Synthesis, Characterization, and Biological Studies of Silver and Gold *N*-Heterocyclic Carbene Complexes Derived from 4,5-Diarylimidazoles

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Abbreviations

CD, circular dichroism;

CT, calf thymus;

COX, cyclooxygenase;

DTNB, dithiobis nitrobenzoic acid;

ETAAS, electrothermal atomic absorption spectroscopy;

ER, estrogen receptor;

5-FU, 5-fluorouracil;

GoPI, 1-phenyl-2,5-di(2-pyridyl)-phosphole]AuCl;

GR, glutathione reductase;

LiHMDS, lithium bis(trimethylsilyl)azide;

LOX, lipoxygenase.

NHC, *N*-heterocyclic carbene;

NSAID, nonsteroidal anti-inflammatory drug;

PARP, poly (ADP-ribose) polymerase;

PBS, phosphate buffered saline;

PTPs, protein tyrosine phosphatases;

ROS, reactive oxygen species;

SAR, structure-activity relationship;

TNB, 2-nitro-5-thionitrobenzoic acid;

TrxR, thioredoxin reductase;

rt, room temperature.

1. Introduction

1.1 Introduction

Despite almost all drugs placed in the market are organic compounds and natural products, during the past four decades scientists are taking a growing interest in the development of metallotherapeutic drugs and metal-based diagnostic agents. Based on their wide spectrum of coordination numbers and geometries as well as kinetic properties, metal complexes offered different mechanisms of drug action compared with organic agents and gained a growing interest as pharmaceuticals for the use as diagnostic agents or as chemotherapeutic drug [1, 2].

The accidental discovery of the antitumor properties of cisplatin by Rosenberg and co-workers was followed by one of the most impressive drug success stories ever and a significant improvement of cancer chemotherapy [1]. Besides cisplatin, several other platinum complexes such as carboplatin, oxaliplatin, nedaplatin, and lobaplatin also have been approved for current tumour therapy (see Figure 1.1) [1, 2].

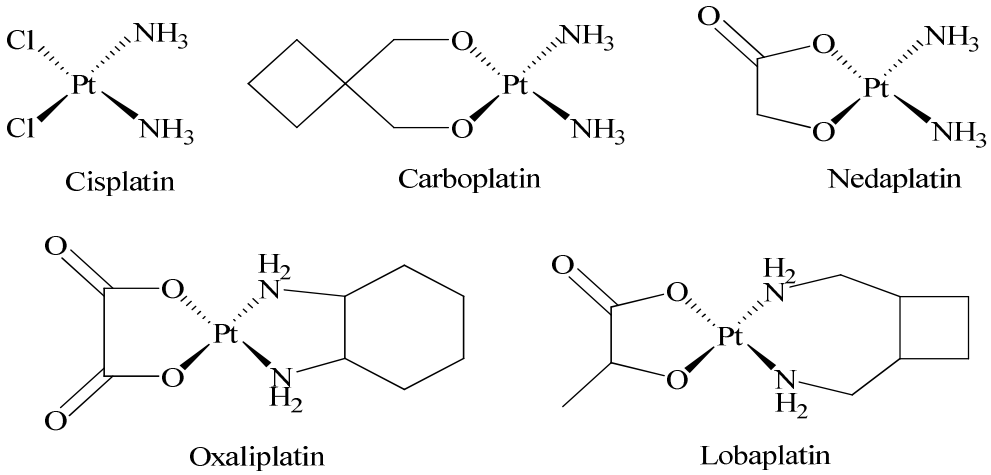


Figure 1.1. Structures of different platinum complexes.

However, it must be noted that only a limited number of tumors could be treated with platinum-based anticancer drugs and the patients suffered from significant side effects (such as gastrointestinal and hematological toxicity). Additionally, drug-resistance phenomena lowered the impact of these agents. In order to overcome these disadvantages, current strategies in the development of novel metallodrugs focused more and more on the use of transition metal complexes containing improved organic ligands [2].

N-heterocyclic carbenes (NHCs) are an interesting class of ligands with donor properties similar to

phosphines. They are generally derived from the so-called persistent carbenes, which are stable compounds of divalent carbons. As they are strongly stabilized by π-donating substituents, NHCs are good σ-donors [3, 4].

Metal-NHC complexes are mostly used in catalytic applications. Recently, NHCs came into the focus as carrier ligands for cytotoxic metal complexes because they perfectly fit prerequisites for an efficient drug design and fast optimization. NHCs are readily accessible in few steps and their substituents can be widely varied allowing an easy fine-tuning of both the physicochemical properties and the reactivity in biological medium of the final metal-NHC complexes. Additionally, their high stability and ease of derivatization make them suitable candidates for drug development [4].

1.2. Silver *N*-Heterocyclic Carbene Complexes

Silver complexes were used as antimicrobial agents for many years and found applications as antiseptics now [3]. Some silver complexes exhibited antitumor activity *in vitro* and *in vivo*, too. It has been reported that silver complexes derived from coumarin exhibited antitumor activity against certain types of cancer [5] and silver carboxylate dimers possessed antitumor potency [6]. Moreover, some silver complexes with phosphine ligands could even against cisplatin resistant cell lines [7] and a novel hydrogen bonded bimetallic supramolecular coordination polymer {[$SnMe_3$(bpe)][$Ag(CN)_2$]·$2H_2O$} exhibited specific *in vivo* and *in vitro* antitumor effects [8].

All silver complexes seem to have the same mode of action which involves the release of Ag^+ ions that enter cell membranes and disrupt their function. However, the big problem with the existing silver drugs (such as silver sulfadiazine) is that they lose their effects quickly due to rapid release of the Ag^+ ions. Thus, it is important for silver complexes with strongly coordinating ligands which can prevent the quick release of silver ions. A very bright strategy to overcome these was followed by employing Ag-NHC complexes [3, 4].

Ag-NHC complexes are synthesized from imidazolium salts and Ag_2O, Ag_2CO_3 or AgOAc and contain relatively strong silver–carbon bonds. Due to their increased stability, they can overcome the problems displayed by conventional silver antibiotics such as fast loss of activity or sulfonamide resistance of pathogens [3]. Since the release of silver ions for Ag-NHC complexes is retarded as compared to ionic silver complexes such as $AgNO_3$, they are expected to act as antimicrobial agents. This research was mainly done by Youngs *et al.* [3].

Encouraging by the results from other groups regarding antitumor activity of silver complexes, Youngs *et al.* described a series of Ag-NHC complexes derived from 4,5-dichloro-1*H*-imidazole (*e.g.* **1**, Figure 1.2) [9]. All complexes exhibited cytotoxic activity against ovarian (OVCAR-3) and breast (MB157) cancer cells *in vitro*. However, they had little effects on cervical (Hela) cancer cells. Similar to this result, a low cytotoxic silver complex (**2**) on HeLa cells (3% proliferation inhibition at a 10 μM concentration) was reported by Panda *et al.* [10]. Further *in vivo* studies showed that [4,5-dichloro-1,3-dimethylimidazol-2-ylidene]silver(I) acetate (**1**) was active against ovarian cancer in mice [9].

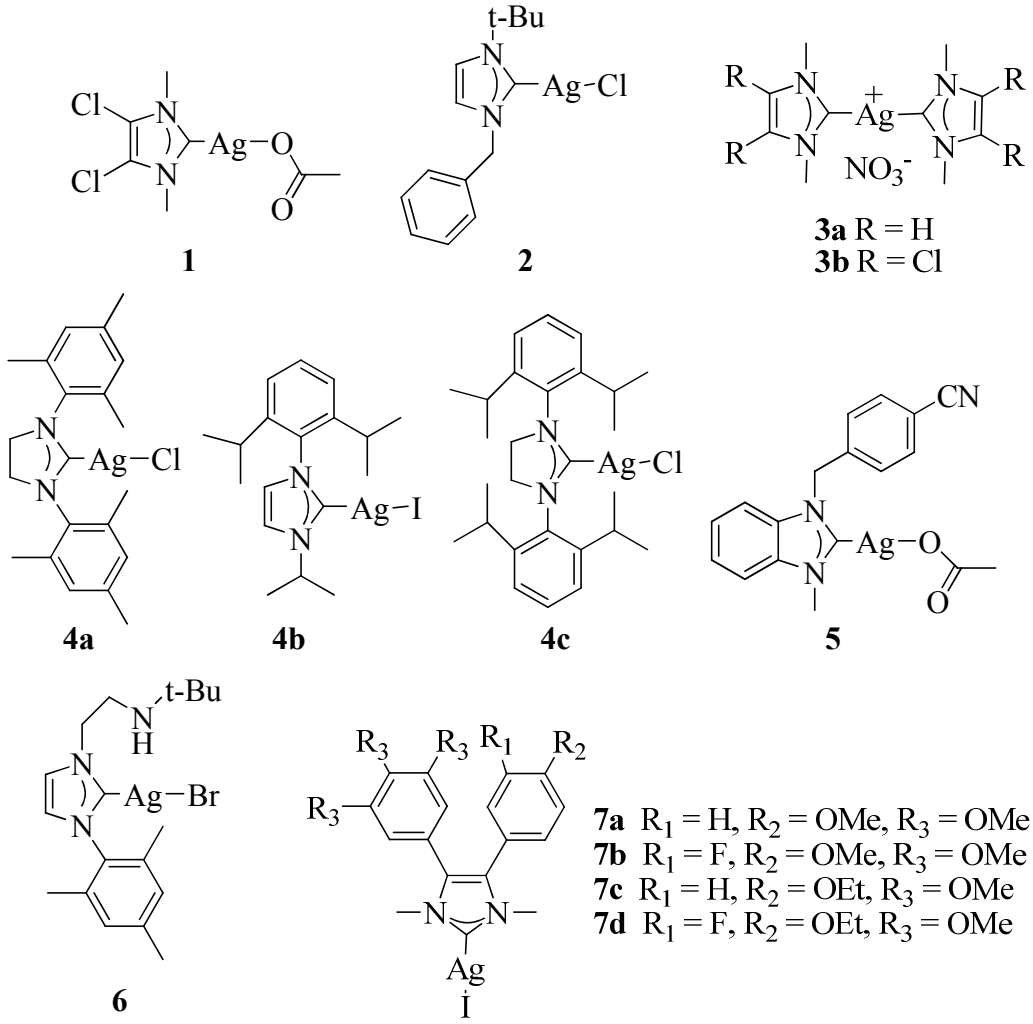


Figure 1.2. Structures of several Ag-NHC complexes.

Later, two Ag-bis(NHC) complexes {[bis(1,3-dimethylimidazol-2-ylidene)]silver(I) nitrate (**3a**) and [bis(4,5-dichloro-1,3-dimethylimidazol-2-ylidene)]silver(I) nitrate (**3b**)} (Figure 1.2) were prepared by the same group and displayed similar antitumor efficacy against H460 lung cancer cells. However, they were less cytotoxic than cisplatin [11].

Recently, the groups of Gautier and Morel reported a series of saturated and unsaturated Ag-NHC complexes (*e.g.* **4a**, Figure 1.2), which exhibited higher cytotoxic effects than cisplatin against various cell lines. This effect was found to be in part from the use of bulky *N*-substituents or variation of saturation between the 4 and 5 position of the imidazole ring [12].

Fourteen Ag–NHC complexes including **4a** with significant antibacterial properties were reported by Ronland *et al.*. Slight differences in the NHC ligand structure induced dramatic changes in the activity. Some complexes were efficient at low concentrations against resistant strains of *S. aureus* that

caused major problems to public health. Due to synergistic effects, these complexes could also restore the activity of ciprofloxacin against resistant *S. aureus* NorA at very low concentrations. Interestingly, the most active complex with the lowest cytotoxicity on the EPC cell line is **4a** which could be a potential candidate for the development of new topical antibacterial drugs. In addition, two complexes (**4b** and **4c**, Figure 1.2) showed high cytotoxicity towards MRC5 and EPC cell lines. The influence of the NHC structure on antibacterial activity and cytotoxicity was further investigated to explore their potential as antibacterial and antitumor agents [13].

More recently, a series of benzyl-NHC silver acetate complexes which displayed antitumor activity toward Caki-1 (a kidney cancer cell line) cells was developed by Tacke and coworkers [14-18]. One of these compounds [1-methyl-3-(4-cyanobenzyl)benzimidazole-2-ylidene]silver(I) acetate (**5**, Figure 1.2) showed the highest cytotoxicity (IC_{50} = 1.2 μM) against Caki-1 cells, up to now for the Ag-NHC complexes synthesized in this research group. This complex was about 3-fold more cytotoxic than cisplatin (IC_{50} = 3.3 μM) [16].

In addition, Li *et al*. developed an Ag-NHC complex bearing an amino-NHC ligand [19]. Unfortunately, this complex was less active than the corresponding Au(I)-NHC and Pd(II)-NHC complexes as well as cisplatin against breast carcinoma (MCF-7 and MDA-MB 231) and glioblastoma (U-87 MG) cell lines.

Schobert *et al.* combined *N*-methyl-4,5-diarylimidazolium salts, which modeled on the naturally occurring vascular disrupting anticancer drug combretastatin A-4 and displayed promising antitumor effects, with silver fragment (**7a-d**) [20]. These silver complexes showed distinct antiproliferative effects in the selected cell lines but less cytotoxicity than the corresponding Au(I)-NHC complexes. In addition, the replacement of the *p*-methoxy group (**7a**, **7b**) by a *p*-ethoxy group (**7c**, **7d**) led to erratic alterations of activities of the silver complexes.

1.3. Gold *N*-Heterocyclic Carbene Complexes

The application of gold in medicine is traceable for several thousand years and gold complexes have been evaluated for many different pharmaceutical purposes, notably cancer and arthritis. The spectrum of gold complexes with described cell growth inhibiting properties comprises a large variety of different ligands attached to gold in the oxidation states +I or +III [21, 22]. Based on the great structural variety of the used ligands, a unique mode of action or pharmacological profile is unlikely to exist. In particular, direct DNA damage, modification of the cell cycle, mitochondrial damage including thioredoxin reductase (TrxR) inhibition, proteasome inhibition, modulation of specific kinases, and other cellular processes affected by gold compounds, which eventually trigger apoptosis, seem to play a major role in the mechanism of action of gold compounds [22].

Two lead structures for antitumor active gold complexes are auranofin and its chloro analogue Et_3PAuCl (Figure 1.3) [22]. Both complexes exhibit their antitumor activities mainly due to the inhibition of the enzyme TrxR. The mammalian TrxR is a family of selenocysteine containing pyridine nucleotide-disulfide oxido-reductases and its major cytosolic forms are known as TrxR1 (cytosolic), TrxR2 (mitochondrial), and TrxR3 (testis specific) [22]. Since this enzyme is relevant for the control of mitochondrial function and the intracellular redox state, it is associated with many cellular processes, such as antioxidant defense and redox homeostasis. Moreover, it is found at elevated levels in human tumor cell lines. Gold complexes are in general good inhibitors of this enzyme. They demonstrated strong affinity for TrxR leading to inactivation due to the formation of a covalent bond at the Se-center of the enzyme [22].

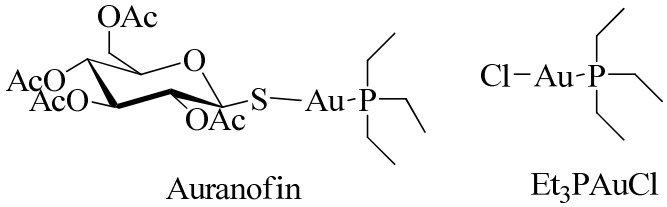


Figure 1.3. Structures of auranofin and Et_3PAuCl complexes.

Indeed, auranofin is in widespread clinical use as an antiarthritic drug and now under investigation as means of reducing the viral reservoir of HIV that lies latent in the body's T-cells [23]. This compound has also been shown to be useful for the treatment of tumors although it has not been approved for applications in this area. This complex inhibited DNA, RNA, and protein synthesis at cytotoxic con-

centrations, but unlike cisplatin it did not directly interact with DNA. The cytotoxic activity, cellular association, and efflux were dose-, time-, and temperature-dependent. Morphological changes (such as surface membrane changes or cell rounding) were observed under exposure of cells to auranofin [24].

Previous structure-activity relationship (SAR) studies on auranofin and Et_3PAuCl indicated that the phosphine ligand is more important for the biological potency than the halide or the thioglucose. Exchange of the carbohydrate ligand of auranofin (or the chlorine ligand of Et_3PAuCl) does not lead to a loss of antitumor activity *in vitro* [25, 26]. Of late, there has been considerable interest in NHCs as alternatives to phosphines as ligands for the soft Au(I) ion. As mentioned above (Section 1.1), NHC ligands have similar donor properties to phosphines, but they are usually more easily synthesized [3, 12].

The application of Au(I)-NHC complexes for targeting mitochondrial cell death pathways was widely studied. Following the successful application of gold phosphine complexes as antitumor agents [27], Berners-Price *et al.* reported the induction of mitochondrial permeability transition in isolated rat liver mitochondria by dinuclear Au(I)-bis(NHC) complexes [28-33]. The wingtip groups were modified in order to adjust the lipophilic character of the complexes, a critical factor for targeting malignant cells. Antimitochondrial effects (induction of Ca^{2+}sensitive mitochondrial swelling) were also observed for a series of mononuclear, linear, cationic Au-NHC complexes which was synthesized by the same group. In accordance with previous studies on Au(I) species, which demonstrated that the bioactivity can be influenced by fine-tuning of the lipophilicity, the onset of mitochondrial swelling was most rapidly induced by the complexes with the highest lipophilicity. A complex (**8**) with significant antimitochondrial property and intermediate lipophilicity was selected for further studies. It was found that **8** could selectively induce apoptosis *via* the activation of caspase 9 and caspase 3 in cancer cells, but not normal cells. Furthermore, **8** selectively inhibited TrxR activity but not glutathione reductase (GR) activity in MDA-MB 231 cells and was accumulated in mitochondria [32].

The same group then took advantage of the luminescence associated with aurophilic interactions in dinuclear Au-NHC complex (**9a** and **9b**) to visualize its intracellular localization [34]. Fluorescence confocal microscopy images of mouse macrophage cancer cells incubated with complex **9b** showed an intracellular localization to lysosomes along with the preservation of cell morphology.

With the same purpose of targeting mitochondria, Raubenheimer *et al.* designed a bis-ferrocenylcarbene Au(I) complex **10**, which exhibited higher growth inhibition potency at lower concentrations than cisplatin against two of the three tested cancer cell lines. It is possible that the antitumor activity

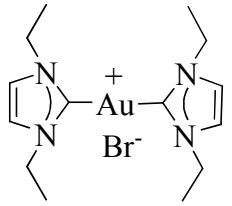
of the complex was enhanced by the presence of the ferrocene fragment [35].

Youngs's group also developed two Au(I)-bis(NHC) complexes (**11a** and **11b**) *via* transmetallation of their silver precursors (**3a** and **3b**) with chloro dimethylsulfide gold [11]. Both complexes showed similar antiproliferative efficacy against H460 lung cancer cell line but less active than cisplatin.

Taking advantage of their developments in the synthesis of novel Au(I)-NHC complexes and the associate synthetic routes facilitated by a versatile Au(I)-NHC synthon, two different series (neutral and cationic) gold complexes were prepared and screened against LNCaP (prostate carcinoma) and MDA-MB 231 cell lines by Nolan and his coworkers [36]. The cationic complexes were more effective than the neutral complexes, possible because they could cause cell death by an apoptotic pathway in cancer cell lines [23]. Among the neutral complexes, **12a** and **12b** are the most effective and were more potent than cisplatin. In addition, both complexes exhibited similar cytotoxic effects on normal prostate, breast epithelial cells and tumor cells from the same tissue.

More recently, a series of Au(I)-NHC complexes was rational designed by Ott *et al*. based on available crystal structure data of the gold phosphole complex chloro[1-phenyl-2,5-di(2-pyridyl)-phosphole]gold(I) (GoPI) in the active site of GR. The target complexes were based on a benzimidazol-2-ylidene core and demonstrated a good stability against the thiol glutathione [37]. TrxR was selectively inhibited by these complexes in comparison to the closely related enzyme GR, and all complexes triggered significant antiproliferative effects in cultured tumor cells. Unfortunately, the expected enhanced activity with increasing the lipophilicity/surface volume of the residues at the benzimidazol-2-ylidene nitrogens could not be observed. The tumor selective behavior was not observed in these complexes as comparative experiments in nontumorigenic cell lines (HEK-293 human embryonic kidney cells and HFF human foreskin fibroblasts) afforded similar activities.

More detailed experiments on chloro[1,3-diethylbenzimidazol-2-ylidene]gold(I) **13a** revealed a distinct pharmacodynamic profile including the high increase of reactive oxygen species (ROS) formation, apoptosis induction, strong effects on cellular metabolism (related to cell surface properties, respiration, and glycolysis), inhibition of mitochondrial respiration and activity against resistant cell lines. It should also be noted that the corresponding free ligand was ineffective in all comparative experiments, which confirmed that the presence of the Au(I) center was necessary to obtain compounds with the described bioactivities.



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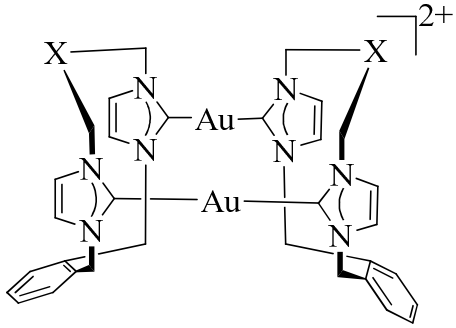
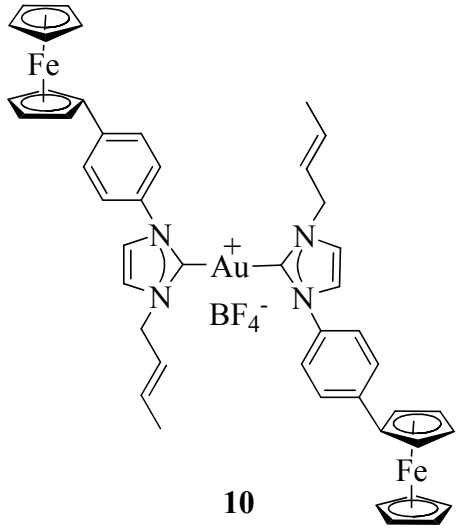


9a X = 1,2-phenyl
9b X = CH_2



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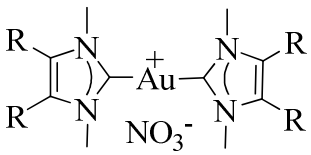
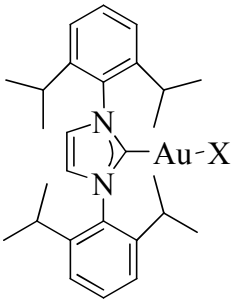
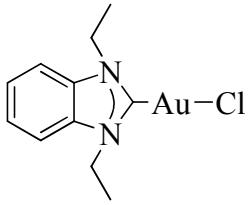


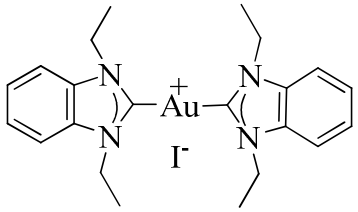
11a R = H
11b R = Cl



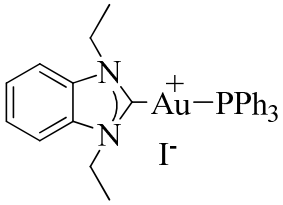
12a X = OH
12b X = Cl



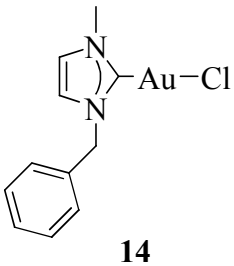
13a



13b



13c



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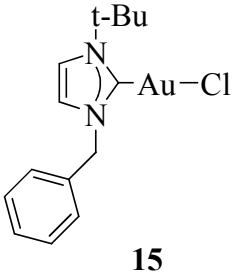

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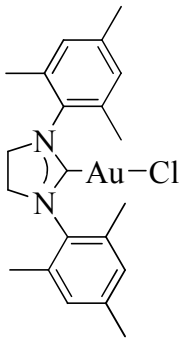

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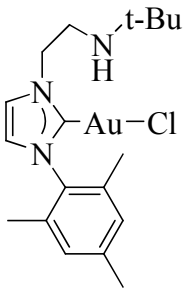

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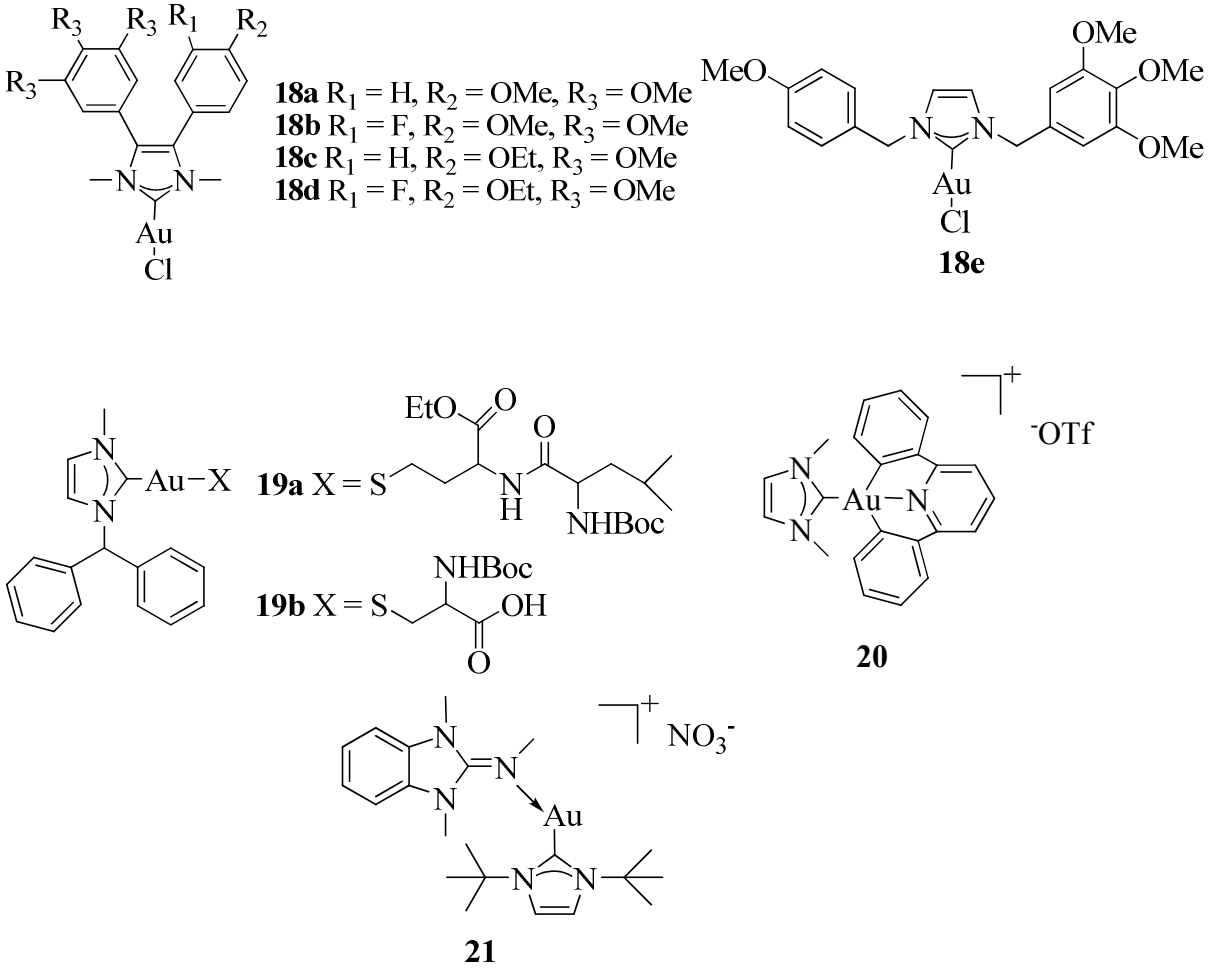


Figure 1.4. Structures of Au-NHC complexes.

Next, to modify the pharmacodynamic properties of **13a**, an additional NHC ligand and a triphenylphosphine were chosen by the same group, respectively [38]. The coordination with these neutral ligands led to the formation of cationic species (**13b** and **13c**). The introduction of a positive charge turned out to be a key feature to increase the cellular uptake, induce mitochondrial accumulation, and improve general cytotoxic properties. This could be related to effects commonly known for delocalized lipophilic cations [27, 39]. The modulation of the stability of the coordinative bonds of the complexes affected the reactivity toward the target enzyme TrxR. These two cationic complexes might provide a useful compromise between good inhibitory effects against TrxR and strong antiproliferative/antimitochondrial properties.

In the course of identifying new potential targets for cancer treatment, a series of Au(I)-NHC complexes were synthesized and characterized by Barrios *et al.* [40]. Along with auranofin, they were tested as inhibitors of the cysteine-dependent protein tyrosine phosphatases (PTPs). PTPs are a family of

enzymes that play integral roles in various physiological processes and they have been shown to be involved in numerous diseases including cancer. The study of the inhibition of PTPs by Au(I)-NHC complexes and auranofin, would take advantage of the high affinity of gold for sulfur. These studies revealed that the Au(I)-NHC complex **14** was a better inhibitor of PTP than auranofin with a IC_{50} range from 10 to 40 μM on four PTP types [40].

A structurally different Au(I)-NHC complex **15** was studied by Panda *et al.* and displayed very low inhibition of HeLa cell proliferation (1.7% at 10 μM) as previously observed for the silver analogue **2** [10].

In contrast to the low inhibitions of complex **15**, the groups of Gautier and Morel developed a more active complex **16**, which displayed IC_{50} in the same range as cisplatin on five cancerous cell lines [12]. However, this complex was less cytotoxic than the corresponding Ag-NHC complex **4a**.

Li *et al.* also developed an Au(I)-NHC complex (**17**) bearing an amino-NHC ligand, which exhibited selective antiproliferative potency against U-87 MG cells [19]. However, this complex did not inhibit human TrxR indicating that it might trigger apoptosis through a DNA dependent mechanism. Further experiment indicated that this complex mediated S-phase arrest *via* down-regulation of cyclin A, cyclin B1, and cdk2. Apoptosis was induced by this substance in U-87 MG cells through a p53-bak pathway. Moreover, the complex participated in an important molecular event that mediated negative regulation of p21. Consequently, it had therapeutic potential in the treatment of glioblastoma in the case of p21-dependent resistance of p53-induced apoptosis.

Five Au(I)-NHC complexes **18a-e** derived from *N*-methyl-4,5-diarylimidazolium salts were prepared by Schobert's group [20]. All complexes showed cytotoxic activity with IC_{50} in the micromolar range and distinct selectivity for certain cell lines. In addition, they were generally more cytotoxic than their respective silver precursors **7a-d**. In contrast to related metal-free 1-methyl-4,5-diarylimidazoles, the complexes did not noticeably inhibit the polymerisation of tubulin to give microtubules. The cellular uptake of complexes **18a-d** occurred mainly *via* the copper transporter (Ctr1) and the organic cation transporters (OCT-1/2). In addition, complex **18e** was accumulated preferentially *via* the organic cation transporters and by Na^+/K^+-dependent endocytosis. These gold complexes seem to operate a mechanism different from that of the parent 1-methylimidazolium ligands.

Given the promise of Au-NHC complexes for medicinal applications, Metzler-Nolte *et al.* extended their efforts in metal–bioconjugate systems to Au-NHC complexes and reported a series of Au-NHC

complexes including Au(III)-NHC species as well as Au(I)-NHC derivatives containing cysteine thiolate ligands (**19a** and **19b**) [41]. Both the Au(III)-NHC complexes and cysteine-modified NHC derivatives showed similar biological activities compared to related Au(I)-NHC complexes without cysteine-derived ligands. These results indicated that the development of structurally diverse bioactive Au-NHC species is possible. The activity as well as pharmacokinetic properties of the Au-NHC complexes can be optimized by appropriate choice of the oxidation state of the metal and more sophisticated ligands. Due to the NHC complexes could be functionalized with peptide ligands, this research opens the possibility of developing metal-NHC derivatives for targeted drug delivery.

To overcome the instability of Au(III) complexes under physiological conditions, Che *et al.* reported a panel of stable $[Au(R–C^N^C)NHC]^+$ (HC^N^CH = 2,6-diphenylpyridine) complexes, which displayed prominent *in vitro* antitumor properties [42]. Among them, [gold(III)(C^N^C)(1,3-dimethylimidazol-2-ylidene)] trifluoromethanesulfonate (**20**) showed high *in vitro* and *in vivo* antitumor activity and induced DNA strand breaks and subsequent cell death presumably in part through the stabilization of TopoI-linked DNA.

Inspired by the scarcity of Au(I) complexes containing ylideneamine ligands and together with the biological activity displayed by ylideneamine metal complexes, Cronje *et al*. reported on the preparation and extensive characterization of a series of pentafluorophenyl-, phosphine- and NHC-containing ylideneamine Au(I) complexes. These complexes were evaluated for their potential as antitumor and antimalarial agents [43]. All complexes including Au(I)-NHC complex **21** exhibited a significant increase in growth inhibition with respect to the free ligands against cervical carcinoma cells and 3D7 strain of *Plasmodium falciparum*. However they were less active than cisplatin in the antitumor test and chloroquine in the antimalarial test, respectively.

1.4. Platinum *N*-Heterocyclic Carbene Complexes

Platinum-based antitumor drugs play the leading role in the treatment of various solid tumors such as genitourinary, colorectal, and non-small cell lung cancers. As an outstanding representative, cisplatin has been used for more than 30 years in standard chemotherapy regimens either as single drug or in combination with other cytotoxic agents or radiotherapy [1].

Cisplatin exerts its antitumor activity through interacting with DNA and forming adducts that interfere with transcription and replication, thereby triggering programmed cell death (apoptosis) [1]. The interactions of cisplatin with DNA have been extensively studied and it is now well known that a cis-Pt-G-G intrastrand crosslink is the critical lesion that leads to cisplatin toxicity. However, as discussed above (Section 1.1) the effectiveness of cisplatin is hindered by the phenomenon of tumor resistance and toxic side effects [2].

With the aim of overcoming these limitations, a huge number of platinum complexes were extensively investigated over the years by giving specific goals. These include reduction in toxicity of cisplatin (such as nausea, vomiting, nephrotoxcity, neurotoxicity and ototoxicity), circumvention of the acquired drug resistance observed in certain tumors, increased spectrum of activity since cisplatin is inefficient against some of the commonest tumors (*e.g.* colon and breast) and oral administration for the new anticancer drugs. In addition, enormous efforts have been directed to understand the mechanism of the cytotoxic activity of cisplatin [1].

Of late, some pioneering ways towards the synthesis of novel platinum antitumor agents have emerged. Those are based on changing the coordinated nitrogen ligand or altering the leaving groups [1, 2]. Other strategies have focused on changing the type of the metal center or applying Pt(IV) complexes that are less reactive, less toxic than Pt(II) complexes and relatively more soluble in water. Researches also have been shifted to discover "nonclassical" drugs that can act in a manner different from cisplatin. Abnormal structures that violate the empirical SAR of platinum complexes lacking NH, NH_2, or NH_3 ligands and multinuclear complexes are examples of these complexes that are designed to circumvent cisplatin resistance and enhance its activity.

Pt-NHC complexes have been highlighted as a promising and original platform for building new cytotoxic drugs of the cisplatin series [44-48]. Mixed NHC-amine Pt(II) complexes were prepared *via* a facile and modular two-step sequence leading to *trans*-configured square planar species by Marinetti *et al.* [47]. Their efficiency against both cisplatin-sensitive (CEM and H460) and -resistant (A2780/DDP,

CH1/DDP, and SK-OV-3) cell lines was demonstrated by *in vitro* experiment.

All novel complexes exhibited cytotoxic activities with IC_{50} at a micromolar range. IC_{50} of the measured complexes against CEM T leukemia cells was in the 0.6-2.7 μM range, generally significantly lower than cisplatin (3.0 μM) under similar experimental conditions. And some of the complexes also outperformed cisplatin against H460 lung cancer cells. In addition, the selective complexes displayed high cytotoxicity against human ovarian A2780/DDP cells (IC_{50} = 1.2-1.8 μM) and CH1/DDP cells (IC_{50} = 2.1-2.4 μM) irrespective of modulations of the NHC scaffold. Moreover, two of the complexes (**22a** and **22b**) were also significantly more potent than cisplatin against SK-OV-3 cells, with IC_{50} of 2.8 and 2.6 μM, vs 6.1 μM for cisplatin.

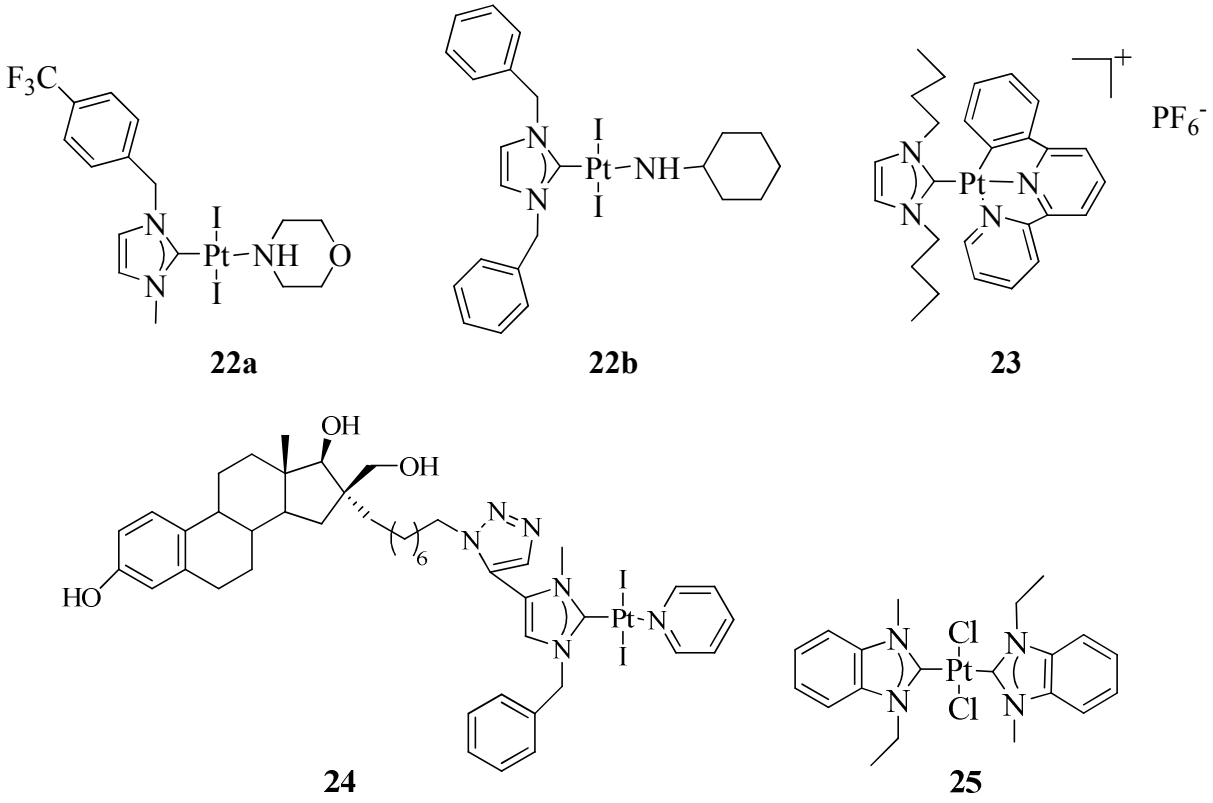


Figure 1.5. Structures of Pt-NHC complexes.

This study afforded a potent and innovative new chemical platform for cytotoxic drugs of the Pt(II) series, based on NHC ligands [47]. It demonstrated the potential antitumor activity of (NHC)PtI_2(amine) complexes and therefore implemented a range of ligand pairs affording platinum complexes. These results also demonstrated that the (NHC)Pt(II) unit is an easily available, highly tunable structure, potentially amenable to tumor-specific drug targeting and/or delivery approach, *via* a specific design of its carbene group.

Contrary to most platinum-based antitumor agents which target DNA, coordination of NHC ligands to cyclometalated Pt(II) complexes conferred these luminescent complexes to other cellular targets. The strong Pt–carbene bond(s) rendered the Pt(II) complexes to display unique photophysical properties and enhanced stability against biological reduction and ligand exchange reactions. Che *et al*. reported some Pt complexes which were highly cytotoxic and displayed high specificity to cancerous cells [49]. Among them, [(C^N^N)Pt(II)(*N*,*N*'-^{n}Bu$_2$NHC)]PF$_6$ (**23**, where HC^N^N = 6-phenyl-2,2'-bipyridine) with a lipophilic carbon chain on the carbene ligand induced apoptosis in cancer cells, demonstrated an enhancing synergistic effect with cisplatin *in vitro*, and displayed potent *in vivo* activities using nude mice models.

As this complex was strongly emissive, its cellular localization could be traced using emission microscopy. In contrast to common platinum-based antitumor agents, **23** did not accumulate in the vicinity of DNA but preferentially accumulated in cytoplasmic structures including sites where active survivin, an inhibitor of apoptosis, is located. More detailed experiments on this complex revealed that it significantly inhibited the expression of survivin, activates poly (ADP-ribose) polymerase (PARP) and induced apoptosis in cancer cells. *In vivo* results showed that injection of **23** at 3 mg kg^{-1} significantly inhibited the NCI-H460 tumor growth, did not cause death of mice, and had no significant weight loss compared to that in the vehicle control group. Given the ease of structural modification of NHC ligand to alter the overall biological activities, this type of [(C^N^N)Pt(II)(NHC)]$^{+}$ complexes having unique photophysical properties provided an entry to a new class of potential anticancer drug leads.

In order to possibly enhance selectivity and specificity towards cancer cells, Bellemin-Laponnaz *et al.* designed an oestrogen functionalized Pt(II) complex **24** as a possible candidate to target hormone dependent diseases (*e.g.* breast cancer) [44]. This complex was obtained by reaction of functionalisation of platinum complex with the oestrogen derived azide *via* using ruthenium-catalyzed azide–alkyne cycloaddition. Despite the ability of **24** to act as potential chemotherapeutic agents is currently under study, they are currently extending the scope of this method to a more diverse set of azides with the aim to generate chemical libraries and later to endow cytotoxic NHC complexes of transition metals with new properties.

Ciftci *et al.* synthesized a Pt–bis(NHC) complex (**25**) and evaluated its effects on oxidative and histological damage in cardiac tissue of rats [45]. Results indicated that **25** caused more oxidative stress, testicular damage and reproductive toxicity in testis tissue than cisplatin. Lately, in odor to evaluate

the reproductive toxicity of **25** and to compare these toxic effects with those of cisplatin, they determined the levels of oxidative, histological, hormonal and spermatological damage in testis tissues of rats after administration of cisplatin and **25** [46]. Both complexes induced oxidative and myocardial damage in rats. On the other hand, **25** caused more oxidative stress and myocardial damage in heart tissue than cisplatin. These results might suggest that therapeutic effects of novel Pt-bis(NHC) complex as an anticancer drug may be superior to cisplatin.

1.5. Palladium and Copper *N*-Heterocyclic Carbene Complexes

Besides the mentioned silver, gold, and platinum derivatives, NHC complexes with palladium and copper have also been recently reported to exhibit antiproliferative properties.

On the basis of the structural and thermodynamic analogy between Pt(II) and Pd(II) complexes, there is also much interest in the study of Pd(II) derivatives as potential anticancer drugs [3, 12, 50-52]. Many new mononuclear, dinuclear and multinuclear palladium complexes with reduced crossresistance to cisplatin, decreased toxicity and high specificity have been developed. Similar to platinum agents, DNA is also their major target in the cell. The Pd(II) ions are capable of interacting with DNA, thus enabling cross bindings and inhibiting its synthesis as well as inducing apoptosis. Palladium complexes might materialize a concept of tumor targeting which would result in drugs with other spectrum of activity and lack of cross-resistance as compared with platinum drugs [51].

Panda *et al.* reported the study of two Pd(II)-NHC complexes: one bis(NHC) complex **26a** and one mixed complex **26b**, with NHC and pyridine ligands, pyridine being a typical ligand of the active *trans* platinum complexes [10]. In both complexes the palladium centre was substituted in a *trans*-geometry.

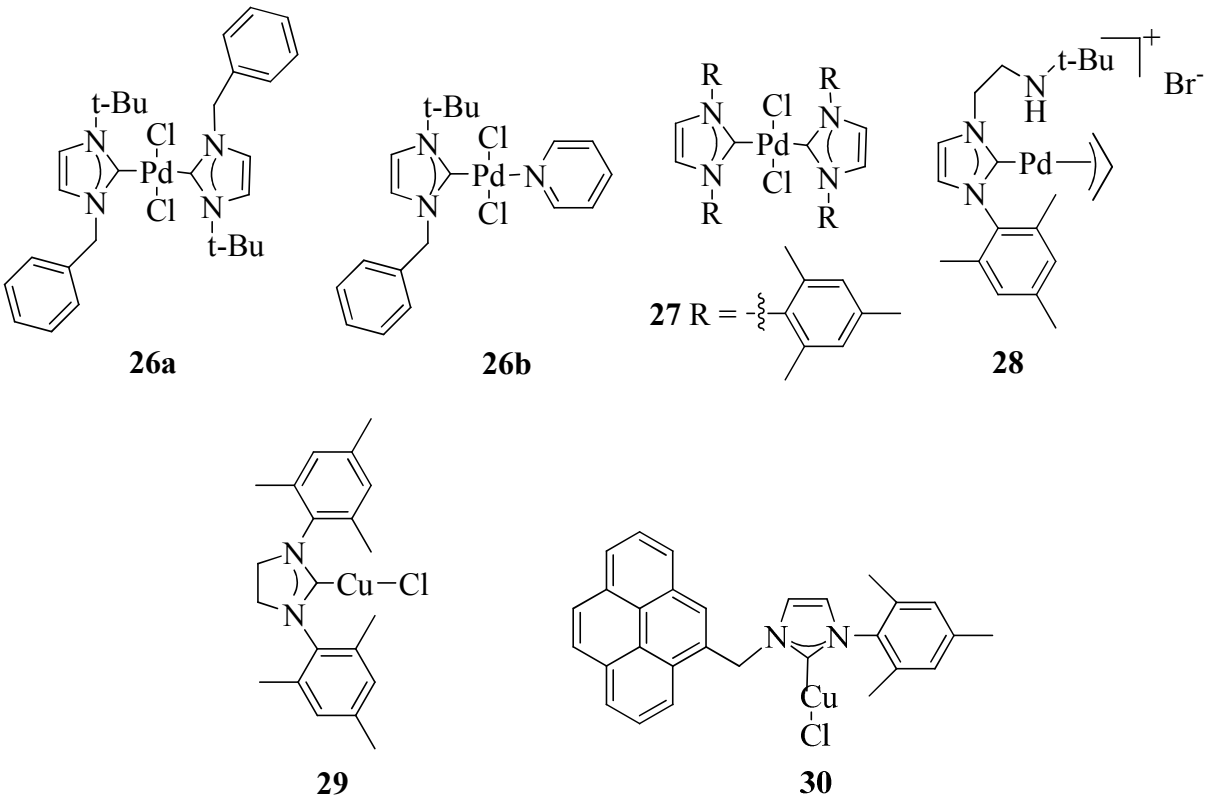


Figure 1.6. Structures of Pd- and Cu-NHC complexes.

Specifically, **26a** showed more cytotoxicity (from 2- to 20-fold) than cisplatin against cervical cancer (HeLa), breast cancer (MCF-7), and colon carcinoma (HCT 116) cell lines. Further studies showed that **26a** inhibited tumor cell proliferation by arresting the cell cycle progression at the G2 phase, preventing the mitotic entry of the cell. This evidence suggested that the treated cells underwent programmed cell death through a p53-dependent pathway. The combination of these results clearly demonstrated that complex **26a** followed the same cellular pathway than cisplatin.

The groups of Gautier and Morel also reported a Pd(II)-bis(NHC) complex (*trans*[$PdCl_2(IMes)_2$]) **27** and briefly evaluated *in vitro* the binding capacity of this complex on guanosine and its genotoxicity on plasmid DNA [12]. However, no adduct of the palladium complex with guanosine could be detected by NMR spectroscopy. The same lack of reactivity was observed with the plasmid which kept its supercoiled form.

In addition, Li *et al.* developed a Pd(II)-NHC complex (**28**) with higher cytotoxic activities than cisplatin and the corresponding Au(I)-NHC and Ag-NHC complexes against breast cancer cells (MCF-7 and MDA-MB 231) [19]. The IC_{50} of **28** (4.50 μM) in MDA-MB 231 cells is 3-fold lower than that of Au(I)-NHC complex **17** (14.22 μM) and approximately 10-fold lower than that of Ag-NHC complex **6** (46.58 μM) and cisplatin (48.43 μM). This finding demonstrated that the antitumor activities of the amino-NHC metal complexes were not solely dependent on molecular hydrophobicity and that activities could be altered by the choice of the metal ion. Interestingly, IC_{50} of the complex displayed similar trends in the estrogen receptor positive (ER+) cell line MCF-7 and estrogen receptor negative (ER-) cell line MDA-MB 231, indicating that the effects on cell viability might be caused by an ER-independent pathway.

The coordination compounds of Cu(I,II) have been investigated as potential antitumor agents only in the last few decades, particularly after the discovery of cisplatin, although copper has a long history of medical application [3, 12, 53-56]. Copper, which is important for the function of several enzymes and proteins and involved a lot of physiological processes, may be less toxic than non essential metals, such as platinum.

Recently, a great variety of copper complexes were screened for their cytotoxicity and some complexes showed good antitumor activity *in vitro* and *in vivo*. It has become clear that copper compounds could act by different mechanisms compared with cisplatin and DNA does not represent the main and/or unique biological target for this class of metal complexes [54, 55, 57]. Indeed, besides DNA binding,

intercalation and cleavage activity, other ways lead to oxidative cell damage (such as superoxide dismutase mimetic activity and generation of ROS by redox-cycling) also have been described. Generally, these intracellular molecular events trigger cancer cell death through an apoptotic mechanism. Moreover, down regulation of antiapoptotic proteins (Bcl-2 and Bcl-XL) and a non-apoptotic form of programmed cell death (paraptosis) have been shown on human cancer cells treated with copper compounds. The later is very stimulating because it suggested the ability of triggering cell death even in cancer cells with multiple defects in the normal apoptotic pathway as, for instance, cisplatin resistant cells.

Cu(I)-NHC complexes were focused on antitumor activity due to their stability in numerous conditions which was evidenced by the different media in which they might be used as catalysts [58]. This relatively high stability might allow them to reach biological targets inside the cell. Cu(I)-NHC complexes could react with intracellular oxygen, produce ROS which would lead to oxidative attack of DNA as reported for bleomycin [12].

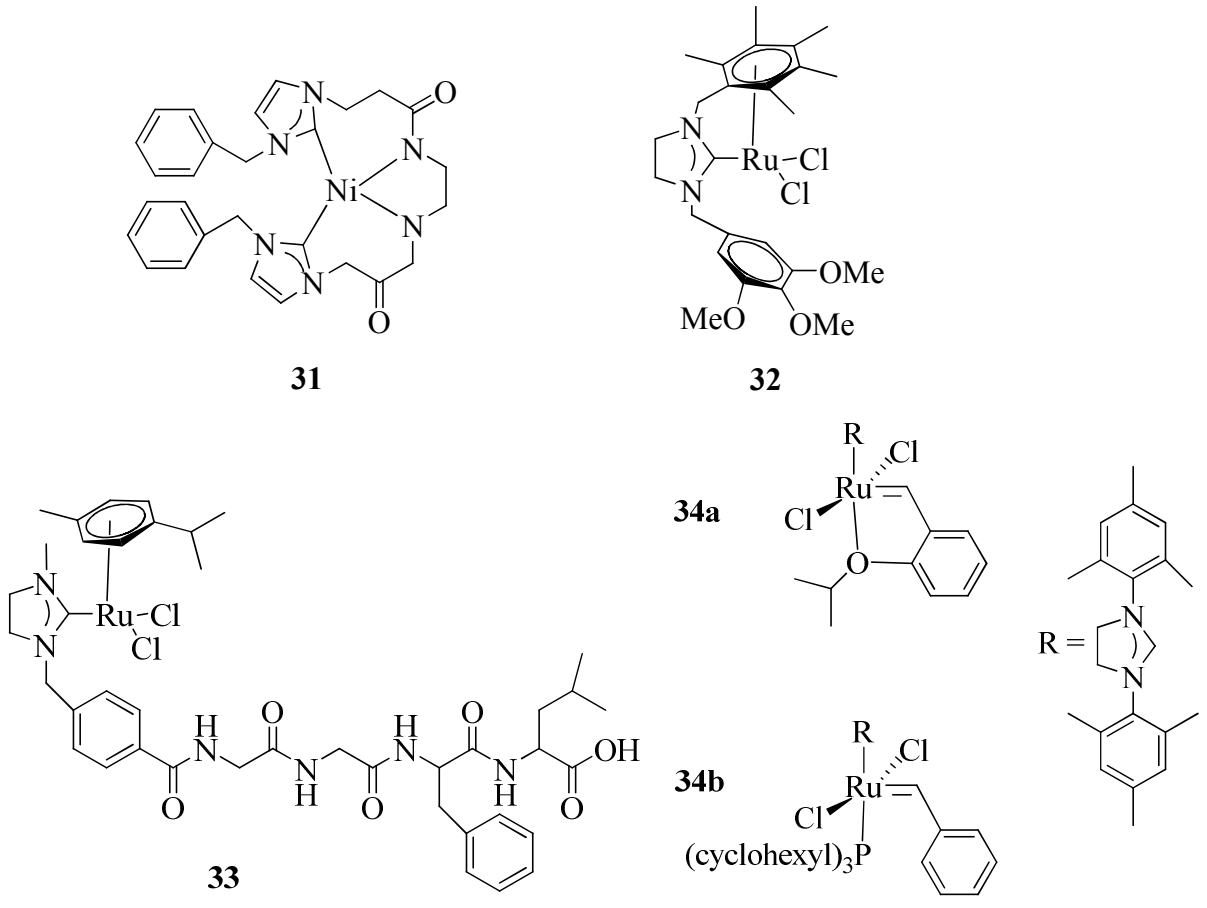
Cu(I)–NHC complex **29** showed high cytotoxicity against human cancer cells and was developed by the groups of Gautier and Morel [59]. An increase of cytotoxicity up to 150-fold compared with cisplatin in the HL60 cell line was observed. This higher cytotoxicity was preserved regardless of the nature of the cell line. Unlike cisplatin, this complex caused arrest of the cell cycle progression at the G1 phase concomitantly with apoptosis induction at low concentration. An aerobic radical process leading to a DNA strand break might be responsible for the observed cytotoxicity.

Later, the same groups reported a series of Cu(I)-NHC complexes which were mostly synthesized according to literature procedures [12]. Almost all complexes exhibited higher cytotoxicities than cisplatin on MCF-7 cells. One of these complexes (**30**) which was designed with the intent of using it for the localization of intracellular targets by fluorescence microscopy presented an intense fluorescence when excited around 360 nm.

1.6. Nickel and Ruthenium *N*-Heterocyclic Carbene Complexes

Nickel is an essential component in different types of enzymes such as urease, carbon monoxide dehydrogenase, and hydrogenase [50]. Recently, the apparent potential of nickel complexes in antitumor studies have been reported [50, 60, 61].

Three Ni-NHC complexes were described by Ghosh *et al*.. They showed less cytotoxic activity than $NiCl_2 \cdot 6H_2O$ against HeLa and MCF-7 human cancer cell lines and the CHO non-tumorigenic cell line [60]. Morphological studies on HeLa cells showed that **31** caused minimum surface abnormality on the cancer cells consistent with its reduced cytotoxic activity. These observations further substantiate the fact that drastic reduction in the cytotoxic activity of nickel was successfully achieved by encapsulation of the metal center in Ni-NHC complexes by employing a new class of tightly binding NHC ligand.



31 **32** **33** **34a** **34b**

Figure 1.7. Structures of Ni- and Ru-NHC complexes.

Ruthenium-based drugs are much less toxic than platinum-based drugs and capable of overcoming the resistance induced by platinum drugs in cancer cells [62]. These activities were attributed to the ability of ruthenium to mimic iron in binding to biological molecules, such as human serum albumin and transferrin, and to the selective activation to more reactive species by the reducing environment of solid tumors as compared to healthy tissues. This type of complexes has been designed to mimic platinum drugs, particularly for DNA-targeting. Unfortunately, the ruthenium complexes interact far more weakly with DNA relative to platinum complexes and the mechanisms of action of ruthenium-based antitumor complexes are comparatively unexplored. However, there is evidence to suggest that ruthenium complexes might directly interfere with specific proteins involved in signal transduction pathways and/or alter cell adhesion and migration processes [62].

Recently, Ciftci *et al.* compared the oxidative damage in rat heart tissue induced by Ru(II)-NHC (**32**) and Au(I)-NHC complexes which showed antitumor effects *in vitro* [63]. The results indicated that both complexes caused oxidative damage by suppressing to antioxidant defense systems and increasing to lipid peroxidation in heart tissue of rats. This effect was changed in a dose-dependent manner for Au(I)-NHC treatment. Additionally, it was observed that Au(I)-NHC complex was more cardiotoxic than Ru(II)-NHC which indicated that Ru(II)-NHC should be preferred to Au(I)-NHC for cancer treatment when they were used clinically.

To improve the solubility and stability of ruthenium complexes, Metzler-Nolte *et al.* described a way to synthesize ruthenium carbene peptide conjugates using solid phase synthesis without decomposition [64]. The synthesis of the first fully characterized metal-NHC peptide conjugate **33** containing a functionalized *p*-cymene Ru(II)-NHC ligand and the pseudoenkephalin peptide was reported. However, the ability of **33** to act as potential chemotherapeutic agents had not been evaluated. Due to the well-documented cytotoxic activity of *p*-cymene ruthenium complexes (such as the RAPTA complexes from Dyson's group [65-68]), the potential properties of this novel Ru(II)–NHC peptide conjugates need further investigation.

Ruthenium complexes of the Grubbs type, consisting of a central benzylidene dichlororuthenium core with additional phosphine or NHC ligands, have had an enormous impact on modern synthetic organic chemistry [69]. However, the possible biological relevance of these catalysts is unknown.

Encouraging by the promising biological activity of ruthenium complexes, Ott *et al.* evaluated the inhibition of enzymatic activities (TrxR, GR, proteases trypsin and cathepsin B) and cell growth

(MCF-7 and HT-29 cells) as well as on cellular metabolism of archetypical examples of Grubbs catalysts (**34a** and **34b**) [70]. The results indicated that **34a** was the most pronounced in terms of enzyme inhibition and antiproliferative effects in tumor cells. This complex interacted with TrxR and albumin considerably strong and followed a time-dependent process. However, both complexes were less active than benzimidazol-2-ylidene gold complexes reported in the same group in inhibition of enzyme TrxR and cell growth [37]. The observed triggering of biological effects might provide a rationale for the use of Ru(II)–NHC fragments in metallodrug design.

2. Background and Aims of the Research Project

Platinum-based drugs such as cisplatin or oxaliplatin are widely used in current tumor chemotherapy. However, severe side effects and frequent development of resistance phenomena complicate and hamper the clinical application [1, 2]. A very promising strategy to overcome these obstacles is the use of specific carriers and the change from platinum to other transition metals [65, 71].

NHCs came into the focus as carrier ligands for cytotoxic metal complexes because they perfectly fit prerequisites for an efficient drug design and fast optimization [3, 4, 12]. NHCs are readily accessible in few steps and their substituents can be widely varied allowing an easy fine-tuning of both the physicochemical properties and the reactivity in biological medium of the final NHC metal complexes. Despite extensive studies on NHC complexes in organometallic chemistry and catalysis, only a restricted array of biomedical applications have been reported so far for copper, gold, nickel, palladium, platinum, ruthenium and silver derivatives [3, 4, 12].

Among novel non-platinum based antitumor agents, gold complexes have recently gained attention because of their strong antiproliferative effects [21, 22, 27, 72]. Two lead structures are auranofin, which is well known for its anti-arthritic properties, and its chloro analogue Et_3PAuCl are very promising lead structures for the design of metal complexes with a mode of action different from cisplatin [23, 27, 72].

Both complexes exhibited their antitumor activities due to inhibition of the enzyme TrxR [22, 72]. In addition, previous SAR studies indicated that the phosphine ligand is more important for the biological potency than the halide or the thioglucose. Exchange of the carbohydrate ligand of auranofin (or the chlorine ligand of Et_3PAuCl) does not lead to a loss of antitumor activity [26]. Of late, there has been considerable interest in NHCs as alternatives to phosphines as ligands for the soft Au(I) ion. The relative ease of systematic modification of the NHC substituents and the comparable donor properties of NHCs to phosphines render NHCs attractive ligands [4, 73].

Encouraged of previously reported results of metal-NHC complexes with high antitumor activity [3, 4, 12], we started the examination of the antitumor activity of Ag-NHC complexes with pharmacologically active 4,5-diarylimidazole ligands. To achieve a high accumulation grade in tumor cells, the aromatic rings were 2-F, 3-F, 4-F, 4-OH, or 4-OCH_3 substituted. Such substitution patterns were already very successfully used in the case of [1,2-diarylethylenediamine]platinum(II) complexes [1, 74, 75]. The related NHC complexes can be considered as plain derivatives of these compounds.

Then, in continuation of the SAR study with NHC-silver-halide complexes derived from 4,5-

diarylimidazole we focused our attention on the design of analogous Au-NHC complexes. We supposed that the replacement of the phosphine in Et_3PAuCl by an NHC ligand with pharmacological activity (*e.g.* hormonal activity, cyclooxygenase (COX) inhibitory properties) could lead to new multi target antitumor agents including the TrxR.

Besides, we decided to investigate the influence of the halide exchange at the Au-NHC complexes on their pharmacological properties. We selected the 4-F and 4-OCH_3 (4-OH) substituted bromo[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I) complexes and exchanged the bromide by a second NHC ligand (Au(I)-bis(NHC)) to realize the concept of bivalent drugs or in relation to auranofin by 2′,3′,4′,6′-tetra-*O*-acetyl-*β*-*D*-glucopyranosyl-1-thiolate as well as a phosphine ligand (triphenylphosphine).

Finally, in order to investigate whether the gold centre is crucial for the antitumor activity of cationic [bis(1,3-diethyl-4,5-diarylimidazol-2-ylidene)]gold(I) bromide complexes, the imidazole ligands were connected by a methylene bridge and screened against cancer and bacterial cell lines.

The results are published as listed below:

Wukun Liu, Kerstin Bensdorf, Ulrich Abram, Ben Niu, Aruljothi Mariappan, Ronald Gust. Synthesis and biological studies of silver *N*-heterocyclic carbene complexes derived from 4,5-diarylimidazole. ***European Journal of Medicinal Chemistry*** **2011**, *46*(12), 5927-5934.

Wukun Liu, Kerstin Bensdorf, Maria Proetto, Ulrich Abram, Adelheid Hagenbach, Ronald Gust. NHC gold halide complexes derived from 4,5-diarylimidazole: synthesis, structural analysis, and pharmacological investigations as potential antitumor agents. ***Journal of Medicinal Chemistry*** **2011**, *54*(24), 8605-8615.

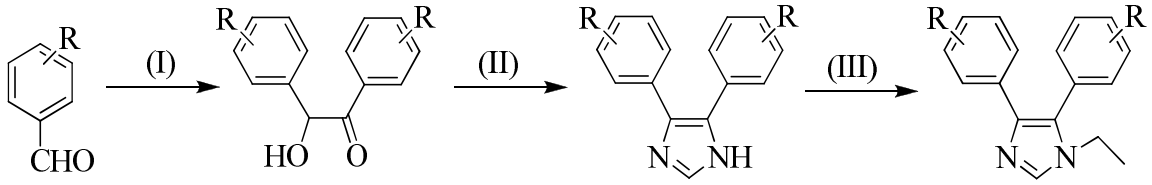
Wukun Liu, Kerstin Bensdorf, Maria Proetto, Adelheid Hagenbach, Ulrich Abram, Ronald Gust. Synthesis, characterization, and in vitro studies on bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I/III) Complexes. ***Journal of Medicinal Chemistry*** **2012**, *55*(8), 3713–3724.

Wukun Liu, Xiaohua Chen, Ronald Gust. Synthesis, pharmacological investigations of bis(4,5-diarylimidazol-2-ylidene) derivatives as antitumor and antibacterial agents ***Archiv der Pharmazie*** **2012** (DOI: 10.1002/ardp.201100474)

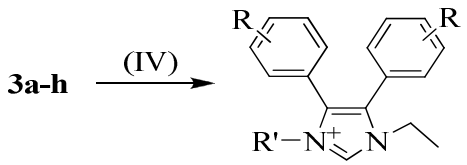
3. Description of the Work

3.1 Synthesis

3.1.1 Synthesis of Imidazolium Ligands



1a-g **2a-g** **3a-g**



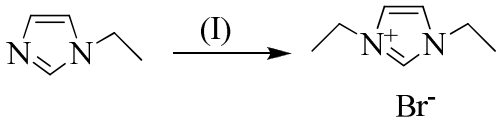
4a-j

Compounds	R	R'	X
1a, 2a, 3a, 4a	2-MeO	Et	Br
1b, 2b, 3b, 4b	3-MeO	Et	Br
1c, 2c, 3c, 4c	4-MeO	Et	Br
1d, 2d, 3d, 4d	2-F	Et	Br
1e, 2e, 3e, 4e	3-F	Et	Br
1f, 2f, 3f, 4f	4-F	Et	Br
1g, 2g, 3g, 4g	H	Et	Br
3h, 4h	4-OH	Et	Br
4i	4-MeO	$PhCH_2$	Cl
4j	4-F	$PhCH_2$	Cl

Scheme 3.1. Synthesis routes and structures of imidazolium compounds.
Reagents and conditions: (I) thiamine hydrochloride, water/ethanol 1:2, rt, 2-7 days, 49-62%; (II) formamide, reflux, 3 h, 61-80%; (III) 95% NaH, ethyl bromide, absolute THF, reflux, 2 h, 68-88%; (IV) ethyl bromide or chloromethyl benzene, CH_3CN, reflux, 48-72 h, 72-84%.

Compounds **1a-g** were obtained from commercially available substituted benzaldehydes *via* the catalysis of thiamine hydrochloride**.** For ring closure, yielding the respective imidazoles **2a-g**, the benzoines **1a-g** were heated in formamide to reflux for 3 h. Reaction of **2a-g** with NaH and ethyl bromide in absolute THF afforded the corresponding *N*-alkylation (**3a-g**) [76]. The hydroxyl-substituted imidazole

3h was generated from **3c** by ether cleavage with BBr_3 [76]. Subsequent reaction of **3a-h** and commercially available **3k** with ethyl bromide in CH_3CN yielded the imidazolium salts **4a-h** and **4k**. The same reaction of **3c** and **3f** with benzylchloride resulted in 1-benzyl-3-ethyl-4,5-diarylimidazolium chlorides **4i** and **4j**. All of the synthetic compounds gave satisfactory analytical and spectral data, which were in full accordance with their predicted structures.

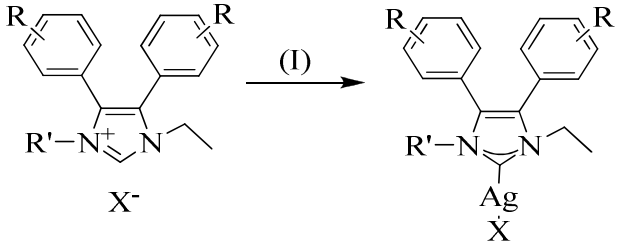


3k **4k**

Scheme 3.2. Synthesis route of imidazolium compound **4k**.
Reagents and conditions: (I) ethyl bromide, CH_3CN, reflux, 48-72 h, 74.2%.

3.1.2 Synthesis of Silver *N*-Heterocyclic Carbene Complexes

The Ag-NHC complexes were synthesized in analogy to the reported procedure by Wang and Lin [77, 78]. The imidazolium salts **4c-f**, **4h** and **4j** were treated with silver oxide in a mixture of CH_2Cl_2 and CH_3OH at rt to form the corresponding Ag-NHC complexes **1-Ag** to **6-Ag** (Scheme 3.3). The complexes were well characterized by IR, NMR and MS spectra. In addition, **3-Ag** was crystallized from a $MeOH/CH_2Cl_2$ solution and characterized by X-ray diffraction.

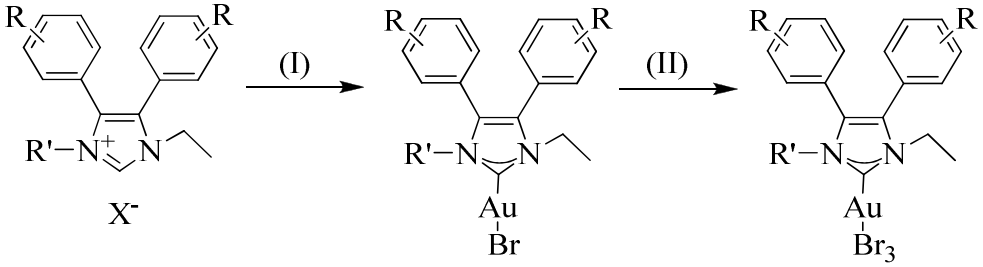


4c-f,4h,4j **1-Ag - 6-Ag**

Compounds	R	R'	X
1-Ag	2-F	Et	Br
2-Ag	3-F	Et	Br
3-Ag	4-F	Et	Br
4-Ag	4-MeO	Et	Br
5-Ag	4-OH	Et	Br
6-Ag	4-F	$PhCH_2$	Cl

Scheme 3.3. Synthesis routes and structures of Ag-NHC complexes.
Reagents and conditions: (I) Ag_2O, CH_3OH/CH_2Cl_2 1:1.2, rt, 12 h, 51-82%.

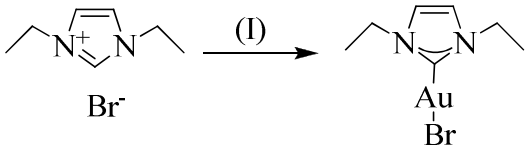
3.1.3. Synthesis of Gold *N*-Heterocyclic Carbene Complexes



4a-j **1-Au - 10-Au** **12-Au, 13-Au**

Compounds	R	R'	Compounds	R	R'
1-Au	2-MeO	Et	**6-Au, 13-Au**	4-F	Et
2-Au	3-MeO	Et	**7-Au**	H	Et
3-Au, 12-Au	4-MeO	Et	**8-Au**	4-OH	Et
4-Au	2-F	Et	**9-Au**	4-MeO	$PhCH_2$
5-Au	3-F	Et	**10-Au**	4-F	$PhCH_2$

Scheme 3.4. Synthesis routes and structures of Au-NHC complexes.
Reagents and conditions: (I) Method A: LiHMDS, Me_2SAuCl, LiBr, DMF, rt, 4 h, 45-84%; Method B: Ag_2O, CH_3OH/CH_2Cl_2 1:1.2, rt, 12 h, then Me_2SAuCl, LiBr, rt, 6 h, 28-48%; (II) Br_2, CH_2Cl_2, rt, 3 h, 83-85%.



4k **11-Au**

Scheme 3.5. Synthesis route of **11-Au**.
Reagents and conditions: (I) Method A: LiHMDS, Me_2SAuCl, LiBr, DMF, rt, 4 h, 64.3%; Method B: Ag_2O, CH_3OH/CH_2Cl_2 1:1.2, rt, 12 h, then Me_2SAuCl, LiBr, rt, 6 h, 48%.

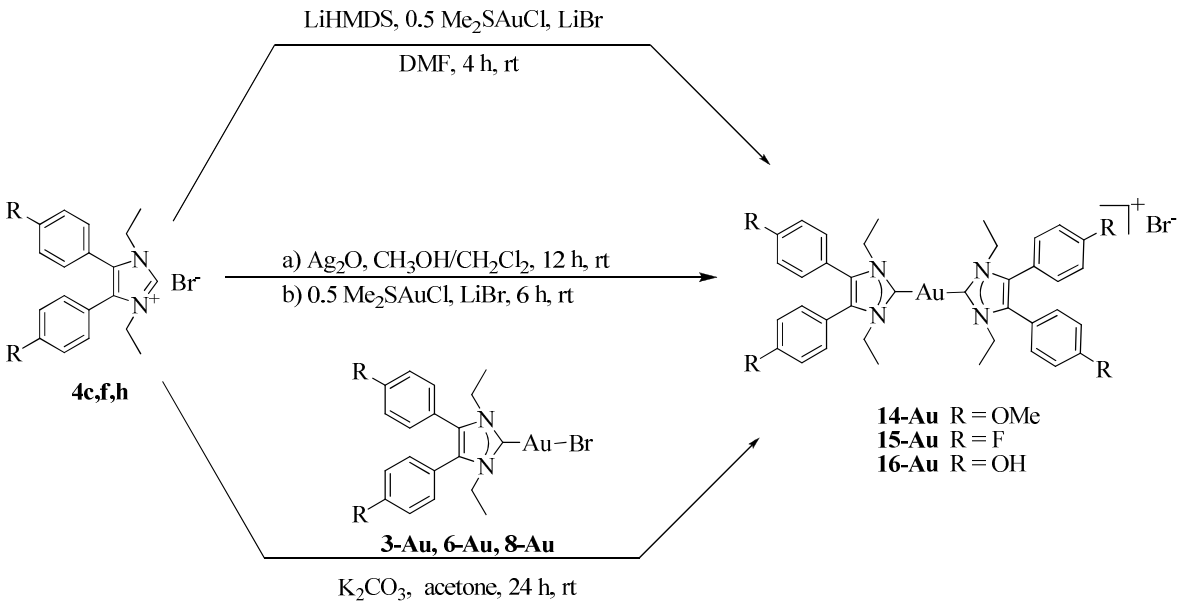
Two synthetic procedures were investigated for the synthesis of the Au(I)-NHC complexes **1-Au** to **11-Au** (Scheme 3.4, 3.5). The first procedure (Method A) involves the treatment of a DMF solution of Me_2SAuCl with a solution containing an equimolar amount of **4a-k** (treated for deprotonation with lithium bis(trimethylsilyl)amide (LiHMDS)). This procedure gave **1-Au** to **11-Au** in high yields [30]. The second procedure (Method B) involves the Ag(I)–NHC transfer method, whereby **4a-k** were treated with silver oxide in CH_2Cl_2, and the resulting Ag(I)–NHC complex was allowed to react with Me_2SAuCl [30]. This procedure gave **1-Au** to **11-Au** in moderate yields. Further amounts of lithium bromide have to be present in both methods to ensure the exclusive formation of NHC-Au-Br.

The oxidation of Au(I) complexes **3-Au** and **6-Au** were performed with bromine in CH_2Cl_2 at rt, resulting in the formation of an orange powder of the desired Au(III) complexes **12-Au** and **13-Au** [41,

[79].

All of the synthetic complexes gave satisfactory analytical and spectral data, which were in full accordance with their predicted structures. In addition, **5-Au** and **13-Au** were crystallized from a $MeOH/CH_2Cl_2$ solution and characterized by X-ray diffraction

3.1.4. Synthesis of Gold Bis(*N*-Heterocyclic Carbene) Complexes And Auranofin Derivatives

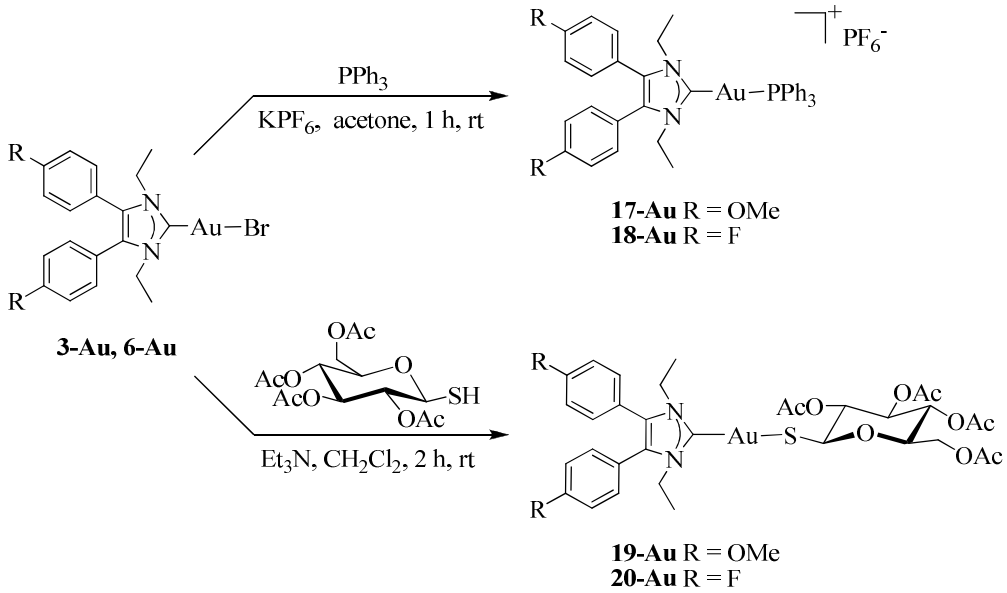


Scheme 3.6. Synthesis routes of **14-Au** to **16-Au**.

Three synthetic routes were investigated for the synthesis of **14-Au** to **16-Au** (Scheme 3.6). In method A [31, 32, 80] a DMF solution of Me_2SAuCl was treated with a solution containing two equivalents of **4c**,**f**,**h** previously deprotonated with LiHMDS. This reaction gave **14-Au** to **16-Au** in high yields (55-80%). The second procedure (Method B) involved the Ag(I)-NHC transfer method, whereby **4c**,**f**,**h** were treated with silver oxide in CH_2Cl_2, and the resulting Ag(I)-NHC complex was allowed to react with a half equimolar amount of Me_2SAuCl [81]. This procedure gave **14-Au** to **16-Au** in moderate yields (30-60%). Additional lithium bromide is necessary in both methods to ensure that sufficient amounts of bromide ions are available for the formation of Au(I)-bis(NHC) bromide. In the third synthetic route (Method C)[82] **3-Au**, **6-Au** or **8-Au** was dissolved with an equimolar amount of corresponding **4c**,**f**,**h** in the presence of K_2CO_3. This procedure was also very effective and gave **14-Au** to **16-Au** in high yields (80-90%).

The cationic NHC-Au(I)-triphenylphosphines **17-Au** and **18-Au** were prepared from **3-Au or 6-Au** by Br/PPh_3 exchange (Scheme 3.7) and were isolated as hexafluorophosphate salts [30]. A modified pro-

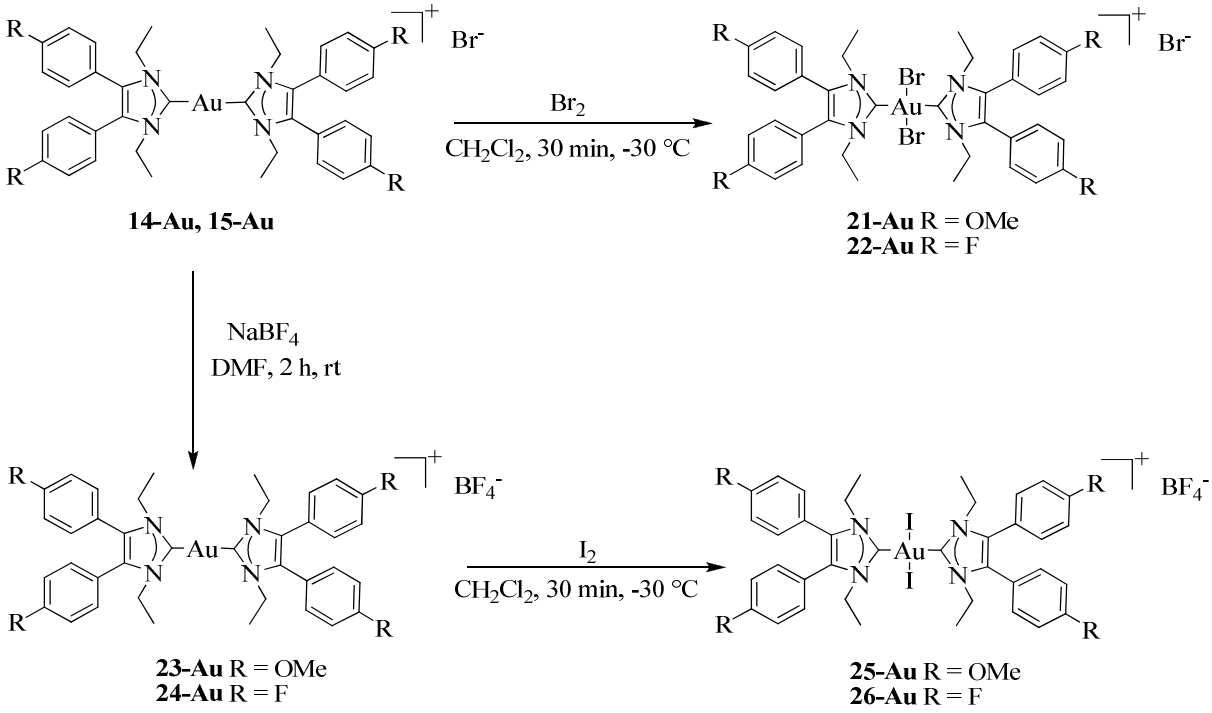
cedure of Berners-Price *et al.*[30] was used for the synthesis of the neutral NHC-Au(I)-2′,3′,4′,6′-tetra-*O*-acetyl-*β*-*D*-glucopyranosyl-1-thiolato complexes **19-Au** and **20-Au**. The NHC-Au(I)-Br complexes **3-Au** or **6-Au** was respectively dissolved in CH_2Cl_2 and reacted with 2′,3′,4′,6′-tetra-*O*-acetyl-*β*-*D*-glucopyranosyl-1-thiol under basic conditions (triethylamine, Scheme 3.7). The crude oily mixtures were purified by flash column chromatography, followed by a recrystallization from EtOH/H_2O to obtain the analytically pure products as white solids.



Scheme 3.7. Synthesis routes of **17-Au** to **20-Au**.

A convenient and gentle method to get the Au(III) complexes **21-Au** and **22-Au** (Scheme 3.8) is the oxidation of the readily available Au(I)-bis(NHC) complexes **14-Au** and **15-Au** with a slight excess of bromine [80]. Dibromo-Au(III)-bis(NHC) complexes **21-Au** and **22-Au** were isolated as orange powders in high yields [80]. Exchange of the bromide counter ion in **14-Au** or **15-Au** by tetrafluoroborate yielding the complexes **23-Au** or **24-Au** was easily performed with an excess of $NaBF_4$ in DMSO. The following treatment with elemental iodine gave the diiodo-Au(III)-bis(NHC) complexes **25-Au** and **26-Au** as reddish-orange powders [82]. Both oxidative addition reactions are straightforward and do not require the exclusion of air or moisture.

The analytical and spectral data of all target gold complexes gave were in full accordance with their predicted structures. In addition, **23-Au** was crystallized from a MeOH/CH_2Cl_2 solution and characterized by X-ray diffraction.



Scheme 3.8. Synthesis routes of **21-Au** to **26-Au**.

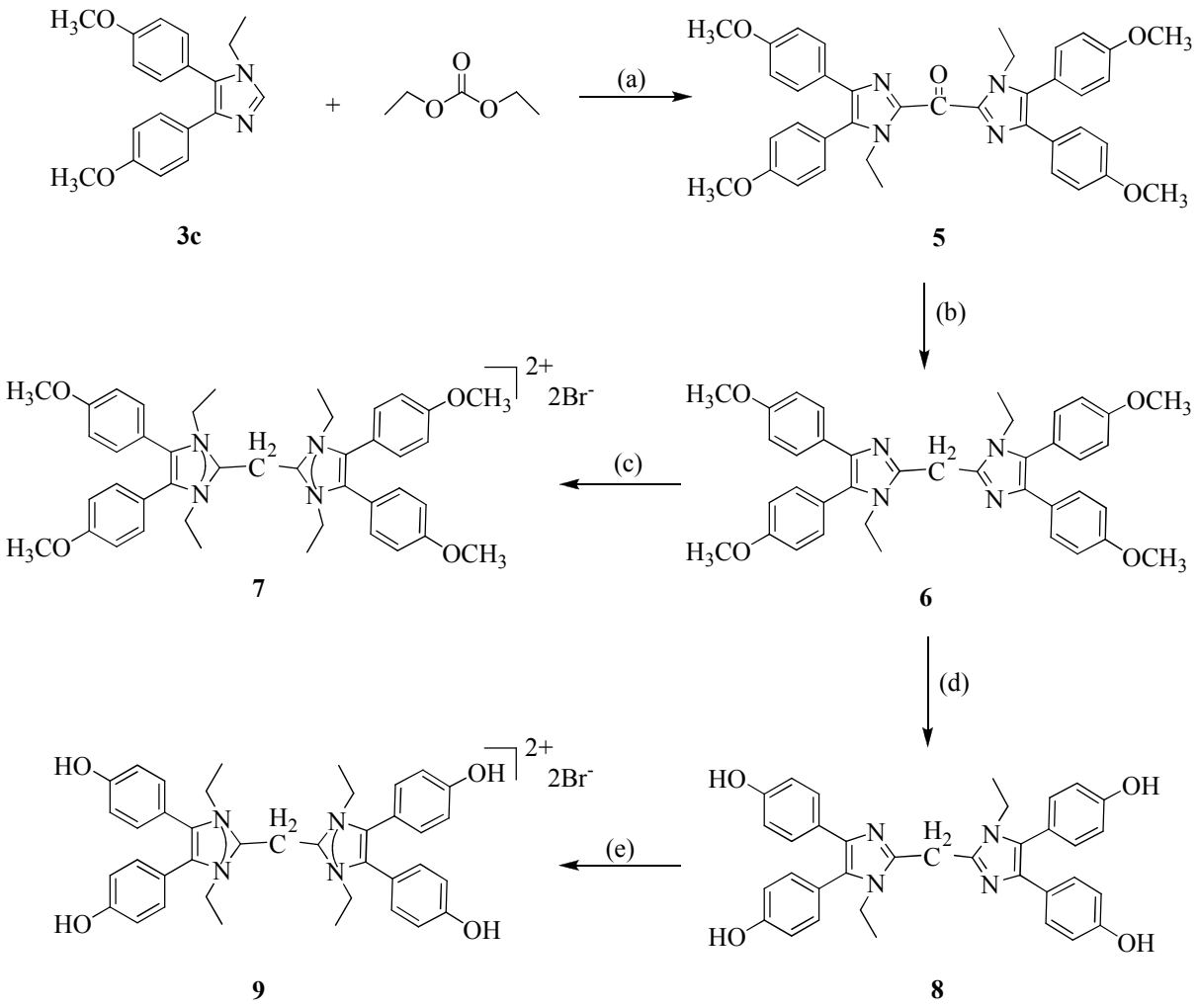
3.1.5 Synthesis of Bis(4,5-diarylimidazol-2-ylidene)methane Derivatives

Deprotonation of **3c** and reaction with diethyl carbonate at low temperature using a modified procedure of Rüther *et al.*[83] yielded the bis[1-ethyl-4,5-bis(4-methoxyphenyl)-1*H*-imidazol-2-yl]methanone **5**. Due to the relatively low acidity of the imidazole, BuLi was used as base.

For reduction to the bis[1-ethyl-4,5-bis(4-methoxyphenyl)-1*H*-imidazol-2-yl]methane (**6**) the keton **5** was heated with hydrazine hydrate and potassium hydroxide [83]. Following this procedure, **6** could be obtained in high yield and analytically pure after recrystallization from THF.

The bis[1-ethyl-4,5-bis(4-hydroxyphenyl)-1*H*-imidazol-2-yl]methane (**8**) was generated from the corresponding compound **6** by ether cleavage with BBr_3. Finally, compounds **6** and **8** were further reacted with ethyl bromide in CH_3CN to yield the bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]-methane derivatives **7** and **9**, respectively.

All of the synthetic compounds gave satisfactory analytical and spectral data, which were in full accordance with their predicted structures.



Scheme 3.9. Synthetic routes of bis[4,5-diarylimidazol-2-ylidene]methane derivatives.

Reagents and conditions: (a) *n*-BuLi, THF, -80 °C to rt, 54.0%; (b) KOH, $NH_2NH_2{\cdot}H_2O$, 120-150 °C, 73.3%; (c) ethyl bromide, CH_3CN, reflux, 48-72 h, 77.6%; (d) BBr_3, CH_2Cl_2, –60 °C, then warm to rt, 48 h, 65.5%; (e) ethyl bromide, CH_3CN, reflux, 48-72 h, 68.2%.

3.2. Antiproliferative Effects

3.2.1. Antiproliferative Effects of Silver *N*-Heterocyclic Carbene Complexes

As outlined in Table 3.1, the substituents at the 4,5-standing phenyl rings of the silver complexes determined the cytotoxicity. The 4-OH substituted complex **5-Ag** showed relatively low antiproliferative activities with IC_{50} values of 9.2 to 16.2 µM, while the fluoro- and methoxy-substituted complexes **1-Ag** to **4-Ag** and **6-Ag** showed strong activities against the tested cancer cell lines with IC_{50} below 10 µM.

Table 3.1. Growth inhibitory effects (IC_{50}, [µM]) against breast and colon cells after 72 h incubation.

Compounds	MCF-7	MDA-MB 231	HT-29
1-Ag	3.4 ± 0.7	3.6 ± 0.2	7.5 ± 0.5
2-Ag	3.5 ± 0.1	4.1 ± 0.7	7.4 ± 0.8
3-Ag	3.9 ± 0.2	3.5 ± 0.3	4.4 ± 0.1
4-Ag	3.7 ± 0.3	8.5 ± 0.8	9.9 ± 0.1
5-Ag	9.2 ± 0.2	12.8 ± 0.2	16.2 ± 0.2
6-Ag	3.6 ± 0.1	3.4 ± 0.2	6.8 ± 1.2
Cisplatin	1.6 ± 0.5	7.8 ± 0.8	4.1 ± 0.3

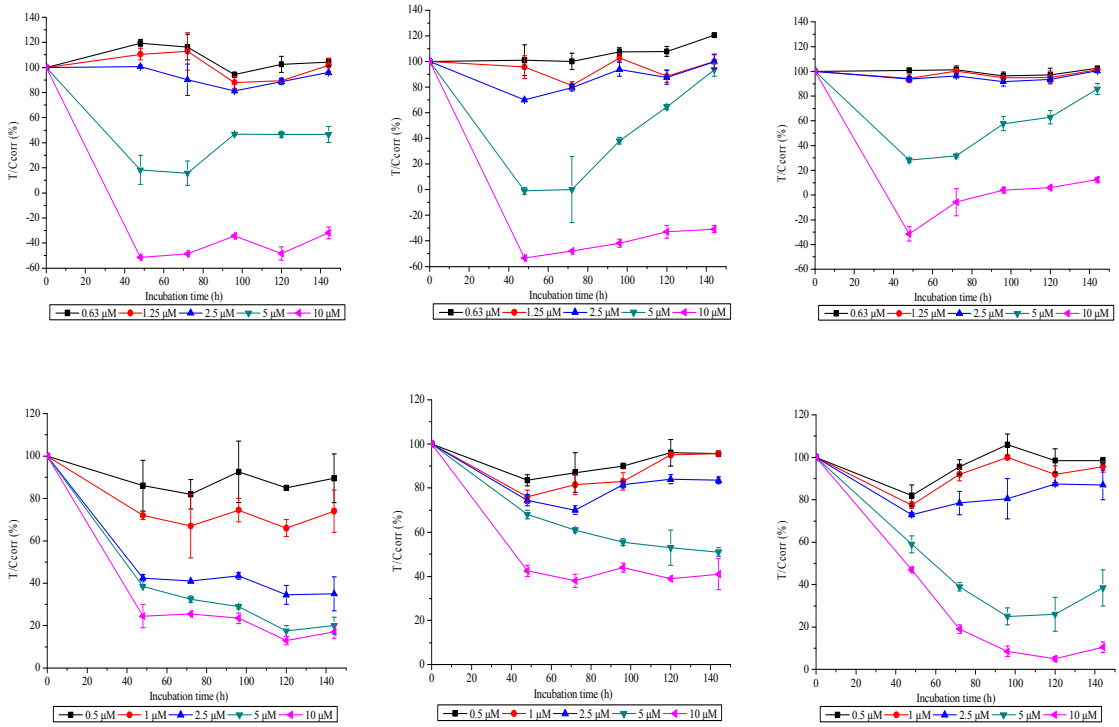


Figure 3.1. Time activity curves of **3-Ag** (above) and cisplatin (below) on MCF-7 (left), MDA-MB 231 (middle) and HT-29 (right) cell lines. Error bars are hidden behind the symbols in some cases.

The time activity curves (Figure 3.1) indicated that complex **3-Ag** caused cytocidal effects (T/C_{corr} < 0%) at 10 μM and cytostatic effects (T/C_{corr} ≈ 0%) at 5 μM, while cisplatin did not reach cytocidal activity. The curves of **3-Ag** at higher concentrations are characterized by a fast onset of activity with maximal effects already after an incubation time of 48 h. Cisplatin achieved its maximal effect at the end of the experiment. In addition, Figure 3.1 showed in the case of **3-Ag** a recuperation of the tumor cells after a prolonged exposition time. Because exponential cell growth is guaranteed for at least 140 h of incubation, the rise of the growth curve could be explained by the development of drug resistance. This effect of **3-Ag** is more pronounced at MDA-MB 231 and HT-29 cells compared to MCF-7 cells.

3.2.2. Antiproliferative Effects of Gold *N*-Heterocyclic Carbene Complexes

Table 3.2. Growth inhibitory effects (IC_{50}, [μM]) against breast and colon cells after 72 h incubation.

Compounds	MCF-7	MDA-MB 231	HT-29
4f	> 50	> 50	> 50
1-Au	1.2 ± 0.1	2.4 ± 0.5	3.1 ± 0.4
2-Au	1.6 ± 0.6	2.9 ± 0.3	4.2 ± 1.0
3-Au	1.4 ± 0.1	3.7 ± 0.9	2.9 ± 0.1
4-Au	0.80 ± 0.06	1.7 ± 0.9	3.3 ± 1.0
5-Au	3.1 ± 0.1	6.4 ± 0.1	4.2 ± 0.3
6-Au	1.1 ± 0.3	3.9 ± 0.1	2.3 ± 0.1
7-Au	0.87 ± 0.07	3.1 ± 0.5	3.3 ± 0.7
8-Au	4.5 ± 0.3	> 20	17.0 ± 2.8
9-Au	2.6 ± 0.3	3.3 ± 0.7	4.3 ± 0.1
10-Au	1.8 ± 0.1	2.4 ± 0.1	3.2 ± 0.3
11-Au	3.0 ± 0.4	6.9 ± 0.8	7.7 ± 0.8
12-Au	1.9 ± 0.1	4.4 ± 1.1	6.2 ± 1.0
13-Au	1.9 ± 0.4	4.4 ± 0.7	7.5 ± 2.9
Cisplatin	1.6 ± 0.5	7.8 ± 0.8	4.1 ± 0.3

According to our previous reports, auranofin caused comparable effects to cisplatin against MCF-7 cells (IC_{50} = 1.1 μM) and was slightly more active against HT-29 cells (IC_{50} = 2.6 μM) [25, 84]. Et_3PAuCl was less active at MCF-7 and HT-29 cell lines (IC_{50} = 3.2 and 5.3 μM, respectively) [84].

Exchange of the triethylphophine ligand by 1,3-diethyl-1,3-dihydro-2*H*-imidazol-2-ylidene in Et_3PAuCl as well as the exchange of the chloride by bromide did not change the activity against MCF-

7 cells (**11-Au**: IC_{50} = 3.0 µM) but reduced the effects against HT-29 cells (**11-Au**: IC_{50} = 7.7 µM). At the MDA-MB 231 cell line **11-Au** caused an IC_{50} = 6.9 µM, similar to cisplatin. Introduction of phenyl rings at C4 and C5 of the heterocycle (**11-Au** → **7-Au**) strongly increased the growth inhibitory effects (IC_{50} = 0.87 µM at MCF-7 cells, IC_{50} = 3.1 µM at MDA-MB 231 cells, IC_{50} = 3.3 µM at HT-29 cells). **7-Au** was even more active than cisplatin.

As outlined in Table 3.2, methoxy substituents at the aromatic rings (**7-Au** → **1-Au** to **3-Au**) did not change the growth inhibitory properties of **7-Au**, while F substituents in the ortho-position (**4-Au**) increased the activity against MDA-MB 231 cells (IC_{50} = 1.7 µM) and reduced the activity at all cell lines in meta-position (**5-Au**: IC_{50} = 3.1 µM at MCF-7 cells, IC_{50} = 6.4 µM at MDA-MB 231 cells, IC_{50} = 4.2 µM at HT-29 cells). The results of **6-Au** bearing a 4-F substituent are comparably active to that of **7-Au** (unsubstituted) and **3-Au** (4-OCH_3).

Exchange of the 4-OCH_3 groups by more hydrophilic 4-OH groups (**3-Au** → **8-Au**) strongly decreased the cytotoxicity (**8-Au**: IC_{50} = 4.5 µM at MCF-7 cells, IC_{50} > 20 µM at MDA-MB 231 cells, IC_{50} = 17.0 µM at HT-29 cells).

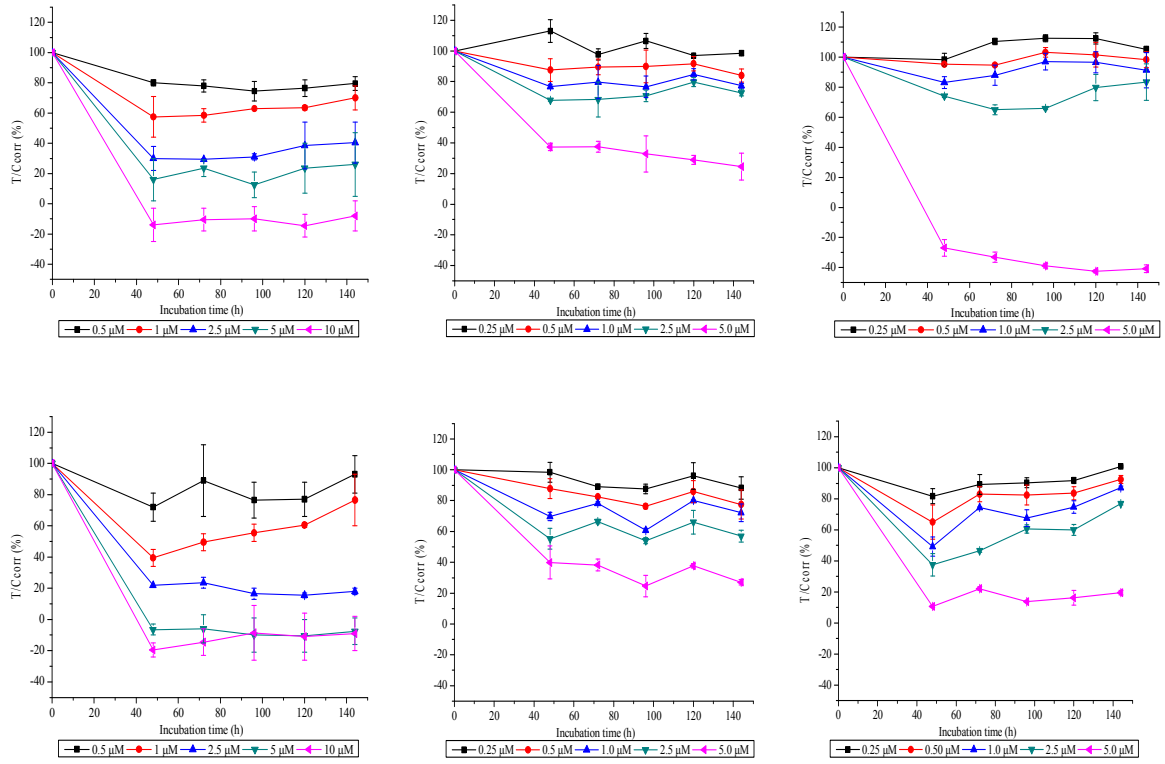


Figure 3.2. Time activity curves of complexes **3-Au** (above) and **6-Au** (below) on MCF-7 (left), MDA-MB 231 (middle) and HT-29 (right) cell lines. Error bars are hidden behind the symbols in some cases.

The substituents at the nitrogen atoms seem to play a subordinate role because complexes with either an N_1-ethyl chain or a bulky N_1-benzyl moiety yielded nearly identical results (compare **3-Au** and **9-Au** as well as **6-Au** and **10-Au**, Table 3.2).

It must be noted that the growth inhibitory effects depended on the presence of a metal center (the imidazoles were inactive; see *e.g.* **4f** in Table 3.2). Interestingly, the relevance of the oxidation state for the growth inhibition of MCF-7 and MDA-MB 231 cells was low. At the HT-29 cell line only, the Au(III) complexes **12-Au** and **13-Au** were less active than their Au(I) congeners **3-Au** and **6-Au**.

Time-dependent cytotoxicity studies were performed on the examples of **3-Au** and **6-Au**. The curves presented in Figure 3.2 indicated for **6-Au** a recuperation of HT-29 cells at concentrations below 2.5 μM and at MCF-7 cells below 1 μM.

3.2.3. Antiproliferative Effects of Gold Bis(*N*-Heterocyclic Carbene) Complexes And Auranofin Derivatives

Exchange of the Br^- ligand in **3-Au** and **6-Au** by 2′,3′,4′,6′-tetra-*O*-acetyl-*β*-*D*-glucopyranosyl-1-thiolate (→ **19-Au** and **20-Au**) marginally increased the growth inhibitory effects at the MDA-MB 231 cell line (IC_{50}= 1.44 and 1.34 μM, respectively) and in the case of **20-Au** at MCF-7 cells (IC_{50} = 0.81 μM), too. The effects at HT-29 cells remain unchanged.

Interestingly, the use of triphenylphosphine ligands led to a 5-fold higher activity despite the formation of cationic species. Complexes **17-Au** and **18-Au** were cytotoxic at nanomolar concentrations more pronounced at MCF-7 ($IC_{50} \approx 0.21$ μM) than at HT-29 ($IC_{50} \approx 0.40$ μM) and MDA-MB 231 cells ($IC_{50} \approx 0.68$ μM), independent of the substituent at the NHC.

A further increase in activity was determined with cationic Au(I)-bis(NHC) complexes: **14-Au** and **15-Au** were about 10-fold higher active than **3-Au** and **6-Au** (**15-Au** to higher extent than **14-Au**). Complex **15-Au** showed the highest activity among the new complexes with the outstanding IC_{50} = 0.10 μM at MCF-7 cells. Unexpectedly, the 4-OH substituted complex **16-Au** also caused strong growth inhibition, with selectivity for MCF-7 (IC_{50} = 0.30 μM) and HT-29 cells (IC_{50} = 0.47 μM). In contrast to **8-Au,** which was inactive at MDA-MB 231 cell, its IC_{50} = 1.55 μM even surpassed that of cisplatin.

Table 3.3. Growth inhibitory effects (IC_{50}, [μM]) against breast and colon cell lines.

Compounds	MCF-7	MDA-MB 231	HT-29
14-Au	0.17 ± 0.05	0.54 ± 0.11	0.43 ± 0.01
15-Au	0.10 ± 0.02	0.34 ± 0.01	0.23 ± 0.01
16-Au	0.30 ± 0.07	1.55 ± 0.04	0.47 ± 0.18
17-Au	0.22 ± 0.01	0.70 ± 0.22	0.42 ± 0.04
18-Au	0.21 ± 0.10	0.67 ± 0.11	0.37 ± 0.02
19-Au	1.26 ± 0.09	1.44 ± 0.20	3.19 ± 0.21
20-Au	0.81 ± 0.12	1.34 ± 0.12	3.22 ± 0.02
21-Au	0.15 ± 0.01	0.49 ± 0.01	0.32 ± 0.01
22-Au	0.13 ± 0.01	0.50 ± 0.10	0.33 ± 0.01
23-Au	0.13 ± 0.01	0.56 ± 0.07	0.36 ± 0.01
24-Au	0.15 ± 0.01	0.40 ± 0.01	0.62 ± 0.02
25-Au	0.15 ± 0.01	0.63 ± 0.01	0.26 ± 0.03
26-Au	0.13 ± 0.02	0.77 ± 0.09	0.30 ± 0.01
Cisplatin	1.6 ± 0.5	7.8 ± 0.8	4.1 ± 0.3

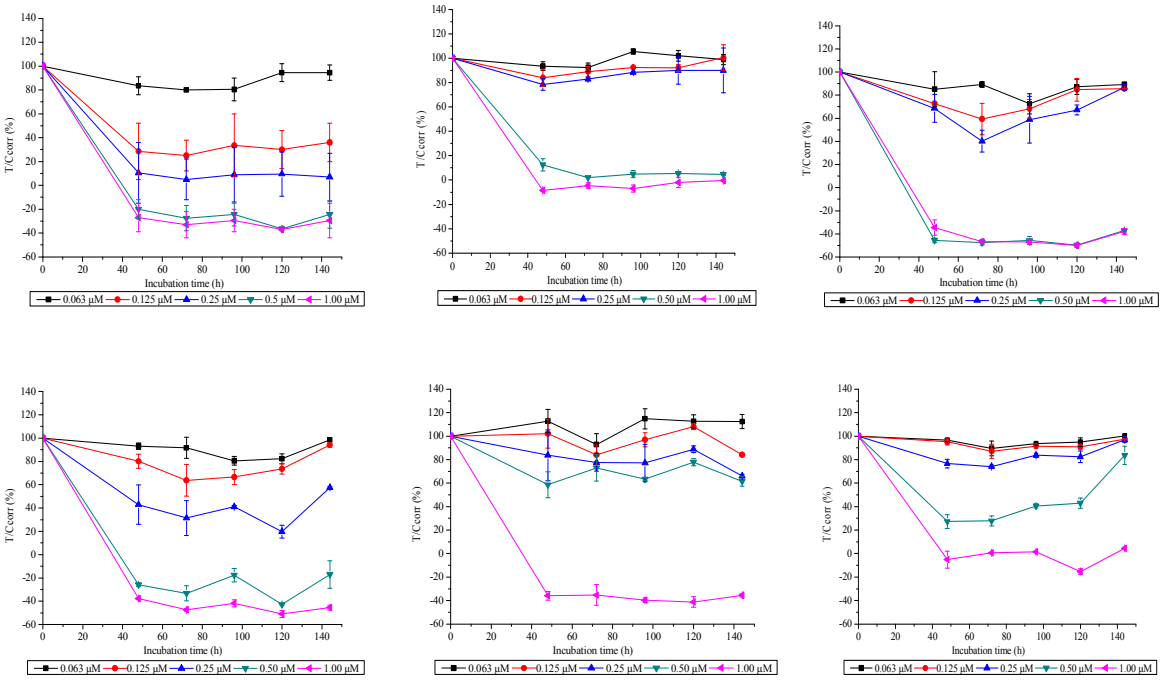


Figure 3.3. Time activity curves of complexes **15-Au** (above) and **18-Au** (below) on MCF-7 (left), MDA-MB 231 (middle) and HT-29 (right) cell lines. Error bars are hidden behind the symbols in some cases.

The influence of the counter ion and the oxidation state on the *in vitro* antitumor effects is low. The IC_{50} of bromides (**14-Au** and **15-Au**) is nearly the same as those of tetrafluoroborate salts (**23-Au** and **24-Au**). Oxidation of **14-Au** and **15-Au** with Br_2 yielding **21-Au** and **22-Au** only slightly reduced the growth inhibitory effects in the case of **15-Au** → **22-Au** (see Table 3.3).

Finally, the exchange of the bromide ligands in **21-Au** and **22-Au** by iodide increased the IC_{50} at MDA-MB 231 cells from about 0.50 µM to 0.63 µM (**25-Au**) and 0.77 µM (**26-Au**). The effects at MCF-7 and HT-29 cells remained unchanged.

As outlined in Figure 3.4, **15-Au** and **18-Au** caused cytocidal effects ($T/C_{corr} < 0\%$) at 1 µM. At higher concentrations their curves are characterized by a fast onset of activity with maximal effects already after an incubation time of 48 h. In addition, Figure 3.4 showed in the case of **15-Au** and **18-Au** only a marginal recuperation of the tumor cells after a prolonged exposition time. Therefore, the development of drug resistance is very unlikely [85].

3.2.4. Antiproliferative Effects of Bis[4,5-diarylimidazol-2-ylidene]methane Derivatives

The methanone **5** was inactive in all cell lines ($IC_{50} > 20$ µM), while its methylene analogon **6** reduced the growth of MCF-7 and HT-29 cells with $IC_{50} = 10.1$ and 14.1 µM, respectively. Interestingly, it was inactive against MDA-MB 231 cells ($IC_{50} > 20$ µM). An additional *N*-ethyl group increased the growth inhibitory potency. Although **7** represents a permanent cation it was more active than 5-FU at MCF-7 cells ($IC_{50} = 2.6$ µM; 5-FU: $IC_{50} = 4.7$ µM) and comparable active at HT-29 cells ($IC_{50} = 7.1$ µM; 5-FU: $IC_{50} = 7.3$ µM). Again, no influence was observed on MDA-MB 231 cells ($IC_{50} > 20$ µM).

Table 3.4. Growth inhibitory effects (IC_{50}, [µM]) against breast and colon cells after 72 h incubation.

Compounds	MCF-7	MDA-MB 231	HT-29
5	> 20	> 20	> 20
6	10.1 ± 1.2	> 20	14.1 ± 4.1
7	2.6 ± 0.2	> 20	7.1 ± 0.4
8	9.7 ± 0.4	11.2 ± 2.2	8.1 ± 0.6
9	5.5 ± 0.4	10.8 ± 1.0	15.5 ± 0.1
5-FU	4.7 ± 0.4	9.6 ± 0.3	7.3 ± 1.0

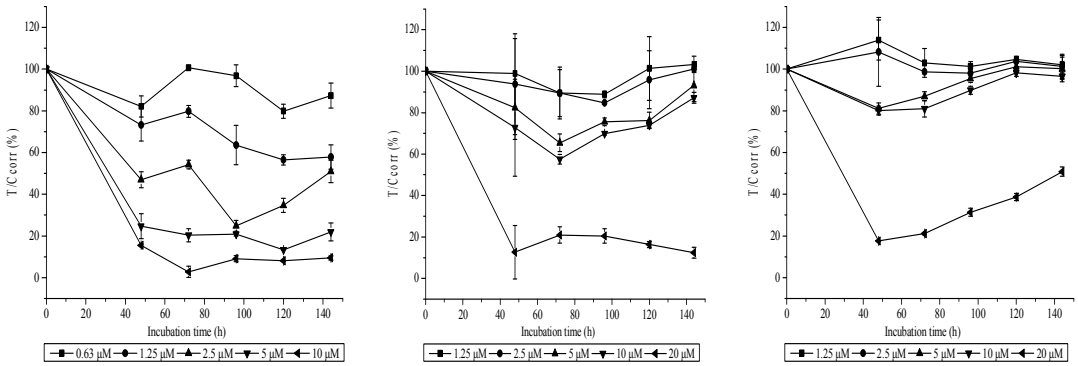


Figure 3.4. Time activity curves of **7** on MCF-7 (left) cell line and **9** on MDA-MB 231 (middle) and HT-29 (right) cell lines. Error bars are hidden behind the symbols in some cases.

Effects at MDA-MB 231 cells could be observed after ether cleavage resulting in compounds **8** and **9** (IC_{50} = 10.2 and 10.8 µM, respectively). At the other cell lines **6** was half as active as its methoxy derivative **7** (IC_{50} = 5.5 and 2.6 µM at MCF-7 cells; IC_{50} = 15.5 and 7.1 µM at HT-29 cells) while **5** (4-OH) and **6** (4-OCH_3) were equipotent at MCF-7 cells (IC_{50} = 9.7 and 10.1 µM). At HT-29 cells, **5** (IC_{50} = 8.1 µM) was more active than **6** (IC_{50} = 14.1 µM).

The time activity curves (Figure 3.4) of **7** and **9** showed marginal recuperation after a prolonged exposition to the drugs, which could be an indication of development of drug resistance [85].

3.3. Reaction with Glutathione.

In the used assay, the positive control Me_2SAuCl (33% 20 min; 56% 60 min) reduced TNB formation under the chosen conditions (Figure 3.5) while auranofin (194% 20 min; 212% 60 min) strongly increased the TNB release during the reaction. A DMSO control was set as 100%.

Interestingly, the complexes determined on the examples of **3-Au** (82%, 20 min; 91%, 60 min) and **6-Au** (85%, 20 min; 90%, 60 min) did not significantly influence the reaction and can thus be considered as sufficiently stable against thiols under biologically relevant conditions.

Similar to **3-Au** (91%) and **6-Au** (90%), the selected gold complexes **15-Au** (111%), **18-Au** (117.5%), **20-Au** (114.5%), and **24-Au** (112.0%) did not significantly induce the oxidation of GSH after 60 min of incubation (Figure 3.6), too.

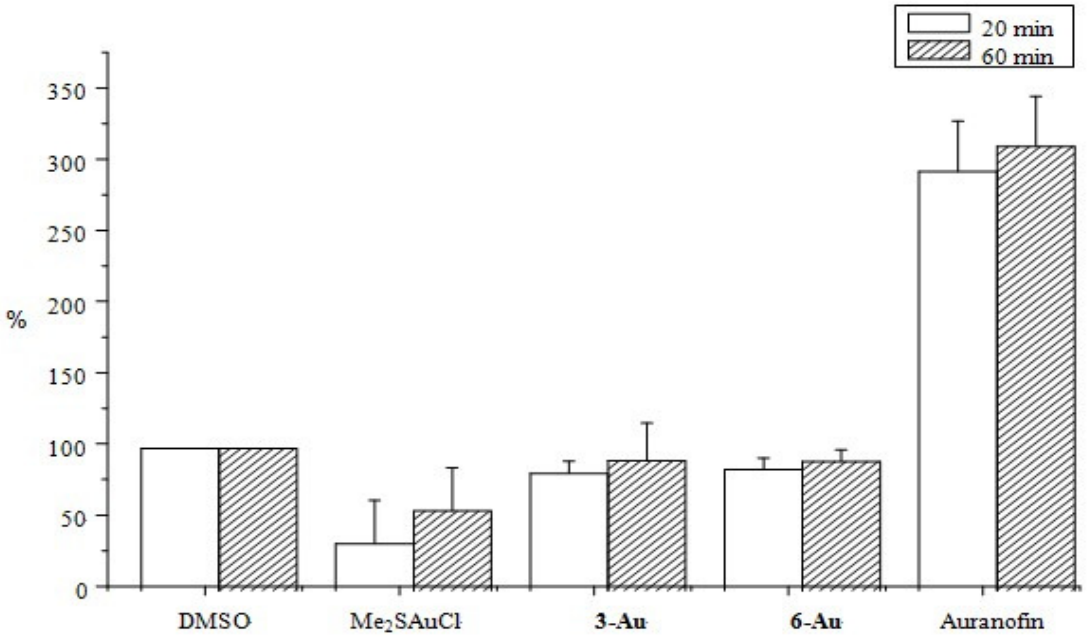


Figure 3.5. Interaction of gold complexes (166.7 μM) with glutathione and dithiobis nitrobenzoic acid (DTNB). Percentage building of 2-nitro-5-thionitrobenzoic acid (TNB) after 20 and 60 min of incubation.

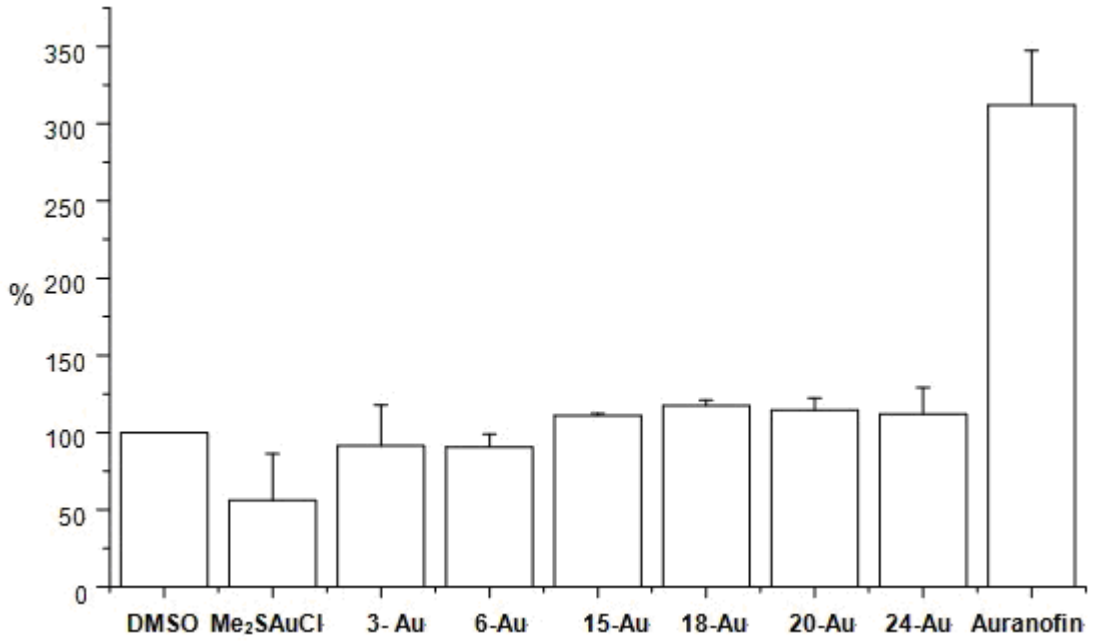


Figure 3.6. Interaction of gold complexes with glutathione and DTNB. Percentage building of TNB after 60 min of incubation.

4. Discussion

Cytotoxicity results against mammary (MCF-7 and MDA-MB 231) as well as colon (HT-29) carcinoma cells indicated that the substituents at the 4,5-standing phenyl rings of the silver complexes determined the activity. The 4-OH substituted complex **5-Ag** was less active than the fluoro- and methoxy-substituted complexes **1-Ag** to **4-Ag**, **6-Ag**. The most active complex bromo[1,3-diethyl-4,5-bis(4-fluorophenyl)imidazol-2-ylidene]silver(I) (**3-Ag**) was slightly less active against MCF-7 cells, more active against MDA-MB 231 cells and comparable active as cisplatin against HT-29 cells.

It is well accepted that the DNA represents the major target of antitumor active metal complexes [2, 65], so we studied the interaction of **3-Ag** with calf thymus (CT) DNA by using circular dichroism (CD) spectroscopy [86]. However, the missing changes of absorbance excluded DNA binding as the mode of action.

Because the NHC ligands described here are derivatives of 2,4,5-triarylimidazoles, they were not only COX-inhibitors which inactivate the COX enzymes dependent on the substituents in the aromatic rings, but also ligands of the ER [87-89]. It makes sense to take both targets into consideration as well. Among the complexes only **6-Ag** induced a low luciferase expression which is an indication of only slight receptor binding. Therefore, an ER-mediated cytotoxicity could be excluded. In addition, the most promising complex **3-Ag** caused no COX inhibition, so it is very unlikely that COX enzymes are involved in the inhibition of the tumor cell growth.

Besides, a number of Ag-NHC complexes possessed good antibacterial activities[3] which induced us to evaluate the effectiveness of **3-Ag** and **6-Ag** as antibacterial agents *in vitro* through a modified agar diffusion test [90]. Interestingly, **3-Ag** and **6-Ag** possessed antimicrobial properties at a level comparable to $AgNO_3$ against *Ervinia amylovora* Ea 273, *E. coli* DH5α, *Bacillus subtilis* 168 and *Bacillus megaterium* cell lines. Further testing of **3-Ag** against other organisms (*Erwinia carotovora, Pseudomonas aeruginosa and Bacillus cereus* ATCC 14579) also indicated positive results in comparison with the negative control.

The novel Au-NHC complexes (**1-Au** to **13-Au)** caused growth inhibitory effects against tumor cell lines. There are at least six Au-NHC complexes (**1-Au** to **4-Au**, **6-Au**, and **7-Au**) among thirteen complexes which were more active than cisplatin and Et_3PAuCl and as active as auranofin against the used mammary and colon carcinoma cell lines. Especially, the most active complex bromo[1,3-diethyl-4,5-bis(2-fluorophenyl)-1,3-dihydro-2*H*-imidazol-2-ylidene]gold(I) **4-Au** (IC_{50} = 0.8 μM,

MCF-7; IC_{50} = 1.7 μM, MDA-MB 231; IC_{50} = 3.3 μM, HT-29) exhibited distinctly higher antiproliferative potency than cisplatin (IC_{50} = 1.6 μM, MCF-7; IC_{50} = 7.8 μM, MDA-MB 231; IC_{50} = 4.1 μM, HT-29).

High growth inhibitory effects depended on the presence of the C4,C5-standing aromatic rings. Methoxy groups and fluorine substituents only marginally changed the cytotoxicity against the used cell lines, while 4-hydroxy groups nearly terminated the activity. The substituents at the nitrogens and the oxidation state of the metal played a subordinate role. In addition, the growth inhibitory effects of the complexes depended on the presence of a metal center (the imidazoles were inactive; see *e.g.* **4f**).

Furthermore, these novel gold complexes (IC_{50} = 0.8-3.1 μM, MCF-7; IC_{50} = 1.7-6.9 μM, MDA-MB 231; IC_{50} = 2.3-7.5 μM, HT-29) were distinctly more active than the silver complexes **1-Ag** to **6-Ag** (IC_{50} = 3.4-9.2 μM, MCF-7; IC_{50} = 3.4-12.8 μM, MDA-MB 231; IC_{50} = 4.4-16.2 μM, HT-29) and the reported benzimidazol-2-ylidene Au(I) complexes (IC_{50} = 4.6-10.3 μM, MCF-7; IC_{50} = 6.4-13.3 μM, HT-29) tested under comparable conditions [37].

Next, exchange of the Br^- ligand in bromo[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I) complexes by 2′,3′,4′,6′-tetra-*O*-acetyl-*β*-*D*-glucopyranosyl-1-thiolate (→ **19-Au** and **20-Au**) almost did not increased the growth inhibitory effects against the tumor cells while the use of triphenylphosphine ligand (**17-Au** and **18-Au**) led to a 5-fold higher activity despite the building of cationic species. A further increase in activity was determined with cationic Au(I)-bis(NHC) complexes. Interestingly, the cytotoxic effect of **14-Au** and **15-Au** was up to 10-fold higher than that of cisplatin. Compared to **3-Au** and **6-Au** the growth inhibitory effects also strongly increased. The anion variation (**23-Au** and **24-Au**) and the oxidation state (Au(III)-bis(NHC) **21-Au**, **22-Au**, **25-Au** and **26-Au**) of the metal for Au-bis(NHC) complexes played a subordinate role.

Importantly, complex **15-Au** showed the highest cytotoxicity (IC_{50} = 0.10 μM) against MCF-7 cells. To the best of our knowledge, this is the lowest IC_{50} of gold complexes tested under comparable conditions, indicating its high potential as an antitumor agent.

All these results indicated that the attempt to increase the antitumor potency of Au-NHC complexes was successful if the bromide ligand was exchanged by PPh_3 or a further NHC ligand. The resulting complexes were more active than cisplatin with an IC_{50} in the low nanomolar range. In this SAR study we could demonstrated that gold complexes bearing two 1,3-diethyl-4,5-diarylimidazol-2-ylidene ligands represented effective cytostatics with growth inhibitory effects at MCF-7, MDA-MB 231 and

HT-29 cells up to 10-fold higher than cisplatin.

Further cytotoxic data clearly demonstrated that the exchange of the gold center by a methylene group decreased the growth inhibitory effects. The cationic complexes **14-Au** and **16-Au** were about 10 to 20-fold higher cytotoxic than the methylene derivatives **7** and **9**. However, unlike its high anti-tumor activity, **14-Au** was less active than compound **7** against *Ervinia amylovora* Ea 273, *Escherichia coli* DH5α, *Bacillus subtilis* 168 and *Bacillus megaterium* cell lines *in vitro* through a modified agar diffusion test.

Thiols such as the tripeptide glutathione played an important role for inactivation of cisplatin and many other metal-based drugs. Auranofin biologically processed in the same way and metabolized in ligand exchange processes. This "thiol-based" metabolism of Au(I) complexes hampered so far the development of novel drug candidates out of this class [22, 37]. Therefore, we studied the interaction of our complexes with glutathione under physiological conditions. In the assay, auranofin strongly increased the TNB release during the reaction while the selective complexes **3-Au**, **6-Au**, **15-Au**, **18-Au**, **20-Au**, and **24-Au** did not significantly influence the reaction and can thus be considered as sufficiently stable against thiols under biologically relevant conditions. Accordingly, the choice of NHC ligands probably provided an useful strategy for the design of Au(I) drugs with enhanced biological stability.

Because the cytotoxic effects of metal complexes in cell culture experiments were strongly influenced by cellular uptake and accumulation, the intracellular drug level in MCF-7 and HT-29 cells was studied on the example of gold complexes (**3-Au**, **6-Au**, **15-Au**, **18-Au**, **20-Au**, and **24-Au**).

The cellular molar concentrations of Au-NHC complexes **3-Au** and **6-Au** were higher in MCF-7 cells than in HT-29 cells. The uptake in HT-29 cells of both complexes corresponded with that of the references cisplatin and Et_3PAuCl, while it strongly differed in MCF-7 cells: **6-Au** ≈ cisplatin < **3-Au** < Et_3PAuCl.

For the complexes **15-Au**, **18-Au**, **20-Au**, and **24-Au**, with exception of complex **24-Au**, for which a 60% reduced intracellular gold content was measured, all complexes hold their Au level in MCF-7 cells over an incubation time of 24 h. In HT-29 cells even a higher metal concentration was measured. In addition, the accumulation depended on the complex structure. Each modification on **6-Au** increased the intracellular gold content. The highest drug level was determined for the Au(I)-bis(NHC) complexes **15-Au** and **24-Au** (50- to 60-fold compared to the extracellular concentration of 10 μM).

The cellular uptake values for complexes are in accordance with their growth inhibitory potency. *In vitro* cytotoxicity assays showed that **15-Au**, **18-Au**, and **24-Au** were more active than **6-Au**, **20-Au**, and Et_3PAuCl at both cell lines, respectively. In addition, it should be noted that comparable cytotoxicity for **15-Au**, **18-Au**, and **24-Au** in both cell lines were obtained.

Prompted by the favorable results of the cellular uptake results, we investigated whether the cytotoxic effects of gold complexes might be a consequence of a metal accumulation in the nucleus, which was already confirmed by Ott *et al.*[25] for the [*N*-(*N*′,*N*′-dimethylaminoethyl)-1,8-naphthalimide-4-sulfide](triethylphosphine)gold(I) complex. Therefore, the nuclei of MCF-7 and HT-29 cells treated with **3-Au**, **6-Au**, **15-Au**, **18-Au**, **20-Au**, **24-Au** or Et_3PAuCl was isolated by a short sucrose gradient and investigated for their gold content using ETAAS.

Similar to the cellular uptake, the nuclear gold content of the Au-NHC complexes **3-Au** and **6-Au** was higher in MCF-7 than in HT-29 cells. However, the uptake results of the complexes did not correlate with their growth inhibitory potencies. Therefore, we generally investigated the DNA binding properties using salmon testes DNA. In this test, **3-Au** and **6-Au** showed a moderate binding to DNA after 4 h, not as high as cisplatin but higher than Et_3PAuCl. On the basis of these results, it is not very likely that DNA interaction caused the cytotoxic effects of the Au-NHC complexes.

Compared with Au-NHC complex **6-Au** caused a gold content in the nuclei of MCF-7 which was a half of that obtained with Et_3PAuCl, the metallation of the nuclei was more efficient with the Au(I)-bis(NHC) complexes **15-Au** and **24-Au** and the auranofin derivatives **18-Au** and **20-Au**. Another situation was found in HT-29 cells. The cationic complexes **15-Au**, **18-Au** and **24-Au** were about 20-fold higher accumulation grade in the nuclei compared to the parent compound **6-Au**.

In conclusion, the accumulation studies point to an active transport of the charged complexes through the cell membrane or by a passive transport as stable ionic pairs. In the nuclei the cationic complexes can bind electrostatically at the negative charged phosphate backbone of the DNA and can participate on the damage of the cells. The exact DNA binding mode is still unclear but will be investigated in detail in a forthcoming study. Nevertheless at this stage of the study it could be demonstrated that the NHC ligands are suitable to direct the complexes in high amounts to the nuclei.

Based on the history of the drug design, the complexes are derivatives of auranofin a competent TrxR inhibitor [22, 23] and the NHC are derivatives of synthetic hormones (ER binder) and COX inhibitors [76, 87, 88], these targets must be considered as targets of the novel complexes, too.

The TrxR inhibition is a well accepted principle to reduce tumor cell growth.[17,26,33-41,43] Auranofin and related gold complexes demonstrated high affinity for TrxR leading to inactivation due to the formation of a covalent bond to the Se-center of the enzyme.

The Au-NHC complexes (**1-Au** to **13-Au**) caused TrxR inhibition with an IC_{50} in the range of 0.374 to 1.505 μM. As expected, the inhibitory effect depended on the presence of a gold center; the selected ligand was completely inactive.

They are comparable active as previously reported benzimidazol-2-ylidene gold complexes (IC_{50} = 0.39 to 4.0 μM) [37], naphthalimide Au(I) phosphine complex (IC_{50} = 0.27 μM) [25], and Au(I) complexes containing imidazole and thiazole-based diphos type ligands (IC_{50}: 0.12 to 1.22 μM) [91] tested under comparable conditions. The inhibition of the enzyme TrxR was already confirmed as one of major targets for these reported Au complexes.

Besides, the IC_{50} of these complexes was much lower than the values from other similar Au-NHC complexes [*e.g.* gold complexes bearing amino linked NHC from Li's group ("did not inhibit human TrxR at 0.5-4 μM") [19] and cationic Au(I)-bis(NHC) complexes from Berners-Price's group ("TrxR activity was inhibited by ~50% with 5 μM") [32]].

Based on these reasons, we concluded that TrxR might be one of major targets of the Au-NHC complexes. However, a clear SAR is not given by the data and a correlation with the growth inhibition was missing. In addition, they were less active than Et_3PAuCl and auranofin. These results induced us to investigate further targets of the Au-NHC complexes besides the TrxR interaction.

For the Au-bis(NHC) complexes and auranofin derivatives, the structure of the complexes clearly determines the TrxR inhibitory effects. While the bivalent Au-bis(NHC) complexes **14-Au** to **16-Au** and **21-Au** to **26-Au** were nearly inactive against TrxR, the Ph_3P and glucopyranosylthiolate derivatives **17-Au** to **20-Au** caused a 50% inhibition of the enzyme at about half of the concentration used for **3-Au** and **6-Au**. Compared to Et_3PAuCl and auranofin, however, all complexes were less active and a correlation between the inhibition of TrxR and cytotoxicity did not exist. So we could exclude TrxR as targets for the Au-bis(NHC) complexes and auranofin derivatives.

As the NHC ligands described here are derivatives of 2,4,5-triarylimidazoles designed as ER ligands [76, 87, 88], we investigated if the ER is involved in the mode of action in hormone-dependent MCF-7 cells. Unfortunately, all complexes did not induce agonistic or antagonistic properties. Therefore, an ER-mediated cytotoxicity can be excluded.

A further possible target of triarylimidazole derivatives are the COX enzymes (COX-1/2). Both subtypes are not only the targets for the design of anti-inflammatory drugs but also in cancer chemotherapy [92]. Recently, it was verified that auranofin inhibited the formation of eicosanoids related to the activity of COX-1 and 12-LOX in human platelets [21]. Interestingly, our results showed that **6-Au** possessed very promising COX inhibitory properties with COX-1 selectivity at 10 μM. Unlike complex **6-Au**, the Au(I)-bis(NHC) complex **15-Au** was inactive at COX-1 and COX-2, so it was decided to renounce on further experiments at that moment. Nevertheless it is worthy to note that **6-Au** is an effective COX-1 inhibitor which makes this class of complexes to an interesting subject of a further SAR study.

5. Conclusions and Outlook

Silver and gold *N*-Heterocyclic carbene complexes derived from 4,5-diarylimidazole were synthesized and fully characterized.

Pharmacological investigations revealed that all silver complexes possessed growth inhibitory effects against mammary (MCF-7 and MDA-MB 231) as well as colon carcinoma (HT-29) cells. Preliminary *in vitro* studies showed that **3-Ag** and **6-Ag** exhibited good antibacterial activities. However, they were only marginally active at the DNA, ER and the COX enzymes, so these targets can be excluded to be involved in the mode of action. Despite the mode of action of Ag-NHC complexes remains unclear, these findings indicated that this type of Ag-NHC complexes may be useful in anticancer and antibacterial chemotherapy.

NHC-Au-halide(s) caused growth inhibitory effects dependent on the substituents at the aromatic rings against MCF-7 and MDA-MB 231 breast as well as HT-29 colon cancer cell lines. The influence of *N*-substituents and the oxidation state of the metal (Au(I) or Au(III)) was relatively low. All complexes inhibited the TrxR, which led to the conclusion that this enzyme might be the main target of these complexes. The missing SAR and the missing correlation with cytotoxic properties indicated the involvement of further targets. Based on the investigations on cellular and nuclear uptake, as well as the binding to the ER, DNA binding and interference in the hormonal system could be excluded. The selective inhibition of the COX-1 enzyme by complex **6-Au** opens a new perspective for use Au-NHC complexes in medicinal chemistry.

In addition, we could demonstrate that gold complexes bearing two 1,3-diethyl-4,5-diarylimidazol-2-ylidene ligands represent effective cytostatics with growth inhibitory effects against MCF-7, MDA-MB 231 and HT-29 cells up to 10-fold higher than cisplatin. Oxidation from Au(I) to Au(III) or variation of the anionic counter ion of the cationic Au(I)-bis(NHC) complexes did not change their cell growth inhibitory capacities. They were effectively accumulated in the tumor cells and located in high amounts in the nuclei. Preliminary studies on the mode of action indicated that TrxR, the ER and the COX enzymes (COX-1/2) could be excluded as targets for the cationic complexes.

Finally, the lower influence of bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]methane compounds compared with the related gold complexes documented that the gold core seems to be responsible for the high cytotoxic activity of cationic bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I) complexes. Antibacterial tests showed that compound **7** exhibited higher activity than $AgNO_3$ and the gold com-

plex **14-Au**.

Overall, this work provided a great number of Ag-NHC and Au-NHC complexes, potentially active as antitumor agents. All these results might guide future development of improved metal-based drugs especially gold complexes for the treatment of human diseases including cancer.

The mode of action of the gold complexes is still unclear. Direct DNA damage, modification of the cell cycle, mitochondrial damage involving TrxR inhibition, proteasome inhibition, modulation of specific kinases, and other cellular processes, which eventually trigger apoptosis must be taken into account. After these initial studies with TrxR, the ER and the COX enzymes which must also be considered as targets based on our drug design, further studies on the mode of action of the gold complexes with expanded biochemical assays are necessary.

Summary

This thesis presented the synthesis characterization, biological studies of silver and gold *N*-heterocyclic carbene (NHC) complexes derived from 4,5-diarylimidazoles.

Six Ag-NHC complexes were synthesized and characterized. Further antiproliferative studies showed that they possessed growth inhibitory effects against mammary (MCF-7 and MDA-MB 231) as well as colon (HT-29) carcinoma cells. They were only marginally active at the DNA, ER and the COX enzymes. So these targets can be excluded to be involved in the mode of action. However, the growth of bacteria was significantly inhibited by the selected complexes and opens a new application of this complex type.

In addition, the neutral Au-NHC complexes were synthesized, characterized, and analyzed for biological effects. High growth inhibitory effects of Au-NHC complexes depended on the presence of the C4,C5-standing aromatic rings. The substituents at the nitrogen atoms and the oxidation state of the metal play a subordinate role. All complexes caused TrxR inhibition distinctly lower than auranofin excluding this enzyme as main target. Because of the low nuclear content, a participation of DNA-interaction on the mode of action is very unlikely. The missing ER-binding and the missing correlation of growth inhibition and inactivation of COX enzymes excluded these targets, too.

Furthermore, Au-bis(NHC) complexes as well as auranofin analogues were synthesized, characterized, and analyzed for biological effects. Compared to Au-NHC complexes, the growth inhibitory effects of Au-bis(NHC) complexes against breast cancer as well as against colon cancer cells strongly increased. This effect, which is more than 10-fold higher than that of cisplatin, is independent of the oxidation state and the anionic counter ion. Besides, the gold core seems to be responsible for the high cytotoxic activity of cationic Au-bis(NHC) complexes, thus, the exchange of the gold center by a methylene group decreased the growth inhibitory effects. Further investigations with selected complexes demonstrated a rapid and high accumulation grade in the tumor cells (50- to 60-fold compared to the extracellular concentration of 10 μM) within the first 6 h, which kept constant for 24 h.

Zusammenfassung

Diese Arbeit beschreibt die Synthese, Charakterisierung und biologische Untersuchungen von N-heterocyclischen Carbenkomplexen (NHC), die von 4,5-Diarylimidazolen abgeleitet wurden. Sechs synthetisierte Silber-NHC Komplexe besaßen wachstumshemmende Wirkung an Brust- (MCF-7 and MDA-MB 231) sowie Colonkarzinom- (HT-29) Zellen. Nachdem sie sich nur als geringfügig aktiv an DNA, ER und an COX-Enzymen erwiesen, wurden die Inhibierung dieser Biomoleküle als Wirkungsmechanismus ausgeschlossen. Interessanterweise hemmen sie das Wachstum verschiedener Bakterienstämme und eröffnet eine neue Anwendung dieser Komplexe.

Darüber hinaus wurden auch neutrale Au-NHC Komplexe synthetisiert, charakterisiert und auf biologische Effekte untersucht. Die wachstumshemmende Wirkung hängt von der Anwesenheit der C4,C5-ständigen aromatischen Ringe ab. Die Substituenten an den Stickstoffatomen und die Oxidationsstufe des Metalls spielen eine untergeordnete Rolle. Alle Komplexe verursachen an TrxR eine deutlich schwächere Hemmung als Auranofin, was nicht mit der zytotoxischen Wirkung korreliert. Aufgrund der geringen Goldmenge in den Zellkernen ist eine Beteiligung von DNA-Interaktion an der Wirkung sehr unwahrscheinlich. Nachdem keine ER-Bindung gefunden wurde und eine Korrelation zwischen Wachstumshemmung und Inaktivierung von COX Enzyme nicht gegeben ist, schließen wir diese Ziele auch aus.

Zudem wurden einige Au-bis(NHC) Komplexe sowie Auranofin-Analoga synthetisiert und getestet. Die Zytotoxizität von Au-bis(NHC) Komplexen ist im Vergleich zu Au-NHC Komplexen stark erhöht und mehr als 10-fach höher als die von Cisplatin. Oxidationsstufe und das Gegenion haben keinen Einfluss. Dagegen verringert der Austausch des Gold-Zentrum durch eine Methylengruppe die wachstumshemmenden Effekte, was die Notwendigkeit des Zentralatoms für die hohe zytotoxische Aktivität von kationischen Au-bis(NHC) Komplexen dokumentiert. Weitere Untersuchungen mit ausgewählten Komplexen zeigten eine schnelle und hohe Aufnahme in die Tumorzellen (50- bis 60-fache in Vergleich zur extrazellulären Konzentration von 10 μM) innerhalb der ersten 6 Stunden. Diese Konzentration blieb konstant für 24 Stunden. Wie bei Au-NHC konnte die Beteiligung von TrxR, den ER-Rezeptoren sowie von COX Enzymen als mögliche pharmakologische Ziele ausgeschlossen werden.

References

1. Ott, I.; Gust, R. Preclinical and clinical studies on the use of platinum complexes for breast cancer treatment. *Anti-Cancer Agents Med. Chem.* **2007,** 7, 95-110.

2. Ott, I.; Gust, R. Non platinum metal complexes as anti-cancer drugs. *Arch. Pharm.* **2007,** 340, 117-126.

3. Hindi, K. M.; Panzner, M. J.; Tessier, C. A.; Cannon, C. L.; Youngs, W. J. The medicinal applications of imidazolium carbene-metal complexes. *Chem. Rev.* **2009,** 109, 3859-3884.

4. Gautier, A.; Cisnetti, F. Advances in metal-carbene complexes as potent anti-cancer agents. *Metallomics* **2012,** 4, 23-32.

5. Thati, B.; Noble, A.; Creaven, B. S.; Walsh, M.; McCann, M.; Kavanagh, K.; Devereux, M.; Egan, D. A. In vitro anti-tumour and cyto-selective effects of coumarin-3-carboxylic acid and three of its hydroxylated derivatives, along with their silver-based complexes, using human epithelial carcinoma cell lines. *Cancer Lett.* **2007,** 248, 321-31.

6. Zhu, H. L.; Zhang, X. M.; Liu, X. Y.; Wang, X. J.; Liu, G. F.; Usman, A.; Fun, H. K. Clear Ag-Ag bonds in three silver(I) carboxylate complexes with high cytotoxicity properties. *Inorg. Chem. Commun.* **2003,** 6, 1113-1116.

7. Liu, J. J.; Galettis, P.; Farr, A.; Maharaj, L.; Samarasinha, H.; McGechan, A. C.; Baguley, B. C.; Bowen, R. J.; Berners-Price, S. J.; McKeage, M. J. In vitro antitumour and hepatotoxicity profiles of Au(I) and Ag(I) bidentate pyridyl phosphine complexes and relationships to cellular uptake. *J. Inorg. Biochem.* **2008,** 102, 303-310.

8. Etaiw Sel, D.; Sultan, A. S.; Badr El-Din, A. S. A novel hydrogen bonded bimetallic supramolecular coordination polymer {[$SnMe_3$(bpe)][$Ag(CN)_2$].$2H_2O$} as anticancer drug. *Eur. J. Med. Chem.* **2011,** 46, 5370-5378.

9. Medvetz, D. A.; Hindi, K. M.; Panzner, M. J.; Ditto, A. J.; Yun, Y. H.; Youngs, W. J. Anticancer activity of Ag(I) *N*-heterocyclic carbene complexes derived from 4,5-dichloro-1H-imidazole. *Met. Based Drugs* **2008,** 2008, 384010.

10. Ray, S.; Mohan, R.; Singh, J. K.; Samantaray, M. K.; Shaikh, M. M.; Panda, D.; Ghosh, P. Anticancer and antimicrobial metallopharmaceutical agents based on palladium, gold, and silver *N*-heterocyclic carbene complexes. *J. Am. Chem. Soc.* **2007,** 129, 15042-15053.

11. Siciliano, T. J.; Deblock, M. C.; Hindi, K. M.; Durmus, S.; Panzner, M. J.; Tessier, C. A.; Youngs, W. J. Synthesis and anticancer properties of gold(I) and silver(I) *N*-heterocyclic carbene complexes. *J. Organomet. Chem.* **2011,** 696, 1066-1071.

12. Teyssot, M. L.; Jarrousse, A. S.; Manin, M.; Chevry, A.; Roche, S.; Norre, F.; Beaudoin, C.; Morel, L.; Boyer, D.; Mahiou, R.; Gautier, A. Metal-NHC complexes: a survey of anti-cancer properties. *Dalton Trans.* **2009**, 6894-6902.

13. Roland, S.; Jolivalt, C.; Cresteil, T.; Eloy, L.; Bouhours, P.; Hequet, A.; Mansuy, V.; Vanucci, C.; Paris, J. M. Investigation of a series of silver-*N*-heterocyclic carbenes as antibacterial agents: activity, synergistic effects, and cytotoxicity. *Chem. Eur. J.* **2011,** 17, 1442-1446.

14. Patil, S.; Claffey, J.; Deally, A.; Hogan, M.; Gleeson, B.; Mendez, L. M. M.; Muller-Bunz, H.; Paradisi, F.; Tacke, M. Synthesis, cytotoxicity and antibacterial studies of *p*-methoxybenzyl-substituted and benzyl-substituted *N*-heterocyclic carbene-silver complexes. *Eur. J. Inorg. Chem.* **2010,** 2010, 1020-1031.

15. Patil, S.; Deally, A.; Gleeson, B.; Hackenberg, F.; Muller-Bunz, H.; Paradisi, F.; Tacke, M. Synthesis, cytotoxicity and antibacterial studies of novel symmetrically and non-symmetrically p-

nitrobenzyl-substituted *N*-heterocyclic carbene-silver-(I) acetate complexes. *Z. Anorg. Allg. Chem.* **2011,** 637, 386-396.

16. Patil, S.; Deally, A.; Gleeson, B.; Muller-Bunz, H.; Paradisi, F.; Tacke, M. Novel benzyl-substituted *N*-heterocyclic carbene-silver acetate complexes: synthesis, cytotoxicity and antibacterial studies. *Metallomics* **2011,** 3, 74-88.

17. Patil, S.; Dietrich, K.; Deally, A.; Gleeson, B.; Muller-Bunz, H.; Paradisi, F.; Tacke, M. Synthesis, cytotoxicity and antibacterial studies of novel symmetrically and nonsymmetrically 4-(methoxycarbonyl)benzyl-substituted *N*-heterocyclic carbene-silver acetate complexes. *Helv. Chimi. Acta* **2010,** 93, 2347-2364.

18. Patil, S.; Deally, A.; Gleeson, B.; Muller-Bunz, H.; Paradisi, F.; Tacke, M. Synthesis, cytotoxicity and antibacterial studies of symmetrically and non-symmetrically benzyl- or p-cyanobenzyl-substituted *N*-Heterocyclic carbene-silver complexes. *Appl. Organomet. Chem.* **2010,** 24, 781-793.

19. Wang, C. H.; Shih, W. C.; Chang, H. C.; Kuo, Y. Y.; Hung, W. C.; Ong, T. G.; Li, W. S. Preparation and characterization of amino-linked heterocyclic carbene palladium, gold, and silver complexes and their use as anticancer agents that act by triggering apoptotic cell death. *J. Med. Chem.* **2011,** 54, 5245-5249.

20. Kaps, L.; Biersack, B.; Muller-Bunz, H.; Mahal, K.; Munzner, J.; Tacke, M.; Mueller, T.; Schobert, R. Gold(I)-NHC complexes of antitumoral diarylimidazoles: Structures, cellular uptake routes and anticancer activities. *J. Inorg. Biochem.* **2012,** 106, 52-58.

21. Ott, I. On the medicinal chemistry of gold complexes as anticancer drugs. *Coord. Chem. Rev.* **2009,** 253, 1670-1681.

22. Nobili, S.; Mini, E.; Landini, I.; Gabbiani, C.; Casini, A.; Messori, L. Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. *Med. Res. Rev.* **2010,** 30, 550-580.

23. Berners-Price, S. J.; Filipovska, A. Gold compounds as therapeutic agents for human diseases. *Metallomics* **2011,** 3, 863-873.

24. Chiellini, C.; Casini, A.; Cochet, O.; Gabbiani, C.; Ailhaud, G.; Dani, C.; Messori, L.; Amri, E. Z. The influence of auranofin, a clinically established antiarthritic gold drug, on bone metabolism: analysis of its effects on human multipotent adipose-derived stem cells, taken as a model. *Chem. Biodivers.* **2008,** 5, 1513-1520.

25. Ott, I.; Qian, X.; Xu, Y.; Vlecken, D. H.; Marques, I. J.; Kubutat, D.; Will, J.; Sheldrick, W. S.; Jesse, P.; Prokop, A.; Bagowski, C. P. A gold(I) phosphine complex containing a naphthalimide ligand functions as a TrxR inhibiting antiproliferative agent and angiogenesis inhibitor. *J. Med. Chem.* **2009,** 52, 763-770.

26. Mirabelli, C. K.; Johnson, R. K.; Hill, D. T.; Faucette, L. F.; Girard, G. R.; Kuo, G. Y.; Sung, C. M.; Crooke, S. T. Correlation of the in vitro cytotoxic and in vivo antitumor activities of gold(I) coordination complexes. *J. Med. Chem.* **1986,** 29, 218-223.

27. Barnard, P. J.; Berners-Price, S. J. Targeting the mitochondrial cell death pathway with gold compounds. *Coord. Chem. Rev.* **2007,** 251, 1889-1902.

28. Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Day, D. A. Mitochondrial permeability transition induced by dinuclear gold(I)-carbene complexes: potential new antimitochondrial antitumour agents. *J. Inorg. Biochem.* **2004,** 98, 1642-1647.

29. Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Skelton, B. W.; White, A. H. Dinuclear

gold(I) complexes of bridging bidentate carbene ligands: synthesis, structure and spectroscopic characterisation. *Dalton Trans.* **2004**, 1038-1047.

30. Baker, M. V.; Barnard, P. J.; Berners-Price, S. J.; Brayshaw, S. K.; Hickey, J. L.; Skelton, B. W.; White, A. H. Synthesis and structural characterisation of linear Au(I) *N*-heterocyclic carbene complexes: New analogues of the Au(I) phosphine drug Auranofin. *J. Organomet. Chem.* **2005,** 690, 5625-5635.

31. Baker, M. V.; Barnard, P. J.; Berners-Price, S. J.; Brayshaw, S. K.; Hickey, J. L.; Skelton, B. W.; White, A. H. Cationic, linear Au(I) *N*-heterocyclic carbene complexes: synthesis, structure and anti-mitochondrial activity. *Dalton Trans.* **2006**, 3708-3715.

32. Hickey, J. L.; Ruhayel, R. A.; Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Filipovska, A. Mitochondria-targeted chemotherapeutics: the rational design of gold(I) *N*-heterocyclic carbene complexes that are selectively toxic to cancer cells and target protein selenols in preference to thiols. *J. Am. Chem. Soc.* **2008,** 130, 12570-12571.

33. Jellicoe, M. M.; Nichols, S. J.; Callus, B. A.; Baker, M. V.; Barnard, P. J.; Berners-Price, S. J.; Whelan, J.; Yeoh, G. C.; Filipovska, A. Bioenergetic differences selectively sensitize tumorigenic liver progenitor cells to a new gold(I) compound. *Carcinogenesis* **2008,** 29, 1124-1133.

34. Barnard, P. J.; Wedlock, L. E.; Baker, M. V.; Berners-Price, S. J.; Joyce, D. A.; Skelton, B. W.; Steer, J. H. Luminescence studies of the intracellular distribution of a dinuclear gold(I) *N*-heterocyclic carbene complex. *Angew. Chem. Int. Ed. Engl.* **2006,** 45, 5966-5970.

35. Horvath, U. E. I.; Bentivoglio, G.; Hummel, M.; Schottenberger, H.; Wurst, K.; Nell, M. J.; van Rensburg, C. E. J.; Cronje, S.; Raubenheimer, H. G. A cytotoxic bis(carbene) gold(I) complex of ferrocenyl complexes: synthesis and structural characterisation. *New J. Chem.* **2008,** 32, 533-539.

36. Weaver, J.; Gaillard, S.; Toye, C.; Macpherson, S.; Nolan, S. P.; Riches, A. Cytotoxicity of gold(I) *N*-heterocyclic carbene complexes assessed by using human tumor cell lines. *Chem. Eur. J.* **2011,** 17, 6620-6624.

37. Rubbiani, R.; Kitanovic, I.; Alborzinia, H.; Can, S.; Kitanovic, A.; Onambele, L. A.; Stefanopoulou, M.; Geldmacher, Y.; Sheldrick, W. S.; Wolber, G.; Prokop, A.; Wolfl, S.; Ott, I. Benzimidazol-2-ylidene gold(I) complexes are thioredoxin reductase inhibitors with multiple antitumor properties. *J. Med. Chem.* **2010,** 53, 8608-8618.

38. Rubbiani, R.; Can, S.; Kitanovic, I.; Alborzinia, H.; Stefanopoulou, M.; Kokoschka, M.; Monchgesang, S.; Sheldrick, W. S.; Wolfl, S.; Ott, I. Comparative in vitro evaluation of *N*-heterocyclic carbene gold(I) complexes of the benzimidazolylidene type. *J. Med. Chem.* **2011,** 54, 8646-8657.

39. Filipovska, A.; Berners-Price, S. J. The design of gold-based, mitochondria-targeted chemotherapeutics. *Aust. J. Chem.* **2008,** 61, 661-668.

40. Krishnamurthy, D.; Karver, M. R.; Fiorillo, E.; Orru, V.; Stanford, S. M.; Bottini, N.; Barrios, A. M. Gold(I)-mediated inhibition of protein tyrosine phosphatases: a detailed in vitro and cellular study. *J. Med. Chem.* **2008,** 51, 4790-4795.

41. Lemke, J.; Pinto, A.; Niehoff, P.; Vasylyeva, V.; Metzler-Nolte, N. Synthesis, structural characterisation and anti-proliferative activity of NHC gold amino acid and peptide conjugates. *Dalton Trans.* **2009**, 7063-7070.

42. Yan, J. J.; Chow, A. L.; Leung, C. H.; Sun, R. W.; Ma, D. L.; Che, C. M. Cyclometalated gold(III) complexes with *N*-heterocyclic carbene ligands as topoisomerase I poisons. *Chem. Commun.* **2010,** 46, 3893-3895.

43. Coetzee, J.; Cronje, S.; Dobrzanska, L.; Raubenheimer, H. G.; Joone, G.; Nell, M. J.; Hoppe, H. C. Novel *N*-heterocyclic ylideneamine gold(I) complexes: synthesis, characterisation and screening for antitumour and antimalarial activity. *Dalton Trans.* **2011,** 40, 1471-1483.

44. Chardon, E.; Puleo, G. L.; Dahm, G.; Guichard, G.; Bellemin-Laponnaz, S. Direct functionalisation of group 10 *N*-heterocyclic carbene complexes for diversity enhancement. *Chem. Commun.* **2011,** 47, 5864-5866.

45. Ciftci, O.; Ozdemir, I.; Vardi, N.; Gurbuz, N. Novel platinum-*N*-heterocyclic carbene complex is more cardiotoxic than cis-platin in rats. *Hum. Exp. Toxicol.* **2011,** 30, 1342-1349

46. Ciftci, O.; Beytur, A.; Cakir, O.; Gurbuz, N.; Vardi, N. Comparison of reproductive toxicity caused by cisplatin and novel platinum-N-heterocyclic carbene complex in male rats. *Basic Clin. Pharmacol. Toxicol.* **2011,** 109, 328-333.

47. Skander, M.; Retailleau, P.; Bourrie, B.; Schio, L.; Mailliet, P.; Marinetti, A. *N*-heterocyclic carbene-amine Pt(II) complexes, a new chemical space for the development of platinum-based anticancer drugs. *J. Med. Chem.* **2010,** 53, 2146-2154.

48. Alves, G.; Morel, L.; El-Ghozzi, M.; Avignant, D.; Legeret, B.; Nauton, L.; Cisnetti, F.; Gautier, A. A platinum Chugaev carbene complex as a potent anticancer agent. *Chem. Commun.* **2011,** 47, 7830-7832.

49. Sun, R. W. Y.; Chow, A. L. F.; Li, X. H.; Yan, J. J.; Chui, S. S. Y.; Che, C. M. Luminescent cyclometalated platinum(II) complexes containing *N*-heterocyclic carbene ligands with potent in vitro and in vivo anti-cancer properties accumulate in cytoplasmic structures of cancer cells. *Chem. Sci.* **2011,** 2, 728-736.

50. Abu-Surrah, A. S.; Kettunen, M. Platinum group antitumor chemistry: design and development of new anticancer drugs complementary to cisplatin. *Curr. Med. Chem.* **2006,** 13, 1337-1357.

51. Gao, E. J.; Liu, C.; Zhu, M. C.; Lin, H. K.; Wu, Q.; Liu, L. Current Development of Pd(II) complexes as potential antitumor agents. *Anti-Cancer Agents Med. Chem.* **2009,** 9, 356-368.

52. van Rijt, S. H.; Sadler, P. J. Current applications and future potential for bioinorganic chemistry in the development of anticancer drugs. *Drug Discov. Today* **2009,** 14, 1089-1097.

53. Tan, S. J.; Yan, Y. K.; Lee, P. P.; Lim, K. H. Copper, gold and silver compounds as potential new anti-tumor metallodrugs. *Future Med. Chem.* **2010,** 2, 1591-1608.

54. Tisato, F.; Marzano, C.; Porchia, M.; Pellei, M.; Santini, C. Copper in diseases and treatments, and copper-based anticancer strategies. *Med. Res. Rev.* **2010,** 30, 708-749.

55. Ruiz-Azuara, L.; Bravo-Gomez, M. E. Copper compounds in cancer chemotherapy. *Curr. Med. Chem.* **2010,** 17, 3606-3615.

56. Bowen, R. J.; Navarro, M.; Shearwood, A. M.; Healy, P. C.; Skelton, B. W.; Filipovska, A.; Berners-Price, S. J. 1 : 2 Adducts of copper(I) halides with 1,2-bis(di-2-pyridylphosphino)ethane: solid state and solution structural studies and antitumour activity. *Dalton Trans.* **2009**, 10861-10870.

57. Drewry, J. A.; Gunning, P. T. Recent advances in biosensory and medicinal therapeutic applications of zinc(II) and copper(II) coordination complexes. *Coord. Chem. Rev.* **2011,** 255, 459-472.

58. Diez-Gonzalez, S.; Marion, N.; Nolan, S. P. *N*-heterocyclic carbenes in late transition metal catalysis. *Chem. Rev.* **2009,** 109, 3612-3676.

59. Teyssot, M. L.; Jarrousse, A. S.; Chevry, A.; De Haze, A.; Beaudoin, C.; Manin, M.; Nolan, S. P.; Diez-Gonzalez, S.; Morel, L.; Gautier, A. Toxicity of copper(I)-NHC complexes against human tumor cells: induction of cell cycle arrest, apoptosis, and DNA cleavage. *Chem. Eur. J.* **2009,** 15, 314-

318.

60. Ray, S.; Asthana, J.; Tanski, J. M.; Shaikh, M. M.; Panda, D.; Ghosh, P. Design of nickel chelates of tetradentate *N*-heterocyclic carbenes with subdued cytotoxicity. *J. Organomet. Chem.* **2009,** 694, 2328-2335.

61. Lee, S. Y.; Hille, A.; Frias, C.; Kater, B.; Bonitzki, B.; Wolfl, S.; Scheffler, H.; Prokop, A.; Gust, R. [Ni^{II}(3-OMe-salophene)]: a potent agent with antitumor activity. *J. Med. Chem.* **2010,** 53, 6064-6070.

62. Levina, A.; Mitra, A.; Lay, P. A. Recent developments in ruthenium anticancer drugs. *Metallomics* **2009,** 1, 458-70.

63. Ciftci, O.; Ozdemir, I.; Cakir, O.; Demir, S. The determination of oxidative damage in heart tissue of rats caused by ruthenium(II) and gold(I) *N*-heterocyclic carbene complexes. *Toxicol. Ind. Health* **2011,** 27, 735-741.

64. Lemke, J.; Metzler-Nolte, N. The synthesis of ruthenium and rhodium complexes with functionalized *N*-heterocyclic carbenes and their use in solid phase peptide synthesis. *Eur. J. Inorg. Chem.* **2008,** 2008, 3359-3366.

65. Gasser, G.; Ott, I.; Metzler-Nolte, N. Organometallic anticancer compounds. *J. Med. Chem.* **2010**, 3-25.

66. Casini, A.; Gabbiani, C.; Sorrentino, F.; Rigobello, M. P.; Bindoli, A.; Geldbach, T. J.; Marrone, A.; Re, N.; Hartinger, C. G.; Dyson, P. J.; Messori, L. Emerging protein targets for anticancer metallodrugs: inhibition of thioredoxin reductase and cathepsin B by antitumor ruthenium(II)-arene compounds. *J. Med. Chem.* **2008,** 51, 6773-6781.

67. Nowak-Sliwinska, P.; van Beijnum, J. R.; Casini, A.; Nazarov, A. A.; Wagnieres, G.; van den Bergh, H.; Dyson, P. J.; Griffioen, A. W. Organometallic ruthenium(II) arene compounds with antiangiogenic activity. *J. Med. Chem.* **2011,** 54, 3895-3902.

68. Ang, W. H.; Casini, A.; Sava, G.; Dyson, P. J. Organometallic ruthenium-based antitumor compounds with novel modes of action. *J. Organomet. Chem.* **2011,** 696, 989-998.

69. Vougioukalakis, G. C.; Grubbs, R. H. Ruthenium-based heterocyclic carbene-coordinated olefin metathesis catalysts. *Chem. Rev.* **2010,** 110, 1746-1787.

70. Oehninger, L.; Alborzinia, H.; Ludewig, S.; Baumann, K.; Wolfl, S.; Ott, I. From catalysts to bioactive organometallics: do grubbs catalysts trigger biological effects? *ChemMedChem* **2011,** 6, 2142-2145.

71. Hartinger, C. G.; Dyson, P. J. Bioorganometallic chemistry--from teaching paradigms to medicinal applications. *Chem. Soc. Rev.* **2009,** 38, 391-401.

72. Bindoli, A.; Rigobello, M. P.; Scutari, G.; Gabbiani, C.; Casini, A.; Messori, L. Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs. *Coord. Chem. Rev.* **2009,** 253, 1692-1707.

73. Casini, A.; Gabbiani, C.; Mastrobuoni, G.; Sorrentino, F.; Dani, B.; Rigobello, M. P.; Bindoli, A.; Cinellu, M. A.; Pieraccini, G.; Messori, L. Thioredoxin reductase, an emerging target for anticancer metallodrugs. Enzyme inhibition by cytotoxic gold(III) compounds studied with combined mass spectrometry and biochemical assays. *Medchemcomm* **2011,** 2, 50-54.

74. Gust, R.; Beck, W.; Jaouen, G.; Schonenberger, H. Optimization of cisplatin for the treatment of hormone-dependent tumoral diseases Part 2: Use of non-steroidal ligands. *Coord. Chem. Rev.* **2009,** 253, 2760-2779.

75. Gust, R.; Beck, W.; Jaouen, G.; Schonenberger, H. Optimization of cisplatin for the treatment

of hormone dependent tumoral diseases Part 1: Use of steroidal ligands. *Coord. Chem. Rev.* **2009,** 253, 2742-2759.

76. Gust, R.; Keilitz, R.; Schmidt, K.; von Rauch, M. Synthesis, structural evaluation, and estrogen receptor interaction of 4,5-bis(4-hydroxyphenyl)imidazoles. *Arch. Pharm.* **2002,** 335, 463-471.

77. Garrison, J. C.; Youngs, W. J. Ag(I) *N*-heterocyclic carbene complexes: synthesis, structure, and application. *Chem. Rev.* **2005,** 105, 3978-4008.

78. Lin, J. C.; Huang, R. T.; Lee, C. S.; Bhattacharyya, A.; Hwang, W. S.; Lin, I. J. Coinage metal-*N*-heterocyclic carbene complexes. *Chem. Rev.* **2009,** 109, 3561-3598.

79. de Fremont, P.; Singh, R.; Stevens, E. D.; Petersen, J. L.; Nolan, S. P. Synthesis, characterization and reactivity of *N*-heterocyclic carbene gold(III) complexes. *Organometallics* **2007,** 26, 1376-1385.

80. Hirtenlehner, C.; Krims, C.; Holbling, J.; List, M.; Zabel, M.; Fleck, M.; Berger, R. J.; Schoefberger, W.; Monkowius, U. Syntheses, crystal structures, reactivity, and photochemistry of gold(III) bromides bearing *N*-heterocyclic carbenes. *Dalton Trans.* **2011,** 40, 9899-9910.

81. Wang, H. M. J.; Lin, I. J. B. Facile synthesis of silver(I)-carbene complexes. Useful carbene transfer agent. *Organometallics* **1998,** 17, 972-975.

82. Jothibasu, R.; Huynh, H. V.; Koh, L. L. Au(I) and Au(III) complexes of a sterically bulky benzimidazole-derived *N*-heterocyclic carbene. *J. Organomet. Chem.* **2008,** 693, 374-380.

83. Braussaud, N.; Rüether, T.; Cavell, K. J.; Skelton, B. W.; White, A. H. Bridged 1-methylbisimidazoles as building blocks for mixed donor bi- and tridentate chelating ligands. *Synthesis* **2001,** 2001, 626-632.

84. Scheffler, H.; You, Y.; Ott, I. Comparative studies on the cytotoxicity, cellular and nuclear uptake of a series of chloro gold(I) phosphine complexes. *Polyhedron* **2010,** 29, 66-69.

85. Gust, R.; Ott, I.; Posselt, D.; Sommer, K. Development of cobalt(3,4-diarylsalen) complexes as tumor therapeutics. *J. Med. Chem.* **2004,** 47, 5837-5846.

86. Hille, A.; Ott, I.; Kitanovic, A.; Kitanovic, I.; Alborzinia, H.; Lederer, E.; Wolfl, S.; Metzler-Nolte, N.; Schafer, S.; Sheldrick, W. S.; Bischof, C.; Schatzschneider, U.; Gust, R. [N,N'-Bis(salicylidene)-1,2-phenylenediamine]metal complexes with cell death promoting properties. *J. Biol. Inorg. Chem.* **2009,** 14, 711-725.

87. Gust, R.; Busch, S.; Keilitz, R.; Schmidt, K.; Von Rauch, M. Investigations on the influence of halide substituents on the estrogen receptor interaction of 2, 4, 5-tris(4-hydroxyphenyl)imidazoles. *Arch. Pharm.* **2003,** 336, 456-465.

88. Wiglenda, T.; Ott, I.; Kircher, B.; Schumacher, P.; Schuster, D.; Langer, T.; Gust, R. Synthesis and pharmacological evaluation of 1*H*-imidazoles as ligands for the estrogen receptor and cytotoxic inhibitors of the cyclooxygenase. *J. Med. Chem.* **2005,** 48, 6516-6521.

89. Gust, R.; Keilitz, R.; Schmidt, K.; von Rauch, M. Synthesis, structural evaluation, and estrogen receptor interaction of 4,5-bis(4-hydroxyphenyl)imidazoles. *Arch. Pharm.* **2002,** 335, 463-471.

90. Chen, X. H.; Scholz, R.; Borriss, M.; Junge, H.; Mogel, G.; Kunz, S.; Borriss, R. Difficidin and bacilysin produced by plant-associated Bacillus amyloliquefaciens are efficient in controlling fire blight disease. *J. Biotechnol.* **2009,** 140, 38-44.

91. Wetzel, C.; Kunz, P. C.; Kassack, M. U.; Hamacher, A.; Bohler, P.; Watjen, W.; Ott, I.; Rubbiani, R.; Spingler, B. Gold(I) complexes of water-soluble diphos-type ligands: synthesis,

anticancer activity, apoptosis and thioredoxin reductase inhibition. *Dalton Trans* **2011,** 40, 9212-20.
92. Ott, I.; Schmidt, K.; Kircher, B.; Schumacher, P.; Wiglenda, T.; Gust, R. Antitumor-active cobalt-alkyne complexes derived from acetylsalicylic acid: studies on the mode of drug action. *J. Med. Chem.* **2005,** 48, 622-629.

Curriculum Vitae

For reasons of data protection, the curriculum vitae is not published in the online version.

List of publications

1. Wukun Liu, Kerstin Bensdorf, Ulrich Abram, Ben Niu, Aruljothi Mariappan, Ronald Gust. Synthesis and biological studies of silver *N*-heterocyclic carbene complexes derived from 4,5-diarylimidazole. ***European Journal of Medicinal Chemistry*** **2011**, *46*(12), 5927-5934.

2. Wukun Liu, Kerstin Bensdorf, Maria Proetto, Ulrich Abram, Adelheid Hagenbach, Ronald Gust. NHC gold halide complexes derived from 4,5-diarylimidazole: synthesis, structural analysis, and pharmacological investigations as potential antitumor agents. ***Journal of Medicinal Chemistry*** **2011**, *54*(24), 8605-8615.

3. Wukun Liu, Kerstin Bensdorf, Maria Proetto, Adelheid Hagenbach, Ulrich Abram, Ronald Gust. Synthesis, characterization, and in vitro studies on bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I/III) Complexes. ***Journal of Medicinal Chemistry*** **2012**, *55*(8), 3713–3724.

4. Wukun Liu, Xiaohua Chen, Ronald Gust. Synthesis, pharmacological investigations of bis(4,5-diarylimidazol-2-ylidene) derivatives as antitumor and antibacterial agents ***Archiv der Pharmazie*** **2012** (DOI: 10.1002/ardp.201100474)

Appendixes

Wukun Liu, Kerstin Bensdorf, Ulrich Abram, Ben Niu, Aruljothi Mariappan, Ronald Gust. Synthesis and biological studies of silver *N*-heterocyclic carbene complexes derived from 4,5-diarylimidazole. ***European Journal of Medicinal Chemistry*** **2011**, *46*(12), 5927-5934.
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Wukun Liu, Kerstin Bensdorf, Maria Proetto, Ulrich Abram, Adelheid Hagenbach, Ronald Gust. NHC gold halide complexes derived from 4,5-diarylimidazole: synthesis, structural analysis, and pharmacological investigations as potential antitumor agents. ***Journal of Medicinal Chemistry*** **2011**, *54*(24), 8605-8615.
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Wukun Liu, Kerstin Bensdorf, Maria Proetto, Adelheid Hagenbach, Ulrich Abram, Ronald Gust. Synthesis, characterization, and in vitro studies on bis[1,3-diethyl-4,5-diarylimidazol-2-ylidene]gold(I/III) Complexes. ***Journal of Medicinal Chemistry*** **2012**, *55*(8), 3713–3724.
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Wukun Liu, Xiaohua Chen, Ronald Gust. Synthesis, pharmacological investigations of bis(4,5-diarylimidazol-2-ylidene) derivatives as antitumor and antibacterial agents ***Archiv der Pharmazie*** **2012** (DOI: 10.1002/ardp.201100474)
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