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POSTER PRESENTATION

Metals in medicine: metal-related diseases

P 20

Lipophilic platinum(II) complexes with dicarboxylates: in vitro antiproliferative activity as well as the mechanism of the suppression of tumour cells growth

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Improving a therapeutic profile of existing platinum(II) complexes, combination of 5,7-diterbutyl-1,2,4-triazolo[1,5-*a*]pyrimidine (dbtp) as N-donor ligand and two types of leaving groups: malonate (mal) **(1)** as well as cyclobutane-1,1-dicarboxylate (CBDC) **(2)** were used. The complexes were characterized in depth by multinuclear magnetic resonance spectroscopy (^{1}H, ^{13}C, ^{15}N, ^{195}Pt) and X-ray. The spectroscopical results indicate that the local geometry around the platinum(II) center approximates a square-planar arrangement with two monodentate nitrogen atom N3 of the dbtp and bidentate dicarboxylate.

To estimate the permeability of [Pt(mal)$(dbtp)_2$] and [Pt(CBDC)$(dbtp)_2$] through the cell membrane, their partition coefficients were calculated using shake-flask method. Both complexes were much more lipophilic (log P = ~2.0) than cisplatin (log P = −1.76) or carboplatin (log P = −1.48). Additionally, the therapeutic potential of the obtained Pt(II) compounds was examined using in vitro cytotoxicity experiments against two human cancer cell lines i.e.: cisplatin-resistance breast (T47D) and lung adenocarcinoma (A549). They exhibited improved cytotoxic activity against T47D cell line, suggesting ability to overcome cisplatin resistance mechanism in that tumour cell line. Furthermore, it ought to be highlighted that presented platinum-based prodrugs, resulted to be over 36-times more active than carboplatin against A549. Promising results encouraged to analyze also the influence of platinum(II) complexes on cell cycle and cell death (subG1) of A549 cell. It was demonstrated that **(1,2)** are capable of arresting the cell cycle at the G0/G_1 phase, whilst cisplatin and carboplatin stopped the cells in G2/M stage, signifying the differences in the mechanism of the suppression of tumour cell growth. Finally, in the quest for low–toxic platinum drugs, the antiproliferative in vitro activity against normal mouse fibroblast cells (Balb/3T3). The title platinum (II) complexes were 1.5-fold less active than cis-DDP, implying that they should be less toxic than the worldwide used cisplatin.

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Visible light-induced annihilation of human tumour cells using novel platinum-porphyrin conjugates

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Despite the extended use of porphyrin complexes in PDT [1,2], tetraplatinated porphyrins have so far not been studied for their anticancer properties upon light irradiation [3]. This is in contrast to ruthenated tetrapyridyl porphyrin complexes [4].

We would like to report about the synthesis of novel tetraplatinated porphyrins as well as their photophysical characterization and in vitro light-induced anticancer properties [5]. The quantum yield of $^{1}O_2$ (Φ) production upon light irradiation was found to be between 0.41 and 0.54. The dark and light toxicity against human cancerous and non-cancerous cell lines (MRC-5, HeLa, A2780 and CP70) was determined by the MTT assay. IC_{50} values were obtained after 4 h incubation, a washing step, followed by 15 min irradiation at either 420 nm (6.95 J cm^{-2}) or 575 nm, (6.23 J cm^{-2}) respectively. These platinum-porphyrin conjugates had only minor dark toxicity, however upon visible light irradiation, IC_{50} values down to 19 ± 4 nM could be observed. These values correspond to an excellent phototoxic index (PI = IC_{50} dark/IC_{50} light) of >5,000. After 4 h incubation in HeLa cells, incubation of a tetraplatinum-porphyrin conjugate led to a concentration of about 105 ng Pt in the nucleus per mg protein in the cell. Strikingly, the use of this conjugate increased the nuclear platinum content by more than 30-fold compared with cisplatin. This is obviously only partially a consequence of the porphyrin conjugate having 4 platinum centers versus one in cisplatin; the main reason being that the conjugate is a more efficient platinum importer into the cell nucleus than cisplatin.

Taken together, all these favourable characteristics imply that tetraplatinated porphyrin complexes may be worth being explored as novel PDT anti-cancer agents in vivo.

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P 22

Water-soluble 1,3,5-triazine platinum(II) complexes as potential candidate of anticancer drugs

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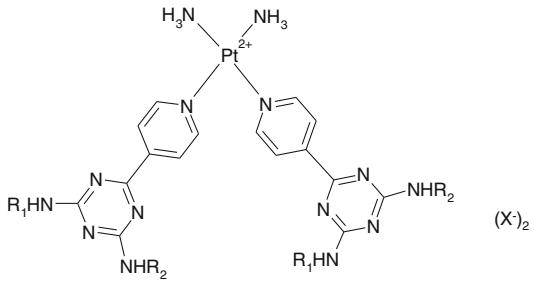
2,4-diamino-1,3,5-triazine and its derivatives have demonstrated anticancer properties [1]. On the other hand, cisplatin, is the first example of anticancer drug, which cross links double-strand DNA and leads to DNA distortion and apoptosis of the tumor cell [2]. Derivatives or other platinum compounds, such as carboplatin, oxaliplatin and AMD473, were later synthesized and developed in order to reduce the side effects of cisplatin [3]. The development of platinum complex as anti-cancer drug goes unrelenting over the years [4].

The water-soluble *cis*-diammine-bis-[2,4-diamino-6-(4-Pyridyl)-1,3,5-triazine]platinum(II) tosylate $[Pt(L_1)_2(NH_3)_2](OTs)_2$ (C1) was synthesized from the ligation of 1,3,5-triazine derivative (L1) with diammine platinum(II) complex (Figure 1). The gel mobility shift assay had been shown a slight decrease in DNA mobility presenting of C1. Besides, the disk diffusion test on ECC agar had been shown C1 had the ability to inhibit the growth of *E. coli*.



X^- = *p*-CH_3-C_6H_4-$SO3^-$ R_1/R_2 = H, or C_6H_5
Ligand (L) 1: R_1=R_2=H; 2: R_1=H, R_2=C_6H_5; 3: R_1=R_2=C_6H_5

Figure 1: Structures of $[Pt(L)_2(NH_3)_2]$ $(OTs)_2$ and its phenyl substituted derivatives.

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P 23

Gold-coated maghemite nanoparticles for magnetic delivery of antitumor platinum(II) complexes

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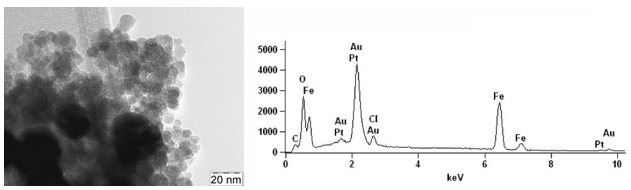
The magnetic nanoparticles represent one of the crucial possibilities of the targeted drug delivery, because their therapeutic application could reduce the chemotherapeutic dose and consequently also negative side effects connected with the drug application. The pharmacologically perspective systems should meet several requirements, such as easy and reproducible preparation with good yields, an effective response to the external magnetic field, high stability and drug release, non-toxicity and significant therapeutic action.

In this work we present the representatives of the maghemite nanoparticles ($\approx$15 nm) functionalized by various cytotoxic platinum(II) complexes. The composites are based on gold-coated maghemite covered by a sulphur-containing carboxylic acid (e.g. thioctic acid) and functionalized by cisplatin or its analogues with different *N*-donor ligands (e.g. 7-azaindoles [1]). The SEM and TEM results showed that the products consist of well-dispersed nanoparticles, while EDS, ICP-MS and XPS proved the presence of the platinum(II) complex within the prepared composites (see figure). The biological experiments researching the stability and drug-release under different conditions as well as in vitro cytotoxicity, were performed or are currently in progress and their results will be discussed within the framework of the presentation.



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P 24

Conjugates of cisplatin and cyclooxygenase inhibitors as potent anti-tumour agents overcoming cisplatin resistance

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Platinum-based anti-tumour therapy is complicated by severe side effects and intrinsic and acquired resistance of tumour cells. Implicated in cisplatin resistance is cyclooxygenase-2 (COX-2), a key enzyme in the biosynthesis of prostaglandins. COX-2 is overexpressed in many tumours and plays a role in tumour initiation and progression [1]. It is also associated with poor outcome in several types of cisplatin-treated cancer [2]. Thus, COX inhibitors are used as chemopreventive and adjuvant chemotherapeutic agents. Clinical studies have shown synergistic effects when COX inhibitors are administered in combination with various anti-tumour agents, such as cisplatin [3]. However, the mechanism by which COX-2 is involved in tumourigenesis is still mainly unknown, and also controversial results have been reported. Prior studies of the influence of COX inhibitors on the efficacy of anti-tumour agents have used combinatorial treatments resulting in potential discrepancies between clinical

and cell culture studies. Due to differential pharmacokinetics, delivery of the drugs to a tumour in vivo may fail to recapitulate administration of the compounds to cells in culture. To address this issue, we report the first covalently linked conjugates of cisplatin with COX inhibitors [4]. Indomethacin or ibuprofen were coordinated at cisplatin as axial ligands, resulting in platinum(IV) complexes. Intracellular reduction allows these conjugates to act as prodrugs and in a dual action mode upon cleavage. The covalent conjugation ensures concerted transport of both drugs into tumour cells and may promote enrichment of the complexes in COX-2-expressing tumours [5]. The platinum(IV) complexes show highly increased cytotoxicity compared to cisplatin and even overcome cisplatin-related resistance in tumour cells. Furthermore, the indomethacin conjugate represents the first highly potent COX-2-selective inhibitor containing a metal centre. Furthermore, these conjugates provide tools for the elucidation of the influence of COX inhibitors on the efficacy of platinum-based anti-tumour agents.

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P 25

Ratiometric delivery of cisplatin and doxorubicin using tumour-targeting carbon-nanotubes entrapping platinum(IV) prodrugs

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Chemoresistance often occur after successive treatment with single agent chemotherapy. Combination therapy, the administration of a cocktail of different anticancer drugs has been employed to overcome chemoresistance and improve the performance of single-agent chemotherapy. However, differences in pharmacokinetic profile of the drugs often complicate treatment regimens. This can be overcome using nanomaterials which allow simultaneous delivery of multiple drugs to the targeted site. In order to deliver the exact stoichiometric amount of cisplatin and doxorubicin, an inert platinum(IV) complex was designed for entrapment in tumor-targeting multiwalled carbon. Upon chemical reduction, equimolar of cisplatin and doxorubicin were released from the hydrophobic carrier thereby achieving synchronous delivery of these two mechanistically complementary drugs.

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On the hydrolysis of Pt(IV) pro-drugs with haloacetato ligands in the axial positions

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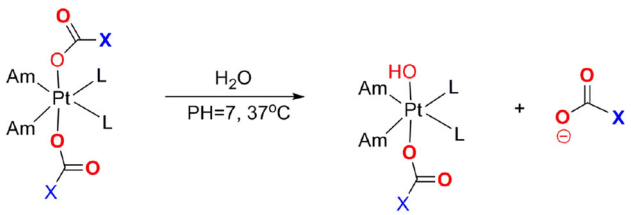
Platinum(II) based drugs are most widely used anticancer agents in chemotherapy. Whilst they are effective, their use is limited by their severe side effects and development of cellular resistance. In attempts to overcome the drawbacks of Pt(II) based drugs, Pt(IV) complexes were designed as prodrugs with the assumption that their high kinetic inertness will minimize undesirable side reactions in the blood but once inside the cancer cell they will be activated by reductive elimination, releasing the cytotoxic square-planar Pt(II) drugs. There were reports that Pt(IV) complexes with either trifluoroaceate (TFA) [1] or dichloroacetate (DCA) [2] axial ligands were potent anticancer agents.

We recently reported that Pt(IV) complexes with either TFA or DCA axial ligands can undergo rapid hydrolysis under physiological conditions [3]. We now report on a systematic study on the hydrolysis of Pt(IV) complexes with haloacetato ligands. Pt(IV) complexes with axial TFA or DCA ligands can undergo hydrolysis and the rates of hydrolysis increase with increasing pH consistent with the Sn1CB mechanism. The half-lives for hydrolysis of the Pt(IV) complexes with two TFA or DCA ligands at pH = 7 and 37 °C range from 6–800 min which is short relative to the duration of cytotoxicity studies that last 24–96 h. With two monochloroacetato (MCA) or acetato axial ligands, hydrolysis is negligible. The rate of hydrolysis depends primarily on the electron withdrawing strength of the axial ligands but also upon the equatorial ligands.



X = CF_3, $CHCl_2$

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Photo-targeted platinum conjugates as selective anticancer drugs

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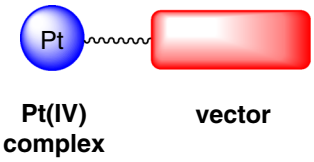
The use of light provides a unique mechanism for triggering drug release from delivery systems or for activating drugs at a desired time and place. In this context, photoactivated Pt(IV) pro-drugs are very attractive compounds since they can be selectively activated by visible light to become highly active species towards a range of cancer cell lines, including cisplatin-resistant cancer cells [1]. Despite the potential of photoactivatable metallodrugs for cancer treatment, the application of the so-called targeted strategies to these complexes can be used to improve their pharmacological properties such as aqueous solubility, cell uptake and tumour selectivity to further optimize their activity and reduce the occurrence of unwanted adverse reactions [2–4]. Here, we report the synthesis, characterization and phototoxicity of new conjugates in which Pt(IV) complexes are covalently bound to tumour-targeting vectors based on peptides or glycosides, either non-modified or caged with photoactivatable protecting groups.



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P 28

Synthesis and characterization of bis-maleimide-functionalized platinum(IV) complexes for tumor-targeted drug delivery

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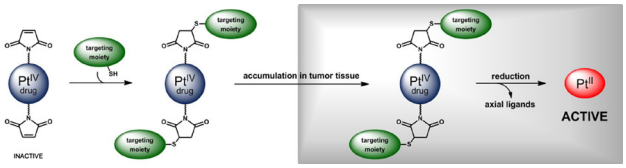
Metal-based cancer therapy using platinum(II) drugs, like cisplatin, is essential in clinical practice. However, therapy is accompanied by severe side effects and ineffectiveness due to development of resistance. Therefore, current research focus is to specifically target the tumor tissue exploiting their unique properties [1]. A well-known strategy is albumin-binding, since albumin is able to accumulate in the tumor tissue due to the enhanced permeability and retention (EPR) effect.

Based on the above-mentioned knowledge, an albumin-targeting, bis-maleimide-functionalized platinum(IV) complex (**1**) was synthesized in this study. As a reference compound, an analogues succinimide-containing platinum(IV) complex (**2**), which lacks the thiol affinity, was prepared. The very fast and quantitative binding of **1** towards simple thiols, like cysteine, was proven by RP-HPLC studies, whereas **2** showed no binding affinity. The binding to albumin was evaluated in detail by different analytical methods. In addition, SEC–ICP–MS measurements using fetal calf serum revealed a high selectivity of **1** for serum albumin. Finally, **1** and **2** were studied in vivo in a syngeneic murine CT-26 colon cancer model. Both compounds showed potent anticancer activity, however with significant higher activity and even disease stabilization for **1**.



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P 29

The development of novel HSP70-1 inhibitor molecules as labile ligands for Pt anticancer compounds

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Colorectal cancer is a major cause of death and disease worldwide. Current treatment options depend on the stage of the cancer but can generally include surgery, radiotherapy and chemotherapy. Oxaliplatin, a platinum (Pt)-based compound for example plays a very important and well documented role in treating colorectal cancer [1]. The cytotoxicity of Pt drugs is attributed to multiple mechanisms but primarily their ability to enter cells, hydrolyse and covalently bind DNA, causing the formation of DNA adducts. These events can lead to DNA damage responses and ultimately programmed cell death, apoptosis. The clinical efficacy of Pt drugs is limited however by drawbacks, such as toxicity, but primarily by the high incidence of chemoresistance (intrinsic or acquired) [2]. Since many colorectal cancers are intrinsically resistant to platinum-based therapies there is an urgent need to develop novel and innovative therapeutic strategies for combating colorectal cancer. The HSP70 family of heat shock proteins are highly conserved molecular chaperones whose expression is increased by cells in response to a variety of cellular stresses. HSP70 is overexpressed in colorectal cancer, amongst other cancers, and is associated with cancer progression, chemotherapy resistance and poor prognosis as it is thought to provide cancer cells with a survival advantage [3]. HSP70 is therefore an exciting and legitimate anti-cancer target. Consequently, we wish to develop novel platinum HSP70 inhibitor drug candidates as potential alternative treatments for colorectal cancer. A summary of a molecular modeling study undertaken to develop novel HSP70 inhibitor molecules as labile ligands for Pt anticancer compounds using Vina and PLANTS will be described.

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Synthesis, characterization and biological evaluation of platinum(II) compounds with carboxy derivatives of boldine

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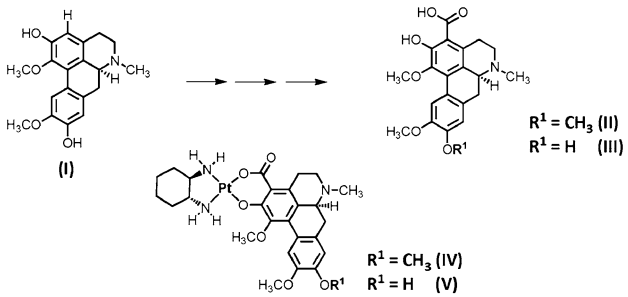
Cisplatin has been employed worldwide during the last three decades for the treatment of different kinds of cancer. To reduce the secondary side effects of this drug, an enormous effort in the design of new drugs has been carried out. The second and third generation analogues, carboplatin and oxaliplatin are now also used worldwide.

Our research group has been focused on the synthesis of new platinum drugs, employing derivatives of the natural product boldine (I), as ligands [1]. Boldine has a powerful antioxidant and cytoprotective activity which was isolated from the bark of the endemic Chilean boldo tree (*Peumus boldus* Molina). Previous work showed a modulating effect on apoptosis induction of ovarian and leukemia tumour cells by some natural products (synergistic in the case of quercetin or anethole), when they were co-administered either with cisplatin or oxaliplatin [2,3]. It was also observed, that the addition of quercetin during cisplatin therapy, reduced the risk of drug induced nephrotoxicity [3]. We are trying to evaluate the effect of using the natural product as a ligand of the platinum analogue.

In vitro cytotoxic assay revealed that compounds IV and V are as potent as the commercial drug oxaliplatin toward three human tumour cells (MCF-7, PC-3 and HT-29) and that their activities are three to four times lower than the analogous commercial drug over the epithelial human colon cell (CCD-841, a non-tumour cell). On the other hand, compounds II and III exhibit no measurable activities (>100 μM) toward all cell lines studied. In order to establish if the coordination of the ligands II and III influence the cytotoxic activity of IV and V on tumour vs non-tumour cells, further studies were developed.



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P 31

Syntheses, characterization and cytotoxicity assays of novel binuclear Cu(II)–Pt(II), and correlated mononuclear copper(II) complexes with oxindolimine ligands

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The design, syntheses and characterization of novel mononuclear copper(II) (species **1** and **2**) and correlated binuclear heterometallic Cu(II)–Pt(II) complexes (species **3** and **4**) acting as DNA-targeting agents are described. In addition to their spectroscopic characterization (UV/Vis, IR, EPR, mass spectrometry), the corresponding formal redox potentials in DMF were determined. Cyclic voltammetry measurements showed that in all cases, one-electron quasi-reversible waves were observed, and ascribed to the formation of corresponding copper (I) species, and to oxindolimine ligand reduction. The Cu(II/I) redox potentials were similar (−0.86 V vs. NHE), except for one of the mononuclear species, which showed a

more negative $E_{1/2}$ value (−1.2 V). The ligand reduction occurred around −1.0 V. Furthermore, all complexes showed characteristic EPR spectral profile and parameters with $g_{\|} > g_{\perp}$ suggesting an axially distorted environment around the copper(II) center [1]. By complementary fluorescence studies, it was shown that glutathione cannot reduce any of these complexes, under our experimental conditions (room temperature, phosphate buffer 50 mM, pH 7.4). Finally, the cytotoxicity of each of these complexes was tested, in comparison with cisplatin (species **5**), towards murine B16F10 melanoma cells, exhibiting low IC_{50} values, in the μM range, after 24 h incubation at 25 °C, as shown in Table below. The obtained results indicate that those complexes can be promising alternative antitumor species.

IC_{50} values (μM)

Complexes	1	2	3	4	5
B16F10 cells (24 h incubation)	1.98 ± 0.18	2.72 ± 1.06	0.63 ± 0.25	1.91 ± 0.20	2.88 ± 0.45

Financial support by FAPESP, NAP-Redoxoma, and CEPID-Redoxoma is gratefully acknowledged.

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P 32

Biological evaluation of light-activatable ruthenium-based anticancer prodrugs

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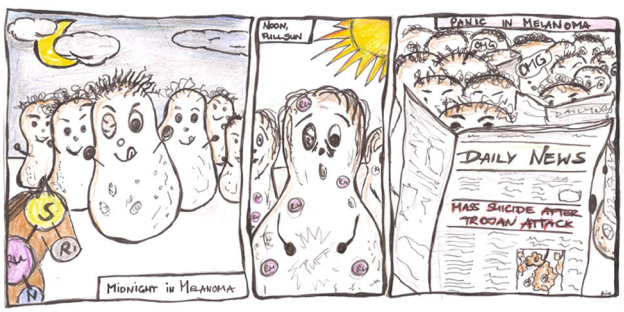
Innovative ideas are necessary to change the *status quo* in anticancer treatment and overcome current limitations e.g. in chemotherapy. One of such promising ideas is the activation of metal-based anticancer prodrugs, also called photo-activatable chemotherapy (PACT). In this approach, a Trojan horse-like prodrug is administered to tumorous tissues followed by light irradiation of the tumor, which generates an active form of the prodrug that selectively kills the irradiated cancer cells.



In this presentation we will show how the cytotoxic $[Ru(tpy)(bpy)(OH)_2]^{2+}$ *complex* can be photochemically released *by irradiation of a prodrug protected by natural sulfur-containing ligands [1].* A light irradiation setup with three different LED sources (455, 528 and 618 nm), a controlled atmosphere, and temperature control, was designed and used for testing the phototoxicity of a series of prodrugs and liposome-supported prodrugs. The concept, experimental pitfalls, and in vitro proof-of-concept data in several human cancer cell lines will be shown and discussed.

Financial support by the European Research Council is gratefully acknowledged.

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P 33

Mixed monodentate tetracarboxylato platinum(IV) complexes featuring a symmetric or unsymmetric coordination sphere

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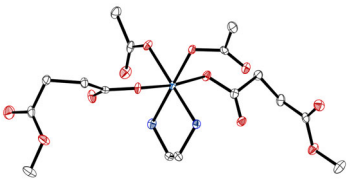
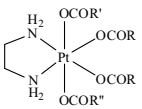
The advantageous pharmacological and chemical properties of platinum(IV) prodrugs turn them into promising candidates for anticancer chemotherapy. They are capable to overcome limitations of currently used platinum(II) cytostatics, which are mainly related to their toxicity and drug resistance mechanisms. In this work, symmetric and unsymmetric platinum(IV) complexes with monodentate carboxylato ligands were developed. In comparison to commonly used bidentate carboxylato ligands in the equatorial position, monodentate ligands are expected to confer an increased reactivity to the platinum(II) species after reduction. The synthesized complexes exhibit a general coordination sphere of $[Pt(en)(OCOR)_2(OCOR')(OCOR'')]$, where the carboxylato ligands are represented by acetato and succinic acid monoester ligands. Platinum(II) complexes have been synthesized according to [1] and symmetrically and unsymmetrically oxidized with corresponding peroxides to obtain platinum(IV) complexes, which were further carboxylated with noncyclic anhydrides. The complexes were investigated by elemental analysis, ESI–MS, FT-IR and multinuclear (^1H, ^{13}C, ^{15}N, ^{195}Pt) NMR spectroscopy as well as by X-ray crystallography in some cases. In addition, cytotoxic properties were evaluated by means of the MTT colorimetric assay in human cancer cell lines.





R = CH_3, $C_4H_7O_2$
R' = CH_3, $C_4H_7O_2$, $C_5H_9O_2$, $C_8H_{15}O_2$
R" = CH_3, $C_4H_7O_2$, $C_5H_9O_2$, $C_8H_{15}O_2$

Reference

1. Rochon FD, Gruia LM (2000) Inorg Chim Acta 306:193–204

P 34

Metal element changes by the effect of cisplatin administration in rats

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Several platinum complexes, such as cisplatin, oxaliplatin, carboplatin are used. Generally serious problem is the excretion of essential metal elements from the body during the treatment, while hardly any data are available on the distribution and metabolism of nonessential elements. Our aim was to study the concentration of both essential and nonessential elements during the treatment with cisplatin. Male Wistar rats (n = 20, 175–190 g) were randomly divided into 2 groups (n = 10/group). The control group received 1 % methyl cellulose at 10 mL/kg body weight, p.o. by gastric gavage twice daily, and cisplatin was injected i.p. at a single dose of 6.5 mg/kg body weight. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used for determination of Al, As, B, Ba, Ca, Co, Cr, Cu, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pt, S, Sb, Si, Sn, Sr, V and Zn concentrations in the plasma, liver and kidney at 14 days after treatment. Total scavenger capacity and diene conjugate content were also determined in the liver. Elevated free radical reactions were observed in the liver of the cisplatin-treated group, although redox balance did not changed significantly. The concentrations of essential elements were decreased in the plasma, kidney and liver, except for Fe, which was elevated in the liver. The concentrations of non-essential elements were also changed, but mainly accumulation could be observed especially for Al, Pb and Pt. According to the results of the study, besides the excretion of essential elements, and the toxic effect of Pt due mainly to induction of free radicals, the side-effects of increased levels of non-essential elements have to be taken into consideration during treatment with cisplatin.

P 35

Diverse cytotoxic behaviour of *cisplatin* and *oxaliplatin*-derived complexes involving kinetin moiety

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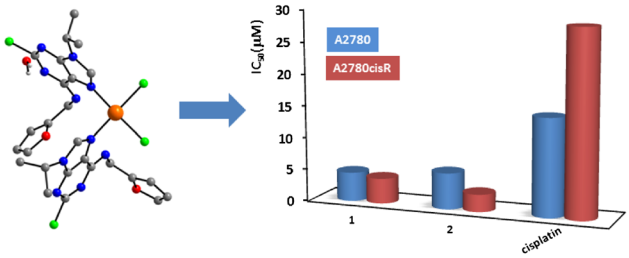
Platinum-containing therapeutics (e.g. *cisplatin*, *oxaliplatin*) have successfully been applied in medicine, however, have also shown undesirable side-effects and their use is connected with resistance of cancer cells [1]. The ongoing research in this field has proven that simple modification in the structure of platinum-based drugs does not guarantee desirable activity of the resulting compounds even in the first panel of testing, in vitro. For significant cytotoxicity, a suitable combination of the leaving and carrier ligands in the studied molecule is crucial.

In this work, *N*-donor carrier ligands (L^n) in the prepared dichlorido and oxalato complexes *cis*-$[PtCl_2(L^n)_2]$ and $[Pt(L^n)_2(ox)]$ were based on a plant hormone kinetin (*N*6-furfuryladenine), which is non-toxic to human cells. Moreover, kinetin has been shown to be a beneficial compound for cells, as it has been applied in medicinal cosmetics ameliorating the signs of aging [2]. The herein reported complexes were fully characterized and screened for their in vitro cytotoxic activity, which identified only the dichlorido complexes as cytotoxic. In order to address the contrasting behaviour of the two groups of complexes, studies of hydrolysis and interactions with sulphur-containing biomolecules were performed. The dichlorido complexes were further tested on a panel of human cancer cells. Notably, the results showed that the complexes are able to circumvent *cisplatin* resistance in A2780cisR ($IC_{50} \approx 3$ μM), while also being more cytotoxic against A2780 and HOS than *cisplatin*, and comparably active against MCF7 and G-361 human cancer cells.



Financial support is gratefully acknowledged (CZ.1.05/2.1.00/03.0058; PrF_2014_009).

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P 36

Synthesis, purification, and characterization of asymmetric Pt(IV) complexes that inhibit the Stat3 signal transduction pathway

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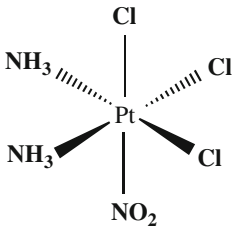
The asymmetric Pt(IV) complex, *fac*-$[PtCl_3(NH_3)_2(NO_2)]$ (CPA-7, Figure 1), has been shown to control tumor cell growth by inhibiting the Stat3 signal transduction pathway [1]. Although an improved synthesis of CPA-7 was reported by Littlefield et al. [2], uncertainty as to the exact nature of the reaction product(s) and the lack of the essential details of its purification and purity warrant a reinvestigation of this system.

We present here (1) an optimized method of synthesis and purification of this complex, (2) its complete characterization by spectroscopic (^1H, ^{15}N & ^{195}Pt-NMR & UV–Vis) and other analytical techniques (ESI–MS, CV) and (3) the results of photo-stability studies in various media.

Additionally, we present a status report on the synthesis and characterization of CPA-7 analogs of the general formula, *fac*-$[PtCl_3A_2(NO_2)]$, wherein A_2 represents two monodentate or one bidentate primary alkyl and aromatic amine by replacing the NH_3 groups in CPA-7. It is anticipated that the synthesis of these analogs will lead to generally enhanced aqueous solubility, potentially

allowing the Pt(IV) analogs of essentially insoluble Pt(II) precursors to be evaluated as Stat3 inhibitors/antitumor agents.



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P 37

Antitumor activity of heterodinuclear ruthenium(II)–platinum(II) complexes as photochemotherapeutic agents

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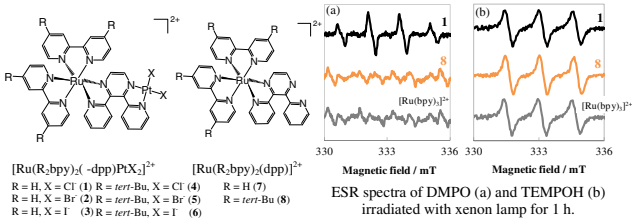
Cisplatin (*cis*-[$PtCl_2(NH_3)_2$]) has been one of the leading anticancer drug for near 30 years. However, cisplatin has several drawbacks such as toxicity and drug resistance. Ru(II)–polypyridine complexes were proposed as potential antitumor substances with non-covalent interactions and available for photodynamic therapy (PDT). In this study, we have synthesized heterodinuclear Ru(II)–Pt(II) (**1**–**6**) and mononuclear Ru(II) (**7** and **8**) complexes, and evaluated DNA photocleavage ability. The interactions of these complexes with DNA have been investigated by spectroscopic (UV–Vis, fluorescence, ESR) and agarose gel electrophoretic methods. In addition, the cytotoxicity of **1**–**8** were also determined using the MTT assay in Hela cell lines. All the complexes can photocleave pBR322 DNA with visible light radiation (xenon lamp, 300 W) through both •OH and $^1O_2$ (**1**–**6**) and $^1O_2$ (**7**, **8**) generation mechanisms [1]. The DNA photocleavage ability of **1**–**6** is higher than that of **7** and **8**. Furthermore, in the series of **1**–**6** DNA photocleavage ability of **1**–**3** (R = H) is higher than that of **4**–**6** (R = *tert*-Bu). **1** and **4** ($X = Cl^-$) can bind covalently to DNA through the dissociation of Cl^- in low Cl^- concentration (0–15 mM). On the other hand, **2**, **3**, **5** and **6** interact with DNA by non-covalent mode. **1**–**6** exhibit higher cytotoxicity compared to **7** and **8**. Moreover, **4**–**6** are found to be more cytotoxic than **1**–**3**, that is, **4**–**6** may be expected to be applied to antitumor drugs that reduce the drawbacks and increase the effects.



ESR spectra of DMPO (a) and TEMPOH (b) irradiated with xenon lamp for 1 h.

Reference

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P 38

Cytotoxicity of palladium(II) complexes with 1,10-phenanthroline derivatives and dithiocarbamate as DNA intercalators

Masashige Fujii[1], Hideaki Furusawa[1], Kana Kondo[1], Naho Iizuka[1], Misaki Nakai[1], Takaji Sato[2], Yoshiki Mino[2], Yasuo Nakabayashi[1]
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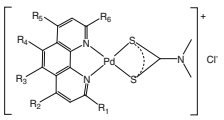
Cisplatin (*cis*-[$PtCl_2(NH_3)_2$]; *cis*Pt) has been one of the leading antitumor drug. However, dose-related nephrotoxicity and a variety of toxic side effects are frequently observed with this drug. In addition, some cancers have been reported to obtain resistance by continuously administering *cis*Pt (*cis*Pt-resistant cancers). Therefore, much attention has been focused on the development of new platinum complexes with decrease of the toxic side effects and effectiveness for *cis*Pt-resistant cancers. In this study, Pd(II) complexes with 1,10-phenanthroline derivatives (R-phen) and dimethyldithiocarbamate (dmdt), [Pd(dmdt)(R-phen)]Cl, were synthesized and the interactions of these complexes with CT-DNA investigated using competitive ethidium bromide (EtBr) studies. On adding the complexes to CT-DNA pretreated with EtBr the fluorescence intensity of CT-DNA-bound EtBr decreased. The apparent DNA binding constants (K_{app}) estimated from relevant fluorescence quenching data were **Pd-1** > **Pd-2** > **Pd-3** > **Pd-4**. Next, the cytotoxic activities of these complexes were determined by MTT assay (IC_{50}). Moreover, the partition coefficients of these complexes (log $P_{o/w}$) were also obtained by 1-octanol/water system. It is estimated that the difference of IC_{50} values are attributed to the hydrophobicity (log $P_{o/w}$). In summary, it is concluded that the cytotoxic activities of these complexes are proportional to the ability of intercalation and hydrophobicity. It is particularly noteworthy that the cytotoxic activity of **Pd-2** was 16 times higher than that of *cis*Pt for *cis*Pt-sensitive L1210 and 110 times higher than that of *cis*Pt for *cis*Pt-resistant L1210.



$R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$ (**Pd-1**)
$R_1 = R_6 = CH_3$ $R_2 = R_3 = R_4 = R_5 = H$ (**Pd-2**)
$R_2 = R_5 = CH_3$ $R_1 = R_3 = R_4 = R_6 = H$ (**Pd-3**)
$R_3 = R_4 = CH_3$ $R_1 = R_2 = R_5 = R_6 = H$ (**Pd-4**)

Complex	$10^{-6}K_{app}$ / M^{-1}	log $P_{o/w}$	IC_{50} / μM		RF[c]
			L1210(0)[a]	L1210(*cis*Pt)[b]	
Pd-1	2.8	-0.5	0.30 ± 0.05	0.28 ± 0.06	0.93
Pd-2	2.4	0.83	0.37 ± 0.02	0.17 ± 0.03	0.49
Pd-3	1.7	-0.27	2.0 ± 0.2	3.0 ± 0.4	1.5
Pd-4	0.93	-0.18	12 ± 1	15 ± 1	1.2
Pt-1[d]	2.7	-0.78	0.53 ± 0.03	0.29 ± 0.13	0.55
*cis*Pt[e]	-	-	4.8 ± 0.3	19 ± 1	4.00

a) *cis*Pt-sensitive L1210. b) *cis*Pt-resistant L1210.
c) RF is defined as the relative ratio of IC_{50} values.
d) [Pt(dmdt)(phen)]
e) Komeda S, *et al.* (2002) J Am Chem Soc 124:4738-4746

P 39

Photoactivatable ruthenium complex for cancer therapy

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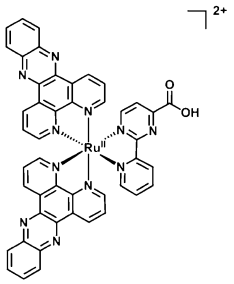
The chemotherapeutical success of platinum-based anticancer agents has driven the exploration of other transition metal complexes as new metallodrugs displaying fewer side- effects. The best examples of these are ruthenium-based compounds. Our work aims at developing a robust mechanism for controlling the anticancer action from a substitutionally-inert polypyridyl Ru(II) complex with very high spatio-temporal resolution with light irradiation.



This bis(dppz)–Ru(II) complex showed cytotoxicity comparable to that of cisplatin, targeted mitochondria, and impaired the mitochondrial membrane potential, leading to apoptosis [1]. Detailed structure–activity relationship analyses on the active Ru(II) complex unraveled the crucial role of the carboxylate group in the cytotoxic activity [2]. This fundamental study underpins the development of an efficient substitutionally-inert metal complex-based prodrug candidate which can selectively respond to activation by light, displaying a significant increase in cytotoxicity against cervical and bone cancer cells upon irradiation with UV-A light (2.58 J cm^{-2}) [3].

Financial support by the Swiss National Science Foundation, the University of Zurich (UZH), the Stiftung für Wissenschaftliche Forschung of the UZH, the Stiftung zur Krebsbekämpfung, the Huggenberger-Bischoff Stiftung and the UZH Priority Program is gratefully acknowledged.

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P 40

Ru(II) polypyridyl complexes showing their potential as novel anticancer PDT agents

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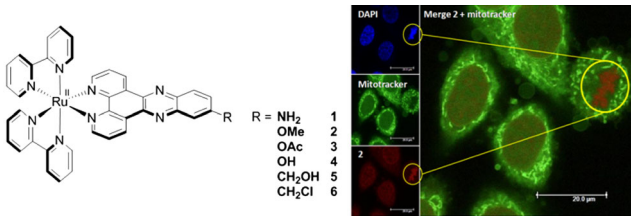
The treatment of cancer cells with a spatial and temporal control is one of the most appealing feature of photodynamic therapy (PDT). This innovative medical technique exploits the synergistic action of light, oxygen and a non-toxic photosensitizer (PS). A lot of interest and research has grown recently around this approach, since side-effects due to the general toxicity of the drugs are minimized. On the other hand, the PSs on the market displayed some other drawbacks, as the prolonged light sensitivity induced in the patient. Here we present an in-depth study in the photochemical and photobiological behaviour of six novel Ru(II) polypyridyl complexes with strong DNA binding affinity. **1** and **2** showed the best phototoxic effect upon irradiation at 420 nm, with a dark/light toxicity ratio of 150 and 40 respectively. The two complexes exhibited high cellular uptake, together with an outstanding nuclear accumulation (as confirmed by microscopy and AAS studies). Furthermore, the ability to photocleave DNA at concentrations comparable with the IC_{50} values upon irradiation and the very efficient binding to DNA via intercalation ($K_b \sim 10^{-6}$–10^{-7} M^{-1}) suggested the involvement of DNA in the mechanism of phototoxic action of these compounds.



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P 41

Synthesis and fluorescent properties of arene–ruthenium(II) complexes

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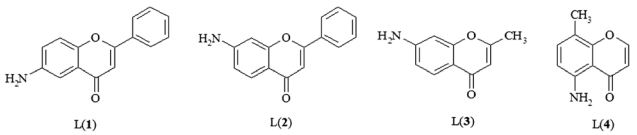
[2]Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz, Nicholaus Copernicus University in Torun, M. Sklodowskiej-Curie 9, 85-094 Bydgoszcz, Poland

In this study we designed and synthesized a series of novel half-sandwich organoruthenium(II) complexes with the general formula $[(\eta^6\text{-arene})RuLCl_2]$ (where L = aminoflavone or amino-methylchromone derivatives and η^6-arene = *p*-cymene, benzene, hexamethylbenzene or mesitylene). The complexes were fully characterized by elemental analysis, MS, UV–Vis, IR and NMR spectroscopy.



The fluorescent properties of ligands and their complexes were examined in series of solvents such as: nonpolar (chloroform), polar aprotic (acetonitrile, DMSO, DMF) and polar protic (ethanol, methanol, glycerol and water). The behavior of complexes in solvents with different polarity and viscosity was investigated in details by total fluorescence spectroscopy (excitation-emission contour maps and 3-D spectra) as well as by synchronous fluorescence spectroscopy SFS (at different wavelength intervals). Ligands exhibit blue fluorescence, whereas for their complexes with organoruthenium(II) blue to green fluorescence was observed with Stokes' shifts in range 40–150 nm. The fluorescence excitation and emission spectra demonstrated significant solvatochromism of all the compounds. Additionally for some complexes excitation-wavelength dependent emission was observed. To describe the effect of polarity of solvents the Lippert-Mataga plot (Stokes' shift vs. polarizability of the solvent) was used.

Financial support from Medical University of Lodz (grant No 503/3-066-02/503-01 to E. Budzisz), grants No 502-03/3-066-02/502-34-025 and 503/3-066-02/503-06-300 to A. Pastuszko.

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Tuning the in vitro cell cytotoxicity of dinuclear arene ruthenium trithiolato complexes: influence of the arene ligand

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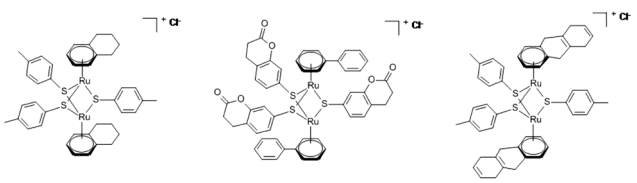
We recently synthesized thiophenolato-bridged *p*-cymene ruthenium complexes of the type $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)_2Ru_2(SR)_3]^+$, which are highly cytotoxic against human ovarian cancer cells, the IC_{50} values being in the nanomolar range [1–3]. Their exact mechanism of action is still unclear, although the cytotoxicity of these complexes could be to a certain extent correlated to the lipophilicity of the corresponding thiophenol ligands [2–3].

In this contribution, the influence of the arene on the in vitro cytotoxicity is investigated. For this purpose, a new series of eight thiolato-bridged arene ruthenium complexes bearing 4-methylthiophenolato or 4-methylcoumarin-7-mercapto bridges and biphenyl, 5,8,9,10-tetrahydroanthracene, indane, and 1,2,3,4-tetrahydronaphtalene as arene ligands have been synthesized and studied for their in vitro cytotoxicity.



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Noncovalent DNA binding and nuclease activity of mixed-ligand ruthenium(II) complexes

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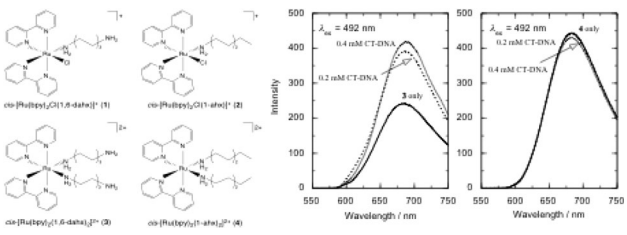
Metal complexes are ideal templates for the design of DNA-interactive systems. In addition to a variety of binding modes, metal complexes that reversibly bind to DNA are becoming of increasing interest. In this study, to design novel anticancer drugs, two type ruthenium(II) complexes *cis*-$[Ru(bpy)_2ClL]^+$ and *cis*-$[Ru(bpy)_2L_2]^{2+}$ (bpy = 2,2′-bipyridine, L = 1,6-diaminohexane (1,6-dahx), 1-aminohexane (1-ahx)) were synthesized, and the interactions of these complexes with DNA were experimentally explored. The ligand 1,6-dahx possesses the potential to form hydrogen-bonding with suitable DNA functionalities, which would be expected to enhance the DNA binding affinity significantly. Moreover, the hydrogen-bonding of 1,6-dahx with the intrastrand nucleobases may exhibit high levels of DNA sequence-specific recognition. The emission spectra have been used to probe the interaction of the present ruthenium(II) complexes with calf thymus DNA (CT-DNA) and artificial DNA ($[poly(dG\text{-}dC)]_2$ (**GC**), $[poly(dA\text{-}dT)]_2$ (**AT**)). The emission intensity decreased on addition of **GC** to **1** and **2**, indicating that **1** and **2** covalently bind at guanine residues. In contrast, the enhancement of emission intensity was observed on addition of **AT** to **1** and **2**. Hence, both **1** and **2** possess no specificity for base-pair binding. The emission intensity increased by factors of 8.7 and 1.3 on addition of **AT** to **3** and **4**, respectively. In addition, the binding of **3** to **AT** led to a blue shift of the emission maximum ($\Delta\lambda = 49$ nm). These findings clearly indicate that **3** possesses the specificity for AT base-pair binding. In the AT-rich regions of the minor groove, **3** is stabilized by hydrogen-bonding to the N3 atoms of adenine and/or O2 atoms of thymine residues. The emission intensity showed about 1.7 times enhancement at a [CT-DNA]/[**3**] ration of 20:1, whereas the emission spectra of **4** were little affected by the addition of CT-DNA. The chemical nuclease activity in the presence of H_2O_2 follows the order: $\mathbf{1} > \mathbf{2} \approx \mathbf{3} > \mathbf{4}$. **1** and **3** possessed hydrogen-bonding ability show more efficient cleavage activity.



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Studies on anticancer properties of molecularly targeted multifunctional ruthenium(II) and iridium(III) complexes

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Cisplatin is one of the most effective anticancer drugs in clinic. However, the applications of platinum-based anticancer agents are hindered by their intrinsic and acquired drug resistance, side effects, and limited spectrum of activity. Recently, other transition metal complexes, such as $Ru^{III/II}$ and Ir^{III} complexes, show great potential as alternatives to platinum-based drugs for anticancer therapy [1].

The major focus of our research is on the rational design of molecularly targeted multifunctional metallo-anticancer agents. Most of the traditional anticancer drugs exert their activities by causing DNA damage or disturbances in mitotic apparatus. Molecularly targeted anticancer strategies offer tremendous hope for greater anticancer activity, fewer side effects as well as personalized therapy by inhibiting or activating cancer-specific biomolecules. On the other hand, as compared with organic molecules, metal complexes are endowed with many unique features, including photophysical, photochemical, redox and magnetic properties, which can be utilized to construct multifunctional platforms for cancer therapy.

The molecular targets that we investigate mainly include histone deacetylases (HDACs) [2], cyclin-dependent kinases (CDKs) [3–5] and the mammalian target of rapamycin (mTOR). The structural–activities relationships for inhibition of the targets by ruthenium(II) and iridium(III) complexes are studied at the molecular level. Additionally, the information on the mechanism of cell death induced by these complexes is investigated in detail [2–8].

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Oxicams as bioactive ligand systems in anticancer Ru^{II}(*p*-cymene) complexes

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Bioorganometallic chemistry provides an excellent platform to incorporate drug-like properties in molecules. In recent years, metal arene complexes have been extensively studies for the design of metallodrugs. The most successful examples are RAPTA-C and RM175 [1]. Despite small difference in their structures, both exhibited contrasting biological activity and modes of action. RM175 displayed in vitro anticancer activity similar to that of cisplatin; while RAPTA-C inhibits metastasis in vivo. As evident from X-ray crystallographic experiments, RM175 prefers to bind to DNA while RAPTA-C has high affinity to histone proteins of the nucleosome core particle [2]. This demonstrates that a small structural variation can dramatically alter the reactivity of compounds with cellular targets and hence alter their biological activity. Coordination of a biologically active ligand to a metal center may result in enhanced activity due to synergetic effects. The use of ligands with anti-inflammatory properties may improve the bioactivity of the Ru arene scaffold. As oxicams are known for their anti-inflammatory properties, herein we report the synthesis and characterization of Ru^{II}(*p*-cymene) complexes of oxicam-derived ligands. Their hydrolytic stability, reactivity with biomolecules and in vitro antiproliferative activity will be discussed.

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P 46

Polynuclear Rh(III), Ir(III) and Ru(II) organometallic complexes: synthesis and biological evaluation as anticancer agents

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Cancer is one of the major diseases of the modern world. Traditional chemotherapeutic approaches have experienced an increasing rise in resistance, thus new strategies to target resistance are being explored. The discovery of cisplatin by Rosenberg and the work by others have pioneered medicinal chemistry and have paved the way in drug design to offer compounds with a broad spectrum of biological activities. Recently, particularly promising anticancer activities have been shown by Rh(III), Ir(III) [1] and Ru(II) complexes [2], with polynuclear complexes showing increased potencies over analogous mononuclear complexes. Recently, a series of polynuclear polyester complexes, based on monodentate ligands, were synthesized in our group that exhibited moderate anticancer activities, in ovarian cancer cells [3].

This presentation reports on the preparation and spectroscopic characterization of new monomeric and trimeric Rh(III), Ir(III) and Ru(II) polyester complexes, Figure 1, based on bidentate ligands. Several model mononuclear analogues have been prepared and their structures determined by single crystal X-ray diffraction analysis. The in vitro cytotoxicities of the ligands and complexes were established on A2780 and A2780cisR, human ovarian carcinoma cells, and human non-tumorous skin cells, KMST-6.

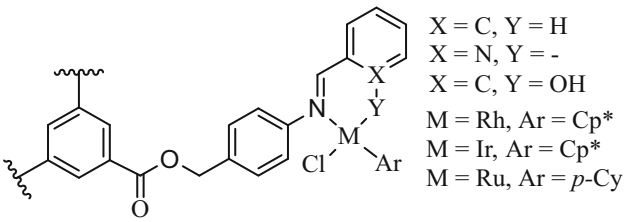

Figure 1 Polyester complexes

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A new complex of Ru(III) with N-(2-pyridyl)salicylideneimine: DNA binding properties and activity against *Staphylococcus aureus*

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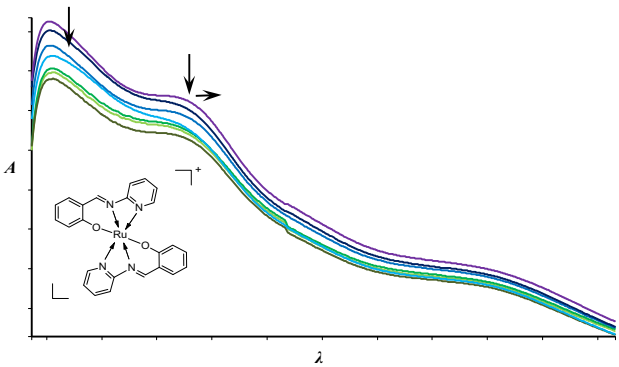
The design and study of new drugs is permanently huge challenge for at least two reasons: (i) the cancer has not been defeated (ii) bacteria strains rapidly develop resistance to existing drugs. Primarily due to the ability of Ru(III) complex to bind DNA as one of key targets, new complexes of ruthenium are subject of growing interest. We present here the ability of a new complex, bis[*N*-(2-pyridyl)salicylideneimine-*ONN*]ruthenium(III) chloride, to bind CT DNA and in vitro activity against *Staphylococcus Aureus (*MRSA). The complex was characterized based on mass spectrometry, infrared and electronic spectra, cyclic voltammetry. Spectrophotometric titration of Ru(III) complex with increasing concentration of CT DNA at 287 nm showed 3 nm red shift and decrease of absorption indicating an intercalative mode of binding ($K_b = 3.61 \times 10^4\ M^{-1}$) as the most probable. Titration of DNA with increasing concentration of Ru(III) compound at 260 nm resulted in close value of constant binding ($K_b = 3.90 \times 10^4\ M^{-1}$). Ru(III) compound has shown significant activity against community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), hospital acquired methicillin-resistant *Staphylococcus aureus* (HA-MRSA), methicillin-sensitive *Staphylococcus aureus* (MSSA) reaching about 76 % of vancomycin activity, the reference antibiotic.



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Synergistic effect of nitric oxide and singlet oxygen originated from light irradiation on nitrosyl ruthenium complex as potentiation for photodynamic therapy. Kinetic, photobiological and cytotoxicity studies

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Nitric oxide (NO) is an important biological messenger. It has been implicated in many physiological processes, including cardiovascular control, neuronal signaling, defense against microorganism and tumors. It can trigger pro- and antitumor responses depending on the concentration of this molecule (NO) in biological system. The antitumor effect is pronounced when there are high levels of NO in tumor cells, which make a challenge develop compounds that can deliver NO under external stimulation. This work describes synthesis, kinetic, photochemical and photobiological studies of some nitrosyl ruthenium complex type $[Ru(phthalocyanine\text{-}R)NO]^{n+}$ and $[RuL_5NO]$—antibody used as NO deliver system. Cell viability decreased to 12 % for Ru–NO–IgG compound while aqueous $[RuL_5NO]^{2+}$ was found 85 %. It may be related to the NO release in an appropriate target to kill cancer cell. Apoptosis was described as the main biological mechanism for the nitrosyl ruthenium complex. It is also being performed in our laboratories a chemical investigation on phthalocyanine ruthenium compounds as nitric oxide releasers. One of this complex is $[Ru(pc\text{-}R)NO(NO_2)]$ (I) (where pc-R = phthalocyanine-$(COO)_n$) as putative system to improve photodynamic therapy (PDT). Once phthalocyanine compounds are widely used in PDT due to their capacity of singlet oxygen generation, cytotoxicity assays against cancer cell lines were evaluated as well. The compound was able to inhibit cellular viability when irradiated at 660 nm, compared with the treatment without photo stimulus. The cytotoxicity is depend on the charge o the ruthenium complex. Similar studies was also conduced with Quantum dot coupled to nitrosyl ruthenium (QD-Ru) system. The generated QD-Ru was able to produce NO by photoinduced eletron transfer as well singlet oxygen by energy transfer. The cell viability were found between 10–25 % with 5 J/cm of potency in ligh irradiation. The cell death is mainly attributed to the apoptosis mechanism. The initial studies suggested us the NO production increases the sensitivity of the cells to singlet oxygen. The synergistic effect of NO and $^1O_2$ may improve Photodynamic Therapy.

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Interaction of ruthenium(II) polypyridyl complexes with some sulfur- and nitrogen-donor ligands

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In the last few decades, a large interest has grown in ruthenium polypyridyl complexes as a possible alternative to the use of classical platinum chemotherapy. We have recently developed a series of new, water-soluble, monofunctional Ru(II) complexes with meridional geometry of the general formula *mer*-$[Ru(L_3)(N\text{–}N)X][Y]_n$, (where L_3 = 2,2′:6′,2″-terpyridine (tpy) or 4′-chloro-2,2′:6′,2″-terpyridine (Cl-tpy); *N–N* = 1,2-diaminoethane (en), 1,2-diaminocyclohexane (dach) or 2,2′-bipyridine (bpy); X = Cl or dmso-*S*; Y = Cl, PF_6 or CF_3SO_3; n = 1 or 2, depending on the nature of X) were synthesized. With the aim of gaining insight into the possible interactions between S-containing amino acids and N-containing heterocycles with Ru(II) complexes, we have studied the ligand substitution reactions of two Ru-tpy complexes, [Ru(Cl-tpy)(en)Cl]Cl **(1)** and [Ru(Cl-tpy)(-dach)Cl]Cl **(2)** with S-containing ligands such as thiourea (Tu), L-cysteine (L-Cys) and L-methionine (L-Met), and with N-containing ligands such as pyrazole (Pz), 1,2,4-triazole (Tz) and pyridine (Py). The kinetics and thermodynamics of the investigated substitution reactions were established quantitatively by UV–Vis spectrophotometry and NMR spectroscopy. The kinetic studies were supported also by theoretical calculations.

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P 50

Investigations on the cytotoxicity, cellular uptake and thioredoxin reductase inhibition of gold(I) alkynyl complexes

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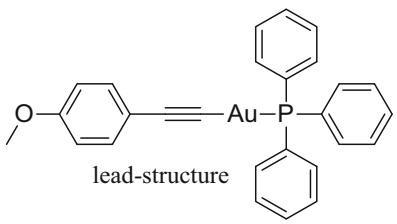
Besides the well-known use in catalysis, gold(I) complexes are also known for their luminescent properties as wells as for their potential as cytotoxic agents [1–3]. Recent work of our group has also shown that compounds out of a series of gold(I) alkynyl phosphane complexes showed anti-angiogenic properties in zebrafish embryos and inhibited thioredoxin reductase, a selenocysteine containing enzyme that is crucial for cell proliferation, in the low-nanomolar range. Additionally these complexes were cytotoxic against MCF-7 breast cancer and HT-29 colon carcinoma cells making them a very promising substance class for tumor therapy [4].

However, a pitfall of this type of organometallics is its poor solubility in aqueous medium caused by the lipophilic triphenylphosphane ligand (see figure). This work presents a new series of novel gold(I) alkynyl phosphane complexes, for which we used the most promising alkynyl ligand of the former work, and modified the phosphane ligand to improve solubility.

After successful synthesis the novel gold(I) alkynyl complexes were tested for their antiproliferative properties using HT-29 adenocarcinoma cells and MDA-MBA-231 breast cancer cells. Additional experiments that were carried out with these compounds were the determination of the cellular uptake into HT-29 colon carcinoma cells using high-resolution continuum source AAS as well as the investigation of the inhibiting potential against isolated thioredoxin reductase.



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P 51

Fluorescent organometallic gold(I) *N*-heterocyclic carbene complexes: synthesis and biological activities

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In modern times, gold complexes have been investigated for their activity against tuberculosis, and several gold containing anti-arthritis drugs have subsequently entered the market and remain in clinical use today. Both gold(I) and gold(III) complexes have also appeared in the last years as promising candidates for new possible anticancer agents [1]. However, the identification of the actual biological targets for those compounds, as well the determination of their distribution in tissues, cells and subcellular compartments still remains a challenge. Among the various strategies to achieve metal compounds imaging in biological environments, fluorescence microscopy is certainly one of the most explored, and an increasing number of publications has appeared reporting on bifunctional metal compounds bearing fluorescent moieties for both therapeutic and imaging applications (so called *theranostic* agents) [2,3]. Within this frame, we have synthetized new gold(I)–NHC complexes bearing a fluorescent coumarin moiety, and characterized their photophysical properties. The compounds were tested as possible anticancer agent against several human tumor cell lines and a model of healthy cells in vitro. Moreover, they were proved to be potent inhibitors of thioredoxin reductase, a seleno-enzyme whose inhibition can lead to apoptosis via mitochondria-related pathways [4]. Finally, we imaged the compounds' uptake into cancer cells using fluorescence microscopy techniques.

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Synthesis and structural characterization of gold(III) complexes with nitrogen-containing heterocycles

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Aromatic nitrogen-containing heterocycles represent an important class of ligands in coordination, supramolecular and bioinorganic chemistry [1]. These ligands have been used for the synthesis of different mononuclear and dinuclear gold(III) complexes, showing remarkable stability under physiological conditions and relevant cytotoxic activity toward different human tumor cell lines [2]. Considering the importance of gold(III) heterocyclic complexes, we have recently investigated the reactions between $KAuCl_4$ and three diazine ligands (az), pyridazine, pyrimidine and pyrazine [3]. It was showed that regardless of different stoichiometric ratio of the reactants, reactions of $[AuCl_4]^-$ with the above mentioned ligands lead to the formation of mononuclear complexes of the general formula $[AuCl_3(az)]$, in which the corresponding diazine acts as a monodentate ligand. This study provided the first crystal structures of $[AuCl_3(az)]$ complexes, allowed to establish the correspondence between basicity of the diazine ligand and its ability to engage the uncoordinated nitrogen atom in intermolecular interactions, and demonstrated the inherent helicity of the investigated molecules in crystals [3]. As a continuation of our ongoing interest towards the coordination chemistry of gold(III) with nitrogen-containing heterocyclic ligands, herein we report the synthesis, NMR spectroscopic and X-ray crystallographic characterization of two mononuclear gold(III) complexes with monodentate coordinated heterocycles, phenazine and quinoxaline, as well as spectroscopic characterization of the dinuclear gold(III) complex with bridging 4,4′-bipyridine ligand. We found that the formation of mononuclear gold(III) complexes with monodentate coordinated diazines and phenazine or quinoxaline has resulted from the strong electron-withdrawing effect of Au(III) ion. However, this effect has not been manifested in the reaction of Au(III) ion with 4,4′-bipyridine (2:1 molar ratio, respectively), which finally lead to the dinuclear $\{[AuCl_3]_2(\mu\text{-}4,4\text{-bipyridine})\}$ complex. The structural properties of gold(III) complexes with the above mentioned nitrogen-containing heterocycles have been discussed in terms on the nature of coordinated ligand.

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P 53

Toward the elaboration of new gold-based optical theranostics for in vivo imaging

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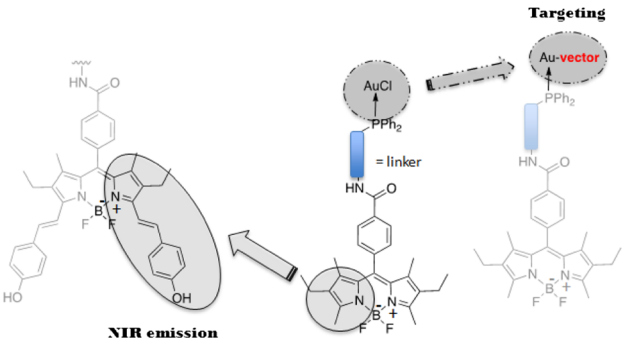
Since the pioneer discovery of cisplatin for biological applications by Rosenberg in the 1960s, metal complexes have become the most currently investigated and used class of compounds in cancer chemotherapy [1]. Gold-based derivatives gave very promising results as anticancer agents [2]. One challenge is to understand their mechanism of action to improve the efficiency and to limit the side effects of such compounds. To deal with this issue, we have drawn our inspiration from theranostics: we attached a fluorophore on metal-based complexes to be able to track them in vitro. More precisely, we recently developed three metal-containing BODIPY-phosphine compounds based on Ru, Os and Au. This first series of complexes showed promising results: interesting IC_{50} in several cancer cell lines, especially for the Au derivative, and the possibility to follow the compounds in vitro by optical imaging [3]. In the present study, we decided to improve the gold(I) complex for it to be suitable for in vivo studies in small animals. First, the conjugation was extended on the BODIPY core, which enables the displacement of the absorption and emission wavelength of the compound to the near infrared region, and then we worked on the introduction of small biovectors for targeting them selectively on several cancer cell lines (Scheme 1). The synthesis and the photophysical studies of the different targeted systems will be discussed. The biological studies will then be presented and compared with the first described compounds.



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P 54

Vitamin B_{12}-gold(III) conjugates for the selective delivery of chemotherapeutics into tumor cells

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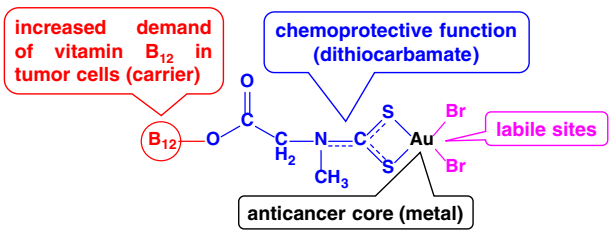
Some gold(III)–dithiocarbamato complexes have recently shown promising antitumor activity, both in vitro and in vivo, together with negligible systemic and organ toxicity [1], although selective tumor targeting is still a major issue.

In order to maximize the impact on cancer cells and minimize side-effects, our latest approach focuses on complexes with tumor targeting properties provided by the coordination of biologically-active ligands, such as vitamin B_{12} (cyanocobalamin). Vitamin B_{12} is an essential nutrient with very low availability. Therefore, rapidly dividing tumor cells, requiring higher amounts of nutrients and energy for cell proliferation, show increased demand of vitamin B_{12} compared to healthy ones [2]. Such avidity of cyanocobalamin can, thus, be exploited for the site-specific delivery of drugs into the tumor by binding vitamin B_{12} (carrier) to an anticancer agent (chemotherapeutics) [3].

We here report on the conjugation of gold(III)–dithiocarbamato derivatives to the 5′-ribose of vitamin B_{12} [4] aimed at combining the anticancer properties and favorable toxicological profile of the gold analogues previously reported with an improved tumor selectivity provided by the conjugated cobalamin acting as delivery carrier, so as to achieve biomolecular recognition and tumor targeting.



Financial support by the National University of Ireland Galway (CoS Scholarship to R.B.) and the COST Action CM1105 (STSM Grant to R.B.) is gratefully acknowledged.

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P 55

Synthesis and preliminary biological investigation of novel NHC gold(I) complexes as potential anticancer agents

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Platinum complexes are widely used in cancer treatment. However, the use of these drugs is limited by severe side effects and the development of resistance during the therapy. Therefore, new metal centers have been investigated to replace platinum. Among these metals, gold has raised particular interest and several complexes of gold(I) and (III) have been synthetized and tested against tumor cells [1].

Of all the possible ligands for gold complexes, N-heterocyclic carbenes (NHCs) are especially promising because of their extremely strong bond to gold(I), leading to complexes with high stability [2,3]. Thus, a new NHC ligand based on an imidazole core bearing a substituted phenyl ring and a methoxy-pyridine ring in positions 4 and 5, respectively, was designed. This carbene precursor is structurally related to a compound proven to be a Janus kinase (JAK) inhibitor [4]. In several solid tumors and most hematopoietic tumors the JAK/STAT pathway is overactivated, suggesting JAK inhibitors as potential anticancer agents [5]. Therefore, including a ligand whose structure is related to a JAK inhibitor could be expected to increase the anticancer activity of the newly designed gold complexes. A versatile pathway to synthesize the imidazolium ligands has been developed, allowing the preparation of a series of derivatives with various substituents. The corresponding gold(I) complexes were prepared and characterized. Preliminary investigations of on cytotoxicity and cellular uptake provided promising results.

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P 56

Titanocene-gold compounds inhibiting AKT and MAPKAP kinases block renal cancer growth in vitro and in vivo

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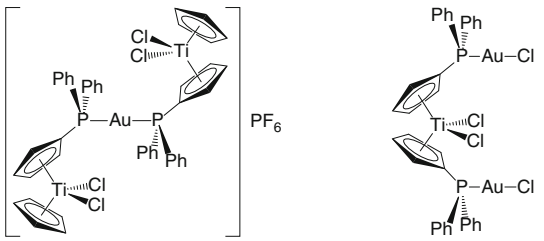
We and others have reported on the cytotoxic effects of gold(I)–titanocene compounds (such as **1**[1] and **2**[2]) on ovarian [1,2] and prostate [2] cancer cell lines. One of the main problems of these complexes is the expected dissociation of the cyclopentadienyl groups in physiological media. This implies that the heterometallic compound will break down into two monometallic components before reaching the target tumor in vivo defeating the purpose of using a single molecule with two different cytotoxic metals.

In order to overcome this problem we have used titanocene derivatives containing gold(I) fragments that are not directly bound to the Cp rings. These compounds have been significantly more effective than monometallic titanocene and gold (I) analogues in renal cancer cell lines indicating a synergistic effect of the resulting heterometallic species. They were also markedly more active than cisplatin and titanocene Y. We will report on initial mechanistic studies in vitro coupled with studies of their inhibitory properties on a panel of 34 kinases of oncological interest. We have found that the compounds inhibit AKT and MAPKAP kinases with a high selectivity for MAPKAP3 ($IC_{50} = 91$ nM). *In vivo* studies on mice with human renal cancer xenografts indicate that the compounds can be excellent candidates for further development as potential renal cancer chemotherapeutics.



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P 57

A glimpse into molecular mechanism of phenolato titanium(IV) anticancer complexes

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In the past decades titanium complexes have emerged as potential anticancer drugs, with two derivatives reaching the clinical trials: titanocene dichloride and budotitate. Due to instability in biological environment, difficulties in identifying the active species and in resolving the mechanism of action for these classes of complexes, a new class of titanium compounds, based on aminophenolato ligands, was developed. This group of complexes exhibits high cytotoxicity towards various cancer cell lines, negligible toxicity towards primary murine cells, high hydrolytic stability, and identified hydrolysis products that are both stable and biologically active.

This study aims to probe into the mechanism(s) of action of the phenolato titanium(IV) complexes. Since these complexes exhibit C_2 or C_1 symmetry, rendering them chiral, the examination of isolated enantiomers is essential both for medicinal use and for gaining

mechanistic insights. We found that for complexes based on bipyrrolidine chiral moiety, generally the racemic mixtures were inactive whereas the pure enantiomers exhibited similarly high cytotoxic activity. This observation supports the assumption on the involvement of polynuclear hydrolysis product as the active species as well as the premise that the biological target is chiral.

To further explore possible molecular mechanism(s) of action for this class of compounds, gene chip array, western blotting, ELISA and FACS methods were applied on leading salan complexes. Gene chip array studies suggested involvement of the following pathways: (1) Systemic Lupus Erythematosus; (2) Disruption to cell cycle; (3) p53 signaling; (4) ECM receptor interaction. Studies of flow cytometry revealed growth arrest in G-1 of the cell cycle within 24 h. ELISA and western blotting revealed increasing levels of apoptotic related proteins, p53 and p21, throughout exposure for 48 h, as well as the expression of caspase3, caspase9 and MDM2 proteins.

Collectively, the present findings are consistent with interaction of phenolato Ti(IV) complexes with DNA and initiation of a DNA damage response culminating in cell cycle arrest and apoptosis. This pathway resembles those of chemotherapeutic drugs that exert their effect through DNA interstrand crosslinking and double strand breaks.

This research was funded by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC Grant agreement (No. 239603)

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Miller M, Tshuva EY (2014) Eur J Inorg Chem 9:1485–1491

P 58

Combination of anti-cancer salan Ti^{IV} complexes with cisplatin: synergistic effects

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The significant drawbacks of cisplatin, namely its high toxicity and development of drug-resistance, initiated an extensive search for other metals that can lead to anti-cancer activity. Ti^{IV} complexes showed promising cytotoxic activity, and two Ti^{IV} based complexes reached clinical trials. Nevertheless, these complexes have failed clinical trials due to instability in aqueous environment. Our group designed a new family of anti-cancer Ti^{IV} complexes based on salan ligands, which showed high cytotoxic activity toward numerous cancer cell lines and enhanced stability in aqueous environment.

Combination therapy is a very common method in clinical treatment of cancer. By combining two drugs or more, the doses that are required to reach the desired effect are reduced, and consequently their side effects and toxicity are reduced as well.

Herein, combinations of salan Ti^{IV} complexes with cisplatin are presented. Non-covalent combination of salan Ti^{IV} complexes and cisplatin often showed a synergistic behavior, depending on the substitution on the salan ligand, the ratio of the combined drugs, the type of the treated cancer cell lines, and the schedule of administration. Additionally, attempts are made to covalently combine salan Ti^{IV} complexes with cisplatin and with steroids. Such combined complexes may enhance the cytotoxicity as well as the selectivity of the complexes to particular cell types. Achievements in these directions will be discussed.

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Reference

Ganot N, Redko B, Gellerman G, Tshuva EY (2014) (submitted)

P 59

Highly active antitumor titanium(IV) phenolato complexes: the influence of structural factors on complex performance

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The anticancer activity of the inorganic compound cisplatin and its worldwide use in the pharmaceutical industry initiated a new field of research, aimed at finding new inorganic complexes of other metals that may lead to improved anticancer drugs. Among others, Ti(IV) complexes titanocene dichloride and budotitane demonstrated high cytotoxic activity but eventually failed clinical trials due to their low hydrolytic stability. We previously introduced cytotoxic Ti(IV) complexes based on salan ligands that demonstrated high activity along with exceptional hydrolytic stability.

Herein we examine the influence of structural aspects on the complex reactivity and stability. We will present the first trans-Ti(IV) complexes of high cytotoxicity based on salen ligands [1–2]. These complexes are highly active towards various cancer cell lines, with improved hydrolytic stability relative to that of the known Ti(IV) complexes. Structure–activity relationships will be discussed. Additionally, we will present salalen-Ti(IV) complexes, which are half salan-half salen, with a fac-mer binding mode that gives a C_1 symmetry. The effect of this geometry on the properties and performance of the Ti(IV) complexes will also be addressed.

This research was funded by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC Grant agreement (No.239603).

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P 60

Highly effective and hydrolytically stable vanadium(V) phenolato antitumor agents: development, analysis and mechanistic investigation

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In the field of anti-cancer chemotherapeutic research, numerous metal compounds are being investigated worldwide in order to resolve the limited activity range and severe toxicity of the antitumor drug cisplatin. One of the most promising metals studied today is vanadium. Vanadium compounds were found to exert favorable properties for use in therapy, but the complicated aquatic chemistry of vanadium compounds impeded the potential of vanadium as an antitumor agent. Therefore, the development of compounds with improved hydrolytic stability is essential for therapeutic utilization. In previous work we developed a new family of vanadium(V) complexes with tetradentate diamino bis(phenolato) "salan" ligands with favorable cytotoxic activity. Nevertheless, theses complexes exhibited mils water stability. Appreciable cytotoxic activity was measured for an isolated hydrolytsis product that was found to exhibit a dimeric structure with no labile ligands [1].

Herein we developed a family of oxo-vanadium(V) complexes with pentadentate diamino tris(phenolato) ligands, which do not contain any labile ligands, and therefore exhibit remarkable resistance towards hydrolysis. Importantly, these compounds display exceptional cytotoxic activity, higher than that of cisplatin by up to

two orders of magnitude, which is preserved during incubation in DMSO for several weeks [2]. These properties, along with promising in vivo results for a representative complex, encourage further development and investigation regarding the mechanism of action of this family of complexes. Preliminary evidence of possible cellular pathways of these complexes will be discussed, as well as structure–activity studies concerning different substituents on the phenolato rings.

This research was funded by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC Grant agreement (No. 239603).

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P 61

Redox properties of iron sucrose

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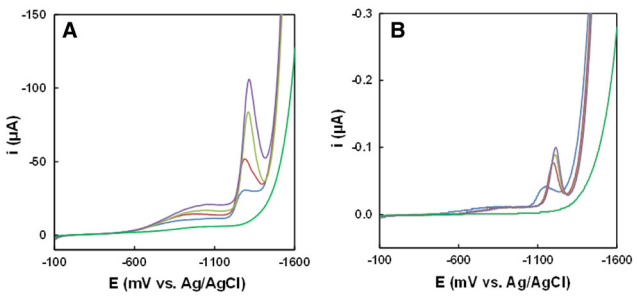
Iron sucrose is a Fe(III) oxide hydroxide colloid stabilized by a layer of sucrose molecules. Some preparations are used in the treatment of iron deficiency anemia [1]. We applied cyclic voltammetry and polarography to investigate their redox properties. The Fe(II) content of iron sucrose is routinely determined by a polarographic method, because it is thought to cause toxic effects [1] in therapeutic applications.

In the potential range of 100–1600 mV vs. Ag/AgCl, two major current waves were detected which increased and changed shape with every cycle. The first broad wave can safely be assigned to the reduction of iron(III) to iron(II). The second wave is conventionally representing iron(II) to iron(0) reduction [2,3]. Because of the 2-electron transfer reaction, the integral of the second wave should be strictly 2 times higher than the first. However, we found a variable ratio depending on exposition time of the electrode to the solution. We conclude that the second wave is also mainly caused by iron(III) to iron(II) reduction, namely of Fe(III) that is located deep inside the particle, compared to the Fe(III) species at the particle surface which should produce the first wave. The figures below show the current evolution in time on a gold (left) and a mercury (right) electrode.



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P 62

Interaction of hybrid polyoxometalates with biological targets

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Polyoxometalates (POMs) are discrete and polyanionic metal-oxides with potential applications in medicine. Due to their redox and structural properties, indeed, they can affect electron transport within the cells and form adducts with macromolecules, via electrostatic interactions and hydrogen bonds. This behaviour has shown to be useful to denature proteins and inhibit enzymes, leading to antiviral, antitumoral and antibacterial activities [1].

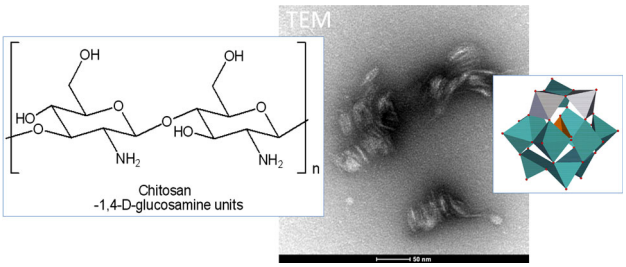
In this field, POMs interaction with biological substrates seems to be favoured, in terms of delivery, stability and biological activity, by the presence of organic domains [2].

The presentation will thus describe:

(i) The synergistic antibacterial effect of POMs and chitosan, forming hybrid nanoaggregates (see figure) [3];

(ii) Cell delivery and tracking of fluorescent hybrid POMs;

(iii) The recognition capabilities by a biotinylated POM, for which spectroscopic and surface plasmon resonance techniques were used to explain the nature of the interaction with avidin.



Financial support by the MIUR–FIRB prot. RBAP11ETKA is gratefully acknowledged.

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P 63

EuroTracker dyes: very bright europium complexes for live cell imaging

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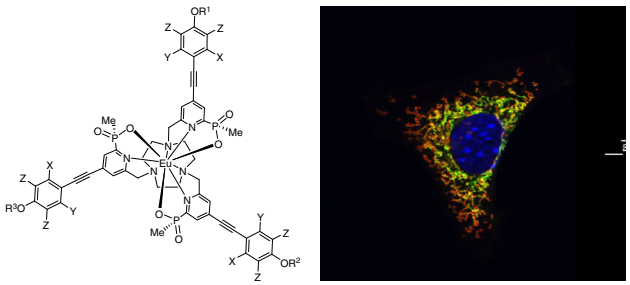
Several exceptionally bright europium complexes have been prepared that exhibit excellent cell uptake behaviour and distinctive localisation profiles within various mammalian cell types. Each complex localises in specific organelles within the cell, including the mitochondria, lysosomes and the endoplasmic reticulum, and is visualised by fluorescence microscopy (*Figure*). The long luminescence lifetimes of these complexes (>1 ms) allow for time-gated spectral imaging and make them an attractive alternative to commercial fluorescent dyes.

The advantages of lanthanide complexes over traditional fluorescence dyes for live cell imaging include: a large Stokes' shift that minimizes self-quenching; an optical signal that conveys information on the local environment through emission spectral form and a long luminescence lifetime allowing time-gated detection that overcomes problems with autofluorescence of biomolecules.

The complexes reported have very high molar extinction coefficients ($\varepsilon = 55$–$60{,}000\ M^{-1}\ cm^{-1}$) and quantum yields ($\varphi \sim 50\ \%$), resulting in exceptionally bright ($\varepsilon \times \varphi$) compounds. They are non-toxic ($IC_{50} > 100\ \mu M$), show high levels of cellular uptake and exhibit selective organelle staining depending on the nature of the macrocyclic co-ordinating ligand.

In summary, this new class of "EuroTracker" dyes as cellular stains offer several improvements over the classical fluorescence organic dyes.



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P 64

Synthesis of water stable $\{M^{(V)}O_2\}^+$-NHC Complexes (M = Re, ^{99}Tc)

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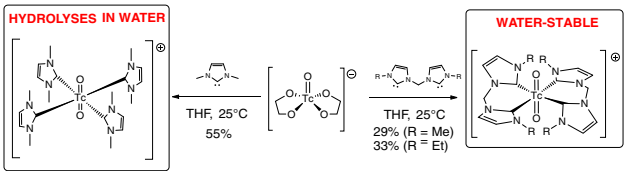
99m-Technetium (^{99m}Tc) is the main nuclide used for diagnosis in nuclear medicine due to its almost ideal properties. Therefore, the search for novel ligand systems suitable for application with ^{99m}Tc is of great interest in this field of research. Recently, the scope of N-heterocyclic carbenes (NHCs) has been extended from catalytic application to the field of bioinorganic chemistry and metals in medicine. In this context, the NHC chemistry of technetium came into our research focus. However, ^{99}Tc-NHC complexes are scarce [1]. While $\{Re^{(V)}O_2\}^+$ complexes, which contain monodentate NHCs, are hydrolytically stable, the corresponding $\{Tc^{(V)}O_2\}^+$-NHC complexes show rapid hydrolysis in the presence of trace amounts of H_2O [2]. We present novel synthetic pathways for the synthesis of water stable $\{M^{(V)}O_2\}^+$-NHC complexes [3]. The key link for these general procedures is $[M^{(V)}O(glyc)_2]^-$ (M = Re, ^{99}Tc; glyc = ethylene glycolato). The high water stability of the products allows conversion of the $\{M^{(V)}O_2\}^+$ core into $\{M^{(V)}OCl\}^{2+}$ with HCl as the H^+ and Cl^- source. The remarkable stability and pH-controllable reactivity of the new complexes underline the potential of NHCs as stabilizing ligands for ^{99}Tc complexes and pave the way for the first ^{99m}Tc-NHC complexes in the future.



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P 67

Does length really matter? Structure–luminescence relationships in Eu^{III} and Tb^{III} complexes

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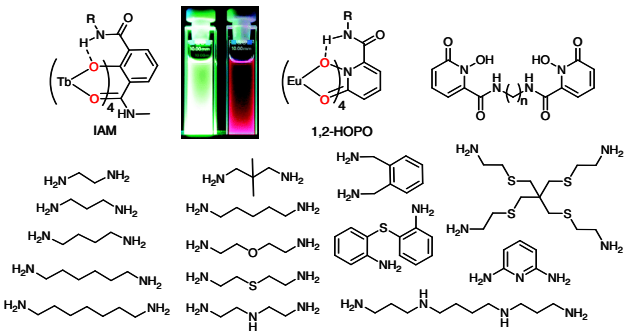
Luminescent lanthanides have attracted attention for their unique photophysical properties making them a vital tool for modern medicinal applications such as diagnostic immunoassays for the detection of cancer markers, blood processing and drug monitoring. In the Raymond group, one current interest is the sensitization of the luminescent visible emission from Tb^{III} and Eu^{III}. To achieve high overall quantum yields, we utilize the 2-hydroxyisophthalamide (IAM) or 1-hydroxypyridin-2-one (1,2-HOPO) chelate groups as antennas, respectively [1] Herein we report a large group of octadentate and tetradentate ligands based on the 1,2-HOPO and IAM moieties with various modifications to not only improve quantum yields, but also increase water solubility and stability in aqueous solution. An example of one set of modifications we have made, is varying the tether length in the tetradentate 1,2-HOPO ligands. We will point out geometric and electronic factors contributing to high quantum yields in Eu^{III} and Tb^{III} complexes. Examples of the ligands that have been prepared are shown in the figure below. Financial support by the Alexander von Humboldt Foundation and a grant from the Department of Energy are gratefully acknowledged.



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P 68

DNA sensing by lanthanide-binding peptides

Christelle Gateau[1,2], Alexandra Botz[1,2], Laetitia Ancel[1,2], Colette Lebrun[1,2], Pascale Delangle[1,2]

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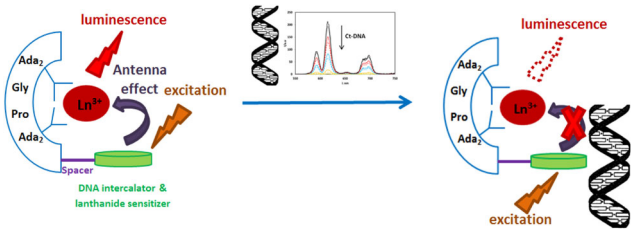
DNA is a fascinating biomolecule, which is storing and dispensing genetic data required for life. Therefore, molecules that bind to DNA are extremely useful as biochemical tools to detect DNA both in vitro and inside the cell. Time-resolved luminescence of lanthanides is particularly attractive for applications of these ions as luminescent DNA probes, since it allows to eliminate the background natural fluorescence. Lanthanides-binding peptides are especially promising molecules for biological applications, since they provide high hydrophilicity and solubility in water to the probe.



In the lab, we demonstrated that hexapeptides containing unnatural amino acids bearing aminodiacetate side chains and a sequence Pro-Gly give stable lanthanide-peptide complexes at physiological pH. Introduction of aromatic moieties, which act both as a lanthanide sensitizer and DNA binding group allows the detection of double stranded DNA thanks to the time-resolved luminescence of the lanthanide ion.

Here we presented the rational design, preparation and evaluation of these new DNA sensing lanthanide binding peptides.

This research was supported by the "Région Rhone-Alpes" and the Labex ARCANE (Grant ANR-11-LABX-0003-01).

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P 69

Structures and biotransformations of potent insulin-enhancing $V^{IV}O$ complexes with picolinate derivatives

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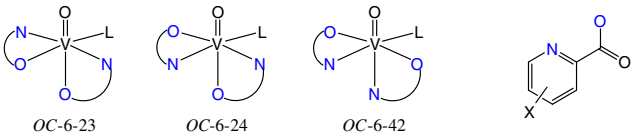
The potential use in the therapy of type 2 diabetic patients is one of the most important applications of vanadium in medicine [1,2]. Promising insulin-like effects have also been found in the case of $V^{IV}O$ picolinato complex and its derivatives [3].

Three new $V^{IV}O$ compounds with the 5-cyanopyridine-2-carboxylic acid, 3,5-difluoropyridine-2-carboxylic acid and 3-hydroxypyridine-2-carboxylic acid have been synthesized and characterized by X-ray, EPR and DFT methods. Their interactions with the blood proteins apo-transferrin and albumin have also been studied by EPR spectroscopy.

Studies in the solid state and in solution have revealed *cis*-octahedral structure of all three compounds with the solvent or monodentate ligand in equatorial position *cis* to the $V^{IV}O$ moiety, while the arrangement of the bidentate picolinate ligands is variable. In the solid state *OC*-6-23 and/or *OC*-6-24 arrangements of the complexes have been determined, but in the solution partial isomerization into *OC*-6-42 complexes has been observed. In the presence of protein apo-hTf at physiological pH complexes decompose and the majority of $V^{IV}O^{2+}$ ions binds to the protein, while in the presence of HSA mixed species with ligands can also be formed.



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P 70

Silver(I) bis(norharmane) compounds: synthesis, 3D structures and cytostatic properties

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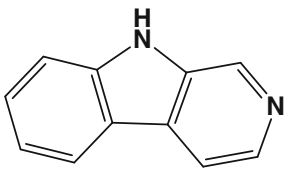
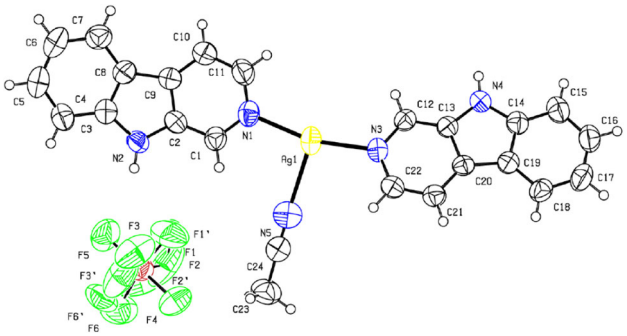
Silver coordination compounds are well known as protectors of the open skin to prevent bacterial infections. They are hardly toxic for human beings, so the question has risen whether such compounds, when the proper ligands are present, can be investigated for their cytotoxic activity [1]. So we have chosen a less well-known ligand that in addition to the nitrogen metal binding site has a remote H-bond donor group which may establish additional hydrogen bonds. The ligand norharmane (*9H*-Pyrido[3,4-*b*]indole, abbreviated as Hnor; see Figure below) is a mixed-ring heterocyclic compound that belongs to an alkaloid family called β-carbolines (βCs).

We report on four new Ag coordination compounds with this ligand. As counter ions we have chosen: NO_3^- (potential metal



coordination), BF_4^-, ClO_4^- and PF_6^- (decreasing tendency to coordinate) also to see whether the NH ligand would bind (intermolecularly) to the anion. In the case of the first three anions the compounds have the formula $[Ag(Hnor)_2](anion)$, while the compound with the PF_6^- anion resulted to be $[Ag(CH_3CN)(Hnor)_2](PF_6)$, with a weakly coordinated CH_3CN (Ag–N 259.2 vs 217.0 pm). See figure below for the hexafluoridophosphate.

These four Ag compounds were tested for their antiproliferative activities in human ovarian (A2780) and lung (A549) cancer cell lines. Surprisingly, only the perchlorate-containing derivative shows promising activity in the selected cancer cells.



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P 71

Zn(II)-triggered cellular uptake and nuclear localization of a near-infrared fluorescent probe

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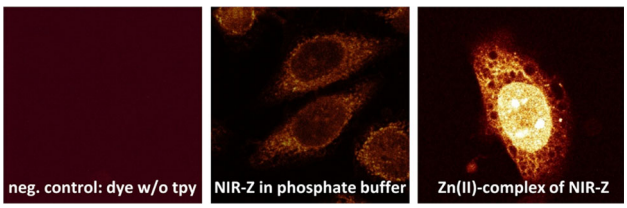
The distribution of Zn(II) ions in tissues is remarkably specific. Amongst others, zinc-rich tissues include the central nervous system, prostate, pancreas, and breast cancer tissue [1]. In contrast, Zn(II) levels are dramatically decreased in prostate cancer tissue [2]. Fluorescent probes for endogenous zinc sensing are promising tools for early detection of, for example, prostate cancer [3].

In earlier studies, our group has demonstrated that the attachment of terpyridine (tpy) to peptide nucleic acids results in significantly elevated cellular uptake of these PNA-tpy conjugates in presence of Zn(II) [4]. We have now developed a novel near-infrared probe (NIR-Z) consisting of a fluorophore and a terpyridine moiety. While NIR-Z is poorly internalized in the absence of Zn(II), we observed a significant enhancement of cellular uptake for Zn(II)-complexes of NIR-Z as well as an influence on the intracellular distribution [5].

This probe might allow tissue-selective labeling and even the detection of potential malignancies. Its advantageous near-infrared absorption and emission that allow imaging with low background fluorescence from endogenous fluorophores as well as its high extinction coefficient and sufficient quantum yield render the probe a highly interesting candidate for potential in vivo applications.



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P 72

Novel multi-functional metallodrug candidates as potential cancer therapeutics

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The first platinum-based anti-cancer chemotherapeutic, cisplatin, was granted clinical approval in 1978. Yet, surprisingly to date, only two further platinum drugs have gained full global approval namely carboplatin and oxaliplatin. Although hugely successful, the widespread application and efficacy of platinum drugs are hindered by their toxic side effects, limited activity against many human cancers and susceptibility to acquired drug resistance. As a consequence, many investigations have been conducted into trying to identify new molecular targets beyond DNA which may present unique opportunities for therapeutic exploitation. In recent years, histone deacetylase (HDAC) enzymes have been identified as novel cancer targets, the inhibition of which suppresses tumour cell proliferation. Our group has designed and synthesised novel anti-cancer bifunctional platinum drug candidates which possess both DNA binding and HDAC inhibitory activity [1–4]. Building on this research, we have been adding to this library of compounds including the development of novel ruthenium HDAC inhibitor derivatives. In doing so, our ultimate aim is to develop novel drug candidates that may overcome the drawbacks associated with classical platinum drugs. A summary of our results to date will be described.

This material is based upon works supported by the Science Foundation Ireland under Grants No. [07/RFP/CHEF570] and [11/RFP.1/CHS/3094]. We also gratefully acknowledge the Programme for Research in Third Level Institutions (PRTLI), administered by the HEA for funding. We thank also colleagues in COST CM1105 for fruitful discussions and collaborations.

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P 73

Coordination abilities of the alloferon 1 mutants containing two histidine residues towards copper(II) ions

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Alloferon is a tridecapeptide $H^1GVSGH^6GQH^9GVH^{12}G$ isolated from the bacteria-challenged larvae of the blow fly *Calliphora vicina* [1]. Alloferon is believed to have similar therapeutic use to interferons and interferon inducers including but not limited to treatment of interferon-sensitive viral and cancer diseases. Alloferon is practically non-toxic, has no teratogenic, embryotoxic or mutagenic properties as has been shown in advanced preclinical studies [1]. Many essential metal ions act as the important factor influencing the structure of natural and synthetic oligopeptides, and as a consequence, they may have a critical impact on their biological activity. It was found that alloferon causes apoptosis in hemocytes of *Tenebrio molitor* [2]. Also, heavy metals are known to be typical stimuli to trigger apoptosis in vertebrate and invertebrate cells [3].

In this study the copper(II) complexes of the (H6,9A), (H6,12A) and (H9,12A) mutants of alloferon 1 were performed by the combined application of potentiometric equilibrium, spectroscopic (UV–Visible, CD, EPR) and MS methods in solution. The CuL complex with $\{NH_2,N_{Im}\text{-}H^1,N_{Im}\text{-}H^{6,9or12}\}$ binding site dominates in wide 4.5–7.5 pH range. At physiological pH 7.4 are present in equilibrium the CuL and $CuH_{-1}L$ $\{NH_2,N^-,CO,N_{Im}\text{-}H^{6,9or12}\}$complexes. The first amide nitrogen deprotonation is suppressed by the coordination of the H^1 residue and the formation of the macrochelate by the H^6, H^9 or H^{12} residues.

The induction of apoptosis in vivo in *T. molitor* cells by the ligands and their Cu(II) complexes was studied. The biological results show that Allo(H6,9A), Allo(H6,12A) and Cu(II)-Allo(H9,12A) show weaker proapoptotic activity than alloferon 1. The Cu(II) complexes of the Allo(H6,9A) and Allo(H6,12A) were practically inactive. These results may confirm our suggestion that an important role in the biological activity of Cu(II) complexes with alloferon play histidine residues at position 1 and 6 through which a macrochelate is created [4,5].

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P 74

Novel bio-inorganic tools for ROS detection in living cells

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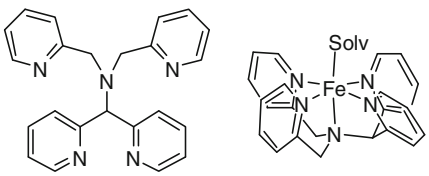
Free radicals and other Reactive Oxygen Species (ROS) are important components in many physiological and pathological processes in the living cell [1]. Still much is unknown about the roles of ROS and oxidative damage in cellular processes. Elevated concentrations of ROS can cause oxidative stress which severely impacts the cellular function by damaging important cellular components [2]. The goal of this project is to design novel catalytic tools for the detection and manipulation of ROS and oxidative stress in both healthy as well as cancer cells with a particular focus on the sub-cellular environment. This will give rise to new insights into the behavior of cancer cells compared to healthy cells under oxidative stress. Ideally suited for this purpose are the bio-inspired iron oxidation catalysts based on N4Py ligands (See Figure) [3]. This transition metal catalyst can convert H_2O_2 and $O_2^{\cdot-}$ into highly reactive oxygen species (hROS), which may include diffusible radicals such as ·OH, or iron-based species such as $Fe^{III}OOH$ or $Fe^{IV}O$, that can easily be detected and are capable of generating controlled oxidative stress inside the (sub-)cellular environment. Current research focuses on the covalent attachment of a fluorophore to the Fe(II)-N4Py catalyst, in order to track the ligand inside living cells using con-focal microscopy. This will give more insight into the mode of action of Fe(II)-N4Py and its role in cell death.



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P 75

Improved reaction conditions for the synthesis of novel KP1339 derivatives and preliminary investigations on their anticancer potential

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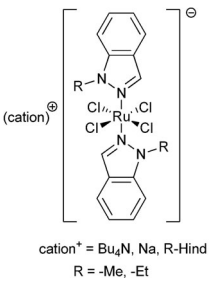
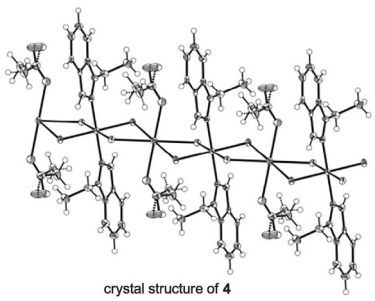
Due to the very promising results of $Na[RuCl_4(1H\text{-indazole})_2]$ (NKP1339) in clinical studies [1], the chemistry of *Keppler-type* ruthenium(III) coordination compounds is still of large scientific interest [2]. By applying a new acid free method on the way to this type of coordination compounds, six different complexes of the general formula (cation)-*trans*-$[RuCl_4(azole)_2]$, where (cation) = tetrabutylammonium (Bu_4N–) (**1**, **2**), sodium (**3**, **4**), azolium (**5**, **6**) and azole = 1-methyl-indazole (**1**–**3**), 1-ethyl-indazole (**4**–**6**), have been synthesized. The new synthetic method allows the introduction of acid-sensitive or -labile ligands and can therefore facilitate the future development of ruthenium(III) based anticancer agents. All synthesized complexes have been characterized by elemental analysis, electrospray ionization (ESI) mass spectrometry, IR-, UV–Vis- and NMR spectroscopy. Furthermore, the influence of the alkyl substituent at indazole ligand on the stability in aqueous media as well as on the biological activity in three human cancer cell lines (CH1, A549 and SW480) will be discussed.



cation$^+$ = Bu_4N, Na, R-Hind
R = -Me, -Et



crystal structure of **4**

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P 76

Surfactant-mediated activation of the lead anticancer ruthenium compound KP1019 by encapsulation into polymeric nanoparticles

Britta Fischer[1], Petra Heffeter[2,3], Kushtrim Kryeziu2,3, Lars Gille[4], Samuel M. Meier[1,3], Walter Berger[2,3], Christian R. Kowol[1,3], Bernhard K. Keppler[1,3]

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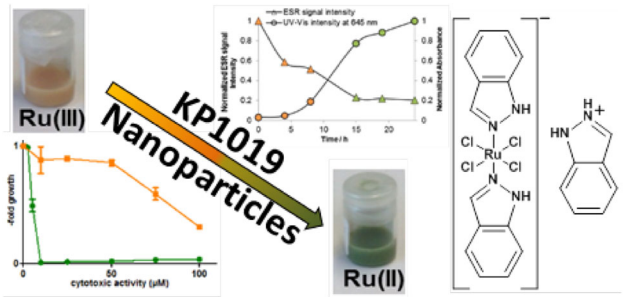
KP1019 is the lead anticancer ruthenium(III) compound and has been successfully tested in a clinical phase I [1]. Since, it is characterized by quite low stability in aqueous solution especially at physiological pH, nanoparticles would offer the possibility to circumvent these limitations [2]. Therefore, highly reproducible poly(lactic) acid (PLA) nanoparticles of KP1019 were prepared by a single oil-in-water (o/w) emulsion. During storage the color of these nanoparticles changed from brown to green. Cytotoxicity measurements comparing different aged nanoparticles revealed that the color change is associated with a 20-fold increased activity compared to "free" KP1019. A reaction between the used surfactant Tween 80 and KP1019 was identified as the cause of the green color. Kinetic studies using UV–Vis, ESI–MS and ESR spectroscopy indicated a coordination of Tween 80 to KP1019, and furthermore the color change was found to correlate with a reduction of the Ru(III) by Tween 80. The results provide a first approach to stabilize a biologically active Ru(II) species of KP1019 in aqueous solution and the nanoparticles probably can be used to selectively generate this activated species in the tumor tissue via passive targeting.



Financial support by the exploratory focus "Functionalized Materials and Nanostructures" of the University of Vienna is gratefully acknowledged.

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P 77

Synthesis, characterization and anticancer activity of (thio)pyrone-derived organometallic complexes

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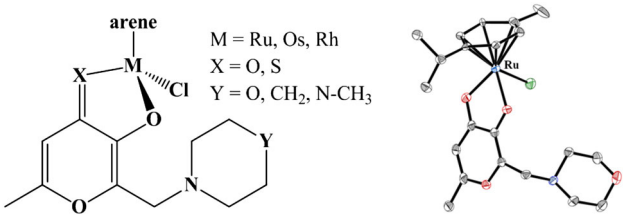
Non-platinum tumor-inhibiting metal and organometallic complexes have gained increasing interest in recent years. One promising approach is the coordination of organometallic fragments to bioactive molecules. Pyrones are known for their bioavailability, favorable toxicity profile and high affinity towards metal ions. This intensively studied ligand system can be easily converted to the respective thiopyrone which offer a potential S,O coordination motif. It has already been shown that thiopyrone-based Ru(II) complexes provide an increased stability under physiological conditions and a different biomolecule interaction profile compared to the pyrone analogues. In addition, these organometallics exhibit promising cytotoxicity in vitro [1,2] and are currently investigated in vivo.

The (thio)pyrone scaffold was modified via Mannich reaction utilizing morpholine, *N*-methylpiperazine and piperidine in order to expand the (thio)pyrone library and to investigate the influence on cytotoxicity of the corresponding metal complexes. Due to the emerging interest in Rh(III) Cp* and Os(II)–arene complexes, the corresponding organometallics were synthesized and characterized by means of 1D and 2D NMR spectroscopy, elemental analysis, ESI–MS and if possible by X-ray diffraction analysis. The anticancer potential was examined by means of the colorimetric MTT assay. The stability in aqueous solution was studied via ^{1}H NMR spectroscopy.



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P 78

A cobalt-based strategy for tumor targeting of EGFR-inhibitors

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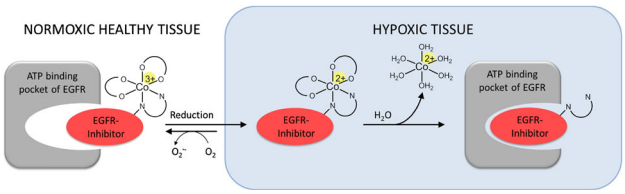
During the last decades the development of receptor tyrosine-kinase inhibitors was a major step forward in cancer treatment. However, despite its success, the therapy is limited by strong adverse effects and resistance development [1,2]. Aim of the here presented study was the design of novel epidermal growth factor receptor (EGFR) inhibitors which are specifically activated in malignant tissue and, thus, are expected to show reduced side effects. To this end, a cobalt(III)-based prodrug strategy was used, which allows targeted release of an active EGFR inhibitor triggered by hypoxia in the tumor tissue [3]. New inhibitors with bis-chelating moieties were synthesized and tested for their EGFR-inhibitory potential. The most promising candidate was subsequently coordinated to Co(III) and the biological activity of this complex was tested in cell culture under hypoxic vs. normoxic conditions. Indeed, hypoxic activation and subsequent EGFR inhibition could be proven. The anticancer activity of the new complex was tested in xenograft models revealing potent anticancer activity also in vivo. Summarizing, this study shows, that Co(III)-based tumor-targeting represents a promising strategy to improve EGFR inhibitor treatment.



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P 79

Fluorophore ATCUN complexes: dual probes for DNA cleavage

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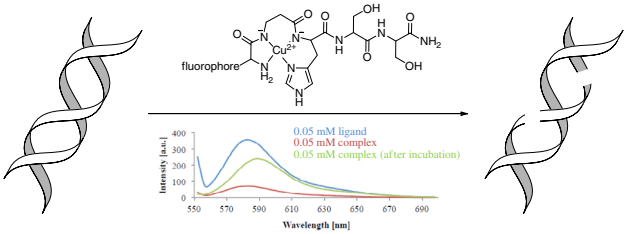
The knowledge about the amino terminal Cu(II)- and Ni(II)-binding motif (ATCUN) has expanded from a small metal binding site in proteins like certain species of albumins to efficient ligands for artificial nucleases [1–3]. In the last years several newly designed amino acid sequences based upon the ATCUN motif have been demonstrated to act as highly selective, fluorescent chemosensors for Cu(II) ions [4]. Based on findings of Imperiali and co-workers we have recently synthesized some fluorophore-ATCUN peptides for investigation of their nuclease activity and fluorescence properties [5]. We could show that the Cu(II)-induced fluorescence quenching can partly be recovered when the Cu(II)–peptide complex undergoes oxidative DNA cleavage involving Cu(I) species. Therefore, our new approach might allow detecting Cu(II)-dependent DNA cleavage not only by commonly used gel electrophoresis but also via fluorescence spectroscopy.



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P 80

Amphiphilic metal complexes of cyclen derivatives for improved protein cleavage via particle formation

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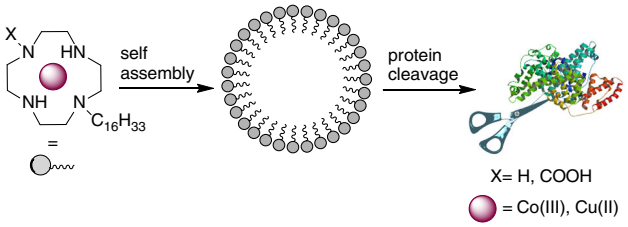
Cleavage of pathogenic proteins, as they appear in amyloidogenic diseases like Alzheimeŕs disease, Parkinson's disease and type 2 diabetes mellitus, is a therapeutic option for their treatment. The moderate protein cleavage activity of cyclen complexes is known

since the nineties and enhancement of this activity and targeting to pathogenic proteins are under investigation ever since [1].

Formation of micelles and vesicles from amphiphilic Zn(II) cyclen derivatives has already been proven as potent strategy for DNA cleavage [2]. Our goal is to improve the protease activity of Cu(II) and Co(III) complexes of cyclen derivatives by a similar strategy, i.e. the self-assembly to micelles. The influence of mono *N*-alkylation and -carboxylation of the cyclen ligand on the protein cleavage activity and particle formation behaviour is described in this work.



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P 81

Copper complexes of novel anthraquinone-substituted cyclen derivatives for DNA cleavage

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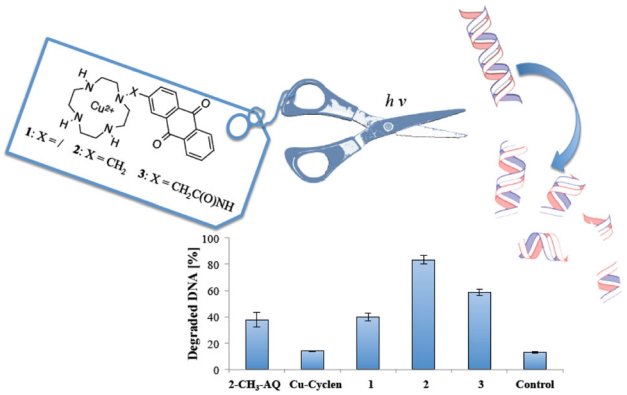
The photolytic cleavage of plasmid DNA by anthraquinone derivatives has been known for years. Such derivatives are promising candidates for future anti-cancer therapeutics and especially for those against skin cancer [1, 2]. We have recently designed three new water soluble copper(II) complexes that link the anthraquinone moiety with the well studied artificial nuclease Cu(II)-1,4,7,10-tetraazacyclododecane, Cu(II)cyclen [3]. We were able to show that these systems can cleave plasmid DNA under irradiation at 365 nm as effectively or even more effectively than anthraquinone itself. The anthraquinone moiety increases DNA affinity and enhances the oxidative cleavage activity, as well. To gain further insight into the redox processes involved in the cleavage mechanism we performed electrochemical studies, indicating synergic effects might exist between the anthraquinone moiety and the copper centre for some of the complexes.



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P 82

HSP70 as a metallodrug target

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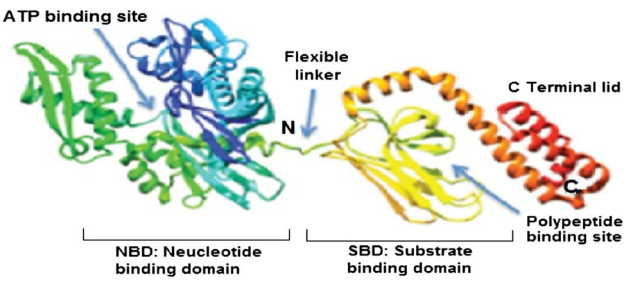
Cancer is a major cause of death and disease worldwide. Over the past 30 years platinum (Pt) compounds have played a very important and well documented role in treating cancer. The cytotoxicity of Pt drugs is attributed to multiple mechanisms but primarily their ability to form DNA adducts. The clinical efficacy of Pt drugs is limited though by toxicity and chemoresistance (intrinsic or acquired)[1]. Since many cancers are intrinsically resistant to Pt-based therapies there is an urgent need to develop novel and innovative therapeutic strategies for combating cancer.

HSP70 is a stress-inducible chaperone, which maintains protein homeostasis during normal cell growth but during a stress response is overexpressed and binds to and stabilises its protein substrates. It is overexpressed in colorectal and prostate cancer amongst other cancers, and is associated with cancer progression, chemotherapy resistance (including against cisplatin) and poor prognosis as it is thought to provide cancer cells with a survival advantage by conferring protection against apoptosis, influencing senescence and inhibiting autophagy for example. In addition given HSP70 is overexpressed in cancer cells relative to normal cells this effect should be selective. Inhibition of HSP70 is therefore an exciting and legitimate anti-cancer target.

Consequently, we wish to develop novel Pt HSP70 inhibitor drug candidates as potential alternative treatments for colorectal and prostate cancer. A summary of results to date will be described.



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P 83

New carbohydrate-bearing 8-hydroxyquinoline compounds as multifunctional chelators of copper(II) and zinc(II) ions

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Mounting evidence suggests a pivotal role of metal imbalances in protein misfolding and amyloid diseases. As such, metal ions represent a promising therapeutic target and therefore, the synthesis of chelators that also contain complementary functionalities to combat the multifactorial nature of neurodegenerative diseases is a highly topical issue Recent investigations have rekindled interest in 8-hydroxyquinolines (OHQs) as therapeutic agents for cancer, Alzheimer's disease and other neurodegenerative disorders. We have recently demonstrated that glycosylation is a versatile and powerful strategy for improving drug features including solubility, pharmacokinetics, drug targeting, and biological activities [1–4]. Here, we report several OHQ glycoconjugates whose multifunctional properties are highlighted, including their Cu(II) and Zn(II) binding abilities, and antioxidant and metal-induced antiaggregant capacity. Glucose, trehalose and cyclodextrin were the carbohydrates of choice because of their interesting properties such as antioxidant, antiaggregant and inclusion abilities.

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P 84

Antimicrobial properties of Cu-based nanoparticles: interaction with DNA, ROS production and lipid peroxidation

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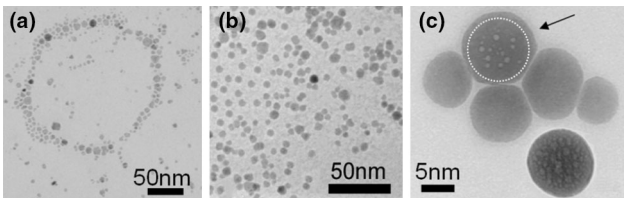
The abuse of antimicrobial drugs has led to increasing number of infections associated with antibiotic-resistance microbes. By using water as a solvent or typical solvothermal synthesis and in the presence of surfactants, copper based nanoparticles (Cu-based NPs) were formed, while synthesis control is achieved to tune their composition, size and shape. The higher biological activity of the NPs combined with lower applicable doses could give rise to the next generation of antimicrobials, namely nano-antimicrobials.

Herein, we solvothermally prepared Cu-based NPs of different composition and sizes capped with the non ionic surfactants tetraethylene glycol, polyethylene glycol 1000, polysorbate 20 and oleylamine. The antimicrobial activity of the synthesized Cu, Cu/Cu_2O, Cu_2O and CuO NPs has been screened against Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*), Gram-negative bacteria (*Escherichia coli*, *Xanthomonas campestris*, *Bacillus subtilis*) and fungus (*Saccharomyces cerevisiae*). The results clearly indicated that the composition of the NPs was the main factor affecting their performance, since Cu_2O NPs found with enhanced antimicrobial effect and specificity against the Gram-positive strains. In an attempt to further explore their mechanism of action, we studied their interaction with DNA and Cu-based NPs found to induce pDNA, ds CT-DNA and fungal DNA degradation in a dose-dependent manner. The ROS production and lipid peroxidation have also been verified, while ionic contribution to the bactericidal activity of NPs cannot be supported as the released ions found below the value of inhibiting bacterial growth.



The research has been co-financed by the European Union and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework—Research Funding Program: Thales. Investing in knowledge society through the EU Social Fund.

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P 85

Cystic fibrosis: new molecular imaging tools

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Cystic Fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which encodes the CFTR protein, a chloride channel expressed in the apical membrane of epithelial cells in the airways, pancreas, intestine and exocrine glands. Therapies based in drugs that correct the trafficking or gating defects of CFTR (termed correctors or potentiators, respectively) are emerging. Although the ultimate endpoints to assess the efficacy of pharmacological correction would be the benefits upon the clinical phenotype, there is no available methodology to detect the presence of normal (or corrected) CFTR at the membrane in living organisms.

Molecular Imaging can be the solution, since it allows the in vivo non-invasive visualization of a target molecule by virtue of its interaction with an imaging probe. Single-photon emission computed tomography (SPECT) and positron emission tomography are the most sensitive imaging modalities available, and allow early disease diagnosis and follow up of therapy. So, the aim of this work is the development of non-invasive radiolabelled imaging probes for the detection of CFTR at the plasma membrane of human cells. The probe reported in this communication was based on a CFTR inhibitor known to interact specifically with CFTR at the region of the channel pore.

The ^{99m}Tc radioisotope, used in SPECT, was the chosen radionuclide due to its physical properties, low cost and easy availability. The CFTR inhibitor was radiolabelled with the *fac*-$[^{99m}Tc(CO)_3]^+$ core, using the bifunctional chelator (BFC) approach. This strategy involved a three-component system constituted by a high affinity biomolecule ($CFTR_{inh}$), a radiometal (99mTc) and a BFC, designed to bind both to the radiometal and the biomolecule. Cellular studies in human bronchial epithelial cells expressing wt-CFTR were performed and the amount of radiolabelled probe that can interact with CFTR assessed. To evaluate if the metal complex of $CFTR_{inh}$ still maintained its ability to interact with CFTR, a non-radioactive surrogate rhenium complex was synthesized and its inhibitory efficacy was assessed through a functional assay in cells expressing CFTR. In the future, these types of probes may have the potential to be used on SPECT imaging to assess early therapy response in drug evaluation.

Acknowledgments: This work was funded by grant EXPL/BIM-MEC/0115/2012 (FCT, Portugal). FCT is also acknowledged for the PhD Fellowship (SFRH/BD/38753/2007 to B Oliveira and for the FCT Investigator grant to F Mendes. The authors would like to thank the *Cystic Fibrosis Foundation Therapeutics* for providing the CFTR inhibitor through the Chemical Compound Distribution Program.

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DNA binding and cytotoxic activity of Zn(II) and Cu(II) complexes with new bis(thiosemicarbazone) derivatives

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Bis(thiosemicarbazone) complexes of Zn(II) and Cu(II) have received considerable attention in the design of metallodrugs, either as anticancer therapeutics or diagnostic radiopharmaceuticals. Moreover, the availability of several medically relevant copper radioisotopes (e.g. ^{62}Cu, ^{64}Cu and ^{67}Cu) makes them potentially useful tools for cancer theranostics, profiting from a versatile chemical modification of the bis(thiosemicarbazone) framework and a stable coordination of radiocopper ions. The mechanism involved in the anticancer activity of Zn(II) and Cu(II) bis(thiosemicarbazonates) is not fully understood. However, it has been shown in a few studies that there is an accumulation of the complexes in the nucleus of tumor cells, indicating that DNA could be a potential target of their action. In this context, we have embarked in the synthesis of Zn(II) and Cu(II) complexes with new bis(thiosemicarbazone) chelators, symmetrically functionalized with protonable cyclic amines of the piperidine and morpholine type (Fig. 1). We have hypothesized that the presence of the cyclic amine groups could enhance the DNA affinity of the compounds and, together with the planarity of the metallic center, could promote some selectivity towards different types of DNA (e.g. G-quadruplex *vs.* duplex DNA). In this communication, we report the DNA binding and cytotoxic activity studies of these new M(II)-bis(thiosemicarbazone) complexes, performed with the aim of assessing their usefulness in the design of metal-based drugs for cancer theranostics.

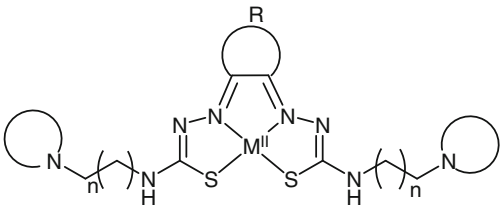

Fig. 1

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P 87

Biological evaluation of zinc(II) chlorido complexes with O^6-substituted 9-deazahypoxanthine derivatives

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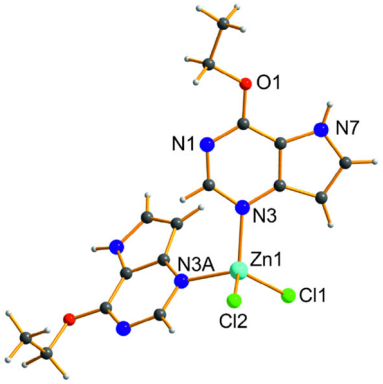
In this work, a series of new zinc(II) complexes with the general formula $[Zn(L_n)_2Cl_2]$ involving O^6-substitued 9-deazahypoxanthine derivatives (L_n) were biologically evaluated for their in vitro cytotoxic and immunomodulating activity. The present study showed that the compounds exhibited no cytotoxic effect on the human prostate (PC3, LNCaP), ovarian carcinoma (A2780) and monocytic leukemia (THP-1) cancer cell lines up to the concentration of IC_{50}>50 μM, and IC_{50}>10 μM, respectively. The effect of these complexes to influence the activity of inflammatory-related zinc-dependent matrix metalloproteinase (MMP-2) and to affect the secretion of pro-inflammatory cytokine IL-1β was determined using the lipopolysaccharide-activated macrophage-like THP-1 cell model. The ability of the complexes to attenuate IL-1β production was not observed. On the other hand, these complexes were able to increase the total amount of MMP-2 protein and significantly elevate the level of the active form of this protease. Increased activity of MMP-2 could be beneficial during wound healing, rheumatoid arthritis or repairing of injured nervous system due to the ability of this protease to remove some pro-inflammatory cytokines, promote neovascularisation and organise tissue remodelling [1–3].



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P 88

Interaction of azide with human neuroglobin and H64Q mutant investigated by solution NMR and molecular dynamics simulation

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Neuroglobin (Ngb), a new member of hemoprotein family, can reversibly bind some small ligands and take part in many biological processes such as signal transduction and hypoxic adaptation. Ngb presents hexacoordinated heme, either in ferrous or ferric forms, having the distal His64(E7) as the internal sixth ligand of heme iron. To explore the mechanism of exogenous ligand binding to hexacoordinated Ngb and the effect of distal key residue His64 on the binding property, two proteins including HNgb and the mutant [H64Q] HNgb were used in the present work, and their interactions with azide were studied by solution NMR. Further, the protein conformation was analyzed through molecular dynamics (MD) simulation. The results showed that the mutant protein had better binding affinity with azide than that of wild type HNgb, which revealed that the mutation of His64 influence the binding property of exogenous ligand. Comparing ^{1}H NMR spectra of HNgb (a), $HNgbN_3$(b), [H64Q] HNgb (c), [H64Q] $HNgbN_3$ (d), [H64Q] MNgb (e) [1], [H64Q] MNgbCN (f), MNgb (g) and MNgbCN (h) [2] may find that different exogenous ligands result in remarkable pseudo-contact shifts of heme methyl protons, implying distinct magnetic axial orientation in these proteins and corresponding complex systems. In accordance with those from NMR observation, the influences of both azide binding and H64Q mutation on heme electronic structure and heme pocket conformation were reflected by the MD analysis as well.

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P 89

pH-Specific structural speciation studies in biologically relevant binary Cr(III)-hydroxycarboxylic acid systems

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Chromium is abundantly present on the earth's crust. Its use includes a) industrial processes in tanneries, cement industries, plating and alloying industries, corrosive paints [1], doping [2] of advanced materials for the modification of the efficiency and lifetime of the photorefractive signals (i.e. the "memory"-type signals in connection with hologram recording) [3], heterogeneous catalysts, electrochromic devices and, more recently, in gas sensors [4], and b) direct or indirect involvement in plants, animals, and humans [5].

In a widely diverse coordination environment, through which Cr(III) develops its activity, a field of avid research activity has emerged. In this field of research, the role(s) of Cr(III) in biological systems is inevitably associated through its aqueous chemistry with lipids, proteins, and amino acids free in the cytosol or as components of peptides and lipid membrane structures. In all such cases, soluble and bioavailable forms of Cr(III) promote (bio)chemical activity, thus reflecting the complexity of the aqueous speciation in binary and ternary systems present in biological media. In view of chromium's involvement in cellular processes, thereby directly or indirectly affecting the physiology of organisms with often deleterious consequences, research efforts have targeted a) the aqueous structural speciation of Cr(III), with metal-complexing carboxylate-containing low molecular mass physiological ligands, and b) the study of the physicochemical properties of arising species potentially bioavailable and eliciting interactions with cellular biotargets.

In our quest to probe and comprehend interactions of chromium with ligands often involved in chemistries of toxic and biologically significant processes, we have looked into the aqueous structural speciation of the binary system of Cr(III)-heida (2-hydroxyethyliminodiacetic acid). Synthesis in aqueous media led to the isolation of three new complexes. The complexes were characterized by elemental analysis, spectroscopic, structural, thermal, EPR and magnetic susceptibility studies. The physicochemical properties of the new species project the fundamental features of the interaction of Cr(III) with (O,N)-containing bio-substrates potentially involved in toxic effects manifested at the cellular level.

Financial support "IKY Fellowships of Excellence for Postgraduate studies in Greece—Siemens Program" is gratefully acknowledged.

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P 90

The therapeutic properties of $VO(dmpp)_2$ as demonstrated by in vivo studies in type 2 diabetic GK rats

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This work aims at confirming the therapeutic properties already demonstrated by the bis(1,2-dimethyl-3-hydroxy-4-pyridinonato)oxidovanadium (IV), $VO(dmpp)_2$ [1,2] in non-obese type 2 diabetic Goto-Kakizaki (GK) rats. An in vivo study was carried out, treating Wistar (W) and GK rats during 21 days with a daily dose of $VO(dmpp)_2$ (44 μmol/kg). This study showed that, $VO(dmpp)_2$ does not affect the normal increase of body weight of both W and GK rats, after 8 days of treatment ameliorates glycaemia in GK rats (8.4 ± 0.3 mM *vs* 10.1 ± 0.2 mM in GK control, $P < 0.001$) but does not interfere with glucose levels in W rats. Moreover, after 21 days of treatment, it improves the glucose intolerant profile of GK rats (13.1 ± 0.5 mM/min vs 20.6 ± 0.7 mM/min in GK control, $P < 0.001$), despite no increase of plasma insulin levels during oral glucose tolerance test (OGTT). The glucose intolerance is also ameliorated in GK rats submitted to an acute treatment (single dose of 44 μmol/kg 30 min before the glucose load) although better results were obtained with the chronic one. Western blotting was used to clarify the mechanism of action at the molecular level, and the results revealed that in W and GK rats $VO(dmpp)_2$ significantly promotes IRS2 ($P < 0.05$) and p-AKT expression ($P < 0.001$ and $P < 0.05$, respectively, relative to the respective controls) and in the diabetic animals reduces the increase of PTP1β expression ($P < 0.001$, relative to GK treated with placebo).

The anti-diabetic properties of $VO(dmpp)_2$ showed in GK rats through its effects on glycaemia and OGTT and explained by its interaction with proteins of the insulin signaling cascade [3] corroborate previous data and suggest the possible use of this compound in the therapy of type 2 diabetes.

Financial support by Swedish Research Council, Swedish Diabetes Association and Karolinska Institutet is acknowledged as well as the synthesis of $VO(dmpp)_2$ by João Costa Pessoa and his Post-doc student Somnath Roy at the Technical University of Lisbon, Portugal. J.Pelletier was supported by a Föreningen för diabetesforskningens främjande grant and N.Domingues by an Erasmus grant during her stay in Karolinska Institutet.

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P 91

Synthesis, spectral characterization and antimicrobial activity of Co(II) and Hg(II) complexes of 1,3-bis(1*H*-benzimidazol-2-yl)-2-oxapropane

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Bis-benzimidazoles are strong chelating agents coordinating through both of the C=N groups nitrogen atoms. In addition, the benzimidazole ring system is present was clinically approved anthelmintics, antiulcers, antivirals and antihistamines [1]. Recently, there have been reports on benzimidazole derivatives exhibiting antitumor and antimicrobial properties and acting as thrombopoietin receptor agonists [2,3].

In this study, $Co(NO_3)_2$ and $Hg(NO_3)_2$ complexes of 1,3-bis(1*H*-benzimidazol-2-yl)-2-oxapropane were synthesized and characterized by elemental analysis, molar conductivity, magnetic moment, TGA, FT-IR, ESI–MS, fluorescence spectroscopy. The complexes are 1:1 electrolytes and they have 1:1 M:L ratio. According to the magnetic moment data ($\mu_{eff} = 1.26$ BM) there is M–M interaction in the Co(II) complex. The complexes fluoresce although weaker compared to the ligand.

In addition, antibacterial activities of the compounds were evaluated using the disk diffusion method against six bacteria and *Candida albicans*. The Hg(II) complex shows superior activity toward *S. epidermidis* and *E. coli* whereas the $Co(NO_3)_2$ complex, $[Co(\mathbf{L})(NO_3)(H_2O)_2]NO_3$ (Fig. 1), showed weak activity toward all of the microorganisms.

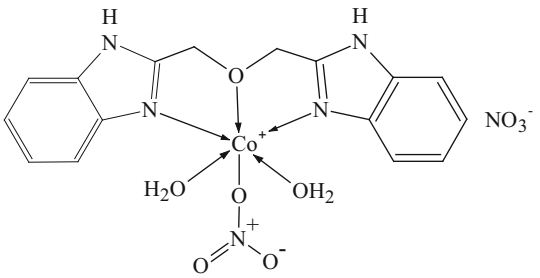

Fig. 1. The suggested structure of the Co(II) complex.

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P 92

New photoactivatable CO-releasing molecules of manganese with imidazoline/benzimidazole ligands

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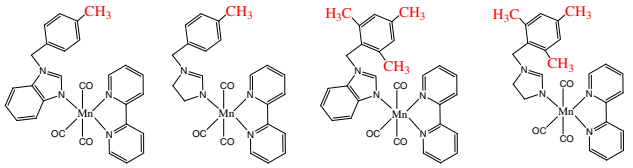
Metal carbonyl complexes are the systems of choice to exploit the beneficial effects of CO for therapeutic applications in medicine [1]. Also photoactivatable CO-releasing molecules (photo-CORMs) are important part of these researches [2]. Imidazolines/benzimidazoles are such bioactive compounds with anti-inflammatory and anti-hypertensive action [3].Therefore; we combined these two kinds of bioactive molecules and synthesized four molecules shown in Scheme. The selected molecules allow of making a comparison with CO-releasing properties of imidazoline and benzimidazoles.

All molecules were fully characterized by NMR, IR MS and elemental analysis. Currently we are investigating the long-term stability, electronic absorption spectra and CO-releasing properties of these complexes.



This work was supported by "The Scientific and Technological Research Council of Turkey (TÜBİTAK) within project 112T320".

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P 93

Antiproliferative effect of polyoxometalates is induced by ROS-mediated DNA damage in human cancer cells

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Polyoxometalates (POMs) are discrete oligomeric anions of early transition metals, such tungsten (W), molybdenum (Mo) and vanadium (V) oxides. Various biological effects of POMs have been studied, like their antitumor, antiviral and antibacterial activities [1]. We studied the mechanism involved in the antitumoral effect of two polyoxometalates, $(NH_3^iPr)_4[Mo_8O_{26}]$ (OCTA) and the commercial polyoxometalate $(NH_4)_6[Mo_7O_{24}]\cdot 4H_2O$ (HEPTA) [2] in a variety of cancer cell lines.

The POMs have been tested against five types of human cancer cell lines and the results showed that POMs promoted morphological changes and repressed the proliferation of all the cancer cell lines studied in a dose-dependent manner. We have observed that these POMs dramatically induced cell cycle arrest in G2/M phase and caused a remarkable DNA damage, DNA double-strand breaks (DSB) which are potentially lethal lesions for the cells. We found an increase of histone H2AX phosphorylation (γH2AX) after treatment, which provides a sensitive probe of the induction of DSBs in nuclear foci, and DNA fragmentation, analyzed by Comet assay. POMs treatment also led to an increased intracellular reactive oxygen species (ROS) formation.

These results show that POMs induce an increased in intracellular ROS formation, that causes oxidative stress and DNA damage, which leads to G2/M cell phase arrest and proliferation inhibition. These observations provide an evidence for a new anticancer mechanism of POMs and suggest a potential therapeutic strategy, targetly the dysfunctional redox regulation in cancer cells. This therapy may tackle the classical problem of intra-tumor heterogeneity and be widely applicable to a wide range of tumors.

Financial support by Universidad Francisco de Vitoria is gratefully acknowledged.

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P 94

Synthesis and kinetic of metalation of 2,3,7,8,12,13,17,18-octakis(propyl), *N*,*N*,*N*',*N*'-tetramethylamino porphyrazines and 2,3,9,10,16,17,23,24-octa substituted phthalocyanine

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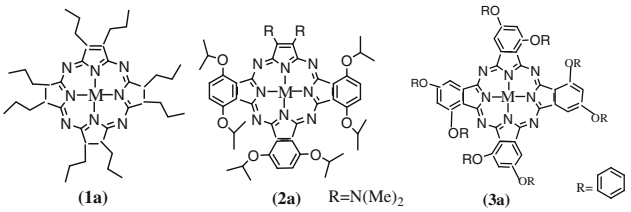
Tetrapyrrole macrocyclic compounds such as phthalocyanine and porphyrazines and their metal complexes have found usage in medicine as potential photosensitisers for photo dynamic therapy in the treatment of cancer and tumour cells, as well as biomedical imaging agent [1]. Tuning of their properties may be achieved by the modification of their peripheral substituents and the incorporation of varied metal ions in their central cavity [1]. Peripheral functionalities may be symmetrical or unsymmetrical. Unsymmetrical substituents include the porphyrazine-phthalocyanine hybrid which combines the electronic character and extended π electron system of porphyrazines and phthalocyanines [2].

In this regard three tetrapyrrole macrocyclic compounds 2,3,7,8,12,13,17,18-octakis(propyl)porphyrazine **1a**, *N*,*N*,*N*',*N*'-tetramethylamino porphyrazine hybrid **2a** and 2,3,9,10,16,17,23,24-octa substituted phthalocyanine **3a** were synthesized and characterized using elemental analysis, FTIR, ^{1}H, ^{13}C NMR and UV–Vis spectroscopic techniques. Kinetics of their metalation with Cu(II) and Co(II) was studied and are been reported for the first time. It is suggested that deformation of the ring, which is a function of their peripheral functionalities, is essential for effective coordination of the ligands.



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P 95

Assessment of the anti-cancer activity of the copper complexes with imidazole and pivalato ligands

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Despite the development of science and technology progress so far there have not been found effective drugs for cancer. Many coordination compounds were investigated due to their antitumor potential. The most known cis-diamminedichloroplatinum(II) is used as anti-cancer therapeutic agent. The copper complexes are the coordination compounds possessing anti-tumour properties. Their capability to kill cancer cells is mainly linked to the induction of oxidative stress [1]. Among various copper complexes tested as potential anti-cancer drugs, strong antitumor activity was found in the bis(acetato)bis(imidazole)copper(II) complex. In the 50 % inhibition dose (ID_{50}) of the cell growth tests using the mouse cancer cell line B16 melanoma, the cytotoxic effect of this compound (20 ng/mL) was equivalent to that of the therapeutic drug cis-DDP (8 ng/mL) and better than that of mitomycin C (100 ng/mL) [2]. Previously investigated copper complexes with imidazole ligand showed moderate, concentration dependent cytotoxic effects [3]. We have synthesized and investigated the anti-cancer effect of copper (II) complex with (4(5)-methylimidazole) and pivalato ligands. The compound is more lipophilic than bis(acetato)bis(imidazole)copper(II) so we have expected greater cytotoxic activity than in case of previously investigated compound. The investigation has been limited due to small solubility of the examined compound in ethanol. The effect of the compound on cell line growth is considerable.

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P 96

Antitumor potential of copper complexes with mitochondrion as the cellular target

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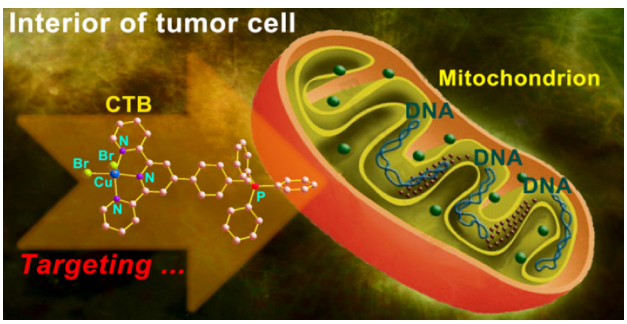
Copper complexes are promising antitumor agents for their redox properties and low toxicity. In this study, the antitumor potential of copper(II) complexes derived from triphenylphosphonium derivatives was investigated. Triphenylphosphine (TPP) was introduced into the complexes for its mitochondrion-targeting ability and lipophilic character. The complexes are able to cross the cytoplasmic and mitochondrial membranes of tumor cells and influence the mitochondrial membrane potential more keenly than anticancer drug cisplatin. The cytotoxicity of the complexes was tested on MCF-7, HeLa, Skov-3, A549 and cisplatin-resistant A549R tumor cells. The complexes are more cytotoxic against these cells than cisplatin; particularly, they are highly effective against cisplatin-resistant tumor cells. The complexes interact strongly with DNA via an intercalation stabilized by electrostatic force, and display a significant cleavage activity towards supercoiled pBR322 DNA and cellular DNA through an oxidative mechanism. The cytotoxicity of the complexes seems to arise from a multiple mechanism of action, including the modification of DNA conformation, generation of reactive oxygen species, scission of DNA strands, and dissipation of mitochondrial membrane potential. This study demonstrates that copper complexes with mitochondrion-targeting group could be efficient antitumor agents free of drug resistance to cisplatin.



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P 97

Cobalt-coordination and oxidation state in a heterobimetallic complex

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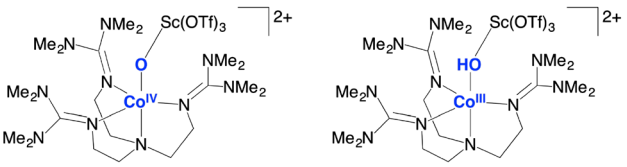
In 2010, a new iron-oxygen species was reported [1], in which an iron-oxygen complex was capped by a Sc^{3+}-moiety. This new species represents a series of complexes where a Lewis acid is binding to an metal-bound oxygen or nitrogen. Through extensive molecular modelling [2] of Fe^{IV}-oxo, Fe^{III}-oxo and Fe^{III}-hydroxo complexes and the new species, it was shown unambiguously that the oxidation state of iron in this Lewis-acid capped metal-bound oxygen system is +3, coinciding with water as secondary axial ligand to scandium. Here we report our results [3] for a related heterobimetallic complex where two proposals [4,5] have been brought forward for the oxidation state of cobalt and its coordinating ligands (see Figure). We have studied both of them and other possible scenarios in the same rigorous computational setup.



Proposal 1: *ACIE* **2011**, *50*, 1711 Proposal 2: *JACS* **2012**, *134*, 17526

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P 98

Peptide-based chelating drugs in diagnosis and treatment of Alzheimer disease

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Alzheimer's disease (AD) is a devastating neuro-degenerative disorder characterized by the progressive and irreversible loss of memory followed by complete dementia. Despite the disease's high prevalence and great economic and social burden, an explicative aetiology or viable cure is still not available. Currently available therapeutics for AD only alleviate its symptoms [1]. The AD-affected brain suffers from metal-ion homeostasis (metallostasis), resulting in redistribution of metals into inappropriate compartments. This metal disorder gives rise to the production of amyloid-β aggregates (SPs) and oxidative stress, which are two associated signs of AD pathology. To date, all clinical trials targeting amyloid β have failed; however, some clinical trials targeting metal interactions with amyloid β have all shown benefit for patients [2]. In addition, recent data indicate that metals play a role more upstream in the disease process than previously thought and might provide new targets for pharmacotherapy [3]. Consequently, targeting metals probably represents a tractable avenue for an AD-modifying therapy.

In this context, early detection of copper ion and the recuperation of its normal trafficking in the brain represent an attractive new therapeutic approach in AD. With the aim of developing biocompatible chelating drugs with sensing properties, we present the synthesis of a series of natural and non-natural fluorescent peptides and preliminary studies on their binding and selectivity towards copper(II).

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P 99

The impact of synthetic analogs of histidine on copper(II) coordination properties to an albumin-like peptide

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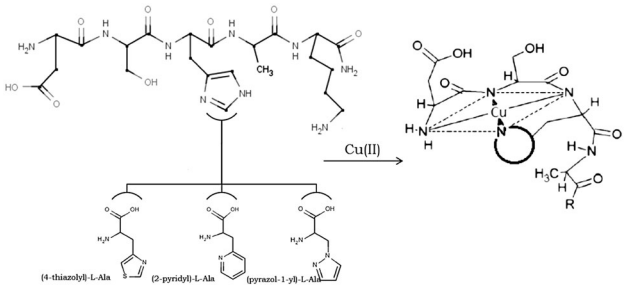
The purpose of our project was to obtain peptidomimetics possessing Cu(II) binding properties, which would be useful for biomedical applications. In this context we used potentiometry, molecular modelling, UV–Vis and CD spectroscopies to characterize the Cu(II) binding properties of pentapeptide analogs of the N-terminal sequence of histatin 5. The peptides investigated had a general sequence DSXAK-am (am stands for N-terminal amide), with X including His and its three synthetic analogues, (4-thiazolyl)-L-alanine (1),(2-pyridyl)-L-alanine (2), and (pyrazol-1-yl)-L-alanine (3). The heterocyclic nitrogens present in these analogs were significantly more acidic than that of the His imidazole.

We found that DSXAK-am peptides were able to bind Cu(II) and form 4 N complexes in a cooperative fashion, with similar affinities. These results indicate that acidic heterocyclic amino acids provide a viable alternative for histidine in peptidomimetics designed for metal ions binding.



This work was supported in part by the project "The impact of synthetic modifications of the histidine side chain on coordination properties of analogs of the N-terminal pentapeptide of Histatin 5 towards copper(II) ions." (Grant No. DEC-2012/05/N/ST5/01499) financed by National Science Centre.

P 100

Probing Cu^{+} binding properties of two toxic mutants of the human prion protein fragment

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Prion proteins are responsible for the transmissible spongiform encephalopathies (TSEs), which is a group of fatal and infectious neurodegenerative diseases that affect human and diverse animal species [1]. Prion diseases result in associated with accumulation of abnormal protein aggregates in diffuse synaptic plaques that result in neurodegeneration [2]. Prion protein (PrP) exist in at least two conformational states, the normal cellular form (PrP^{C}) and an abnormal infective form (PrP^{Sc}) having higher content of β-sheet structure than the native protein [3]. Human Prion Protein ($hPrP^{C}$) is able to sequester transition metal ions like Cu^{2+} with binding atoms allocated within octarepeat domain, a part of unstructured N-terminal domain or outside it to His-96 and His-111 residues of so called "toxic" domain [4,5]. Study on some electrochemical reports suggested that Cu(I) binding site was located in the region between residues 90 and 114, and X-ray techniques were employed to show that Cu(I) was coordinated via S, N and O ligands [6]. Using spectroscopic techniques find that the $PrP_{91–124}$ region encompassing His-96 and His-111 can bind a Cu(I) ion in a favourable tetrahedral environment comprising His-96, His-111, Met-109 and Met-112, but mutation of histidine residues reduce the Cu(I) affinity of PrP fragment [7]. Two peptidic analogues of the $PrP_{91–115}$ fragment were synthesized with His-96 ($PrP_{91–115}$ $H_{111}A$) and His-111 ($PrP_{91–115}$ $H_{96}A$) residues substituted by alanine residues. In this work we have used Ag(I) to probe the interactions of Cu(I) with peptidic analogues of histidine residues. Our aim was to evaluate the binding mode, coordination geometry and affinity of the studied analogues.

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P 101

Binding of Cu^{2+} to the islet amyloid polypeptide (IAPP)

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Metal ions may induce protein aggregation and amyloid formation associated with several medical disorders, for example Alzheimer's disease [1,2]. Similarly, islet amyloid polypeptide (IAPP) may be involved in the etiology of type II diabetes, as amyloid plauqes rich in IAPP are often found in the pancreas of type II diabetics [3]. However, the knowledge compiled on metal ion–IAPP interactions [4–7] is much less than for A-β and Alzheimer's disease. In contrast to human IAPP (hIAPP), the corresponding peptide from rat (rIAPP) does not appear to aggregate, probably due to the differences in the amino acid sequences displayed below. In terms of metal ion binding capacity, the R18H substitution from rIAPP to hIAPP implies that hIAPP may display stronger binding of metal ions such as Zn^{2+} and Cu^{2+}, and this indeed appears to be the case for Cu^{2+} [4], although literature data are scarce. In this work we aim to explore the fundamental properties of the interaction of Cu(II) with IAPP, i.e. to determine the dissociation constant of the metal ion–peptide complex, and to identify amino acids coordinating to the metal ion at physiological pH, with a particular focus on the role of His18. To this end we compare the binding of Cu^{2+} to rIAPP and the R18H variant of rIAPP.

hIAPP	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	H	S	S	N	N	F	G	A	I	L	S	S	T	N	V	G	S	N	T	Y
rIAPP	K	C	N	T	A	T	C	A	T	Q	R	L	A	N	F	L	V	R	S	S	N	N	L	G	P	V	L	P	P	T	N	V	G	S	N	T	Y

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Islet amyloid polypeptide (IAPP)—Structural effects of Zn^{2+}

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Aggregation of Islet amyloid polypeptide (IAPP), a peptide hormone secreted by the pancreatic β cells, cause increased β cell apoptosis and loss of pancreatic mass, together with the buildup of amyloid deposits, in type 2 diabetes[1]. As binding of metal ions may induce aggregation and oligomerization of several other amyloidogenic proteins, among them amyloid β peptides [2,3] it is of interest to investigate the structural effects of metal ions on IAPP. We investigate the effects of the Zn^{2+} ion, being highly prevalent in vivo, with concentrations ranging up to mM during co-storage with IAPP in the β-granules prior to secretion [4]. The effects of Zn^{2+} on IAPP fibrillation have been described previously [5,6], though much remains to be elucidated regarding the specifics of the metal ion–peptide interaction. We explore the structural effects of Zn^{2+} through a CD spectroscopic analysis. Also, via NMR spectroscopy the role of individual side-chains for the metal ion interaction is explored. Previous investigations on human IAPP indicated His 18 as important for IAPP's ability to bind Zn^{2+} [5]. Here, In order to work with a non-fibrillating system, we as an initial study work with the truncated rat IAPP 9–37 sequence, comparing it with the corresponding R18H variant.

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Metal ion catalyzed oxidation of a human prion fragment

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Metal-catalyzed oxidation (MCO) can lead to damage of bio-molecules and this is implicated in oxidative stress, biological aging and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease [1]. MCO of proteins is mainly a site-specific process in which only one or a few amino acids at the metal-binding sites of the protein are preferentially oxidized. The amino acid residues of histidine and methionine have been proposed to play important roles in metal mediated oxidative stress. Histidine oxidation predominantly forms oxo-histidine [2], methionine oxidation forms methionine sulfoxide and, under extreme conditions, sulfone [3].

Oxidation of Hu-PrP(103–112) fragment (Ac-SerLysProLys ThrAsnMetLysHisMet-NH_2, SKPKTNMKHM) and its systematically synthesised mutants (SKPKTNAKHA, SKPKTNAKHM, SKPKT NMKHA) were studied. Potentiometric and spectroscopic techniques (UV–Vis, CD and EPR) were used to study the speciation, and bonding details of copper(II) complexes, the oxidation of the peptide in the presence of copper(II) ions were studied by HPLC–ESI–MS.

Only 1:1 complexes are formed at any copper(II) ion to ligand ratios. The histidine residue is the anchoring binding site and the successive deprotonation and coordination of amide functions takes place toward the N-termini. In the case of peptides containing Met109, the thioether donor atom of methionine residue may be equatorially involved in the binding. Cu(II)/hydrogen peroxide system as oxidizing agent were applied at pH 7.4 where 2 N and 3 N complexes are formed. Oxidation of the peptides leaded the cleavage of peptide bonds. Oxidation products were identified by MS/MS.

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Cu ligands with high selectivity over Zn to combat Alzheimer's disease

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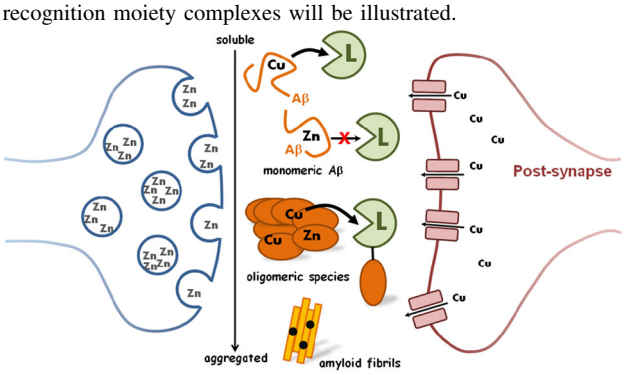
The aetiology of Alzheimer's disease is linked to the aggregation of the amyloid-β peptide, a central event of the so-called amyloid cascade. The intervention of Cu and Zn ions in the amyloid cascade is widely acknowledged. Cu can (i) form oligomeric aggregates considered as the most toxic species of the aggregation process and (ii) be involved in Reactive Oxygen Species (ROS) production due to its redox ability [1]. Because these two effects are deleterious, Cu is a target of choice for therapeutic approaches based on chelation [2,3]. Recent results on Cu(II) [4,5] and Cu(I) removal and impact on ROS production and Aβ aggregation will be described as well as the interplay of Zn in such processes [6]. Several strategies such as Cu(II) and Cu(I) ligation or use of bi-functional ligands encompassing an Aβ recognition moiety complexes will be illustrated.



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