BioMet 14

XIV PharmacoBioMetallics

Pisa, 24-25 ottobre 2014

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Volume a cura di Michelangelo Scopelliti

Sommario

Plenary Lecture

Copper in Myocardial Regeneration

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Depressed activity of hypoxia-inducible factor (HIF)-1 in myocardial ischemic infarction, although it is activated in the early response to ischemia, is a key factor for the pathogenesis. Copper is required for HIF-1 activation and under ischemic conditions copper effluxes from the heart. In an attempt to examine the efficacy of localized copper supplementation on myocardial regeneration, we produced a monkey model of myocardial ischemic injury by coronary artery ligation. After the establishment of myocardial infarction, an ultrasound contrast copper-loaded microbubble targeted delivery procedure was conducted to supplement copper specifically to the infarct area of the myocardium. This treatment significantly increased the density of blood vessels, and importantly, caused regeneration of the infarct area, as determined by increased myocardial cells, ventricular wall thickening, and recovery of contractility. The expression of vascular endothelial growth factor was significantly increased and the activation of HIF-1 was observed. In addition, stromal cell-derived factor-1 was overexpressed along with increased accumulation of endothelial progenitor and hemotoprogenitor cells. There were no adverse effects of the copper-microbubble on cardiac structure or function, as monitored by morphology and electrocardiogram. This study thus demonstrates that targeted delivery of copper to the copper depressed infarct area of the myocardium significantly activates HIF-1 transcriptional activity and promotes the recovery of myocardial infarction. This procedure is highly applicable to human patients with ischemic heart disease.

Comunicazioni Orali

Biomineralizzazione e biocristallografia

Higher Crystallization Success Obtained for TTR-ligand Complexes Using a New Approach

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Transthyretin (TTR) is a homotetrameric protein that transports thyroxine and retinol, through its association with retinol binding protein, in plasma and cerebrospinal fluid. Recently TTR has been classified as a metallopeptidase with an inducible active site. [1] Under certain conditions it aggregates to form fibrils associated with TTR amyloidosis. The X-ray analysis of the complex between the protein and the small molecules can be a valuable method to obtain useful information that can guide the design of new compounds able to stabilize the protein. Unfortunately, for some ligands, the commonly used crystallization precipitants, high ionic strength salts, may be poorly adapted to give crystals of certain complexes of interest. We report here the results from the use of a new simplified procedure developed to obtain well diffracting crystals of TTR ligand complexes without the need of a cumbersome soaking step. [2] The use of polyethylene glycol instead of ammonium sulphate or citrate has been evaluated as an alternative to obtain new TTR complexes with good results both with natural and synthetic compounds. [3] This new approach avoids soak periods as long as 5 weeks with a 10-fold molar excess of the ligand to achieve full saturation of both binding sites. [4] This method uses commercial protein and crystallization screens, yields crystals that are isomorphous with those for other complexes grown with ammonium sulphate or citrate based precipitants, within 3 days using a small amount of protein.

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Nuovi farmaci inorganici in oncologia e malattie vascolari

Mechanistic Thermodynamic and Kinetic Studies to Enlighten the Details of Small Molecules Binding to Biosubstrates

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Non-covalent interaction between planar molecules and nucleic acids (NA) takes place mainly through two processes denoted as intercalation and groove binding. The interest for these molecules is very high owing to their applications in biochemistry, biology and medicine. Actually, small NA-binding molecules can be used both as staining agents and probes for NA as well as antitumour, antivirus and antibacterial drugs. In this context, a remarkable role is played by metal complexes and metallo-intercalators. [1,2,3]

The knowledge of the details of the binding mode of the small molecules to NA is crucial to understand the effects that can be exerted on those biological processes where nucleic acids are involved. The analysis of the thermodynamic and kinetic aspects of the interaction can provide important information for a deep understanding (and optimization) of the binding process. [4,5] This presentation will focus on the strength of a coupled thermodynamic and kinetic study to enlighten the details of the interaction. In particular: 1) repetition of binding experiments under different experimental conditions (reactant concentrations, temperature, added salt concentration) gives information on the exact type of binding; 2) the kinetic technique enables to characterize the steps of the mechanism of binding to the nucleic acid; 4) reaction forward and backward rates, equilibrium constants, ΔG, ΔS and ΔH can be obtained; 5) the site size (n) can be known where n is defined as the number of ligand molecules per site under saturation conditions; 6) the binding mode can be evidenced by viscometric experiments and fluorescence quenching experiments; 7) breaking of the phosphodiester bond can be put into evidence by gel electrophoresis experiments.

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Possible Use of Hydroxyapatite Nanocrystals in the Delivery of Phosphaplatins

Michele Benedetti;[a] Federica De Castro;[a] Chiara R. Girelli;[a] Danilo Migoni;[a] Alessandro Romano;[a] Tiziano Verri;[a] Marco Lelli;[b] Norberto Roveri;[b] Francesco P. Fanizzi.[a]

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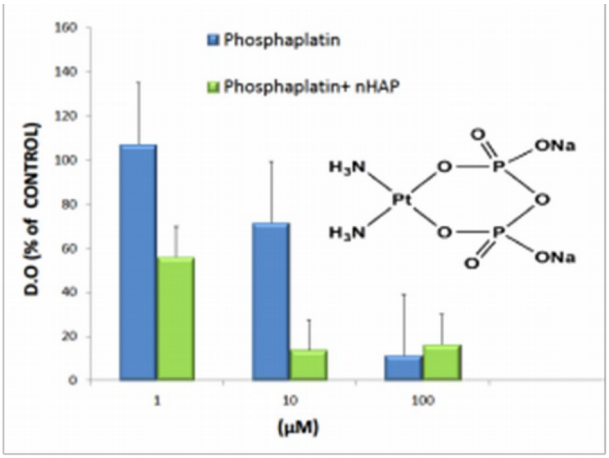
Platinum based anticancer drugs, e.g. cisplatin, carboplatin, and oxaliplatin, are widely used for the treatment of a variety of cancers. Moreover, a significant percentage of tumors become resistant to the treatment with classical platinum drugs. This constitutes one of the most important problems, generally leading to a poor survival rate of treated patients. [1]

Recently, phosphaplatins, monomeric Pt complexes with phosphates, were studied for their strong antitumor activity, even on cisplatin resistant cell lines. [2] Previous works have shown that generally hydroxyapatite, $[Ca_5(PO_4)_3(OH)]$, nanocrystals (nHAP) can be internalized by tumor cells, by endocytosis, and that some antitumor drugs can be adsorbed on nHAP. [3] In this context we studied the modulation of *in vitro* cytotoxicity of a model phosphaplatin, *i.e.* $Na_2\{cis\text{-}[Pt(NH_3)_2(P_2O_7)]\}$, 1, tested on HeLa cells, by MTT assay, adsorbed on nHAP, see Figure. The adduct 1-nHAP showed a significant increase of cytotoxicity with respect to complex 1 or nHAP alone, suggesting the activation of alternative mechanism(s) of uptake and/or cytotoxicity induction, due to the combined action of 1 and nHAP, see Figure.



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New Antiproliferative Platinum(II) Complexes Based on Imidazole Moiety

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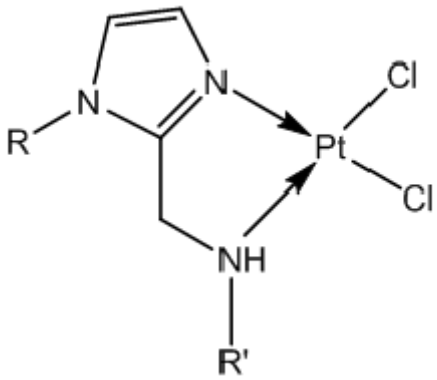
The synthesis of a series of Pt-complexes chelating diamines derived from variously substituted 2-methylaminoimidazoles was realized. The different substituents on the basic amines of the imidazole ring and of aliphatic chain were carried out with the aim of either modulating the so called "trans-effect", which the activation of the platinum complexes is strictly related to, or improving the lipholicity. The initial screening study about Pt-compounds cytotoxicity on different cancer cells lines revealed, among the different platinum complexes, Pt-4a as the lead compound. [1]



On the basis of these results in order to increase lipophilicity and the consequent solubility of Pt(II) complexes across biological membranes it was then decided to introduce at the N1 of the imidazole moiety differently-long saturated and unsaturated aliphatic chains. Moreover, a comparison with cisplatin uptake mechanism was developed by using the octapeptide Mets7 [2] as a probe to investigate the interaction of the synthesized platinum compounds with the N-terminal domain of Ctr1.

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Biological Activity of Bis(carboxylato) Cisplatin-based Pt(IV) Prodrug Candidates: How Long the Axial Ligands Should Be?

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The pharmacological properties of the Pt(II) antitumor drugs may be improved exploiting Pt(IV) complexes, *i.e.* prodrugs activated *in vivo* in the hypoxic, reducing and acidic tumor environment, that converts the inert octahedral Pt(IV) compounds into their active square planar Pt(II) metabolites. The choice of the axial ligands is essential to modulate the water solubility, the lipophilicity (and the related cellular uptake) and the redox properties of these complexes. Despite the growing interest, a limited number of consistent information about their cellular uptake and DNA platination is available. The amount of Pt-DNA adducts is a consequence of an intricate equilibrium between intracellular influx, kinetics of Pt(IV) reduction and aquation of the Pt(II) metabolites, efflux and DNA interaction. To investigate these relationships, the cellular accumulation ratio (AR), the DNA platination and the antiproliferative activity of a series of cisplatin-based Pt(IV) prodrug candidates, *trans*,*cis*,*cis*-[Pt(carboxylato)$_2$Cl$_2$(NH$_3$)$_2$], where carboxylato = $CH_3(CH_2)_nCOO^-$ (n = 0, 2, 4, 6; Figure 1), were studied.

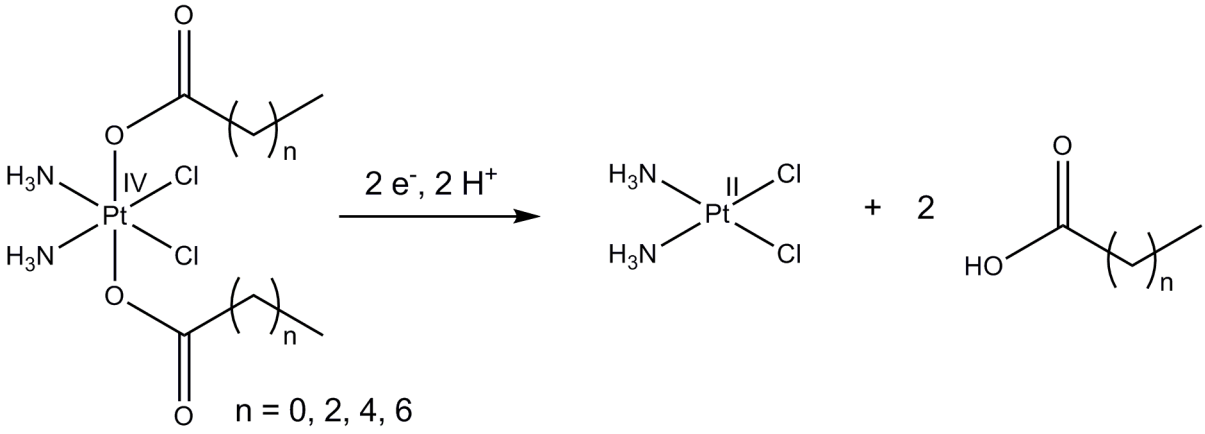

Figure 1. Activation by reduction of bis(carboxylato) cisplatin-based Pt(IV) prodrugs

The AR of these Pt(IV) complexes increased with lipophilicity (*i.e.* with the chain length) and remained almost unchanged during the recovery period in fresh complete medium, at least for the most lipophilic compounds. DNA platination increased with AR and lipophilic Pt(IV) complexes continuously platinated DNA even during the recovery.

Moreover, the cytotoxicity was evaluated on tumor cells following different treatments. Also in this case the recovery time was almost unimportant for the lipophilic Pt(IV) complexes. Finally, the effect of the Pt(IV) complexes on multicellular tumor spheroids, that allow prolonged treatments *in vitro*, was also evaluated: the Pt(IV) prodrug candidates exerted a prolonged antiproliferative action even when the drug was removed from the culture medium. [1]

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Further Insight into Iodido Platinum Complexes as Potential Anticancer Drugs

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Cisplatin is a leading anticancer drug in wide clinical use for the treatment of several types of malignancies. Since the discovery of CDDP, research has been largely focused on the characterization of its DNA-adducts according to the concept that DNA is its primary biological target. Yet, nucleobases are not the only targets for this drug; its interaction with proteins is underscored by a number of studies that include the determination of the complexes between this important molecule and several proteins. [1,2] Despite its huge clinical success, CDDP still presents many drawbacks such as intrinsic resistance, acquired resistance during the treatment and several, heavy side effects.

In this frame iodido analogues of CDDP represent an interesting family of new potential anticancer drugs with a non-conventional mode of action. Our study started with the characterization of the parent complex *cis*-$Pt(NH_3)_2I_2$. Based on the results previously obtained by ESI-MS, UV-Vis and X-ray crystallography, showing that *cis*-$Pt(NH_3)_2I_2$ forms stable adducts with model proteins, [3] herein we moved to extent studies on this class of compounds investigating also the correlation existing between the cytotoxic effect produced by *cis*-$Pt(NH_3)_2I_2$ and its analogues, and the nature of the amine ligand. A number of unexpected results were obtained and their implications evaluated to elucidate in more detail the reactivity and the mechanistic aspects of this novel class of promising anticancer platinum complexes.

Acknowledgments

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New Heterodimetallic Gold(I)-Platinum(II) Compound as Potential Anticancer Agents

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Following the success of platinum based anticancer complexes, several other metal compounds based on ruthenium, gold, titanium, copper, have been prepared and evaluated as experimental anticancer agents. It is now well established that the reactions of anticancer metallodrugs with protein targets, are of paramount interest; these interactions might feature processes that are crucial for the biodistribution, the toxicity, and even the mechanism of action of this important group of anticancer agents.

Various tactics and some new approaches have been employed to improve the physicochemical and biological properties of metal complexes. A relatively new concept in medicinal inorganic chemistry is represented by the chance of joining two or more bioactive metals within the same molecular entity. Accordingly, a number of polynuclear platinum, ruthenium and gold compounds have been developed and biologically characterized with some interesting outcomes. [1-3]

A few examples derived from the research activities carried out in MetMed lab in Florence will be illustrated. Particular attention will be paid to the design, preparation and characterization of a novel heterodimetallic complex containing a platinum(II) center coupled to a gold(I) center.

The cytotoxic properties of the compound was evaluated *in vitro* against A2780 ovarian cancer cells.

The ultimate aim of these studies is to elucidate the cooperative effects of metals when associated in the same molecular entity in order to improve the activity as antitumor agents, to obtain multiple biological targets, to improve the affinity and specificity of compounds towards targets.

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Protein Targets for Anticancer Metallodrugs

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Following the clinical success of platinum based anticancer complexes, several metal compounds based on different metal centers were prepared and evaluated as prospective anticancer agents. Beyond their interactions with DNA, it is now well accepted that reactions of anticancer metallodrugs with protein targets are of great relevance to define the overall pharmacological and toxicological profile of this group of compounds. The focus of this presentation will be on the "protein metalation" processes. Strategies developed in the MetMed Laboratory, in Florence, during the last years to characterize the resulting metallodrug-protein adducts at the molecular level will be described. In particular, major achievements were obtained through joint implementation of ESI mass spectrometry and X-ray diffraction methods. [1-3] On the other hand, strategies based on metalloproteomics and metallomics will be presented that may be valuable to elucidate in more detail the mechanism of the cytotoxic actions.

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Antimony Bisthiosemicarbazone Complexes for Medical Applications: a Preliminary Study

Poster

Giorgio Pelosi;[a] Franco Bisceglie;[a] Guido Catina;[a] Rossella Alinovi;[b] Silvana Pinelli;[b] Charlotte Sommer;[c] Christine McKenzie.[c]

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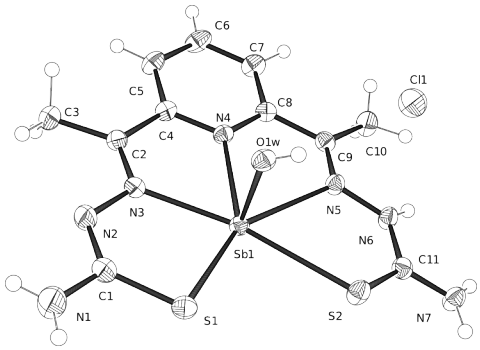
The development of metal-based radiopharmaceuticals is a dynamic and steadily growing research area. Two antimony isotopes (namely ^{119}Sb and ^{117}Sb) have recently been suggested as radionuclides for therapy [1] The first one decays by electron capture emitting low energetic Auger electrons and is suitable for radionuclide therapy. The other isotope, ^{117}Sb, decays by EC and β^+ emission and can be used for Single Photon Emission Computed Tomography (SPECT) imaging. [2] For diagnostic or therapeutic medical applications, the metal ion must not be released from the carrier ligand through which it is introduced into the human body and the complex stability must be guaranteed by a high denticity and by a negative charge on the ligand.

Thiosemicarbazones, in addition to their versatile chelation properties, present also biological properties. In particular, one of the most interesting effects exerted by their metal complexes on many cell lines is selective proliferation inhibition. This capacity makes them interesting potential antitumor, antibacterial, antiviral and antimalarial drugs. [3]

In this contribution we report the syntheses, characterizations and structures of a series of antimony coordination compounds obtained starting from antimony(III), chloride and acetate, using 2,6-diacetylpyridine bisthiosemicarbazone and 2,6-diacetylpyridine bis(N^4-phenylthiosemicarbazone) as ligands, with the aim to verify if these molecules can be used as antimony carriers or anticancer compounds. For the two most soluble compounds, a preliminary test of proliferation inhibition on leukemic cell U937 was also carried out.



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Au(III)-dithiocarbamato Complexes Loaded in Targeted Sterically Stabilized Micelles as Anticancer Agents

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Cisplatin is one of the most efficient anticancer drug used in the clinics so far. It is highly effective against a number of human tumors, especially testicular and ovarian cancer. Anyway, its application is limited due to severe side effects as well as the problem of developing drug resistance, which is very common during cisplatin treatment. These limitations have motivated an extensive research towards the design of alternative platinum or non-platinum based drugs with less side effect, and in the development of new drug delivery systems for improving drug efficacy, reducing unwanted side effects and circumventing cellular accumulation mediated drug resistance.

Among new metal-based anticancer agents, Gold(III) dithiocarbamato complexes have proved to be very promising drugs for their biological behavior notwithstanding their low water solubility. [1,2] Pure sterically stabilized micelles (SSM) of DSPE-PEG2000, and sterically stabilized mixed micelles (SSMM) containing PC or DOPC phospholipids (5, 10 or 20% mol/mol with respect to DSPE-PEG2000) are developed as delivery systems for the gold based cytotoxic drug Au(III)-dithiocarbamato complex AuL12. The effect of PC or DOPC phospholipids on the AuL12 loading and on the physicochemical properties of micelles was also investigated. The gold complex remains stable up to 72 h when incorporated in the aggregate, as indicated by UV–vis measurements. Target-selective micelles were also prepared by adding a small amount of the amphiphilic peptide MonY-BN-AA1 in micelle composition. BN-AA1 peptide is an analogue of [7-14]Bombesin able to selectively target GRP receptors, overexpressed by several cancer cells, such as prostate cancer and ovarian cancer cells. [3]

Preliminary *in vitro* cytotoxicity studies on PC-3 cells overexpressing GRP receptors show a decrease of cell viability, ~50%, is obtained in cells treated with AuL12-targeted micelles at 10 μM drug concentration for 48 h with respect to untargeted micelles. [4]

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[Pt(O,O'-acac)(γ-acac)(DMS)]: *in vivo* Studies of a Promising Anticancer Drug in Cancer Therapy

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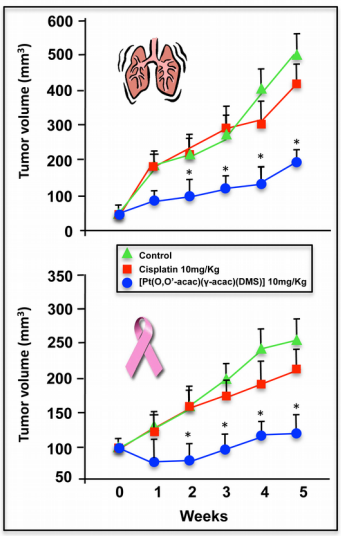
Despite the wide use of cisplatin in oncology, it is associated with significant dose-limiting toxicities including nephrotoxicity and neurotoxicity. There is correspondingly a clear incentive to develop new strategies for safer and more effective platinum drugs-based therapies.



SCID mice carrying mesothelioma or breast carcinoma received intravenous Pt(O,O'-acac)(γ-acac)(DMS)] or cisplatin.

A new platinum drug for non genomic targets, [Pt(O,O'-acac)(γ-acac)(DMS)] was specifically designed and synthetized by some of us [1] to overcome the cisplatin related problems. [2] This compound has recently gained increasing attention as potential anticancer agent because of its high and selective cytotoxicity towards cancer, as observed in immortalized cell lines and confirmed in breast cancer cells in primary cultures. [2-6] The selectivity of [Pt(O,O'-acac)(γ-acac)(DMS)] stimulates a more detailed study aimed at pre-clinical investigation of its therapeutic potential in vivo. In this context, we employed a preclinical model based on the subcutaneous injection of MCF-7 breast cancer and ZL55 malignant pleural mesotelioma cell lines in SCID mice. Remarkably, [Pt(O,O'-acac)(γ-acac)(DMS)] stands out for higher anticancer activity than cisplatin toward both the murine tumor models examined, inducing up to 50% inhibition of tumor growth. We also demonstrated enhanced in vivo pharmacokinetics (PK), biodistribution and tolerability of [Pt(O,O'-acac)(γ-acac)(DMS)] when compared to cisplatin administered in Wistar rats. PK studies with [Pt(O,O'-acac)(γ-acac)(DMS)] revealed prolonged Pt persistence in systemic blood circulation and decreased nephrotoxicity and hepatotoxicity, two major target sites of cisplatin toxicity.

Altogether, these findings suggest that [Pt(O,O'-acac)(γ-acac)(DMS)] is a promising therapeutic agent for preventing growth of cancer, thus providing a solid starting point for its validation as a suitable candidate for further pharmacological testing.

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Radiofarmaci nella diagnostica e terapia tumorale

In vitro Targeting and Imaging the Translocator Protein TSPO 18-kDa Through new Coordination Complexes of Transition metals

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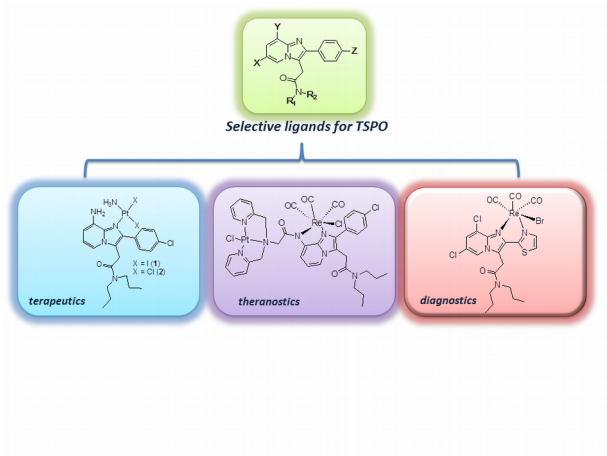
Among the intracellular organelles, mitochondria represent an attractive subcellular target due to their function particularly important for oxidative damage, calcium metabolism and apoptosis. Therefore, it is not surprising that damage to mitochondria can contribute to various human disorders. However, for several reasons, including the lack of identification of suitable outer mitochondrial membrane (OMM) biomarkers, the targeting of mitochondria has been a neglected area. Discovered in 1977 as an alternative binding site in the kidney for the well known benzodiazepine diazepam, the translocator protein (TSPO), previously known as peripheral-type benzodiazepine receptor, is an 18-kDa high affinity cholesterol- and drug-binding protein found mostly in the OMM as part of a mitochondrial cholesterol transport complex. In normal conditions, TSPO is present minimally in healthy human brain and liver; vice versa, TSPO reaches high levels in steroid synthesizing and rapidly proliferating tissues, and its biological role has been mainly linked to mitochondrial function, steroidogenesis and cell proliferation/apoptosis. Aberrant TSPO levels have been linked to multiple diseases, including cancer, endocrine disorders, brain injury, neurodegeneration, ischemia-reperfusion injury and inflammatory diseases. Thus, TSPO has become an extremely attractive subcellular target not only for imaging disease states overexpressing this protein, but also for a selective mitochondrial drug targeting. [1] Although a wide number of TSPO ligands have been synthesized, only few of them have the ability to deliver metal-based drugs. In particular, very recently we reported some potent and selective imidazopyridine-based TSPO ligands, which can carry both a cytostatic platinum species and a rhenium complex as a model of ^{99m}Tc imaging agent. [2-5] In the compounds so far investigated, atoms already present in the TSPO ligand were used as donors for anchoring the metal core. A further development is represented by the use of conjugates in which the TSPO-targeting moiety is covalently linked with an appropriate chelating system, such as di(2-picolyl)amine, forming a strong coordination compound with metal ions in the pertinent oxidation state. [6,7] The goal of these studies is to provide new TSPO ligands that can be used for preparing coordination complexes of a metallo drug to be employed in diagnosis and therapy.



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Ruolo degli ioni metallici nelle patologie degenerative croniche

Modulation of Ubiquitin Interaction with Metal Nanoparticles: Implication for Health Impact of Nanotechnology

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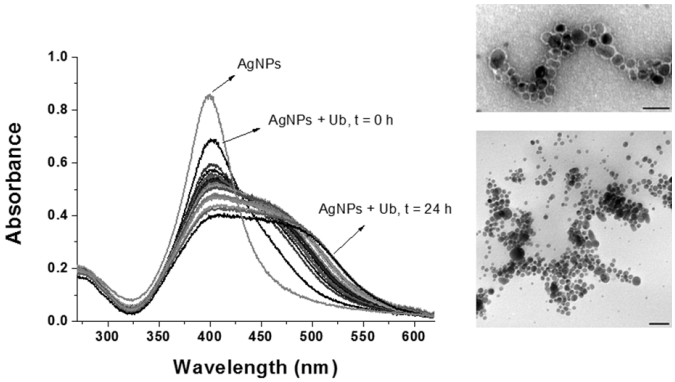
The interest in nanomaterials stems from the dependence of their properties on particle size and shape at the nanoscale. Furthermore, the 1-100 nm scale is critical for the interaction with the biological environment. It is not surprising that the biomedical applications of nanomaterials are increasing [1] and the Food and Drug Administration has already approved nanotechnology-based drugs.[2,3] Although the interaction of nanoparticles (NPs) with proteins has emerged as a key issue in addressing nanotoxicity, [4] information about the effects of nanomaterials on biological systems are rather scarce. Several studies show that, once entered in biological systems, NPs immediately encounter the abundant plasma proteins which form the so-called protein corona. [5] This process is governed by molecular interactions between the NP surface and the amino acid residues of the protein. [6] When proteins interact with NPs, their native conformation can be altered and new epitopes can be exposed on the surface, giving rise to unexpected biological responses. [7]

We investigated the interaction of silver nanoparticles (AgNPs), produced by laser ablation with human ubiquitin (Ub), a protein essential for degradative processes in cells. [8] The surface plasmon resonance peak of AgNPs indicates that Ub is rapidly adsorbed on the AgNP surface yielding a protein corona; the Ub-coated AgNPs then evolve into clusters held together by an amyloid form of the protein, as revealed by binding of thioflavin T fluorescent dye. Transthyretin, an inhibitor of amyloid-type aggregation, [9] impedes aggregate formation and disrupts preformed AgNP clusters. In the presence of sodium citrate, a common stabilizer that confers an overall negative charge to the NPs, Ub is still adsorbed on the NP surface, but no clustering is observed. Ub mutants bearing a single mutation at one edge β strand (*i.e.* Glu16Val) or in loop (Glu18Val) behave in a radically different manner. [10]



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Structural Characterization of Cu(I)-β-Synuclein Interactions

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Synucleins (α-synuclein (αS), β-synuclein (βS) and γ-synuclein (γS)) are a family of intrinsically disordered proteins, that are involved in numerous neurodegenerative pathologies (αS and βS), as well as in various types of cancers (γS). [1] αS has been widely studied because of its neurotoxic role in Parkinson's disease, in addition the inhibitory effect of wild-type βS on αS aggregation, was discovered several years ago. [2] It is well accepted that Cu(II) and Cu(I) ions play a critical role in the aggregation process of synucleins and might represent the link between the pathological mechanism of protein aggregation and oxidative damage. [3] Many efforts were applied to understand copper (I)/(II) interaction with αS, [4-6] on the other hand very little is known about βS. In this work the characterization of Cu(I) binding to the N-terminal region of βS has been obtained by means of different spectroscopic techniques. The metal coordination spheres of the two proteins (αS and βS) have been also compared.

Acknowledgments

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System biology per lo studio dei metalli

Multifunctional Glycoderivatives of Carnosine: Metal-binding and Functional Characterization

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Carnosine is an endogenous dipeptide widely and abundantly distributed in muscle and nervous tissues of numerous animal species. Many functions have been proposed for this compound, such as antioxidant, antiaggregant, antiglycating agent and metal ion-chelator, especially for copper(II) and zinc(II). The administration of carnosine provides benefits in Alzheimer's disease and other neurodegenerative disorders. However, the main limitation on therapeutic use of carnosine on pathologies related to increased oxidative stress and/or metal ion dyshomeostasis is associated with the hydrolysis by the specific dipeptidase carnosinase. Several attempts have been made to overcome this limitation. The glycoconjugation has been found to be a promising approach to protect the dipeptide moiety in this respect. A number of glycoside derivatives of carnosine have also been characterized in terms of their binding features for copper(II). [1-4] Here, we report the structural and functional characterization new carnosine derivatives with linear sugars such as trehalose, a multifunctional sugar tested for the treatment of Huntington's disease, Parkinson disease and several tauopathies. The copper(II) binding properties, as well as the antiaggregant and antiglycating actions make the new carnosine conjugates suitable for the treatment of a wide class of degenerative disorders.

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Copper(II) Ions and Angiogenin: Mutual Interaction in Human Endothelial Cells

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Angiogenin (ANG) is a member of the ribonuclease family that act as a potent angiogenesis stimulator, and interacts with endothelial cells inducing a wide range of responses (angiogenesis, cells proliferation, cells migration and pro-survival effects). It is known that vascular pathologies are present in neurodegenerative diseases and Angiogenin is down-regulated in Alzheimer and Parkinson diseases, as well as it has been found as one of the mutated genes in amyotrophic lateral sclerosis (ALS). [1]

In the complex puzzle of neurodegenerative disease onset the metal ions dyshomeostasis emerged as a key actor. In particular, copper has been implicated directly or indirectly in the pathogenesis of numerous neurological diseases and has been also involved in angiogenesis processes. [2] Copper (II) induces an increase of Angiogenin binding to endothelial cells [3] but, so far, the relationship between copper-Angiogenin and angiogenesis induction remain unclear. Thus, the effects of copper (II) ions on Angiogenin activity and expression were evaluated.

In the present study, the binding of copper was demonstrated to affect the intracellular localization of Angiogenin decreasing its nuclear translocation. Moreover, the copper (II)-Angiogenin complex negatively affects the protein-induced angiogenesis, as well as endothelial cells migration. Surprisingly, copper also reveals the ability to modulate the Angiogenin transcription. These results highlight the tight relationship between copper and Angiogenin, pointing out the biological relevance of ANG-copper complex in the regulation of endothelial cell function, and revealing a possible new mechanism at the basis of vascular pathologies.

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Sessione Poster

Diagnostici innovativi in oncologia e malattie cardiovascolari

FTIR Characterization of Nasal Polyps Lesions

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Nasal polyps are polypoidal masses arising mainly from the mucous membranes of nose and paranasal sinuses. They are freely movable and non-tender overgrowths of the mucosa that frequently accompany allergic rhinitis. Usually they show an inflammatory nature, but sometimes they can evolve to tumoral pathologies, with different degree of malignancy. In these cases, there is an objective difficulty in distinguishing, with routinely immunohistochemical assays, inflammatory zones from benign and malign neoplasia.

On continuing our research on oral cavity lesions, selected sections of benign, inflammatory and dysplastic nasal polyps have been analyzed by using FPA FTIR Imaging Spectroscopy, and compared with ones of palate and maxillary carcinoma. The multivariate analysis (HCA and PCA) carried out on the raw data of each section afforded to segregate different kinds of epithelium (transitional, respiratory, dysplastic and tumoral) according to the nature of the lesion, as suggested by the histopathological investigation. Their average spectra have been used as loading standards in custom maps to reconstruct the topological distribution and to confirm the results from the histopathological analysis.

Definite band area ratios have also been checked, to define specific spectral markers for a rigorous and objective characterization of the development of this pathology. In particular, meaningful biochemical modifications have been detected in tumoral samples, as confirmed by the following findings: (a) a more permeable plasma membrane with an increase in the acyl chain peroxidation (3013/2959, ν = CH/ν_{asym} CH_3); (b) an enhancement of transcriptional cellular activity (1121/1020, νC-O RNA/νC-O DNA); (c) an higher of carbohydrate consumption (1045/1545, glycogen/AII) and (d) a decreased transducing signals (1516/1240, tyrosine/ν_{asym} PO_2-) due to an increase of tyrosine phosphorylation.

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Metalloproteine come catalizzatori biologici

2-(Phenylsulphonamido)-Pyrazole Derivatives as Carbonic Anhydrase Inhibitors

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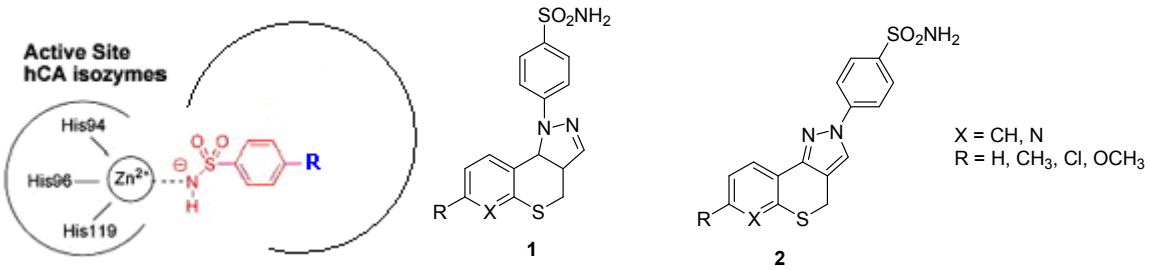
Carbonic anhydrases (CAs, EC 4.2.1.1) are a superfamily of metalloenzymes which catalyze the interconversion between CO_2 and bicarbonate by using a metal hydroxide nucleophilic mechanism. Five genetically distinct CA families are known to date, the α-,β-,γ-,δ-, and ζ-class enzymes. CAs are ubiquitous isozymes involved in crucial physio-pathological events, and represent the targets of inhibitors with several therapeutic applications (obesity, epilepsy, glaucoma, and tumours). [1]

Recently we described a new class of CA inhibitors, based on the thiopyrano-fused pyrazole scaffold featuring a N1-pendant 4-sulfamoylphenyl moiety. Many new compounds showed excellent inhibition profiles against pharmacologically relevant isoforms. [2]

In this study small libraries of pyridothiopyranopyrazoles (**2**, X = N) and benzothiopyranopyrazoles (**2**, X = C) were developed to investigate the effect on the CA inhibitory activity of shifting the sulphonamide moiety from the 1- to the 2-position of the core scaffold. Synthesis and biological results will be discussed.



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Structural Basis for the Rational Design of New Antibrucella Agents

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Brucella is a causative agent of brucellosis, which is the most widespread zoonosis worldwide. In humans there are three pathogenic *Brucella* species, *Brucella suis*, *B. abortus* and *B. melitensis.* [1] They are intracellular pathogens that can survey and multiply within the phagocytic cells of the mammalian host. The pathogen is capable of establishing persistent infections in humans which are difficult to eradicate, even with antibiotic therapy.

The enzyme hystidinol dehydrogenase (HDH) is essential for intramacrophagic replication. [2] In particular, it is a zinc-enzyme which catalyzes the last two steps in the biosynthesis of L-histidine: sequential NAD-dependent oxidations of L-histidinol to L-histidinaldehyde and then to L-histidine. During evolution the HDH sequence has been well conserved and since it is absent in mammals, has become a novel target for the development of anti-*Brucella* agents.

To date, only the enzymes from *Salmonella typhimurium,* [3] *Escherichia coli* [4] and *B. suis* [5] have been cloned, whilst the *E. coli* HDH is the only enzyme of this family which has so far been structurally characterized. [4]

Aiming at the development of a strategy targeting *B. suis* virulence, we have focused our attention on HDH from *B. suis* (BsHDH). In particular, here we report the crystallographic structure of a mutated form of BsHDH both in its unbound form and in complex with a nanomolar inhibitor. [6] These studies provide the first structural background for the rational design of potent HDH inhibitors, thus offering new hints for clinical applications.

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Structural and Inhibition Studies of Carbonic Anhydrase Inhibitors Containing the Sulfamide Zinc Binding Group

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Carbonic anhydrases (CAs) are ubiquitous metalloenzymes, which catalyze the reversible hydration of carbon dioxide to bicarbonate ion and proton. These proteins are present in prokaryotes and eukaryotes, and are encoded by five evolutionarily unrelated gene families. [1] Human CAs are widely distributed in many tissues and organs. Since at these sites CAs play a crucial role in various physiological processes, they have recently become interesting targets for pharmaceutical research. Indeed, several CA inhibitors (CAIs) incorporating a sulfonamide/sulfamate/sulfamide moieties are currently clinically used for the treatment or prevention of a multitude of diseases such as glaucoma, solid tumors, and epilepsy. [2] However, most of the CAI based drugs present various non-desired side-effects, mainly because of their lack of selectivity for the different CA isoforms. Thus, the identification of selective CAIs is one of the main purpose for the development of new pharmacological agents. Here we report a new series of compounds containing a sulfamide moiety as zinc-binding group (ZBG) (Figure 1).

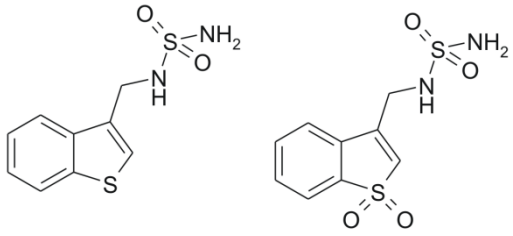

Figure 1. Schematic representation of chemical structures of CA inhibitors 1-2

These compounds have been synthesized and tested for determining their inhibitory action against human CA I and II. The X-ray structures of isoform hCA II in complex with I1-I2 have also been solved providing further insights into sulfamide binding mechanism and confirming that such ZBG, if conveniently derivatized, can be usefully exploited for obtaining new effective and selective CAIs.

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Asymmetric Imine Reductase Based on Human Carbonic Anhydrase II as Host Protein

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In the context of dative anchoring strategies, [1-5] hCAII is an attractive protein scaffold for the creation of artificial metalloenzymes for the asymmetric transfer hydrogenation of imines, using aryl-sulfonamide-bearing IrCp* pianostool complexes. [6,7]

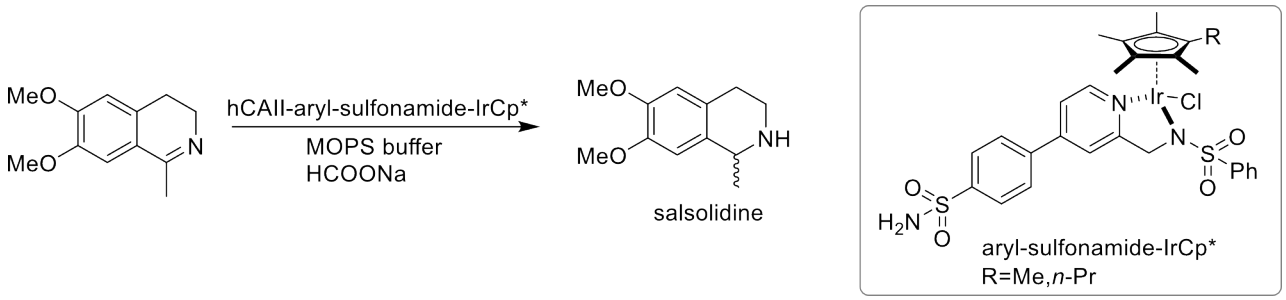

Fig.1 Artificial transfer hydrogenase for imine reduction and iridium cofactors used in the study.

Guided by the X-ray structure of complex [(η^5-Cp*)Ir ⊂ WT hCA II] (PDB ID 3ZP9), [7] a chemogenetic optimization strategy was used to improve activity and selectivity of the ATHase. Mutations around the putative catalytic site were introduced based on design models generated by means of the Rosetta design suite. This *in silico* screening identified 8 mutations (L60V-A65T-N67W-E69Y-Q92F-L140M-L197M-C205S) which were combined to afford a total of 50 hCA II mutants. The resulting ATHases showed significantly improved performance both in terms of activity and of selectivity: up to *ee* 90 (S) and TON up to 50.

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Biomineralizzazione e biocristallografia

An Advanced Material to Efficiently Crystallize Protein Molecules

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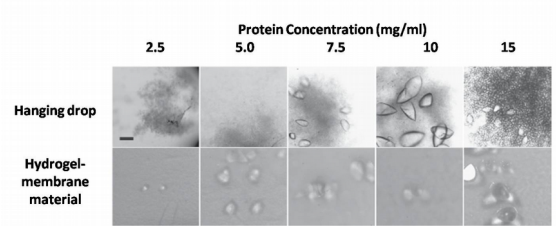
X-ray diffraction techniques allow obtaining of molecular structures at a resolution level at the moment not still surpassed by other techniques. However, obtaining crystal samples having quality suitable for diffraction experiment represents the bottleneck in the structure determination process, particularly in the case of protein molecules due to their weak intermolecular interactions, flexibility, and to the contribution of solvent on their aggregation. A turning point in this field could be represented by the use of new materials which could be able to extend the range of the experimental conditions able to trigger nucleation. [1] An example is represented by gel-mediated protein crystallization that is able to produce high quality crystals [2] that are particularly suitable for soaking experiments with drugs of foreign molecules due to their resistance to the osmotic stress. [3] However, the low consistency and fragility of the gel materials restricts the use of such technique for which a support for gel is required. Hydrophobic membranes have been already tested as support for protein crystallization, showing ability to modulate the solvent exchange and to trigger heterogeneous nucleation. [4] In addition, their flexibility makes them suitable as support for gels during crystallization experiments.



The fundamental idea of this study is to improve current approaches to protein crystallization by development a new material made of hydrogel and membrane able to combine the advantages of crystallization in gel with those of membrane-assisted crystallization. Crystallization by hydrogel-membrane material has been tested on two proteins, leading to crystals that grow at lower protein concentration and having improved diffraction properties than those produced by means of conventional technique. As prospective, the material can be optimized to promote membrane proteins crystallization, for biomineralization, and non-classical mesocrystal structures.

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Potential Applications of Drug/Calcite Hybrid Crystals: from Targeted Delivery Carriers to Active Scaffolds

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Calcium carbonate ($CaCO_3$) crystals show wide perspectives as smart carriers for drugs due to their capability to adsorb, and more importantly to entrap, molecules. Moreover, $CaCO_3$ is a biocompatible and biodegradable material, which preparation is easy, low cost, organic solvent-free and the size of the $CaCO_3$ particles can be easily controlled. [1]

CaCO3 solubility is pH-sensitive and the use of $CaCO_3$ crystals entrapping molecules allows their release only where the dissolution of crystal occurs. This carrier is particularly suitable for the selective release of drugs in tissues that are more acidic than normal physiological pH (tumors, inflamed tissues). The feasibility of such a system for anticancer therapy was tested *in vitro* by releasing the drug doxorubicin (DOX), an anthracycline widely used in chemotherapy, from calcite/DOX hybrid crystals.

In addition doped calcite hybrid crystals can be used to store efficiently unstable drugs. Minocycline, an anti-inflammatory drug sensitive to light, heat and pH, was incorporated in calcite crystals and its chemical properties remained unchanged for months.

In regenerative medicine molecule-doped calcite crystals can be used to create active scaffolds. $CaCO_3$ crystals entrapping retinoic acid (a differentiating agent) were synthesized. *In vitro* tests demonstrated that the controlled release of retinoic acid via crystal dissolution allowed the differentiation of stem cells into astrocytes.

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Delivery and *in vitro* Test of Platinum-based Antitumor Drugs by Hydroxyapatite Nanocrystals

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Calcium-containing matrices based on synthetic hydroxyapatite (HA) nanocrystals [1,2] have been used to load platinum(II)-bisphosphonate complexes specifically designed to act as prodrugs in the local treatment of bone tumors.

The inorganic matrix itself can activate the Pt-complexes into their active forms. The inorganic composite materials can be implanted locally, at the site of an osteosarcoma, after surgery and act both as bone substitutes and as platinum drug releasing agents. The final goal is that of inhibiting locally the tumour re-growth and of reducing the systemic toxicity typical of cisplatin and other platinum-based antitumor drugs. [3]

In the present work we have extended the investigation to nanocrystalline apatites which can be administered by injection. The role of the Ca/P ratio in influencing the superficial adsorption, as well as the release of the active platinum complexes from the nanocrystals, has also been investigated at different pH value. [4] In particular, we are interested in colorectal cancer, which is at the top of the list of the most common cancers worldwide with around 1 million new cases diagnosed every year. Presently, apart from oxaliplatin, there are no other metallo-drugs in advanced clinical development which appear to be active against colorectal cancer and that could be used for the treatment of patients with oxaliplatin-refractory colorectal cancer. We found that the oxaliplatin analog [$PtCl_2$(cis-1,4-DACH)] (kiteplatin) is very effective in circumventing cisplatin and oxaliplatin resistance in oxaliplatin-resistant colorectal cancer cells, [5] and in this work we have pursued the embodiment of [$PtCl_2$(cis-1,4-DACH)] (or its analogs) into HA nanocrystals to be delivered at colorectal cancer site.

Acknowledgments

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Multi-target-directed Ligands in Alzheimer's Disease Treatment

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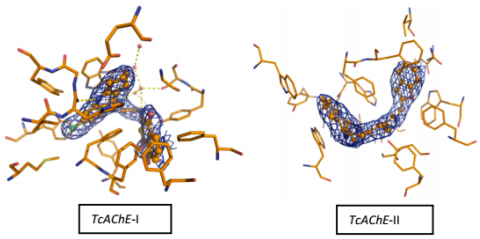
Among the various drug discovery methods, a very promising modern approach consists in designing multi-target-directed ligands (MTDLs). This methodology has been specifically developed for treatment of disorders with complex pathological mechanisms. One such disorder is Alzheimer's disease (AD), currently the most common multifactorial neurodegenerative disease. AD is related to increased levels of the amyloid β peptide (Aβ) and the hyperphosphorylated tau protein, along with loss of neurons and synapses. Moreover, there is some evidence pointing to the role of oxidative stress, metal ion deregulation, inflammation and cell cycle regulatory failure in its pathogenesis. There are many attractive targets for the development of anti-AD drugs, and the multi-factor nature of this disease calls for multi-target-directed compounds which can be beneficial for AD treatment. We report on the structure-activity relationships of two novel multitarget anti-Alzheimer compounds designed by combining a tacrine fragment and a juglone (I) [2] or benzofuran (II) function respectively with a linker of a suitable length. *In vitro*, both compounds displayed excellent acetylcholinesterase (AChE) inhibitory potencies and interesting capabilities to block amyloid-βaggregation. The X-ray analysis of the Torpedo californica AChE – inhibitor complexes allowed a structure-based rationale for the outstanding activity data.



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Physisorption of GHHPH Tetra Repeat of HPRG on hydroxyapatite nanocrystals as tunable angiogenic nanoplatform

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Histidine-Proline Rich Glycoprotein (HPRG) is a known modulator of angiogenesis, that is a major but transient event during the formation and repair of wound tissues. [1,2]

In a tissue-engineered scaffold the angiogenesis process can be triggered by the loading and/or the surface tailoring with growth factors, such as VEGF, FGF, BMP and/or genetically modified cells. Nevertheless, inorganic angiogenic regulators, such as copper ions, are of great interest, due to their low cost, higher stability, and potentially superior safety compared with recombinant proteins or genetic engineering approaches. [3]

This study tackles the fabrication and physicochemical characterization of hybrid bio-composite scaffolds based on nano-hydroxyapatite (nHAp), functionalized with both copper and the GHHPH peptide, that is the known tetra-repeat sequence in HPRG. [4]

Specifically, the nHAp nanocrystals were modified by physical adsorption processes of: i) Cu(II) ions, ii) fluorescein amidite-labeled tetra-repeat (GHHPH-FAM) and iii) GHHPH-FAM/Cu(II) complexes.

The functionalized composite scaffolds (nHAp/GHHPH-FAM, nHAp/Cu and nHap/GHHPH-FAM/Cu) were characterized by AFM, UV-Vis, fluorescence and FT-IR spectroscopies. Preliminary cell assays with neuroblastoma cells were carried out by confocal microscopy. The dynamic processes of cellular internalization were scrutinized in live imaging experiments by tracking the peptide fluorescent tag and copper chemosensor, respectively.

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Nuovi farmaci inorganici in oncologia e malattie vascolari

Cisplatin Handover from the Copper Transporter ATOX1 to ATP7A in Near Physiological Conditions

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A growing number of studies reveals that copper (Cu) transporters are involved in the biological response to antitumor platinum (Pt) drugs, which are among the most used chemotherapeutics. [1] The soluble chaperone ATOX1, which physiologically brings Cu(I) from CTR1 to the metal binding domains (MBDs) of Cu(I)-ATPases ATP7A and ATP7B, can bind cisplatin (cis-PtCl2(NH3)2) and participate to the intracellular distribution of Pt-drugs. [2] Also ATP7A and ATP7B can bind and efflux actively Pt-drugs through the vesicles of the trans-Golgi network, hence contributing to the development of tumor cell resistance. [3]

Here we report a tandem ESI-MS and NMR spectroscopic characterization of cisplatin binding to ATOX1 and MNK1, the first MBD of ATP7A. In the absence of any reducing agent, we found that Pt binds to the metal binding motif (CXXC) of both proteins, but with significant differences. MNK1 forms quite long lasting monofunctional adducts (*cis*-$[PtCl(NH_3)_2]^+$-MNK1) which evolve to bifunctional adducts (*cis*-$[Pt(NH_3)_2]^{2+}$-MNK1), while ATOX1 reacts slowly by forming directly bifunctional adducts (*cis*-$[Pt(NH_3)_2]^{2+}$-ATOX1). We also studied the reactivity of these proteins towards cisplatin in conditions mimicking the cellular environment, that is millimolar concentration of the physiological reducing agent glutathione (GSH). It was found that MNK1, but not ATOX1, competes successfully with GSH for binding to cisplatin. Finally, no transfer of cisplatin from ATOX1 to MNK1 occurs in our experimental setting. The latter result appears to be in contrast with literature data reporting the occurrence of such a transfer, although always an exogenous reducing agent, such as tris(2-carboxyethyl)phosphine (TCEP), exerting a strong *trans*-labilizing effect, was present. Our study highlights the complexity of Pt-loading reactions with a special focus on how different platinophiles can influence each other: only a careful molecular investigation of the speciation taking place under physiological conditions can clarify key issues, such as resistance of tumor cells to cisplatin. [4]

Acknowledgments

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A New Class of Unsymmetric Pt(IV) Antitumor Prodrugs

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The Pt(II) complexes are the most important drugs in anticancer chemotherapy. Since the discovery of cisplatin, several Pt(II) candidates have been studied. In recent years more attention has been paid to Pt(IV) complexes as anticancer prodrugs. In fact, this octahedral compounds can be reduced *in vivo* in the hypoxic, reducing environment of the tumour tissue. Their active square-planar Pt(II) metabolites through a two electron reduction which leads to loss of the axial ligands. Pt(IV) complexes produce fewer side reactions with biomolecules and exhibit greater chemical inertness than their Pt(II) derivates.

The choice of the ligands is essential to modulate their redox properties and lipophilicity (and related cellular uptake). Moreover these complexes can undergo further functionalizations useful for drug targeting and delivery strategies (DTD) through its axial ligand.

Pt(IV) complexes are usually prepared by oxidation (typically using hydrogen peroxide or chlorine) of the corresponding Pt(II) counterparts. A different way to oxidize the Pt(II) compounds is represented by the use of N-chlorosuccinimide as oxidant. This reaction can be carried out in different coordinating solvent (e.g. glycol) to obtain complexes with one axial chloride and one solvent molecule, in high yield and purity (Figure 1).

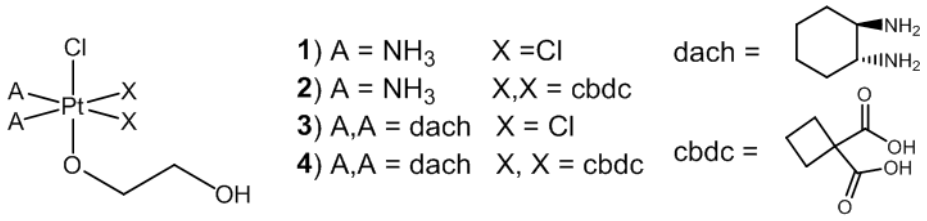

Figure 1. Pt(IV) complexes with axial glycol molecule.

Moreover the oxidative reaction can be conducted in a non coordinating solvent in presence of another nucleophile acting as ligand. The synthesis, characterisation and stability of the studied complexes is reported.

Finally, since the coordinated glycol may be used to link the complexes to a suitable vector for DTD, the coupling of the complexes with model molecules is also presented. [1]

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Chemistry and Biology of Two Novel Gold(I) Carbene Complexes as Prospective Anticancer Agents

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Two novel gold carbene compounds, namely, chlorido (1-butyl-3-methylimidazole-2-ylidene) gold(I) and bis(1-butyl-3-methyl-imidazole-2-ylidene) gold(I), were prepared and characterized as prospective anticancer drug candidates. These compounds consist of a gold(I) center linearly coordinated either to one N-heterocyclic carbene (NHC) and one chloride ligand or to two identical NHC ligands. N-Heterocyclic carbenes (NHCs) are very interesting gold(I) ligands as they manifest donor properties similar to phosphines, thus affording very stable gold(I) complexes; in addition, their imidazolium salt precursors are often more easily synthesized than similarly functionalized phosphines. The azoles and azolium salts used in the synthesis of NHCs are generally air stable species, and their synthesis and purification is, in most cases, relatively straightforward. Hydrophilic/lipophilic properties can be readily fine-tuned by the incorporation of appropriate functional groups.

Even though several studies have been carried out so far on the cellular effects of gold carbene compounds and valuable mechanistic information has been gathered, the precise mode of the gold carbene complex action, at the molecular level, is still unclear. This led us to prepare and characterize two novel gold carbene complexes and investigate in depth their main chemical and biological features through a variety of physicochemical and biochemical tests.

The obtained results support the view that the investigated gold carbene complexes work – most likely – through selective metalation of a few proteins bearing specific structural motifs for metal recognition.

Pt(IV) Derivatives as Dual Action Anticancer Agents

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Cisplatin, the keystone of metal-based antitumor drugs, is one of the most efficient anticancer agents used for the treatment of a great variety of solid tumors. Unfortunately, its effectiveness has been hampered by inherited setbacks, such as the unfavourable toxicological profile and the tumour resistance phenomena. Although huge efforts have been undertaken in the last four decades in order to develop innovative platinum anticancer drugs, the issue of reducing toxicity over normal cells and to widen the spectrum of action toward additional and refractory tumors has been only partially addressed. [1] More recently several research groups oriented their endeavors towards the development of Pt(IV) complexes. The chemical properties of octahedral Pt(IV) complexes offer a unique opportunity for the design of dual action pro-drugs. Pt(IV) complexes are kinetically inert and thermodynamically stable and are expected not to react with nucleophiles in the blood. They are however activated by reduction inside the cancer cells yielding the square planar cytotoxic Pt(II) complex as well as the two axial ligands. [2] Thus, a fine tuning of the axial ligands can confer favorable pharmacological properties to the drugs, allowing for the enhancement of cellular uptake and of the selectivity towards cancer cells.

In this perspective, we have prepared a series of Pt(IV) derivatives of cisplatin and oxaliplatin with in the axial positions two Histone deactylase (HDAC) inhibitors, *i.e.* valproate and 4-phenylbutyrate. All complexes were evaluated for their *in vitro* antitumor activity against a panel of human cancer cells, some of which suitably selected for their resistance to cisplatin. Furthermore, parameters such as cell accumulation, levels of platination of nuclear DNA, inhibition of HDAC activity and cell death induction mode were detected, in an attempt to gain insights into the mechanism of action of these dual action pro-drugs.

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Cell Uptake and Binding Studies of Some Copper Complexes

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Copper is the third most abundant transition metal in biological systems where it plays many crucial roles in a variety of biological processes essential for life. [1] Indeed, the ability of copper to cycle between its oxidized and reduced forms makes it a suitable cofactor for redox active metalloenzymes. [2] Nevertheless, when intracellular copper concentration is too high, the same redox properties can lead to cellular oxidative damage. [3] Interestingly, as a consequence of their altered metabolism, cancer cells show an enhanced copper uptake with respect to normal ones. This property prompted us to synthesize copper complexes with the aim to develop new potential antitumor agents.

In the present study we report the biological properties and the intracellular copper uptake of some Cu(II) dithiocarbamates designed as novel anticancer agents. These complexes show interesting antiproliferative activity on a panel of human tumor cell lines and demonstrate the ability to induce cell death through the apoptotic pathway. The intracellular copper uptake was demonstrated and quantified by ICP-OES technique on cancer cells incubated with different concentrations of copper complexes.

Moreover, cell binding studies were carried out with the aim to formulate a preliminary hypothesis on the interaction mode of examined complexes with the whole cell. For this purpose a binding model previously developed in our laboratory [4] was applied.

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Polymeric Cyclodextrin-based Nanoparticles as Carriers of the Pt(IV) Complex LA-12

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One of the most active area of pharmacological research is aimed to design new drug delivery systems (DDS) in order to decrease adverse effects of antitumor agents. [1] Among the wide set of different classes of molecules, cyclodextrins (CyD) are known to be particularly suitable for this purpose. This is due to their unique properties to protect drugs included in their cavity from physical, chemical, and enzymatic degradation. [2]

Here, we report the synthesis of a novel water-soluble amino β-CyD polymer (poly-β-CyD3NH2) through a multi-step reaction. Furthermore, we have functionalized this polymer with folic acid (FA) through an amide coupling reaction obtaining poly-β-CyD3FA to vehicle the nanocarrier to folate receptors (FRs) on the tumor cell surface. [3]

Moreover, to evaluate the targeting ability of the synthesized polymers, preliminary cell proliferation assays on different cancer cell lines have been performed using LA-12 [(OC-6-43)-bis(acetato)(1-adamantylamine)amminedichloroplatinum(IV)] as a cytotoxic agent. [4]

Results show that poly-cyclodextrin functionalized with folic acid improves the aqueous solubility of LA-12 and it could be used as a promising DDS in anti-tumor treatment of FR(+) cancer.

Acknowledgments
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Gold Complexes as Antimicrobial Agents

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The antimicrobial properties of gold and its compounds are known since the late XIX century, following the discovery of the antitubercular activity of $K[Au(CN)_2]$ by Robert Koch. Further studies on gold complexes showed that their antimicrobial action is generally very fast, and the short exposure time required should avoid the development of some resistance among various sensitive bacteria and yeasts. Nowadays, resistance phenomena to available antibiotics represent a crucial issue in view of the lack of promptly available alternatives, and, in this framework, gold based compounds could be a convenient opportunity, as they may act on non-classical targets of microbial cells. Furthermore, reactivity, stability and toxicity of the metal centre can be easily tuned by the choice of the appropriate ligands. Most of the published papers concern the antimicrobial properties of Au^{I} derivatives, while only a few reports on Au^{III} are present.[1] Interestingly, a recent example reports on the reproposal of the well-known antiarthritic drug Auranofin against some penicillin resistant *Staphylococci.* [2]

In the light of these findings, a collection of gold complexes bearing polydentate heterocyclic nitrogen ligands has been assayed against a variety of Gram-negative/positive bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus atrophaeus*, *Staphylococcus aureus*, *Streptococcus intermedius*, respectively) and yeast (*Candida albicans*, *Candida kruseii*, *Candida glabrata*) with very promising results. The most of the tested compounds displayed severe inhibition of microbial cells proliferation during a short time of exposure. Analysis of their bacteriostatic/bactericidal action was carried out in vitro on both monolayer cultures and three-dimensional grown biofilms. Results of this study besides initial structure/activity correlations will be illustrated.

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The Angiosuppressive Effects of [Pt(O,O′-acac)(γ-acac)(DMS)]

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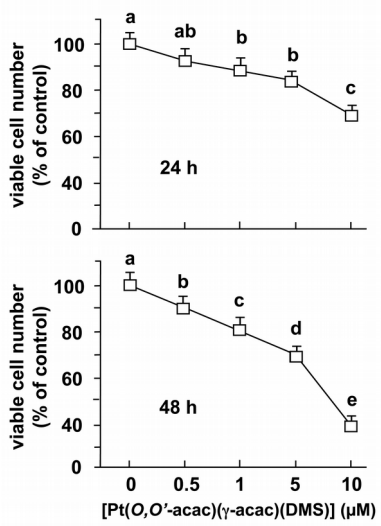
Breast cancer development is a complex multi-step process in which angiogenesis (or neovascularization) plays a very essential role. A large amount of anti-angiogenic drugs have been studied in order to find a drug which can have an effect on the angiogenesis pathway and vascular endothelial cells. However, the majority of these drug studies have had to be halted due to their serious side-effects and poor efficacy. We showed that [Pt(O,O'-acac)(γ-acac)(DMS)], a new platinum drug for non genomic targets, specifically designed and synthesized by some of us, [1] exerts antimetastatic responses in vitro, decreasing metalloproteases production and tumor breast cells migration. [2] In the present study, we aimed to investigate the anti-angiogenesis effects of [Pt(O,O'-acac)(γ-acac)(DMS)].



Concentration-dependent inhibition of HUVECs proliferation.

[Pt(O,O′-acac)(γ-acac)(DMS)] significantly inhibited human umbilical vein endothelial cell (HUVEC) proliferation. Then, the ex vivo rat aortic ring capillary-network sprouting has been carried out. It was observed that after one week of treatment under regular growth conditions, [Pt(O,O′-acac)(γ-acac)(DMS)] potently inhibited the sprouting as well as capillary-network formation from rat aortic ring in a dose-dependent manner. Compared to control 0,5 and 1 μM doses of [Pt(O,O′-acac)(γ-acac)(DMS)] suppressed capillary-network formation by 68 to 100%, respectively. In addition, in mouse xenograft model of breast cancer, treatment of mice with [Pt(O,O′-acac)(γ-acac)(DMS)], decrease MMP-1 content in tumours. These observations demonstrated that [Pt(O,O′-acac)(γ-acac)(DMS)] could inhibit angiogenesis, which warrants further studies in other in vivo models.

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Azolate Gold(I) Phosphane Complexes as Innovative Therapies for the Treatment of HER2-driven Breast Cancer

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Gold(I) compounds have been known as cytotoxic agents since 30 years ago. [1] Lastly, the inhibition activity studies on compounds (such as LAuL', where L is a phosphane and L' a co-ligand) led to the individuation of a likely molecular target, [2] renewing the interest on the field of these metallodrugs. In the design of active gold compounds, the proper hydro / lipophilic balancing provides the lowering of the overall toxicity, maintaining both a good cellular uptake and anticancer properties. Imidazoles and pyrazoles as co-ligands afford to gold(I) phosphane compounds having cytotoxic activity, but enough polarity to be soluble in physiological media. Different azolate gold(I)phosphane complexes have been synthesized. They contain substituents on imidazole or pyrazole ligands such as R = NO_2, CF_3, CN, Cl, CH_2OH) or substituents such as COOH or $COONHEt_3$ in the phosphane moiety. Some of them have been already tested as antitumoral in some panels of cancer cells, resulting active. [3] In this work we present the study of the cytotoxic effects of several gold(I) compounds and a natural compound on an *in vitro* model of HER2-overexpressing breast cancer. We tested the effectiveness of these compounds as potential anticancer agents on SKBR-3 cell line, a human breast cancer cell line that overexpresses the HER2 (Neu/ErbB-2) gene product. [4] These cells display an epithelial morphology in tissue culture and are a useful preclinical model to screen for new therapeutic agents which could overcome the drawback of resistance to HER2-targeted therapies. [5] In order to screen the cytotoxic activity of these new compounds on SKBR-3 cells we performed different cell viability assays. As conclusion we observed a detrimental effect on the cytotoxicity for those compounds having an ionic structure or highly hydrophilic polar substituents on the azolate or phosphane ligands and a remarkable activity for those compounds having the Ph_3PAu^+ moiety and substituted imidazolate as co-ligands.

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Silica Nanoparticles as Vectors for Pt(IV) Prodrugs

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The platinum(II) complexes are the most important drugs in anticancer chemotherapy but their low selectivity of action leads to serious side effects. In recent years, therefore, the research has moved towards Pt(IV) compounds: their reduction to their parental Pt(II) complexes is the basis of their antitumor activity. [1] Moreover, in order to selectively accumulate drugs to the tumor site, a strategy of drug targeting and delivery (DTD) can be exploited. [2] In particular, to pursue a passive DTD method, the so-called "enhanced permeability and retention effect" can be exploited: the solid tumors tissue, in rapid and uncontrolled growth, shows high permeability of blood vessels and inefficient lymphatic drainage from the cell interstices, thus circulating macromolecules (e.g. proteins, nanoparticles, liposomes, etc.) can extravasate and accumulate in tumor tissue.

The main purpose of this work has been to synthesize a cisplatin-based Pt(IV) prodrug, having one axial ligand suitable for the binding to a vector for DTD and the other axial ligand inert during the following synthetic phases (1). This kind of unsymmetric complex should avoid cross-links between vectors. To corroborate this choice, a bis-functionalized complex (2) has been also used for comparison. Aminopropyl-functionalized core-shell silica nanoparticles have been chosen as vectors (Figure 1).

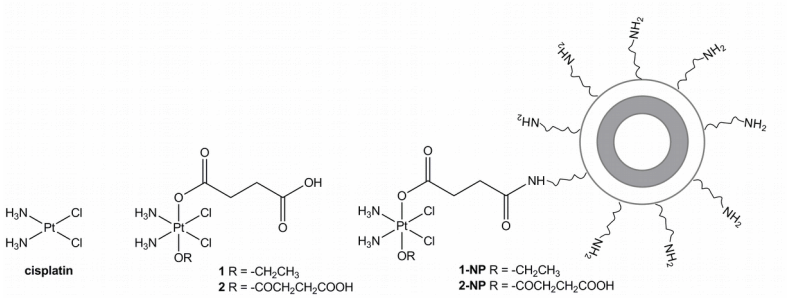

Fig. 1. Scheme of the complexes under investigation

Finally, Pt cellular accumulation and antiproliferative activity of both complex 1 and its conjugates have been carried out on the very Pt-sensitive ovaric A2780 cell line.

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Thioredoxin Reductase Inhibition by Gold N-heterocyclic Carbene Compounds: an ESI-MS Investigation on the Synthetic Linear Selenocysteine Containing C-terminal Peptide hTrxR(488-499)

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Thioredoxin reductase (TrxR) is an important and ubiquitous enzyme critically involved in the regulation of intracellular redox metabolism. A number of recent reports suggest that thioredoxin reductase constitutes an important "druggable target" for the development of new anticancer agents. [1] Indeed, modulation of TrxR activity through selective inhibitors may contrast effectively cancer cell proliferation. Owing to the presence of a functional selenolate group, it was suggested that thioredoxin reductase might represent a primary target for experimental Gold compounds. [2]

In fact, gold-based drugs typically behave as strong inhibitors of the enzyme thioredoxin reductase (hTrxR), possibly as the consequence of direct Gold(I) coordination to its active site selenocysteine. To gain a deeper insight into the molecular basis of enzyme inhibition and prove gold-selenocysteine coordination, the reactions of three parent Gold(I) NHC compounds with the synthetic C-terminal dodecapeptide of hTrxR containing Selenocysteine at position 498, were investigated by electrospray ionization mass spectrometry (ESI-MS). [3] Formation of 1:1 Gold-peptide adducts, though in highly different amounts, was demonstrated in all cases. In these adducts the same $[Au\text{-}NHC]^+$ moiety is always associated to the intact peptide. Afterward, tandem MS experiments, conducted on a specific Gold-peptide complex, pointed out that Gold is coordinated to the selenolate group. The relatively large strength of the Gold-selenolate coordinative bond well accounts for potent enzyme inhibition typically afforded by these Gold(I) compounds. In a selected case, the time course of enzyme inhibition was explored. Interestingly, enzyme inhibition turned out to show up very quickly and reached its maximum just few minutes after mixing. Overall, the present results offer some clear insight into the process of thioredoxin reductase inhibition by Gold-based compounds.

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Studies on the Antimicrobial Activity of Silver Nanoparticles as Additive for Several Kind of Materials

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Silver nanoparticles (AgNPs) have attracted extensive research interest due to their attractive optical, electronic properties and excellent antimicrobial activities. AgNPs exhibit strong cytotoxicity towards a broad range of microorganisms and are widely used as an antibacterial agents. [1] The advantage of AgNPs compared to bulk metal or salts is the slow and regulated release of silver from nanoparticles, thereby causing long lasting protection against bacteria. The antimicrobial activity of AgNPs is comparatively better than most prominent antibiotics used worldwide. [2] Numerous methods have been developed for the preparation of AgNPs. The most common method is the chemical reduction of silver salt by a reducing agent in the presence of a stabilizing agent. In this work AgNPs have been prepared by reducing silver cations with $NaBH_4$ and using as stabilizer sodium citrate, PVP (polivinylpirrolidone) or polysaccharides. AgNPs so obtained were characterized as average 10 nm particles by DLS and UV-vis spectroscopy. This work has the aim to verify the biocide action of silver nanoparticles mainly in plasters but also in other substrates occurring in a civil environment to reduce exposure to risk of infection by people with weak immune system. The study was focused to develop a method of study for each kind of material both in the AgNPs dispersion's method and on the antimicrobial activity of the resulting substrate treated with AgNps. The antimicrobial activity has been led on *Escherichia coli* cells cultured in Luria Broth. [3] The minimum concentration needed to have effects has been determined in each case. Moreover a comparative study of the inhibitory effect of AgNPs and silver salts such as $AgNO_3$ and $AgCF_3SO_3$ on the regards of DHFR (DeHydroFolateReductase) from *E. coli* has been performed.

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Copper(I) and Gold(I) Phosphane Complexes: Biological Activity, Neurotoxicity and Photon Activation Therapy Effect

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Cisplatin (CDDP) is an anticancer drug widely used in clinic for the treatment of several solid tumours, despite its high effectiveness treatment is still limited by severe side effects and by inherited or acquired resistance phenomena. These drawbacks have stimulated the search of alternative strategies based on different metals offering a better toxicity profile while maintaining the same level of efficacy as platinum-based treatments. [1,2] Recently we evaluated the cytotoxicity and neurotoxicity of our newly developed promising water soluble anticancer complexes ($[Cu(PTA)_4]PF_6$, $[Cu(thp)_4]PF_6$, $[Au(PTA)_4]PF_6$, $[Au(thp)_4]PF_6$) using CDDP as reference drug. The cytotoxicity was evaluated on A549 non-small cell lung cancer (NSCLC) and IGROV-1 ovarian human cancer cells while the neurotoxicity was tested on dorsal root ganglia organotypic cultures. In our model CDDP resulted neurotoxic at concentrations achievable in plasma of patients treated with the same drugs. Similarly the gold-based compound $[Au(PTA)_4]PF_6$ was neurotoxic at lower concentration than IC_{50} calculated for the tested cancer cell lines. On the contrary, both copper-based compounds and $[Au(thp)_4]PF_6$ were neurotoxic at higher concentrations with respect to the IC_{50} obtained in tumor cell lines tested. We then tested at the ID17 beamline of the ESRF the efficacy of synchrotron radiation (SR) to trigger the Auger effect in IGROV-1 cells containing a high Z-number element. Irradiation of cells pre-treated with CDDP or $[Cu(PTA)_4]PF_6$ concentrations allowing roughly 90 % of cell survival induced an enhancement in cellular death with respect to drug and irradiation alone. With the other compounds no cell death enhancement was observed. Our results suggest that SR-enhanced CDDP activity might allow the use of a reduced dose of CDDP thus achieving side effects minimization due to the exposure of normal cells/tissues to less toxic doses. Furthermore, considering the anticancer activity and the neurotoxic profile of $[Cu(PTA)_4]PF_6$, our data suggest that copper-based drugs represent new and promising compounds in anticancer treatment also in combination with SR.

Acknowledgments
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Water-soluble Porphyrazine Macrocycles for Potential Application in the Field of Photodynamic Therapy

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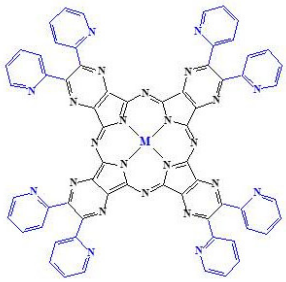
The pyrazinoporphyrazine macrocycles having formula [Py_8TPyzPzM] (Figure) were extensively investigated by our group. [1] Due to the presence of external electron-withdrawing dipyridinopyrazine fragments, these compounds behave as strongly electron-deficient macrocycles. Quaternization of pyridine N atoms leads to the formation of related water soluble supercharged mono- or bimetallic species. [1b-d,2] These compounds (neutral or positively charged) were proved to be excellent photosensitizers in a non-aqueous solvent (dimethylformamide) for the generation of singlet oxygen, $^1O_2$, the cytotoxic agent in photodynamic therapy (PDT).



Currently, our research group is performing singlet oxygen quantum yield (ΦΔ) measurements in water solution using already known and new positively charged water soluble porphyrazine macrocycles, under experimental conditions which guarantee the preponderant presence of the macrocycles in their monomeric form; this aspect is relevant for the best ΦΔ response. Data will be anticipated and discussed.

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Ruolo degli ioni metallici nelle patologie degenerative croniche

Copper(II) Complexes of Oxidized Derivatives of 1,10-phenanthrolines: Synthesis and UV Investigation

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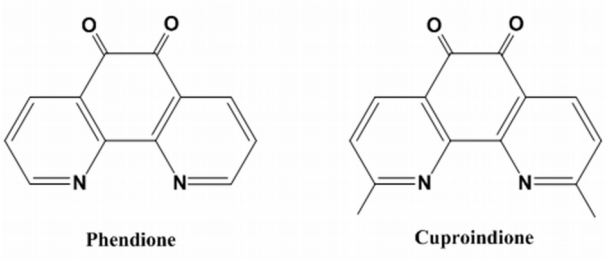
Phenanthrolines and their metal complexes are intercalating agents which interact with DNA by aromatic π-stacking with base pairs. The phenanthroline derivate 1,10-phenanthroline-5,6-dione (phendione; Figure 1) displays a significant anticancer activity, both as a free ligand and coordinated to many metal ions, [1,2] together with antibacterial and antifungal properties. [3]

Moreover, copper complexes of 1,10-phenanthroline (phen) and its derivatives are known to bind and cleave DNA. [4,5] In this work, we have investigated the complexation of phendione and 2,9-dimethyl-1,10-phenanthroline-5,6-dione (cuproindione; Figure 1) with Cu(II) by UV-Vis titration experiments. We have also synthesized and characterized the complex species for which there was evidence of formation in the UV experiments.



Our results allowed to gain information on the effect of the methyl substituents in position 2 and 9 of the phenanthroline moiety on both geometric and redox properties of the corresponding Cu(II) complexes. In particular, our results show a different behavior between the two ligands in the complexation with copper(II) and the formation of complexes with different stoichiometry depending on the presence of the methyl substituents.

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Inorganic Features of Copper(II) Interactions with Neurotrophin-3 N-Terminal Peptide Fragments

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Neurotrophins (NTs) are secreted proteins essential for the differentiation and the wiring regulation of the central and peripheral nervous system during the development. [1] They also ensure neuronal maintenance in the adult organism and modulate synaptic transmission. The neurotrophin family comprises nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and neurotrophin-4 (NT4). The NTs are structurally and functionally related proteins which exert their biological action as noncovalent dimers and specifically bind the TrK receptor family.

Although a neurotrophin hypothesis of AD has been proposed, the link between neurotrophic factor, the amyloid cascade and biometals has not been taken into account so far. As a matter of fact, there is a significant overlap between brain areas featured by metal ion dyshomeostasis and those where the neurotrophins exert their biological activity. Metal ions can directly modulate their activity through conformational changes and/or by activating their downstream signaling in a neurotrophinin dependent mode. [2]

We have recently investigated the interaction of copper(II) and zinc(II) ions with NGF and BDNF. [3,4] In the present communication we report on the synthesis of a NT3 N-terminus peptide fragment encompassing the residues 1–13 blocked at the C-terminus (YAEHKSHRGEYSV-NH_2, NT3 1-13) and the coordination features of its copper(II) complexes by means of potentiometry and spectroscopy (UV/Vis and CD). The binding properties of the NT3 1-13 peptide were critically compared with those of the acetylated analogous (Ac-YAEHKSHRGEYSV-NH_2, AcNT3 1-13) and of the two shorter Ac-KSHRGEYSV-NH_2 (AcNT3 5-13) and YAEHK-NH_2 (NT3 1-5) residues.

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Cu(II)/Cu(I) Interactions with the Amyloidogenic Region of Prion Protein

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The prion proteins (PrPs) seems to play an important role in copper homeostasis and its biological functioning (for reviews see [1,2,3]). The basic region which binds Cu(II) ions is the octa-repeat domain consisting of four (-Pro-His-GlyGly-Gly-Trp-Gly-Gln-) peptide fragments. Mammalian and Avian PrP contains also another effective Cu(II) binding motif in the so called amyloidogenic region, human PrP91-127. [3] The octa-repeat domain in human is able to bind up to four Cu(II) ions and the amyloidogenic region two additional ones. Moreover, human PrP contain [M(X)nM] motifs which are well known to act as Cu(I) binding sites. [4] In this study we have investigated Cu(II) and Cu(I) interactions with the amyloidogenic human PrP region, hPrP91-127, by means of CD, NMR, FT-IR, cyclic voltammetry and MD simulations. The Cu(I) coordination sphere is discussed with a particular emphasis to the role played by Met and His residues.

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Antiretroviral Activity of Metal-chelating HIV-1 Integrase Inhibitors

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Metal chelating agents represent an important class of enzyme inhibitors and the metal binding strategy is of particular interest for the design of effective antivirals. [1,2] In the last decade, HIV-1 integrase (IN) has been validated as an important pharmacological target for the development of new drugs. IN has a crucial role in the viral life-cycle, since it catalyses the integration of proviral cDNA into the host cell genome. This enzyme contains a catalytic core domain with an amino acidic triad, which coordinates two divalent Mg2+ cofactors that are essential to the catalytic process. [3] Chelation of the magnesium cofactors of IN has proven to be a successful strategy in the design of IN inhibitors, and it resulted in the approval by FDA of the chelating inhibitors raltegravir (Isentress®) and dolutegravir. [4,5] A great number of chelating compounds have been studied as IN inhibitors. [1] In previous studies, we demonstrated that the diketoacids (DKAs) and some ligands synthesized as model of well-known potent IN inhibitors (Fig.1), effectively chelate divalent metal ions in solution, forming metal complexes with different stoichiometric ratios. [6-8] We isolated some metal complexes with these ligands and different divalent metal ions (Mg, Mn, Co, Ni, Cu Zn) and tested them for their ability to inhibit IN in enzymatic assays.

HIV-1 replicates through the process of reverse transcription, that is accomplished through the enzyme reverse transcriptase (RT). RT has a ribonuclease (RNase) H domain that shows structural homologies with IN.[1] For these reasons, we evaluated the activity of the most active compounds also toward HIV-1 RNase H enzymatic activity.

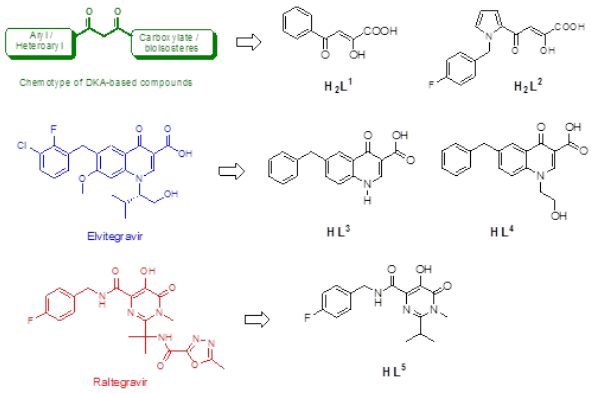

Fig. 1 Chemical structures of model ligands H_2L1, H_2L^2, HL^3-HL^5 and their parent compounds

Finally, we tested the anti-HIV activity and cytotoxicity of the ligands and of the corresponding metal complexes in HIV-infected MT4 cells.

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System biology per lo studio dei metalli

8-Hydroxyquinoline Derivatives and Anticancer Therapy: Differential Potentiation of Cytotoxic Effects by Copper and Zinc

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8-Hydroxyquinoline derivatives are metal-binding compounds with cytotoxic properties that could be exploited in novel anticancer therapies. In the present study we compared the effects of 5-chloro-7-iodo-8-hydroxyquinoline (Clioquinol, CQ), 5-chloro-8-hydroxyquinoline (ClHQ) and 8-hydroxyquinoline (OHQ) and their glycoconjugates: 5-chloro-7-iodo-8-quinolinyl-β-D-glucopyranoside (GluCQ) and 5-chloro-8-quinolinyl-β-D-glucopyranoside (GluClHQ). MTT assays were performed in order to test the effect on cell growth and toxicity in two colon carcinoma cell lines (Caco-2 and HT29). In the presence of physiological concentration of copper (Cu2+) the three compounds decreased cell growth after a short 2-hours or a prolonged 72-hours-treatment. Addition of copper or zinc (20 μM copper nitrate or 50 μM zinc chloride) to the culture medium potentiated the cytotoxicity of the compounds but with marked differences. CQ effect was potentiated in similar way by the addition of Cu^{2+} and Zn^{2+}, while ClHQ and OHQ effects were more sensitive to the addition of Cu2+. Glucosylated forms of CQ and ClHQ were ineffective after 2-hours incubation and required a prolonged 72-hours-incubation to show cytotoxicity, suggesting that hydrolysis of glucoconiugates was necessary for the their action. However a marked potentiation was again observed in the presence of Cu^{2+}, but not Zn^{2+}, in the case of 5-chloro-8-quinolinyl-β-D-glucopyranoside (IC_{50} without added metals: 5.17 μM, IC_{50} with added copper: 0.10 μM, IC_{50} with added zinc: 7.9 μM). Such data suggest differences in the metal-dependent mechanism of action of 8-Hydroxyquinoline derivatives and shed light on their potential as anticancer agents.

ATOX1 Gene Silencing Increases Susceptibility to Anticancer Therapy Based on Copper Ionophores or Chelating Drugs

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Copper is a catalytic cofactor required for the normal function of many enzymes involved in fundamental biological processes but highly cytotoxic when in excess. Therefore its homeostasis and distribution is strictly regulated by a network of transporters and intracellular chaperones. The mutational profiles associated to cancer could be responsible for deficiency in physiological functions that makes the cancer cells highly sensitive to specific drug treatments. In this sense the protein network involved in copper homeostasis could be altered in cancer and represent a "Achille's heel" of cancer cells. Previously we analyzed the presence of somatic mutations and copy number variations in copper homeostasis genes in colorectal cancer reporting a frequent deletion of ATOX1 gene. In the present study the Caco-2 colon carcinoma cell line was used as *in vitro* model to evaluate if Atox-1 deficiency could affect sensitivity to experimentally induced copper dyshomeostasis. In this cell line, ATOX1 gene showed a normal diploid copy number and a downregulation of its expression was induced by siRNA. Silencing of ATOX1 increased toxicity of a short treatment with high concentration of Cu^{2+}. Copper ionophores, such as hydroxyquinoline derivatives, induced a copper-dependent cell toxicity. A significant potentiation of 5-chloro-8-hydroxyquinoline toxicity was observed after ATOX1 silencing. On the contrary the copper chelator T-PEN (N,N,N',N-tetrakis (2-pyridylmethyl) ethylenediamine) produced a form of cell toxicity that was reversed by the addition of Cu^{2+}. ATOX1 silencing increased Caco-2 cells sensitivity to T-PEN toxicity. Our results suggest the possibility of a copper-chelating therapy in a subtypes of tumors showing specific alterations in ATOX1 expression.

Nanostrutture di interesse biomedico e ambientale

Development of New Theranostic Nanosystems Composed of Semiconducting-Magnetic Heterostructures and Cyclic RGD Peptide for Integrin Targeting

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Nanosystems combining therapeutic and diagnostic properties give new and improved opportunities to overcome limitations associated with conventional cancer diagnosis and therapy. In particular, multifunctional nanoparticles based on inorganic heterostructures, able to integrate several features within a single construct, can be successfully conjugated with targeting ligands, such as antibodies, folate and peptides onto nanostructure, to achieve multi-targeting nanoplatforms potentially useful for selective drug delivery to the tumor cells. One efficient strategy to realize a cancer-targeted drug delivery is based on the exploitation of molecular markers i.e membrane receptors that are overexpressed. [1-3]

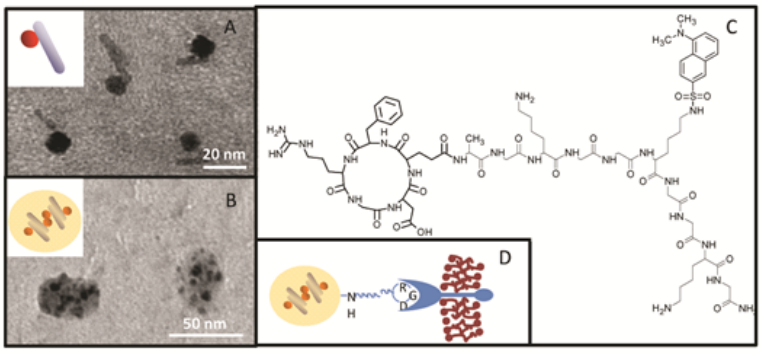

Figure 1. TEM micrograph of BNCs before (A) and after PEG-modified phospholipid functionalization (B). Molecular structure of cyclic RGD peptide (C). Scheme for BNC micelles bioconjugated with peptide for targeting of alphavbeta3 integrin (D).

Here we reported the preparation of binary asymmetric nanocrystals, formed by a spherical ɣ-Fe2O3 magnetic domain epitaxially grown onto a lateral facet of a rodlike anatase TiO2 (BNCs), and their inclusion into water dispersible block copolymer micelles composed of polyethylene glycol modified phospholipids (PEG lipids). [4] A properly designed peptide containing the RGD motif for targeting of αvβ3, expressed on several types of cancer cells, has been successfully conjugated with the BNC incorporated in lipid micelles. Each step has been thoroughly monitored by using optical and structural techniques, and the peptide/BNC conjugates, characterized by an average hydrodynamic diameter smaller than 100 nm, have resulted homogeneously dispersed and sufficiently stable in aqueous solution to perform in vitro experiments. The cytotoxicity of the peptide/BNC conjugates has been also assessed. These systems have a large potential for cancer treatment, since the RGD motif can target the nanostructures to tumor area, where magnetically induced hyperthermia could be combined with TiO2 induced photodynamic therapy.

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Indice degli autori

The First Three-Dimensional Molecular Structure of a a-Lithiated Oxirane Finally Revealed

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Epoxides, strained three-membered ring heterocycles, are among the most versatile intermediates in organic chemistry.[1] Among metalated oxiranes, α-lithiated aryloxiranes have been widely investigated in the last years in terms of solvent, temperature and bases, [2] but until now no evidence in the solid state has been obtained. On one hand it is difficult to crystallize reaction intermediates as organo lithium with small 3 member rings as epoxides and on the other it is hard to handle crystals at low temperature and sensitive to the air. Herein we report the first α-metalated oxirane crystal structure that we obtain by reacting ortho-trifluoromethylstyrene oxide with 1.4 equiv of sec-butyl lithium in diethyl ether. The organolithium-epoxide complex has been isolated and characterized by single-crystal X-ray diffraction at 100 K by using XTEMP-2 techniques. [3] The complex crystallizes in a monoclinic system (space group $P2_1/n$) with chemical formula $C_{30}H_{44}F_6Li_2N_4O_2$ and Z = 2. Cell dimensions $a = 9.5926(5)$ $b = 11.3718(6)$ $c = 15.7795(8)$ $\beta = 106.108(16)°$. The structure consists of a dimeric aggregate coordinating TMEDA and Lithium (Figure 1).

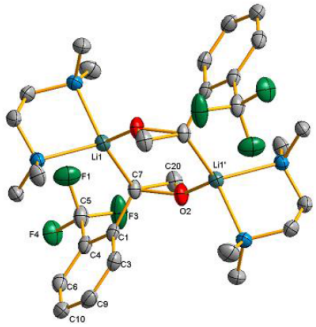

Figure 1: Molecular Structure of $(TMEDA)_2(Li_2(C_9H_7F_3Li_1O_1)_2$.

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