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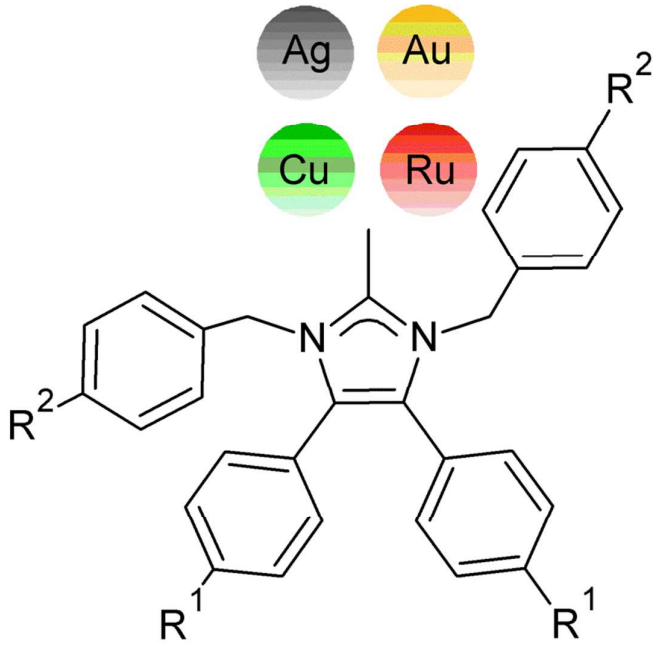

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Benzyl-substituted metallocarbene antibiotics and anticancer drugs

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Benzyl-substituted metallocarbene compounds synthesised by our group and others during the past 5 years give a new perspective on their activity as antibiotic and antitumoral drugs. *N*-Heterocyclic carbenes containing an imidazole core were functionalised and their transition metal complexes (M = Ag, Au, Cu, Ru) have shown promising antibacterial, as well as anticancer activity *in vitro* and *in vivo*. IC_{50} values in the nanomolar region in addition to antibacterial activity comparable to conventional antibiotics lead the way towards novel possible drug candidates.

Introduction

Silver-based *N*-heterocyclic carbenes

Silver-based *N*-heterocyclic carbenes for the treatment of bacterial infections have gained a huge rise in interest since the discovery of the small-molecule silver(I) carbene complexes **SCC1**[1] and **SCC10**[2] (Figure 1) by the group of W.J. Youngs in 2008/9. **SCC1** and **SCC10** were tested extensively *in vitro* for their antibacterial activity against a panel of highly resistant opportunistic pathogens recovered from the respiratory tract of patients with cystic fibrosis.

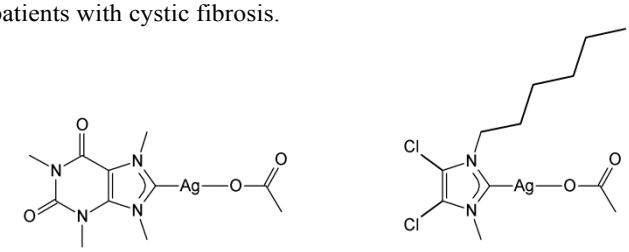

Figure 1: Structures of **SCC1** and **SCC10**

SCC1 was as well tested against *E. coli* J53 strains with and without the silver resistance plasmid pMG101 and it showed MIC values between 1 and 8 µg / mL against all pathogens, but did not show any activity against the silver resistant strain of *E. coli*. Furthermore, *in vivo* studies on mice bearing a *P. aeruginosa* infection were carried out with **SCC1** to find a 100% survival rate[3]. Since **SCC1** and **SCC10** are easily nebulised or aerolised, therapeutic outcomes with higher local drug concentration due to high doses delivered to the lung, a proportionally lower systemic drug concentration, and therefore decreased systemic toxicity can be achieved. However, the small size of **SCC10** and its quick diffusion across lung epithelium results in rapid clearance from the lungs following nebuliser administration. Resulting from these findings the efficacy of inhaled nanoparticles loaded with **SCC10** is currently under investigation. First results showed that core-loaded and dual-loaded shell cross-linked nanoparticles (SCK NPs) are effective against the cystic fibrosis relevant bacteria *P. aeruginosa* due to the ability of these nanoparticle formulations to provide a sustained release of the encapsulated silver carbene complex[4]. This approach seems to be a promising step towards an efficient antibacterial treatment for patients with cystic fibrosis.

Besides the antimicrobial activity of silver, a lot of interest arose around the possible anticancer properties of silver complexes during the last years. Some of them exhibited antitumor activity *in vitro* and *in vivo*, for example silver complexes derived from coumarin[5] exhibited antitumor activity against certain types of cancer and silver carboxylate dimers[6] possessed antitumor potency. Nevertheless, the first reports on the evaluation of anticancer activity of NHC-Ag(I) complexes only occurred in 2008 and are still relatively rare.

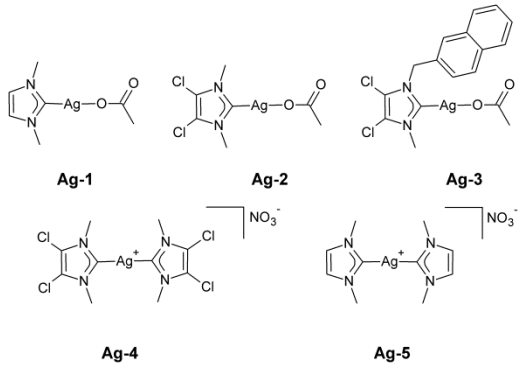

Figure 2: Structures of Ag-NHC complexes **Ag-1** to **Ag-5**

The group of W. Youngs reported the anticancer activities of several monomeric and dimeric imidazol-2-ylidene and 4,5-dichloroimidazol-2-ylidene silver acetate complexes (Figure 2)[7]. Complex **Ag-1** proved not to be stable enough to be evaluated but the IC_{50} values of **Ag-2** and **Ag-3** were found to be in the same range like cisplatin against OVCAR-3 (ovarian) and MB157 (breast) cancer cells and only little activity against HeLa (cervical) cells was observed. On the other hand the dimeric complexes **Ag-4** and **Ag-5** are 10 fold less active against cisplatin than against H460 (lung) cancer cells and no significant activity was observed against HeLa cells.

Gautier *et al.* and Roland *et al.* reported the IC_{50} values of **Ag-6** to **Ag-9** (Figure 3) against MCF-7 (breast) cancer cells and MCR5, a non-cancerous cell line in rapid proliferation and EPC, a quiescent cell line. A 350-fold increase in activity if compared to cisplatin was found against MCF-7 for **Ag-9** and the compound remains until now the most active NHC-Ag(I) complex. However, no discrimination between cancerous and non-cancerous cells in rapid proliferation could be found. The activity seems to be directly correlated with the proliferation rate, since it was shown that the NHC-Ag(I) complexes displayed a 2- to 18-fold lower cytotoxicity for the quiescent EPC cell line. In addition, Gautier and Roland reported that NHC-Ag compounds induce apoptosis independent of the caspase cascade via the mitochondrial AIF pathway[8].

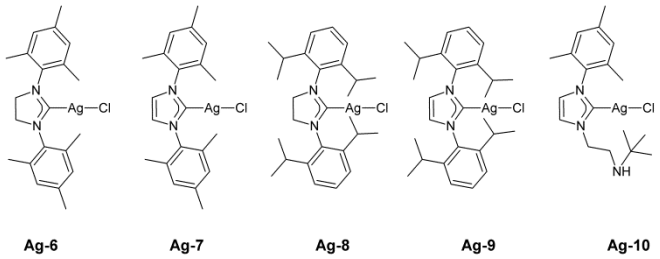

Figure 3: Structures of Ag-NHC complexes **Ag-6** to **Ag-10**

Li *et al.* studied the cytotoxic behaviour of amino-linked heterocyclic silver (I) bromide complex (**Ag-10**) against the human breast cancer cell lines MCF-7 and MDA-MB-231 as well as the glioblastoma cells U-87 MG. In comparison to cisplatin it showed similar activity but the investigated Au and Pd analogues showed much better activity[9].

Gold-based *N*-heterocyclic carbenes

One of the currently best studied gold-based complexes in medicine is the thioredoxin reductase (TrxR) inhibitor auranofin and some of its derivatives. 30 years ago, it was originally developed for the treatment of rheumatoid arthritis as a substitution for the injectable gold compounds aurothiomalate and aurothioglucose, but despite its efficacy in the treatment of both rheumatoid arthritis and psoriasis, auranofin is seldom used since more novel anti-rheumatic medications have become available. Nevertheless, the interest in potential new applications of auranofin never died and during the last years more and more insight was gained into the possible treatment of other diseases with auranofin. Klegeris *et al.*[10] recently summarized the vast studies on the biological activity of auranofin. Besides the anti-neoplastic, anti-parasitic, anti-bacterial and anti-viral activity of auranofin also its cytoprotective effects have been studied. It was found that auranofin shows a good balance between anti-inflammatory and protective activities, which make it a good candidate for the treatment of several diseases associated with inflammation and tissue damage[11]. During the past 7 years a considerable amount of reports on potential new Au(I/III)-based anticancer drugs with micro- to nanomolar cytotoxic activity has been published. One alternative to the use of phosphines are NHCs and in the last years considerable interest has arose around these ligands for the synthesis of biological active Au(I) complexes. Berners-Price *et al.* reported dinuclear Au(I)-bis(NHC) complexes which induced mitochondrial permeability transition in isolated rat liver mitochondria. In order to adjust the lipophilic character of the complexes, a critical factor for targeting malignant cells, the wingtip groups were adjusted and a further mononuclear, linear, cationic Au-NHC complex series was synthesised leading to Ca^{2+}-sensitive anti-mitochondrial effects. The Ca^{2+}-sensitive mitochondrial swelling is highly influenced by the lipophilicity of the Au(I)-complexes and therefore the fine-tuned bis(1,3-diisopropyl-2H-imidazole-2-ylidene) gold(I) bromide complex (Figure 4, **Au-1**), showing intermediate lipophilicity and significant anti-mitochondrial activity was selected for further studies. Apoptosis induction via the activation of Caspase 9 and Caspase 3 in cancer cells as well as selective inhibition of TrxR activity but not GR activity in MDA-MB 231 cells and accumulation in mitochondria was found for the above mentioned compound. Since dinuclear Au(I)-NHCs show luminescence associated to their aurophilic interactions, it was used to visualize (via FCM) the intracellular distribution of two Au(I)-bis(NHC) complexes. The obtained images of mouse macrophage cancer cells showed intracellular localization to lysosomes of one complex while the cell morphology was preserved[12].

In 2008 Raubenheimer *et al.* synthesised a bis-ferrocenylcarbene Au(I) complex (Figure 4, **Au-2**) with the aim to target mitochondria. While they found enhanced antitumor activity compared to cisplatin against two cancer cell lines, it remained unclear if this was due to the presence of the ferrocenylgroups[13].

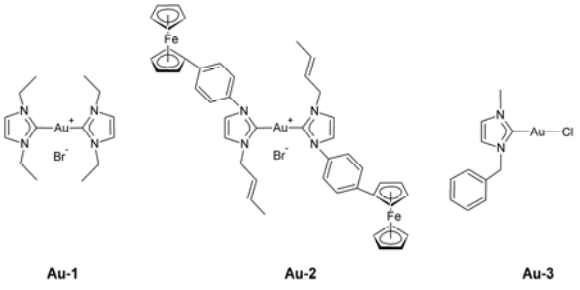

Figure 4: Structures of Au-NHC complexes **Au-1**, **Au-2** and **Au-3**

Barrios *et al.* synthesised a series of Au(I)-NHCs with the aim to find new potential targets for cancer treatment, in this case the inhibition of cysteine-dependent protein tyrosine phosphatases (PTPs). PTPs have shown to be involved in numerous diseases including cancer and therefore this family of enzymes became of

interest. In a comparative study with auranofin, the new developed monomeric, neutral Au(I)-NHCs inhibited PTP activity in Jurkat T leukaemia cells and primary mouse thymocytes. (1-methyl-3-benzyl-imidazole-2-ylidene) gold chloride (Figure 4, **Au-3**) was an even better inhibitor of PTP than auranofin with an IC_{50} range from 10 to 40 μM on four PTP types[14].

Extensive studies on the structural optimisation and identification of possible targets of Au(I)-NHCs have been carried out by Ott *et al.* A series of benzimidazole-2-ylidene gold(I) complexes have been synthesised based on the gold phosphole complex GoPI (chloro[1-phenyl-2,5-di(2-pyridyl)-phosphole] gold(I)) in the active site of GR and they showed selective TrxR inhibition as well as anti-proliferative effects in cancer cells. Unfortunately, neither selectivity towards tumour cells over non-tumour cells (HEK-293 and HFF) was observed nor improvement of activity with enhanced lipophilicity and / or surface volume of the side arms in position 1 and 3 of the benzimidazole were achieved. Comparison of the corresponding free ligand with the (1,3-diethyl-benzimidazole-2-ylidene) gold(I) chloride complex (Figure 5, **Au-4a**) showed that the biological activities, namely i) high increase of ROS formation, ii) apoptosis induction, iii) inhibition of mitochondrial respiration and iv) activity against resistant cell lines, are dependent on the presence of the Au(I) centre. Moving on from monomeric, neutral Au(I)-NHCs to cationic $[NHC\text{-}Au(I)\text{-}X]^+$ (X = NHC or triphenylphosphine) complexes (Figure 5: **Au-4b** and **Au-4c**) led to general improved cytotoxic properties, an increase in cellular uptake, induced mitochondrial accumulation and alternate reactivity towards the target enzyme TrxR[15].

On the search for metal-NHCs with targeted drug delivery routes, Metzler-Nolte *et al.* developed a series of Au(I)- and Au(III)-NHCs bearing cysteine-based thiolato ligands (**Error! Reference source not found.** 5: **Au-5a** and **Au-5b**) [16]. These metal-bioconjugates showed similar biological activity if compared to the Au(I)-NHCs without cysteine-derived ligands which shows that the activity as well as pharmacokinetic properties of Au-NHC complexes can be varied and optimized by choosing the appropriate oxidation state of the metal core and ligand system.

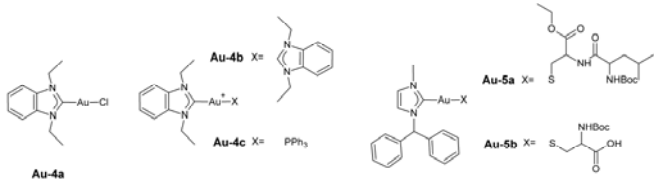

Figure 5: Structures of Au-NHC complexes **Au-4a-c** and **Au-5a** and **Au-5b**

Besides classical benzimidazole-derived NHC ligands, Huynh *et al.* also investigated a series of Au(I) and Au(III) mono-, homobis- and heterobis(carbene) complexes with non-classical pyrazole-derived NHC ligands. Again, the cationic Au-bis(NHC) complexes (Figure 6, **Au-10a** and **Au-10b**) showed higher activity against non-small lung cancer cells (NCI-H1666), with IC_{50} values in the nanomolar range, than the neutral Au-NHC complexes and cisplatin[17]. Four cytotoxic imidazole and benzimidazole-based Au(I)-NHC complexes (Figure 6, **Au-11a-d**) have been selected for screening for their TrxR inhibition properties both on the purified enzyme and on cell extracts *in vitro*, after initial screening for their cytotoxic activity against the cisplatin-sensitive and -resistant human ovarian (A2780S/R) and non-tumourigenic human embryonic kidney (HEK-239T) cell line by Mohr *et al.* Cytosolic TrxR1 was better inhibited by these four complexes than mitochondrial TrxR2 and even to a much lesser extend GR.

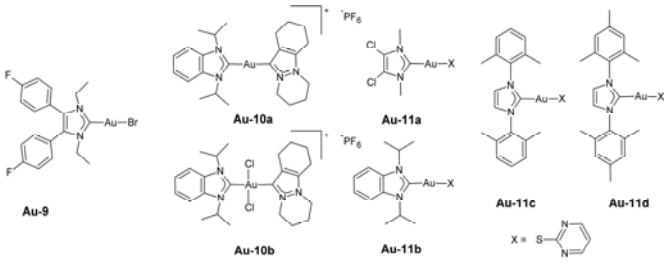

Figure 6: Structures of Au-NHC complexes **Au-9**, **Au-10a**, **Au-10b** and **Au-11a-d**

Interestingly, the inhibition of TrxR by these complexes seems to be relevant in cancerogenic cells, but not in non-tumourigenic cells, which is not the case for auranofin. In the presence of these complexes in tumourigenic cells a correlation between cytotoxicity and thioredoxin oxidation via TrxR inhibition was observed and biochemical assays on glutathione systems and ROS formation displayed great differences compared to auranofin[18]. In 2012, Schobert *et al.* reported their results on the biological evaluation of five *N*-methyl-4,5-diarylimidazole-2-ylidine gold(I) chlorides which showed micromolar cytotoxic activity and selectivity against human colon carcinoma (HT-29), leukaemia (HL-60), melanoma (518A2), cervix carcinoma cells (KB-V1/Vbl) and non-malignant foreskin fibroblasts (HF). The cellular uptake of the complexes occurred mainly via the copper transporter (Ctr1) and the organic cation transporters (OCT-1/2). Furthermore, the complex ((1-*p*-methoxybenzyl-3-(3,4,5-methoxybenzyl)imidazole-2-ylidene) gold(I) chloride (Figure 7: **Au-12**) was preferentially accumulated by Na^+/K^+-dependent endocytosis and via the OCT-1/2. The Au(I)-NHC complexes did not inhibit polymerisation of tubulin to give microtubules, a performance observed for the metal free 1-methyl-4,5-diarylimidazole precursors, therefore their mechanism of action appears to be different from that of their free ligands[19].

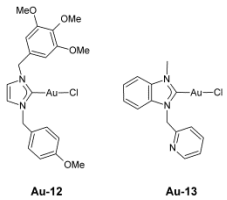

Figure 7: Structures of Au-NHC complexes **Au-12** and **Au-13**

Dinda *et al.* synthesised two mononuclear, neutral, benzimidazole-based Au(I)-NHC complexes, but the evaluation of the (1-methyl-3-

(2-pyridylmethyl)benzimidazole-2-ylidene) gold(I) chloride (Figure 7**Error! Reference source not found.**, **Au-13**) showed no improvement of cytotoxic behaviour against mouse melanoma (B16F10), human hepatocarcinoma (HepG2) and human cervical carcinoma (HeLa) cells when compared to cisplatin. When treated with the complex, the cells showed interesting morphological changes (cell rounding and shrinkage, nuclear fragmentation) which indicate an induction of apoptotic cell death. Whereas cisplatin showed 75% cytotoxicity at 10 μM against normal human peripheral blood mononuclear cells, the complex did not show any significant cytotoxic effect at 100 μM[20].

Ruthenium-based anticancer drugs

Ru(II) and Ru(III) compounds have been widely explored in respect to their potential uses in medicine as chemotherapeutics against different diseases. In particular, they have demonstrated high potentiality for the development of drug candidates for cancer therapy being promising alternatives to platinum complexes. Novel targets like different modes of DNA binding are offered by either classical coordination ruthenium compounds or organoruthenium compounds and they show potential to overcome resistances and side-effects exhibited by cisplatin and platinum-based antitumor drugs. Additionally, the design of ruthenium compounds targeting relevant cellular proteins and cellular signalling pathways overexpressed in tumour cells, mimicking organic enzyme inhibitors or other organic bioactive compounds and those suitable for photodynamic therapy are currently underway[21]. The advantages of ruthenium in biological applications are the following: Ru(II) as well as Ru(III) both show preferences for N and S donors, which are found in biomolecules like DNA, serum, cellular proteins and enzymes. Ru(II) and Ru(III) form six coordinated octahedral compounds allowing the *in vivo* interconversion between both oxidation states without extra energy requirements for structural rearrangements. This octahedral geometry offers wider possibilities of coordination to relevant molecular targets than the four coordinated Pt centre. These additional coordination sites could be used to fine-tune the pharmacologically relevant properties of the compounds. Moreover, Ru complexes are kinetically as inert as Pt(II) complexes while ligand exchange determines the activity in biological systems. Furthermore, ruthenium compounds show low toxicity in humans, probably due to metabolic similarities with iron. It can mimic iron in binding biomolecules (e.g. serum albumin and transferrin) and this contributes to a decrease in toxicity and helps the transport of the ruthenium complexes into the cancer cells. Since cancer cells show higher iron requirements than normal cells and therefore exhibit an increased number of transferrin receptors on the cell surface the uptake of ruthenium-compounds is increased in cancer cells[21]. Another advantage is that the redox potential Ru(II)/Ru(III) in many complexes is available in the biological redox potential window. This behaviour can be then exploited to develop ruthenium complexes which can be activated in hypoxic tumour tissues by bioreduction, allowing for achieving selectivity by reoxidation in healthy normally oxygenated cells. Nevertheless, the kinetic lability of ruthenium ions is highly dependent on the types of bound ligands and substitution rates can vary in many orders of magnitude[22]. These various chemical and biological properties have led to the discovery of potent Ru-based pro-drugs[23]. Three of the four most prominent complexes have successfully entered clinical trials (Figure 8).

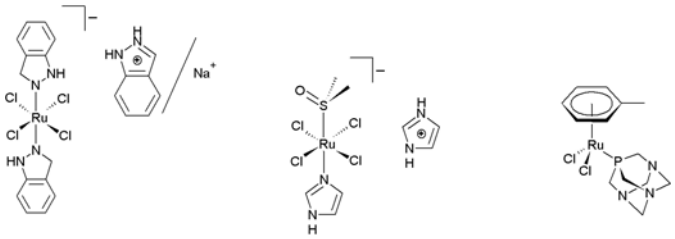

Figure 8: **KP1019**[23]/ **KP1339**[23], **NAMI-A**[23] and **RAPTA-T**[23] (from left to right)

The first ruthenium compound which entered clinical trials was **NAMI-A** and dose finding was carried out in phase I. PK determination and toxicity evaluation were performed in 24 patients with diverse tumour histotypes and previously treated with surgery and chemotherapy with or without radiotherapy, according to the different tumour type[24]. Today **NAMI-A** is studied for use as a second line therapy in the metastatic non-small-cell lung carcinoma (NSCLC) in combination with Gemcitabine. Although the study is still ongoing, preliminary data of dose finding show the possibility to treat these patients with the combination **NAMI-A** + Gemcitabine. Main toxicities are phlebitis at the injection site, for which reason the drug is infused through a central venous catheter (port-a-cath), general malaise and cutaneous blister formation[25]. The biological *in vitro* and *in vivo* effects of **KP1019** can be summarised as follows: *in vivo* activity against autochthonous chemically-induced colorectal cancer in mice[26], *in vitro* activity on colorectal colon carcinoma[27], *in vivo* activity on the chemo-resistant MAC15A colon carcinoma[28], unable to modify the metastatic cell behaviour[29], transferrin[30] and HSA[31] binding, more cytotoxic after reduction, causes apoptosis via the mitochondrial pathway[30], generation of ROS species[27] and at last solubility problems[30] (which are now overcome by its Na^+ analogue **KP1339**[31]).

Another ruthenium-based complex which shows some selectivity of anti-tumour activity towards metastases after the discovery of **NAMI-A** is the **RAPTA-T** complex by the group of Dyson. **RAPTA-T** ($Ru(\eta^6\text{-}C_6H_5Me)(PTA)Cl_2$) with PTA = (1,3,5-triaza-7-phosphoadamantane), which is similar to nearly all other derivatives of the **RAPTA** series[23], shows better cytotoxicity against tumour cells compared to non-tumour cells but is only weakly cytotoxic. Nevertheless it has shown *in vitro* to have significant influence on cell behaviour by modifying invasion and metastasis[32].

In general ruthenium-based drugs are much less toxic than platinum-based drugs and are furthermore often capable of overcoming platinum-induced resistances in cancer cells[21,22,33]. The ability of ruthenium to mimic iron in binding to biological

molecules, for example in HSA and transferrin, and the selective activation to more reactive species by the reducing environment of solid tumours seem to be the reasons for these activities[33,34].

Copper-based *N*-heterocyclic carbenes

Following the assumption that endogenous metals may be less toxic, considerable efforts have been devoted to copper(I) complexes. Copper is an essential trace nutrient. In mammals it is mainly found in the bloodstream as a co-factor in various enzymes. Since copper is only toxic when outside its normal metabolic pathways, complexes of Cu(I/II) may serve as Fenton-type reagents and effective cytotoxic agents[35]. Recently published reports of anticancer properties of copper complexes may be roughly divided into two sets. The main set contains complexes of copper(II)[36] which sometimes are reduced *in vivo* to yield more toxic copper(I) species. Yet it seems more logical to use copper(I) and omit the *in vivo* reduction phase. Santini *et al.* reported promising activity of phosphine-copper(I) complexes against a panel of tumour cell lines[37]. Since phosphine ligands can be successfully replaced with NHCs to stabilise the sensitive copper(I) ion, this idea was quickly picked up. The majority of attention on these new complexes was focused onto establishing their catalytic properties[38], but studies of their anticancer properties have recently been reported. Gautier *et al.*[39] showed that the cytotoxicity of copper(I) complexes against human cancer cells lies well with that of cisplatin and other metal–NHCs. Therefore, the use of copper(I) against human cancer cells is, in our opinion, a valuable strategy.

Since our last review in 2011[40] concerning NHC-silver(I) acetates as bioorganometallic anticancer and antibacterial drugs, we investigated several series of other NHC-metal complexes for their possible biological activities.

Substituted (benz)imidazole-derived NHC-silver(I) acetates

In 2010, the silver(I)-NHC lead structure with remarkable cytotoxicity **SBC1**[41] was discovered in our group; **SBC1** has been further evaluated *in vitro* and *in vivo* in the following years in cooperation with our group. **SBC1** has shown activity against several cancer cell lines (MCF-7 (breast), Caki-1 (renal), PC-3 (prostate), UKF-NB-3 and UKF-NB-6 (neuroblastoma)) in the low micromolar range and exhibited HSA and DNA binding[42].

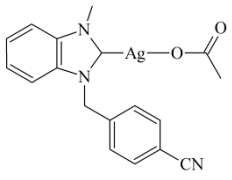

Figure 9: Structure of **SBC1**

Unfortunately, *in vivo* tests in zebrafish resulted in high mortality at concentrations of 10 μM and above. Also, the *in vivo* experiment using non-tumour bearing mice showed that the toxicity of SBC1 was under- and the MTD overestimated. This led to the situation that three out of eight tumour bearing mice in the xenograft experiment died when they were exposed to daily dosages of 50 mg/kg. Even in the group which received the lower dosage, two out of eight mice died during the treatment. The relatively high T/C values of 78% and 75% combined with the significant toxicity of **SBC1** demonstrated that the compound is toxic and does not have a useable therapeutic index in its given formulation.

Attempts to synthesise more selective and active (benz)imidazole-derived NHC-silver(I) acetates[43] showed, that substitution of the methyl group in position 1 at the imidazole ring with further benzyl-ligands does not lead to better cytotoxic activity against Caki-1 cells, if compared to **SBC1**. Furthermore, the stability of these compounds was not suitable for them to become potential drug candidates, and without further vectorisation (anchoring **SBC1** to a targeting molecule) no improvement in activity can be found.

Substituted 4,5-diarylimidazole-derived NHC-silver(I) acetates

Another possible mode of action of these NHC-silver(I) acetates is their potential antibacterial activity. **SBC3**[41], also discovered in 2010, shows significant activity against the *M. bovis BCG Pasteur* and *M. smegmatis* as well as against *Salmonella typhimurium*, *MSSA* and *MRSA*. The best activities are found for *E. coli* and *P. aeruginosa*, which makes **SBC3** already as active as conventional β-lactam antibiotics against these two bacterial strains. Especially breaking the resistance in *MRSA* is a good argument for the further development of the silver-based antibiotic drug candidate **SBC3** *in vivo*[44].

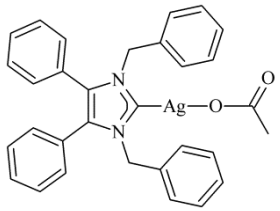

Figure 10: Structure of **SBC3**

The next step in optimising the thermal and UV stability in the following three years was the design of **SBC3**-like silver acetate complexes. These newly synthesised compounds, varied in their lipophilicity due to different substitution patterns in R^1 and R^2 (see Figure 11)[41,45]. All complexes were obtained in high purity and excellent yields and show high thermal and UV stability. Nevertheless, some trends have been observed while synthesising this class of compounds. While the imidazolium salts with electron donating groups in para position on the

benzyl and phenyl substituents react much quicker in the metallation process (2-3 days), the ones with electron withdrawing substituents in these positions need up to 7 days, to form the NHC-silver(I)acetate complexes; it might be that the electron density distribution over the imidazole ring is slightly influenced by the different substitution pattern.

Antibacterial tests against *E. Coli* and *MRSA* resulted in lower or comparable activity to **SBC3**, but IC_{50} evaluations against the cancer cell lines Caki-1 and MCF-7 led to surprising findings. While the substitution patterns R^1 = OMe and Cl as well as R^2 = COOMe, CN and NO_2 gave moderate to low activity against the selected cell lines, especially substituting R^1 and R^2 with methyl groups (**Ag2b**[45]) led to up to nanomolar cytotoxicity.

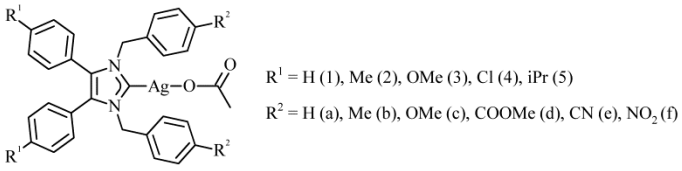

Figure 11: Structures of Ag-NHC complexes **Ag1a-f**, **Ag2a-e**, **Ag3a-e**, **Ag4a-e** and **Ag5a-e**

Ag2b, better known as **WBC1**, is the most promising candidate. Here the activity of an already cytotoxic imidazolium is strongly enhanced by deprotonation and coordination to silver acetate resulting in a water-soluble and neutral drug molecule. This indicates its high potential as an anticancer drug and gives a good insight into possible further substitution patterns.

Other Metal-NHC Compounds

In 2010, our group synthesised three novel mononuclear, neutral benzimidazole-based Au(I)-NHC complexes (Figure 7, **Au-14a-c**) by transmetallation *in situ* from the NHC-Ag(I)Br complexes. The Au(I)-NHC complexes showed good solubility in DMSO and biological medium and were tested against the cancer cell line Caki-1 (renal), but unfortunately no higher activity than cisplatin was observed[46].

During the following years the most promising NHC-silver(I) acetates have been used as templates to investigate the influence of other transition metals (namely copper[47], ruthenium and gold[48]) on the cytotoxic activity against Caki-1 and MCF-7 (see Figure 12). Exchanging the silver acetate moiety against ruthenium (*p*-cymene) dichloride (**Ru1-6**) or gold chloride (**Au4-6**), gold acetate (**Au7-9**) and gold thioglucopyranose (**Au10-12**) resulted in an overall weaker cytotoxic profile (IC_{50} values (μM) for silver acetate analogues of **SBC1**: 1.2 (± 0.6) (Caki-1)[41] and 23 (± 1) (MCF-7) (unpublished results); **Ru4**: 4.6 (± 0.6) (Caki-1) and 2.1 (± 0.8) (MCF-7); **Ru6**/**Au6**/**Au9**/**Au12**: 5.5 (± 0.6) (Caki-1) and 3.4 (± 0.6) (MCF-7)). Interestingly, upon transmetallation of **SBC1** to its ruthenium containing analogue **Ru1**, the cytotoxic activity is completely lost. Nevertheless, some interesting results have been obtained which might lead to further investigation of the mode of action and cellular targets of these complexes. Especially the Ru(II)-NHC complexes **Ru5** and **Ru6** which show 24-fold and 16-fold better activity against MCF-7 than Caki-1, indicate that there might be different mechanisms of cellular uptake and/or of cell death induction present in the two different cell lines.

Furthermore, the **SBC3** gold chloride (**Au4**) and gold thioglucopyranose (**Au10**) analogues exhibited good IC_{50} values against MCF-7 with 8-fold and 2-fold better activity than against Caki-1, respectively.

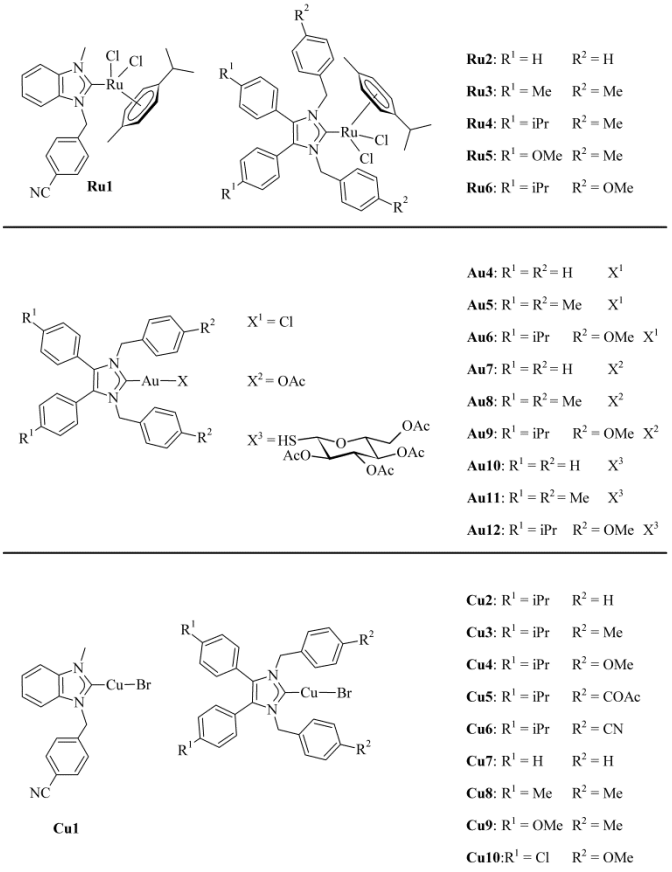

Figure 12: Structures of metal-NHC complexes **Ru1** to **Ru6**, **Au4** to **Au12** and **Cu1** to **Cu10**

One unsymmetrically substituted (**Cu1**) and nine new symmetrically substituted (**Cu2-Cu10**) NHC–copper(I) bromide complexes were synthesised in the transmetallation reaction of *p*-benzyl substituted imidazolium halides with dimethylsulfido copper(I) bromide over silver(I) oxide[47]. The newly synthesised NHC–copper(I) bromide compounds show relatively high biological activity with compound **Cu4** being the most active. With an IC_{50} value of 0.60 (±0.09) μM against human breast carcinoma MCF-7 and an IC_{50} = 0.65 (±0.08) μM

against human renal cell carcinoma Caki-1, it is a promising candidate for further biological studies.

Interestingly, the IC_{50} values of **Cu4** are approximately ten times better compared to its previously reported NHC–silver(I) acetate derivative[45] (IC_{50} of 3.4 (±0.6) μM against MCF-7 and IC_{50} = 5.5 (±0.6) μM against Caki-1). At the same time compound **Cu7** is almost twenty times less active against MCF-7 cell line and 40 times less cytotoxic when tested against Caki-1 renal cell carcinoma than its previously reported silver(I) acetate equivalent[45]. Compared to cisplatin (IC_{50} values of 10.4 (±0.2) μM against MCF-7 and 3.3 (±0.2) μM against Caki-1), **Cu4** shows significant improved activity and it also compares well with the most active NHC–Cu(I)Cl complex (IC_{50} value of 0.075 (±0.002) against MCF-7) reported by Gautier and co-workers[39].

It is therefore visible that the influence of the metal ion on the toxicity of the overall complex is significant. Compounds based on both transition metals can reach the single digit micromolar cytotoxicity level, provided the substitution pattern of the carbene ligand is appropriate.

Additionally, no metal-to-activity relationship pattern can be clearly concluded as in our opinion, both NHC–silver(I) and NHC–copper(I) complexes induce cell death via different mechanisms. Therefore, a particular substitution pattern of the carbene ligand can prove itself to be very active for the copper complex (as shown above for NHC–copper(I) bromide complex **Cu4**) while exhibiting average cytotoxic activity in the form of NHC–silver(I) acetate and vice versa. Most active NHC–silver(I) acetate complexes reported previously, showed average biological activity when transmetallated to NHC–copper(I) bromide. It is therefore recommended that when screening a library of the candidate compounds, no predictions are to be made based on the activity of the precursor's analogues with other transition metals. This indicates that certain previously reported substitution patterns of the imidazolium halide precursors, that showed no particular biological activity combined with silver, may still become an interesting cytotoxic agents if transmetallated to copper or other transition metals and tested against appropriate cancerous cell lines.

With its low IC_{50} values against both cancer cell lines, compound **Cu4** belongs to the group of highly biologically active NHC–metal complexes synthesised by our research group. Future studies will focus on establishing the potential of **Cu4** (**WBC4**) as an anticancer drug *in vivo*, as well as further investigation and structure optimisation of biologically active NHC–copper(I) complexes.

Conclusions

Benzyl-substituted metallocarbene compounds are accessible drug-like molecules, which are tuneable for lipophilicity, solubility and activity through use of the appropriately substituted *p*-benzyl bromide. It can be said that several highly cytotoxic complexes have been found in this compound class, identifying **WBC4** as a new lead structure for further investigations, but unfortunately none of the complexes **Ag1a** to **Ag5e** can be considered as an improved antibacterial drug candidate since all highly antibacterial acting complexes are also found to be highly cytotoxic. So, **SBC3** remains to be the antibiotic lead compound and is chosen for *in vivo* evaluation. In conclusion, several NHC-silver(I) acetates with IC_{50} values in the micromolar region have been synthesised and the identified lead structures were successfully transmetallated to their ruthenium, gold and copper analogues. Besides **WBC1**, evaluation of the obtained derivatives for their *in vitro* cytotoxic activity revealed two promising Ru(II)-NHC (**Ru5** and **Ru6**), two Au(I)-NHC (**Au5** and **Au10**) and one Cu(I)-NHC (**WBC4**) complex (Table 1), which hopefully will show interesting results in further studies.

Complex	IC_{50} Caki-1 [μM]	IC_{50} MCF-7 [μM]
Ag2b/WBC1	0.51 ± 0.07	1.4 ± 0.1
Ag4e	140 ± 10	50 ± 2
Au5	16 ± 2	30 ± 3
Au10	14 ± 2	6.1 ± 1.5
Ru1	> 500	> 500
Ru5	39 ± 5	2.4 ± 0.7
Ru6	13 ± 2	7.0 ± 1.2
Cu1	126 ± 10	134 ± 16
Cu4/WBC4	0.65 ± 0.08	0.60 ± 0.09

Table 1: Exemplary IC_{50} values against Caki-1 and MCF-7

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