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Inorganic Anticancer Agents: Their Chemistry and Antitumor Properties

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In the last two decades, the development of new tumor-inhibiting metal complexes has received increasing attention owing to the successful therapy of testicular carcinomas with cisplatin. Here, a short overview is presented with special consideration of platinum compounds and of two new substances: a titanium complex, *cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV) (budotitane, International Nonproprietary Name), and a ruthenium complex, *trans*-HInd[$RuCl_4(ind)_2$]. Some aspects of the mode of action of cisplatin, the first metal complex in cancer therapy, are summarized in this article because it provides a background for all subsequent investigations. Activation and activated species, binding to and transport by plasma proteins, DNA interactions and the formation of metal-nucleotide adducts are chemical aspects discussed in the context of a new generation of tumor-inhibiting metal complexes.

Key Words: *cancer therapy, drug targeting, tumor-inhibiting metal complexes, cisplatin, budotitane,* trans-*indazolium-tetrachlorobis(indazole)ruthenate(III)*

I. INTRODUCTION

A Sunday afternoon excursion to the "German pharmacy museum" in Heidelberg's famous castle gives a nice survey of how the use

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of pharmaceutical agents has developed and changed through the centuries. For an inorganic chemist, the fact is impressive that a some hundred years old experience with inorganic drugs and minerals was nearly completely given up at the beginning of this century, as organic chemists started to dominate therapeutical chemistry as well as biochemical practice. A renaissance of such inorganic topics can be observed during the last decade, during which new investigations of the role of metal ions in many biochemical processes have spread interest in bioinorganic research and led to new therapeutic strategies.

In this article we want to present a summary of research concerning new inorganic anticancer agents which has been carried out during the last 10 years. The development and success of cisplatin (International Nonproprietary Name) for the therapy of testicular and ovarian carcinomas among others have helped to promote interest in other tumor-inhibiting metal complexes. Barnett Rosenberg discovered the tumor-inhibiting qualities of diammine platinum compounds by chance in 1964 during his investigations into the influence of an electric field on bacterial growth.[1] The observed filament growth of *Escherichia coli* bacteria was caused by *cis*-diamminetetrachloroplatinum(IV), which had formed in the nutrient solution by platinum electrodes. Subsequent synthesis of some simple platinum complexes, together with a check of efficacy in experimental tumor models, gave evidence that *cis*-diamminedichloroplatinum(II) led to a significant influence on tumor growth.[2] Even though at first there were considerable reservations regarding these heavy metal compounds, cisplatin entered clinical studies and soon showed amazingly good effects on various advanced tumors. Today, cisplatin is used in combination with other antitumor agents, mainly against testicular and ovarian carcinomas, bladder tumors and tumors of the head and neck. The dose-limiting factor in therapy is caused by numerous side-effects, including nephro-, neuro-, and ototoxicity, nausea, and a mild myelotoxicity. The reduction of side-effects and an extended therapeutical spectrum gives reason to look for further inorganic drugs against cancer.

In this article we want to call attention to the distribution and the mode of action of some of these new drugs. The numerous investigations of cisplatin possess a leading character for all subsequent tumor-inhibiting metal complexes; therefore a short overview is given in the following section.

II. CISPLATIN AND ITS MODE OF ACTION

Metals form coordination compounds by binding ligands firmly or loosely, depending on the chemical nature of both the metal ion and the ligands. A loosely coordinated ligand, such as chloride in the case of platinum(II) complexes, will exchange for other ligands, especially nucleophilic sites of biomolecules, and thereby produce a biological lesion. Often the substitution reactions start directly after administration of the drug and lead to metastable complexes or toxic species, depending on a following reaction, which leads to a therapeutic effect or a toxic side-effect. For *cis*-diamminedichloroplatinum(II) (cisplatin), the activation occurs by hydrolysis. The rate constant for the hydrolytic replacement of the first chloro ligand at 37°C is 7×10^{-3} min^{-1}, corresponding to a half-life of 2 h[3]; hydrolysis of the second chloro ligand is about two times slower. Hence the *cis*-$[PtCl(H_2O)(NH_3)_2]^+$ cation is the biologically important species; the firmly bound, conserved ammine ligands are responsible for the reactivity as well as for the target selection. The hydrolysis leads to pH-dependent equilibria, which have been studied in detail.[4-6] Under physiologically relevant conditions of 150 mM free chloride, as in most extracellular fluids, the level of hydrolyzed cisplatin is negligible, whereas under conditions of 3 mM free chloride, as within most cells, close to 50% will be in the form of *cis*-diamminechloroaquaplatinum(II), provided the pH of the solution is low (pH < 6). These results seem to give evidence that a reaction of proteins with cisplatin in the blood is unlikely to be a mechanism for growth inhibition.

Nevertheless the interactions of cisplatin with serum proteins like albumin and the different globulins play an important role for its distribution inside the organism.[7] Comparative pharmaco-kinetics of cisplatin and second-generation analogues like carboplatin (INN) showed that a high percentage of cisplatin is bound to serum proteins in humans (Table I).[8] A number of platinum compounds that are in advanced preclinical and clinical studies are summarized in Fig. 1.

Carboplatin shows a similar spectrum of indication as cisplatin, with reduced nephro- and increased myelotoxicity. Also, the reversibility of binding has been studied *in vitro*[9] by equilibrium dialysis. Carboplatin shows that there are major differences in protein binding compared to cisplatin. Extensive dialysis after 12 hours incubation

TABLE I

Comparative pharmacokinetics of cisplatin and carboplatin after 1 hour infusion (Ref. 8). In general, the disappearance of Pt from plasma in humans showed a rapid initial phase (α-clearance) followed by a more prolonged later phase (β-clearance).

Complex	Total Platinum $t_{1/2}$ α (min)	Total Platinum $t_{1/2}$ β (h)	Free Platinum $t_{1/2}$ α (min)	Free Platinum $t_{1/2}$ β (min)	% of Platinum Found Protein-Bound (3–4 h Post-infusion)	24 h Urinary Excretion (% of dose)
cisplatin	8.7–22.5	30.5–106	22	not seen	>90	30–35
carboplatin	98	6.7–>24	87	354	24	65

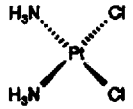
$t_{1/2}$ α (min), $t_{1/2}$ β (h) = half-life of the α- and β-clearance of the drug from the blood plasma.

of the complexes with plasma proteins demonstrated that after 3 hours 60% of cisplatin, but only 20% of carboplatin, was still bound to the proteins, a value that stayed constant during the following 6 hours.

These results support the idea that a major amount of cisplatin binds irreversibly to serum proteins within the first two hours after injection, and there seems to be no mechanism involving release of cisplatin from the protein bound species in the blood plasma. However, due to the antitumor activity of serum protein-bound platinum at high doses,[10] a pinocytic uptake of the latter and subsequent release of active platinum species within the tumor cells seems likely but does not contribute significantly to the antitumor activity of cisplatin.

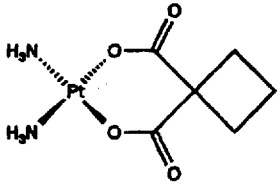
DNA is believed to be one of the major targets for this drug and many other anticancer chemotherapeutics.[11] Other targets are receiving increasing attention now, like the cell membrane with its wide fund of different receptors for intracellular communication,[12] but this cannot be discussed extensively in this compact overview. In this respect, the reader is referred to recent excellent reviews.[13–18]

Due to the high extracellular concentration of 150 mM chloride ions, platinum(II) can pass the membrane as a neutral compound and then become activated 2–4 mM chloride concentration prevailing in the cytoplasm and the organelles. The drug spreads in the cell and



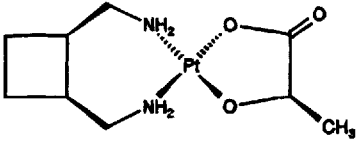
Cisplatin

cis-Diamminedichloroplatinum(II)



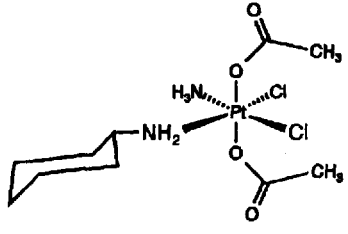
Carboplatin

cis-Diammine(cyclobutane-1,1-dicarboxylato)platinum(II)



Lobaplatin

cis-(cis-1,2-Bis(aminomethyl)-cyclobutane)platinum(II)lactate



JM 216

cis-(ammine)(cyclohexylamine)dichloro-trans-(diacetato)platinum(IV)

FIGURE 1 Structures of cisplatin and a few second generation analogues.

can penetrate other membrane barriers before activation is completed. Due to their positive charge(s) acquired during the activation reaction, platinum(II) complexes react eagerly with the purine bases of nucleoside diphosphates and triphosphates.[19] Most primary products are formed under kinetic rather than thermodynamic control; the negatively charged DNA-backbone influences the target choice. To date, the adducts of platinum(II) complexes with DNA have been the only platinum-biomolecule adducts[20] isolated directly from treated cells; the reactions with all other biomolecules are assumed on the basis of cell-free reactions.

Distinct types of DNA products of platinum(II) complexes have been demonstrated by HPLC analysis after nuclease digestion, and by ^{1}H, ^{31}P and ^{195}Pt NMR spectroscopy of platinum(II)-oligonucleotides.[17] These are mainly bifunctional adducts that crosslink guanine–guanine residues in the sequences d(GpG) (relative abundance 40–70%) and d(GpNpG) or adenine–guanine residues in the sequences d(ApG) (20–25%) or d(ApNpG), and monofunctional adducts.[18] Figure 2 shows an X-ray crystal structure of such a dinucleotide as a model compound.[15] Bifunctional adducts (1%) between opposite DNA strands are detected by alkaline elution and gradient centrifugation techniques.

The distribution of adducts in DNA can be studied by DNA mapping with nucleases[21,22] or by replication mapping with DNA polymerases.[23–25] By various techniques, the structural changes have been investigated also (for recent reviews, see Refs. 17, 26 and 27). The formation of adducts disrupts the local structure of double-stranded DNA. The structural features of double-stranded DNA inferred by intrastrand crosslinks, especially the "kink" in the position of the crosslink at d(GpG), have also been elucidated by molecular modelling.[28] The results of pulse polarography suggest a delicate distortion of the DNA double helix around monofunctional adducts, a suggestion at variance with the results by most other techniques.[17,26] Some new findings show that an interstrand linking of DNA by cisplatin might be important for therapeutic efficacy.[29–31]

For the reaction mechanism, an attack by activated platinum(II) complexes at guanine residues of DNA in position N7 is supposed, in double-stranded DNA from the side of the major groove. The initial attack of DNA by activated cisplatin is followed by the replacement of the remaining chloro ligand before the adduct performs an

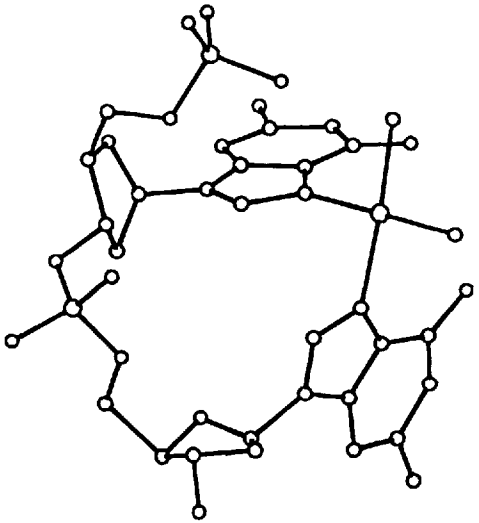


FIGURE 2 X-ray crystal structure of an "intrastrand" dinucleotide cisplatin adduct: *cis*-{$Pt(NH_3)_2$d(pGpG)]. (Figure prepared from parameters tabulated in Ref. 15.)

intramolecular attack on the second purine residue (either guanine or adenine).[32] The formation of crosslinks is followed by a local, partial unwinding of double-stranded DNA together with a second wave of crosslink formation (Fig. 3). The half-life of the first wave of crosslink formation is 200 s, and for the local distortion of the double-stranded DNA (second wave of crosslink formation) it is 30 min. It is interesting to note that the rates of formation of the various crosslinks are indistinguishable.[32]

Under physiological conditions, the DNA monofunctional adducts have considerable lifetimes due to the release of the second chloro ligand before the crosslinks can be formed. It is not known whether the kinetic mechanism is the same *in vivo*, where the binding of chromatin proteins and of various inorganic anions can interfere with the reaction of platinum(II) complexes.

III. OTHER PLATINUM COMPOUNDS

Among the cisplatin analogues, currently undergoing early clinical testing,[33] are complexes including (i) diaminocyclohexane deriva-

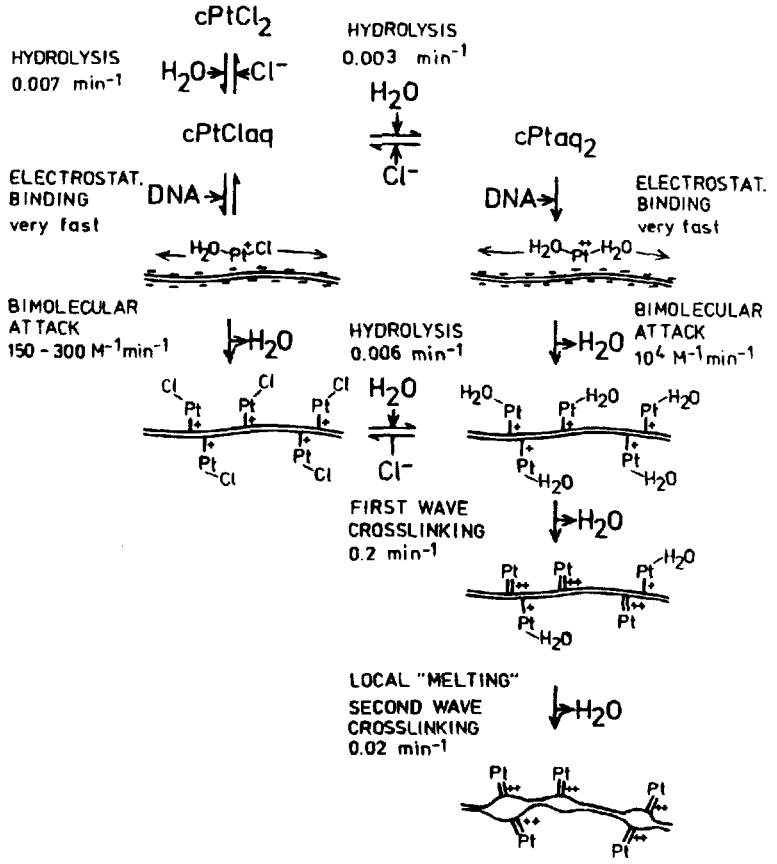


FIGURE 3 Kinetic mechanism of cisplatin binding to DNA *in vitro*. Double stranded DNA is symbolized by two parallel lines. Monofunctional adducts by cisplatin are indicated by single "bonds", bifunctional adducts irrespective of their type of crosslink by double "bonds". Symbols "−" refer to negative and "+" to positive charges. Ammine ligands are omitted from the graph. The arrows in the electrostatic complex indicate one-dimensional diffusion of the platinum(II) complex along the DNA backbone. The rate constants refer to pH 5–6 and 37°C. The results of the conformational change ("local melting") are exaggerated (Refs. 32 and 91).

tives, (ii) platinum(II) complexes with dicarboxylate cyclobutane or related oxygenated leaving groups, and (iii) an orally active mixed ammine/amine platinum(IV) complex,[34] JM 216 (Fig. 1).

In the past, research was basically centered on the production of cisplatin analogues and, in a few cases, also on similar complexes with other central metals. These, however, were often designed in structural analogy to the active platinum compounds. The tumor-inhibiting properties of the new compounds were always compared to those of cisplatin, and every effort was made to achieve a higher antitumor activity of platinum compounds or a lower toxicity level. Clinicians had long since realised that cisplatin was a specific drug that is exceedingly active in certain special tumors but it is inefficient in other, very common, tumors. For this reason, scientists working in this experimental field must first of all aim at finding new compounds that supplement the narrow cisplatin spectrum of indication. This applies to precisely those tumors that are responsible for the major share of cancer mortality today, e.g., tumors of the lung and adenotumors of the gastrointestine.

The concept of a directed development of new tumor-inhibiting metal complexes ("drug targeting") is basically characterised by the three following procedures[35]:

—synthesis and activity screen of "direct" cisplatin analogues (see Section II).
—linking of cancerotoxic platinum compounds or of other tumor-inhibiting metal complexes with carrier molecules or carrier systems in order to achieve an accumulation in certain tissues.
—trials with new metal complexes that do not have platinum as their central metal.

In this section we will discuss some new platinum compounds to show actual strategies to obtain new indication spectra. The concept of drug targeting can only be applied to tumors that contain biochemical targets different in structure or quantity from those of normal tissues. Some research groups started this way by the synthesis of platinum compounds with a specific activity against hormone-dependent tumors.[36] Carcinomas, possessing steroid hormone receptors, include malignancies of the breast, the endometrium and the prostate. Therefore, some medicinal chemists have tried to develop

platinum complexes with binding affinity for the estrogen receptor and selective action on estrogen receptor positive tumors. From these studies, two important structures emerged: 1,2-diphenylethylenediamines as neutral ligands, and other complexes based on the 2-phenylindole system (Fig. 4).

In the evaluation of these agents, it is often difficult to discriminate between their endocrine activity and their specific cytotoxic action due to the lack of suitable models. An important question is whether the amount of cytotoxic agent accumulated in the cell via a receptor is high enough to be lethal in a cell. However, it cannot be ruled out that the hormonal properties of these particular complexes make the tumor cells more susceptible to chemotherapeutics.[36]

A similar concept related to this topic of drug targeting consists of linking cytotoxic platinum structures to osteotropic phosphonic acids in order to achieve accumulation of platinum compounds in bone tumors and bone metastases. Osteosarcoma is the commonest bone malignancy.[37] The osteotropic properties of phosphonic acids are already being used for numerous medical purposes such as technetium scintigraphy, the use of phosphonic acids in metabolic disorders of the bone and the therapy of tumor-induced hypercalcemias, among others. The major representatives of platinum complexes recently synthesized by us in this context include the compounds AMDP, *cis*-diammine[nitrilotris(methylphosphonato)(2-)-O^1,N^1]platinum(II), and DBP, *cis*-diammine[(bis(phosphonatomethyl)amino)acetato)(2-)-O^1,N^1]platinum(II) (Fig. 5).[38]

These compounds are considerably less toxic than cisplatin[39] and show marked activity in a transplantable osteosarcoma.[38] Phosphonic acid groups which are linked to platinum can be distinguished from free phosphonic acids by the difference in chemical shifts in the ^{31}P NMR.[40] For those complexes, the formation of GN7, GN7 chelates is observed, together with the release of the phosphonic acid ligand. First, the platinum phosphonate bond is broken, probably by direct attack of the first G-base.[41]

A more general concept was pursued with the synthesis of the first platinum crown ether. It was not a specific cell receptor or high affinity towards a selected tissue that was taken into consideration in the development, but the possible use of general transport mechanisms into the cell. Figure 6 shows the X-ray crystal structure of this new type of platinum(II) complex.[42]

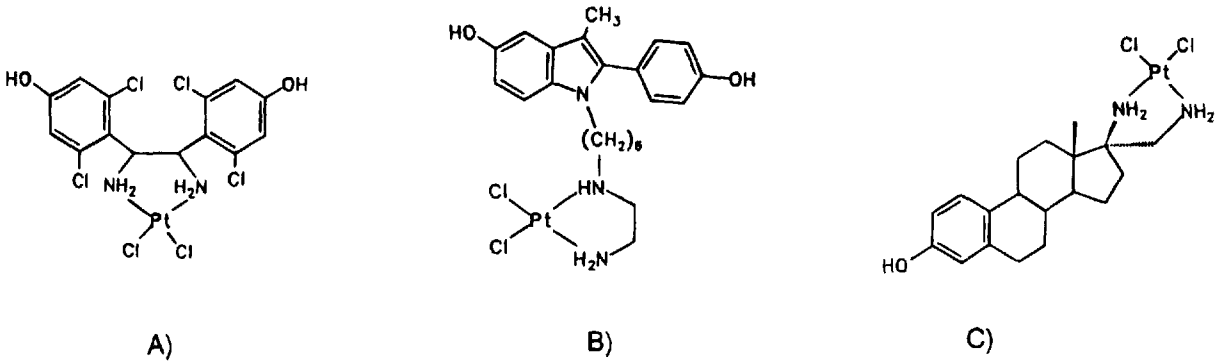
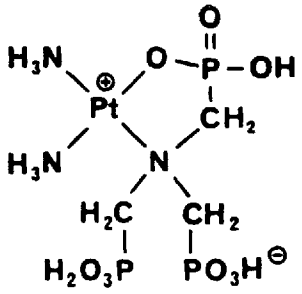
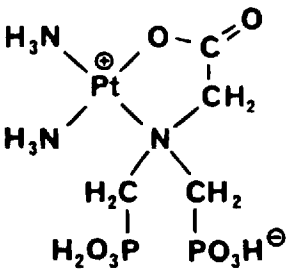


FIGURE 4 Examples of three different classes of hormone-active new platinum complexes (Ref. 36): (A) with a high activity against estrogen dependent tumors; (B) with a high estrogen receptor affinity; (C) with a steroidal hormone as a functional group.



AMDP



DBP

FIGURE 5 Structures of two examplary platinum(II) phosphonato complexes: AMDP, *cis*-diammine[nitrilotris(methylphosphonato)(2-)-O^{1},N^{1}]platinum(II) and DBP, *cis*-diammine[(bis(phosphonatomethyl)amino)acetato)(2-)-O^{1},N^{1}]platinum(II). Of DBP, the X-ray crystal structure is known (Ref. 38).

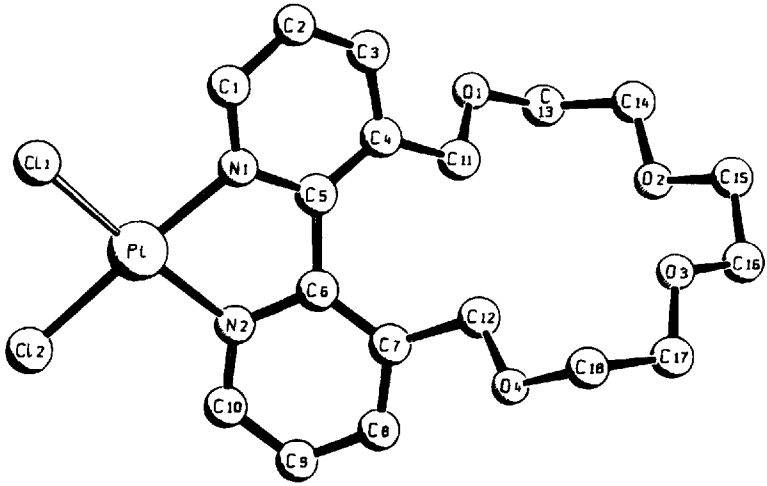


FIGURE 6 X-ray crystal structure of a platinum(II) linked crown ether: dichloro(5,7,8,10,11,13,14,16-octahydro[1,4,7,10]tetraoxacyclohexadecino-[13,12-b:14,15-b']-dipyridine-N^{1},N^{20})platinum(II) (Ref. 42).

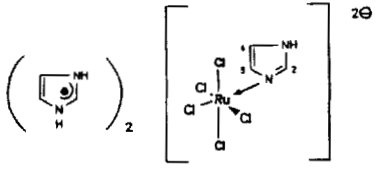
Another idea was to synthesize a new class of orally absorbable drugs. Oral application is cheaper and easier to handle than the normal intravenous administration of a drug. The requirements for such drugs are: stability in the acid conditions of the stomach and in the alkaline conditions of the gut and nearly complete absorption in the gastrointestine. An important example of such orally absorbable drugs is the previously mentioned platinum complex JM 216 (cf. Fig. 1).[34]

IV. TUMOR-INHIBITING RUTHENIUM COMPLEXES

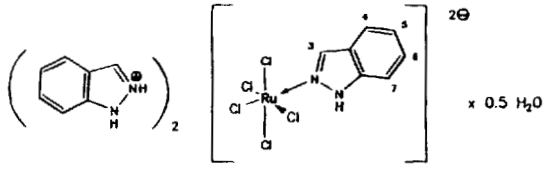
Some classic ruthenium complexes such as "ruthenium red," $[(NH_3)_5Ru^{III}\text{-}O\text{-}(NH_3)_4Ru^{IV}\text{-}O\text{-}Ru^{III}(NH_3)_5]^{6+}$, *cis*-$[Ru(DMSO)_4Cl_2]$, or *cis*-$[Ru(NH_3)_4Cl_2]Cl$ are well known for their antitumor activity, but so far none of them and none of their derivatives have been able to qualify for clinical trials.[43–48] Nevertheless, heterocycle substituted ruthenium DMSO complexes show interesting antimetastatic properties.[49]

In different antitumor screenings,[50] we found that complexes of the general formulas *trans*-$LH[RuCl_4L_2]$ and $(LH)_2[RuCl_5L]$ show promising antitumor activity. Figure 7 shows the structures of the imidazole and indazole complexes. These compounds exhibited the best activity in these antitumor tests. The two compounds *trans*-$HInd[RuCl_4(ind)_2]$ and *trans*-$HIm[RuCl_4(im)_2]$ also showed the best results in the final, autochthonous tumor model.[39] Figure 8 illustrates the results obtained with these two compounds in comparison to cisplatin and 5-fluorouracil.

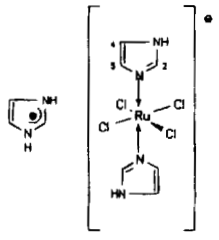
Three different experiments are summarized in Fig. 8. Cisplatin turned out to be completely inactive in this model, just as it is in the clinical tumor type. 5-Fluorouracil, at present the only drug in clinical use to produce a certain reduction of tumor volume, showed the same positive effect it has on comparable clinical tumors. Tumor volume decreased to 40% relative to that of controls. $HIm[RuCl_4(im)_2]$, given at a dose of 7 mg/kg twice a week over ten weeks, reduced tumor volume to 10%. The indazole derivative had already turned out to be less toxic in chronic application, and hence it could be applied at a higher dose. The result was a decrease in



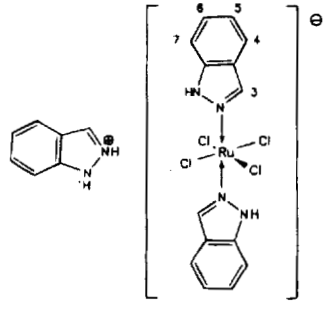
Bisimidazolium-pentachloro(imidazole)ruthenat(III),
$(HIm)_2[RuCl_5(im)]$



Bisindazolium-pentachloro(indazole)ruthenat(III),
$(HInd)_2[RuCl_5(ind)]$



trans-Imidazolium-tetrachlorobis(imidazole)ruthenat(III),
trans-$HIm[RuCl_4(im)_2]$



trans-Indazolium-tetrachlorobis(indazole)ruthenat(III),
trans-$HInd[RuCl_4(ind)_2]$

FIGURE 7 Structures of ruthenium imidazole and indazole complexes of the general structures *trans*-$LH[RuCl_4L_2]$ and $(LH)_2[RuCl_5L$

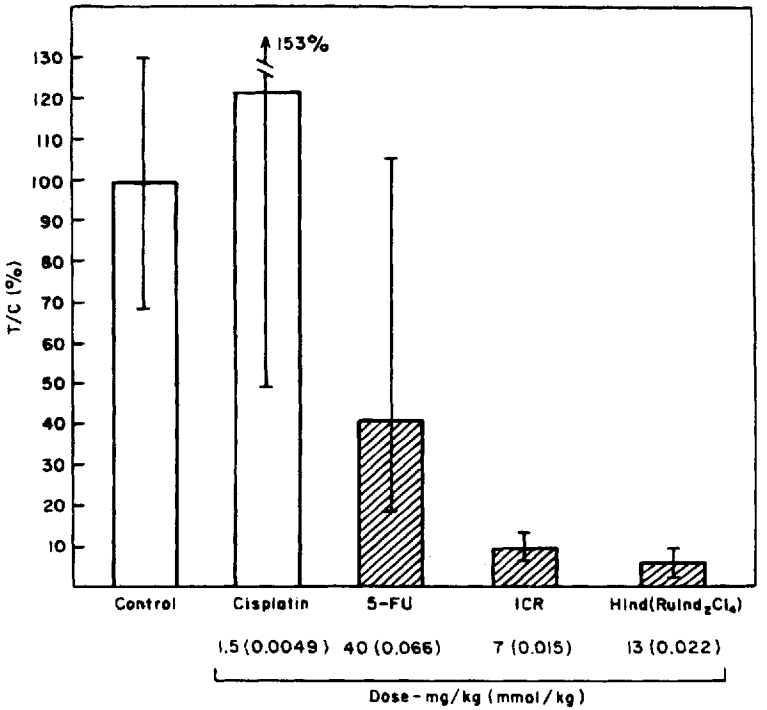


FIGURE 8 Test results of the two ruthenium compounds, *trans*-HInd[$RuCl_4(ind)_2$] and *trans*-HIm[$RuCl_4(im)_2$] in autochthonous colorectal tumors of the rat, compared to cisplatin and 5-fluorouracil. Doses were applied twice a week over ten weeks. The reduction of tumor volume represented by the shaded columns is statistically significant, in comparison to the control group. T/C (%) = (median tumor weight of treated animals vs. median medium tumor weight of control animals) × 100. In this case, the lower the T/C values, the better the antitumor activity.

tumor volume to even 5%. Final evaluation showed that in this group about one third of the animals were even tumor-free.

In the case of cisplatin, the kinetic stability of the complex is an outstanding criterion for its use as a therapeutic agent. Prevention of fast hydrolysis reactions led our attention to other metals that show a hydrolysis stability similar to that of platinum(II). Ruthenium(III) is well known for its kinetic stability in aqueous solution; for the tumor-inhibiting complexes like HInd[$RuCl_4(ind)_2$] and HIm[$RuCl_4(im)_2$], a slow exchange of their ligands can be predicted.[51]

HPLC investigations with $HInd[RuCl_4(ind)_2]$ in H_2O indeed showed slow decomposition (in physiological saline at 22°C) with a rate of hardly 1% per hour. The initial formation of an aqua complex could also be confirmed by the isolation of the analogous complex aqua-trichloro(1-methylindazole)ruthenium(III), $[RuCl_3(H_2O)(1MeInd)_2]$ (Fig. 9).[52] The imidazole complex hydrolyzes faster in physiological saline (22°C); after one hour nearly 3% of the original complex has been substituted in solution. The respective imidazolium cation is the weaker acid and leads to a less acidic milieu, so that the pK-dependent concentrations of the other nucleophiles in solution (imidazole, chloride and H_2O) are raised. The result is the observed loss of kinetic stability of the imidazole complex in physiological saline.

A general decrease in kinetic stability can be observed when $HInd[RuCl_4(ind)_2]$ is added to a physiological buffer solution, similar to the blood buffer (0.1 M NaCl, 0.004 M NaH_2PO_4, 0.025 M $NaHCO_3$). A blue-green precipitate is formed after 10 minutes (37°C). These investigations were done in preparation of experiments to explore forms of $HInd[RuCl_4(ind)_2]$ and $HIm[RuCl_4(im)_2]$ in human serum.

To obtain an insight into the forms of intravenously administered antitumor metal complexes, it is important to study their interactions with plasma proteins. For example, the differences in efficacy, activity and toxicity between cisplatin and carboplatin are mentioned in relation to the measured differences in the reversibility of plasma binding of both compounds as shown in Section II.

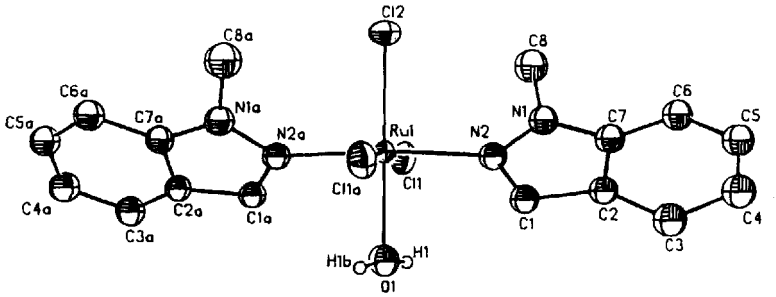


FIGURE 9 X-ray crystal structure of $[RuCl_3(H_2O)(1MeInd)_2]$. Until now we could obtain a structure of the methyl-substituted indazole complex only (Ref. 52).

LPLC studies of $HInd[RuCl_4(ind)_2]$ have shown that the major amount of the complex is bound to albumin in serum (about 80%).[53] This is not surprising when we consider the amount present in serum (approximately 42 weight per cent of the solutes) and the size of albumin. The remaining amount of $HInd[RuCl_4(ind)_2]$ is bound to transferrin. Ultrafiltration experiments have given evidence that no binding to low molecular species (M.W. $< 30{,}000$) occurs. HPLC studies of the reaction showed that binding to transferrin occurs within 5 minutes.

When it became clear that albumin and apo-transferrin are involved in the binding of HInd-$[RuCl_4(ind)_2]$, the next step was to investigate whether binding was specific or nonspecific. By CD spectroscopy, a change in the optically active amino acid groups could be followed in the visible region, showing that binding is specific.[53] Albumin can take up 5 moles of the complex, whereas saturation of apo-transferrin is reached after only 2 moles.

Crystallographic experiments were carried out to obtain more detailed information on the binding sites for the two ruthenium indazole complexes.[54] Apo-lactoferrin was chosen rather than apo-transferrin because of the availability of suitable crystals, but the binding behaviour should be similar owing to the strong similarities between lactoferrins and transferrins.[55] Although the results are only preliminary, difference Fourier maps show very clearly that the ruthenium(III) complexes fit into the iron-binding site of the apo-lactoferrin and are coordinated to histidine 253 of the protein, presumably following the loss of one or more chloride ligands. The two indazole ligands remain coordinated to the ruthenium atom (Fig. 10).[54]

If $HInd[RuCl_4(ind)_2]$ is transported into the tumor cells by apo-transferrin, it has to be released again inside the cells to exert its antitumor effect. The release should occur at low pH-values (4–6) like the endocytotic uptake of iron into the cell tissue. Figure 11 shows the results after a small amount of citrate was added to the $(HIndRuCl_4(ind)_2)_2$-transferrin adduct. After 2 hours of incubation nearly all of the complex is released from transferrin, and a new "free" ruthenium complex that is observed at 10.2 min retention time might have coordinated citrate. This finding suggests that the protein-mediated transport into cells and the reversibility of binding might be reasons for the good activity of the ruthenium complex in colon cancer models and the limited number of observed side-effects. Many

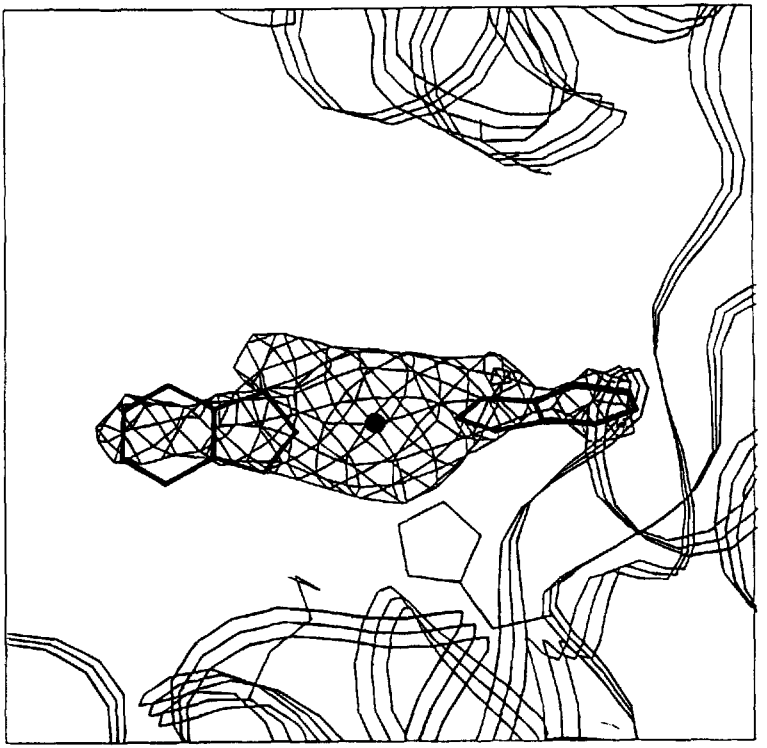
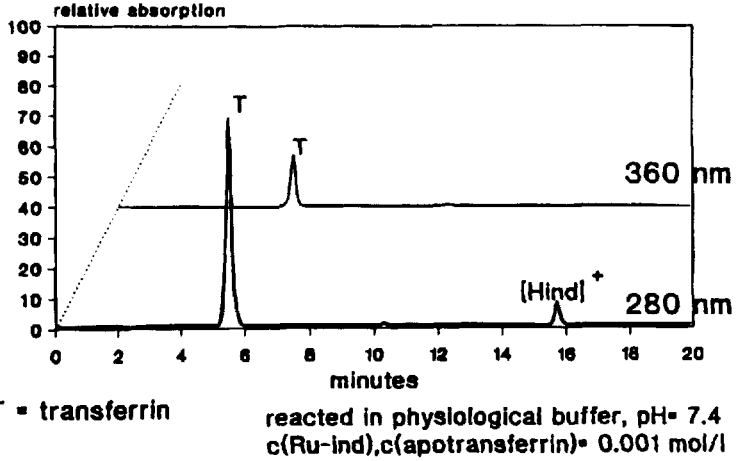


FIGURE 10 Difference electron density for the ruthenium indazole complex in the N-terminal lobe of human apo-lactoferrin, showing that the two indazole ligands are retained. The Ru atom binds to His 253, and the nearby side chain of Lys 301 may help stabilize binding (Ref. 54).

solid tumors express a high level of transferrin-receptors. This is why the metal-transferrin adducts should easily accumulate in the tumor to exert their antitumor activity after release, thus making selective treatment possible.[56]

As for the intracellular mode of action, we started to look at the binding affinity towards DNA. The kinetics of the metal-complex–DNA binding have been measured with the help of an ICP-AES (Induced Coupled Plasma–Atomic Emission Spectrometer).[57] The binding behaviour of $HInd[RuCl_4(ind)_2]$ and $HIm[RuCl_4(im)_2]$ towards salmon testes DNA gives evidence that similar amounts of



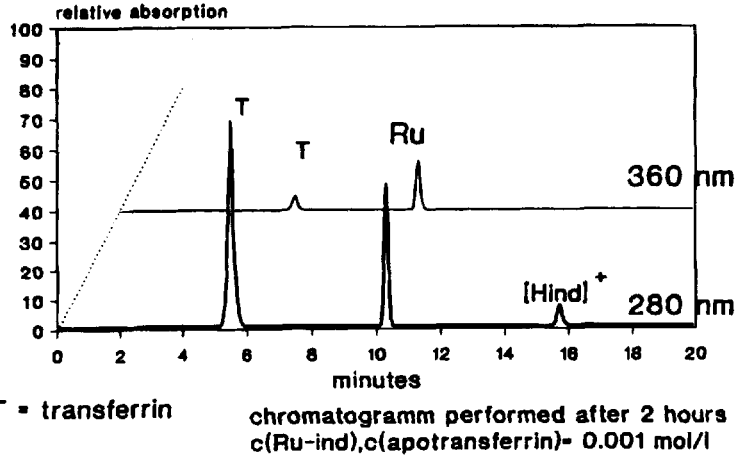


FIGURE 11 Chromatograms of $HInd[RuCl_4(ind)_2]$ bound to apo-transferrin (above) and after incubation with citrate for 2 hours (pH 4). (Column: Bio-Sil SEC (300 × 7.8 mm) from Bio-Rad; mobile phase: 0.15 M NaCl, 0.01 M NaH_2PO_4, 5% CH_3CN).

metal bind to DNA at similar reaction rates (Fig. 12). It should be noted that both the new tumor-inhibiting ruthenium complexes show high affinity to DNA; especially after one day the measurements show a better binding to DNA than in the case of cisplatin. These results correlate with the published data of activity in the experimental tumor models.[39]

As to the mechanism of the DNA interaction, electrostatic binding of highly hydrolyzed, positively charged species has to be taken into consideration as well as coordinative linkage as in the case of cisplatin. The ruthenium imidazole complex $HIm[RuCl_4(im)_2]$ reacts with DNA and inhibits template-primer properties for DNA synthesis catalysed by *Escherichia coli* DNA polymerase I.[58] As far as model complexes are concerned, the first NMR experiments and HPLC investigations have given evidence that the reaction with single nucleotides is rather slow; this work is still in progress.

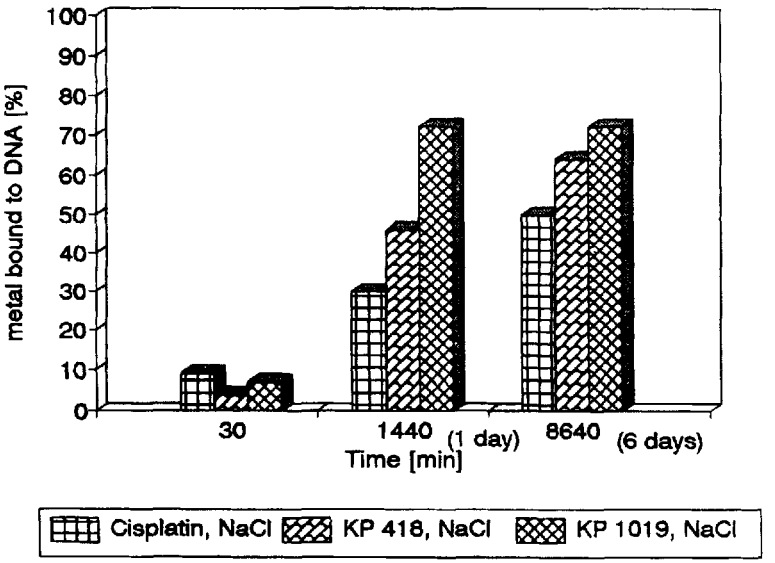


FIGURE 12 Binding behaviour of $HInd[RuCl_4(ind)_2]$, KP 1019, and $HIm[RuCl_4(im)_2]$, KP 418, towards DNA, in comparison with cisplatin (10 mM NaCl, Salmon Testes DNA). The binding (%) was measured by induced coupled plasma atomic emission spectrometry (ICP-AES) at different times: 30 min, 1440 min (1 day), 8640 min (6 days) (Ref. 57).

So today a nucleotide adduct, related to the "ruthenium red", 9-[(7-MeGua)$(NH_3)_5Ru^{III}$] mentioned in this section, is the best characterized example of a purine-linked ruthenium(III) complex (Fig. 13).[59]

V. BUDOTITANE (TITANIUM COMPOUNDS)

For more than 10 years now, titanium complexes have been investigated because of the tumor-inhibiting properties of some structures within this class of complexes.

The new transition metal complex *cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV), budotitane (INN) (Fig. 14), has demonstrated antitumor activity in several experimental tumor models.[60]

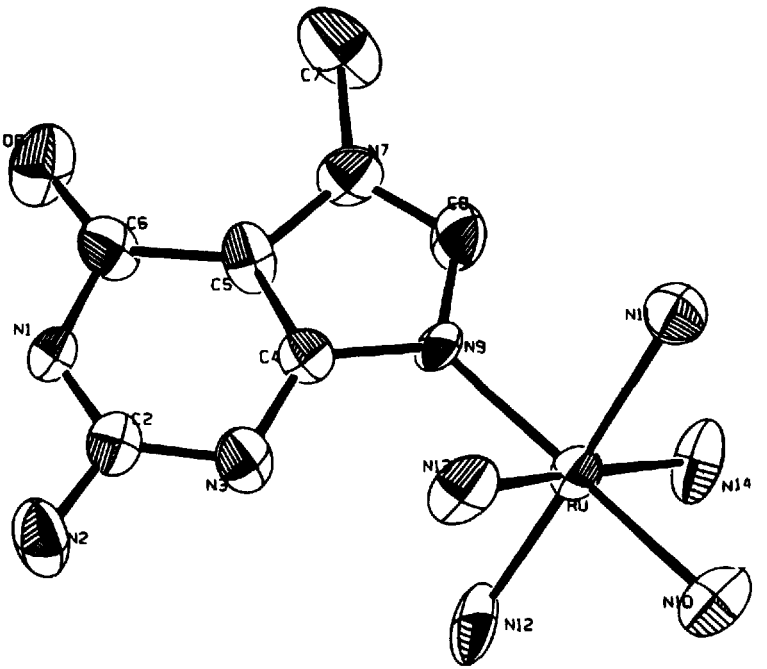
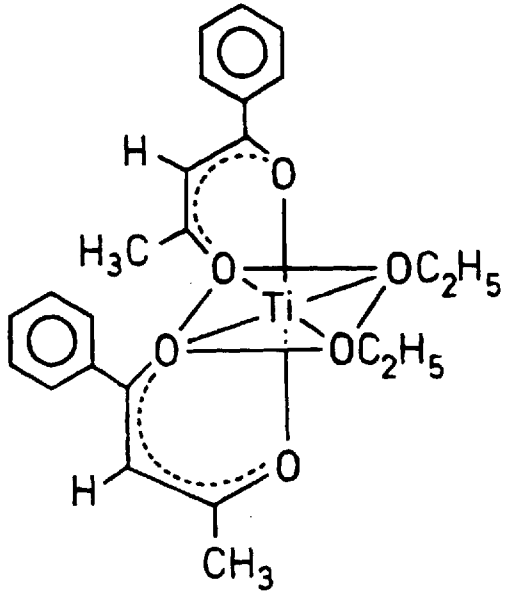
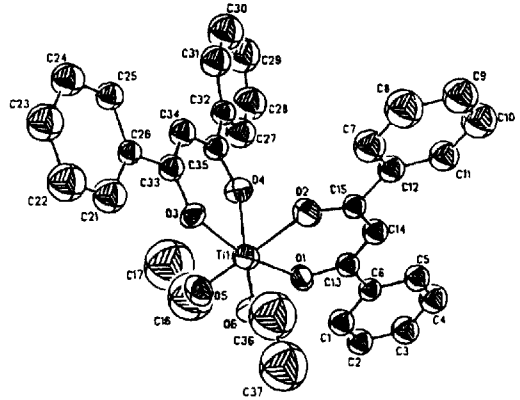


FIGURE 13 X-ray crystal structure of 9-[(7-MeGua)$(NH_3)_5Ru]^{3+}$ (according to M. Clarke, Ref. 59).





Selected bond distances (Å) and angles (°):

Ti(1)-O(1)	1.999 (9)	O(1)-Ti(1)-O(2)	82.6 (4)
Ti(1)-O(2)	2.057 (10)	O(2)-Ti(1)-O(4)	82.3 (4)
Ti(1)-O(4)	2.074 (11)	O(4)-Ti(1)-O(3)	81.9 (4)
Ti(1)-O(3)	1.996 (9)	O(3)-Ti(1)-O(5)	98.2 (4)
Ti(1)-O(5)	1.792 (11)	O(5)-Ti(1)-O(6)	98.4 (5)
Ti(1)-O(6)	1.793 (12)	O(6)-Ti(1)-O(1)	100.1 (5)

FIGURE 14 Structure of budotitane (INN), *cis*-diethoxybis(1-phenylbutane-1,3-dionato)-titanium(IV), $Ti(bzac)_2(OEt)_2$, and the X-ray crystal structure of the analogous model complex *cis*-diethoxybis(1,3-diphenylpropane-1,3-dionato)titanium(IV), $Ti(bzbz)_2(OEt)_2$, supplemented by selected structural data.

Surprisingly good results were achieved in the autochthonous AMMN-induced colorectal tumor model (Table II). The high predictivity of this tumor model, where budotitane was significantly more active (reduction of initial tumor weight to 20%) than 5-fluorouracil (40%) and cisplatin, has already been described in the literature.[39]

The six-coordinate "octahedrally" configurated bis(β-diketonato) titanium compounds can have both the *cis*- and the *trans*-configuration. The *cis*-configuration is usually energetically favoured, even though the *trans*-isomer should be favoured for steric reasons.[60,61] The number of possible isomers in the *cis*- and *trans*-form depends on whether the bound diketone in the 1- and the 5-position has the same or different substituents. For budotitane, an unsymmetrically substituted diketone complex, there exist three isomers in the *cis*-form and two in the *trans*-form.

The formation of isomer equilibria of the titanium(IV)(β-diketonato)$_2$X$_2$ complexes could be observed in temperature-dependent NMR studies,[60] but this will not be discussed here in detail. NMR experiments have shown that budotitane has mainly a *cis*-structure at room

TABLE II

Survey of the most important results of budotitane therapy in transplantable tumors. When survival time is used as parameter in the calculation of T/C values, these should be high, i.e., they should exceed 125%. T/C values > 300% mean that a high percentage of animals are cured. When tumor weight is used as parameter, T/C values should be low for a promising compound. In this case, they should fall below 45%.

Tumor Model	Evaluation Parameter	Optimum T/C Value (%)
Sarcoma 180 ascitic tumor	ST	>300
Sarcoma 180 tumor, subcutaneously growing	TW	0
Sarcoma 180 tumor, intramuscularly growing	TW	30
Walker 256 carcinosarcoma	ST	200
P 388 leukemia	ST	130
Stockholm ascitic tumor	ST	>300
Ehrlich ascitic tumor	ST	>300
MAC 15A colon tumor	ST	>300
AMMN-induced colorectal tumors	TW	20

ST = median survival time; TW = median tumor weight. AMMN = Acetoxymethyl methylnitrosamine.

temperature. So far it has not been possible to obtain suitable crystals for an X-ray analysis with budotitane, probably due to the presence of the three different *cis*-isomers. Thus we selected for crystallisation a symmetrically substituted dibenzoylmethanatotitanium(IV) complex, $Ti(bzbz)_2(OEt)_2$, as a model compound (Fig. 14, right). Most phenyl rings are nearly coplanar with the metal enolato chelate, as found for $Ti(bzbz)_2(OEt)_2$. Therefore, it may be assumed that in the budotitane molecule the phenyl rings are coplanar with the metal enolate ring.

Budotitane belongs to the class of rapidly hydrolyzing metal complexes. If a 10^{-3} M solution of $Ti(bzac)_2(OEt)_2$ is dissolved in absolute acetonitrile, the addition of 20 vol.% H_2O leads to decomposition of the complex within a few minutes. The primary product of the hydrolysis of the two ethoxy groups of budotitane, obtained in a pure form by reaction of the complex with water in 1:2 H_2O:acetonitrile, has the composition $[Ti(bzac)_2O]_n$. The product is probably produced by oligomerisation of the intermediate $Ti(bzac)_2(OH)_2$ that is formed on the reaction of budotitane with water. The molecular weight of $[Ti(bzac)_2O]_n$, (between n = 2 and n = 3) has been confirmed by vapour pressure osmometric measurements.[60]

The much higher decomposition rate of budotitane has consequences for the galenic formulation of the complex. A drug used in the clinic or in preclinical toxicological and pharmacological studies must be relatively insensitive to hydrolysis; if it is not, a formulation must guarantee these properties. In the case of budotitane, the use of CremophorEL, a glycerinepolyethylene-glycolericinoleate (BASF), is successful. Budotitane, CremophorEL and propylenelglycol are dissolved in water-free ethanol (weight ratio 1:9:1); the evaporated product is known as a coprecipitate. The major amount (80%) of the micellar emulsion with CremophorEL, after a rapid decomposition of 20% of the titanium complex, is stable over many hours in the region from 1 to 2% coprecipitate by weight in physiological saline.

Because of the high affinity of titanium(IV) towards oxygen containing ligands, the influence of plasma proteins in the blood cannot be investigated easily. The use of CremophorEL and the formation of a micellar emulsion lead to a more complex entrance form of the drug inside the organism; hence a direct synthesis of possible adducts with biological targets has been carried out. In addition to binding to DNA, other modes of action have been taken into consideration

that can explain the high activity of this compound in the experimental tumor models. Because of the hardness of titanium(IV) and its affinity towards oxygen, biomolecules are of interest that make metal coordination to an oxygen containing ligand possible. For this reason, numerous steroid, sugar and nucleoside derivatives of budotitane (Fig. 15) have been synthesized. The adducts have been characterized by spectroscopic methods, and in each case coordination by oxygen was found.[62] Synthesis was possible with sterans like cholesterol, stigmasterine or 5α-cholestane-3β-ol, as well as with the bile acid dehydrocholic acid. Also, complex binding occurred with derivatives of the steroid hormones testosterone, 4,5-dihydroxotestosterone, the corticoid 11-deoxycortisterone, and with the gestagen 5-pregnene-3β-ol-20-on.

From the nucleosides we characterized stable adducts with cytidine, uridine and thymidine. In each case, relatively fast binding to the hydroxy functions of the sugar moieties of the nucleosides took place; coordination to the basic nitrogen of the DNA nucleobases was not possible. Thus, we have grounds for the assumption that the biological activity is mediated by the complexed sugar- and phosphate-hydroxy groups in the case that DNA adducts *are* involved in the mode of action.

Activity tests with these model compounds in the sarcoma-180 ascitic tumor model as well as in colon carcinoma cell lines have shown a similar or even better efficacy against these tumors in comparison to budotitane. This fact gives evidence that adducts of budotitane with hydroxy-linked biomolecules can function as transport forms for this new drug under physiological conditions without any activity loss.

The binding behaviour towards DNA has been investigated again by measurements with an ICP-AES. The budotitane coprecipitate (CremophorEL) was incubated with double-stranded salmon testes DNA at 37°C for various times. After separation of the DNA from the reaction milieu, the amount of titanium was measured at 334.94 nm. At this wavelength, interference of other elements is not to be expected. The titanium complex shows, in comparison to cisplatin and to *trans*-HInd[$RuCl_4(ind)_2$], the fastest reaction rates with DNA; after 30 minutes 56% of the complex is already bound to DNA (30 min: 56%, 1 d: 94%, 6 d: 100%).[57,63] The nature of the DNA interac-

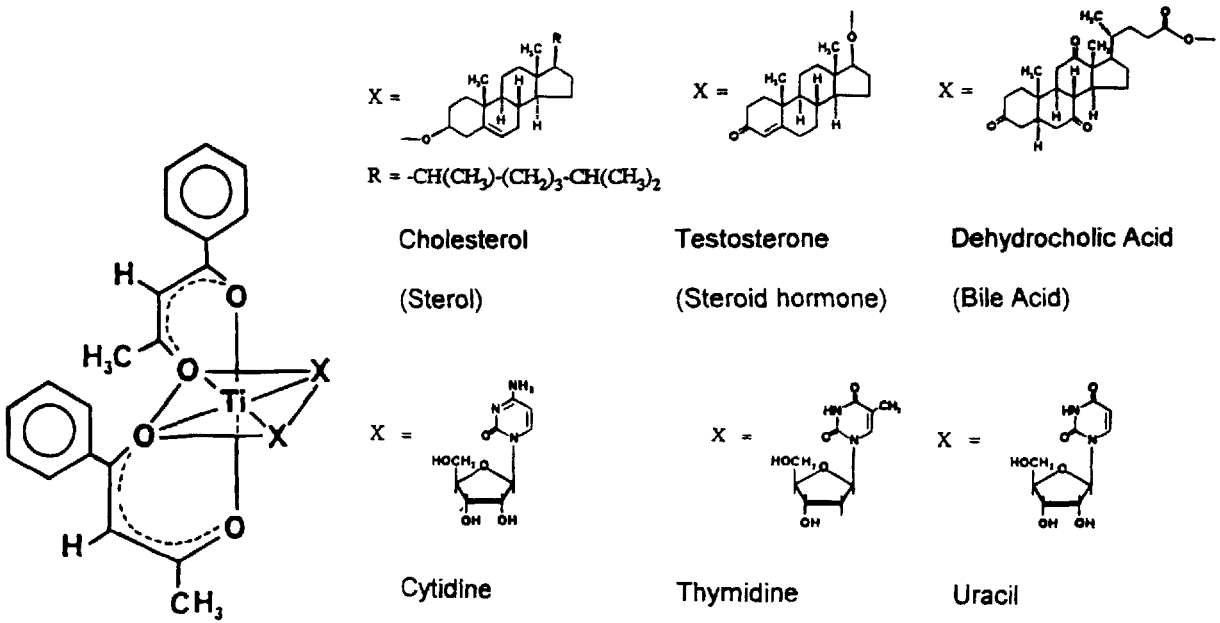


FIGURE 15 Derivatives of budotitane with biomolecules: the upper row shows budotitane steroid complexes; underneath there are some adducts with nucleosides.

tion is still unknown, but due to the previously discussed budotitane–nucleoside adducts oxygen–titanium links can be assumed.

Titanocene, $[C_5H_5]_2Ti^{IV}Cl_2$, is another titanium compound with well-known tumor-inhibiting properties. It is also the leading substance in the class of antitumor bis(cyclopentadienyl) metal complexes. The molecular geometry of these metallocene diacido complexes of early transition metals is that of a distorted tetrahedron.[64,65] These complexes show little activity in the normal first-line screens for anticancer activity (leukemias P 388 and L 1210), but they exhibit systemic activity against numerous other experimental tumors.[66,67] Titanocene complexes are also readily hydrolyzed in aqueous media.[67,68] By ICP spectroscopy, it was shown that DNA-bis(cyclopentadienyl)titanium adducts are formed at pH 5.3, whereas at pH 7.0 DNA-mono(cyclopentadienyl)titanium adducts occurred. The DNA-titanium adducts once formed were stable at neutral pH for up to 48 h.[69]

VI. OTHER METAL COMPLEXES

Although gold(I) complexes are best known for their activity against primary chronic polyarthritis (PCP), some gold(I) complexes also exhibit antitumor activity. These include tetrahedral gold(I) diphosphine complexes and auranofin, [1-thio-β-D-gluco-pyranosato-2,3,4,6-tetraacetato-S)-(triethylphosphine)gold(I)].[70,71] The mechanisms of action of auranofin are not clear, but the phosphine is eventually liberated and excreted as the oxide, $(CH_3CH_2)_3P{=}O$. The high cytotoxicity of auranofin *in vitro* can be attributed to the presence of the phosphine, which can be cytotoxic by itself; and the low potency of auranofin *in vivo* is probably due to its high reactivity towards thiolate ligands. In contrast, tetrahedral gold(I) complexes such as $[Au(dppe)_2]Cl$,[72,73] where dppe is 1,2-diphenylphosphinoethane, are much less reactive toward ligand exchange and exhibit a wider spectrum of anticancer activity. The free ligands $Ph_2P(CH_2)_nPPh_2$ and dppe-bridged digold(I) complexes exhibit a similar pattern of activity towards P388 leukemia,[74] although the complexes are much more potent than the free ligands. With respect to antitumor activity, auranofin is only active in the P388 leukemia[75] of mice when it is injected intraperitoneally (*i.p.*), i.e., into the

peritoneal cavity of the animal, and not when it is administered intravenously (*i.v.*).

Within the transition metal complexes, there are also known rhodium and rhenium carboxylates[76,77] and *cis*-configured palladium complexes[78]; they exhibit some antitumor activity.

Apart from these important classes of new tumor-inhibiting transition metal complexes, there are some tumor-inhibiting main group complexes that should be mentioned, too. Gallium was first used for diagnostic bone scans because of the metal's affinity towards osseous tissue. Gallium nitrate, $Ga(NO_3)_3$, has inhibitory effects on several transplantable tumors in rodents.[79] While the exact mechanism of cytotoxicity is unknown, the observed effect is probably related to the metal's ability to concentrate in malignant tumors. It is hypothesized that the uptake of gallium into the cell is mediated by a transferrin receptor. Investigations concerning the clinical value of gallium nitrate seems limited, because tumor-induced hypercalcemias can be treated successfully with newer bisphosphonates, and many efficient drugs are available for the treatment of malignant lymphomas, a type of tumor where gallium is seen to be active. Recent investigations have shown that a combination therapy of $GaCl_3$, used for diagnostic bone scans, and cisplatin against lung carcinomas may be successful.[80]

Spirogermanium, N-(3-dimethylaminopropyl)-2-aza-8,8-diethyl-8-germaspiro-4,5-decane-dihydrochloride produces, *in vitro*, a significant inhibition of DNA, RNA and protein synthesis. It shows cytotoxic activity in numerous cell cultures and had activity *in vivo* against a few carcinomas.[81]

Some recent reviews summarize research efforts with new tin(IV) compounds.[82–88] Antitumor and antileukemia screening tests have been done with diorganotin(IV) derivatives of dipeptides and mercaptoamino acids,[89] as well as with tin derivatives of salicylic acid.[90] For the first group of complexes, no simple or clear correlations between the observed activities and the structural features are available at the moment. Thus, different coordination modes (sulfur, nitrogen, oxygen) of the amino acids, which behave like inorganic ligands, and the different organic groups (Me, Et, Ph) lead to observable activities in P 388 and L 1210 leukemia activity tests.[89] The tin complexes with salicylic derivatives showed antitumor activity against different cell lines.[90] Here, the salicylic chelate seems to

influence the delivery of the active part $[RR'Sn(IV)]^{2+}$ into the cell. Only a few organo-tin(IV) moieties have been tested so far.

VII. SUMMARY

Research in the field of inorganic anticancer agents has intensified in the last two decades, because the success of cisplatin in the therapy of testicular and ovarian carcinomas meant that the development of new tumor-inhibiting metal complexes could be a promising strategy in the therapy of cancer. Since cisplatin, which is among the most sold anticancer drugs today, is only effective against very few tumors, scientists began to synthesize numerous Pt derivatives, with the aim of finding substances that might show activity against a wider range of tumors. The most important cisplatin analogues include diaminocyclohexane derivatives, platinum(II) complexes with dicarboxylate cyclobutane or related oxygen donor leaving groups, an orally active mixed ammine/amine platinum(IV) complex, and Farell's dinuclear platinum complexes, reviewed in this volume, which show interesting activity in various models. The spectrum of indication of "direct" cisplatin analogues is very similar to the parent compound. This is why new compounds with other central metals are also being synthesized, because it is hoped that these may have a different spectrum of indication and thus might be suited to treat the most common cancers.

The mode of action of cisplatin has been investigated in a large number of experiments, and it is thought that DNA is the major target. These examinations possess a leading function for all other investigations, which this article has pointed out. New platinum compounds are now being developed to obtain new indication spectra. The concept of drug targeting plays an important role in this field. Substances with activity against hormone-dependent tumors and others active against bone tumors and bone metastases, platinum crown ethers and orally absorbable platinum compounds are among the most interesting examples.

The search for new tumor-inhibiting complexes with other central metals turned up ruthenium, titanium and a number of other metals. In the field of ruthenium compounds, those with the general formulas

trans-LH[$RuCl_4L_2$] and $(LH)_2$[$RuCl_5L$] have shown promising antitumor activity. The compounds with imidazole and indazole as ligands are effective in an autochthonous colorectal tumor model, which has a high predictivity for the clinical situation. As for their mode of action, a selective transport mechanism into the cell via transferrin is being discussed. They also seem to bind to DNA.

Among titanium compounds, budotitane features prominently. This compound has shown activity against autochthonous, AMMN-induced colorectal tumors. It is in clinical studies today. Budotitane shows fast reaction rates with DNA, but the mode of binding is still unknown.

Gold complexes, rhodium and rhenium carboxylates, gallium and germanium complexes and tin compounds have also been synthesized, characterized and tested for their antitumor activity, but so far these compounds have failed to attain real clinical significance and use. They are more or less at an advanced preclinical stage of development, and their value for clinical cancer therapy cannot be determined yet.

In this article, we have tried to give an overview of the current state of research in the field of inorganic anticancer agents. There are numerous metal complexes at a preclinical state of development, but we also find a few that have entered clinical studies or are about to do so. Much work is still necessary to investigate chemical features, elucidate modes of action, and carry out antitumor activity tests. We may assume, though, that inorganic chemistry will make an important contribution to the therapy of cancer in future.

Acknowledgments

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