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Non-Medical Applications of Inorganic Medicines. A Switch Based on Mechanistic Knowledge

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Dedication: this article is dedicated by TM to little Alessandro. Hoping you can look at the world, always with imagination and curiosity.

Metals have been used in medicine for centuries. However, it was not until much later that the effects of inorganic drugs could be rationalized from a mechanistic point of view. Today, thanks to the technologies available, this approach has been functionally developed and implemented. It has been found that there is probably no single biological target for the pharmacological effects of most inorganic drugs. Herein, we present an overview of some integrated and multi-technique approaches to elucidate the molecular interactions underlying the biological effects of metallodrugs. On this premise, selected examples are used to illustrate how the information obtained on metal-based drugs and their respective mechanisms can become relevant for applications in fields other than medicine. For example, some well-known metallodrugs, which have been shown to bind specific amino acid residues of proteins, can be used to solve problems related to protein structure elucidation in crystallographic studies. Diruthenium tetraacetate can be used to catalyze the conversion of hydroxylamines to nitrones with a high selectivity when bound to lysozyme. Finally, a case study is presented in which an unprecedented palladium/arsenic-mediated catalytic cycle for nitrile hydration was discovered thanks to previous studies on the solution chemistry of the anticancer compound arsenoplatin-1 (AP-1).

1. Introduction

Nowadays, the leading role of inorganic chemistry in medicine, either for diagnostic or therapeutic purposes, is widely recognized.[1] Though in the last years we assisted to a significant expansion of the investigation on the fate that metal(loid)-based drugs undergo in the biological environment;[2a,b] yet for several of them it is still difficult to formulate a clear description of their reactivity towards biological targets.[3] This is mainly due to their peculiar properties and their tendency to be highly reactive towards multiple biologically relevant species.[4]

However, using integrated multi-technique strategies, it is possible to exploit customized - and simplified - environments to study the activation and subsequent adduct formation of inorganic molecules such as cisplatin and its derivatives.[5–7]

In this context, it is convenient to use biological model targets (by the word "model" we mean substrates that are not the real biological target), which are cheap, well known and have a high versatility in terms of their possible exploitation, using different biophysical methods ranging from mass spectrometry to spectrophotometry to X-ray diffraction.[8] This condition, although simplified, allows the evolution of the system under physiological-like conditions -and thus the formation of adducts between the model target and the inorganic drug- to be followed in a dynamic, time-dependent manner. The use of model biological substrates is convenient because it allows a reliable generalization of the results obtained to real targets, thus providing fundamental information for the study and characterization of the mechanism of action (and activation) of the inorganic drug itself.[8] This approach can be further supported by theoretical chemistry, which, together with experimental observation, is crucial for the overall analysis and validation of the results.

In this article, we focus on how this mechanistic knowledge of experimental or established inorganic drugs can be valuable for the implementation of these complexes in fields other than medicine.

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Based on the above considerations, we propose an overview of the potential impact of known metallodrugs for innovative applications through a series of practical examples.

In fact, even established or well-known inorganic compounds that are widely used as experimental or clinical drugs are, first of all, coordination compounds whose properties can be (re)evaluated in a kind of new concept of "drug repurposing", significantly expanding the "chemical space" for their use. In this context, repurposing refers to the use of the original medicinal complex for applications far beyond the medical field.

2. Getting Information into the Mechanism of Action/Activation of Inorganic Drugs

One of the greatest challenges facing chemists, biologists and physicians today is to fully describe the behavior and the fate of metallodrugs in the biological environment. This is crucial because a complete understanding of the reactions that take place in the patient's body not only makes it possible to optimize the use of a drug, but also to avoid - or reduce/overcome - the side effects that often occur. This is the case, for example, with platinum-based chemotherapeutics.[9a,b] From a mechanistic and molecular point of view, we know that the main target for the anti-cancer activity of platinum drugs is nuclear DNA. However, we also know that only a very limited amount (approximately 2%) of the administered dose of cisplatin reaches the genomic target in the nucleus and induces apoptosis. As a result, most of the drug reacts with other biological substrates, including proteins.[10] In this context, the DNA paradigm of cisplatin (and platinum drugs in general) has been profoundly revised in recent decades, and the so-called "protein metalation process" has rightly received increasing attention.[11] Indeed, given that metals are excellent binding partners for several proteins, it is necessary to delineate the Pt coordination process in detail. Proteins play an essential biological role in several cellular processes, and their impairment as a consequence of coordination of metallic fragments derived from medicinally used complexes may significantly affect both the pharmacological outcome and the emergence of undesired side effects.[8] However, the detailed description of protein metalation by metallodrugs remains a very ambitious and challenging goal for researchers.

Typically, these studies require the use of appropriate experimental methods and models to describe the behavior of the inorganic drugs under investigation in the biological environment and their binding to cellular proteins. In recent years, we have developed a specific protocol encompassing the integrated use of high-resolution mass spectrometry and X-ray crystallography.[8] The protocol typically involves the use of small model proteins, such as lysozyme (HEWL) and bovine RNase A.[12a] Although highly simplified, this type of experimental approach can highlight significant differences in the reactivity of structurally related metallodrugs. The case of Pt drugs reacting with model proteins is exemplary. By incubating cisplatin, carboplatin and oxaliplatin with RNase A under appropriate conditions and taking ESI-MS spectra at regular time intervals, we observed that cisplatin and carboplatin behave in a very similar way, i.e. they have similar binding kinetics and can coordinate the model protein at the level of

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Alessandro Pratesi earned his Chemistry Laurea degree from the University of Florence (Italy) in 2006. He completed his PhD in Chemical Sciences at the same university in 2011, supervised by Prof. M. Ginanneschi and L. Messori. Since 2019, he has served as an Assistant Professor at the Department of Chemistry and Industrial Chemistry at the University of Pisa. His research focuses on synthesizing new metal-based drugs, particularly those involving platinum, gold, and palladium, and investigating their interactions with proteins and nucleic acids. Recently, he has delved into studying new Pt(IV)-based complexes with bioactive axial ligands and their delivery in PLGA-PEG nanoparticles.

Tiziano Marzo received his Ph.D. in bioinorganic chemistry from the University of Florence in 2016. In the same year, he received a 3-year financial support from Italian Association for Cancer Research (AIRC) and moved to the Department of Chemistry and Industrial Chemistry, University of Pisa. In 2018, he obtained a position at the Department of Pharmacy, University of Pisa, where he currently serves as an Associate Professor. He had a stint at the Universidad Autónoma de Madrid and, in 2024, at the University of Calgary as visiting professor. His research interests are focused on inorganic, bioinorganic and coordination chemistry.

the same amino acid residues through the same fragment $[Pt(NH_3)_2]^{2+}$. Indeed, the ESI-MS spectra allowed us to unambiguously determine the nature of the protein-bound metal fragments and the degree of protein metalation. Conversely, we found that oxaliplatin exhibits a profoundly different binding mode compared to cisplatin and carboplatin, both kinetically and in the nature of the protein-bound fragments (Figure 1). These findings were well supported by crystallographic studies carried out on the same systems, which gave consistent results, as shown in Figure 1, despite the inevitable differences in the applied experimental conditions.

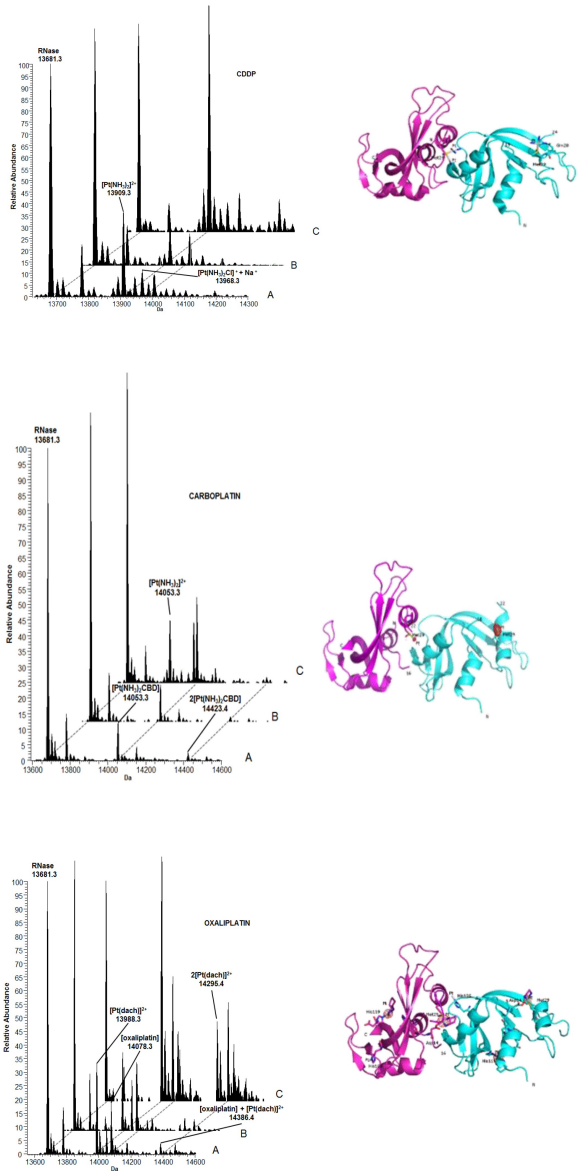


Figure 1. Deconvoluted ESI MS spectra of RNase A treated respectively with 3×10^{-4} M of cisplatin (top), carboplatin (middle) and oxaliplatin (bottom), recorded after 24 h (A), 72 h (B), 168 h (C) of incubation at 37 °C. Experimental conditions: metal:protein ratio = 3:1; buffer: 20 mM ammonium acetate pH 6.8.The ribbon representation represent the asymmetric unit of the RNase A–drug structures obtained for cisplatin (top), carboplatin (middle) and oxaliplatin (bottom). Reproduced from ref. [12a], Copyright (2015), with permission from Elsevier Inc.

The different reactivity of cisplatin and carboplatin with respect to oxaliplatin was further confirmed in other model proteins, including HEWL,[13a,b] taking advantage of the same experimental approach. Overall, these results are consistent with previous evidence that metal ligands play a leading role in governing protein-metal drug interactions, driving the drug-biological target recognition process and stabilizing the structure of the final adduct.[8] The binding of the three Pt drugs to methionine residues is relevant also because Pt binding to S-donor ligands (e.g. methionine and glutathione) occurs in physiological conditions playing a relevant role in the Pt–drugs metabolism. S-donor binding of Pt-drugs was suggested to be involved as an intermediate in transport to the DNA in nucleus and as a pathway of drug inactivation.[12a–c]

Such a significant difference in reactivity between cisplatin and carboplatin, on the one hand, and oxaliplatin, on the other, is also consistent with the different clinical uses of the three drugs. In fact, cisplatin and carboplatin are currently used to treat the same malignancies with almost overlapping results, while oxaliplatin is used almost exclusively to treat colorectal cancer, a tumor type where the first two drugs are poorly effective.[14] Remarkable independent evidence obtained by Lippard and coworkers further confirmed that oxaliplatin has a different mechanism of action and activation of apoptosis than cisplatin and oxaliplatin and is specifically DNA-independent.[14] In summary, by applying the integrated strategy described above, it is possible to obtain precise information on the metalation of proteins caused by anticancer metallodrugs at the molecular level, to highlight differences and to shed light on the possible mechanistic implications.

3. Mechanistic Information Drives New Applications of Metallodrugs: Some Relevant Examples

Effective methods to obtain relevant information on the mode of action of inorganic drugs are of paramount importance to improve/implement their clinical use. However, this knowledge could also be used to exploit these compounds in contexts other than medicine. This possibility could allow the emergence of a kind of "unconventional drug repurposing", where selected inorganic entities are used in fields other than medicine because of their specific chemical properties. Some case studies are summarized below to illustrate this concept.

3.1. The Mixed-Valence Diruthenium Adduct of Lysozyme [HEWL/$Ru_2(OAc)_2$] is a Chemoselective Catalyst

Paddlewheel compounds with a bimetallic core are of great interest for medicinal applications.[15a,b] Indeed, their peculiar chemical structure allows the coordination of up to four ligands to the bimetallic center, and provided the ligand is a pharmacologically active releasable molecule, the resulting complex is potentially capable of multiple delivery (up to four

equivalents).[16a,b] In addition, due to the presence of the M–M core, it is possible to combine the ligand action with the medicinal properties of the metals.[17] In this context, mixed valence Ru_2 (II, III) complexes have been widely investigated for their biological application, in particular as potential anticancer agents.[18a,b] Interestingly, the study of the reactivity of Ru_2 (II, III) complexes towards model proteins is a valuable tool to elucidate their mode of action. Accordingly, the model protein HEWL was used to study the behavior of $[Ru_2(\mu\text{-}O_2CCH_3)_4Cl]$ and its derivatives.[19] It was found that the cationic species of $[Ru_2(\mu\text{-}O_2CCH_3)_4]^+$ is able to form stable adducts with HEWL. More interestingly, the adduct was found to carry two diruthenium centres firmly anchored to two aspartate groups in positions accessible to the solvent. The reaction is mediated by the formation of two coordination bonds between the two oxygen atoms of the side-chain carboxylate group of Asp and the two ruthenium atoms of the diruthenium motif.[19] This peculiar finding led to the speculation that the known catalytic properties of $[Ru_2(\mu\text{-}O_2CCH_3)_4Cl]$ might be retained upon binding to the protein, preserving the intact Ru_2 core[20] (Figure 2).

A comparative evaluation of the complex and its counterpart immobilized to lysozyme was carried out. Experiments were based on the ability of $[Ru_2(\mu\text{-}O_2CCH_3)_4Cl]$ and [HEWL/$Ru_2(OAc)_2$] (i.e. its adduct with HEWL, see Figure 2) to induce the catalytic oxidation of hydroxylamines to nitrones. Indeed, these latter attract wide interest for various applications, including spin traps and agents for drug-related diseases.[21] All the experiments were carried out from a green chemistry point of view. Overall, a new green method for the preparation of nitrones via the aerobic oxidation of the corresponding N,N-disubstituted hydroxylamines was developed upon exploring the catalytic activity of [HEWL/$Ru_2(OAc)_2$] in aqueous or alcoholic solution under mild reaction conditions (0.1–1 mol% catalyst, air, 50 °C). Interestingly, [HEWL/$Ru_2(OAc)_2$], which can be classified as a new artificial metalloenzyme, imparted complete chemoselectivity to the oxidation of cyclic hydroxylamines, in contrast to the diruthenium catalyst $[Ru_2(\mu\text{-}O_2CCH_3)_4Cl]$. Indeed, the immobilisation of the catalytically active fragment on the HEWL structure allowed the reaction to proceed with high selectivity towards the oxidised species.

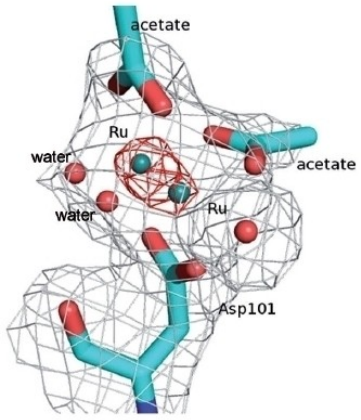


Figure 2. Details of the binding sites of $[Ru_2(\mu\text{-}O_2CCH_3)_4Cl]$ in HEWL. Reproduced from ref. [19], Copyright (2014), with permission from Wiley-VCH.

3.2. Use of Established Inorganic Drugs to Solve Protein Structures

The mechanistic information coming from the study of approved platinum-based anticancer drugs -through the investigation of their interaction with model biological targets- allows their application to solve problems of protein structure resolution. Indeed, in the absence of suitable electron-rich scatterers already present in the crystal, the introduction of a heavy atom in the protein structure represents a reliable option. This procedure is well known as isomorphous replacement.[22] Basically, it involves the soaking of preformed crystals in the presence of a solution containing a heavy element in order to derivatize the protein and change the X-ray diffraction intensities in a manner that allows the estimation of the phases.[23] Several compounds containing heavy atoms have been tested for the derivatization of crystals; among the most commonly used are the so-called 'magic seven', namely K_2PtCl_4, $KAu(CN)_2$, K_2HgI_4, $UO_2(C_2H_3O_2)_2$, $HgCl_2$, $K_3UO_2F_5$ and para-chloromercurybenzoic sulfate. However, although the use of high concentrations of these compounds for short soaking times could generally ensure good results, if they fail it is imperative to try another element. The main limitation of this approach lies in its inherent 'trial and error' nature.

To face and overcome this limitation, it is possible to use established inorganic drugs, such as those based on platinum, for which the mode of binding to proteins is well characterised.[24a,b] The different metalation exerted by cisplatin, carboplatin and oxaliplatin can be conveniently exploited. Indeed, given the specific protein for which the X-ray structure is to be solved, it is possible to select the best metallodrug on the basis of the metalation processes characteristic of the different drugs. In this way, the optimal coordination can be determined, leading to the best possibility of solving the protein structure. In this context, starting from the available information, it is possible to conveniently expand the use of Pt-based approved drugs for solving phase problems.[24b] Furthermore, this method is less time-consuming and, based on the large amount of available data, more reliable. Especially when the prediction is supported by theoretical data.

Indeed, computational chemistry methods are able to support and complete the characterisation of inorganic drugs in their capacity as protein crystallisation stabilisers. In particular, it is possible to accurately visualise the reactivity of the metal complex and generate the data characterising it. This makes it possible to select the best candidate complex for the selective coordination of a particular residue or functional moiety. In addition, it is possible to gain a preliminary understanding of the residue selectivity of inorganic complexes and thus to obtain indications of how protein crystallisation experiments should be configured to address the metalation of particular protein residues. Theoretical methods can also be used to underpin the synthetic process and the rational design of

inorganic complexes, i.e. to improve the properties of existing compounds and to develop new ones. Computational methods can shed light on the packing process, which is the key feature of protein crystallisation. They are also essential for assessing the effects of thermodynamic versus kinetic control on the binding of metal scaffolds to the surface of target proteins.

Overall, by using the huge amount of data available on the "protein metalation process" exerted by established metallodrugs, it is possible to ensure the use of these medicinal compounds for solving protein structures by accurately predicting the metalation site(s). This unconventional application of inorganic drugs has been successfully explored by some of us, confirming its reliability. Specifically, the readers are invited to refer to a recently published article which summarizes some relevant experimental results obtained on transthyretin (TTR), the structure of which was successfully solved at high resolution using approved anticancer platinum drugs.[24b]

3.3. From Cancer Treatment to the Discovery of a New Catalytic Cycle for Nitriles Hydration. The Case of AP-1

Heterobimetallic compounds are very attractive in the field of bioinorganic chemistry because, at least in principle, it is possible to combine two metal-based molecules in a "dual action" prodrug. Among these, the platinum/gold tandem compounds, which combine attractive transition metals in the field of inorganic medicinal chemistry, have been widely studied. Notably, various heterobimetallic compounds bearing iron, ruthenium, palladium, copper, titanium and many others are currently under investigation.[25a–e] AP-1 (Figure 3), a chimeric compound with a covalent bond between a platinum and an arsenic atom, well represents the philosophy of this approach.[26]

To the best of our knowledge, upon interaction with its biological targets, AP-1 can release arsenic trioxide, which is a leading compound employed in the treatment of ATRA-resistant acute promyelocytic leukemia.[27a,b] Notably, the slow intracellular release of arsenic trioxide from the AP-1/DNA adduct may improve the activity of the arsenic moiety against solid tumours. Indeed, one of the known drawbacks of arsenic trioxide is its rapid clearance, which renders it ineffective against solid tumours.[28] For these reasons, some of us have devoted efforts to the synthesis of novel "arsenic-based" heterobimetallic compounds, with the aim of discovering other molecular carriers capable of delivering arsenic trioxide to solid tumours. In this frame, the most straightforward approach seemed to synthesize AP-1 analogues with different transition metals, to gain insight into the structure-activity relationship. The first attempt was to synthesize a palladium(II) derivative of AP-1, given the large amount of literature highlighting the promising cytotoxic properties of the coordination complexes based on this metal center.[29a,b] In addition, the d^8 electronic configuration of Pd(II) ensures the square planar coordination geometry, similar to that of Pt(II). The synthesis attempt was carried out by replacing K_2PtCl_4 with $PdCl_2$, with reference to the reported procedures,[26] which used a water/acetonitrile mixture as solvent. In our case, the described reaction did not yield the desired compound; rather, we observed a massive formation of acetamide as the product of the hydration reaction of acetonitrile. Notably, the hydration process was reproduced several times under the same conditions becoming ineffective without the simultaneous presence of $PdCl_2$ and As_2O_3. Further studies demonstrated the existence of a co-catalytic cycle (Scheme 1), that can be exploited for the hydration of aromatic or aliphatic nitriles under very mild conditions and with high yields.[30]

To elucidate the precise mechanism of this new reaction, we took advantage of the previous crystallographic information available for AP-1. More specifically, the X-ray structure of AP-1 allowed us to postulate the key adduct shown in the third step of Scheme 1. Interestingly, the reaction has recently been verified with several palladium cocatalysts, such as palladium acetate, Pd(dppe)Cl_2 (cas number: 19978-61-1) and Pd(dtbpf)Cl_2 (cas number: 95408-45-0), confirming the general reactivity value of this serendipitous discovery. Finally, we recall other examples of cisplatin or other related compounds used as catalysts in the synthesis of small molecules.[31] In any case, to the best of our knowledge, the above reaction is the first example of nitrile hydration promoted by a heterobimetallic catalytic cycle.

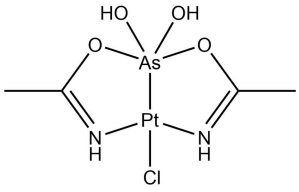
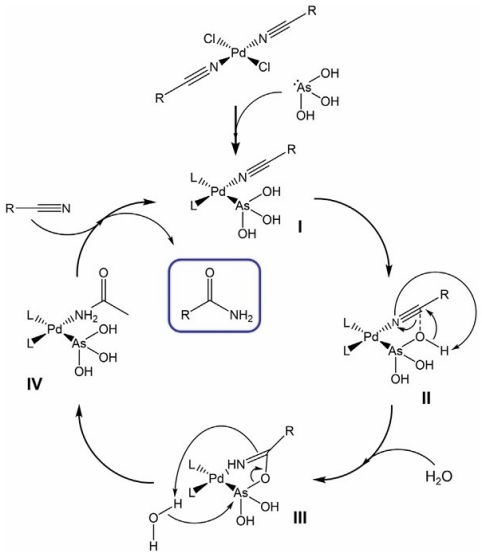


Figure 3. AP-1 structure.



Scheme 1. Proposed reaction mechanism for the discovered catalytic cycle. Reproduced from ref. [30], Copyright (2023), with permission from Frontiers Media S.A.

4. Summary and Outlook

We have shown here that inorganic drugs can be considered for their beneficial repositioning in fields other than medicine. In fact, based on the mechanistic knowledge gained on established metallodrugs thanks to extensive biological studies - the latter being essential for defining their mechanism of action and consequently for improving clinical protocols - they can be conveniently used to address chemical problems that are not strictly related to medicine. This leads to a kind of alternative meaning of "drug repurposing", where the the term "repurposing" is used to indicate a novel application of an established inorganic drug to solve non-medical problems. In this context, the proof of concept for the validity of this approach has been offered using some relevant and consistent examples.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Metallodrugs · Drug repurposing · Mass spectrometry · X-ray crystallography · Cancer

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