P1

The Biological Activity of Ruthenium-PTA Complexes

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Ruthenium compounds are of interest in the clinic due to the highly successful anticancer activity of other platinum group metal compounds, such as cisplatin, and the relatively toxicity of ruthenium compared to platinum itself. Ruthenium-arene organometallic compounds are postulated to interact with DNA targets, although protein interactions have also been shown. These organometallic species are more stable than inorganic metal compounds, which facilitates pharmokinetic studies, thereby increasing the chance of an active compound entering the clinic.

In this study we describe the biological characterisation of complexes in the **R**uthenium-**A**rene-**PTA** (RAPTA) series. The RAPTA complexes have a pH dependent cytotoxicity, such that they are not toxic in normal cells, but become activated in the low pH environment of rapidly dividing cells. In contrast with other pH activated drugs, the mechanism does not involve 'activation-by-reduction', as the Ru metal in the RAPTA compounds is already in the Ru(II) oxidation state. Thus, the mechanism of RAPTA compounds is thought to centre on other events, possibly including ionisation or ligand exchange.

P2

Synthesis of Binary and Ternary Derivatives of Divalent Metal Ions with O-Iodohippuric Acid and Acyclovir

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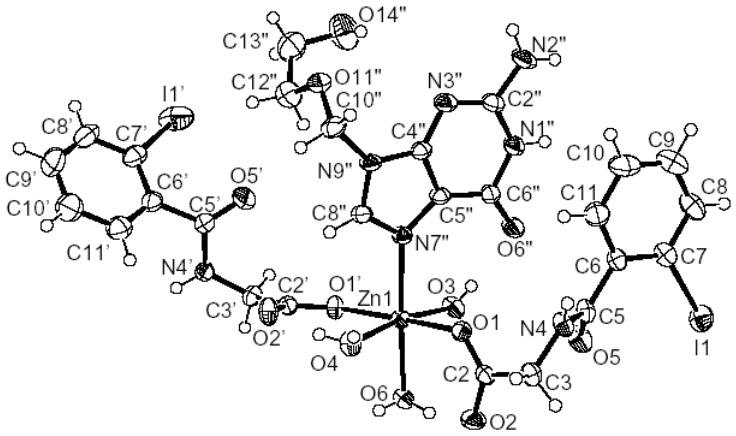
Orto-iodohippuric acid is used as imaging contrast agent [1]. We have explored the synthesis of binary compounds with Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) which are not described in the literature [2]. The obtained results shown that the metal co-ordinates only through the carboxylic group and deprotonation of peptidic bond is not present in any case. On the other hand, the *o*-iodohippurate ligand presents the aromatic ring almost orthogonal to the amide bond contrarily to the total planarity of the system Ph-CO-NH in the hippurate anion. Acyclovir (ACV) ternary complexes with different metal ions have been prepared and the corresponding Zn(II) derivative has been structurally solved by X-ray diffraction studies. Different recognition factors are present in this ternary complex: i) Hydrogen bonds between coordinated water molecules and the organic ligands. ii) "stacking" between aromatic rings (almost parallels with a distance between the planes about 3.5Å).



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P3

What's New from Metal Complexes: the Experience of Linfa Laboratory

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LINFA laboratory was established on 1999 aimed at capitalizing the experience acquired with the studies of the anti-metastasis activity of Ru(III)-dmso complexes, a class of metal complexes to which belongs NAMI-A, the first ruthenium compound to complete a phase I clinical trial. Since the beginning LINFA laboratory received more than 50 metal-based complexes to be tested for their biological and pharmacological properties. First line experiments are carried out on *in vitro* systems and consist of the evaluation of anti-proliferative activity in tumour cell lines, in comparison to a non tumorigenic counterpart, by the MTT test; recently also an endothelial-derived cell line was added in this test to allow the detection of differential activity depending on cellular istiotype. Migratory ability of treated tumour cells is also determined, in this phase of the study, using a transwell chamber, as well as the activity on cell cycle distribution, by flow cytometry after staining with propidium iodide. These effects may be related to the ruthenium uptake by tumour cells, measured by atomic absorption spectroscopy, on whole cells or on macromolecules such as DNA. More advanced experiments are designed to detect the inhibition of tumour cell ability to invade a matrigel barrier, mimicking the extracellular matrix invasion carried out by tumour cells when they metastasise. In parallel, biochemical tests, aimed at analysing the enzymes involved in such degradation, i.e. MMP-2 and MMP-9 gelatinases, are performed. Other advanced researches *in vitro* concern modifications, at a molecular level, of parameters evaluated on the basis of the results obtained in the experiments previously described. After a careful *in vitro* analysis, some compounds may reach the final phase of *in vivo* studies. Experimental models are solid tumours that spontaneously metastasise to the lungs or lymphoproliferative tumours which give CNS metastases: the choice of the best model, to highlight the potential of the compound under examination, is made on the basis of the *in vitro* results. This experimental design is not stiff: some compounds may show properties that make them unsuitable to follow the experiments listed above. Adjustments are currently done under experimentation to explore other pharmacological properties worthy to be studied. Correspondingly modifications of the chemical structure, that ameliorate the terapeutic potential of the compounds and informations about physico-chemical features, are welcome to grow up with a good work into a profitable research.

Acknowledgements: Work done with the contribution of MIUR and supported by grant from LINFA (Laboratorio per Investigare Nuovi Farmaci Antimetastasi). *Metal complexes described in this work are the contribution of many researchers as will be indicated in detail.

P4

Synthesis and Structure of New Ru(II)-dmso Complexes with Dicarboxylate Ligands

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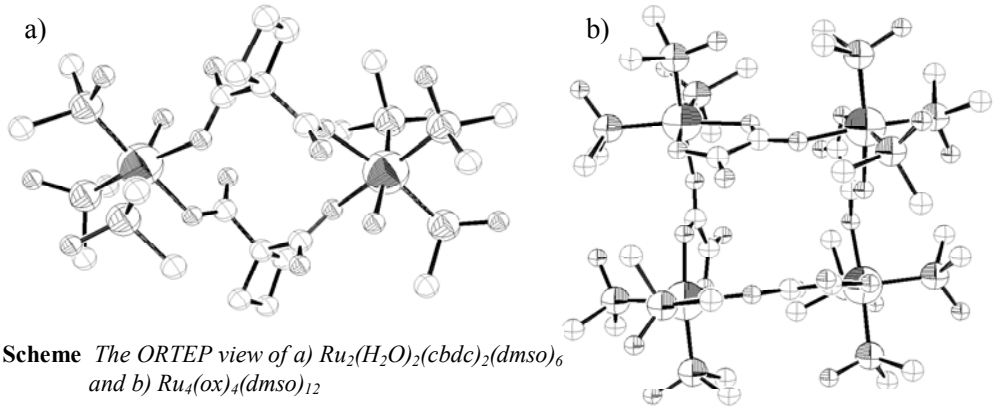
Several works have shown that Ru-dmso complexes have antitumor activity.[1] Although a number of Ru-dmso derivatives with various ligands have been synthesized,[2] there are very few examples with carboxylate ligands[3] and none with dicarboxylates. In attempt to study the properties of such complexes, we have synthesized a series of Ru(II)-dmso complexes containing dicarboxylate ligands, such as 1,1 cyclobutane dicarboxylate (cbdc), malonate (mal) and oxalate (ox).

Treatment of *cis*-$RuCl_2(dmso)_4$ or $[Ru(dmso)_6](OTf)_2$ with the potassium salt of cbdc yielded a very stable dinuclear complex, $Ru_2(H_2O)_2(\mu\text{-cbdc})_2(dmso\text{-}S)_6$ (**1**), bridged by two molecules of cbdc (scheme a). The molecular structure derived from X-ray diffraction study shows the coordinated water molecules involved in intermolecular hydrogen bonding with the cbdc ligands. Similarly, *cis*-$RuCl_2(NH_3)(dmso\text{-}S)_3$ yielded a very similar binuclear complex (**2**), with NH_3 instead of H_2O. Treatment of *cis*-$RuCl_2(dmso)_4$ with $K_2(mal)$ led to the formation of the mononuclear complex $K[RuCl(mal)(dmso\text{-}S)_3]\bullet 2H_2O$ (**3**). Conversely, treatment of *cis*-$RuCl_2(dmso)_4$ with $Ag_2(mal)$ yielded, after removal of the 2 Cl^- ions, a dinuclear complex $Ru_2(H_2O)_2(\mu\text{-mal})_2(dmso\text{-}S)_6$ (**4**) similar to **1**. When $[Ru(dmso)_6](OTf)_2$ was reacted with $K_2(ox)$ a tetranuclear complex $Ru_4(ox)_4(dmso\text{-}S)_{12}$ (**5**) was obtained. In **5** the oxalate acts as a chelating ligand and, at the same time, as a bridge between two Ru ions through one of the carbonyl oxygens (scheme b).



Scheme *The ORTEP view of a) $Ru_2(H_2O)_2(cbdc)_2(dmso)_6$ and b) $Ru_4(ox)_4(dmso)_{12}$*

The hydrolysis and the interaction with imidazole of those complexes have been investigated. The cytotoxic properties will be investigated.

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P5

Thermodynamic Analysis of DNA Containing Monofunctional Adducts of Platinum Compounds

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Anticancer bifunctional platinum(II) compounds bind covalently to DNA forming various types of monofunctional adducts and cross-links. Interactions of these compounds with DNA occur in two steps. The first step includes a formation of monofunctional adducts of the platinum complex preferentially at the N7 position of guanine residues. In this study we focused on platinum compounds, which form monofunctional adducts as the major lesions. These compounds could be also used as models for the first step of bifunctional binding of other platinum compounds.

The effect of platinum monofunctional adducts on thermodynamic stability of 15-mer DNA oligonucleotides was studied by differential scanning and isothermal titration microcalorimetry. Changes in thermodynamic parameters (enthalpy, entropy, free energy) were related to conformational changes induced by these adducts in the host DNA. Biological implications of these results will be discussed.

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P6

In Vitro and *In Vivo* Antiangiogenic Effects of NAMI-A Related to its Nitric Oxide-Scavenger Property

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Introduction: Nitric oxide (NO) has been demonstrated to play a central role in vascular biology and pathobiology and has been implicated as a critical signaling molecule of angiogenesis. During angiogenesis, new blood vessels emerge and grow from preexisting ones through an invasive process that requires proteolysis of the extracellular matrix, migration and proliferation of endothelial cells. Neovascularization is strictly regulated by a balance between stimulatory and inhibitory factors. Disregulation of these control mechanism may lead to abnormal growth of newly generated blood vessels, during pathologic states such as tumor growth. Antiangiogenic therapy is probably one of the most promising strategies to restrict tumor growth and prevent metastasis. The ruthenium complex NAMI-A has been investigated as a possible NO-scavenger in relation to its antiangiogenic activity.

Materials and methods: The generation of NO in culture medium was determined by Griess colorimetric method: DAF-2DA fluorescent probe was used to evaluate NO in living cells. Aortic rings from Wistar rats and Matrigel plugs implanted subcutanously in C57/BL mice were used as *in vivo* angiogenesis assay.

Results: Murine macrophages were used as a model to evaluate NAMI-A interference with NO synthesis. Macrophages were pre-treated 2 hours with 10^{-4}, 10^{-5} and 10^{-6}M NAMI-A and then stimulated with 10 μg/ml LPS in order to increase NO production by iNOS activation. NAMI-A decreased NO production in culture medium at all doses used indicating its negative modulation in NO biosynthesis (10^{-4}M NAMI-A 0,026±0,002, 10^{-5}M 0,029±0,002, 10^{-6}M 0,029±0,001 *vs* controls 0,046±0,003 O.D., $p<0,001$). The EA.hy926 endothelial cell line was used in order to evaluate the effects on a cell hystotype implicated in vascular tube formation during angiogenesis. Cells were treated for 2 hours with 10^{-4}M NAMI-A in combination with an equal concentration of the NO-donor SNAP. NAMI-A significantly decreased NO production in culture medium (10^{-4}M SNAP 0,265±0,004 *vs* SNAP+NAMI-A 0,021±0,002 O.D., $p<0,001$) and in the intracellular compartment (10^{-4}M SNAP 28343,25±550,81 *vs* SNAP+NAMI-A 310,75±142,44 RFU, $p<0,001$). *In vivo,* Matrigel plugs containing $2,4*10^{-4}$M NAMI-A were implanted subcutanously in C57/BL mice. NAMI-A significantly suppressed capillary ingrowth into Matrigel plugs as determined by the reduction of haemoglobin content (0,04±0,02 *vs* controls 0,43±0,09, $p<0,05$). Aortic rings from Wistar rats were treated with $2,4*10^{-4}$M NAMI-A for 6 days. Endothelial cell growth around rings was markedly inhibited after NAMI-A treatment. These data suggest the possibility of NAMI-A to interfere with NO and this effect correlates with the antiangiogenic properties of NAMI-A *in vivo* and contributes to explain its selective and potent antimetastatic effect on solid tumours.

P7

G2/M Premitotic Arrest and Expression of Cell Cycle Regulator Proteins on KB Cells Treated with NAMI-A

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NAMI-A, [ImH] [*trans*-$RuCl_4$(DMSO)Im], is a ruthenium complex endowed with remarkable anti-metastasis effect on solid experimental tumours.

The compound, tested *in vitro* on different tumour cell lines, is not cytotoxic, but it tends to transitory accumulat the treated cells in the G2/M phase of cell cycle. Moreover, it increases β-actin polimerisation and consequently it modifies the structure of cell cytoskeleton. The aim of this study is to unravel the molecular changes responsible for the cell cycle block. The study is conducted on the human oral carcinoma KB cell line, with 0.1 mM NAMI-A for 1 hour in PBS. After treatment cells are washed, to remove the unreacted compound, and cultured in complete medium from 4 to 48 hours to verify the time course of the phenomenon.

At each time point cell cycle analysis is performed by flow cytometry after propidium iodide staining, whereas western blot analysis is used to detect the levels of cell cycle regulation proteins: MPF (cyclin B1, cdk1 and its phosphorilated status), their positive (cdc25C) and negative (wee1, Myt1, P53 and 14-3-3) regulators. Also the steady state levels of cyclin A, cyclin E, cdk2 (that drive microtubule-organizing centrosome duplication in mammalia cells) and nucleophosmin (a centrosomal substrate of cdk2) were investigated. The levels of m-RNAs are determined by RT-PCR, and subcellular localisation of Cyclin B1 is analysed by immunocytochemistry. MCF7 cell line is used as negative control since, in this cell line, NAMI-A is devoid of effects on cell cycle distribution.

Flow cytometry analysis of P.I. stained KB cells shows NAMI-A to cause a statistically significant increase of cells in the G2/M phase starting 12 hours and lasting up to 24 hours after treatment, this effect is completely abolished by 48 hours after treatment.

Protein expression, analysed by immunoblotting techniques, shows the increase of the steady state levels of Cyclin B1 starting from 12 hours and persisting up to 24 hours; this effect is not due to an increased synthesis, as shown by RT-PCR analysis, nor to an incorrect localization of cyclin B1 and cdk1, as detected by immunocytochemistry. In the same experimental conditions the expression of the other proteins tested does not significantly vary.On the contrary the evaluation of the Thr 14 and Tyr 15 phosphorilated status (inactive) of cdk1 revealed an increase starting from 8 hours and persisting up to 16 hours, wherease the total levels of the same protein do not change. Also the m-RNA levels of P21 are increased at 4 and 8 huors after treatment, in line with the time of the G2/M block.

Flow cytometry analysis, conducted on the negative control of MCF7 cell line, does not show any difference between treated and control cells, and the expression of cyclin B1 is not changed. These results show that the interference of NAMI-A with the regulation mechanism of cell cycle progression, causing the transitory accumulation of the treated cells in the G2/M phase, is accompained by the increase of Cyclin B1 level,

presumably due to the reduction of its degradation rather than to increased synthesis of this protein as suggested by RT-PCR analysis.

Further investigations of molecules upstream to cdk1 and on the cell cyle control by cytoskeleton components are in progress.

P8

Characterisation of Ciprofloxacin Binding to the Linear Single and Double Stranded DNA

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Ciprofloxacin is a member of fluoroquinolone antibacterial agents wich prevent the multiplication functions of the cell by inhibiting one of the enzyme reaction phases between the gyrase and DNA. The detailed mechanism of inhibition of the catalytic activity of gyrases still remains unknown. There are many different theories on the mechanism of the activity of fluoroquinolones, but it was confirmed that fluoroquinolones can bind to single stranded, double stranded and super coiled structure of DNA with different binding affinity. The binding affinity of fluoroquinolone to double stranded DNA could be increased by the presence of gyrases, or by the presence of ATP. One of the proposed mechanisms also suggests that fluoroquinolones bind to double stranded DNA in the presence of magnesium(II) ions and therefore cause the destabilisation of the double helix.

To examine the influence of the ionic strength to the affinity of ciprofloxacin to natural and synthetic polymeric DNAs, the binding was investigated at different solvent conditions using a combination of spectroscopic (intrinsic fluorescence emission, UV- and CD-spectroscopy) and hydrodynamic techniques.

Our results show that ciprofloxacin's apparent mode of binding, structure and sequence preferences significantly depend on solution conditions. In 10 mM cacodylate buffer (pH 7.0) containing 108.6 mM Na^+, no sequence preferences in the interaction of ciprofloxacin with DNA was detected, while in 2 mM cacodylate buffer (pH 7.0) containing only 1.7 mM Na^+, a significant binding of ciprofloxacin to natural and synthetic linear double stranded DNA was observed. At low ionic strength of solution, ciprofloxacin binding to DNA duplex containing alternating AT base pairs is accompanied by the largest enhancement in thermal stability and the most pronounced red shift in the position of the maximum of the fluorescence emission spectrum. Similar red shift in the position of λ_{max} is also observed for ciprofloxacin binding to dodecameric duplex containing five successive alternating AT base pairs in the row. On the other hand, ciprofloxacin binding to poly[d(GC)]· poly[d(GC)], calf thymus DNA and dodecameric duplex containing a mixed sequence is accompanied by the largest fluorescence intensity quenching. Addition of NaCl does not completely displace ciprofloxacin bound to DNA, indicating the binding is not entirely electrostatic in origin. The intrinsic viscosity data suggest some degree of ciprofloxacin intercalation into duplex.

P9

Differential *In Vitro* Pro-Adhesive Effect of the Ruthenium Complex NAMI-A in Carcinoma and Neuroblastoma Cells

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Imidazolium *trans*-imidazoledimethylsulfoxidetetrachlororuthenate (NAMI-A) was repeatedly shown to display *in vivo* antimetastatic effect with a mechanism that seems to exclude unspecific DNA interactions. In this work we tested the effects of NAMI-A on the pro-adhesive effects *in vitro* on human tumour cell lines, KB and HeLa carcinomas and IMR-32 neuroblastoma.

NAMI-A increase the ability of KB and IMR-32 cell lines to adhere onto the plastic substrate at 0.001 mM and that of HeLa cell line at 0.01 mM.

The increase in cell adherence with IMR-32 cell line is proportional to the increase of the dose tested, whereas, with KB cell line, the intensity of the pro-adhesive effect is independent on the concentration of NAMI-A used, and is evident immediately after 5 minutes of cell challenge with 0.001 mM NAMI-A exposure, increases up to 48-72 h after NAMI-A withdrawal, and declines only after 96 from treatment. The increase in cell adherence does not involve any modification of β1-integrin chain, at either points: immediately after treatment and at peak (48 h). The ruthenium amount associated with tumour cells after 60 min challenge are rapidly released by the same cells in the medium in the first 5 min after drug withdrawal and washing of the treated cells with fresh medium. The compound released by the treated tumour cells is still able to induce pro-adhesive effects when tested in a *vitro-vitro* bioassay experiment.

These data show the strong ability of NAMI-A (or of its metabolities) to increase cell pro-adhesive properties. Since adhesion is associated to cell motility and invasion, which in turn are associated to tumour malignancy and metastasis, the ability of NAMI-A to reduce solid tumour metastases might be correlated, at least in part, to its pro-adhesive effect.

P10

Ethynylestradiol Derivatives as Carriers for Selective Vehiculation of Cytotoxic Pt(II)-Malonate and Radioactive Tc Fragments Towards Cancer Cells

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Cisplatin, a worldwide approved antitumor drug, lacks of selectivity. In order to improve this drawback of the widely used alkylating cisplatin-like drugs, we synthesized two Pt-complexes anchored to hormones. The anchoring of steroidal skeleton could impart a certain degree of selectivity towards cancer cells, like breast cancer cells, that overexpresses estradiol receptors (ER+).

Ethynylestradiol derivatives functionalised for platinum coordination were obtained according to the Mannich condensation reaction of ethynylestradiol, formaldehyde and 1-methylpiperazine or N,N,N'-trimethylethylenediamine, respectively. CI-MS confirmed the coupling. The hormone-ligands were characterized by means of ^{1}H and ^{13}C NMR spectroscopy.

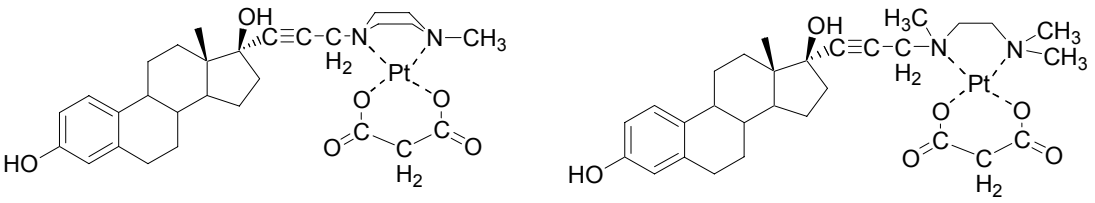
The two ligands were coordinated to platinum(II)-fragments following the Dhara's reaction affording PtI_2-complexes. These complexes are good synthones for further leaving group substitution. Indeed, they exchanged the two iodide ligands with dicarboxylate anion according the following reaction:

$$\text{cis-[EEPtI}_2\text{]} + \text{Ag}_2\text{SO}_4 \xrightarrow{\text{H}_2\text{O}} \text{cis-[PtEE(H}_2\text{O)}_2\text{](SO}_4\text{)} + 2\ \text{AgI}$$

$$\text{cis-[PtEE(H}_2\text{O)}_2\text{](SO}_4\text{)} + \text{Ba(mal)} \xrightarrow{\text{H}_2\text{O}} \text{cis-[PtEE(mal)]} + \text{BaSO}_4$$

EE = *N*-[prop-2-ynyl-3-(17α-estradiol)]-*N'*-methylpiperazine (EE1), *N*-methyl-*N*-[prop-2-ynyl-3-(17α-estradiol)]-*N',N'*-dimethylethylenediamine (EE2)
mal = malonate

The dicarboxylate anion was chosen as the leaving group for the platinum fragment in order to decrease the rate of hydrolysis and to improve water-solubility of the complexes. Thus, we obtained the following stable complexes:



Platinum coordination was checked by ESI-MS in water/DMSO (1:1) for the iodide intermediates and in water/methanol (1:1) for the malonate complexes.

^{1}H, ^{13}C and ^{195}Pt-NMR are reported for these hormone-platinum(II) complexes.

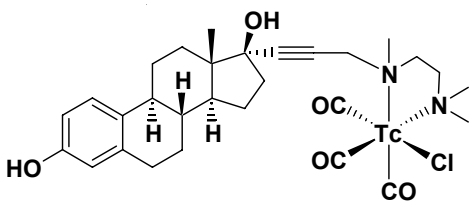
Their biological activity is under investigation with respect to:

(i) aspecific cytotoxicity of the model homologues;

(ii) anti-estrogenic *vs* estrogenic properties and cytotoxicity toward ER+ and ER- cell lines;

(iii) inhibition of the gene-promoter for hTERT component of telomerase.

Beside chemotherapy, early stage diagnosis or monitoring after treatment is a further important issue; EE2 is suitable for ^{99m}Tecnethium coordination to get a complex, *N*-methyl-*N*-[prop-2-ynyl-3-(17α-estradiol)]-*N',N'*-dimethylethylenediamine [$^{99m}Tc(CO)_3Cl$], that can be subjected to biological testing.



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P11

Carboxylation of 2-Hydroxyethyl Substituted Tetrachloro(Ethane-1,2-Diamine)Platinum(IV) Complexes. A New Synthetic Approach to Anticancer Platinum Compounds

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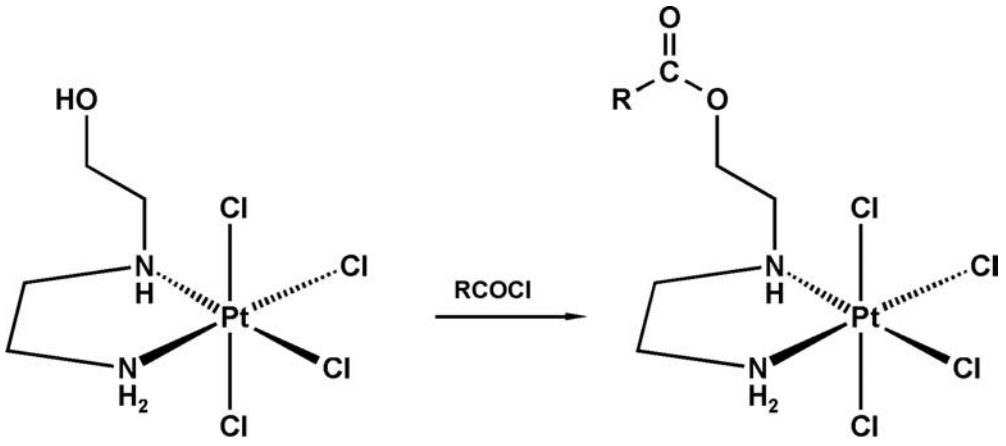
In an attempt to overcome severe limitations of a platinum based chemotherapy, it is desirable to develop new anticancer drugs which show an improved clinical effectiveness and a reduced general toxicity. A class of platinum compounds which could fulfill these requirements are kinetically inert platinum(IV) complexes which display a reduced toxicological profile dependent on their axial ligands. Moreover, such compounds are also suitable for oral administration.

Whereas platinum(II) chemistry is based on ligand exchange reaction, platinum(IV) chemistry made considerable progress through the carboxylation of hydroxide coordinated to the platinum(IV) center. Reduction and release of axial ligands could turn out to be a problem when trying to couple carrier molecules via the coordinated hydroxo group or when important properties such as lipophilicity are depending on the nature of the axial ligands. Therefore, a new strategy was developed for the coupling of carrier molecules to kinetically inert tetrachloroplatinum(IV) complexes taking advantage of peripheral functional groups rather than hydroxo ligands. The typical class of carboxylation reagents widely applied in organic chemistry, the acyl chlorides, were introduced for analogous derivatization of 2-hydroxyethyl-substituted tetrachloro(ethane-1,2-diamine)platinum(IV) complexes. To set up a general reaction procedure and to optimize the reaction conditions, the before mentioned kinetically inert tetrachloroplatinum(IV) compounds and quite simple acyl chlorides have been chosen.



P12

Bis(Aminoalcohol)Dichloroplatinum(II) Complexes and their Singly and Doubly Ring-Closed Alcoholato Species. Novel Prodrugs for Platinum Based Antitumor Chemotherapy

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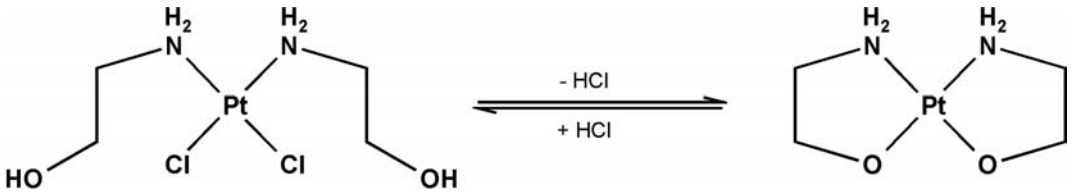
Solid tumors are often hypoxic due to an insufficient blood supply. They draw their energy mainly from glycolysis, which results in production of large amounts of lactate. Therefore, the intracellular pHi and the extracellular pHe are decreasing with increasing tumor size. A high concentration of H^+ outside the tumor cells is a problem for weak base drugs. On the other hand a major breakthrough in chemotherapy could be possible when taking advantage of the acidic pH in many tumors. An interesting class of compounds showing pH dependent behavior are diamineplatinum(II) complexes with *N*-hydroxyalkyl substituents. It was observed by NMR spectroscopy that cis-dichlorobis(2-hydroxyethylamine)platinum(II) and corresponding derivatives undergo intramolecular ligand exchange reactions in aqueous solution resulting in platinum species with one chelating ethanolatoamine ligand. Under more basic conditions, the reaction afforded selectively the double ring closed platinum(II) species.

NMR spectroscopic studies and investigations using CZE-ESI-MS have shown that the binding behavior of *cis*-dichloro*bis*(2-hydroxy-ethylamine)platinum(II) towards 5'-GMP is dramatically increased by decreasing the pH from 7.4 to 6.0. The half life of adduct formation with 5'-GMP was found to be 4.5 h at pH 6 compared to 28.5 h at pH 7.4. This fact is of great interest with regard to the before mentioned lower pH in solid tumors and is in accordance with a pH dependent ring closing/opening reaction of N-hydroxyethyl substituted cis-diaminedichloroplatinum(II) complexes. Fine tuning of important properties such as lipophilicity and reactivity can be driven by selection of the hydroxyalkylamine ligand.



P13

Ruthenium Complexes with Creatinine and Related Ligands

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The selective antimetastatic properties and lack of significant side effects of several ruthenium complexes like NAMI-A [ImH][trans-$Ru^{III}Cl_4$(DMSO-S)(Im)] (Im=imidazole) which is in phase I trials are well known [1]. We have explored the synthesis of Ru(II) and Ru(III) complexes of the type Na[$RuCl_4$(DMSO)L], [$RuCl_3(DMSO)_2$(L)] or HL[$RuCl_4L_2$] where L is a nitrogenated heterocicle related to imidazole, purine or pirimidine ligands. Thus, we have obtained: i) compounds of the type (HL)[$Ru^{II}Cl_3(DMSO)_3$] or (HL)[$Ru^{II}Cl_4(DMSO)_2$] (L = methylisocytosine or creatinine) with X-ray diffraction characterization of (H-creatinine)[$RuCl_3(DMSO)_3$], ii) complexes of the type [$Ru^{III}Cl_4$(DMSO)(HL)] (L = N^6-buthyladenine, nicotine and histamine) obtained in acidic conditions (HCl 0,1N/ethanol), with X-ray diffraction characterization of [$Ru^{III}Cl_4$(DMSO)(N^6-buthyladeninium)]. In addition, Ru(II) compounds, {Ru[$(C_6H_5)_3P$ or $(C_6H_5)_3Sb]_xCl_yL_z$} (L = creatinine) have been also obtained [2]. Preliminary experiments to check the possible cytotoxic activity and other biological properties of these compounds are in progress.

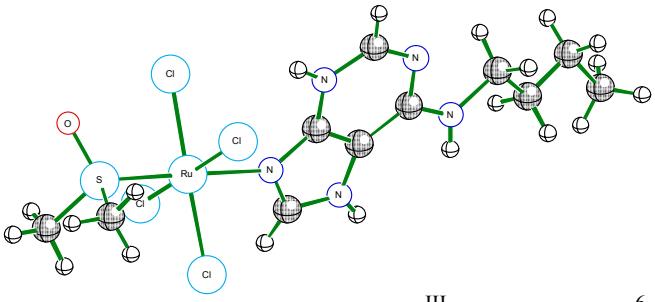

Figure of the molecular structure of the [$Ru^{III}Cl_4$(DMSO)(N^6-buthyladeninium)].

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P14

In Vitro and *In Vivo* Comparisons Between NAMI-A, Cisplatin and Doxorubicin on Biological Markers Involved in the Metastatic Spread

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The development of metastasis is one of the main problem related to the pharmacological treatement of tumors, leading to the failure of antitumor chemotherapy, and it is the major causes of death in tumor bearing patients.

Murine melanoma B16-F10 and B16 cell lines were used to study the antimetastatic activity of compounds *in vitro* and *in vivo* respectively.

In vitro analysis on invasion, adhesion, CD44 expression and MMPs release were performed on B16-F10 cell line treated with (1-100 μM) NAMI-A, (0.5-2 μM) Cisplatin (CDDP) and (20-80 nM) Doxorubicin (DOXO). 24 hrs after sowing cells were treated for 1 hr with compounds in PBS Ca^{2+}-Mg^{2+} saline solution and analysis were performed 24 or 48 hrs after the end of the treatment except for adhesion assay. NAMI-A was able to reduce invasion on transwell chamber at a great extend at the doses of 10 μM and 100 μM (50.1%±5.8; 14.2%±3.1, $p<0.001$ *vs* controls), to reduce MMPs release in dose dependent manner, but no significant modification on the expression of CD44 or β_3 integrin were found. NAMI-A reduced the adhesion of pretreated cells on Matrigel (20 μg/ml) and on fibronectin (10 μg/ml) at all tested doses.

Invasion process was not affected by CDDP or DOXO: only cells treated with 0.5 μM CDDP gave an increased of about 23% *vs* controls and a reduction of invasion was found in cells treated with

2 μM CDDP, but this dose gave also a reduction on cell viability. CDDP and DOXO statistically increased the adhesion of cells at all the tested doses and increased the percentage of CD44 positive cells.

For *in vivo* studies, $0.5x10^6$ B16 tumor cells were implantes *intra*foot pad into B6D2F1 female mice. When primary tumor reached approximately 200 mg (range 150-220 mg) mice were treated intraperitonealy with (35 mg/Kg/day) NAMI-A (pre-surgery treatment), for six consecutive days, with (4 mg/Kg) CDDP at day 1 and with (5 mg/Kg) DOXO at days 1 and 4. Controls received vehicle alone. 24hrs after the end of treatment with NAMI-A, mice were anesthetized and primary tumor was surgically removed proximal to popliteal lymphonode. 24 hrs later another group was treated with NAMI-A (post-surgery) according to the previous schedule. Primary tumor growth was monitored by caliper measurement during the treatment. At surgery cells from primary tumor were processed and CD44, CD54 and β_3 integrin expression was determined by flow cytometer analysis. Lung metastasis evaluation was performed at sacrifice at day 35^{th}. Both kind of treatment with NAMI-A statistically reduced metastasis weight (34.45% ±7.34 and 34.97% ±7.36 respectively pre- and post-surgery, $p<0.05$ *vs* controls). Also in mice treated with DOXO we found a statistical inhibition of metastasis weight (15.70% ±3.20, $p<0.05$ *vs* controls) and a reduction in metastasis number, even if not significant. These data should be correlated to the statistical reduction of primary tumor growth (27.60% ±3.20, $p<0.05$ *vs* controls) in mice treated with DOXO. CDDP treatment didn't reduce neither the number or the weight of lung metastasis. CD44 or CD54 expression on primary tumor cells was not

affected by NAMI-A treatment. On the other hand, after NAMI-A treatment we found a significant reduction of the density of β_3 integrin receptor *per* cell. DOXO gave a statistical increased of CD44 and CD54 positive cell and had no effect on β_3 integrin expression. CDDP gave an increased in CD54 positive cells. MMPs activity detemined by zimography analysis on primary tumor cell lysate and on plasma fraction obtained at sacrifice revealed a reduction in NAMI-A treated mice.

The comparison between *in vivo* and *in vitro* data, obtained with these cell lines, allows us to conclude that NAMI-A is the only compound clearly active on solid tumor metastases and to conclude that the studies on invasion and gelatinases activity seems to be the most useful to identify an antimetastatic compound in comparison to cytotoxic drugs.

P15

Cytotoxicity Studies of Pt(II) Complexes with N-(3-Pyridyl)-2-(4-(Trifluoromethyl)Phenyl)Diazencarboxamide

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Combination drug chemotherapy is often used to overcome inconveniences of cisplatin such as nephrotoxicity and drug resistance. Among others, one form of drug resistance results from the elevated levels of GSH in tumors. Diazenecarboxamides as selective oxidants (for GSH) may be useful in the treatment of certain tumor types [1-4].

We prepared the following platinum (II) complexes with *N*-(3-pyridyl)-2-(4-(trifluoromethyl)phenyl)diazencarboxamide (**L**): *cis*-$[Pt(ipa)_2(\mathbf{L}\text{-}N1)_2](NO_3)_2$, $[Pt(en)(\mathbf{L}\text{-}N1)_2]Cl_2$ and $[PtCl_2(\mathbf{L}\text{-}N1)_2]$. *N*-(3-pyridyl)-2-(4-(trifluoromethyl)phenyl)diazencarboxamide as ligand in the Pt(II) complexes preserves its oxidation properties [5]. The complexes were tested against different human cancer cell lines.

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P16

Some Metal Ion Complexes of the Uridine 5'-Monophosphate Analogue 5-Uracilmethylphosphonate (5Umpa^{2-}) Show in Aqueous Solution an Enhanced Stability in Contrast to Those of Its 6-Isomer (6Umpa^{2-})

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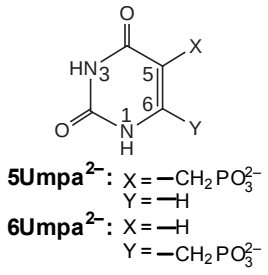
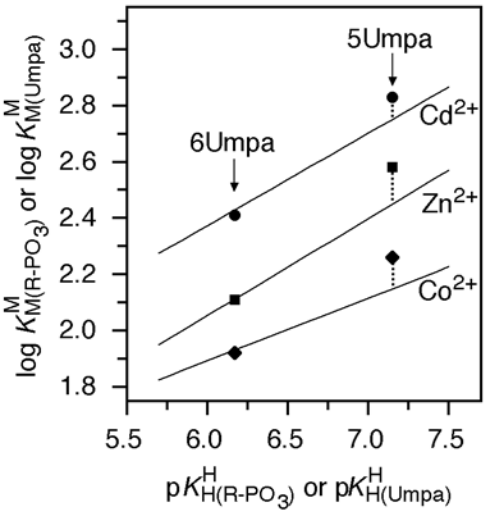
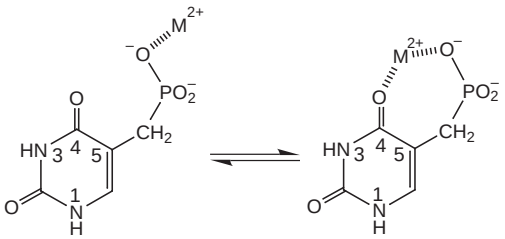
Phosphonate derivatives in combination with nucleobase residues may be considered as mimics of nucleotides; indeed, this type of compounds is synthesized with the aim to develop useful therapeutic agents [1] and receives therefore much attention. The uridine 5'-monophosphate (UMP^{2-}) analogues 5Umpa^{2-} and 6Umpa^{2-} (see Structure) belong in the indicated category of compounds and in fact, in combination with *Cisplatin* they prolong the survival time of mice with lymphoid leukemia [2].



Since the metabolic pathways which involve nucleotides and their analogues also depend in general on the presence of divalent metal ions (M^{2+}) [3], we studied by potentiometric pH titrations in aqueous solutions (25 °C; I = 0.1 M, $NaNO_3$) the stability of several M(5Umpa) and M(6Umpa) complexes with the aim to reveal their structures in solution. With previously established straight-line plots of log $K^{M}_{M(R\text{-}PO_3)}$ *versus* p$K^{H}_{H(R\text{-}PO_3)}$ (where R-PO_3^{2-} represents simple phosphate monoester or phosphonate ligands with a non-interacting residue R) [4] we were able to define the expected stability of these complexes for a pure phosphate-M^{2+} coordination because the acid-base properties of H(5Umpa)$^-$ and H(6Umpa)$^-$ are known [5]. Some results are shown in the Figure: It is evident that the data points for the three M(5Umpa) complexes are above their reference lines whereas those for the M(6Umpa) species fit within the error limits on these lines. In other words, 6Umpa^{2-} coordinates like a simple phosph(on)ate ligand to transition metal ions which means that the uracil residue does not participate in metal ion binding and therefore 6Umpa^{2-} behaves in this respect like its parent compound UMP^{2-} [6]. The increased





stability of the M(5Umpa) complexes means that the phosphonate-coordinated metal ion must interact with a further binding site [7]; for steric reasons this interaction has to occur with the (C4)O unit since this is the only accessible site and consequently 7-membered chelates are formed (see the Equilibrium Scheme). From the stability enhancements of 0.11, 0.13 and 0.08 log units for the Co(5Umpa), Zn(5Umpa) and Cd(5Umpa) complexes (see Figure) follows [7] that the formation degrees of the chelated isomers amount to 22%, 26% and 17%, respectively.

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P17

Binding Properties of Λ- and Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ Enantiomers with the Deoxynucleotide Duplex d(5'-$C_1G_2C_3G_4A_5A_6T_7T_8C_9G_{10}C_{11}G_{12}$-3')$_2$

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The synthesis and characterization of the enantiomeric complexes Λ- and Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$, (GHK = glycine-histidine-lysine) as well as their binding properties to the deoxynucleotide duplex d(5'-CGCGAATTCGCG-3')$_2$ studied by means of NMR, ESI-MS and CD spectroscopy, are reported. The ROESY spectrum of the Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ shows intramolecular crosspeaks between the *bpy* H3 or H3' protons and the aromatic H2 and H5 of the histidine imidazole ring, indicating that the peptide adopts an orientation with the imidazole ring close to *bpy* ligand, possibly interacting by π-stacking. The absence of intramolecular crosspeaks between the peptide and the bipyridine ligands in the NOESY spectrum of Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ on the other hand, shows that in this case the peptide is far from the two *bpy* ligands, having different orientation from the Λ-enantiomer.

The enantiomers interact with the oligonucleotide duplex enantioselectively. The Δ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ binds in the oligonucleotide major groove close to the central part of the sequence, interacting with both the peptide and the bipyridine ligands of the complex. The Λ-[Ru(bpy)$_2$(m-bpy-GHK)]Cl$_2$ binds loosely, approaching the helix from the minor groove. The NMR analysis shows that the peptide (GHK) binding leads the rest of the enantiomer to interact with the oligonucleotide.

P18

Steroids as Delivery Vehicles for Platinum Based Therapies

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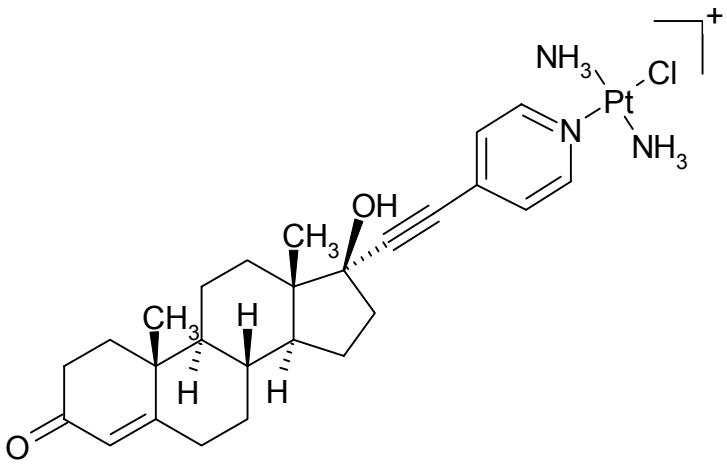
Carboplatin and oxaliplatin, together with cisplatin, are currently licensed worldwide for cancer treatment. However several problems still remain with these platinum based drugs including toxicity, poor localisation, resistance. Moreover only a small range of cancers responds to platinum based therapies. The side effects associated with current metallo-drug treatments make the concept of targeting / localising drugs to tumour site attractive. We are developing a new set of compounds, steroidal metal complexes,[1] combining testosterone and estrogen with platinum centers with the aim of localising delivery to tumours rich in estrogen (ER) and androgen (AR) receptor proteins. Several tumour types including breast, testicular, ovarian and prostrate express high levels of ER and / or AR proteins. By targeting the ER and AR with the steroidal components of our complexes we hope to localise the steroidal metal complex within these types of tumour cells.



Cis- and trans-isomers of our steroidal metal complexes are being developed alongside neutral and positive species in an attempt to investigate structure-function relationships and increase water solubility. The interactions between the steroidal metal complexes and DNA is investigated by atomic force microscopy (AFM), circular (CD) and linear (LD) dichromism and gel electrophoresis and such data will be presented. Initial synthetic and DNA binding results will be shown.

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P19

New Insights in the Mechanism of Resistance in A2780 Human Ovarian Carcinoma Cells with Acquired Resistance to Cisplatin

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In this study, the cellular processing of the fluorescent N,N'-bis(aminoalkyl)-1,4-diaminoanthraquinones and their dinuclear platinum complexes in A2780 human ovarian carcinoma cell line and the derived A2780 cell line with acquired resistance to cisplatin has been monitored by time-lapse fluorescence microscopy. The observations for the two cell lines were compared. The cellular distribution pattern for the free ligands is similar in sensitive and resistant cells, whereas for the platinum complexes significant differences were observed. In cisplatin-resistant cell lines the platinum complexes were found to be sequestrated in acidic vesicles in cytosol from the very beginning of incubation. This was not observed in the case of sensitive cells. Thus, platinum accumulation in vesicles presents a mechanism of resistance to platinum compounds. Elevated levels of glutathione in cisplatin-resistant cell line appear to play a negligible role in this process. Encapsulation of the dinuclear platinum complexes in lysosomal vesicles explains drastically decreased activity of these compounds in resistant cells as compared to sensitive.

P20

Interaction of DNA Modified by Trinuclear Antitumor Compound BBR3464 with Tumor Suppressor Protein p53

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Multinuclear platinum complexes represent a new class of anticancer drugs that are characterized by different DNA binding profile with respect to that of their mononuclear counterparts. BBR3464 has been identified as the most active member of this class of compounds. It is more potent than cisplatin, and retains significant activity in human tumor cells lines and xenografts refractory or poorly responsive to cisplatin.

The molecular mechanisms by which BBR 3464 is able to overcome cisplatin resistance are not understood. However, its ability to form DNA adducts such as long-distance intra- and interstrand crosslinks which are not produced by conventional mononuclear platinum compounds suggest that BBR3464 may escape, at least in part, the classical mechanism of cisplatin resistance related to DNA damage recognition and repair. Moreover, due to its ability to modify DNA in way, which is different from cisplatin, BBR3464 could differently evoke pathways of cellular response to DNA damage such as triggering of the apoptotic pathway.

It has recently been demonstrated that unlike cisplatin, which is generally less active against tumor models carrying a mutated p53 gene, BBR3464 displays high activity in human tumor cell lines characterized by mutant p53 gene, probably as a consequence of its ability to induce p53-independent programmed cell death (apoptosis).

The p53 gene encodes a nuclear phophoprotein that is known to be crucial for the prevention of tumor formation in humans. The ability of p53 to bind to specific, consensus DNA segments and to act as a transcriptional factor in response to genotoxic stresses (including treatment with anticancer platinum drug) is essential for its protective function.

This study was undertaken to examine DNA interaction of active and latent p53 protein with DNA fragments and oligodeoxyribonucleotide duplexes modified by BBR3464 and "classical" cisplatin in cell free medium. It was found that DNA adducts of both cisplatin (1) and BBR3464 reduced binding affinity of the consensus DNA sequence to active p53, but the inhibition was much more effective in the case of BBR3464 compared to cisplatin. The results also demonstrate an increase of the binding affinity of active and latent p53 to DNA lacking the consensus sequence and modified by cisplatin, but not by BBR3464. The results support the hypothesis that differences in biological activities of cisplatin and BBR3464 may also be associated with their different efficiency to affect the binding affinity of platinated DNA to p53 protein.

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P21

Synthesis and DNA Binding Interactions of Supramolecular Cylinders

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A variety of supramolecular cylinders have been prepared by the interaction of metal ions with imine- or azo-based ligands. These imine-based ligands are prepared by mixing a suitable aldehyde with a suitable diamine (Figure 1).

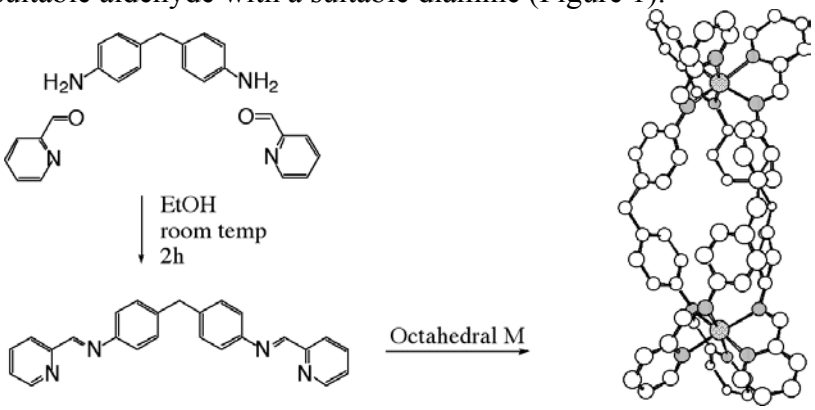

Figure 1. Synthesis of a supramolecular cylinder

The azo-based ligands are prepared in a similar way by mixing an appropriate nitroso-derivative with a diamine. The prepared cylinders bind to the major groove of DNA and by doing this they induce intramolecular coiling in the DNA (Figure 2). By the substitution of the cylinders with different functionalities these DNA binding abilities could be changed. To introduce only one functionality per helicate, helicates with mixed ligands are synthesized. The separation of the different helicates formed by mixing two appropriate ligands is currently under investigation.

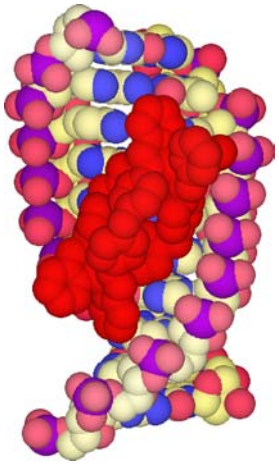

Figure 2. Binding of the cylinder to the major groove of DNA

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P22

Metal Ion Complexes of Uridine (Urd) and Related Ligands Including Uridine 5'-*O*-Thiomonophosphate ($UMPS^{2-}$)

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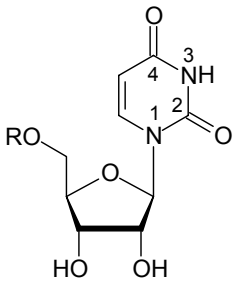
The uracil moiety is an important part of nucleic acids, their components and derivatives. Well known examples are the nucleoside uridine (Urd) and the nucleotide uridine 5'-monophosphate (UMP^{2-}). The thio analogue of the latter, $UMPS^{2-}$, is like other thiophosphate derivatives of interest for biochemical investigations, for example in enzymatic studies, in studies of ribozymes, and in the antisense strategy. Since divalent metal ions (M^{2+}) often participate in biological processes of nucleic acids as well as in those of their components and derivatives [1], we measured the acidity constants of the denoted ligands and the stability constants of their M^{2+} complexes by potentiometric pH titration in aqueous solution (25 °C; I = 0.1 M, $NaNO_3$).



Urd	R = H
UMP^{2-}	R = PO_3^{2-}
$UMPS^{2-}$	R = PO_2S^{2-}

The monoprotonated thiophosphate group in $H(UMPS)^-$ is by about 1.4 pK units more acidic than the phosphate group in $H(UMP)^-$ [2]. Regarding the stability of the 1:1 complexes with most metal ions, *e.g.* Ca^{2+} or Co^{2+}, the thiophosphate group of $UMPS^{2-}$ behaves like a phosphate group of the same basicity, which could be shown by comparison of the measured equilibrium data with log $K^{M}_{M(R\text{-}PO_3)}$ *versus* p$K^{H}_{H(R\text{-}PO_3)}$ straight-line plots [3] (R-PO_3^{2-} = phosphate monoester or phosphonate ligands with a non-coordinating residue R). On the other hand M(UMPS) complexes with highly thiophilic metal ions, like Zn^{2+} or Cd^{2+}, are considerably more stable than the corresponding M(UMP) species.

After deprotonation of its (N3)H site at high pH values M(UMPS) may coordinate a second metal ion to form dinuclear $M_2(UMPS–H)^+$ complexes. Though these studies were hampered in part by the formation of metal ion-hydroxo species, we could measure the stability constants for some $M_2(UMPS–H)^+$ and $M(Urd–H)^+$ complexes. These constants are, within their error limits, identical. For instance, we determined the log stability constants 1.40 ± 0.22 and 3.22 ± 0.22 for the $Mn_2(UMPS–H)^+$ and $Cd_2(UMPS–H)^+$ complexes, respectively [4], and for the corresponding $Mn(Urd–H)^+$ and $Cd(Urd–H)^+$ complexes we measured 1.36 ± 0.05 and 3.15 ± 0.04, respectively [5].

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P23

Drug Targeting Approach in the Synthesis of New Platinum Compounds with Activity Against Bone Tumors

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Cisplatin[1] is probably the most important metal-based drug widely used in tumor therapy, however it suffers for severe side effects and limited spectrum of activity. The aim of the present research was to find analogous platinum compounds with improved antitumor activity but less severe side effects. We have followed the so-called *drug targeting approach*,[2,3] that is bind the platinum to a carrier which will bring the drug only where it is needed, without damaging healthy cells and tissues. In this context, Keppler has already proposed some platinum complexes with aminobisphosphonate ligands. These compounds could be preferentially adsorbed by bones and calcified tissues and therefore be active against bone tumors.[4] Since the phosphonate group is a rather weak donor for platinum, we have planned to link the phosphonate group to a much better donor group for platinum, such as a sulphoxide. The used ligand, diethyl (methylsulphinyl)methylphosphonate (SMP)[5] is a bidentate ligand which should act as S—O donor towards platinum. Moreover the sulphur atom is a stable chiral center, therefore the ligand is obtainable in two enantiomeric forms. We have synthesised and characterized via NMR a series of complexes of general formula PtL_2(SMP), in which L_2 stands for a bidentate (dimethylmalonate, malonate, cyclobutandicarboxylate, cyclohexanediamine and ethylenediamine) or two monodentate (chloride) ligands. The formation of a five-membered chelate ring contributes to the chemical stability of the SMP complexes and bidimensional NMR experiments have allowed determination of the ring puckering.

In preliminary studies, performed in physiological medium, these compounds have shown to have a reasonable stability which is a prerequisite for future pharmacological tests.

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P24

Functionalised Metallo Cylinders: Stability and DNA Interactions

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Metal based drugs, such as cisplatin, bind through metal-nitrogen coordinate bond to DNA and have proved to be among the most effective pharmaceuticals used to treat cancers. Nevertheless challenges remain to be overcome (side effects, toxicity, targeting, delivery and resistance). To circumvent such problems, drugs with different molecular-level actions are required. Natural biomolecule recognition of DNA relies primarily on non-covalent binding, usually via motifs on proteins which slot into the grooves of the DNA double-helix and whose quite large surfaces interface with and recognise the bases within these grooves. Such binding is frequently sequence-specific and the major groove is a particularly attractive target since its size and shape vary more with the base sequence. Inspired by this natural approach we have recently prepared tetracationic supramolecular cylinders (triple-helicates) and shown that they bind non-covalently and strongly to the major groove of DNA and induce DNA binding and intramolecular coiling [1-3].

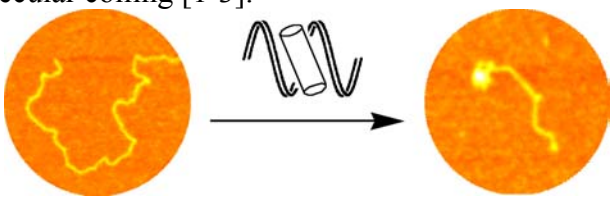

Fig. AFM image of DNA coiling induced by iron(II) cylinder

The cylinders span at least 5 base pairs affording a large surface for potential selective recognition as well as the scaffold for extending the recognition motif. A series of supramolecular cylinders bearing different functional groups on the surface have now been prepared in our laboratory and screened for stability and DNA binding. Results show that modifications of the cylinders cause changes in their binding. Small substituents at the ends of the cylinders lead to retention of major groove binding and DNA coiling. Introduction of very bulky substituents weakens the DNA binding of the cylinders and disables the DNA coiling effects since the cylinders are sterically prevented from binding in the major groove. Substituents especially at the imino carbon can be used to enhance or reduce cylinder stability.

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[2] *Supramolecular chemistry and self-assembly special feature: Intramolecular DNA coiling mediated by metallo-supramolecular cylinders: Differential binding of P and M helical enantiomers* I. Meistermann, V. Moreno, M.J. Prieto, E. Moldrheim, E. Sletten, S. Khalid, P.M. Rodger, J.C. Peberdy, C.J. Isaac, A. Rodger, M.J. Hannon, *Proc. Natl. Acad. Sci.*, 2002, 99, 5069-5074

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P25

Assessing Inter-Nucleot(S)Ide Interactions by Conformational Studies of the Adducts Between Platinum Complexes with Carrier Ligands Unable to Form H-Bonds and Guanine Derivatives

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The complexes phen-PtG_2 and Me_2phen-PtG_2 (phen = 1,10-phenanthroline, Me_2phen = 2,9-dimethyl-1,10-phenanthroline, and G = guanine derivative) represent useful systems for assessing inter-nucleotide interactions without the complications arising from possible hydrogen-bond interactions between the carrier ligand and either the O6 or the phosphate group of the coordinated G's. The latter type of interactions are present in analogous complexes with aliphatic amines widely investigated as models of the major DNA adduct formed by cisplatin.[1,2] The two aromatic diamines used in the present study are characterized by a large in-plane steric bulk which hinders rotation about the Pt-G bonds so that different rotamers (two *Head-to-Tail*, Λ*HT* and Δ*HT*, and one *Head-to-Head*, *HH*) are observable via ^{1}H-NMR. Moreover, since the steric bulk is confined in the platinum coordination plane, the aromatic carrier ligands do not influence the stability of the different rotamers which, in their ground states, have the planes of the guanines orthogonal to the coordination plane.

Noticeable differences have been observed between the phen and Me_2phen complexes. In particular, with respect to the phen species, the Me_2phen complexes exhibit: i) a smaller difference in chemical shift between the two H8 signals of the *HH* rotamer and between the H8 signals of the two *HT* rotamers; ii) an average value of the H8 signals of the *HH* rotamer shifted at higher field and an average value of the H8 signals of the *HT* rotamers shifted at lower field. These observations are in accord with: i) a canting of the guanine bases favouring the leaning of the six-membered ring of each guanine towards the *cis*-guanine (such a conformation ensuring a better dipole/dipole or stacking interaction between the two bases); ii) an average canting of the guanine bases which is greater in the less-hindered phen complex than in the more hindered Me_2phen species.

The effect of the phosphate group upon the stabilization of the various conformers has also been investigated.

The authors thank the University of Bari, the "Ministero dell'Istruzione Università e Ricerca (MIUR)", and the EC (COST Chemistry Projects D20/0001/2000 and D20/0003/01) for financial support.

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P26

Synthesis and Characterization of Novel Multifunctional Platinum(II) Complexes with Dendrimer and Cyclodextrin Ligands

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Following the discovery of the anticancer activity of cisplatin, a large number of analogue complexes has been synthetized and investigated in order to test their binding ability to the natural target, i.e. DNA. Recently, starting from the versatile precursor *trans*-[PtMeCl(DMSO)$_2$], we prepared a series of mono and binuclear organometallic platinum(II) complexes able to interact with a variety of nucleophilic agents,[1] including nucleobases.[2] The diamine-bridged complex [PtMe(DMSO)Cl]$_2$(μ-1,6-diaminehexane) reacts with DNA much faster than analogous coordination compounds, forming mainly monofunctional adducts and minor quantities of interstrand cross-links.[3]

Dendrimers and cationic amphiphilic cyclodextrins (CDs) are currently investigated as potential vectors for gene delivery and could represent novel and "intriguing" ligands for platinum(II). Here we report on the interaction of complexes containing labile dimethylsulfoxide ligands, (*cis*-[PtCl$_2$(DMSO)$_2$)] or *trans*-[PtMeCl(DMSO)$_2$), with polyamidoamine (PAMAM of generation 0, amine terminated). Furthermore a novel organometallic amphiphile was prepared by reacting, under different experimental conditions, the cationic complex [PtMe(phen)DMSO]$^+$ with (heptakis-[2-(ω-amino-oligo ethylene glycol)-6-deoxy-6-hexylthio]-β-cyclodextrin, having seven potential binding sites for platinum(II) moieties. Due to its amphiphilic character, the free ligand is known to self-organize forming vesicles or liposomes.[4]

The synthesis and characterization of the new compounds by MALDI and ESI-MS and by NMR spectroscopy will be discussed.

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P27

Cytotoxicity of Pt Complexes Attached to a Low Molecular Weight Protein Inhibitor of Tumor Cell Growth

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Potato Carboxypeptidase Inhibitor (PCI) is a small molecular weight protein (about 4.000 dalton) with a sequence of 39 amino acids, isolated from potato. PCI is an epidermal growth factor antagonist that inhibits tumor cell growth [1]. This protein is very similar in size, composition and morphology to the Epidermal Growth Factor (EGF) which has a receptor (EGFR). Both proteins are involved in many aspects of the development of carcinomas, including tumor cell growth and metastasis. The amino acid sequence does not contain any methionine and only two histidines are present in the core but not in the periphery of the protein. Six cysteine residues form the three sulphur-sulphur bridges that give the spherical form to the protein. In order to introduce a binding site for a platinum ion, the protein was properly synthesised introducing a terminal methionine in substitution of the terminal glycine in the native protein.

We have followed the binding of cisplatin and other water soluble Pt compounds to the PCI via sulphur atom of methionine, or other possible coordination sites, by HPLC/MS.

The fractions collected allowed us to identify the different adducts formed. Antiproliferative tests were performed in order to compare the citotoxicity of the platinated proteins with that of the the platinum complexes.

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P28

Structure Activity Relationships, Synthesis and Cytotoxic Activity of Metallated and Coordination Complexes from Palladium and Platinum Derivatives with Thiosemicarbazones

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Following the project that our group of research has been developing since many years,[1-2] regarding the coordination and metallation of thiosemicarbazones using palladium and platinum as metals, we describe in this poster presentation the synthesis, structure and properties of TSCN derivatives, selected for their chemical and pharmacological interest. We also evaluate the different coordination sites of the selected TSCNs, comparing their structures and capabilities in order to find the relationship between structure and antitumor activity for continuing investigations in this track.

Tetranuclear orthometallated complexes are the preferential formation for most of the selected TSCNs. Those complexes were the first tetranuclear metallated complexes with TSCNs published in the arts. Binuclear complexes of TSCNs are chloro-bridged complexes where the ligands do not afford orthometallation. Mononuclear complexes, which show analogous structure to cisplatin, are also isolated but no orthometallation has been observed for those mononuclear derivatives.

Structure variations in the TSCN ligands can modulate the coordination and metallation site of the metal affording different derivatives; by contrast electronic effects of different sustituents are not an important factor to change reactivity. Not every derivative shows antitumor activity, and moreover the interaction studies of these complexes against DNA indicate that polinuclear complexes act in a different way to mononuclear complexes.

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P29

DNA Monofunctional Adducts of the Novel Organometallic Ruthenium(II) Antitumor Complexes. Conformation, Recognition by HMG-Domain Proteins and Nucleotide Excision Repair

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Organometallic ruthenium(II) arene complexes of the type [(η^6-arene)Ru(II)(en)Cl][PF_6] (en = ethylenediamine) constitute a relatively new group of anticancer compounds. Modifications of natural DNA in a cell-free medium by antitumor monodentate Ru(II) arene compounds of the general formula [(η^6-arene)Ru(en)Cl]$^+$ (arene = biphenyl, dihydroanthracene, tetrahydroanthracene or *p*-cymene; en = ethylenediamine) were studied using synthetic deoxyribooligonucleotide duplexes containing a single, site-specific monofunctional adduct of these compounds formed at a central guanine residue. Distortions induced in these duplexes by the ruthenium compounds were first characterized by chemical probes of DNA conformation. The results indicate that the distortion extends over 4-6 base pairs around the adduct being most pronounced around the adduct formed by the complex containing *p*-cymene ligand. The structural perturbation due to the monofunctional adducts of all ruthenium compounds tested was also investigated by differential scanning calorimetry. The formation of the monofunctional adducts of all Ru(II) compounds decreases the change of enthalpy of duplex formation. This enthalpic destabilization is partially, but not completely compensated by the entropic adduct induced stabilization of the duplex. The net result of these enthalpic and entropic effects is that the structural perturbation due to formation of the monofunctional adducts of Ru(II) arene compounds induces a decrease in duplex thermodynamic stability with this destabilization being enthalpic in origin. Importantly, the greatest thermodynamic destabilization was observed for the adduct of the complex containing *p*-cymene. In addition, "phasing" assay using electrophoretic retardation as a quantitative measure of the extent of planar curvature of DNA longitudinal axis revealed no bending induced by the adducts of all ruthenium compounds. Consistent with the latter observation were the results of gel-mobility-shift assay. These results demonstrated that the adducts of the ruthenium compounds did not attract HMG-domain proteins that mediate antitumor effects of several platinum compounds. On the other hand, we have observed removal of these adducts from DNA by nucleotide excision repair system *in vitro*. Interestingly, this excision repair was most effective in the removal of the adduct of the Ru(II) arene compound containing *p*-cymene. Thus, further studies aimed at correlating cytotoxicity of Ru(II) arene compounds with their efficiency to destabilize and distort DNA and with the recognition of these distortions and their repair are warranted.

P30

Electrochemical and Biochemical Studies of the Interaction of the Antimetastatic Ruthenium(III) Complex NAMI-A With DNA, Albumins and Telomerase Enzyme

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The interaction of biomolecules with drugs is a major aspect in drug discovery and pharmaceutical development processes. A variety of techniques from molecular biology have been used to study this interaction. However, most of the existing methods require various labeling strategies. In recent years, there has been a growing interest in the electrochemical investigations of interactions between drugs and biomolecules. Electrochemistry offers great advantages over the existing devices based on optical techniques, because it provides rapid, simple and low-cost information on the interaction. The method is based on the observation of the changes in the electrochemical response of the interacting molecules, mainly the decrease of the peak current as a consequence of the lower diffusion coefficient and the shift in the formal electrode potential.

It is commonly believed that the main target for anticancer metal drugs is nucleobases of DNA, mainly guanine N(7). However, a DNA-independent mechanism, such as the inhibition of metalloproteinases (MMP), seems to be responsible for the biological activity of imidazolium *trans*-tetrachlorodimethylsulfoxideimidazoleruthenate complex, $[Ru^{(III)}Cl_4(DMSO)(Im)]^-[ImH^+]$, NAMI-A. Moreover, a number of physico-chemical investigation suggests that NAMI-A is really a *prodrug*, which can be turned to the corresponding Ru(II) reactive species depending on the redox potential of the environment. Finally, it is also interesting to consider the interaction of NAMI-A with plasma proteins, and in particular with serum albumin (by far the most abundant protein in the plasma) as it represents the possible vehicle for the Ru - transport through biological fluids.

Within this frame, we report an application of the electrochemical techniques as a tool for the study of interactions of NAMI-A with DNA, and bovine (BSA) and human (HSA) serum albumin. Moreover, we report also the biochemical studies on the interaction with telomerase enzyme, which activity has been measured using a highly sensitive PCR-based TRAP assay. Telomerase is a specialised ribonucleoprotein that have been shown active in around 85% of human cancers but not in most somatic cells: for this reason telomerase-directed therapy could represent a good target for new anticancer strategies.

P31

Cytosine Deamination to Uracil as Brought about by Pt(II)

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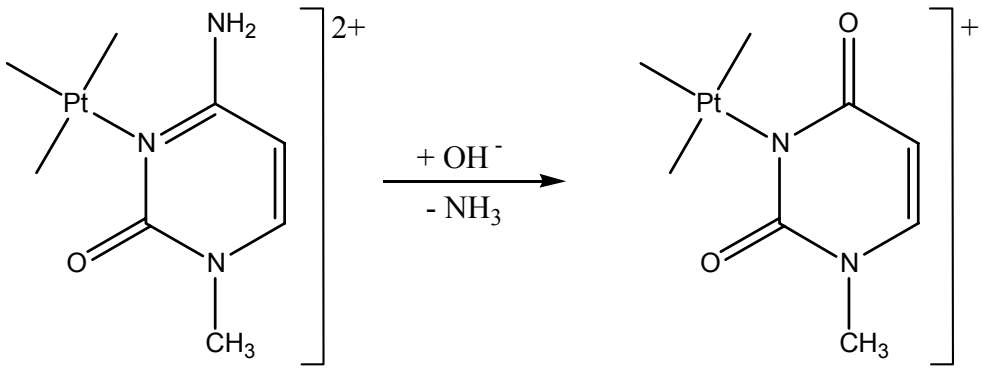
It has been estimated that within 1 day, DNA in every cell will be spontaneously damaged more than 10000 times [1]. Among these insults, hydrolytic deamination of cytosine to uracil as well as of 5-methylcytosine to thymine belong to the most frequent ones. If not repaired, these processes give rise to mutations.

With isolated bases, e.g. 1-methylcytosine (1-MeC), deamination to 1-methyluracil under strongly basic conditions does not occur within a reasonable time frame (several days, pH>12). However, it has previously been observed [2] that *trans*-$[(CH_3NH_2)_2Pt(1,5\text{-DimeC-}N3)]^{2+}$ and *trans*-$[(NH_3)_2Pt(1\text{-MeC-}N3)]^{2+}$ are smoothly converted into the corresponding bis(1-methylthymine) and bis(1-methyluracil) complexes when kept in alkaline solution (pH>12).

Here we demonstrate that $[(dien)Pt(1\text{-MeC-}N3)]^{2+}$ undergoes an analogous reaction to the 1-methyluracilato complex $[(dien)Pt(1\text{-MeU-}N3)]^{+}$.



In parallel reactions we also observe Pt migration from N3 to N4.

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[2] M. Krumm, Ph. D. Thesis, University of Dortmund (1992)

P32

Ternary Complexes of Palladium(II) with Thioether and Imidazole Ligands

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Peptides containing thioether or thiol side chains are generally considered as the primary ligating sites and transporting agents of platinum anticancer drugs under physiological conditions. The thermodynamic equilibrium of multicomponent systems and the biological activity of platinum complexes are, however, connected to the metal ion coordination of the nitrogen donor ligands including guanine and/or imidazole. Ternary palladium(II) complexes are frequently used models to mimic the binding properties of various platinum drugs.

The solution equilibria, formation kinetics and structure of the ternary complexes formed in the reaction of monofunctional palladium(II) species ($[Pd(dien)]^{2+}$, $[Pd(terpy)]^{2+}$, $[Pd(bpma)]^{2+}$ and $[Pd(dipeptideH_{-1})]$) with monodentate thioether (Ac-Met) and imidazole (Ac-His, Ac-Hm) ligands were studied by potentiometric, stopped flow kinetic and ^1H NMR spectroscopic measurements [1-2]. It was found that the coordination of imidazole-N donor atoms results in the highest thermodynamic stability of ternary complexes under physiological conditions, while the thioether complexes were characterised by much faster formation kinetics. On the other hand, both kinetic and thermodynamic parameters of thioether complexes were very much influenced by the other donor functions present in the coordination sphere of palladium(II) ion. The stability constants of thioether complexes were obtained by indirect potentiometric measurements using uridine as competing ligand. The following stability order can be given for the coordination of Ac-Met: $[Pd(dien)]^{2+} > [Pd(GlyGlyH_{-1})] > [Pd(terpy)]^{2+} \sim [Pd(bpma)]^{2+} > [Pd(GlyMetH_{-1})]$, while the rate of substitution reactions was the highest in case of $[Pd(terpy)]^{2+}$ and $[Pd(bpma)]^{2+}$ for both nitrogen and sulfur donor ligands. On the other hand, by changing the monofunctional species from $[Pd(dien)]^{2+}$ to $[Pd(bpma)]^{2+}$ the increasing of rate constants for N-donors is about 40%, while in case of thioether donors it is more than 3 orders of magnitude.

The metal ion coordination of imidazole nitrogen donors is more interesting, becasue both N(1) or N(3) donor atoms can act as a metal binding site. As a consequence, linkage isomers are formed which can be investigated by ^1H NMR spectroscopy. It was found that the ratio of the linkage isomers is very much affected by the tridentate ligands present in the coordination sphere, while in case of Ac-His this ratio also has been affected by the pH of the solution. These findings can be explained by the different steric and electronic effects caused by the tridentate ligands. It also should be mentioned that at higher pH values deprotonation and coordination of pyrrol-type –NH group occur and the system can be characterised by formation of a dinuclear species containing deprotonated imidazolato bridge.

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P33

Cisplatin Binding to DNA Oligomers from Hybrid Car-Parrinello/Molecular Dynamics Simulations

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Structure and binding of cisplatin to DNA in aqueous solution is investigated by QM/MM methodologies based on X-ray structures of platinated DNA (in the free form, cispt-d(CCTCTG*G*TCTCC) -d(GGAGACCAGAGG)[1] (**A**) and in complex with HMG protein domain A, cispt-d(CCUCTCTG*G*ACCTTCC)-d(GGAGAGACCTGGAAGG)[2] (**B**)as well as on molecular docking. In our approach, the platinated moiety is treated at the density functional level and biomolecular frames with the AMBER force field.

During the dynamics, the structure of the platinated DNA dodecamer rearranges significantly towards structural determinants of the solution structure as obtained by NMR. The calculated ^{195}Pt chemical shifts of the QM/MM structure relative to cisplatin in aqueous solution are in qualitative agreement with the experimental data. In contrast, the QM/MM structure of the platinated/DNA HMG complex remains rather similar to the X-ray structure, consistently with its relatively small flexibility.

Docking of $Pt(NH_3)_2^{2+}$ onto DNA in its canonical B-conformation (**C**) causes a large kink (Fig. 1) and a rearrangement of DNA as experimentally observed in the platinated adducts, with NMR chemical shifts in qualitative agreement with the values in aqueous solution. Thus, the QM/MM approach presented here reveals itself as a predictive tool to construct models of platinated DNA.

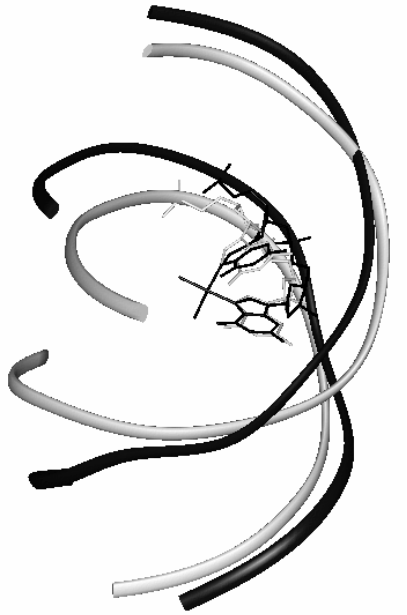

Figure 1: final structures of **A** (light grey) and **C** (black).

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P34

The Antitumor Ruthenium Compound NAMI-A Affects α1 Coupled Mechanisms in Vascular Smooth Muscle In Vivo and In Vitro

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Chronic administration of NAMI-A to CBA mice *in vivo* exert toxic effects on kidneys accompanied by anuria with dilated urinary bladders replenished of urine, suggesting a possible NAMI-A effect on smooth muscle contraction. Considering that micturition is under α-adrenergic control, we investigate the interaction of NAMI-A with the adrenergic system. Experiments are performed on rat aorta rings and short-term effects of NAMI-A on vascular smooth muscle cells are studied. Phenylephrine (PE) (0.01-30μM) cumulative concentration response curves are obtained in the absence and in the presence of NAMI-A. When aorta rings are pre-incubated for 10 min with 0.01, 0.1, 1 and 10μM NAMI-A, we observe an increase of maximal PE contraction. 10 μM NAMI-A also causes a significant shift of the PE curve, with a reduction of EC_{50} from 0.175±0.095 to 0.095±0.039 μM. Conversely, 10μM NAMI-A does not affect either the contraction induced by 5-100 mM KCl, nor the maximal relaxation induced by acetylcholine. The involvement of α1adrenoceptor in mediating NAMI-A effects on smooth muscle cells is suggested by the ability of the drug to reduce the Bmax value of [3H]prazosine bound in renal homogenates from 34 fmol/mg proteins to 24 fmol/mg proteins. Conversely, Kd values do not change dependently of the presence of NAMI-A. The involvement of α_1 adrenoreceptor and its transduction mechanism in mediating NAMI-A effects on smooth muscle cells is further suggested by the ability of the drug to inhibit [^{3}H]-Prazosine and [^{3}H]-IP3 binding by 25% and 42 %,respectively.

In vivo, on the vascular system, NAMI-A, after single *ip* dose (105 mg/kg) and after a multiple administration (17mg/kg/die for six consecutive days) on Winstar rats, causes variations of systolic pressure and of contraction of aorta rings *in vitro*. Systolic pressure increases 1hour after treatment (single dose) and peaks at the third day of treatment (six days treatment). The increase in blood pressure (single dose) is paralleled by significant increase of responsiveness to PE of isolated aorta rings *in vitro* while a significant decrease of contractility to PE is obtained after 6-day treatment *in vitro.* These findings demonstrate that NAMI-A can affect adrenergic response both *in vitro* and *in vivo* witha clear correlation between *in vivo* effects of NAMI-A on blood pressure and *in vitro* responsiveness of α-adrenergic receptors.

P35

The Effect of Carboxylate Group on the Complexation of Amino Acid Derivatives of Bis(Imidazol-2-yl) Group

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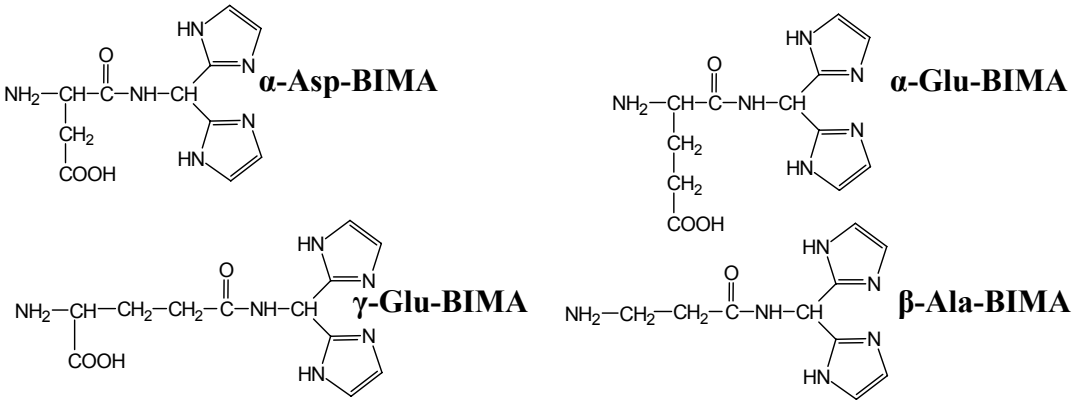
The ligands containing chelating bis(imidazol-2-yl) groups can potentially mimic the active site of metalloenzymes, in which two or more imidazole and/or negatively charged imidazolato groups bind the metal ions. The two imidazole nitrogens of bis(imidazol-2-yl) group forming a six membered chelate are stable binding sites for metal ions studied (Cu(II), Ni(II), Zn(II)). The potential donor atoms of the amino acid chain connected to the bis(imidazol-2-yl) groups are, however, able to change this coordination mode, if the ligand has free terminal amino group in chelatable position with the amide and imidazole nitrogens.

The carboxylate group in the side chain of amino acid has effect on the complexation of ligands. The results of the copper(II) and nickel(II) complexes of four amino acid derivatives containing bis(imidazol-2-yl) group (Figure) are summarized.



The data reveal, that the bis(imidazol-2-yl) group is the main binding site in very acidic solution in all systems studied. The presence of amino acid binding sites on the N-termini of α-Asp-BIMA and γ-Glu-BIMA results in formation of $[Cu_2L_2]$ species via ligand bridging. The terminal amino group, however, behaves as an anchor group inducing the deprotonation and coordination of amide nitrogen in chelatable position and $[Cu_2H_{-2}L_2]$ complexes with imidazole bridge are present in physiological pH. The deprotonation of the imidazole N(1)H group at higher pH leads to formation of di- or polynuclear species with negatively charged imidazolato groups. These complexes could be potential models of superoxide-dismutase enzyme.

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P36

Contribution to the Spectroscopic Studies of Metalloporphyrins Used in Photodynamic Therapy

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Porphyrin derivatives were irradiated with N_2 laser beams due to their strong absorption along a large spectral range, which makes it possible to excite their fluorescence by absorption of radiation at 337.1 nm (the wavelength at which the N_2 laser radiation is emitted). We have chosen to study the behaviour in the irradiated tissues of a group of lypophile TNP type unprotonated porphyrins, which form complexes with metal ions: Mn, Zn, and Cd. From the study of both absorption and fluorescence spectra, it results that, if the excitation is made using a N_2 laser beam, only Zn-porphyrin is suitable for PDT. The obtained data show that the UV radiation emitted by a N_2 pulsed laser may be used for PDT applications; among other dyes having interesting characteristics, Zn-TNP and Zn-TPP in DMSO at 0.5% concentration seem to be more relevant. We have made in vitro irradiation experiments of tumour tissues obtained from different cerebral tumours. The dye impregnation was done immediately after operation.