Novel Carbene-Metal Complexes as Anticancer Drugs and Antibiotics - Potential and Limitations

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ABSTRACT

Benzyl-substituted metallocarbene compounds synthesised during the past 5 years give a new perspective on their activity as antibiotic and antitumoral drugs. N-Heterocyclic carbene (NHC) containing Au and Ru compounds have shown promising anticancer activity in vitro and the Cu derivative WBC4 showed strong cytotoxic efficacy in vivo xenograft studies against difficult to treat renal cell cancer. While the carbene-silver acetate derivative SBC1 failed in vivo as an anticancer drug, the antibacterial derivative SBC3 convinced in vivo and this compound may lead the way towards novel injectable emergency antibiotics against resistant bacteria and fungi.

KEY WORDS

N-Heterocyclic carbene; silver, copper, gold, ruthenium, antibiotic, anticancer drug.

INTRODUCTION

Groundbreaking research by Wiley J. Youngs and coworkers established the synthesis and medicinal use of covalently bonded silver in NHC-silver acetate compounds [1]. One derivative called SCC1, which is derived from caffeine, became the antibiotic lead compound [2]; the synthesis and molecular structure of SCC1 is shown in Fig. 1. This bioorganometallic molecule enables antibiotic treatment of pneumonia when used in a suitable formulation [3]. In the meantime several research groups worldwide have taken up the topic of synthesis and
medicinal application of NHC-metal complexes and recent review articles show the progress [4-9].

![Synthesis and molecular structure of the NHC-silver acetate derivative SCC1.](image)

Fig. 1: Synthesis and molecular structure of the NHC-silver acetate derivative SCC1.

**ANTICANCER ACTIVITY**

The Tacke group contribution started with the idea to make carbene-metal complexes more lipophilic than SCC1 by introducing benzyl groups onto the imidazole nitrogen atoms and add extra stability by substituting the imidazole ring in the 4,5 positions with aryl groups [10-22]. Systematic synthesis and biological testing against human renal cell cancer (CAKI-1) and partly against human breast cancer (MCF-7) led to two preferred structural motifs for further drug development [23-24]; benzimidazoles and 4,5-diphenyl-imidazoles carrying at least one N-benzyl group, which can be seen in Fig. 2.

![Preferred NHC-AgX structures for further drug development.](image)

Fig. 2: Preferred NHC-AgX structures for further drug development.

Relatively early, the research group identified (1-methyl-3-(4-cyanobenzyl) benzimidazole-2-ylidene) silver(I) acetate (SBC1) as the silver-based anticancer lead compound showing an IC50 value of 1.2 μM against CAKI-1 [13]. SBC1 was also tested at the NCI 60 cell line panel, where it showed good activity against breast, prostate and renal cell cancer. More detailed studies against resistant cancer lines exhibit that SBC1 is able to break platinum-resistance in UKF-NB-3 (neoblastoma) and HCT8 (colon cancer) as well as
paclitaxel-resistance in PC3 (prostate cancer) cells [25]. The molecular structure of SBC1 is shown in Fig. 3.

![Molecular structure of SBC1](image)

This is the molecular structure of the NHC-silver acetate derivative SBC1.

In a fluorescence assay SBC1 is well able to bind to albumin, which is a potential way for SBC1 of selective delivery into cancer cells [25]. Furthermore, melting curve experiments and CD spectroscopy reveal that SBC1 targets DNA in cancer cells [25] without being able to say, whether this is the main mechanism of apoptosis induction. Here, results from Gaultier and Roland indicate that carbene-silver complexes induce caspase-independent apoptosis via the mitochondrial pathway and AIF release by forming lipophilic metalallocarbene cations as the reactive species [26]. This group also reports that carbene-silver complexes do not produce significant ROS concentrations and do not modify the cell-cycle distribution of HL60 cells, which implies that these compounds are therefore not genotoxic [26].

All these positive in vitro properties of SBC1 encouraged in vivo testing in mice. In a first experiment non-tumor bearing nude mice were treated with increasing doses of SBC1 and 50 mg/kg were determined as the maximum tolerable dose. In a second experiment CAKI-1 tumors were inoculated under the skin of immune-deficient nude mice and after these tumors reached palpable size the mice were treated with 25 or 50 mg/kg of SBC1 for 5 times, while a control cohort received no treatment. Surprisingly, no tumor reduction effect could be seen between the treatment and the control groups [25]; the tumor volume graph can be seen in Fig. 4.
Fig. 4: Tumor growth curves of CAKI-1 xenografts in nude mice comparing two SBC1 treated cohorts against a control cohort (from [25]).

This rather unexpected and disappointing in vivo testing of SBC1 led to a change in direction and the best carbene ligands were transmetallated from Ag to other metals with promising anticancer properties. So, imidazolium bromides were reacted with silver oxide in DCM and dimeric (p-cymene)RuCl₂ was added to the intermediate carbene-silver bromide [20]. The resulting carbene-Ru(p-cymene)Cl₂ could be isolated in 40 – 76% yield and the crystal structure shows the expected pseudo tetrahedral Ru complex with two chloride and two organometallic ligands [20]. The best derivative, which is shown in Fig. 5, exhibits good activity against the human breast cancer cell line MCF-7 with an IC₅₀ value of 2.4 ± 0.7 μM.

Fig. 5: Synthesis and structure of the most promising NHC-Ru(p-cymene)Cl₂ derivative.
A similar synthetic strategy is applied for gold; here the intermediate carbene-silver bromide is reacted with dimethylsulfido gold monochloride and delivers carbene-gold chloride derivatives in yields ranging from 49 to 83%, which are shown in Fig. 6. Further reactions with silver acetate can exchange the anion and results in carbene-gold acetates in yields ranging from 53-69%. The crystal shows a linear carbene-Au-Cl moiety with a short C-Au bond of 199 pm and a longer Au-Cl bond of 229 pm [20].

Further anion exchange experiments with tetraacetato thioglucose led to carbene-gold thioglucoside derivatives [20]. Such compounds are seen as biocompatible and they might even benefit from overexpressed glucose transporters in cancer cells, which can lead to selective uptake. The gold derivative with the highest anticancer activity is made from 1,3-dibenzyl-4,5-diphenyl-imidazolium and shows good activity against the human breast cancer cell line MCF-7 with an IC50 value of 6.1 ± 1.5 μM [20]; its synthesis and structure is shown in Fig. 7.
In a similar synthesis the intermediate carbene-silver bromide is able to transmetallate its carbene ligand to copper when dimethylsulfido copper monobromide is used. The resulting carbene-copper bromide derivatives are isolated in yields ranging from 32 to 74% and the best derivative WBC4 shows impressive nanomolar activity against Caki-1 (0.60 ± 0.09 μM) and MCF-7 (0.65 ± 0.08 μM) [22]. The synthesis and molecular structure of WBC4, which is isostructural to the silver and gold derivatives, is shown in Fig. 8.

Cytotoxicity of chemotherapeutic agents in the upper nanomolar region is generally seen as ideal and WBC4 was therefore chosen for further in vitro and in vivo testing. Systematic cell testing on the NCI 60 cell panel led to an average GI50 (growth inhibition 50%) value of 288 nM. Particular activity of better than 200 nM was found against leukemia and melanoma as well as lung, colon, prostate and breast cancer [27]. Due to our long-standing interest in difficult to treat human renal cell cancer WBC4 was chosen for such a xenograft mouse model. In a first experiment non-tumor bearing nude mice were treated with increasing doses of WBC4 and 10 mg/kg were determined as the maximum tolerable dose. In a second experiment CAKI-1 tumors were inoculated under the skin of immune-deficient nude mice and after these tumors reached palpable size, the mice were treated with 5 or 10 mg/kg of WBC4 for 5 times, while a control cohort received no treatment [27]. In both treatment groups tumor growth was strongly inhibited compared to the control cohort and the optimal T/C (treatment over control) value of 0.38 was reached on day 32 of the xenograft experiment. The toxicity as measured by the loss of body weight was mild and reversible and the high dose treatment group shows anti-angiogenic effects in the tumor tissue. This mouse experiment, for which the tumor volume graph is shown in Fig. 9, very much encourages clinical testing of WBC4 in future.
Fig. 9: Tumor growth curves of CAKI-1 xenografts in nude mice comparing two WBC4 treated cohorts against a control cohort (from [27]).

ANTIBIOTIC ACTIVITY

After the disappointing in vivo performance of SBC1 we revisited our existing stock of carbene-silver acetate compounds, which were also tested for their antibacterial activity against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli, and identified 1,3-dibenzyl-4,5-diphenyl-imidazol-2-ylidene silver(I) acetate (SBC3) as the potential lead compound for antibiotic development. SBC3 can be made from commercially available 4,5-diphenyl-imidazole in high purity and good yield of 68%; the molecular structure of SBC3 is shown in Fig. 10.

Fig. 10: Molecular structure of SBC3

SBC3 shows the best activity of all derivatives in Kirby-Bauer tests against S. aureus, but it also exhibits significant cell toxicity of 14 μM against CAKI-1 [13]. Nevertheless,
systematic minimum inhibitory concentration (MIC) experiments were initiated against *Mycobacterium bovis BCG Pasteur* and *Mycobacterium smegmatis*, which gave MIC values of 20 and 5 μg/ml. Further tests against *Salmonella* and Methicillin-sensitive or Methicillin-resistant *S. aureus* MSSA/MRSA gave a MIC value of 12.5 μg/ml for all three bacteria. The best performance of SBC3 was noted against *Pseudomonas aeruginosa* and *E. coli* with MIC values of 3.13 and 6.25 μg/ml [28]. The antibiotic activity of SBC3 against *P. aeruginosa* and *E. coli* is already comparable to typical antibiotics and therefore *in vivo* testing of SBC3 became the obvious next step.

For the *in vivo* testing of SBC3 *Galleria mellonella* larvae were chosen. In a first experiment increasing amounts of SBC3 were injected as DMSO/water solutions via a proleg into the larvae and 250 μg of SBC3 were determined as the maximum tolerable dose. In a second experiment the haemocyte density in *Galleria mellonella* larvae was measured after increasing doses of SBC3 were given; doses from 250 μg of SBC3 or higher reduced the haemocyte density, which is a sign of toxicity. Further *in vivo* experiments were testing the antibiotic activity of SBC3 in *Galleria mellonella* larvae; for the antibiotic treatment a dose of 10 μg, which is 1/25 of the MTD, was chosen. In two independent experiments this dose was given to larvae, which were either infected by a lethal dose of *S. aureus* or *Candida albicans* and time-dependent survival was monitored against infected but untreated control cohorts [29]; the survival graphs can be seen in Fig. 11.

**Fig. 11:** Survival of SBC3 treated and non-treated *Galleria mellonella* larvae, which were infected either with *S. aureus* or *C. albicans* (adapted from [29]).
In both experiments the treated cohorts had a significant treatment advantage, which shows that SBC3 can be used as an injectable antibacterial and antifungal antibiotic at a non-toxic concentration.

CONCLUSION

Generally, one can say that Ag, Cu, Au and Ru NHC complexes are generally stable enough in biological media, if the substitution pattern on the NHC ligands is chosen appropriately. At a first glance the preselected silver compound SBC1 looked like the ideal candidate for in vivo testing against renal cell cancer, but failed in this xenograft mouse model due to lack of efficacy. Here, one can speculate that carbene-silver acetate are quickly deactivated by sulfur-containing biomolecules like glutathione and end up as silver sulfide, which is biologically inert. Nevertheless, the high MTD and therefore low toxicity of SBC1 triggered experiments using the more lipophilic derivative SBC3 in vitro and in vivo as a potential antibiotic. SBC3 showed impressive activity against MRSA, E. coli and particularly against P. aeruginosa in vitro; in vivo experiments in G. mellonella larvae showed an antibacterial and antifungal survival advantage using a non-toxic concentration of SBC3 for injection. So, compounds like SBC3 can deliver silver ions bacterial membranes at low concentration causing the bacteria to die [30] before human cells feel the toxic effects [31].

While the best carbene-Ru and carbene-Au complexes are waiting for in vivo testing the carbene-copper bromide WBC4 showed good activity in combination with low toxicity in a xenograft experiment against renal cell cancer; therefore WBC4 has become a potential clinical testing in humans against this notoriously difficult to treat cancer.

REFERENCES

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