



## Review

## On the medicinal chemistry of gold complexes as anticancer drugs

Ingo Ott

Institute of Pharmaceutical Chemistry, Technische Universität Braunschweig, Beethovenstr. 55, 38106 Braunschweig, Germany

## Contents

1. Introduction .....	1670
2. From antirheumatic drugs to anticancer drugs .....	1671
3. The mechanism of action of auranofin and related gold complexes .....	1671
4. Side effects of gold metallodrugs .....	1672
5. Cellular biodistribution of gold compounds .....	1673
6. Other pharmacological uses of gold drugs .....	1673
7. Gold(I) complexes .....	1673
7.1. Auranofin and close analogues .....	1673
7.2. Gold phosphole compounds .....	1674
7.3. Gold(I) complexes with multiple phosphine ligands .....	1674
7.4. Gold(I) carbene complexes .....	1676
7.5. Various other gold(I) complexes .....	1676
8. Gold(II) complexes .....	1677
9. Gold(III) complexes .....	1677
9.1. Gold(III) complexes with Au–N bonds .....	1677
9.2. Gold(III) complexes with Au–S bonds .....	1679
9.3. Gold(III) complexes containing Au–C bonds .....	1679
10. Summary .....	1680
References .....	1680

## ARTICLE INFO

## Article history:

Received 1 November 2008

Accepted 13 February 2009

Available online 27 February 2009

## Keywords:

Gold complexes

Thioredoxin reductase

Metallodrugs

Mitochondria

## ABSTRACT

Metal complexes have shown interesting preclinical and clinical results as antitumor drugs and platinum compounds are well established in current cancer chemotherapy. However, the platinum based treatment of tumoral diseases is massively hampered by severe side effects and resistance development. Consequently, the development of novel metallodrugs with a pharmacological profile different from that of the platinum drugs is in the focus of modern medicinal chemistry and drug design.

Among the non-platinum antitumor drugs, gold complexes have recently gained considerable attention due to their strong antiproliferative potency. In many cases the cell growth inhibiting effects could be related to anti-mitochondrial effects making gold species interesting drug candidates with a mode of action different from that of the platinum agents. The spectrum of gold complexes described as antiproliferative compounds comprises a broad variety of different species including many phosphine complexes as well as gold in different oxidation states.

This presentation gives an overview of the relevant medicinal chemistry of known gold complexes with *in vitro* and *in vivo* tumor growth inhibiting properties.

© 2009 Elsevier B.V. All rights reserved.

## 1. Introduction

Cancer and tumoral malignancies remain among the most widespread and difficult to treat diseases often causing poor gen-

eral conditions of patients and are characterised by a high lethality rate. Despite tremendous efforts to improve therapy and recent advances, the spectrum of available effective drugs is comparably limited and there is a considerable need for the development of new drugs and treatment alternatives. Since their serendipitous discovery, platinum based antitumor agents (e.g. cisplatin, carboplatin or oxaliplatin) have been among those agents, which significantly influenced and shaped current tumor chemotherapy.

E-mail address: [ingo.ott@tu-bs.de](mailto:ingo.ott@tu-bs.de).

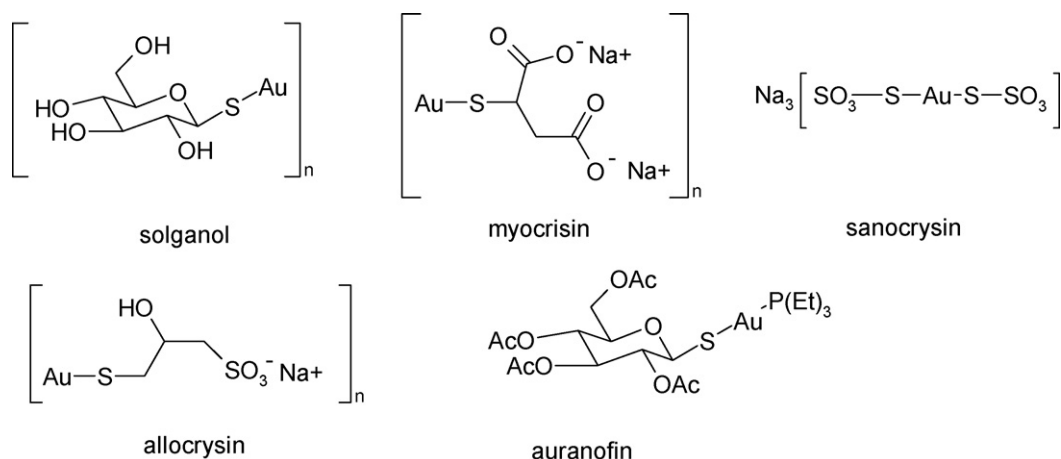


Fig. 1. Gold complexes used for chrysotherapy.

However, severe side effects and frequent development of resistance phenomena complicate and hamper the clinical application [1–3].

From the viewpoint of pharmaceutical and medicinal chemistry the platinum anticancer drugs are of special interest as metal complexes are rarely used as therapeutics. Based on their different kinetics, geometries and reactivities compared to classical non-metal organic drugs, however, metal compounds might offer a huge unexplored chemical space concerning modern drug design and development, which has probably been underestimated by both academic and industrial drug research so far. Consequently, in recent years the interest in non-platinum metal complexes for cancer chemotherapy has been rapidly growing and been stimulated by the possibility to develop new agents with a mode of action and clinical profile different from the established platinum metallodrugs [4–7].

Among the new non-platinum drugs especially gold species have gained more and more attention due to their generally strong tumor cell growth inhibiting effects and the observation that many of the compounds inhibit the enzyme thioredoxin reductase (TrxR) with high potency and specificity. TrxR is relevant for the proliferation of tumor tissues and its inhibition is related to the triggering of anti-mitochondrial effects. Thus, gold species might give access to a class of non-platinum metal compounds with non-cisplatin-like pharmacodynamic and pharmacokinetic properties, which are major goals of bioinorganic and bioorganometallic medicinal chemistry research.

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. Based on the great structural variety of the used ligands a unique mode of action or pharmacological profile is unlikely to exist and also pharmacodynamic effects not related to TrxR inhibition are likely to have high relevance for the pharmacology of gold metallodrugs.

Several reviews on the use of gold complexes have appeared in the recent years highlighting the special interest in this class of metal complexes for antitumor drug development [8–11]. This review aims to give an overview of the medicinal chemistry of gold complexes as antitumor compounds and to present relevant examples with described pharmacological activity.

## 2. From antirheumatic drugs to anticancer drugs

Gold compounds have been playing an important medical role since ancient times and their use can be dated back more than 2000 years [12–14].

Today still symptoms of rheumatoid arthritis are treated with various gold drugs (see Fig. 1) including aurothioglucose (solganol), aurothiomalate (myocrisin) aurothiosulfate (sanocrysin), aurothiopropyl sulfonate (allocrysin) and triethylphosphine-gold(I)tetraacetylthioglucose (auranofin). Rheumatoid arthritis is a painful and disabling chronic autoimmune disease causing inflammatory conditions and progressive joint erosion. The cause of the disease is so far unknown. Current treatment strategies mainly focus on alleviating the symptoms and preventing the progressive destructive processes. The pharmacological therapy uses anti-inflammatory agents, analgetics and so-called disease modifying antirheumatic drugs (DMARDs). Antirheumatic gold complexes belong to the class of DMARDs, which are used to halt or slow down disease progression and lower bone and cartilage damage. The application of DMARDs is of special importance as the mentioned damages are generally irreversible and cannot be influenced by anti-inflammatory agents and analgesics. The application of gold complexes in medicine has been called “chrysotherapy” and aims at reducing inflammation and disease progression in patients with rheumatoid arthritis. Of the above-mentioned gold salts auranofin is of special interest since it can be administered orally in contrast to the other gold salts, which are usually given by injection [15,16].

Experimental research on auranofin revealed that it also displayed promising cell growth inhibiting effects *in vitro* and some efficacy in experimental *in vivo* models. Thus, auranofin showed an increased life span correlating with the administered dose in mice inoculated with P388 leukemia cells [17]. As it showed only limited efficiency in other *in vivo* studies it is so far unclear if auranofin itself might be a suitable antitumor drug candidate. In a variety of mouse tumor models auranofin was only active against i.p. P388 leukemia and only when administered i.p. [18]. However, these studies have definitely triggered an immense interest in the research on gold complexes for cancer chemotherapy.

## 3. The mechanism of action of auranofin and related gold complexes

The mechanism of action of antirheumatic or antiproliferative gold complexes had been under question for a long time. Concerning its antirheumatic properties various enzymes such as cyclooxygenases (COX) had been considered as possible targets and the suppression of the activities of these enzymes as well as others has been reported (see below). Similarly, for auranofin, and aurothioglucose the inhibition of selenium-glutathione peroxidase had been reported [19,20].

However, reports that auranofin and other gold(I) complexes potentially inhibited the enzyme TrxR have most probably answered the question on the biological main target of gold complexes [21]. Auranofin inhibited TrxR with high potency and approximately 1000-fold selectivity compared to other related enzymes (glutathione reductase and glutathione peroxidase) [21]. Based on the different ligand structures of the many gold complexes, for which cell growth inhibiting properties have been observed, a unique mode of action of the agents is not likely to exist but an increasing number of reports on gold complexes with significant TrxR inhibitory properties underlines the relevance of this enzyme in the pharmacology of gold metallodrugs. In this context it is of interest to note that the inhibition of TrxR has been reported not only for different gold(I) complexes but also for various gold(III) compounds [22].

TrxR is a homodimeric protein belonging to the family of glutathione reductase like enzymes. It catalyzes the NADPH-dependent reduction of thioredoxin (Trx) disulfide and many other oxidized cell constituents. TrxR has been identified in different species, such as the malaria parasite *Plasmodium falciparum*, *Drosophila melanogaster*, and humans. The enzyme shows a broad substrate specificity and is involved in numerous metabolic pathways (e.g. antioxidative network, nucleotide synthesis) and pathophysiological conditions (tumors, infectious diseases, rheumatoid arthritis, etc. [23]) Due to its antioxidant properties the thioredoxin system is regarded as preventing cells from oxidative stress, which is a key factor for DNA damage. Overexpression of TrxR has been observed in numerous tumor cell lines and potent cytostatics, like carmustine and cisplatin, are effective inhibitors of TrxR [23]. Furthermore, high levels of the substrate Trx have been associated with the resistance to the platinum anticancer drug cisplatin [23–29].

The active site of TrxR contains a selenocysteine (Sec) containing Gly-Cys-Sec-Gly motif involved in the catalytic mode of action of the enzyme. During enzyme catalysis reducing equivalents are transferred from the substrate NADPH to thioredoxin by means of the FAD prosthetic group [25].

Based on the high affinity of the electrophilic gold center of gold(I) complexes to the nucleophilic sulfur and selenium containing residues a covalent interaction seemed likely as a mode of drug action. The preferred binding of auranofin to the selenocysteine residue was suggested based on the fact that the agent inhibited glutathione reductase, an enzyme which is structurally and functionally closely related to TrxR but lacks the Sec residue in the active site, with significantly lower affinity [21].

Recently, for the gold phosphole complex GoPI covalent binding to cysteines was indicated by the results of mutation experiments and confirmed by crystallography experiments on the enzyme glutathione reductase. Interestingly, upon exposure to the enzyme the gold atom had lost its former ligands and was coordinated by two cysteine residues of the active site of glutathione reductase (for a more detailed description of the binding mode see the below section). This suggests that the “undressing” of gold complexes might represent a general mode of interaction of gold agents with cysteine or selenocysteine containing enzymes [30,31].

In this context the importance of cysteine containing proteins in the pharmacology of auranofin and analogues is additionally underlined by the observations that metallothioneins (which are thiol-rich metal binding proteins) are involved in the resistance of cells towards gold compounds [32,33].

The inhibition of the mitochondrial form of the above-mentioned enzyme TrxR by auranofin is in good agreement with early reports on anti-mitochondrial effects of gold compounds in several *in vitro* studies.

TEPAuCl ((triethylphosphine)gold(I)chloride), an analogue of auranofin containing the triethylphosphine fragment of auranofin

but a chlorine ligand instead of the thiocarbohydrate moiety, caused anti-mitochondrial effects such as mitochondrial swelling or increased permeability of the inner membrane in isolated rat liver mitochondria. These effects could be reversed or weakened by the thiol reducing agent dithiothreitol suggesting an involvement of mitochondrial thiols [34,35]. Studies on freshly isolated rat hepatocytes exposed to TEPAuCl similarly indicated that mitochondria might be the target organelles for TEPAuCl [36]. Auranofin itself induced the mitochondrial membrane permeability transition observed as swelling and loss of membrane potential. Both events could be completely reversed by cyclosporin A, a specific inhibitor of mitochondrial permeability transition [37].

Besides the inhibition of TrxR and related enzymes different mechanisms most likely contribute to the pharmacological profile of gold complexes and based on their different ligands, different kinetic properties, geometries and other features a unique mode of action is most unlikely to exist.

Gold(I) species with labile ligands (such as the Cl in TEPAuCl) interact readily with isolated DNA and therefore this biomolecule cannot be excluded as a possible target for gold drugs [38]. However, auranofin, myochrisin and other gold(I) complexes with tight binding ligands did not bind to the DNA. Interestingly it was observed that 2-mercaptoethanol caused a dissociation of gold–DNA complexes and a regeneration of closed circular superhelical pBR322 DNA [39,40]. Interaction with the DNA has also been confirmed for the gold phosphole complex GoPI or several gold(III) complexes [31,41].

Other biological targets have also attracted attention. Auranofin and arsenic trioxide caused the inhibition of cellular selenoprotein synthesis, which indicates that gold agents may not only inhibit their main biological target TrxR directly but also perturb the related biochemistry concerning protein expression [42]. Auranofin and TEPAuCl stimulated phospholipase C activity, an enzyme which is increased in rheumatoid patients [43].

Cyclooxygenases (COX) and lipoxygenases (LOX) represent another class of enzymes important for the treatment of inflammation and cancer. Recently it was demonstrated that auranofin inhibited the formation of eicosanoids related to the activity of COX-1 and 12-LOX in human platelets [44]. In human synovial fibroblast-like cells auranofin inhibited the biosynthesis of the COX enzymatic product prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and suppressed COX-2 expression [45]. In rat peritoneal macrophages dual effects were observed with auranofin: in unstimulated cells the formation of PGE<sub>2</sub> was increased but in TPA stimulated cells inhibited [46,47]. Auranofin stimulated COX-1-dependent PGE<sub>2</sub> production but inhibited COX-2-dependent PGE<sub>2</sub> production [48].

Based on molecular modeling and mutation experiments for aurothiomalate the selective targeting of Cys-69 in the PB1 domain of protein kinase C  $\iota$  was suggested as a possible mode of action in human lung cancer cells [49].

For gold(III) complexes the partial reversible inhibition of ribonuclease A (RNase A) and deoxyribonuclease I (DNase I) has been reported [50].

As an overall result these studies suggest that besides inhibition of TrxR, which may represent the most relevant target for most gold complexes, the interaction with various biomolecules seems to be important for the pharmacology of antiproliferative gold complexes.

#### 4. Side effects of gold metallodrugs

Similar to the mode of action the occurrence of side effects is strongly dependent on the concrete structure and the ligands of the respective gold agents. However, some major side effects have been reported and need to be addressed in the development of gold

metallo drugs. Generally side effects occur after the accumulation of gold in the body and can affect skin, blood, kidneys or other organs. Of the highest relevance are mucutaneous side effects (e.g. skin rashes, dermatitis or stomatitis). Other major reported side effects include proteinurie, thrombocytopenia or nephropathy [51–58].

Toxic effects on the brain have also been observed. However, long-term treatment of mice with tetrachloroaurate showed that toxic effects did not readily develop and may be of relevance only at higher dosage. Investigation of the gold content of the brain resulted in gold levels below the detection limit of the used atomic absorption spectrometer and may explain the non-brain toxicity of this gold salt [59].

## 5. Cellular biodistribution of gold compounds

Besides the concrete interaction of a drug with its biological target other parameters regulating its absorption and biodistribution play important roles.

The cellular association and uptake of auranofin and TEPAuCl did not require metabolic energy and were temperature dependent. It was suggested that the rate-limiting step for the uptake and distribution is the exchange of the tetraacetylthioglucose or chloride with a sulfhydryl group [60]. Experiments in RAW 264.7 macrophages showed that the cellular association of auranofin was concentration, time and temperature dependent and that the uptake was reduced with increasing amounts of fetal calf serum or albumine in the media. An interesting observation of the same study was that no tetraacetylglucose from auranofin was associated to the cells but Au and the triethylphosphine moiety were internalized and distributed between nuclear, cytosolic and membrane fractions of the cells. This underlined the importance of the model of a ligand exchange process being involved in the mode of action [61].

The cellular uptake of auranofin can in general be rated as high if compared to other metallo drugs and could be correlated with the triggered cytotoxic effects [62]. Thus, in HT-29 colon carcinoma cells the intracellular gold concentration exceeded the exposure level approximately 10–50-fold (for comparison: with the platinum anticancer drugs cisplatin and carboplatin only up to 6-fold accumulation can be reached [63,64]) and the gold uptake increased with increasing exposure concentrations of auranofin [62].

## 6. Other pharmacological uses of gold drugs

Besides their use as antirheumatic drugs and their potential application in tumor therapy gold drugs could be of interest for the treatment of various diseases. Thus, future applications are also envisaged in infectious and parasitic diseases. For example, during *in vitro* studies on auranofin and other gold complexes a significant inhibition of *P. falciparum* growth was noted indicating that there might be an additional use as antimalarial drug [65,66]. However, the use of gold drugs as antiparasitic and antiinfective agents is out of the scope of this article and is addressed in another contribution in the same issue of this journal.

## 7. Gold(I) complexes

Starting from the gold(I) species used in chrysotherapy for the treatment of rheumatoid arthritis the research on gold complexes has so far focused mainly on complexes with gold in the oxidation state +1.

### 7.1. Auranofin and close analogues

As noted above the lead structure for antiproliferatively active gold complexes is the gold glucose derivative auranofin,

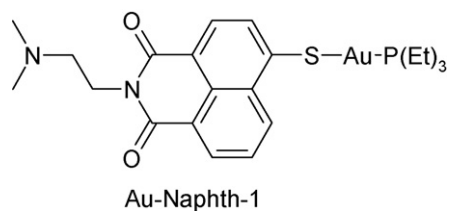


Fig. 2. Au-Naphth-1.

which represents a neutral, linear two coordinate gold phosphine complex containing a carbohydrate ligand. Consequently, an increasing number of studies on the potential of gold complexes as anticancer drugs deals with analogues of this compound.

An early structure–activity relationship study on 63 auranofin analogues had demonstrated the importance of the phosphine ligand and as derivatives lacking this moiety were significantly less active. This study demonstrated that *in vitro* inactive compounds also were not active *in vivo*. However, activity *in vitro* did not necessarily translate into activity *in vivo* [67].

Pharmacological research on auranofin and closely related derivatives is still the focus of a huge number of ongoing studies shedding light on the complicated pharmacodynamic and pharmacokinetic profile of these drugs.

Thus, it was observed that apoptosis induction by auranofin and TEPAuCl in Jurkat T cells appeared to be mediated by the inhibition of the cytosolic and mitochondrial forms of TrxR and that it was accompanied by an increase in cellular hydrogen peroxide levels. In contrast aurothiomalate was only little effective concerning TrxR inhibition and apoptosis induction [68].

Moreover, drug resistance as one of the major problems of current cancer chemotherapy might be addressed by the use of gold(I) complexes as auranofin was also effective in cisplatin-resistant human ovarian cancer cells, which exhibited elevated levels of TrxR activity [69].

Au-Naphth-1 (see Fig. 2) is a gold(I) species, which contains the triethylphosphine moiety of auranofin and a naphthalimide ligand replacing the carbohydrate ligand of auranofin [70]. The drug design strategy for this novel gold complex was motivated by the aim to replace the auranofin carbohydrate ligand, which is supposedly more relevant for the biodistribution of the compound than for its pharmacodynamic effects, by an other bioactive ligand. As bioactive ligand the naphthalimide moiety was chosen based on the promising preclinical results of the naphthalimide class of antitumor drugs [71]. Au-Naphth-1 displayed promising cell growth inhibiting effects, induced apoptosis and inhibited TrxR. Mass spectrometric investigations on a cysteine containing model peptide showed that Au-Naphth-1 bound covalently to the cysteine residue under loss of its thionaphthalimide ligand, which indicates that the interaction with TrxR might also be based on a covalent binding mechanism. Evaluation of the biodistribution of Au-Naphth-1 by fluorescence microscopy and atomic absorption spectroscopy showed that the compound was taken up into the compartments of tumor cells and that the transport of gold into the nuclei was increased in comparison to the chloro analogue TEPAuCl. Interestingly, Au-Naphth-1 also exhibited strong inhibitory effects on angiogenesis in zebrafish embryos. However, this effect could be related to the presence of the naphthalimide ligand as TEPAuCl was not active in this assay [70].

An increasing number of research reports also describes linear gold(I) phosphine species with ligands structurally more distant from auranofin and its close analogues. Some relevant examples are described in the following sections.



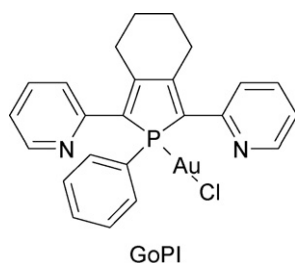


Fig. 3. The gold phosphole complex GoPI.

## 7.2. Gold phosphole compounds

Exceptional biological properties have also been reported recently for gold phosphole complexes. These gold agents are characterised by a phosphacyclopentadiene ligand attached to the central metal and are potent inhibitors of TrxR and the related glutathione reductase. The complex GoPI (see Fig. 3) shows  $EC_{50}$  values for enzyme inhibition in the low nanomolar range and is probably the most potent inhibitor of glutathione reductase reported so far. For the interaction with glutathione reductase the covalent binding to the enzyme was confirmed by crystallographic studies. Interestingly, GoPI bound to a surface exposed cysteine residue (Cys-284) of the enzyme and cross-linked two cysteine residues (Cys-58 and Cys-63) of the active site under formation of a Cys-Au-Cys bridge and loss of its ligands (see Fig. 4). Concerning inhibition of TrxR the interaction with the relevant Sec residue in the active center was confirmed by mutation experiments. The strong enzyme inhibitory potential was accompanied by  $IC_{50}$  values for cell growth inhibi-

tion in the low micromolar range in several glioblastoma cells. A thiophene analogue in which one of the 2-pyridyl rings of GoPI was replaced by a 2-thiophenyl showed comparable activity concerning both enzyme inhibition and antiproliferative effects. Analogous platinum complexes were similar effective in inhibiting TrxR but significantly less active in inhibition glutathione reductase and glioblastoma cell growth [30,31].

A sugar analogue of GoPI, in which the chlorine ligand was exchanged by the carbohydrate moiety of auranofin, displayed improved stability, inhibited TrxR *in vitro* and triggered marked cytotoxic and cytostatic effects in MCF-7 breast cancer cells [72].

## 7.3. Gold(I) complexes with multiple phosphine ligands

A broad range of reports exists describing active agents with multiple phosphine ligands attached to the gold(I) central atom. The lead compound for this class of compounds is  $[Au(dppe)_2]^+$  (see Fig. 5).

$[Au(dppe)_2]^+$  is a tetrahedral bischelated gold complex, which showed antitumor activity *in vitro* and *in vivo* and induced DNA protein cross-links and DNA strand breaks in cells. The complex is kinetically stable in the presence of thiols and its pharmacological profile appears in general to be different from that of auranofin. Structure–activity relationship studies suggested that the optimal length of the bridge connecting the two phosphorus atoms is 2–3 C atoms [73].

Free bisphosphines related to the ligand dppe were active in mice bearing i.p. P388 leukemia. When coordinated as gold(I) complexes the activity was similar but at a lower dose than free dppe. Replacement of the phenyl groups with ethyl or benzyl groups led to inactive compounds whereas by substitution with cyclohexyl or

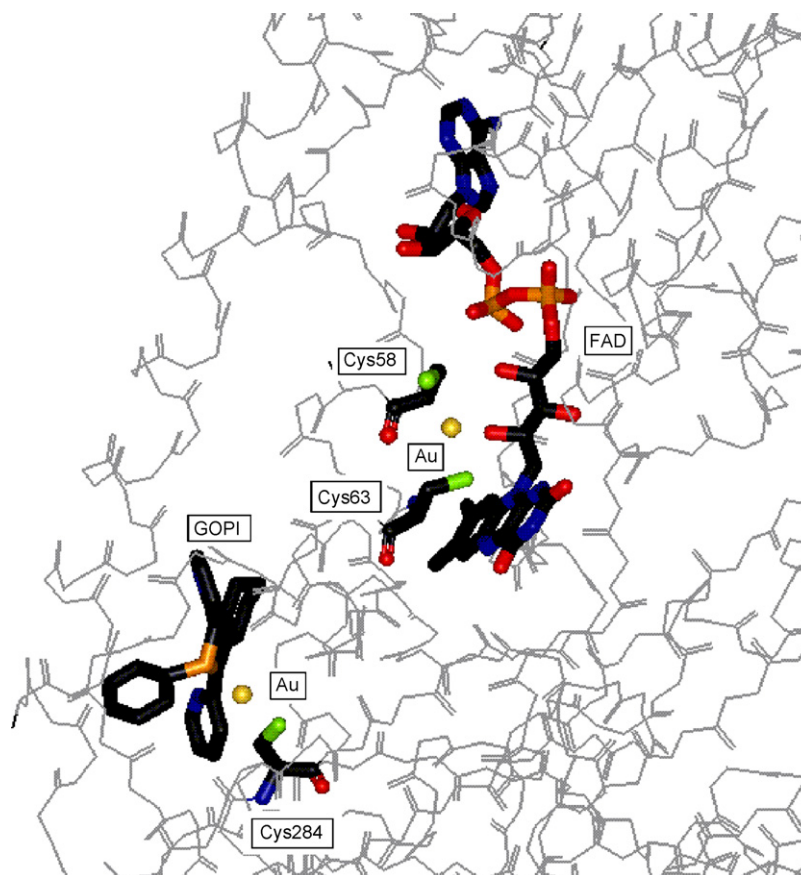


Fig. 4. Interaction of GoPI with glutathione reductase; the picture was generated from the RCSB protein database entry 2AAQ [30] ([www.pdb.org](http://www.pdb.org)) using ViewerLite 4.2; the protein backbone is depicted with grey lines; relevant cysteine residues, GoPI, the gold atoms and FAD are highlighted in colored stick display.

heterocyclic rings active analogues were obtained. Activity was also observed with complexes containing phenyl rings with fluoro or methylthio groups but various analogues with methyl, methoxy, dimethylamino or trifluoromethyl groups were marginally active or inactive. Concerning the bridge linking the two phenyl rings optimum activity was found in this *in vivo* model for ethane and cis-ethylene derivatives [74].

However,  $[\text{Au}(\text{dppe})_2]^+$  triggered cardiotoxic effects in rabbits. The authors of the study also reported a series of *in vitro* experiments on isolated rabbit myocytes showing a disruption of mitochondrial function (as indicated by mitochondrial swelling or a rapid dissipation of the mitochondrial inner membrane electrochemical potential) [75]. Swelling of mitochondria, dissipation of mitochondrial membrane potential and other anti-mitochondrial effects were also observed in isolated rat hepatocytes [76,77].

Physicochemical studies on  $[\text{Au}(\text{I})(\text{dppe})_2]^+$  and some analogues revealed a correlation between drug lipophilicity, cellular uptake and activity [78]. Furthermore, the influence of the anion on the antitumor activity was negligible [79].

In this context,  $[\text{Au}(\text{dppe})_2]^+$  and its analogues belong to the group of delocalized lipophilic cations (DLCs), which can pass through cellular membranes and accumulate in the mitochondria. Consequently, the lipophilicity of the compounds is a very important parameter in optimizing the biodistribution and activity.

Structurally related bidentate phenyl and pyridyl phosphine complexes showed activity against cisplatin-resistant human ovarian cancer cells. Concerning the cytotoxicity the phenyl- and 2-pyridyl analogues were superior to the 3- and 4-pyridyl complexes. During exposure to the dinuclear 2-pyridyl derivative SMIV gold was mainly found in the mitochondrial fractions of CH-1 cells compared to nuclear and cytosolic fractions highlighting the special role of mitochondria for the antitumor potency of gold compounds. Interestingly, the *in vitro* hepatotoxicity of the complexes correlates with the lipophilicity and cellular uptake of the agents. The authors concluded that complexes with a medium lipophilicity might be optimal for further development [80,81].

The  $[\text{Au}(\text{dppe})_2]^+$  propylene analogue  $[\text{Au}(\text{d2pypp})_2]\text{Cl}$  showed selectivity against cancer cells in comparative experiments using normal and cancer breast cells and selectively induced apoptosis in breast cancer cells but not in normal cells. Apoptosis was

induced via the mitochondrial pathway involving mitochondrial membrane potential depolarisation, depletion of the glutathione pool and caspase-3 and caspase-9 activation. The accumulation in the mitochondria was confirmed for the compound. Furthermore, the complex inhibited both thioredoxin and thioredoxin reductase activities [82,83].

The mixed gold phosphine compound chlorotriphenylphosphine-1,3-bis(diphenylphosphino)propanegold(I)  $[\text{Au}(\text{dppp})(\text{PPh}_3)\text{Cl}]$  was investigated in the national cancer institute (NCI) 60 cell panel screening and showed  $\text{LC}_{50}$  values in the low micromolar range in 29 cell lines. The results were most marked in MCF-7 breast cancer cells with the  $\text{LC}_{50}$  value being below the lowest test concentration used ( $0.01 \mu\text{M}$ ). Unexpectedly, the compound was not active in leukemia cells [84]. Further studies on this agent were performed in a panel of human melanoma cell lines, in which the complex displayed  $\text{IC}_{50}$  values in the low micromolar range. An apoptotic response was noted with alterations in the nuclear morphology, loss of mitochondrial membrane potential, cytochrome C and Smac/DIABLO release from mitochondria into cytosol and enhanced caspase-9 and caspase-3 catalytic activity as well as a decreased expression of anti-apoptotic proteins [85]. However,  $[\text{Au}(\text{dppp})(\text{PPh}_3)\text{Cl}]$  decomposes in solution under formation of mono- and dinuclear complexes making the nature of the pharmacologically active species unclear [86].

The dinuclear cationic complex  $[\{\text{AuCl}(\text{PPh}_3)\}_2(\mu_2\text{-DIPHOS})]$  is the unexpected outcome of a study aiming at developing an ethylene analogue of  $[\text{Au}(\text{dppp})(\text{PPh}_3)\text{Cl}]$ .  $[\{\text{AuCl}(\text{PPh}_3)\}_2(\mu_2\text{-DIPHOS})]$  showed activity against JR-8, SK-Mel-5 and 2/60 melanoma cell lines, however, it was less effective than  $[\text{Au}(\text{DPPP})(\text{PPh}_3)\text{Cl}]$  [86].

Four phosphine ligands at the gold center are realised in tetrakis((trishydroxymethyl)phosphine)gold(I) chloride  $[\text{Au}(\text{P}(\text{CH}_2\text{OH})_3)_4]\text{Cl}$ . The compound showed excellent activities (with  $\text{IC}_{50}$  values in the low nanomolar range!) against prostate cancer, gastrointestinal and coloncarcinoma cells and prolonged the G1 phase of the cell cycle. *In vivo* studies in mice confirmed the positive results from the *in vitro* experiments showing a significantly prolonged survival of treated mice compared to the untreated control [87]. These and preliminary pharmacokinetic data in dogs suggested the further development of the agent in clinical phase I trials [88].

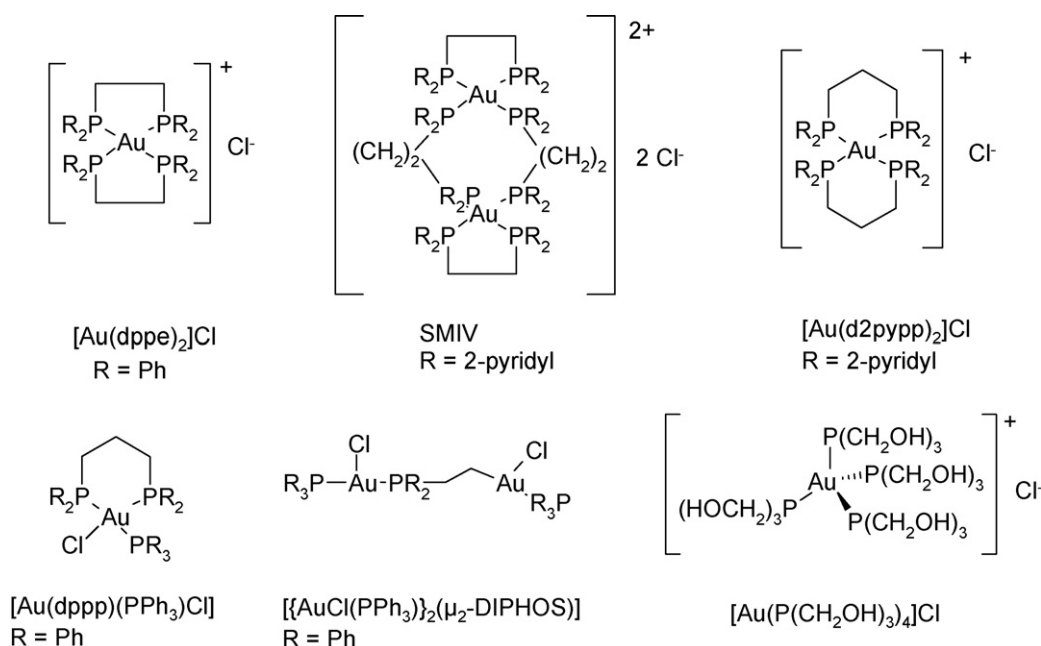


Fig. 5. Gold(I) complexes with multiple phosphine ligands.

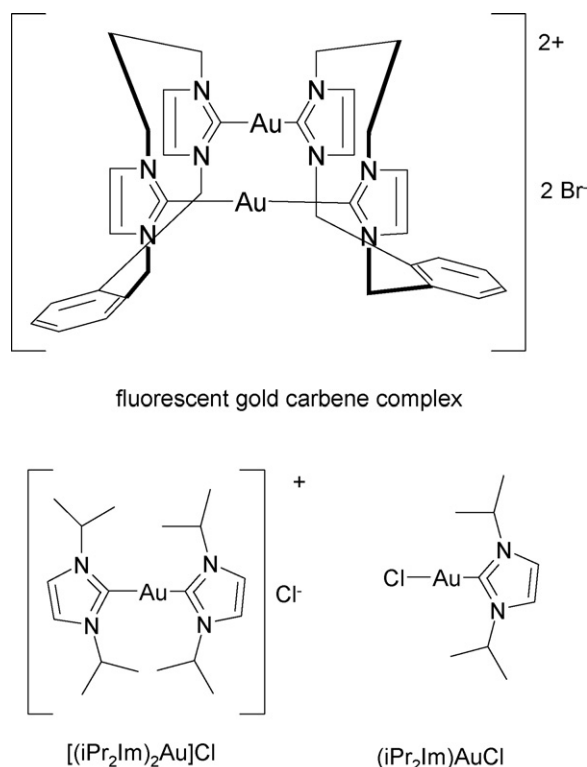


Fig. 6. Gold(I) carbene complexes.

#### 7.4. Gold(I) carbene complexes

N-heterocyclic carbenes are an interesting class of ligands with donor properties similar to phosphines. They are mostly used in catalytic applications but a number of recent papers also deals with their biological functions.

A series of dinuclear gold(I) complexes of bidentate heterocyclic carbene ligands induced mitochondrial swelling in a  $Ca^{2+}$ -dependent manner. Studies on the gold content of the mitochondria did not reveal a correlation between the uptake into the mitochondria and the extent of mitochondrial swelling indicating the presence of a specific mode of action [89]. For one luminescent derivative (see Fig. 6) the uptake into lysosomes of RAW264.7 cells rather than the mitochondria was observed by fluorescence confocal microscopy [90].

Mononuclear, cationic, linear Au(I) N-heterocyclic carbene species induced dose-dependent mitochondrial swelling in isolated rat liver mitochondria, which increased with increasing the

lipophilicity of the alkyl residue at the carbene nitrogen [91]. For a derivative with medium lipophilicity containing an isopropyl residue further going experiments have been reported.  $[(iPr_2Im)_2Au]Cl$  (see Fig. 6) showed selective cytotoxicity in tumorigenic liver progenitor cells in comparison to non-tumorigenic cells. In contrast its neutral chloro analogue  $(iPr_2Im)AuCl$  reduced the cell growth of the tumorigenic and non-tumorigenic cells in a comparable manner indicating that its lipophilic, cationic properties are of high relevance for the bioactivity. Furthermore,  $[(iPr_2Im)_2Au]Cl$  was accumulated in the mitochondria and induced apoptosis via the mitochondrial pathway [92].

The selectivity towards tumorigenic cell lines and apoptosis induction via the mitochondrial pathway (as indicated by induction of caspase-3 and caspase-9) was further confirmed by studies in breast cancer cells. The inhibition of cellular TrxR activity was measured and in a model reaction with cysteine and selenocysteine it was demonstrated that the carbene ligands can be replaced by thiols or selenols [93].

Interestingly, for gold(I) complexes containing N-heterocyclic carbene ligands the inhibition of protein tyrosine phosphatase activity, which is involved in many diseases, was determined in Jurkat T leukemia cells [94]. This is of special interest as protein tyrosine phosphatases contain a cysteine residue in the active site, which supposedly is the target structure of the agents and might be also of high relevance for other bioactive gold species.

#### 7.5. Various other gold(I) complexes

Various other gold species, which cannot be grouped into the above described categories showed promising bioactivities. Some relevant examples are listed in the following paragraphs.

$IC_{50}$  values in the low micromolar range were obtained with triphenylphosphinegold(I) complexes with different sulfanylpropenoate ligands in HeLa and A2780 cells (see  $[Au(PPh_3)(HCLpspa)]$  in Fig. 7 for a relevant example). The free ligands were devoid of activity [95]. The gold(I) 7-azacoumarin species  $[Au(TS)(PEt_3)]$  displayed superior cytotoxic effects compared to cisplatin in A2780 cells and was also strongly active in a cisplatin-resistant variant of the cell line [96]. Similar results had been observed with the vitamin K3 derivative  $[AuPEt_3(K_3TSC)]$ , which represents the first gold(I) complex with a thiosemicarbazone ligand [97].

$Et_3PAu(S_2CNET_2)$  was the most active agent within a series of dithiocarbamate and xanthate complexes in a panel of seven human cancer cell lines [98].

Water soluble cyclodiphosphazane gold(I) complexes exhibited antiproliferative effects in cultured HeLa cells, induced apoptosis and activated the p53 protein [99].

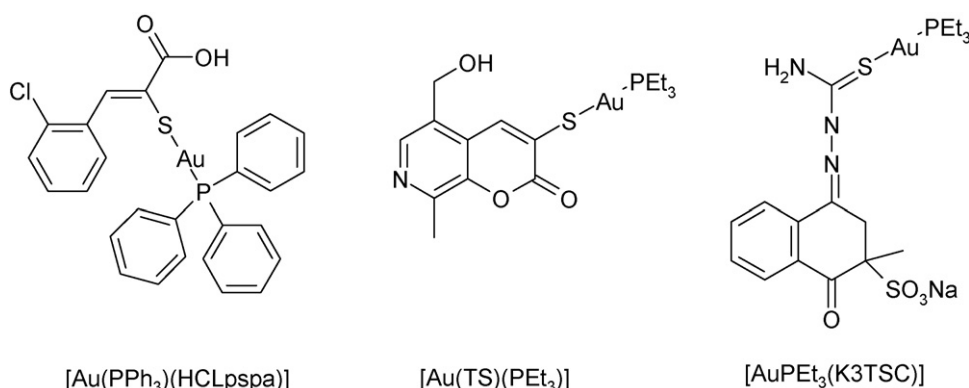
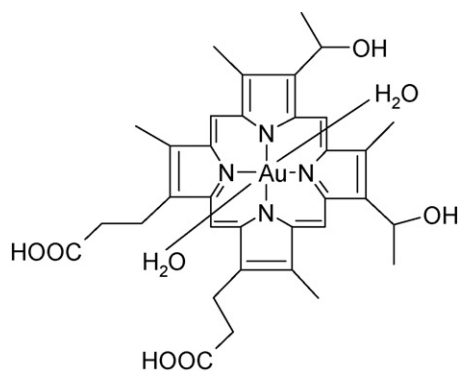


Fig. 7. Various bioactive gold(I) species.

Fig. 8.  $[\text{Au(II)HP-2H(H}_2\text{O)}_2]$ .

## 8. Gold(II) complexes

One report describes the biological properties of a novel stabilized gold(II) complex with hematoporphyrin IX ( $[\text{Au(II)HP-2H(H}_2\text{O)}_2]$ , see Fig. 8) [100]. The complex exhibited cytotoxic effects and triggered apoptosis (DNA fragmentation).  $[\text{Au(II)HP-2H(H}_2\text{O)}_2]$  showed less toxicity than cisplatin against cultured kidney cells, which indicates that its nephrotoxicity might also be lower.

## 9. Gold(III) complexes

Based on their structural and electronic similarity to cisplatin and cisplatin-related antitumor drugs gold(III) species represent a promising class of potential anticancer agents. However, the development of gold(III) complexes as therapeutic drugs has been hampered by their low stability under physiological conditions and remains a critical parameter in the drug development of these species.

Gold(III) complexes with various ligands have been prepared and biologically investigated. Most of them are complexes with Au–N bonds (eventually containing additional Au–O and Au–Cl bonds) but also some species with Au–S or Au–C bonds and their bioactivities have been described.

### 9.1. Gold(III) complexes with Au–N bonds

Two gold chloride species with pyridine ligands ( $\text{AuCl}_3(\text{Hpm})$  and  $\text{AuCl}_2(\text{pm})$ ) showed good cytotoxic activity in T-lymphoblastoid and human ovarian cell lines, which was however comparable to that of  $\text{NaAuCl}_4$  (see Fig. 9 for structures). Binding to the DNA was confirmed for both complexes. Both  $\text{AuCl}_3(\text{Hpm})$  and  $\text{AuCl}_2(\text{pm})$  were relatively stable in organic solvents but underwent hydrolysis of the chloride ligand in aqueous buffer media, a fact which might limit their practical application [101].

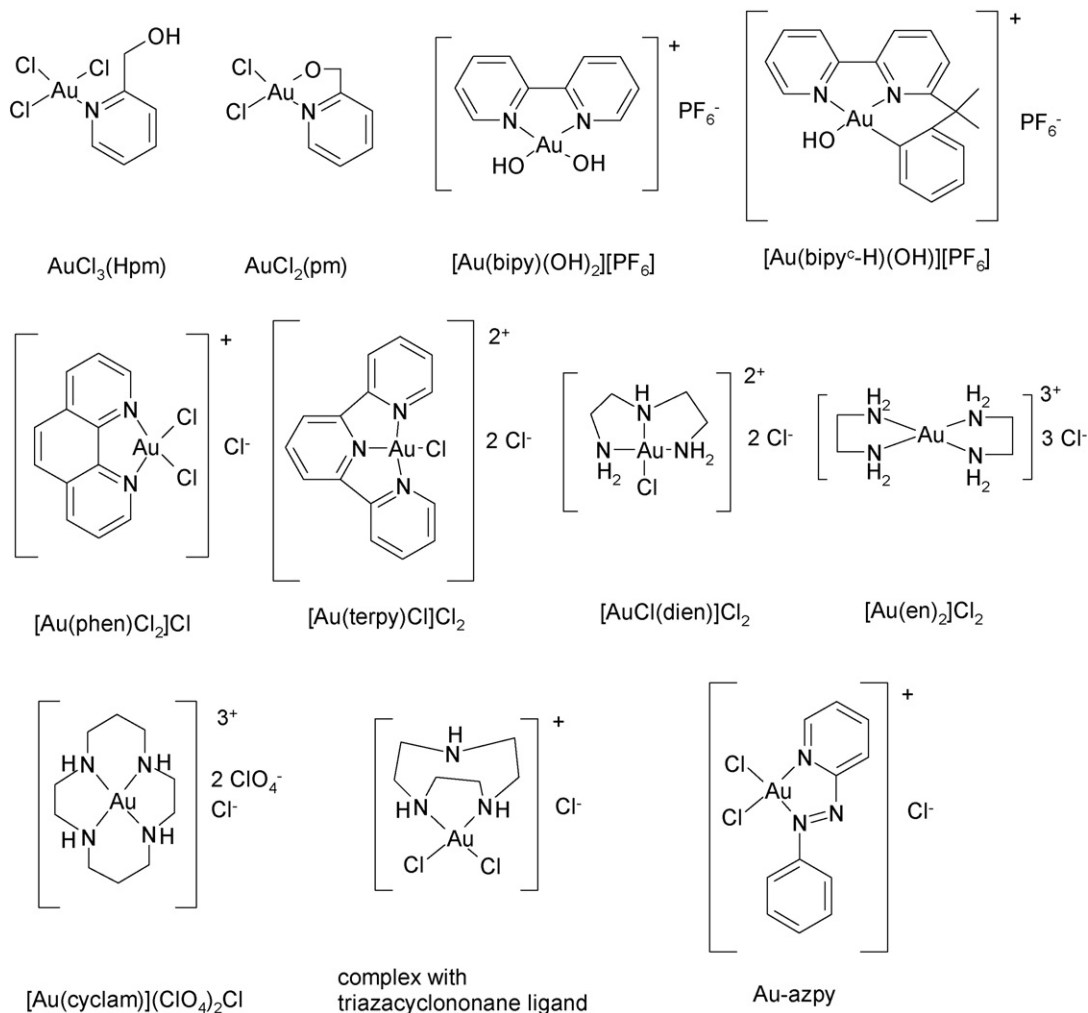


Fig. 9. Gold(III) complexes with Au–N bonds 1.



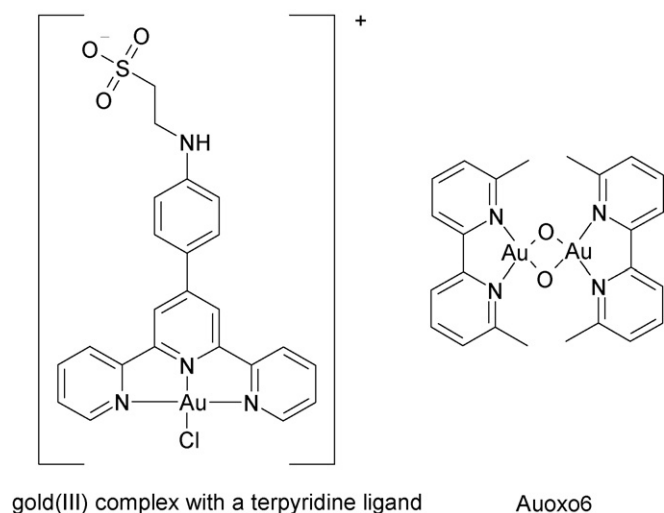


Fig. 10. Gold(III) complexes with Au–N bonds 2.

The bipyridine complexes  $[\text{Au}(\text{bipy})(\text{OH})_2][\text{PF}_6]$  and  $[\text{Au}(\text{bipy}^{\text{c}}\text{-H})(\text{OH})][\text{PF}_6]$  were stable in physiological buffer at  $37^\circ\text{C}$  and cytotoxic. Studies on calf thymus DNA indicated that the interaction of the compounds with DNA is weak and reversible [102].

A series of cationic gold complexes with multidentate ligands, namely  $[\text{Au}(\text{phen})\text{Cl}_2]\text{Cl}$ ,  $[\text{Au}(\text{terpy})\text{Cl}]\text{Cl}_2$ ,  $[\text{AuCl}(\text{dien})]\text{Cl}_2$ ,  $[\text{Au}(\text{cyclam})](\text{ClO}_4)_2\text{Cl}$  and  $[\text{Au}(\text{en})_2]\text{Cl}_2$ , showed reasonable stability in physiological-like environment. The coordination to the polyamine ligands caused a marked stabilization of gold in the  $3^+$  oxidation state as indicated by measurements of the reduction potentials. The stabilization was less evident for the less basic phenanthrene and terpyridine ligands. With exception of the cyclam species  $[\text{Au}(\text{cyclam})](\text{ClO}_4)_2\text{Cl}$  all complexes exhibited good cytotoxic activities in A2780 human ovarian cancer cells. Furthermore, results obtained in cisplatin-resistant A2780 cells indicated that the compounds might overcome resistance phenomena. The free ligands were also investigated in the same assay. Whereas the free ethylenediamine derived ligands were devoid of any activity the potency of free phenanthrene and terpyridine was comparable to that of the respective gold(III) complexes making the results of these two compounds difficult to interpret. Overall this study pointed out that the cytotoxicity of the ethylenediamine derived complexes was related to the presence of the gold(III) central atom, the activity was not related to good leaving groups (such as chlorine), no direct correlation of the antiproliferative effects and the reduction potentials was present and the strong stabilization of the gold(III) center led to a loss of activity (as indicated by the results with  $[\text{Au}(\text{cyclam})](\text{ClO}_4)_2\text{Cl}$ ). For the compounds  $[\text{Au}(\text{phen})\text{Cl}_2]\text{Cl}$  and  $[\text{Au}(\text{terpy})\text{Cl}]\text{Cl}_2$ , which had also shown good stability under physiological conditions, an easy reduction was observed in the presence of ascorbate or thiosulfate. This indicates that for these two complexes the release of their toxic ligands upon *in vivo* reduction might be the reason for the bioactivity [103].

A structurally related gold(III) complex with a triazacyclononane ligand was more active than cisplatin and bound efficiently to the DNA [104].

Au-azpy, a complex containing a bidentate ligand, exhibited promising cytotoxic activity in cisplatin sensitive and cisplatin-resistant ovarian carcinoma and leukemia cells. Interestingly, solutions of Au-azpy showed a cyclisation reaction under formation of a tricyclic cationic organic compound, which also exhibited good cytotoxic activity [105].

For several gold(III) complexes with terpyridine ligands (see Fig. 10 for an example) promising cytotoxicities were observed, which were however comparable to that of the free terpyridine lig-

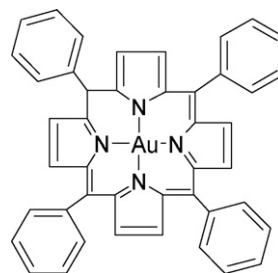


Fig. 11. Gold(III) complexes with Au–N bonds: gold porphyrin 1a.

ands. The gold species were stable in the presence of glutathione and bound to the DNA. Measurement of the gold content of DNA isolated from tumor cells exposed to the complexes showed a level comparable to the DNA platination caused by cisplatin [106]. For structurally closely related compounds luminescence properties and DNA binding have been described [107].

Related cyclometalated gold(III) compounds were stable in solution and in the presence of glutathione. Compounds containing non-toxic N-donor auxiliary ligands exhibited cytotoxicity similar to that of cisplatin. However, for some other ligands the toxicity of the metal free ligand was in the same range as that of the metal complex. A 1-methylimidazole derivative triggered apoptosis, interacted with the DNA in an intercalative manner and caused an arrest of the cell cycle in the S-phase. In contrast the triphenylphosphine and dppp analogues bound only weakly to the DNA and did not arrest the cell cycle [108].

Gold(III) complexes with various amidrazone ligands of the general formula  $\text{AuX}_3(\text{L})$  ( $\text{X} = \text{Cl}, \text{Br}$ ,  $\text{L} = \text{amidrazone}$ ) showed low cell growth inhibiting activities in HT-29 colon carcinoma and MCF-7 breast cancer cells [109].

Out of a series of six dinuclear compounds containing the common structural motif  $[\text{Au}_2\text{O}_2]$  and bidentate bipyridyl ligands Auoxo6 showed the highest cytotoxic potency. The complexes exhibited good stability in aqueous media. In contrast to a low active analogue Auoxo6 bound efficiently to calf thymus DNA and reacted faster with selected model proteins under loss of the bipyridyl ligands [110].

Gold(III) tetraarylporphyrins exhibited excellent cytotoxic activities mediated by apoptotic pathways [111]. Detailed pharmacological studies have been performed on gold(III) porphyrin 1a (see Fig. 11). It was found that the apoptosis inducing effects were related to caspase-dependent and caspase-independent mitochondrial pathways including the activation of caspase-3 and caspase-9. Gold(III) porphyrin 1a treatment led to the release of cytochrome C, which itself activated caspase-3 and caspase-9 [112]. A proteomic analysis indicated the involvement of multiple pathways and showed that cellular structure and stress related chaperone proteins, proteins involved in the formation of reactive oxygen species, proteins related to cell proliferation and others were in the altered clusters [113]. The toxicity of the porphyrin complex was not related to phototoxicity, it interacted with the DNA in a non-covalent manner and partly abrogated the cell cycle at G(0)–G(1). The effects on apoptosis and on the cell cycle were p53 dependent [114]. More detailed studies on the mechanisms underlying the induction of apoptosis revealed that the mitogen activated protein kinases  $\text{p38}^{\text{MAPK}}$  and ERK were transiently activated upon exposure to gold(III) porphyrin 1a, multiple phosphotyrosine proteins (namely cytoskeleton and cytoskeleton-like proteins, kinase proteins and proteins involved in signal transduction) were involved in apoptosis related to  $\text{p38}^{\text{MAPK}}$  and mitochondrial permeabilisation played an important role [115]. In a rat hepatocellular carcinoma model gold(III) porphyrin 1a significantly prolonged survival but did not lead to a drop down of the body weight of the rats or

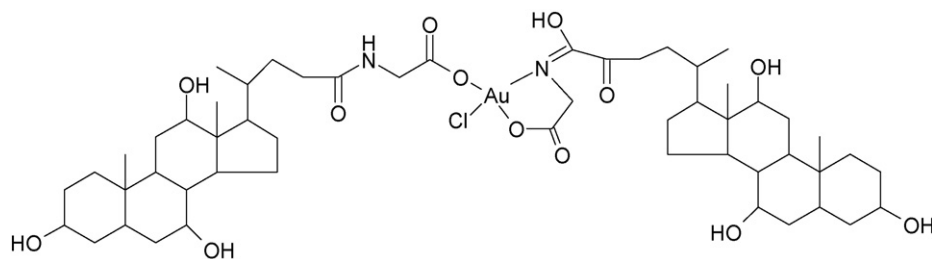


Fig. 12. Gold(III) complexes with Au–N bonds: Bamet-A1.

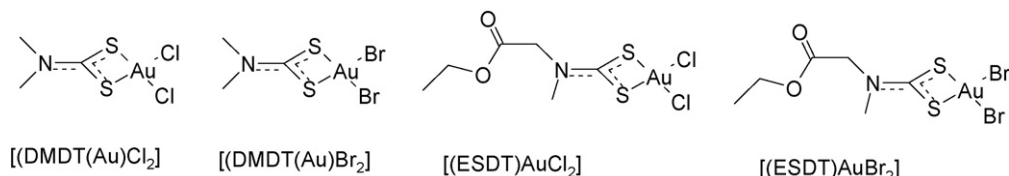


Fig. 13. Gold(III) complexes with Au–S bonds.

affected the plasma aspartate aminotransferase level. Furthermore, it induced necrosis and apoptosis in the tumor tissue but not in normal liver tissue [116].

Bamet-A1 (see Fig. 12) represents a gold(III) bioconjugate with a bile acid. The compound showed antiproliferative effects in a variety of tumor cell lines [117].

### 9.2. Gold(III) complexes with Au–S bonds

Gold(III) dithiocarbamate complexes (see Fig. 13 for some relevant examples) exhibited superior cytotoxic effects if compared to cisplatin, were also active in resistant cells and induced apoptosis [118,119]. The compounds showed good stability under physiological conditions, bound readily to the DNA, inhibited both DNA and RNA synthesis and induced fast DNA lesions. Experiments on red blood cells indicated that hemolytic properties might contribute significantly to the bioactivity of the agents [120]. The complexes triggered cancer cell death via apoptotic and non-apoptotic pathways, affected mitochondrial functions, generated free radicals, increased ERK1/2 phosphorylation and inhibited TrxR [121,122].

For [(ESDT)AuBr<sub>2</sub>] the inhibition of the activity of purified 20S proteasome and 26S proteasome in MDA-MB-231 breast cancer cells was confirmed. Targeting the ubiquitin proteasome pathway led to an accumulation of ubiquitinated proteins and the proteasome target protein p27 and induction of apoptosis. The free ligand ESDT did not exhibit proteasome inhibitory activity. The gold salts KAuCl<sub>4</sub> and KAuBr<sub>4</sub> also showed inhibitory activity but were weaker in inhibiting the chymotrypsin-like activities than [(ESDT)AuBr<sub>2</sub>]. Furthermore, they showed less selectivity in targeting chymotrypsin-like and trypsin-like activities, which are more relevant for growth arrest or apoptosis induction in cancer cells. Interestingly, the treatment of breast cancer bearing nude mice with [(ESDT)AuBr<sub>2</sub>] caused tumor growth inhibition, induction of apoptosis associated with *in vivo* proteasome inhibition [123].

### 9.3. Gold(III) complexes containing Au–C bonds

The TrxR inhibiting and antiproliferative properties of a series of gold(III) complexes including many species with gold–carbon bonds was studied. The complex depicted in Fig. 14 top left showed

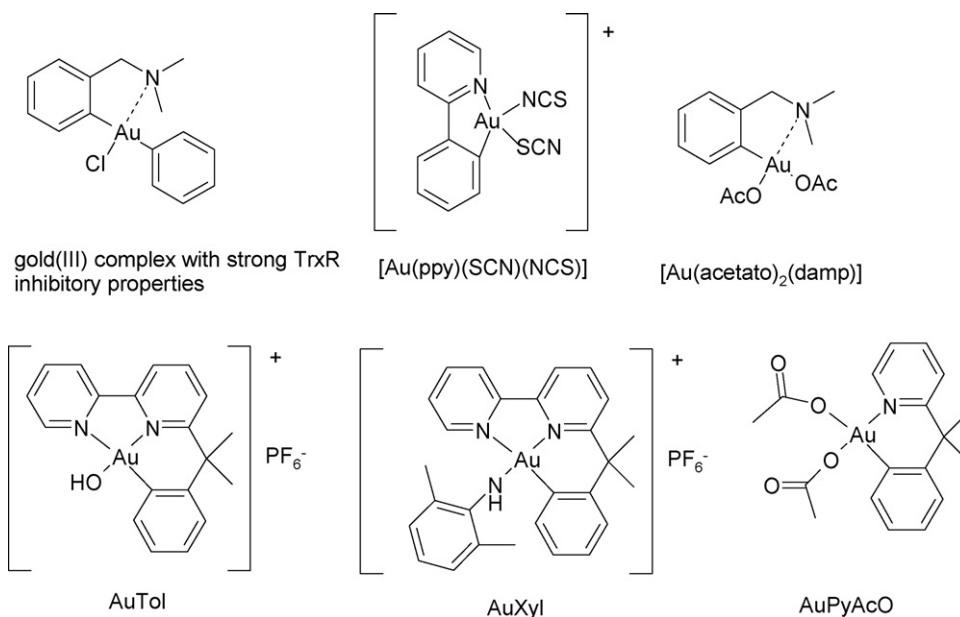


Fig. 14. Gold(III) complexes with Au–C bonds.

the best cytotoxicity results and inhibited TrxR activity. However, the extent of TrxR inhibition did not correlate with the antiproliferative properties [124].

A series of 2-phenylpyridine Au(III) complexes of the general formula [Au(ppy)X] with various thiolate ligands ([Au(ppy)(SCN)(NCS)] is exemplarily depicted in Fig. 14) were *in vitro* more cytotoxic than cisplatin [125].

Gold(III) complexes with 2-[(dimethylamino)methyl]phenyl ligands were active *in vitro* and *in vivo*. More detailed investigations on [Au(acetato)<sub>2</sub>(damp)] showed that the compound did not cause DNA interstrand cross-links and induced only minor cell cycle alterations [126].

Solution studies on the gold–carbon complexes AuTol, AuXyl and AuPyAcO revealed that the complexes underwent hydrolysis of the labile ligands while the gold carbon-bond and the oxidation state remained intact. In cytotoxicity experiments IC<sub>50</sub> values in the low micromolar range were determined. The agents also induced anti-apoptotic effects. Interestingly, this was accompanied by only modest perturbations of the cell cycle [127,128].

## 10. Summary

Gold(I) complexes related to auranofin have been used for treatment of rheumatoid arthritis in the so-called chrysotherapy. Research on this class of complexes also revealed a considerable potential for the chemotherapy of tumoral diseases.

So far a broad variety of various gold(I) and gold(III) (as well as one gold(II)) agents have been investigated for their antitumor effects *in vitro* and *in vivo* mostly showing promising preclinical results. Based on the different ligands and structures a unique mode of action most probably does not exist. However, some trends can be considered as general biological effects of antiproliferative gold species. The most relevant biochemical property is the inhibition of the enzyme TrxR demonstrated for many compounds, which is supposedly based on the covalent binding of the gold center to a selenocysteine residue in the active site of the enzyme. Other properties common for gold drugs are the triggering of anti-mitochondrial effects and the induction of apoptotic events. The interaction with other biological targets (e.g. protein tyrosine phosphatase or DNA) has also been confirmed for different gold compounds and contributes to the pharmacological profile of the complexes.

So far a broad variety of ligands have been investigated as ligands for bioactive gold(I) species. Of those mainly phosphine ligands play a major role. However, recent reports demonstrate that also the use of different ligands can lead to promising preclinical results. For example N-heterocyclic carbene complexes have shown interesting biological properties.

Studies on gold(III) compounds mainly use chelating N-donor or S-donor ligands such as bipyridine or dithiocarbamates. Interestingly, also the use of gold complexes with Au–C bonds with good bioactivities has been reported.

## References

- [1] B. Rosenberg, I. VanCamp, T. Krigas, *Nature* 205 (1965) 698.
- [2] B. Rosenberg, I. VanCamp, J.E. Trosko, V.H. Mansour, *Nature* 222 (1969) 385.
- [3] M. Galanski, M.A. Jakupiec, B.K. Keppler, *Curr. Med. Chem.* 12 (2005) 2075.
- [4] I. Ott, R. Gust, *Arch. Pharm. Chem. Life Sci.* 340 (2007) 117.
- [5] E. Meggers, *Curr. Opin. Chem. Biol.* 11 (2007) 287.
- [6] L. Ronconi, P.J. Sadler, *Coord. Chem. Rev.* 251 (2007) 1633.
- [7] P.C.A. Bruijninx, P.J. Sadler, *Curr. Opin. Chem. Biol.* 12 (2008) 197.
- [8] P.J. Barnard, S.J. Berners-Price, *Coord. Chem. Rev.* 251 (2007) 1889.
- [9] X. Wang, Z. Guo, *Dalton Trans.* (2008) 1521.
- [10] E.R.T. Tiekink, *Bioinorg. Chem. Appl.* 1 (2003) 53.
- [11] E.R.T. Tiekink, *Crit. Rev. Hematol. Oncol.* 42 (2002) 225.
- [12] G.B. Kauffman, *Gold Bull.* 18 (1985) 31.
- [13] G.B. Kauffman, *Gold Bull.* 18 (1985) 69.
- [14] G.B. Kauffman, *Gold Bull.* 18 (1985) 109.
- [15] C.F. Shaw, *Chem. Rev.* 99 (1999) 2589.
- [16] R. Eisler, *Inflamm. Res.* 52 (2003) 487.
- [17] T.M. Simon, D.H. Kunishima, G.J. Vibert, A. Lorber, *Cancer Res.* 41 (1981) 94.
- [18] C.K. Mirabelli, R.K. Johnson, C.M. Sung, L. Faucette, K. Muirhead, S.T. Crooke, *Cancer Res.* 45 (1985) 32.
- [19] J. Chaudiere, A.L. Tappel, *J. Inorg. Biochem.* 20 (1984) 313.
- [20] J.R. Roberts, C.F. Shaw, *Biochem. Pharmacol.* 55 (1998) 1291.
- [21] S. Gromer, L.D. Arscott, C.H. Williams, R.H. Schirmer, K. Becker, *J. Biol. Chem.* (1998) 20096.
- [22] M.P. Rigobello, L. Messori, M. Giordano, M.A. Cinellu, M. Bragadin, A. Folda, G. Scutari, A. Bindoli, *J. Inorg. Biochem.* 98 (2004) 1634.
- [23] K. Becker, S. Gromer, R.H. Schirmer, S. Müller, *Eur. J. Biochem.* 267 (2000) 6118.
- [24] S. Gromer, S. Urig, K. Becker, *Med. Res. Rev.* 24 (2004) 40.
- [25] C.H. Williams, L.D. Arscott, S. Müller, B.W. Lennon, M.L. Ludwig, P.F. Wang, D.M. Veine, K. Becker, R.H. Schirmer, *Eur. J. Biochem.* 267 (2000) 6110.
- [26] G. Powis, D.L. Kirkpatrick, *Curr. Opin. Pharmacol.* 7 (2007) 392.
- [27] J.D. Pennington, K.M. Jacobs, L. Sun, G. Bar-Sela, M. Mishra, D. Gius, *Curr. Pharm. Des.* 13 (2007) 3368.
- [28] E.S.J. Arner, A. Holmgren, *Semin. Cancer Biol.* 16 (2006) 420.
- [29] S. Urig, K. Becker, *Semin. Cancer Biol.* 16 (2006) 452.
- [30] M. Deponte, S. Urig, L.D. Arscott, K. Fritz-Wolf, R. Reau, C. Herold-Mende, S. Konkarevic, M. Meyer, E. Davioud-Charvet, D.P. Ballou, C.H. Williams, K. Becker, *J. Biol. Chem.* 280 (2005) 20628.
- [31] S. Urig, K. Fritz-Wolf, R. Reau, C. Herold-Mende, K. Toth, E. Davioud-Charvet, K. Becker, *Angew. Chem. Int. Ed.* 45 (2006) 1881.
- [32] B.P. Monia, T.R. Butt, C.K. Mirabelli, D.J. Ecker, E. Sternberg, S.T. Crooke, *Mol. Pharmacol.* 31 (1987) 21.
- [33] T.R. Butt, E.J. Sternberg, C.K. Mirabelli, S.T. Crooke, *Mol. Pharmacol.* 29 (1986) 204.
- [34] G.D. Hoke, G.F. Rush, C.K. Mirabelli, *Toxicol. Appl. Pharmacol.* 99 (1989) 50.
- [35] G.F. Rush, P.F. Smith, G.D. Hoke, D.W. Alberts, R.M. Snyder, C.K. Mirabelli, *Toxicol. Appl. Pharmacol.* 90 (1987) 391.
- [36] G.F. Rush, P.F. Smith, D.W. Alberts, C.K. Mirabelli, R.M. Snyder, S.T. Crooke, J. Sowinski, H.B. Jones, P.J. Bugelski, *Toxicol. Appl. Pharmacol.* 90 (1987) 377.
- [37] M.P. Rigobello, G. Scutari, R. Boscolo, A. Bindoli, *Br. J. Pharmacol.* 136 (2002) 1162.
- [38] C.E. Blank, J.C. Dabrowiak, *J. Inorg. Biochem.* 21 (1984) 21.
- [39] C.K. Mirabelli, J.P. Zimmermann, H.R. Bartus, C.M. Sung, S.T. Crooke, *Biochem. Pharmacol.* 35 (1986) 1435.
- [40] C.K. Mirabelli, C.M. Sung, J.P. Zimmermann, D.T. Hill, S. Mong, S.T. Crooke, *Biochem. Pharmacol.* 35, 1427.
- [41] L. Messori, P. Orioli, C. Tempi, G. Marcon, *Biochem. Biophys. Res. Commun.* 281 (2001) 352.
- [42] S. Talbot, R. Nelson, W.T. Self, *Br. J. Pharmacol.* 154 (2008) 940.
- [43] R.M. Snyder, C.K. Mirabelli, M.A. Clark, J.T. Ziegler, S.T. Crooke, *Mol. Pharmacol.* 32 (1987) 437.
- [44] I. Ott, T. Koch, H. Shorafa, Z. Bai, D. Poekel, D. Steinhilber, R. Gust, *Org. Biomol. Chem.* 3 (2005) 2282.
- [45] R. Yamada, H. Sano, T. Hla, A. Hashiramoto, W. Fukui, S. Miyazaki, M. Kohn, Y. Tsubouchi, Y. Kusaka, M. Kondo, *Eur. J. Pharmacol.* 385 (1999) 71.
- [46] M. Yamashita, H. Niki, M. Yamada, M. Watanabe-Kobayashi, S. Mue, K. Ohuchi, *Eur. J. Pharmacol.* 325 (1997) 221.
- [47] M. Yamashita, G. Ichinowatari, K. Yamaki, K. Ohuchi, *Eur. J. Pharmacol.* 368 (1999) 251.
- [48] M. Yamada, H. Niki, M. Yamashita, S. Mue, K. Ohuchi, *J. Pharmacol. Exp. Ther.* 281 (1997) 1005.
- [49] E. Erdogan, T. Lamark, M. Stallings-Mann, J. Lee, M. Pellecchia, E.A. Thompson, T. Johansen, A.P. Fields, *J. Biol. Chem.* 281 (2006) 28450.
- [50] T. Maruyama, S. Sonokawa, H. Matsushita, M. Goto, *J. Inorg. Biochem.* 101 (2007) 180.
- [51] L.A. Taukumova, V. Mouravjoy, S.G. Gribakin, *Adv. Exp. Med. Biol.* 455 (1999) 367.
- [52] R. Eberl, *Acta Med. Aust.* 2 (1975) 125.
- [53] C. Hoffmann, G. Burg, C. Jung, *Z. Rheumatol.* 45 (1986) 100.
- [54] P. Clark, P. Tugwell, K. Bennett, C. Bomardier, *J. Rheumatol.* 16 (1989) 442.
- [55] S. Tosi, M. Cagnoli, G. Guidi, M. Murelli, K. Messina, B. Colombo, *Int. J. Clin. Pharmacol.* 5 (1985) 265.
- [56] T. Vlask, I. Jajic, *Reumatizam* 39 (1992) 25.
- [57] P. Davis, *J. Rheumatol. Suppl.* 5 (1979) 18.
- [58] F. Rainer, *Wien. Klin. Wochenschr. Suppl.* 156 (1984) 40.
- [59] M. Suwalski, P. Zambenedetti, E. Carpena, M. IbnLkayat, W. Wittkowski, L. Messori, P. Zatta, *J. Inorg. Biochem.* 98 (2004) 2080.
- [60] R.M. Snyder, C.K. Mirabelli, S.T. Crooke, *Biochem. Pharmacol.* 36 (1987) 647.
- [61] R.M. Snyder, C.K. Mirabelli, S.T. Crooke, *Biochem. Pharmacol.* 35 (1986) 923.
- [62] I. Ott, H. Scheffler, R. Gust, *ChemMedChem* 2 (2007) 702.
- [63] R. Gust, B. Schnurr, R. Krauser, G. Bernhardt, M. Koch, B. Schmid, E. Hummel, H. Schonenberger, *J. Cancer Res. Clin. Oncol.* 124 (1998) 585.
- [64] A.R. Ghezzi, M. Aceto, C. Cassino, E. Gabano, D. Osella, *J. Inorg. Biochem.* 98 (2004) 73.
- [65] A.R. Sannella, A. Casini, C. Gabbiani, L. Messori, A.R. Bilia, F.F. Vincieri, G. Majorie, C. Severini, *FEBS Lett.* 582 (2008) 844.
- [66] C. Gabbiani, L. Messori, M.A. Cinellu, A. Casini, P. Mura, A.L. Sannella, C. Severini, G. Majori, A.R. Bilia, F.F. Vincieri, *J. Inorg. Biochem.* 103 (2009) 310.
- [67] C.K. Mirabelli, R.K. Johnson, D.T. Hill, L.F. Faucette, G.R. Girard, G.Y. Kuo, C.M. Sung, S.T. Crooke, *J. Med. Chem.* 29 (1986) 218.

- [68] M.P. Rigobello, A. Folda, B. Dani, R. Menabo, G. Scutari, A. Bindoli, *Eur. J. Pharmacol.* 582 (2008) 26.
- [69] C. Marzano, V. Gandin, A. Folda, G. Scutari, A. Bindoli, M.P. Rigobello, *Free Radic. Biol. Med.* 42 (2007) 872.
- [70] I. Ott, X. Qian, Y. Xu, D.H. Vlecken, I.J. Marques, D. Kubat, J. Will, W.S. Sheldrick, P. Jesse, A. Prokop, C.P. Bagowski, *Angew. Chem. Int. Ed.* 48 (2009) 1160.
- [71] M.F. Brana, A. Ramos, *Curr. Med. Chem. -Anticancer Agents* 1 (2001) 237.
- [72] E. Viry, E. Battaglia, V. Deborde, T. Müller, R. Reau, E. Davioud-Charvet, D. Bagrel, *ChemMedChem* 3 (2007) 1667.
- [73] S.J. Berners-Price, C.K. Mirabelli, R.K. Johnson, M.R. Mattern, F.L. McCabe, L.F. Faucette, C.M. Sung, S.M. Mong, P.J. Sadler, S.T. Crooke, *Cancer Res.* 46 (1986) 5486.
- [74] C.K. Mirabelli, D.T. Hill, L.F. Faucette, F.L. McCabe, G.R. Girard, D.B. Bryan, B.M. Sutton, J.O. Bartus, S.T. Crooke, R.K. Johnson, *J. Med. Chem.* 30 (1987) 2181.
- [75] G.D. Hoke, R.A. Macia, P.C. Meunier, P.J. Bugelski, C.K. Mirabelli, G.F. Rush, W.D. Matthews, *Toxicol. Appl. Pharmacol.* 100 (1989) 293.
- [76] P.F. Smith, G.D. Hoke, D.W. Alberts, P.J. Bugelski, S. Lupo, C.K. Mirabelli, G.F. Rush, *J. Pharmacol. Exp. Ther.* 249 (1989) 944.
- [77] G.D. Hoke, G.F. Rush, G.F.G.F. Bossard, J.V. McArdle, B.D. Jensen, C.K. Mirabelli, *J. Biol. Chem.* 263 (1988) 11203.
- [78] M.J. McKeage, S.J. Berners-Price, P. Galetti, R.J. Bowen, W. Brouwer, L. Ding, L. Zhuang, B.C. Baguley, *Cancer Chemother. Pharmacol.* 46 (2000) 343.
- [79] S.J. Berners-Price, G.R. Girard, D.T. Hill, B.M. Sutton, P.S. Jarrett, L.F. Faucette, R.K. Johnson, C.K. Mirabelli, P.J. Sadler, *J. Med. Chem.* 33 (1990) 1386.
- [80] S.J. Berners-Price, R.J. Bowen, P. Galetti, P.C. Healy, M.J. McKeage, *Coord. Chem. Rev.* 186 (1998) 823.
- [81] J.L. Liu, P. Galetti, A. Farr, L. Maharaj, H. Samarasingha, A.C. McGechan, B.C. Baguley, R.J. Bowen, S.J. Berners-Price, M.J. McKeage, *J. Inorg. Biochem.* 102 (2008) 303.
- [82] A.S. Humphreys, A. Filipovska, S.J. Berners-Price, G.A. Koutsantonis, B.W. Skelton, A.H. White, *Dalton Trans.* 43 (2007) 4943.
- [83] O. Rackham, S.J. Nichols, P.J. Leedman, S.J. Berners-Price, A. Filipovska, *Biochem. Pharmacol.* 74 (2007) 992.
- [84] F. Caruso, M. Rossi, J. Tanski, C. Pettinari, F. Marchetti, *J. Med. Chem.* 46 (2003) 1737.
- [85] F. Caruso, R. Villa, M. Rossi, C. Pettinari, F. Paduano, M. Pennati, M.G. Daidone, N. Zaffaroni, *Biochem. Pharmacol.* 73 (2007) 773.
- [86] F. Caruso, C. Pettinari, F. Paduano, R. Villa, F. Marchetti, E. Monti, M. Rossi, *J. Med. Chem.* 51 (2008) 1584.
- [87] N. Pillarsetty, K.K. Katti, T.J. Hoffman, W.A. Volkert, K.V. Katti, H. Kamei, T. Koide, *J. Med. Chem.* 46 (2003) 1130.
- [88] M.L. Higginbotham, C.J. Henry, K.V. Katti, S.W. Casteel, P.M. Dowling, N. Pillarsetty, *Vet. Ther.* 4 (2003) 76.
- [89] P.J. Barnard, M.V. Baker, S.J. Berners-Price, D.A. Day, *J. Inorg. Biochem.* 98 (2004) 1642.
- [90] P.J. Barnard, L.E. Wedlock, M.V. Baker, S.J. Berners-Price, D.A. Joyce, B.W. Skelton, J.H. Steer, *Angew. Chem. Int. Ed.* 118 (2006) 6112.
- [91] M.V. Baker, P.J. Barnard, S.J. Berners-Price, S.K. Brayshaw, J. L/Hickey, B.W. Skelton, A.H. White, *Dalton Trans.* 30 (2006) 3708.
- [92] M.M. Jellicoe, S.J. Nichols, B.A. Callus, M.V. Baker, P.J. Barnard, S.J. Berners-Price, J. Whelan, G.C. Yeoh, A. Filipovska, *Carcinogenesis* 29 (2008) 1124.
- [93] J.L. Hickey, R.A. Ruhayel, P.J. Barnard, M.V. Baker, S.J. Berners-Price, A. Filipovska, *J. Am. Chem. Soc.* 130 (2008) 12570.
- [94] D. Krishnamurthy, M.R. Karver, E. Fiorillo, V. Orru, S.M. Stanford, N. Bottini, A.M. Barrios, *J. Med. Chem.* 51 (2008) 4790.
- [95] E. Barreiro, J.S. Casas, M.D. Couce, A. Sanchez, A. Sanchez-Gonzalez, J. Sordo, J.M. Varela, E.M.V. Lopez, *J. Inorg. Biochem.* 102 (2008) 184.
- [96] J.S. Casas, E.E. Castellano, M.D. Couce, O. Crespo, J. Elena, A. Laguna, A. Sanchez, J. Sordo, C. Taboada, *Inorg. Chem.* 46 (2007) 6236.
- [97] J.S. Casas, E.E. Castellano, M.D. Couce, J. Ellena, A. Sanchez, J. Sordo, C. Taboada, *J. Inorg. Biochem.* 100 (2006) 1858.
- [98] D. deVos, S.Y. Hoo, E.R.T. Tiekink, *Bioinorg. Chem. Appl.* 2 (2004) 141.
- [99] D. Suresh, M.S. Balakrishna, K. Rathinasamy, D. Panda, S.M. Mobin, *Dalton Trans.* (2008) 2812.
- [100] G. Momekov, D. Ferdinandov, S. Konstantinov, S. Arpadjan, D. Tsekova, G. Gencheva, P.R. Bontchev, M. Karaivanova, *Bioinorg. Chem. Appl.* (2008) 1.
- [101] P. Calamai, S. Carotti, A. Guerri, L. Messori, E. Mini, P. Orioli, G.P. Speroni, *J. Inorg. Biochem.* (1997) 103.
- [102] G. Marcon, S. Carotti, M. Coronello, L. Messori, E. Mini, P. Orioli, T. Mazzei, M.A. Cinelli, G. Minghetti, *J. Med. Chem.* 45 (2002) 1672.
- [103] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell, P. Zanelli, *J. Med. Chem.* 43 (2000) 3541.
- [104] P. Shi, Q. Jiang, J. Lin, Y. Zhao, L. Lin, Z. Guo, *J. Inorg. Biochem.* 100 (2006) 939.
- [105] A. Garza-Ortiz, H. denDulk, J. Brouwer, H. Kooijman, A.L. Spek, J. Reedijk, *J. Inorg. Biochem.* 101 (2007) 1922.
- [106] P. Shi, Q. Jiang, Y. Zhao, Y. Zhang, J. Lin, J. Ding, Z. Guo, *J. Biol. Inorg. Chem.* 11 (2006) 745.
- [107] H.Q. Liu, T.C. Cheung, S.M. Peng, C.M. Che, *J. Chem. Soc., Chem. Commun.* (1995) 1787.
- [108] C.K. Li, R.W. Sun, S.C. Kui, N. Zhu, C.M. Che, *Chemistry* 12 (2006) 5253.
- [109] G. Ponticelli, M. Mitewa, *Trans. Met. Chem.* 31 (2006) 707.
- [110] A. Casini, M.A. Cinelli, G. Minghetti, C. Gabbiani, M. Coronello, E. Mini, L. Messori, *J. Med. Chem.* 49 (2006) 5524.
- [111] C.M. Che, R.W. Sun, W.Y. Yu, C.B. Ko, N. Zhu, H. Sun, *Chem. Commun.* (2003) 1718.
- [112] Y. Wang, Q.Y. He, R.W. Sun, C.M. Che, J.F. Chiu, *Cancer Res.* 65 (2005) 11553.
- [113] Y. Wang, Q.Y. He, C.M. Che, J.F. Chiu, *Proteomics* 6 (2006) 131.
- [114] Y. Wang, Q.Y. He, R.W. Sun, C.M. Che, J.F. Chiu, *Eur. J. Pharmacol.* 554 (2007) 113.
- [115] Y. Wang, Q.Y. He, C.M. Che, S.W. Tsao, R.W.Y. Sun, J.F. Chiu, *Biochem. Pharmacol.* 75 (2008) 1282.
- [116] C.T. Lum, Z.F. Yang, H.Y. Li, R. Wai-Yin Sun, S.T. Fan, R.T. Poon, M.C. Lin, C.M. Che, H.F. Kung, *Int. J. Cancer* 118 (2006) 1527.
- [117] J. Carrasco, J.J. Criado, R.I.R. Macias, J.L. Manzano, J.J.G. Marin, M. Medarde, E. Rodriguez, *J. Inorg. Biochem.* 84 (2001) 287.
- [118] L. Giovagnini, L. Ronconi, D. Aldinucci, D. Lorenzon, S. Sitran, D. Fregona, *J. Med. Chem.* 48 (2005) 1588.
- [119] L. Ronconi, L. Giovagnini, C. Marzano, F. Bettio, R. Graziani, G. Pilloni, D. Fregona, *Inorg. Chem.* 44 (2005) 1867.
- [120] L. Ronconi, C. Marzano, P. Zanelli, M. Corsini, G. Miolo, C. Macca, A. Trevisan, D. Fregona, *J. Med. Chem.* 49 (2006) 1648.
- [121] D. Saggioro, M.P. Rigobello, L. Paloschi, A. Folda, S.A. Moggach, S. Parsons, L. Ronconi, D. Fregona, A. Bindoli, *Chem. Biol.* 14 (2007) 1128.
- [122] D. Aldinucci, D. Lorenzon, S.L. Stefani, L. Giovagnini, A. Colombatti, D. Fregona, *Anti-cancer Drugs* 18 (2007) 323.
- [123] V. Milacic, D. Chen, L. Ronconi, K.R. Landis-Piwowar, D. Fregona, Q.P. Dou, *Cancer Res.* 66 (2006) 10478.
- [124] L. Engman, M. McNaughton, M. Gajewska, S. Kumar, A. Birmingham, G. Powis, *Anticancer Drugs* 17 (2006) 539.
- [125] D. Fan, C.T. Yang, J.D. Ranford, J.J. Vittal, P.F. Lee, *Dalton Trans.* (2003) 3376.
- [126] R.G. Buckley, A.M. Elsome, S.P. Fricker, G.R. Henderson, B.R.C. Theobald, R.V. Parish, B.P. Howe, L.R. Kelland, *J. Med. Chem.* 39 (1996) 5208.
- [127] L. Messori, G. Marcon, M.A. Cinelli, M. Coronello, E. Mini, C. Gabbiani, P. Orioli, *Bioorg. Med. Chem.* 12 (2004) 6039.
- [128] M. Coronello, E. Mini, B. Caciagli, M.A. Cinelli, A. Bindoli, C. Gabbiani, L. Messori, *J. Med. Chem.* 48 (2005) 6761.