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Coinage Metal NHC Complexes as Novel Antibiotics and Anticancer Drugs

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The synthesis and biological evaluation against cancer cells and pathogenic bacteria as well as fungi of five coinage metal NHC complexes derived from copper (WBC4), silver (SBC1/SBC3) and gold (NHC-Au-Cl/NHC-Au-SR) is reviewed. The NHC ligand for these compounds is 1,3-dibenzyl-4,5-diphenylimidazol-2-ylidene or derivatives closely related, since this ligand is proven suitable for drug-like molecules. The NHC-silver acetate complex SBC1 failed as an anticancer drug candidate *in vivo*, while its highly related compound SBC3 succeeded *in vivo* as an experimental antibiotic in *Galleria mellonella* larvae showing survival advantage against pathogenic bacteria and fungi. The corresponding gold complexes of NHC-Au-Cl and NHC-Au-SR (R = thioglucoside) as well as the NHC-copper bromide derivative WBC4 exhibited significant growth inhibition, when tested against xenografted human renal-cell cancer Caki-1 in nude mice; WBC4 showed tolerable toxicity in the form of reversible body weight loss, while the two gold compounds did not induce body weight loss in the xenograft mouse model experiment.

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

NHC-Cu(I) based anticancer drugs

The anticancer drug candidate 1,3-di(*p*-methoxybenzyl)-4,5-di(*p*-isopropylphenyl)-imidazol-2-ylidene copper(I) bromide (WBC4) was synthesised from the corresponding imidazolium bromide, silver oxide and dimethylsulfido copper monobromide in 74% yield as shown in figure 1 [9].

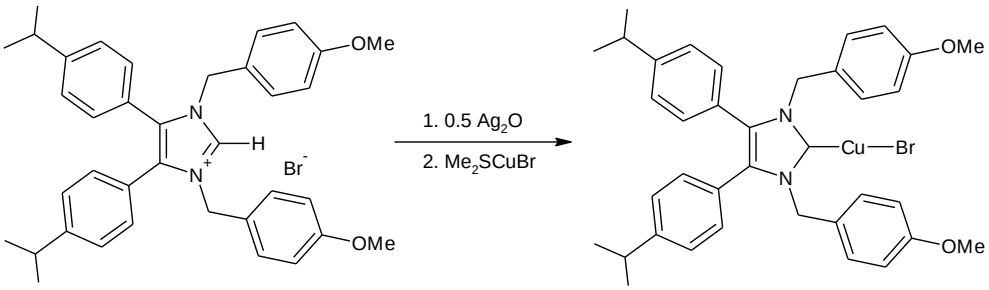

Figure 1: Synthesis of 1,3-di(p-methoxybenzyl)-4,5-di(p-isopropylphenyl)-imidazol-2-ylidene copper(I) bromide (WBC4).

WBC4 is a surprisingly stable Cu(I) complex with respect to water and air, which allowed for its evaluation in biological media. WBC4 was tested on the NCI 60 cancer cell panel *in vitro*; the compound showed very good activity against a wide range of human cancer cell lines inclusive renal cell cancer with an average GI50 value of 288 nM. This encouraged maximum tolerable dose (MTD) experiments in mice, where a MTD value of 10 mg/kg was determined with single injections to groups of 2 mice. In the following tumor xenograft experiment WBC4 was given at 5 and 10 mg/kg in 5 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 [10]. At the higher dose of 10 mg/kg WBC4 showed borderline toxicity leading to 2 mortalities, while a significant T/C value of 0.38 was observed on day 32. At the lower dose of 5 mg/kg WBC4 induced mild and reversible body weight loss with no toxic deaths. At this dose WBC4 showed an identical significant T/C value of 0.38 on day 32, when compared to the other treatment group as shown in figure 2. Immunohistochemistry for the proliferation marker Ki-67 did not show significant changes due to WBC4 treatment in the animals. However, anti-angiogenic effects by WBC4 treatment were observed in CD31 immunohistochemistry. Here, significant reduction in microvessel number, area and ratio was determined in tumors treated with 10 mg/kg of WBC4.

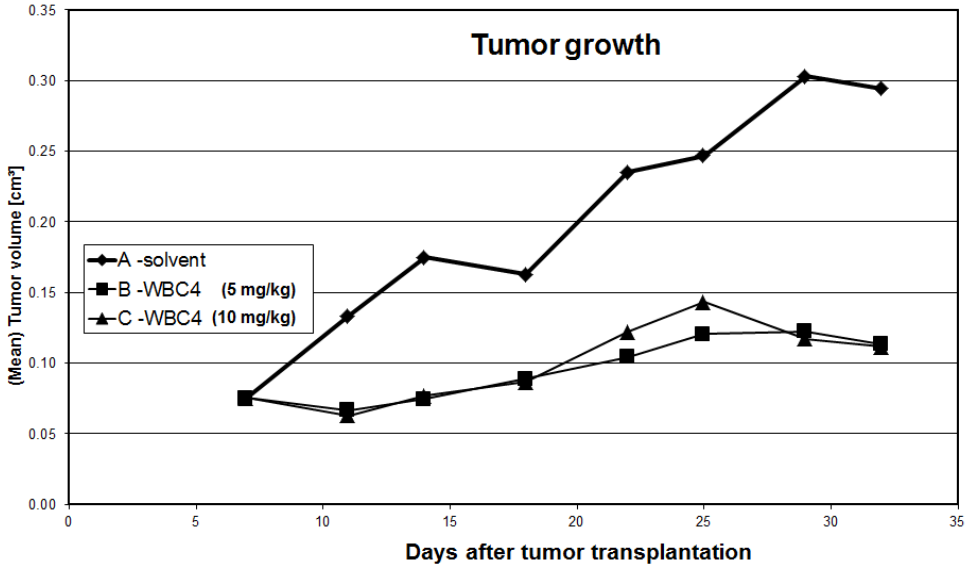

Figure 2: Influence of WBC4 on growth of CAKI-1 xenotransplant tumors in NMRI nu/nu mice; adapted from [10] with permission.

NHC-Ag(I) based anticancer drugs

The anticancer drug candidate 1-methyl-3-(*p*-cyanobenzyl)-benzimidazole-2-ylidene silver(I) acetate (SBC1) was synthesised from the unsymmetrically substituted benzimidazolium bromide and two equivalents of silver acetate in 74% yield as shown in figure 3 [11].

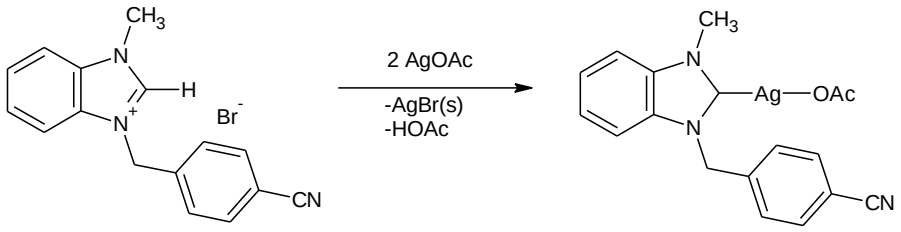

Figure 3: Synthesis of 1-methyl-3-(p-cyanobenzyl)-benzimidazole-2-ylidene silver(I) acetate (SBC1).

SBC1 was tested *in vitro* against human neuroblastoma cells, UKF-NB-3 and UKF-NB-6, delivering IC50 values of 29 +/- 5 and 29 +/- 4 µM, while further testing against cisplatin-,

carboplatin- and oxaliplatin-resistant UKF-NB-3/6 sub-lines showed no cross-resistance with respect to SBC1. A similar trend was found for SBC1 against the human colon carcinoma cell line HCT8 with an IC50 value of 3.1 +/- 0.9 μM; SBC1 was again able to break cisplatin- and carboplatin-resistance in the corresponding sub-lines. SBC1 was also tested against the prostate cancer cell line PC-3 and its paclitaxel-resistant sub-line, which gave IC50 values of 14.1 +/- 0.9 and 14.5 +/- 0.8 μM, which indicated no cross-resistance with paclitaxel. In order to test the possible transport of SBC1 via albumin the binding of SBC1 against this transport protein was measured using a fluorescence titration, which gave a ΔG value of 28 +/- 3 kJ/mol. In circular dichroism and DNA denaturation assays SBC1 proved to be a strongly DNA-binding drug candidate. SBC1 was then given at 25 and 50 mg/kg/d, in four injections to two cohorts of eight CAKI-1 tumor-bearing NMRI:nu/nu mice, while a further cohort was treated with solvent only [12]. At these two dosages SBC1 showed borderline toxicity leading to mortality and body weight loss, while no significant tumor growth reduction or influence on blood parameter with respect to the solvent-treated control group was observed. Further *in vivo* testing against zebrafish larvae revealed significant toxicity of SBC1 at micromolar concentrations; no useable anti-angiogenic dosage was observed.

NHC-Ag(I) based antibiotic drugs

The antimicrobial drug candidate 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene silver(I) acetate (SBC3) was synthesised from 1,3-dibenzyl-4,5-diphenyl-imidazolium bromide and 2 equivalents of silver acetate in 81% yield as shown in figure 4 [11].

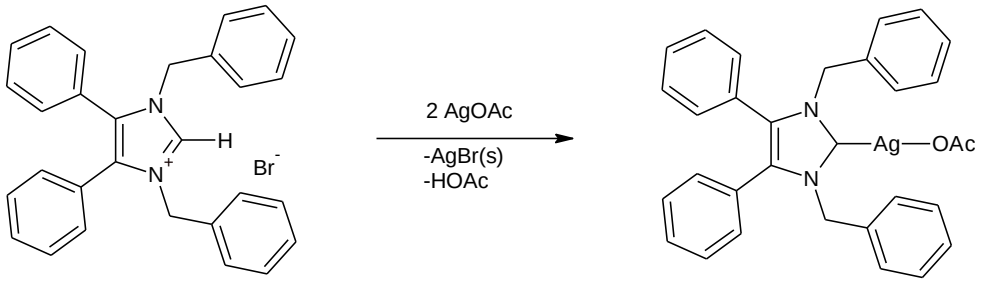

Figure 4: Synthesis of 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene silver(I) acetate (SBC3).

Preliminary *in vitro* evaluation showed that SBC3 showed antibacterial activity comparable to clinically used antibiotics [13], which encouraged further investigations. SBC3

was evaluated for its ability to function *in vivo* using larvae of *Galleria mellonella*. A SBC3 concentration of 25 μg/ml inhibited the growth of *S. aureus* by 71% and *C. albicans* by 86% *in vitro*. Larvae inoculated with 20 μl of SBC3 solution showed no ill effects up to a concentration of 250 μg/ml but administration of 500 μg/ml resulted in a 40% reduction in larval survival and administration of a dose of 1000 μg/ml resulted in total larval death at 24 h [14]. Larvae inoculated with *S. aureus* or *C. albicans* and subsequently administered SBC3 showed increased survival. Administration of SBC3 to larvae did not boost the insect immune response as indicated by lack of an increase in the density of circulating haemocytes (immune cells). The abundance of a number of proteins involved in the insect immune response was reduced in larvae that received 20 μl SBC3 solution of 100 μg/ml. This is the first demonstration of the *in vivo* activity of SBC3 against *S. aureus* and *C. albicans* and demonstrates that SBC3 does not stimulate a non-specific immune response in larvae.

NHC-Au(I) Based Anticancer Drugs

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) [15,16], which is a potential ligand for glucose transporters, were made analogously to WBC4 as shown in figure 5.

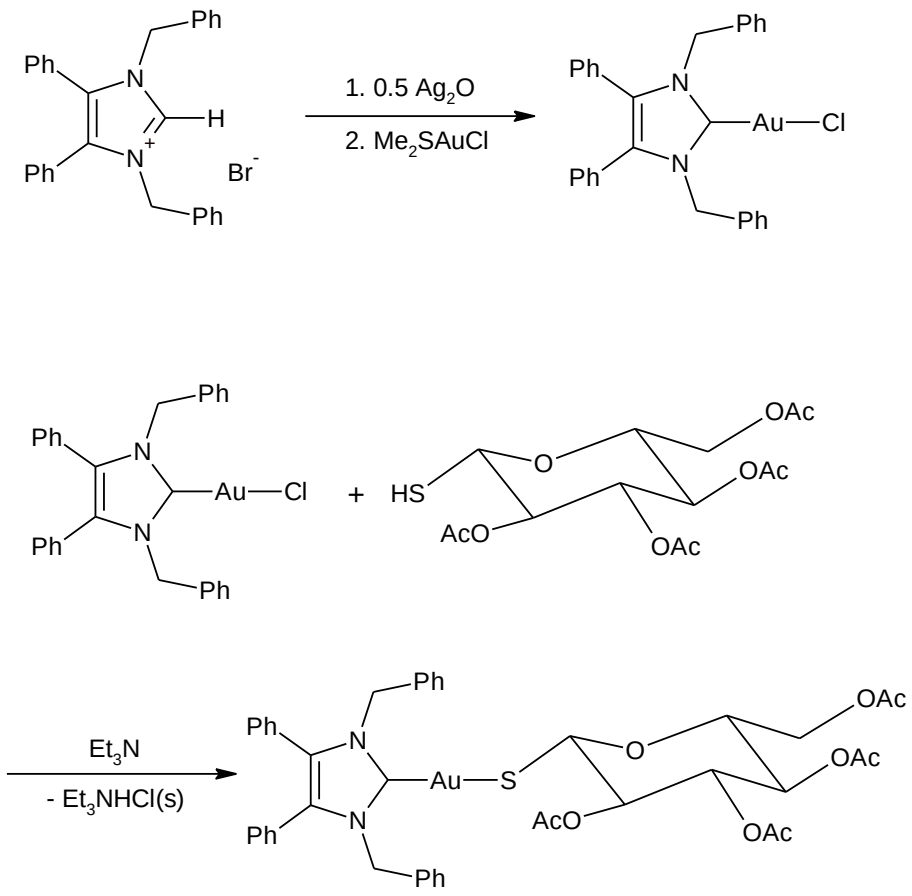

Figure 5: Synthesis of 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene gold(I) chloride (NHC-AuCl) and its 2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl-1'-thiolate derivative (NHC-AuSR).

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 [17]. 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 6. 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.

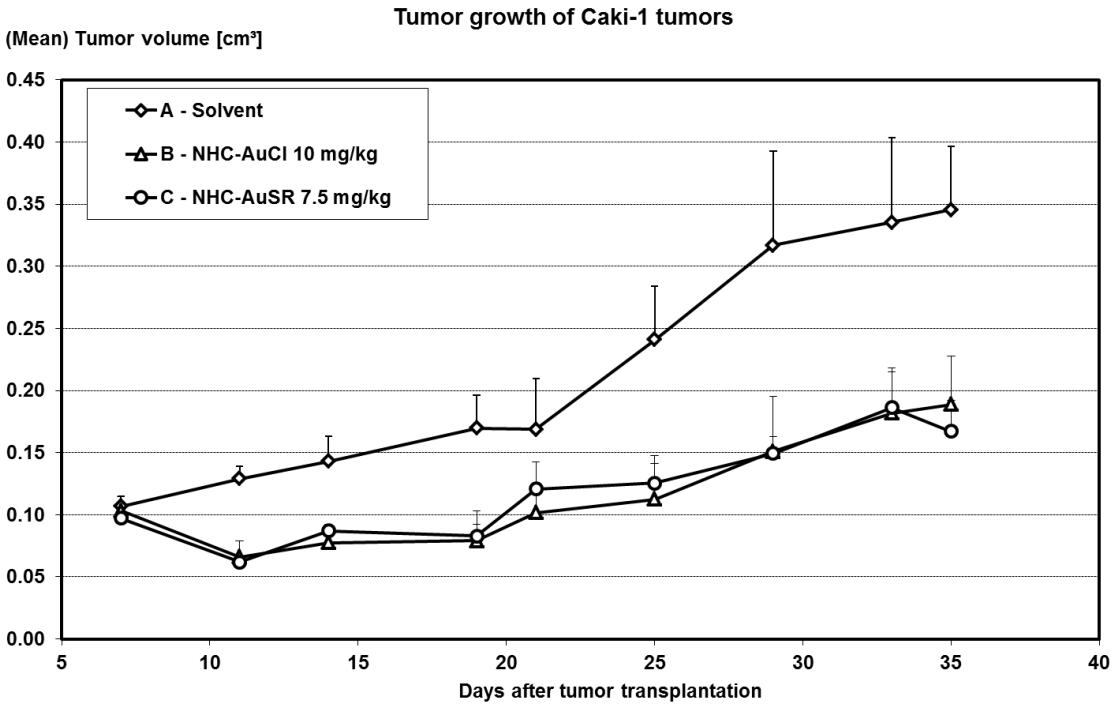

Figure 6: Influence of NHC-AuCl and NHC-AuSR on growth of CAKI-1 xenotransplant tumors in NMRI nu/nu mice; adapted from [17] with permission.

CONCLUSIONS

Monovalent benzyl-substituted NHC coinage metal halides and acetates are air- and moisture-stable compounds that have the right lipophilicity and solubility to act as potential drug candidates.

NHC-silver acetates like SBC1 looked in their *in vitro* evaluation as ideal anticancer drug candidates, but *in vivo* testing showed that xenografted human renal-cell could not be treated by SBC1. Nevertheless, SBC1 was tolerated in high doses in the mouse showing its low toxicity. A similar NHC-silver derivative SBC3 was then successfully tested *in vitro* against a variety of pathogenic bacteria inclusive MRSA and showed already at low dosage a significant survival effect in *Galleria mellonella* larvae, which were infected with *S. aureus* or

C. albicans. It is likely that ligand-stabilised silver(I) complexes will be deactivated by glutathione or other defense mechanism in mammalian cells, but bacteria respond already to low concentration of such species, which means that NHC-silver complexes are antibiotics but not anticancer drugs.

The NHC-copper bromide species WBC4 was a successfully tested drug candidate in xenografted Caki-1 tumors in nude mice; after five injections with a dose of 5 mg/kg the mice responded with mild and reversible body weight loss and a good T/C value of 0.38. Very similar results were found for the two NHC-gold chloride and thioglucoside derivatives NHC-Au-Cl and NHC-Au-SR; six injections of 10 mg/kg (NHC-Au-Cl) or 7.5 mg (NHC-Au-SR) led in xenografted Caki-1 tumors in nude mice to identical T/C values of 0.47. Both compounds induced no body weight loss and can therefore be classified as mild chemotherapy.

Summarising, one can say that silver compound SBC3 has the potential to go into Phase I clinical trials in humans as an emergency antibiotic possibly against sepsis caused by pathogenic bacteria resistant to conventional antibiotics. Here, the unusual mechanism of destruction of bacterial cell walls may become the reason for success. The NHC-copper and NHC–gold complexes WBC4, NHC-Au-Cl and NHC-Au-SR look very promising when it comes to difficult to treat forms of cancer like renal-cell cancer, where all three compounds exhibit significant T/C values in the xenograft experiments. All three species have potential for tests in humans, since one can see very good growth reduction like in WBC4 and low toxicity like in the gold species in combination with a new mechanism of mitochondrial membrane depolarisation and thioredoxin reductase inhibition, which is connected to the NHC-gold derivatives.

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