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Review

Metallomics: An Essential Tool for the Study of Potential Antiparasitic Metallodrugs

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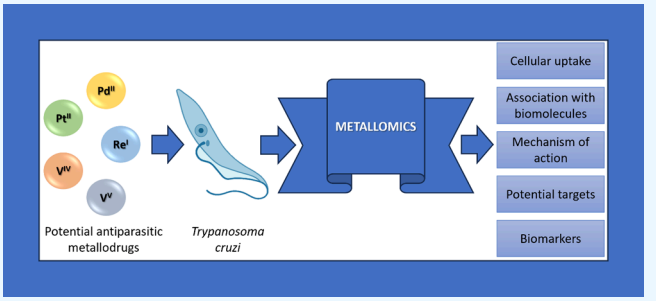
ABSTRACT: Metallomics is an emerging area of omics approaches that has grown enormously in the past few years. It integrates research related to metals in biological systems, in symbiosis with genomics and proteomics. These omics approaches can provide in-depth insights into the mechanisms of action of potential metallodrugs, including their physiological metabolism and their molecular targets. Herein, we review the most significant advances concerning cellular uptake and subcellular distribution assays of different potential metallodrugs with activity against *Trypanosoma cruzi*, the protozoan parasite that causes Chagas disease, a pressing health problem in high-poverty areas of Latin America. Furthermore, the first multiomics approaches including metallomics, proteomics, and transcriptomics for the comprehensive study of potential metallodrugs with anti-*Trypanosoma cruzi* activity are described.



1. INTRODUCTION

The potential use of metal-based compounds in medicine is an area of bioinorganic chemistry of great interest, especially after the discovery of the anticancer activity of cisplatin in 1965.[1] Although cisplatin and the second-generation platinum compounds are still used worldwide, several adverse side effects and resistance have been observed. This led to research for the development of novel metal-based anticancer compounds, challenging the work of modern medicinal inorganic chemistry.[2,3] In a similar way, various metal-based antiparasitic compounds have been developed through the years.[4−15] In this regard, "omics" approaches constitute essential tools providing new insights into the mode of action of potential metal-based drugs. The term omics refers to a comprehensive analysis, typically using advanced high-throughput analytical strategies, data analysis, and informatics to study the roles, relationships, and actions of various types of analytes in biological systems. It includes several fields, such as genomics, proteomics, transcriptomics, metabolomics, and metallomics, among others.[16,17]

Metallomics is an emerging area of omics approaches that has grown enormously since its conception as an academic discipline in 2004. It integrates research related to metals in biological systems, in symbiosis with genomics and proteomics, since the syntheses and metabolic functions of genes and proteins cannot be performed without the help of various metal ions and metalloenzymes. This discipline is defined as the study of the "metallome", which involves the interactions and functional connections of metal ions or species with genes, proteins, metabolites, and other biomolecules in biological systems. The study of the metallome of a species can provide information on the distribution of an element between cellular compartments, on the coordination environment in which a biomolecule is incorporated, or on the concentration of individual metal species present. In this regard, it plays a very important role in providing integrated information that connects metallomics with other omics disciplines.[18−20] However, the ionic state of a given metal is the one that is usually functionally important. To overcome this limitation of the word metallomics, the term ionomics has also been defined, including metals, metalloids, and nonmetals.[21] A more general term in lieu of ionomics is elementomics since not all elements occur in an ionic form, and some of them may be present in biological systems as covalent compounds.[22] In addition, chemical speciation for the identification of chemical species in biological systems using omics approaches has become a remarkable area of study called speciomics. It is proposed as an "umbrella" term containing all omics approaches devoted to speciation analysis, ranging from the evaluation of oxidation states or isotopes of a given element as part of a biological molecule to the evaluation of different protein isoforms in a biological tissue.[23]

The term metallomics was coined for the first time in June 2002 during the Tokushima Seminar on Chemical Engineering

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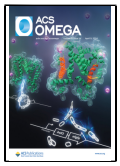

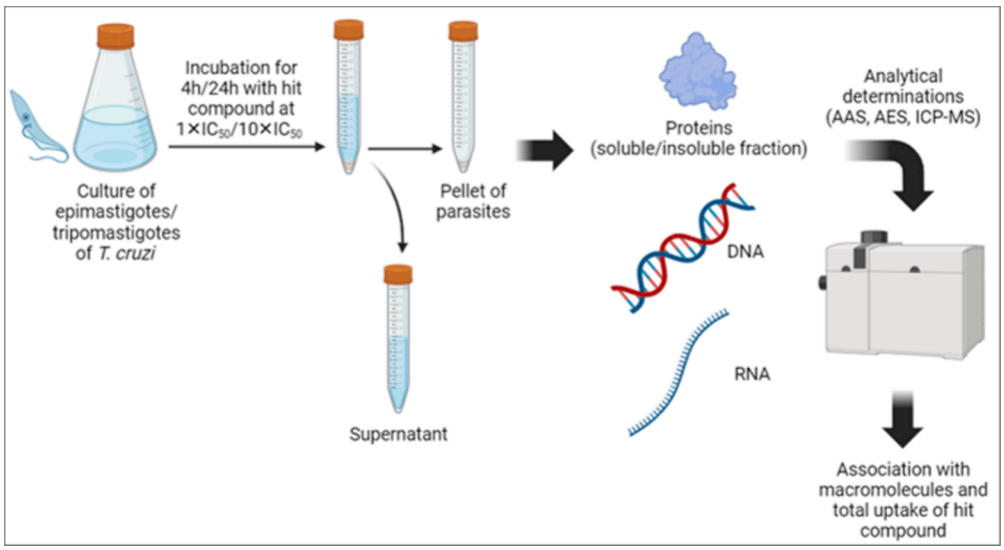

Figure 1. Experimental workflow for cellular uptake assays in metallomics. AAS: atomic absorption spectrometry. AES: atomic emission spectrometry. ICP-MS: inductively coupled plasma mass spectrometry.

held in Tokushima, Japan. During the event, Professor Hiroki Haraguchi (Nagoya University) held an invited lecture where the development of this new omics discipline was suggested, which was closely influenced by the progress of analytical atomic spectrometry, in particular by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES).[24] This omics approach has received great attention as an emerging scientific field since then, and great progress has been achieved.[25−29]

2. ANTIPARASITIC METAL-BASED COMPOUNDS

Among infectious diseases, neglected tropical diseases (NTDs) encompass a variety of 20 conditions primarily found in tropical regions, affecting over 1 billion people in impoverished communities. These diseases result from various pathogens, including viruses, bacteria, parasites, and fungi. These NTDs, linked to poverty, impose a considerable health burden in extensive regions of the world. The existing drugs for these conditions fall short of meeting clinical requirements. For the past decades academic groups and public−private partnerships have dedicated efforts to developing novel drugs, supported by the recent active involvement of some pharmaceutical companies.[30,31]

The WHO classification of neglected illnesses applies in particular to three poverty driven diseases caused by trypanosomatid parasites: Human African Trypanosomiasis (caused by *Trypanosoma brucei*), Chagas disease or American trypanosomiasis (caused by *Trypanosoma cruzi*), and Leishmaniasis (caused by different species of *Leishmania*). Currently, the available therapeutic options to address these infections have low effectiveness and are highly toxic. Over the past decades, this challenge has been exacerbated by the rise and dissemination of drug-resistant strains.[32−36]

In this context, Gambino's group has been working during the last 20−25 years on the rational design of novel metal-based compounds bearing activity against these trypanosomatid parasites, with a particular focus on the causative agent of Chagas disease, *Trypanosoma cruzi*.[5−9,14] Although metallomics and omics studies in general have been performed in the development of anticancer metal-based drugs, an omics strategy had not been explored for unraveling the mechanism of action of antiparasitic metal-based prospective drugs. Knowing the significance of omics studies in drug development, metallomics, proteomics, and transcriptomics of several metal-based compounds were performed for the first time in *Trypanosoma cruzi* by us.[37−47] The current review involves the comparative study of the new knowledge obtained by our group through this approach.

3. CELLULAR UPTAKE ASSAYS

Cellular uptake assays of potential metallodrugs constitute very useful approaches within the field of metallomics. Using an adequate analytical technique, the metallic center of a given metallodrug can be monitored, and thus the fraction capable of entering a cell can be evaluated and quantified. Likewise, the distribution at the subcellular level and the association of the studied metallodrug with biomacromolecules of interest may be studied. In this context, our research group has been working on the optimization and validation of different bioanalytical methods for monitoring potential antiparasitic metallodrugs with activity against *Trypanosoma cruzi*. To the best of our knowledge, no other group has been working on cellular uptake assays of metal-based bioactive compounds regarding *Trypanosoma cruzi*. It should be noted that the procedures to be outlined next are based on bulk studies, in contrast to other cell-to-cell studies described in the literature.[48]

The procedure for the metal-based compound uptake determination has been previously described by our group.[44,47] Briefly, epimastigote or trypomastigote life cycle forms of *Trypanosoma cruzi* in a density of 1×10^7 parasites mL^{-1} are incubated with concentrations corresponding to 1 ×, 5 ×, and 10 × IC_{50} (half maximal inhibitory concentration) previously calculated using the hit compound. Parasites are typically collected at 4 and 24 h after incubation with the studied compound. Each sample, containing 1×10^7 parasites, is then centrifuged, and the supernatant containing the uncaptured compound is separated from the pellet of parasites. The parasites in the pellet fraction are washed with phosphate-buffered saline and resuspended with it to an exact volume. Both fractions (pellet and supernatant) are analyzed separately by an adequate spectrometric technique. Finally, the uptake percentage in the parasites is calculated according to the following equation: %

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Uptake = [P/(P + S)], where P corresponds to ng of metal in the parasites (pellet), S corresponds to ng of metal in the supernatant, and (P + S) corresponds to ng of the metal incorporated in the experiment (supernatant + pellet). Once the metal is uptaken by the parasites or strongly bound (not removable by washing) and the metal remaining in the culture medium is determined, the corresponding mass balance is performed to evaluate the accuracy of the analytical method.[44,47]

Metal association with different macromolecules, namely, desoxyribonucleic acid (DNA), ribonucleic acid (RNA), soluble proteins (SPs), and insoluble fraction (IF) (insoluble proteins + membrane lipids + other insoluble molecules), can be evaluated, providing an idea of the main biological targets of the studied compound. For this task, mid exponential phase parasites are incubated with 1 ×, 5 ×, and 10 × IC_{50} previously determined on trypomastigotes of *Trypanosoma cruzi*. After 24 h of incubation, macromolecules are isolated for further analysis. For DNA isolation, 3×10^7 parasites are collected using specific purification kits. For soluble protein isolation, 3×10^7 parasites are resuspended in a parasite lysis buffer containing detergents, salts, buffering agents, reducing agents, and protease inhibitors. After 30 min of stirring on ice, the lysate is centrifuged. Soluble proteins are isolated from the supernatant, while the insoluble fraction is isolated from the pellet, which is then resuspended in phosphate-buffered saline for further analysis. For RNA isolation, 3×10^7 parasites are collected using a specific reagent. Three independent experiments are typically performed. Analytical determinations are then performed using an appropriate analytical technique.[44,47] A scheme of the experimental workflow is shown in Figure 1.

4. ANALYTICAL TECHNIQUES EMPLOYED IN METALLOMICS

Since the 1970s, ICP-MS and ICP-AES techniques have been positioned as highly sensitive analytical tools with excellent possibilities for simultaneous quantification of multiple elements. Nowadays, it is possible to carry out analyses of basically all the elements in any type of sample using one of these two techniques. Likewise, the use of several other techniques for metallomic studies has been reported, such as flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), laser-induced plasma spectroscopy (LIPS), and energy dispersive X-ray fluorescence spectrometry (EDXRF), among others.[18] An interesting technique that has reemerged in the past few years with several improvements is microwave plasma atomic emission spectrometry (MP-AES), and it can be considered as a good strategy for metallomics studies of highly refractory elements such as rhenium.[47] This technique employs a nitrogen plasma that operates at around 5000 K. This gas can be generated online resulting in cost savings and in the removal of flammable gases from the laboratory. The high plasma temperature provides a higher sample matrix tolerance, while lower detection limits and an expanded working concentration range can be achieved, when compared to FAAS.[49] The choice of the right analytical technique often involves a careful consideration of many factors to ensure that the chosen method aligns with the goals and constraints of the analysis. Some of these factors are sample nature, sample matrix characteristics, sample size, analyte concentration, analysis speed required, need for qualitative or quantitative information, information depth, costs, instrumental availability, need for a nondestructive analysis, research objectives, expertise, and safety considerations.[47]

In addition to metal determination in single cells, spatially resolved elemental concentration and imaging in biological samples have become one of the current challenges in metallomics. Recent analytical advances have enabled the mapping of elements in different types of cells, allowing high-resolution imaging, that can help understand the roles of a given element in relation to intracellular molecules such as proteins, nucleic acids, lipids, sugars, and other metabolites. This is essential for clarifying cellular functions such as proliferation, differentiation, aging, and stress responses. In this regard, scanning X-ray fluorescence microscopy (SXFM) can reliably determine the cellular distribution of multiple elements by a sub-100 nm focusing approach. Visualizing intracellular elements and understanding their dynamics at the single-cell level may provide great insight into their behaviors. Moreover, the use of synchrotron radiation allows the high-resolution spatial speciation analysis of cellular compartments, as it produces extremely intense and focused X-ray beams, which can be essential for achieving high sensitivity in detecting trace elements within a given sample.[50] Also, coupled techniques such as LA-ICP-MS (LA, laser ablation) have been successfully applied to the analysis of complex metal-associated proteomes.[51]

Confocal Raman microscopy (CRM) is another versatile tool in metallomics that allows researchers to investigate the complex interplay between metal ions and biological systems at a microscopic and molecular level. This technique provides information about the structure of chemical components present in biological samples, with the advantage of minimal sample preparation, without the need of previous labeling and relatively free from water interference.[52] The high penetration of infrared radiation makes this technique suitable for tissue imaging.[53] Particularly, rhenium(I) tricarbonyls present vibrational properties suitable for monitoring by this technique. They present intense bands in the range 1800−2200 cm^{-1}, a range in which there is practically no absorption in biological samples.[54] Jaouen's group employed these spectral characteristics in a biological context for the first time by implementing a drug testing method based on the use of metal carbonyls coordinated to them as tracers.[55] Later, this technique was used to track rhenium(I) tricarbonyls in breast cancer cells[56] and to explore the molecular properties of lipids within *Trypanosoma cruzi*.[52] More recently, Raman microscopy has been used for metallomics studies regarding the distribution within *Trypanosoma cruzi* of potential rhenium(I) tricarbonyl compounds against Chagas disease.[46]

5. COMPARISON OF POTENTIAL ANTIPARASITIC METALLODRUGS FROM A METALLOMICS PERSPECTIVE

A large number of metal-based compounds with anti-*Trypanosoma cruzi* activity have been synthesized by our research group, using as a strategy the coordination of metal ions or organometallic centers of pharmacological importance with bioactive organic ligands that have proven activity against *Trypanosoma cruzi*. Binding to a metal center can modify properties such as the solubility, lipophilicity, stability, and electronic and transport properties of the organic ligand, generating compounds that may be more active and/or less toxic. These metal-based compounds can act by affecting two or more targets in the parasite: that of the ligand itself and others resulting from the presence of the metal. The biological properties of the metal-bioactive ligand compound will depend on the nature of the metallic center and the bioactive ligand, the

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Table 1. Uptake of Metal-Based Compounds by a Parasite after 24 h Incubation on Epimastigotes of *Trypanosoma cruzi* (CL Brener Strain) at 1 × IC_{50} and 1 × IC_{50} Doses[a]

Compound	% Uptake		Reference
	1 × IC_{50}	10 × IC_{50}	
$[Pd^{II}(dppf)(mpo)](PF_6)$ with dppf = 1,1′-bis(diphenylphosphino)ferrocene and mpo = pyridine-2-thiolate-1-oxide	4.0	6.0	37
$[Pt^{II}(dppf)(mpo)](PF_6)$ with dppf = 1,1′-bis(diphenylphosphino)ferrocene and mpo = pyridine-2-thiolate-1-oxide	75.0	19.0	38
$[V^{IV}O(5Brsal)(aminophen)]$ with aminophen = 5-amino-1,10-phenanthroline	2.4	2.4	39
$[V^{IV}O(L1\text{-}H)_2]$ with L1 = 5,7-dichloro-8-hydroxyquinoline	17.6	19.7	42
$[V^{V}O(8HQ\text{-}H)(L4\text{-}2H)]$ with 8HQ = 8-hydroxyquinoline and L4 = salicylaldehyde semicarbazone derivative	68.3	78.9	43
$[V^{V}O(IN\text{-}2H)(L2\text{-}H)]$ with IN = Schiff base ligand derived from isoniazid and L2 = 5-chloro-7-iodo-8-hydroxyquinoline	4.4	3.6	44
$[V^{IV}O(L2\text{-}H)(mpo)]$ with L2 = 5-chloro-7-iodo-8-hydroxyquinoline and mpo = pyridine-2-thiolate-1-oxide	2.2	1.4	45
fac-$[Re^{I}(CO)_3(tmp)(CTZ)](PF_6)$ with CTZ = clotrimazole and tmp = 3,4,7,8-tetramethyl-1,10-phenanthroline	1.2	1.2	47

[a]% Uptake: % of metal uptaken by the parasite (pellet) relative to total metal added to the parasite culture.

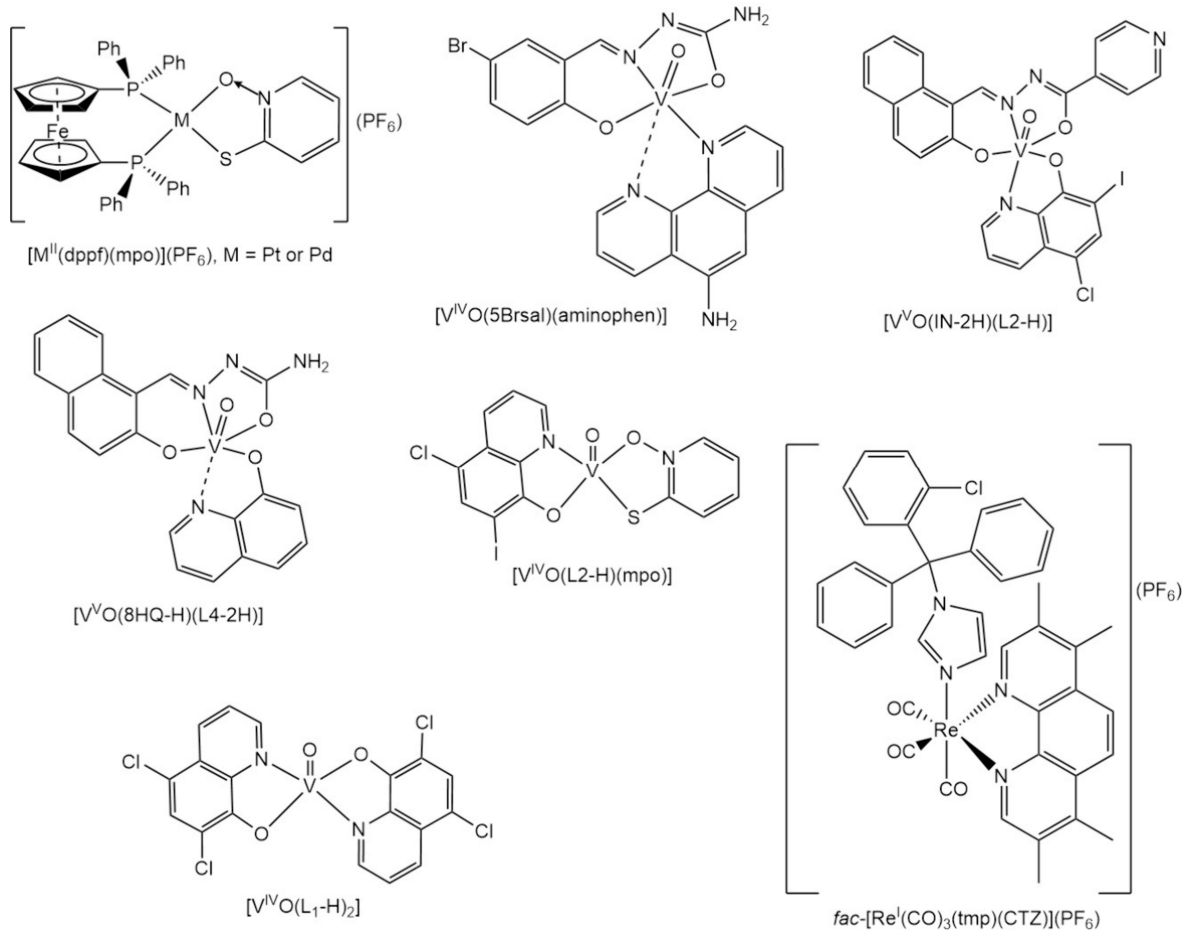

Figure 2. Molecular structures of the metal-based compounds designed as anti-*Trypanosoma cruzi* agents studied by omics techniques. dppf: 1,1′-bis(diphenylphosphino)ferrocene; mpo: pyridine-2-thiolate-1-oxide; 5Brsal: 5-bromo salicylaldehyde semicarbazone; aminophen: 5-amino-1,10-phenanthroline; 8HQ: 8-hydroxyquinoline; tmp: 3,4,7,8-tetramethyl-1−10-phenanthroline; CTZ: clotrimazole.

presence of other coligands, and the physicochemical-structural properties of the whole entity. In this regard, we have focused our attention on the rational design of antiparasitic metal compounds based on the relationships between chemical structure, physicochemical properties, and biological activity obtained in our investigations. This research line has led to important contributions that have been transferred to the scientific field, showing the vital importance of cellular uptake metallomic studies to understand the fate of potential metallodrugs and elucidate targets and mechanisms of action.[37−47] Table 1 summarizes the uptake results obtained after metallomics studies of different metal-based compounds performed on epimastigotes of *Trypanosoma cruzi*. The molecular structures of the bioactive compounds are shown in Figure 2.

As can be observed in Table 1, % uptakes widely vary from 1.2 to 75.0%, depending on the different chemical characteristics of each compound. Cellular uptake of metal-based compounds is a complex process influenced by multiple factors, including the metal center, the physicochemical properties of the final compound, the specific cell type, and the presence of specific transporters and binding targets. These factors can result in

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Table 2. Association (%) of Metal-Based Compounds with Parasite Macromolecules after 24 h Incubation with Epimastigotes of *Trypanosoma cruzi* (CL Brener Strain) at a 1 × IC_{50} dose

Compound	SP	IF	DNA	RNA	Reference
$[Pd^{II}(dppf)(mpo)](PF_6)$ with dppf = 1,1′-bis(diphenylphosphino)ferrocene and mpo = pyridine-2-thiolate-1-oxide	1.0	10.0	75.0	14.0	37
$[Pt^{II}(dppf)(mpo)](PF_6)$ with dppf = 1,1′-bis(diphenylphosphino)ferrocene and mpo = pyridine-2-thiolate-1-oxide	6.0	21.0	66.0	7.0	38
$[V^{IV}O(5Brsal)(aminophen)]$ with aminophen = 5-amino-1,10-phenanthroline	3.8	96.2	-	-	39
$[V^{IV}O(L1\text{-}H)_2]$ with L1 = 5,7-dichloro-8-hydroxyquinoline	50.2	10.5	12.0	27.3	42
$[V^{V}O(8HQ\text{-}H)(L4\text{-}2H)]$ with 8HQ = 8-hydroxyquinoline and L4 = salicylaldehyde semicarbazone derivative	60.0	6.0	23.0	11.0	43
$[V^{V}O(IN\text{-}2H)L2\text{-}H)]$ with IN = Schiff base ligand derived from isoniazid and L2 = 5-chloro-7-iodo-8-hydroxyquinoline	90.3	8.7	0.8	0.2	44
$[V^{IV}O(L2\text{-}H)(mpo)]$ with L2 = 5-chloro-7-iodo-8-hydroxyquinoline and mpo = pyridine-2-thiolate-1-oxide	85.0	10.5	3.5	1.0	45
fac-$[Re^{I}(CO)_3(tmp)(CTZ)](PF_6)$ with CTZ = clotrimazole and tmp = 3,4,7,8-tetramethyl-1,10-phenanthroline	82.1	16.3	0.9	0.7	47

different metallodrugs exhibiting varying levels of cellular uptake even within the same cell type. The charge and coordination environment of the metal ion in the metallodrug can greatly affect its cellular uptake. Cells have ion channels and transporters that selectively allow the entry of specific ions. The charge and coordination of the metal ion can thus determine whether it is recognized by these transport systems. Understanding these factors is critical for optimizing the design and efficacy of metallodrugs in potential medical applications. The differential uptake within the vanadium-based compounds shown in Table 1, ranging from 2.4 to 68.3%, could also arise from the different chemical nature of the compounds and/or different speciation in the incubation media.[57] Interconversion between vanadium(IV) and vanadium(V) oxidation states is one of the main features of vanadium biochemistry. While vanadium(V) predominates in neutral aerated aqueous solutions, it can be reduced to vanadium(IV) by biological compounds. Both vanadium species bind to a wide variety of biological ligands, but none of them act as a strong binder, which leads to complex speciation in biological media.[57]

Also, when comparing platinum and palladium compounds, both including the same ligands, an uptake of 75% was observed for $[Pt^{II}(dppf)(mpo)](PF_6)$ in contrast to the 4.0% observed for $[Pd^{II}(dppf)(mpo)](PF_6)$. This much higher uptake observed for platinum could be explained by the fact that platinum compounds like cisplatin can be transported into cells through specific uptake mechanisms, such as copper transporters like CTR1.[58] These transporters may have a higher affinity for platinum than palladium ions, contributing to the preferential uptake of platinum compounds.[59]

When comparing the uptake results shown in Table 1 for 1 × IC_{50} with the respective results obtained after 24 h incubation at 10 × IC_{50}, the metal incorporation percentages did not change significantly, except for $[Pt^{II}(dppf)(mpo)](PF_6)$, where a statistically significant decrease was observed from 75% (1 × IC_{50}) to 19% (10 × IC_{50}), showing a potential saturation effect.[38] This differential uptake could partially explain the much higher activity on the parasite of the Pt compound in respect to the Pd analogue. A similar behavior was observed for $[V^{IV}O(L2\text{-}H)(mpo)]$ with L2 = 5-chloro-7-iodo-8-hydroxyquinoline and mpo = pyridine-2-thiolate-1-oxide.[45]

For $[V^{V}O(IN\text{-}2H)(L2\text{-}H)]$ the vanadium uptake on the infective trypomastigote form of the parasite was higher than on the epimastigote life cycle form present in the infected bug responsible for the parasite transmission. This correlates well with a higher activity of the compound on the biologically relevant trypomastigote form circulating in the mammalian host blood.[45]

Table 2 summarizes the % association results obtained after subcellular distribution analysis of the same metal-based compounds described in Table 1, performed on epimastigotes of *Trypanosoma cruzi*. It shows the mean associations between the compounds with DNA, RNA, SP, and IF. As can be observed, the main association deeply varies according to the studied compound. For instance, a higher association with DNA was observed for both $[Pt^{II}(dppf)(mpo)](PF_6)$ (66%) and $[Pd^{II}(dppf)(mpo)](PF_6)$ (75%). On the other hand, while a higher association with SP was observed for $[V^{V}O(8HQ\text{-}H)(L4\text{-}2H)]$ (60%), $[V^{IV}O(L1\text{-}H)_2]$ (50.2%), $[V^{V}O(IN\text{-}2H)L2\text{-}H)]$ (90.3%), *fac*-$[Re^{I}(CO)_3(tmp)(CTZ)](PF_6)$ (82.1%), and $[V^{IV}O(L2\text{-}H)(mpo)]$ (83%), a higher association with IF was observed for $[V^{IV}O(5Brsal)(aminophen)]$ (96.2%). Moreover, negligible associations (<1%) with nucleic acids were observed for compounds $[V^{V}O(IN\text{-}2H)L2\text{-}H)]$, *fac*-$[Re^{I}(CO)_3(tmp)(CTZ)](PF_6)$, $[V^{IV}O(5Brsal)(aminophen)]$, and $[V^{IV}O(L2\text{-}H)(mpo)]$ which allowed us to discard these biomolecules as the main targets of action, despite the known capacity of the ligands to intercalate within nucleic acids and the observed *in vitro* interaction of the complexes with DNA.[60] The deeply high association with IF fraction suggests that $[V^{IV}O(5Brsal)(aminophen)]$ could be interacting with membrane-associated proteins. The fact that the association pattern between vanadium-based compounds is quite different indicates that despite the fact that the complexes probably undergo changes/speciation in the incubation media and several fractions of the parasite the nature of the ligands also poses significant effects, showing that the species in both cases are different.[44]

When comparing the behavior of platinum and palladium compounds, a preferred association with DNA for both $[Pt^{II}(dppf)(mpo)](PF_6)$ and $[Pd^{II}(dppf)(mpo)](PF_6)$ was observed. Although both platinum and palladium can form coordination complexes with DNA, platinum compounds are more commonly known for their ability to interact with this biomolecule. Such are the cases of cisplatin and oxaliplatin used in chemotherapy to treat cancer. Cisplatin, for example, forms covalent bonds with the purine bases in DNA, leading to the cross-linking of DNA strands and disruption of the DNA structure.[61] This interference with DNA replication and transcription can inhibit the growth of cancer cells. On the other hand, the specific interactions and mechanisms of DNA binding can vary depending on the particular studied compound since DNA binding is structure-dependent.[62]

Additionally, the data from Table 2 are described in a graphical form in the bar chart displayed in Figure 3.

In order to deepen the localization of the novel *fac*-$[Re^{I}(CO)_3(tmp)(CTZ)](PF_6)$ compound within *Trypanosoma cruzi*, metallomics studies employing confocal Raman microscopy were additionally performed.[46] As previously stated, it can be a very useful strategy for rhenium(I) tricarbonyl monitoring.

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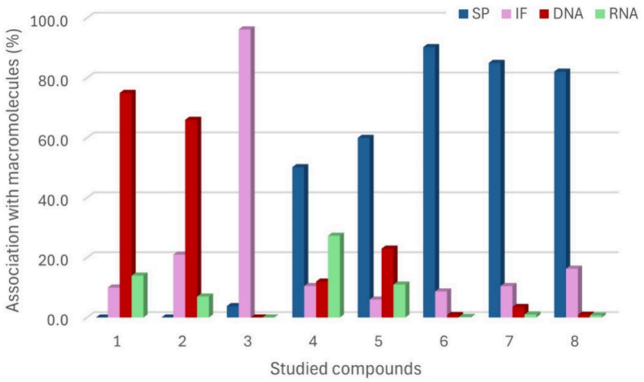

Figure 3. Association (%) of studied compounds with parasite macromolecules after 24 h incubation with epimastigotes of *Trypanosoma cruzi* (CL Brener strain) at 1 × IC_{50} dose: (1) $[Pd^{II}(dppf)(mpo)](PF_6)$, (2) $[Pt^{II}(dppf)(mpo)](PF_6)$, (3) $[V^{IV}O(5Brsal)(aminophen)]$, (4) $[V^{IV}O(L1\text{-}H)_2]$, (5) $[V^{V}O(8HQ\text{-}H)(L4\text{-}2H)]$, (6) $[V^{V}O(IN\text{-}2H)L2\text{-}H)]$, (7) $[V^{IV}O(L2\text{-}H)(mpo)]$, and (8) *fac*-$[Re^{I}(CO)_3(tmp)(CTZ)](PF_6)$.

However, the bands associated with $\nu(CO)$ could not be detected probably due to the low concentration assayed. The main bands of the studied rhenium(I) tricarbonyl compound overlapped with signals coming from lipids, proteins, and DNA from the parasites (Figure 4). Signals coming from DNA were modified due to the interaction with the rhenium compound, indicating a possible weak interaction of the compound with this biomolecule.[46]

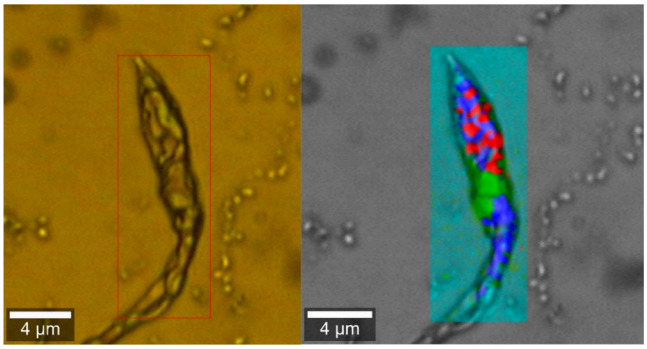

Figure 4. Epimastigotes of *Trypanosoma cruzi* incubated with *fac*-$[Re(I)(CO)_3(tmp)(CTZ)](PF_6)$ at 1 × IC_{50} for 24 h: (a) optical image and (b) overlay between optical and confocal Raman images. The red and green labels correspond to mitochondrial and nuclear DNA, and the blue label corresponds to lipids from the membrane.

6. MULTIOMICS APROACHES

The identification of molecular targets and unveiling the mode of action of potential metallodrugs are mandatory for their clinical development.[51] Cellular responses to a given metallodrug are usually manifested by changes in relative protein activity by either direct or indirect interactions. Therefore, proteomics allows monitoring interactions between a metallodrug and its diverse molecular targets, while overexpressed or under-expressed proteins can be identified and quantified. In this regard, several multiomics approaches combining proteomics, transcriptomics, and metallomics have been successfully implemented in the past few years for elucidating mechanistic aspects regarding the behavior of potential metallodrugs.[16] Proteomics information together with metallomics and transcriptomics can provide a deeper insight into what happens inside the cells after administering a given metallodrug.

In order to identify key molecules and possible pathways involved in the mode of action of the $[V^{IV}O(5Brsal)(aminophen)]$ compound depicted in Tables 1 and 2, our group studied the main omics changes induced in *Trypanosoma cruzi*.[39] Comparative transcriptomic analysis revealed minimal changes on mRNA steady state levels upon treatment, with less than 0.008% of the transcripts differentially expressed, most of them codifying for hypothetical proteins with no known function or functional information or domains. This agreed with the preferred association of the compound with IF rather than with nucleic acids, previously shown by metallomics studies. On the other hand, proteomic analysis of insoluble protein fraction revealed a total of 248 overrepresented and 110 underrepresented proteins when compared to control untreated parasites, with cytochrome P450 being the most downregulated insoluble protein. This protein is involved in mediating detoxification processes, which may explain the lack of an effective detoxification response by the parasites. Soluble proteins that appear differentially expressed included acetyltransferases, reductases, and hydrolases, suggesting a major role of the compound in driving early energy and redox metabolic disorders. Finally, the fact that many ribosomal proteins were differentially expressed in treated parasites, despite the negligible association with mRNA, suggested that the translation process could be affected. In sum, this multiomics approach allowed the identification of the affected processes that drove a very good inhibition of parasite growth even at the low uptake of 2.4% observed.[39]

A multiomics approach including proteomics and transcriptomics was also performed on the compounds $[Pd^{II}(dppf)(mpo)](PF_6)$ and $[Pt^{II}(dppf)(mpo)](PF_6)$ previously described. A similar pattern of distribution between the four analyzed fractions was found for both compounds after metallomics, as aforesaid, with a preferential association with DNA. Also, a similar number of differentially expressed proteins was noticed after proteomics.[40] However, differentially expressed transcripts were identified in parasites after the incubation with each hit compound, resulting in more modulated transcripts for the treatment with $[Pd^{II}(dppf)(mpo)](PF_6)$ (2327 transcripts out of the 10,785 identified) when compared to the treatment with $[Pt^{II}(dppf)(mpo)](PF_6)$ (201 transcripts out of the 10,773 identified), suggesting a mechanism of action at the transcriptome level for the former. Differentially expressed transcripts turned out to be involved in DNA binding, protein metabolism, transmembrane transport, oxidative defense, and ergosterol biosynthesis, demonstrating a potential multimodal mechanism of action for the two studied compounds. This multiomics approach allowed us to unravel significant differences between the analogous hit compounds, highlighting once again the importance of the nature of the metallic core on the biological behavior of metallodrugs.[40] Since ergosterol biosynthesis turned out to be modulated by the two hit compounds, a subsequent study comprising the determination of sterol levels in treated parasites was performed, which confirmed the involvement of $[Pd^{II}(dppf)(mpo)](PF_6)$ and $[Pt^{II}(dppf)(mpo)](PF_6)$ in this biosynthetic pathway.[41] Moreover, two enzymes of the pathway, namely, phosphomevalonate kinase (PMK) and lanosterol 14α-demethylase (CYP51), were selected as targets for a further study by molecular docking, which showed an energetically favorable interaction with the

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compounds. These observations were experimentally verified after the study of PMK and CYP51 overexpressing parasites, confirming that the hit compounds were involved in the inhibition of both enzymes, leading to a decrease in ergosterol levels in the parasites.[41]

These represent the sole multiomics investigations reported thus far on metal-based potential anti-*Trypanosoma cruzi* compounds. Other multiomics approaches have been performed in the search for the elucidation of the metabolic pathways of *Trypanosoma cruzi* related to clinical drug resistance and to identify promising molecular targets for the development of new drugs for treating Chagas disease. For instance, a transcriptomic analysis of benznidazole-resistant and susceptible *Trypanosoma cruzi* populations was performed, generating a robust set of transcripts involved in different metabolic pathways associated with the benznidazole-resistant phenotype of the parasite, which is of utmost importance, since benznidazole is one of the two drugs currently approved for the treatment of the disease.[63] Furthermore, other authors have conducted a genome-wide multidimensional data integration strategy including genomic, transcriptomic, metabolic, and protein structural data sources, to identify candidate proteins with relevant features for target selection in drug development against *Trypanosoma cruzi*, *Trypanosoma brucei*, and *Leishmania spp*, which gave rise to a list of 319 common candidates.[64] Other similar approaches have also been described in the past few years.[65,66]

7. CHALLENGES AND OPPORTUNITIES OF METALLOMICS

While there are exciting opportunities in the interdisciplinary field of metallomics, there are also several challenges that researchers may face. These challenges are mainly related to the development of highly sensitive and selective analytical methods (especially challenging when dealing with trace metal concentrations in complex biological samples), the biological complexity comprising a dynamic nature of metal ions in biological systems (metal ions can change rapidly in response to various stimuli), and the data analysis comprising the processing and interpretation of large data sets and the integration with other omics data for a comprehensive understanding of biological processes. However, the opportunities in metallomics are vast and can significantly impact fields ranging from medicine to environmental science.[67] Especially in the field of medicine, metallomics has the potential to identify metal ion signatures associated with various diseases (leading to the development of novel diagnostic biomarkers), while understanding metal-related processes in diseases can uncover new therapeutic targets for drug development.[68] Collaborative efforts between scientists from various disciplines are crucial for addressing these challenges and fully realizing the potential of metallomics. It is important to develop efficient metallomic strategies during metal-based antiparasitic studies considering the complexity of parasitic targets. Metallomics plays a crucial role in understanding the behavior of potential metallodrugs inside the parasites and in developing antiparasitic strategies to achieve more efficient interventions.

8. CONCLUSIONS

Metallomics has become an essential interdisciplinary field that provides the necessary tools to assess the fate, possible targets, and mechanisms of action of metal-based compounds with specific activities against biological agents that cause different types of diseases. It promotes the key role of bioanalytical chemistry in supporting medicinal inorganic chemistry during the development of new potential metallodrugs, in the search for answers to important Public Health issues that affect different populations. Furthermore, the combination of metallomics with other omics studies allows consideration of the whole changes that occur in a biological system upon treatment. These multiomics approaches constitute an indispensable tool for the development of antiparasitic compounds and the discovery of novel drugs in general. Unraveling the targets and mechanisms of action of each compound is essential to move to the clinical phase and the subsequent regulatory approval for medical use.

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Notes

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