# Metallodrugs as protein modulators

Batista de Almeida, Andreia Filipa

# 3. Aim and Outline

Incorporation of metals into drug scaffolds offers a vast potential for creating promising metal-based drug candidates with unique chemistry and biological activity of clinical significance. As demonstrated by the numerous examples of metal-based complexes with enzyme inhibitory activity (or more in general able to modulate protein activities) cited in the literature, research in medicinal inorganic chemistry, as well as in the investigation of metal compound-protein interactions, has great potential for drug design. As an example, the possibility for metal compounds to alter zinc-finger domains is very attractive in the development of novel anticancer compounds.

In this context, protein modulation (inhibition or activation) by metal compounds for different therapeutic and/or imaging purposes is an intriguing research topic. The integration of chemistry and molecular biology with imaging techniques is providing exciting opportunities in the early detection and treatment of different pathologies. Indeed, the design of ***theranostic*** compounds, where diagnosis is combined with therapy, is highly valuable, for example, to address a disease as complex as cancer.

The interplay between metal/metalloid compounds and aquaporins (AQPs), possible biological targets for metal compounds, has been shown to be very important from different points of view. For example, the uptake of certain inorganic compounds by aquaglyceroporins may have physiological significance, which has not been fully investigated yet. Potential consequences include efficacy of metallodrugs and sensitivity to environmental metals/metalloids, as in the case of arsenic leading to toxic effects. Similarly, the effectiveness of inorganic compounds as drugs may depend on the level of expression of specific AQPs isoforms that may facilitate transport into the cells, which may differ from one cell/tissue type to another.

In addition, the intriguing properties of metal complexes as inhibitors of AQPs are worth exploring to develop novel possible therapeutic agents and/or chemical probes to study AQPs functions in cells. Thus, the use of coordination and organometallic gold compounds as aquaglyceroporins inhibitors holds great potential to reduce cell proliferation in cancer cells, as well as to understand the roles of AQPs in cancer development. However, more research efforts are necessary to elucidate the mechanisms of interactions of inorganic compounds with AQPs at a molecular level, which should be conducted via various methods in the frame of a highly interdisciplinary approach.

In this context, knowledge of inorganic chemistry is essential to explain the chemical speciation of metallodrugs and metal ions in a physiological environment, as well as their reactivity with biomolecules, to elucidate the mechanism of transport of inorganic compounds, as well as their inhibition properties of different protein isoforms.

Overall, from the point of view of Medicinal Chemistry, the exploration of the periodic table presents exciting challenges, where inorganic compounds, and metal complexes in particular, exert drug-like actions by mechanisms that can be quite distinct from those of organic drugs. In fact, metallodrugs show unique modes of activity based on the choice of the metal, its oxidation state, the types and number of coordinated ligands and the coordination geometry.

## Outline of the thesis

Based on the above mentioned considerations, **the main aim** of the work described in this thesis was:

• To investigate the properties of **metal-based compounds** as possible **anticancer agents** or as **chemical probes** to study protein functions in biological systems.

In fact, the understanding of the biological effects induced by different families of inorganic compounds, and achieving structure-activity relationships, are crucial for new drugs development. In addition, validating possible protein targets for metal compounds, will assist identifying their mechanism of pharmacological action and toxicity and, finally will help achieving selectivity via appropriate modifications of the chemical scaffolds.

Based on this primary aim, the work described in this thesis focuses on:

**A)** The study of gold complexes as aquaporin inhibitors.

**B)** The characterization of the properties of different families of gold compounds as anticancer agents *in vitro*.

Accordingly, the manuscript is divided into two main parts:

In **Part A**, the membrane water and glycerol channels aquaporins are studied as targets for metal compounds. Specifically, aquaglyceroporin isoforms are investigated. Thus, in **Chapter A1**, the inhibitory effects of several coordination gold(III) compounds, with N-donor ligands or organometallics, on human aquaglyceroporin-3 (AQP3) are characterized by stopped-flow methods in selected cellular models. In addition, several computational methodologies, such as non-covalent docking, QM/MM and DFT calculations, performed in collaboration with other groups, were applied to study the mechanisms of AQP3 inhibition by gold complexes at a molecular level.

This study revealed initial structure-activity relationship, crucial to improve design of more potent and selective inhibitors.

Following these interesting results on AQP3, human aquaglyceroporin-7 (AQP7) was studied, which is mainly expressed in adipocytes. Hence, in **Chapter A2** the inhibition of water and glycerol permeability of human AQP7 by the gold(III) complex $[Au(phen)Cl_2]Cl$ (phen=1,10-phenantroline; Auphen), transfected in a murine adipocyte model was studied. The mechanism of inhibition of hAQP7 by Auphen, was investigated using *in silico* methodologies, including homology modelling and non-covalent docking. The proposed mechanism of inhibition appears to be different from that observed in the case of hAQP3. This information is useful to improve inhibitor's design and isoform-selectivity.

As described in the introductory chapter, several aquaporin isoforms are known to be inhibited (unselectively) by mercury ions. Several authors studied the inhibition of orthodox aquaporins by mercury using different techniques, such as X-ray crystallography and molecular dynamics (MD) simulations. In **Chapter A3**, for the first time, the mechanism of inhibition of an aquaglyceroporin, AQP3, by mercury is described, using MD. Interestingly, the reported results are

useful to understand the mechanism of inhibition of the gold compounds developed by us, since gold has a similar affinity to mercury for binding amino acids. From these studies it appeared that metal coordination to specific amino acid residues induces protein conformational changes which lead to channel blockage and inhibition of glycerol transport.

MD simulations also can provide insights into other mechanisms of inhibition of water and glycerol transport in AQPs, such as channel gating by different stimuli (e.g. pH). In fact, it has been previously described that AQP3 is gated by pH, however no clear insight into the mechanisms of pore closure at a molecular level have been provided so far. Therefore, **in Chapter A4**, the pH gating of human AQP3 in red blood cells, as well as the pH gating of rat AQP3, in a yeast cell model, respectively, was studied using stopped-flow spectroscopy. Moreover, the same phenomenon of pH gating was described for human AQP7, expressed in a yeast cell model. Thus, in this chapter, using computational tools, we propose an hypothesis for the different mechanisms of pH gating in AQP3 and AQP7.

**Part B** focuses on the study of metal compounds as anticancer agents. Selected results out of the screening of different families of coordination metal compounds performed in the last four years are presented. Specifically, in **Chapter B1** the synthesis and biological activity of a series of gold(I) organometallic compounds are reported. The synthesis and structural characterization of the compound was performed within our group by an other PhD candidate, while my work was focused on the biological characterization. The complexes under investigation are gold(I) *N*-heterocyclic-carbenes (NHC) with different ancillary ligands aimed at fine-tuning their hydrophilic/lipophilic character. All the complexes were tested for their antirpoliferative effects in several cancerous and non-cancerous cell lines. Additionally, the cellular uptake and distribution of the complexes with a fluorescent coumarine moiety was evaluated by fluorescence microscopy. Moreover, in order to get mechanistic insights on the activity of the gold(I) complexes, these were evaluated for their inhibition effects on different types of targets such as the zinc finger protein PARP-1 and enzymes involved in maintaining the intracellular redox balance such as thioredoxin reductase (TrxR), glutathione reductase (GR) and glutathione peroxidase (GP), in collaboration with the group of Prof. Maria Pia Rigobello (University of Padova, Italy)

In **Chapter B2** a series of luminescent polynuclear metal complexes with biological activity in cancer cells is reported. These metal-based compounds bear two different moieties: a ruthenium(II) centre with bipyridyl ligands, with luminescent properties that can be used to track the compound in cells, and a "therapeutic centre" consisting of Au(I), Ru(III) or Rh(III) ions. Moreover, a few of these complexes were conjugated with a thioglucose moiety, aimed at improving cellular uptake via active transport mediated by glucose transporters. The synthesis of these complexes was achieved in collaboration with the group of Prof. Pierre Le Gendre (University of Burgundy, France). In this chapter we describe the anticancer activity of the complexes in different cell lines, which allowed us to select the most promising compounds in our series, as well as the cellular distribution of representative compounds. Moreover, investigation of the possible uptake of the complexes by the glucose transporter GLUT-1 was conducted.

The contents of each chapter are summarized and discussed in the **Summary and Discussion Chapter** of this thesis, where general considerations on the different biological properties of the various investigated families of metal compounds are presented, as well as perspectives for future design of metal complexes for applications in chemical biology and medicine.

# Part A

## Aquaporins as Drug Targets