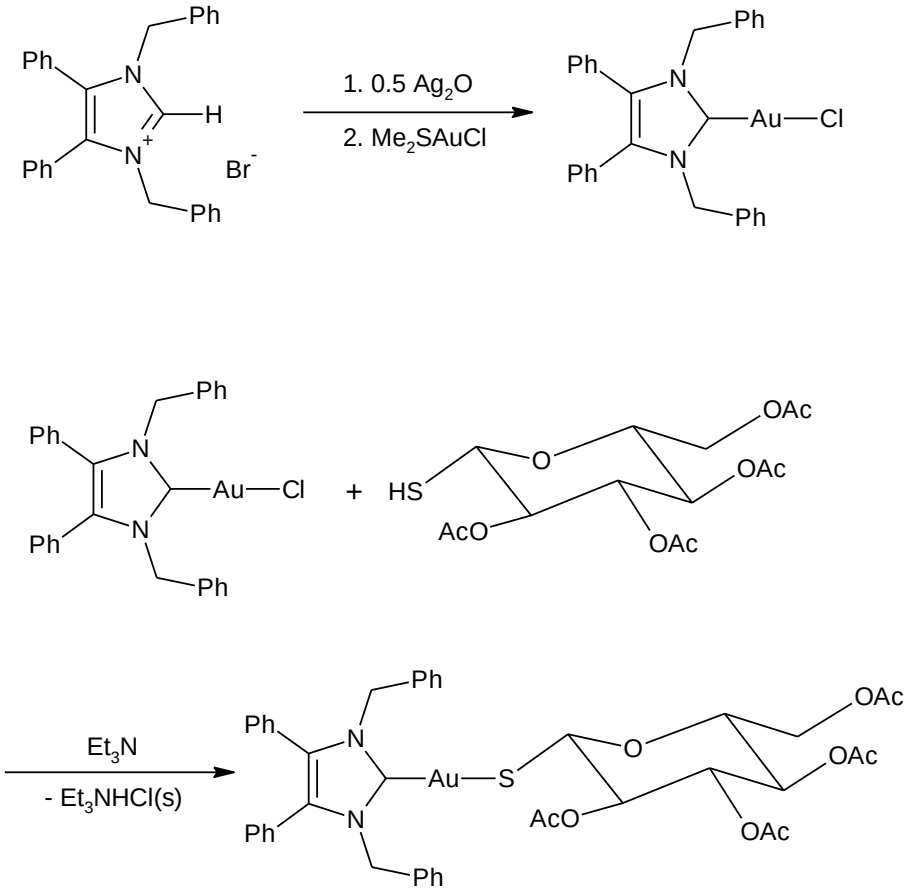

Figure 1: Synthesis of 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene gold(I) chloride (NHC*-Au-Cl) and its 2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl-1'-thiolate derivative (**1**).

NHC*-AuCl and NHC*-AuSR were tested on the NCI 60 cancer cell panel *in vitro* and both compounds showed very good activity against a wide range of human cancer cell lines inclusive renal cell cancer with similar average GI50 values of 1.78 and 1.95 μM, respectively. This encouraged maximum tolerable dose (MTD) experiments in mice, where MTD values of 10 mg/kg for NHC*-AuCl and 7.5 mg/kg for NHC*-AuSR were determined with single injections to groups of 2 mice. In the following tumor xenograft experiment NHC*-AuCl and NHC*-AuSR were given at MTD in 6 injections to two cohorts of 6 CAKI-1 tumor-bearing NMRI:nu/nu mice, while a control cohort of 6 mice was treated with solvent only [12]. NHC*-AuCl at the dose of 10 mg/kg and NHC*-AuSR at the lower dose of 7.5 mg/kg induced both low toxicities in the form of abdominal swelling but no significant body weight loss was seen in both groups. The tumor volume growth reduction was significant and almost identical; optimal T/C values of 0.47 were observed on day 19 for NHC*-AuCl and on day 29 for NHC*-AuSR as shown in figure 2. Immunohistochemistry for the proliferation marker Ki-67 and the angiogenesis marker CD31 did not show significant changes due to NHC*-AuCl or NHC*-AuSR treatment in the animals. However, thioredoxin reductase (TrxR) inhibition with IC50 values of 1.5 μM for NHC*-AuCl and 3.1 μM for NHC*-AuSR seem to indicate that apoptosis induction through elevated oxidative stress is the main mechanism for the two gold compounds.

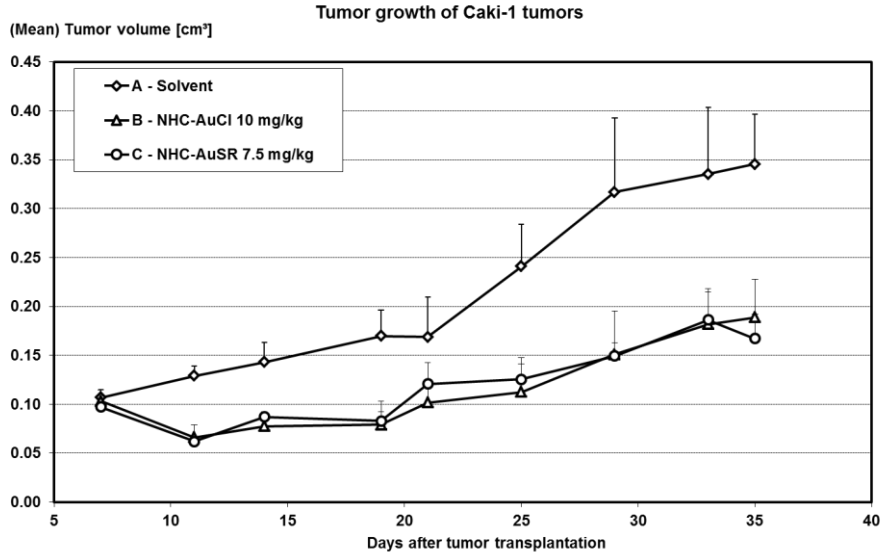

Figure 2: Influence of NHC*-AuCl and NHC*-AuSR (**1**) on growth of CAKI-1 xenotransplant tumors in NMRI nu/nu mice; adapted from [12] with permission.

Additional synthesis involving thioglucose, thiogalactose and thiolactose derivatives allowed access to further NHC*-Au complexes (**2**-**5**) in yields ranging from 79-91%; their structures are shown in figure 3 [13]:

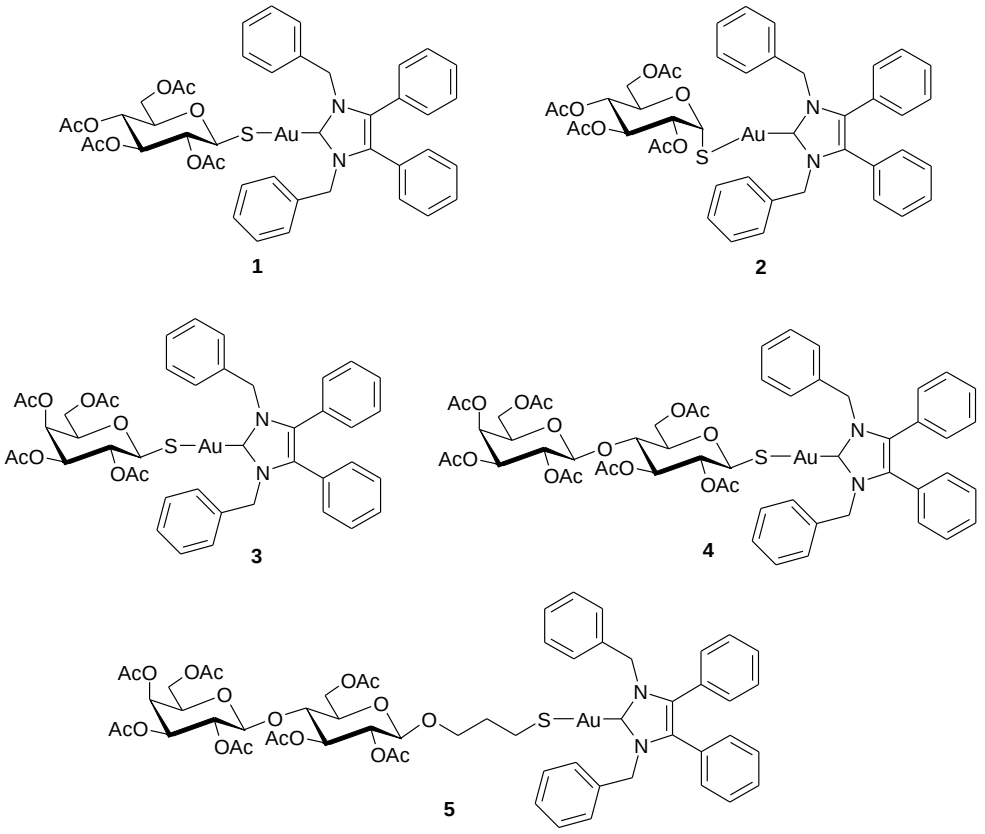

Figure 3: Structures of the NHC*-Au(I)-SR complexes **1**-**5**.

The *in vitro* cytotoxicity of the four synthesized complexes **2-5** were tested against the National Cancer Institute (NCI) 60 cancer cell panel including the Adriamycin-resistant ovarian cancer cell line NCI/ADR-RES. A concentration of 10 μM was used in the initial one dose experiments. All the four compounds showed pronounced activity and reached the full evaluation of the five dose experiments in a second set of experiments. All compounds were tested against 6 breast, 2 prostate, 7 renal, 7 ovarian, 9 melanoma, 6 CNS, 7 colon, 9 NSC lung and 6 leukemia cancer cell lines. The average GI_{50} value of 3.31 μM obtained for **5** was similar to the GI_{50} value of 2.75 μM obtained for **3**. However, an average GI_{50} value of 1.62 μM was obtained for compound **4**, i.e better than both **3** and **5** by a factor of two. An average GI_{50} value of 0.68 μM was obtained for **2** and thus the best in the four synthesized NHC*-Au(I) complexes and better than the previously synthesized β-counterpart **1** which had GI_{50} value of 1.95 μM by a factor of three. Compound **2** showed increased activity than **3** by a factor of four, enhanced activity than **4** by a factor of 2 and better than **5** by a factor of five and on all tested cell lines, **2** showed better activity than its beta counterpart **1**. The obtained GI_{50} values of the six tested CNS and leukemia cell lines for compound **2** were all in the low micromolar region with similar GI_{50} value of 0.29 μM for SF-295 (CNS) and CCRF-CEM (leukemia). GI_{50} values of the tested cell lines of compound **4** were in the low micro molar region with obtained GI_{50} values of 0.40 μM in MCF-7 (breast) cell line and 0.56 μM in K-562 (leukemia) in comparison to that observed for compound **3** with GI_{50} values of 1.65 μM in MCF-7 (breast) cell line and 2.59 μM in K-562 (leukemia) cell line and for compound **5** with GI_{50} values of 2.44 μM in MCF-7 (breast) cell line and 3.27 μM in K-562 (leukemia). Interestingly, complex **4** exhibited activity against these specific cell lines (MCF-7, K-562 and HCT-15) by a factor of four when compared to complex **3** and by a factor of six or seven when compared to complex **5**. Compound **2** exhibited its best activity against prostate cancer cell line PC-3 with GI_{50} value of 215 nM and renal cancer cell line SN 12C with GI_{50} value of 221 nM while **3** showed its best activity against the breast cancer cell line MDA-MB-468 with GI_{50} of 1.63 μM and colon cancer cell line HCC-2998 with GI_{50} of 1.64 μM. Compound **4** showed its best activity against melanoma cancer cell line LOX IMVI with GI_{50} of 236 nM and NSC lung cancer cell line NCI-H522 with GI_{50} of 276 nM while **5** showed its best activity against melanoma cancer line SK-MEL-5 with GI_{50} of 1.97 μM and NSC lung cancer cell line NCI-H522 with GI_{50} of 1.72 μM. Particularly interesting is the fact that the two lactose-containing complexes exhibited their best activity against melanoma and NSC lung cancers. However, for NCI/ADR-RES an Adriamycin-resistant ovarian cancer cell, GI_{50} values of 1.67 μM was obtained for **2**, 6.35 μM for **3**, > 100 μM for **4** and 19.50 μM for **5** with compound **2** demonstrating the best activity against the resistant cell line. This was the only tested cell line where GI_{50} value obtained for **2** was significantly compromised. In all tested cell lines, **2** had better activity in comparison to the other tested compounds and is therefore a potential new anticancer drug candidate awaiting xenograft testing.

In addition, using 1 equivalent of NHC*-Au-Cl, and 1 equivalent of Recombumin® (rHSA, Albumedix Ltd.) full conversion to the corresponding thiol-gold adduct was observed. NHC*-Au–rHSA proved to be stable up to 48 h in human plasma, as observed by LC–MS analysis. This is an important consideration for protein conjugates that are expected to be administrated i.v. and remain stable in circulation. Moreover, analysing surface plasma resonance (SPR) results, it was confirmed that NHC*-Au–rHSA retains its ability to bind to the neonatal FcRn receptor, which is an indication that the biological function of bonded rHSA is kept. And NHC*-Au–rHSA maintains its anticancer activity, when compared to NHC*-Au-Cl as proven on the cancer cell lines CT26 and HUH-7. The synthesis and structure of NHC*-Au-rHSA is shown in figure 4 [14].

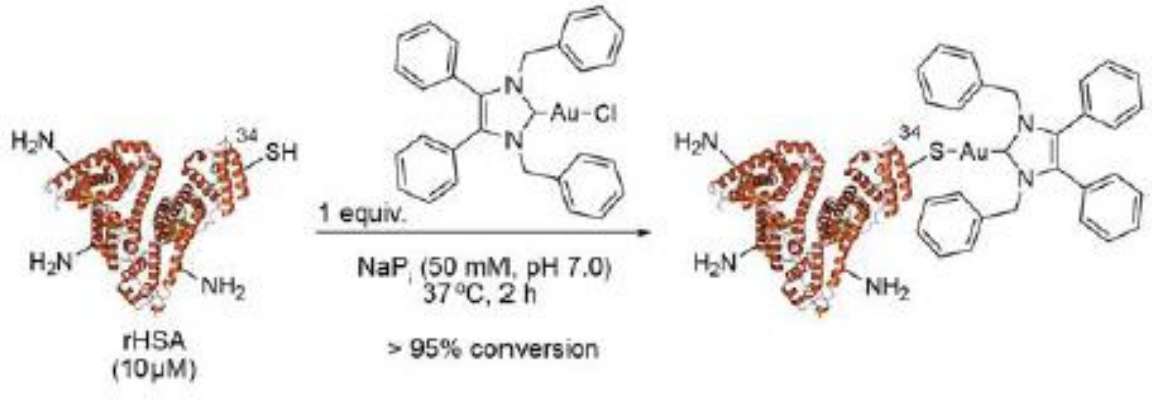

Figure 4: Bioconjugation of rHSA; general reaction of rHSA with the NHC*-Au-Cl.

And a final bioconjugation experiment allowed the cysteine coupling of NHC*-Au-Cl with an engineered monoclonal antibody; using 10 equivalents of NHC*-Au-Cl and 1 equivalent of the Trastuzumab (Herceptin®) derivative Thiomab LC-V205C (Genentech, Inc.) full conversion to the corresponding thiol-gold adduct is observed. The antibody carries selectively two NHC*-Au groups *via* sulfur bonds; synthesis and structure of the antibody-drug conjugate is shown in figure 5 [14].

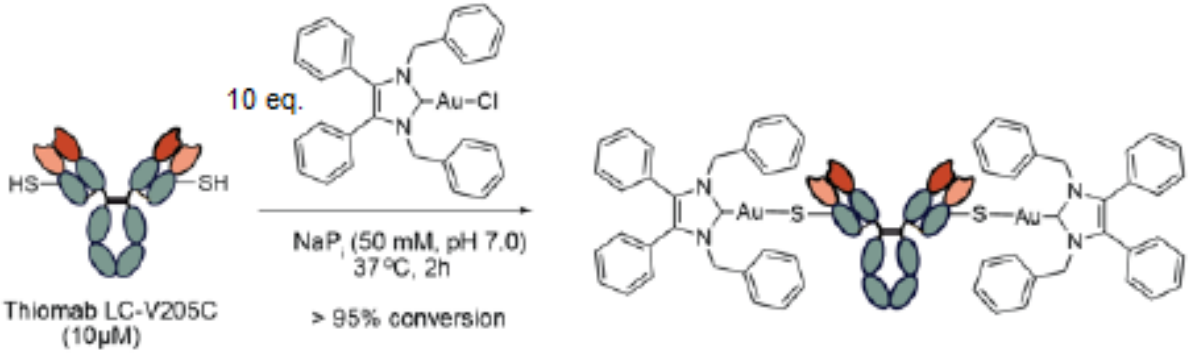

Figure 5: Bioconjugation of Thiomab LC-V205C with two moles of NHC*-Au-Cl.

The antibody-drug conjugate retains the capacity to bind to the Her2 antigen in SKBR3 breast cancer cells that overexpress Her2, as demonstrated by flow cytometry analysis. And the modified antibody exhibited the cytotoxic activity at therapeutic levels (between 20 and 30 mM) when compared to the naked drug, as assessed by CellTiter Blue assay in SKBR3 cells.

CONCLUSIONS

Monovalent benzyl-substituted NHC* gold chloride and thiolates are air- and moisture-stable compounds that can be tuned to have the right lipophilicity and solubility to act as potential anticancer drug candidates.

NHC*-gold(I) bioconjugates with thiosugars are promising starting points; especially alpha and beta tetraacetyl D-thioglucosides show promising activity possibly due to the active uptake into cancer tissue

via the Warburg effect. The beta derivative exhibited already significant anticancer activity *in vivo*, while the alpha derivative is even more active *in vitro* and is awaiting *in vivo* application in the nearby future.

NHC*-gold(I) bioconjugates with cysteine-containing peptides and proteins carry additional interest. Here, reactions with engineered Trastuzumab sees double addition of NHC*-Au substituents, while the biological activity of the monoclonal antibody is retained. Reaction of recombinant human serum albumin leads to NHC*-Au-rHSA *via* a cysteine-link in position 34 of the protein. This NHC*-Au albumin conjugate promises selective uptake into cancer tissue *via* the EPR (enhanced permeability and retention) effect and this bioconjugate is also expecting *in vivo* testing soon.

Summarising, one can say that NHC*-Au-Cl has already the potential to enter clinical trials against selected solid tumors, since the compound combines a novel mechanism of apoptosis (TrxR inhibition) with good antitumoral activity and mild side effects. The aim of further research is to keep these side effects low in combination with enhanced anticancer activity. For this purpose, targeted NHC*-Au-SR derivatives with R being a targeting sugar, peptide or protein are the way to go forward in anticancer research.

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