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Novel Metals and Metal Complexes as Platforms for Cancer Therapy

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Abstract

Metals are essential cellular components selected by nature to function in several indispensable biochemical processes for living organisms. Metals are endowed with unique characteristics that include redox activity, variable coordination modes, and reactivity towards organic substrates. Due to their reactivity, metals are tightly regulated under normal conditions and aberrant metal ion concentrations are associated with various pathological disorders, including cancer. For these reasons, coordination complexes, either as drugs or prodrugs, become very attractive probes as potential anticancer agents. The use of metals and their salts for medicinal purposes, from iatrochemistry to modern day, has been present throughout human history. The discovery of cisplatin, *cis*-$[Pt^{II}(NH_3)_2Cl_2]$, was a defining moment which triggered the interest in platinum(II)- and other metal-containing complexes as potential novel anticancer drugs. Other interests in this field address concerns for uptake, toxicity, and resistance to metallodrugs. This review article highlights selected metals that have gained considerable interest in both the development and the treatment of cancer. For example, copper is enriched in various human cancer tissues and is a co-factor essential for tumor angiogenesis processes. However the use of copper-binding ligands to target tumor copper could provide a novel strategy for cancer selective treatment. The use of nonessential metals as probes to target molecular pathways as anticancer agents is also emphasized. Finally, based on the interface between molecular biology and bioinorganic chemistry the design of coordination complexes for cancer treatment is reviewed and design strategies and mechanisms of action are discussed.

Keywords

Cancer; metal; zinc; copper; platinum; metal complex; DNA; proteasome

1. INTRODUCTION

> "Everything is poisonous, and nothing is harmless. The dose (amount) alone defines whether something isn't poison"[i] Paracelsus, 1493-1541.

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[i]Alle Ding' sind Gift, und nichts ohn' Gift; allein die Dosis macht, daß ein Ding kein Gift ist. Translation by Dr. Claudio Verani, Wayne State University

The current toolbox of active anticancer agents is broad in scope, and targets multiple cellular and biological properties across several tumor types. Over the last fifty years, the development of anticancer drugs moved away from conventional cytotoxicity and towards the rational design of selective agents that act on specific cellular targets [1, 2]. However, significant challenges remain, and the interface between structural biology and chemistry may provide the most productive means for discovering and improving upon novel anticancer agents [3].

In nature, many biological systems make extensive use of metal ions, such as zinc and copper, which play critical roles in the normal functioning of organisms [4]. Transition metals such as copper, iron, and manganese, among others, are involved in multiple biological processes, from electron transfer to catalysis to structural roles, and are frequently associated with active sites of proteins and enzymes [4]. However, dysregulation of some of these essential metals during normal biochemical processing has been implicated in the development of various pathological disorders, such as cancer [5]. These cellular functions only require the “trace metals” in miniscule but tightly regulated amounts. By comparison, other metals such as arsenic, cadmium, chromium, and nickel are less beneficial since they produce a wide range of toxic side-effects, including carcinogenesis [4, 6].

Many metal-containing compounds have been utilized throughout history to treat a wide variety of disorders [7]. In medicinal chemistry —traditionally dominated by organic chemistry— metal complexes have gained favor as diagnostic tools and anticancer agents [8]. Research in anticancer agents was stimulated by the accidental discovery of cisplatin, *cis*-$[Pt^{II}(NH_3)_2Cl_2]$ (Fig. (**1**)). However, its clinical use is restricted due to dose-dependent toxicity and resistance coupled with a narrow spectrum of activity [9, 10]. These limitations have triggered a search for platinum-based compounds that show lower toxicity, higher selectivity, and a broader spectrum of activity [11, 12]. The complexes known as carboplatin and oxaliplatin, among others seen in Fig. **1** arose from this search. Nevertheless, in addition to numerous platinum analogs, other metal complexes containing metal ions such as zinc(II), copper(II), gold, and copper chelating agents have received considerable attention as potential anticancer agents [13-16]. Additionally, the investigation of ruthenium-containing compounds in clinical trials attests to the rich potential of utilizing non-platinum metal-based compounds in the treatment of cancer [17, 18]. Furthermore, metals that take advantage of their unique physiochemical properties have been utilized as powerful tools in cancer diagnosis [19]. This review discusses the role of selected metals in biological processes in cells as they pertain to malignancy and to highlight the medicinal applications of the metals and their complexes in the design and development of metallodrugs for the treatment of cancer. This article also places considerable emphasis on their cellular targets and mechanisms of action.

2. UNIQUE PROPERTIES OF METAL IONS AND METAL-BASED COMPLEXES

The field of medicinal inorganic chemistry encompasses, but is not limited to, the administration (or removal) of a metal ion into (or from) a biological system for either therapeutic or diagnostic purposes [20]. An important property of metals is that they form positively charged ions in aqueous solution that can bind to negatively charged biological molecules. Thus, the charge can be fine-tuned depending on the coordination environment involved, leading to the generation of a species that can be cationic, anionic, or neutral [4, 21]. Additionally, metal ions with high electron affinity can significantly polarize groups that are coordinated to them, fostering the generation of hydrolysis reactions [21].

In recent years, the field of medicinal inorganic chemistry has received considerable attention in the design of anticancer agents [22, 23]. Although metals have been used throughout human history in treating various pathological disorders, it has only been since the landmark discovery of cisplatin (Fig. (**1**)) in the 1960's that the full impact of metal-based compounds in the treatment of cancer has been fully realized. Because the presence of metals under cellular conditions is a tightly regulated process, appropriate administrations of metal-containing drugs must be carefully defined to achieve an optimal therapeutic response [24, 25]. Otherwise, both excess and deficiency of metals may result in undesirable toxicity.

Metal-containing compounds offer many advantages over conventional carbon-based compounds in the development of new medicinal compounds. These advantages are due to their ability to coordinate ligands in a three dimensional configuration, thus allowing functionalization of groups that can be tailored to defined molecular targets [26, 27]. Metal-based complexes offer a rich environment to build upon a variety of distinct molecular structures that confer a wide spectrum of coordination numbers and geometries, as well as kinetic properties, that cannot be realized with conventional carbon-based compounds [8, 28, 29]. The partially filled *d* orbitals in transition metals impart interesting electronic properties that can act as suitable probes in the design of anticancer agents [30]. The oxidation state of a metal is also an important consideration in the design of coordination compounds, given that it allows the participation in biological redox chemistry and plays an influential role in optimal dose and bioavailability of the agent administered [4, 6]. Furthermore, the ability to undergo ligand exchanged reactions offers a myriad of opportunities for metals to interact and coordinate to biological molecules, as demonstrated by the widely used drug cisplatin [26]. Furthermore, when designing metal-based therapeutics, one is not restricted solely by metals selected by nature and can take advantage of the unique properties of nonessential metals, including other 1st and 2nd row transition metals and metals that can impart additional utility not found naturally [21]. Most notable is the design of radiopharmaceuticals that exploit the radioactive properties of metals that are commonly used in the diagnosis of cancer and other medicinal applications [21].

3. PLATINUM-BASED ANALOGS IN CANCER THERAPY

The discovery of cisplatin (Fig. (**1**)) more than four decades ago represented a significant achievement in cancer therapy and stimulated efforts to investigate other platinum and non platinum metal-containing compounds for their potential use in the treatment of cancer [29, 31]. Cisplatin has been widely employed to treat a variety of tumors including ovarian, cervical, head and neck, non-small cell lung carcinoma, and testicular cancers, and is commonly used in combination regimens [32, 33]. However, its widespread clinical use has been hampered by increased toxicity, and the appearance of intrinsic and acquired resistance [34, 35]. In an effort to address these shortcomings, 2nd and 3rd generation platinum analogs, namely carboplatin and oxaliplatin (Fig. (**1**)), have been designed and clinically approved that maintain a more manageable toxicity profile [36]. Carboplatin is effective in the treatment of ovarian carcinoma, lung, and head and neck cancers [37], while oxaliplatin is clinically approved for the treatment of colorectal cancer, which is resistant to cisplatin [38].

A key factor underlying the antitumor effect of platinum-based compounds is related to its ligand exchange kinetics [39]. Although the platinum-ligand bond exhibits similar thermodynamic durability (less than 100 kJ/mol) and is much weaker than typical coordination bonds, such as C-C, C-N, or C-O single and double bonds (between 250 and 500 kJ/mol) [40], the ligand exchange behavior is rather slow. This gives them a high kinetic stability and allows much slower ligand exchange reactions on the order of minutes to days rather than seconds [39, 41]. Additionally, in the case of Pt(II) compounds, ligands oriented in the *trans* position are more rapidly substituted than those in the *cis* position [42]. These

important insights are believed to play a significant role in the antitumor efficacy of platinum(II) compounds. Cisplatin (Fig. (**1**)) governed by a square planar complex is kinetically inert, so it does not easily expand its coordination number and undergoes ligand substitution reactions [9, 43]. Cisplatin is taken up through cells by either passive or active transport [32, 44], where its chloride ions are replaced with water molecules before reacting with DNA, forming coordinative bonds to nitrogen atoms of DNA, specifically 1,2-intrastrand d(GpG) cross links [32, 45]. The high concentration of chloride ions present blood plasma (100 mM) renders cisplatin stable toward hydrolysis. However, the significantly lower intracellular chloride concentrations (4-23 mM) facilitate rapid hydrolysis ($T_{1/2}$ 2 h) to the activated cationic species capable of binding DNA [46-48]. On the other hand, carboplatin (Fig. (**1**)) has a more favorable pharmacokinetic profile, which is primarily predicated on its slower rate of conversion to its reactive species [49]. Studies indicate that the reaction proceeds *via* ring opening in carboplatin and subsequent binding to DNA bases [39, 50]. Due to a more favorable toxicity profile, several-fold higher doses of carboplatin are required compared to cisplatin, and the rate of adduct formation is ten-fold slower [33, 49]. However, carboplatin appears to have a similar spectrum of activity as cisplatin based on its similar mechanism of action, and thus problems persists when used against many cisplatin-resitant tumors [26]. The Replacement of the chloride group of cisplatin by cyclobutanedicarboxylate ligand of carboplatin (Fig. (**1**)) confers good aqueous solubility and greater stability because it forms a six-membered ring with the platinum atom [11]. This in turn leads to diminishing side effects, while retaining a similar level of clinical activity and cross resistance to cisplatin [51].

Upon binding of platinum-based compounds, various signal transduction pathways are activated, which interfere with different cellular processes including transcription and DNA replication, thereby triggering apoptotic cell death [52]. The antitumor activity of cisplatin and carboplatin is derived from the formation of identical 1,2-intrastrand DNA cross-links [53, 54]. In contrast to cisplatin and carboplatin, the bulky diaminocyclohexane (DACH) carrier ligand of oxaliplatin (Fig. (**1**)) is thought to confer less cross-resistance and a more favorable toxicity profile [51]. From a molecular standpoint, mismatch repair (MMR) recognition proteins do not recognize the formation of oxaliplatin-induced DNA adducts, due to the nature of the 1,2 intrastrand cross links [33]. This has been suggested as a critical determinant in its retention of antitumor activity across cisplatin-resistant cells [55, 56]. Oxaliplatin was approved in 2002 by the FDA for the treatment of advanced colon cancer often in combination with chemotherapy [38].

Over the last several decades, investigation into platinum-based drugs has been dominated by the clinical use of cisplatin, carboplatin, and more recently, oxaliplatin [57]. However, due to persistent problems including undesirable toxicity and resistance, investigators have been pursuing innovative strategies in designing the next generation of platinum drugs [58, 59]. The underlying knowledge gained from platnum(II) compounds, including cellular processing and resistance mechanisms, coupled with a further understanding of the mechanisms of action could help translate the next generation of analogs into clinical practice.

Poor solubility and low bioavailability of clinically approved platinum compounds are two important considerations that prompted the search for novel platinum-based coordination compounds with emphasis on oral administration [60]. Whereas platinum(II) chemistry is based on ligand exchange reactions, the octahedral gemometry of platinum(IV) presents two extra ligand sites and their high kinetic inertness lowers reactivity, which can minimize off target effects [61]. The preponderance of evidence suggests that platinum(IV) complexes are reduced *in vivo* to platinum(II), which is the species responsible for its activation, and can be considered as a pro-drug [62, 63]. The most prominent example from this group is

satraplatin, an octhahedral platinum(IV) complex that is administered orally and is currently in advanced clinical stages for treatment of hormone refractory prostate cancer (Fig. (**1**)) [64]. Platinum(IV) complexes display advantages owing to their greater stability and bioreductive activation, thus allowing a greater proportion of drug to reach its biological target. The two axial acetate groups play a pivotal role in increasing its lipophilic character and providing a more bioavailable agent [60, 65]. Upon metabolic activation, staraplatin becomes structurally similar to cisplatin except for the replacement of one of its amine groups with a cyclohexylamine group [65]. The anti-tumor activity of satraplatin has been shown to operate in a similar manner as cisplatin, as evidenced by formation of intra and interstrand DNA cross links [60, 66].

The reduction and intracellular modification of these complexes offer many opportunities to modify bioactive ligands as tumor targeting moieties to better facilitate the delivery of platinum to its intended site of action [67]. Based on the observation that estrogen receptor (ER)-positive breast cancer cells treated with estrogen are sensitized to cisplatin [68], Barnes *et al.* formulated a Pt(IV)-estrogen (Fig. (**1**)) compound to sensitize ER(+)-breast cancer cells and overcome cisplatin resistance [69]. The intracellular reduction of the compound led to the release of one equivalent cisplatin and two equivalents of estrodiol. Its mechanism entails upregulation of the high mobility group protein (HMGB1), a protein which is important in blocking platinum-DNA adduct repair [67, 69]. Another design strategy afforded the use of ethacrynic acid, a known inhibitor of glutathione S-transferase [70], which has been found to be overexpressed in many cisplatin-resistant tumors, also appended to a platinum(IV) center (ethacraplatin; Fig. (**1**)). Intracellular reduction results in the simultaneous effect of blocking GST by ethacrynic acid while exerting the cytotoxic effect of platinum(II) [70]. Another strategy pursued relies on photo-activation of platinum(IV) complexes rather than the traditional mode of intracellular reduction [71, 72]. This can provide advantages in tumor selectivity by eliciting activation of the complex at the site of the tumor, therefore potentially mitigating the collateral damage to normal tissue [48]. These platinum(IV) complexes were designed as dihydroxy in the *trans* position and are appended with two azide ligands, either positioned *cis* or *trans*. (Fig. (**1**)) [71, 72]. These prodrugs are relatively nontoxic under darkened conditions, but activation of these complexes by irradiation results in growth inhibition of human bladder cancer cells while avoiding toxicity to human skin cells. It is also notable to mention that the *cis*-azide complex does not exhibit cross resistance to cisplatin and different platinum-DNA adducts appear responsible for their cytoxicity [67, 71, 72].

Significant progress has been made in the field of platinum-containing anticancer agents, particularly with respect to design strategies and mechanistic understanding of their pharmacological effects. Building upon our current understanding of platinum coordination compounds, including structure-activity-relationship (SAR), tumor uptake and resistance mechanisms can help facilitate the clinical introduction of the next generation of platinum-based anticancer agents.

4. ZINC, APOPTOSIS, AND CANCER

Zinc is an indispensable trace element that plays a critical role in a wide range of cellular processes including cell proliferation, differentiation, and defense against free radicals [73, 74]. Zinc acts as a key structural component in many proteins and enzymes, including transcription factors, cellular signaling proteins, and DNA repair enzymes [75, 76].

It has also been well established that zinc plays a critical role in the regulation of apoptosis in mammalian cells [77-79]. However the precise role of zinc in modulating this response appears to be cell specific, highly complex, and lacking firm conclusion [79]. In many cell

types, including prostate epithelial, glial cells, ovarian epithelial cells, and others, zinc has been reported to induce apoptosis [79]. However, in breast cells, lung epithelial cells, renal cells, macrophages, and Hela cells, zinc exhibits antiapoptotic effects [79]. This has been supported by evidence showing that in some cells, exposure to low levels of zinc induces apoptosis, whereas exposure to high zinc levels inhibits apoptosis [80]. These seemingly contradictory results have been the subject of intense investigation and currently remain unanswered.

Given the indispensible role of Zn in a myriad of biochemical processes, it is not surprising that altered levels of Zn are associated with systemic abnormalities, including the development of cancer [81]. Although the levels of zinc have been found to be compromised in cancer patients as compared to normal subjects, the relationship between tumor development and Zn levels appears to lack discernable conclusions and is dependent on tumor type [79, 82, 83]. Zinc levels have been found to be reduced in patients afflicted with cancer of the liver, gallbladder, digestive tract, or prostate [84-86], whereas breast cancer patients showed decreased and elevated Zn levels in serum and malignant tissues [84, 87, 88]. Emerging evidence suggests that expression levels of Zn transporters are associated with cancer progression [81, 89]. Compelling evidence has been provided that links the zinc uptake transporter ZIP4 with the development of pancreatic cancer [79, 90]. Both *in vitro* and *in vivo* evidence points to an increase in ZIP4 expression is associated with increased zinc uptake, increased cell proliferation, and increased tumor growth [90]. Additionally, ZIP1 has been reported to act as a tumor suppressor of prostate cancer [91], while ZIP6 has been implicated in breast cancer progression and metastasis [92, 93]. Studies have also shown positive results in establishing a molecular link between STAT3, ZIP6 and Snail during EMT (Epithelial Mesenchymal Transition), which may be a contributing factor in tumor cell invasion and metastasis [94]. It was recently shown that the zinc transporter ZIP10 is implicated in the migration and metastasis of breast cancer cells, and consequently this invasive behavior could be inhibited by knock down of ZIP10 expression [81]. These studies were correlated with findings from clinical samples showing that breast cancers with lymph node metastases expressed significantly higher levels of ZIP10 than those without lymph node metastases [81, 95]. Thus, altered Zn transporter expression may play a key role in the development of cancer by disrupting normal intracellular distribution and function. In addition to the critical role that zinc plays in biological systems, the unique properties of zinc have allowed it to gain favor as potential anticancer agents (discussed in sections 5.1 and 7.3).

5. THE UBIQUITIN-PROTEASOME PATHWAY AS A TARGET FOR METAL-BASED COMPOUNDS IN CANCER THERAPY

Protein homeostasis is critical to biological processes that are fundamental to cancer cell survival. Therefore, targeting the key features responsible for protein production and destruction, particularly factors responsible for the growth and proliferation of cancer cells, has been the focus of intense investigation [96]. Empirical findings indicate that many types of actively proliferating tumor cells are more sensitive to proteasome inhibitors than non cancerous cells, and that blockade of tumor proteasome activity results in cancer cell apoptosis [97]. Thus, targeting the ubiquitinproteasome pathway has emerged as a favorable strategy in the treatment of human cancers.

The ubiquitin-proteasome pathway (Fig. (**2**)) plays a crucial role in maintaining cellular homeostatic function by selectively degrading proteins involved in critical cellular functions. These include selective degradation of oxidatively damaged, mutated, or misfolded proteins, as well as those involved in cell proliferation, cell cycle progression, and apoptosis [98]. Proteins destined for degradation are first tagged with a chain of ubiquitin molecules by a

multi-enzymatic system consisting of Ub-activating (E1), Ub-conjugating (E2), and Ub-ligating (E3) enzymes (Fig. (**2**)) [99]. The ubiquitin-tagged protein is then translocated to the 26S proteasome where it undergoes protein degradation, and the ubiquitin molecules are subsequently recycled [100, 101]. The 20S proteasome constitutes the proteolytic core of the 26S proteasome complex and mediates at least three distinct enzymatic activities, which function as a catalytic machine [102]. These activities include the chymotrypsin-like, trypsin-like, and peptidylglutamyl peptide hydrolyzing-like (PGPH) activities [103].

The possibility of targeting the ubiquitin-proteasome pathway therapeutically was initially met with great skepticism in the scientific community owing to its importance in normal cellular development [104]. However, the demonstration that proteasome inhibitors were well tolerated and were found to be active in several cancer models *in vivo*, the proteasome inhibitor bortezomib (Velcade, PS-341) entered into clinical trials [105]. Based on clinical trial data that showed an acceptable therapeutic index with significant clinical benefit, bortezomib was ultimately approved by the FDA for the treatment of myeloma [106, 107]. Bortezomib has been studied extensively as a single agent and in combination with glucocorticoids, cytotoxic agents, immunomodulatory drugs and radiation as treatment for multiple myeloma and other hematological malignancies [108]. The results in some cases have been impressive. However, there is less evidence of botezomib's efficacy *versus* solid tumors. Overall, these studies provided proof-of-principle that targeting the proteasome is a valuable target in cancer therapy.

In order to achieve a higher cytotoxicity profile with a wider spectrum of activity compared to that of platinum-based compounds, the anticancer activity of gold coordination compounds has been investigated [109-111]. Studies have shown that interactions of gold(III) complexes with DNA, the favorable target of platinum, failed to pose a favorable binding mode, precipitating the investigation into gold-protein interactions [112, 113]. Because gold(III) is isoelectronic to platinum(II), and tetracoordinate gold(III) complexes are in the same square-planar geometries as cisplatin, this prompted the investigation of gold compounds as potential anti-cancer agents [111]. Initial studies focused on a series of synthetic gold(III) diothiocarbamate derivatives that were shown to be 1-to-4-fold more cytotoxic than cisplatin and were able to significantly overcome both intrinsic and acquired resistance [110]. However, these initial studies failed to establish a molecular link between these gold compounds and their anticancer activity. Studies from our laboratory highlight the proteasome as a molecular target for gold(III) dithiocarbamate derivatives, one such is referred as Au(DMDT)Br_2 (Fig. (**3**)). We showed for the first time that this gold(III) dithiocarbamate analog inhibits the chymotrypsin-like activity of a purified rabbit 20S proteasome (IC_{50} = 7.4 μmol/L) and the 26S proteasome in highly metastatic MDA-MB-231 breast cancer cells [16]. Inhibition of proteasomal activity results in accumulation of proteasome target protein p27 and induction of apoptosis [16]. Interestingly, treatment of nude mice bearing breast cancer xenografts with this gold(III) compound caused inhibition of tumor growth, associated with proteasome inhibition and apoptosis induction [16].

Recently our lab investigated the mechanism of two novel gold dithiocarbamate derivatives that differ in the oxidation state of the metal, namely (AUL12) with a trivalent oxidation state, and (AUL15) with a monovalent oxidation state (Fig. (**3**)). We found that both gold dithiocarbamate species were able to inhibit the chymotrypsin-like activity of purified 20S proteasome and 26S proteasome in human breast cancer cells, resulting in accumulation of ubiquitinated proteins, proteasome target proteins, and induction of cell death, but at significantly different levels [114]. Additionally, our studies found that inhibition of proteasome activity by gold(I) and gold(III) compounds were completely reversed by the addition of a reducing agent, N-acetyl-L-cysteine, suggesting the involvement of redox activity [114]. Interestingly, treatment of breast cancer cells with a gold(III) (AUL12), but

not gold(I) (AUL15) derivative resulted in the significant production of reactive oxygen species [114]. Our study highlights the proteasome as an important molecular target of gold compounds, but shows distinct mechanisms of action responsible for their underlying biological activities, which depend on the oxidation state of the metal. A further in-depth analysis on the therapeutic potential of gold complexes as anticancer drugs has recently been published [104].

Our lab has also investigated the effects of well defined complexes of pyrrolidine diothiocarbamate complexes containing zinc, $Zn(PyDTC)_2$, and copper, $Cu(PyDTC)_2$, as potential proteasome inhibitors (Fig. (**4**)) [15]. Interestingly, these complexes were found to inhibit the chymotrypsin-like activity of cellular 26S, with much less inhibition of the 20S proteasome [15]. Based on these findings, we have proposed that inhibition of the JAMM domain in the 19S proteasome as a putative target for these metal compounds [15]. Since zinc is required for the catalytic activity of human deubiquitinating isopeptidase located on 19S, it is possible that this enzyme can be targeted by these complexes [115-117]. Further evidence to support this hypothesis comes from our findings comparing two synthetic diethyldithiocarbamate complexes containing copper and zinc [118]. We demonstrated that both $Cu(EtDTC)_2$ and $Zn(EtDTC)_2$ showed a much higher potency to inhibiting the 26S proteasome in intact breast cancer cells compared to their effects toward a purified 20S proteasome (Fig. (**4**)) [118]. While significant progress has been made in investigating the effects of metal complexes toward the ubiquitin-proteasome system, more detailed studies are necessary, especially to validate direct binding of the metal compounds to the JAMM domain of 19S proteasome.

6. COPPER CHELATING LIGANDS ACTING AS A TUMOR PROTEASOME TARGETING STRATEGY

Copper is another essential trace metal that has been selected by nature to be a driving force in many biochemical processes including chemical redox reactions, cellular growth, development, and angiogenesis [7, 119]. Under biological conditions, copper exists in both (Cu^+) and (Cu^{2+}), which allows it to serve as a cofactor in redox reactions, such as cytochrome c oxidase (involved in the mitochondrial electron transport chain) and superoxide dismutase (involved in the detoxification of reactive oxygen species) [120, 121]. The acquisition and distribution of copper is a tightly regulated process to assure proper uptake, distribution, and to avoid unnecessary binding to biomolecules [122, 123].

Depending on the oxidation state, the coordination chemistry of copper is often distinct: Cu^+ shows a preference for sulfur donor ligands, such as cysteine or methionine, whereas Cu^{2+} prefers nitrogen donors such as histidine or oxygen donors such as glutamate or aspartate [121]. Copper in its reduced form (Cu^+) has a filled d^{10} configuration with no preference for geometry based on no LFSE (ligand field stabilization energy) and thus can exist in a wide range of geometries [124, 125]. The d^9 configuration of Cu^{2+} favors a four to six coordination arrangement due to Jahn-Teller distortions [124, 125]. Geometries include four-coordinate square-planar, five-coordinate trigonal bipyramidal and six-coordinate axially distorted octahedral arrangements [125].

6.1. Copper, Angiogenesis, and Cancer

The role of copper in the growth and progression of malignancy has been the subject of intense investigation for the last two decades. This was born out of the realization that copper levels are altered in tumor bearing mice and humans [126, 127]. Additionally, high serum and tissue levels of copper were found in various human tumors including breast [128, 129], prostate [130, 131], colon [131], lung [132], and brain [133], compared to

healthy subjects. The reasons for this elevation have not been fully elucidated and no firm conclusions could be established.

It was first noticed nearly 30 years ago that copper plays a critical role in angiogenesis [134, 135]. Angiogenesis is a complex process that is essential for the growth, invasion, and metastasis of tumor cells [136]. It has been shown that tumors cannot grow larger than 1-2 mm^3 without the formation of new blood vessels [137]. *In vitro* studies have shown that copper acts as a critical angiogenic effector by stimulating the proliferation and migration of endothelial cells [138]. Based on the findings regarding the importance of angiogenesis and copper in tumor development, the concept of antiangiogenic therapy using copper chelators in cancer therapy has gained considerable interest [139, 140]. Indeed, a number of clinical trials have been initiated and have shown promising results [141, 142]. The following section will be devoted to a series of copper-chelating agents being investigated in our lab with special emphasis toward their cellular targets as a promising anticancer strategy.

6.2. Copper Chelating Ligands

Disulfiram (tetraethylthiuram disulfide, DSF) (Fig. (**5**)) is a clinically employed drug used to treat patients afflicted with chronic alcoholism [143]. Upon consumption, alcohol is metabolized to acetaldehyde by alcohol dehydrogenases (ADH) and further converted to acetic acid by aldehyde dehydrogenase (ALDH) [144]. The chemical structure shows that DSF is able to react to copper with its *thiol* group [145]. To test this hypothesis, we mixed DSF with $CuCl_2$ and found a dramatic color change that indicates that a chemical reaction between DSF and copper has occurred [146]. Based on findings that tumors contain higher levels of copper compared to normal tissue, our lab has undertaken an innovative strategy of targeting elevated copper levels with DSF, and hypothesized that the resultant DSF-copper complex could possess the potent tumor-specific killing activity by selectively inhibting the proteasome in tumor cells [122, 146].

To verify our hypothesis that copper ions can be used as a novel, selective target for potential anticancer drugs, we examined the effect of DSF on human breast cancer cells [146]. Our studies found that in the presence of copper, DSF could inhibit proteasomal activity and induce apoptosis in cancer cells. However, in the absence of copper under the same experimental conditions, the effect of DSF was minimal [146]. We extended our studies further by testing whether DSF could react with endogenous levels of copper present in the tumor and convert the pro-angiogenic copper to an anticancer complex *in vivo*. We treated mouse-bearing breast cancer xenografts with DSF for 30 days and found that DSF significantly inhibited tumor growth by 74%, tightly associated with the inhibition of proteasome activity and induction of apoptosis in tumor tissues treated with DSF [146].

Along with DSF, diethyldithiocarbamate (DDTC) and pyrrolidine dithiocarbamate (PDTC) (Fig. (**5**)) are also members of the dithiocarbamate family that were found to act as potent metal chelators [7, 15, 122, 147, 148]. We found that DDTC was capable of binding copper and forming a new complex that potently inhibited the proteasomal chymotrypsin-like activity, induced apoptosis, decreased expression of androgen receptor (AR) and estrogen receptor α and (ERα and ERβ) proteins in both human prostate and breast cancer cells [148]. We also reported that after binding to copper, PDTC could inhibit the proteasomal chymotrypsin-like activity, suppress proliferation and induce apoptotic cell death in cultured human prostate cancer cells [147].

Along the same lines, we selected and tested clioquinol, another copper chelator, for its potential anticancer properties (Fig. (**5**)). Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline), known as CQ, is an 8-hydroxyquinoline derivative that is clinically used for treatment of Alzheimer's disease [149] and Huntington's disease [150]. According to the chemical

structure, CQ is similarly capable of forming stable complexes with copper(II) [151]. To test whether CQ can react with copper, we mixed a solution of CQ with a solution of $CuCl_2$ at 1:1 molar ratio, and subsequently, a dramatic color change was observed [152]. To further verify whether the color change of the CQ-copper mixture indicated a formation of a stable CQ-copper complex, a series of samples was analyzed by X-ray absorption near-edge spectroscopy (XANES) and extended X-ray absorption fine structure spectroscopy (EXAFS) [152]. The results showed that the CQ-copper mixture had a different copper oxidation state than those of $CuCl_2$ and the copper metal, confirming that a chemical reaction occurred between CQ and copper [152].

We further tested biological effects of the CQ-copper complex in human prostate cancer LNCaP (androgen-dependent) and C4-2B (androgen-independent) cells [152]. The results showed that inhibition of proteasome activity, reduction of androgen receptor (AR) expression, suppression of cell proliferation, and induction of apoptotic cell death occurred in both prostate cancer cell lines treated with CQ-copper complex, but not with CQ alone [152]. Furthermore, animal studies of mice bearing human C4-2B xenografts showed that treatment with CQ (10 mg/kg) for 30 days resulted in significant inhibition of tumor growth (66%) compared to the control [152]. The results also showed that proteasome inhibition, induction of apoptosis, suppression of AR expression and inhibition of angiogenesis occurred in the tumors tissues treated with CQ [152].

Elevated proteasome activity and high concentration of copper are unique features in human tumor tissue. Our findings have demonstrated that these unique features of tumor cells can be used as specific targets by already clinically approved drugs that, when complexed to copper, act as potent tumor cell killers.

7. TRIDENTATE [NN'O]-CONTAINING METAL COMPLEXES AS A SUITABLE PLATFORM IN THE DESIGN OF NOVEL ANTICANCER DRUG CANDIDATES

7.1. Tridentate [NN'O]-Containing Ligands with Ga^{3+} as Potential Tumor Proteasome Inhibitors and Anticancer Drug Candidates

Our groups have been actively pursuing a strategy of developing novel complexes of well defined stoichiometry formed between asymmetric [NN'O] tridentate ligands-containing metals as potential anticancer drug candidates (Fig. (**6**)). Such ligands are an evolution from terbutylated analogues used as biomimetic models for galactose-oxidase [153, 154]. Moreover, a secondary amine in this ligand allows for the design of complexes with appended moieties to enhance water solubility [155, 156] or lipophilicity [157-159] to address concerns for drug design purposes. Our studies initially focused on the development of a series of gallium complexes described as $[Ga^{III}(^{Lx\text{-}NN'O})_2]ClO_4$, with asymmetric NN'O-containing pyridine amino phenolate ligands (Fig. (**6**)) [160]. The phenolate moiety groups were appended with electron withdrawing and donating groups such as methoxy (**1**), nitro (**2**), chloro (**3**) bromo (**4**), and iodo (**5**) positioned at the 4^{th} and 6^{th} positions (Fig. (**6**)). The geometry of these complexes appears to be distorted octahedrally, but owing to the flexibility of the ligands, facial coordination takes place [160].

The therapeutic efficacy of these complexes was first tested against cisplatin-resistant neuroblastoma cells [160]. Our studies reveal, in ranking order, methoxy (**1**) = nitro (**2**) < chloro (**3**) < bromo (**4**) < iodo (**5**), that the species containing halogen substituents showed preferential growth inhibition in human neuroblastoma cells with activity superior to that of cisplatin (Fig. (**6**)). Additionally, neuroblastoma cells treated with gallium chloride rendered cells predominantly viable, attaining similar activity as the methoxy substituted complexes [160]. Interestingly, these gallium (III) complexes were found to be relatively nontoxic at 25

μmol/L, only showing an increase in aberrant morphological changes above 50 μmol/L against normal human fibroblasts [160]. Thus, the formation of a coordination complex appended with halogen substituents appears to influence the cytotoxicity of these complexes [160].

We next set out to investigate a possible molecular mechanism for these gallium complexes that could provide insight into their growth inhibitory properties. Our studies reveal that complexes (**3** Chloro) < (**4** Bromo) < (**5** Iodo) are able to target and inhibit proteasomal activity and induce apoptosis in various prostate cancer cell lines (Fig. (**6**)) [161]. The ability to inhibit proteasome activity was indicated by accumulation of ubiquitinated proteins and the proteasomal target protein p27. As observed with cisplatin-resistant neuroblastoma cells, complex (**5** Iodo) achieved superior therapeutic efficacy in a human prostate cancer model. Accordingly, complex (**5**) exhibited potent proteasome inhibitory activity against both 26S proteasome (IC_{50} = 17 μmol/L) and purified 20S proteasome (IC_{50}= 16 μmol/L) [161]. Consistently, this effect was associated with higher indices of apoptosis. It is notable to point out that treatment with the iodo-substitued ligand (HL^I) alone showed only minimal levels of cytotoxicity in our cell culture system. Importantly, complex (**5**) was able to exert the same effect *in vivo* by inhibiting the growth of PC-3 bearing mice xenografts (66%), associated with proteasome inhibition and apoptosis induction [161]. The increased potency of complex (**5**) suggests that the iodo-substituted ligand attains certain physiochemical properties that after coordination with gallium, impart significant biological activity [161, 162]. It is possible that their biological activity is governed by the strong *pi* electron-donating group. Considering that all the ligands used in the coordination complexes of (**3**)-(**5**) contain electron-withdrawing appendages, it is reasonable to suggest that only their *pi*-donating ability could influence their anticancer properties (I > Br > Cl). Additionally, whether the coordination mode of the metal and geometries are instrumental in influencing its biological activity is rather speculative at this point and no firm conclusions could be established [161, 162].

7.2. Tridentate [NN'O]-Containing Ligands with Cu^{2+} as Potential Tumor Proteasome Inhibitors and Anticancer Drug Candidates

Since one of the important focuses in our labs involves investigating the use of copper chelating ligands and other transition metal-based complexes as proteasome inhibitors (discussed above) [122, 146, 152], we decided to extend our work by investigating the role of bivalent transition metals complexed to our same HL^I platform to gain insight into their potential as anticancer agents.

Furthermore, we observed that *in situ* complexation of HL^{Br} or HL^I with Cu^{+2} salts leads to the generation of complexes that display superior anticancer activity when compared to equivalent gallium species (data unpublished). Based on the significantly higher potency conferred by the HL^I ligand when formed in a coordination complex with gallium, subsequent studies relied on this model architecture with bivalent transition metals, such as copper and zinc, with emphasis on mechanistic properties. Spectrometric evaluation of the stoichiometric HL^I:$CuCl_2$:DMSO mixture led to the identification of both monomeric and dimeric fragments that may act as pharmacophores responsible for their biological activity [163]. These initial studies formed the basis of developing a series of copper(II) complexes with the ligand HL^I, which were synthesized and characterized as (**6**), (**7**), and (**8**) (Fig. (**6**)). These complexes, with distinct stoichiometries, protonation states, and nature of the monodentate ligand, were designed considering that a metal ion coordinating to the ligand could bind to the proteasome, possibly *via* the N-terminal threonine or some other coordinating residue [163].

These copper complexes were first tested for their ability to impart cell death in human leukemia cells and compared to the activity of HL^I and copper salt, independently [163]. Consequently, these complexes achieved a comparable IC_{50} (concentration at 50% cell death) value in the range of 3.8-4.0 μmol/L after 18 h treatment in human leukemia cells [163]. In comparison, treatment with either HL^I or copper salt alone showed minimal proteasome inhibitory activity and cytotoxicty as high as 25 μmol/L [163]. Next, we tested whether the cellular proteasome was a molecular target for these copper complexes by using a human prostate cancer model [163]. When these complexes were tested in cultured prostate cancer cells, significant proteasome inhibition associated with higher levels of ubiquitinated proteins and massive apoptosis was apparent [163]. Since either HL^I- or copper salt-treated cells failed to exhibit any considerable biological activity, we hypothesize that the formation of a coordination complex with copper is imperative for imparting biological activity [163]. However, when immortalized, nontransformed breast cells were treated with these copper complexes, they did not demonstrate loss of viability, suggesting resistance to the proteasome inhibitor effects [163]. Although a threshold level of selectivity was reached with these complexes that distinguish normal from tumor cells, further studies are warranted to provide more conclusive evidence [163]. Furthermore, our studies suggest the pharmacophore or active species as $[CuL^I]$ is needed for inhibition of proteasomal activity. Along this line it is possible that the 2:1 ligand to metal complex (**8**) (Fig. (**6**)) may act as a prodrug, and the loss of one of its ligands might occur to free up the metal for binding to coordinating residues on the proteasome. Although more detailed studies are needed, it is tempting to suggest that this pharmacophore would present an open coordination that facilitates interaction with available amino acids, potentially the N-terminal threonine, with high affinity for copper [163].

7.3. Comparative Activities of Nickel(II) and Zinc(II) Complexes of Tridentate [NN'O]-Containing Ligands as Proteasome Inhibitors

To further build upon our studies and further investigate our hypothesis that species $[ML^{IA}]^+$ is the necessary pharmacophore for proteasome inhibition, we compared the proteasome inhibition capabilities of two divalent transition metals as coordination complexes utilizing the same HL^I ligand as a platform [164]. These complexes were synthesized and characterized as (**9**) and (**10**) (Fig. (**6**)) using various spectroscopic, spectrometric, and structural methods [164]. DFT calculations considering different isomers of (**9**) and (**10**) were carried out and show good agreement with the nickel complex, but fail to predict the appropriate geometry for the zinc-containing species because it lacks ligand field stabilization energy [164]. Furthermore, initial comparison studies considering coordination of a 1:1 $[Zn(L)]^+$ fragment with threonine suggest a favorable coordination through the terminal hydroxyl group [164].

The pharmacological effects of these metal complexes along with those of the salts, $NiCl_2$ and $ZnCl_2$, were evaluated *in vitro* and in cultured prostate cancer cells in relation to their proteasome inhibiting and apoptosis-inducing capabilities [164]. We first tested the ability of these species to impart cell death in human leukemia cells. We found that cells treated with (**10**) exhibited superior cell death-inducing properties, with an IC_{50} of ~8 μmol/L. However, (**9**) and both of the metal salts failed to impart any level of cytotoxicity at as high as 20 μmol/L [164]. We next tested whether the proteasome is a target for these metal complexes *in vitro* and in cultured prostate cancer cells. Our results showed that that neither (**9**) nor $NiCl_2$ have the ability to inhibit the proteasome activity at any significant levels. However, under cell-free conditions, $ZnCl_2$ and (**10**) showed significant proteasome inhibiting *versus* the chymotrypsin-like activity of both the 26S proteasome (IC_{50} = 5.7 and 4.4 μmol/L, respectively) and the purified 20S proteasome (IC_{50} = 16.6 and 11.7 μmol/L, respectively) [164]. The observation that Zinc-containing dithiocarbamate derivatives as

mentioned above showed only minimal inhibition toward 20S proteasome, suggests the nature of the dithiocarbamate ligand formed in a coordination complex with zinc may be influencing its targeting capabilities. However, no firm conclusion could be established and is purely speculative at this point. Additionally, decreased proteasome activity by (**10**) was associated with higher levels of ubiquitinated proteins and massive apoptosis, whereas treatment with (**9**) or either metal salt resulted in minimal effects. These interesting results coupled with their nontoxic effect toward nontransformed breast cells, present a compelling rationale for further investigating (**10**) as a potential anticancer drug candidate [164].

As observed with other metal complexes being investigated from our labs, considerable proteasome inhibition can be attained through 1:1 ligand-to-metal species, which we hypothesize to be the pharmacophore in all of these complexes under investigation. Therefore, it is tempting to suggest that an equilibrium $[M(L^{IA})_2] \leftrightarrow [M(L^{IA})_2]^+ + L^{IA-}$ seems necessary to free up the pharmacophore with available coordination sites capable of interaction with the 20S proteasome. It is observed from the molecular structures and DFT calculations that covalent interactions prevail with (**9**), while the interactions in (**10**) (Fig. (**6**)) is governed by ionic bonds [164]. Taken together, these results strengthen the current working hypothesis that fast ligand exchange is necessary to generate the $[Zn[L^{IA}]^+$ fragment along with its gallium(III) and copper(II) congeners necessary to inhibit proteasome activity [164]. Further mechanistic understanding into the role of these metals and ligands and how their coordination and geometry influences their biological activity could lead to the development of next generation drug candidates using asymmetric [NN'O] tridentate metal complexes as selective tumor proteasome inhibitors.

8. CONCLUSION

The critical role that metals play in the functioning and maintenance of life highlights the extensive role that nature plays in regulating these vital components. The clinical success of cisplatin provided the "proof of concept" for investigating metals, essential or nonessential, and their coordination complexes as potential anticancer agents. Since the discovery of cisplatin, thousands of platinum analogs have been synthesized, with only carboplatin and oxaliplatin achieving widespread clinical use. Design strategies of novel platinum complexes have been under intense investigation to address shortcomings of previous generation platinum compounds. Targeting the ubiquitin-proteasome pathway with metal-based compounds is an emerging concept in developmental therapeutics. These include, but are not limited to, copper-, zinc- and gold-containing complexes which have made significant progress in the pursuit of developing novel anticancer drugs. Since higher concentrations of copper is a common trademark of many human tumors, targeting tumor cellular copper with copper chelating agents *via* inhibition of cancer cell proteasome has emerged as an exciting new avenue in cancer therapy. Along this line, metals including gallium(III), Zinc(II), and copper(II) when combined with asymmetric tridentate appended ligands represent an innovative new class of proteasome inhibitors. Since metals are endowed with unique properties that are absent in conventional carbon-based drugs, the positive trend in anticancer drug discovery can be continued by building on the underlying knowledge gained from medicinal inorganic chemistry.

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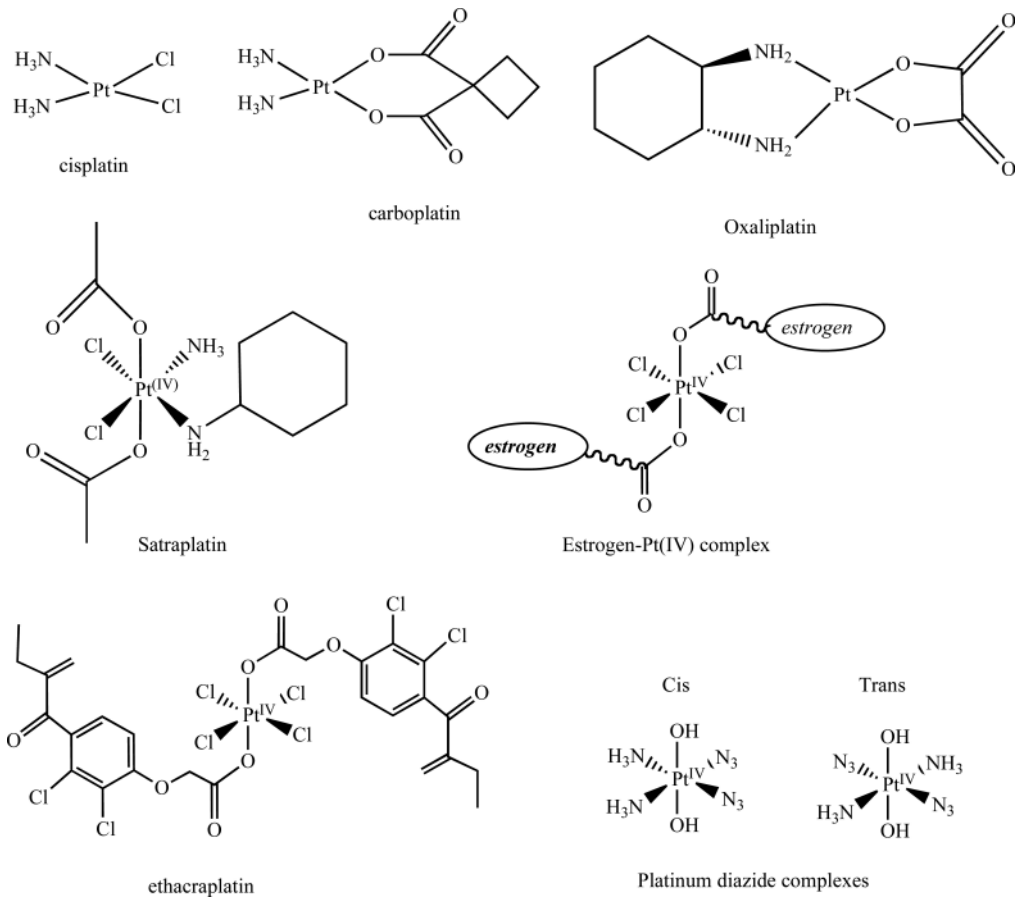

Fig. (1).

Chemical structures of platinum(II) complexes, Cisplatin, Carboplatin, and Oxaliplatin. The platinum(IV) complex Satraplatin has been used to overcome concerns of low bioavailability. Platinum(IV)-complexes have been designed with appended moieties (estrogen and the GHT inhibitor ethacraplatin) to optimize the anticancer effects of cisplatin. Platinum(IV) diazide complexes have been designed that are activated upon irradiation.

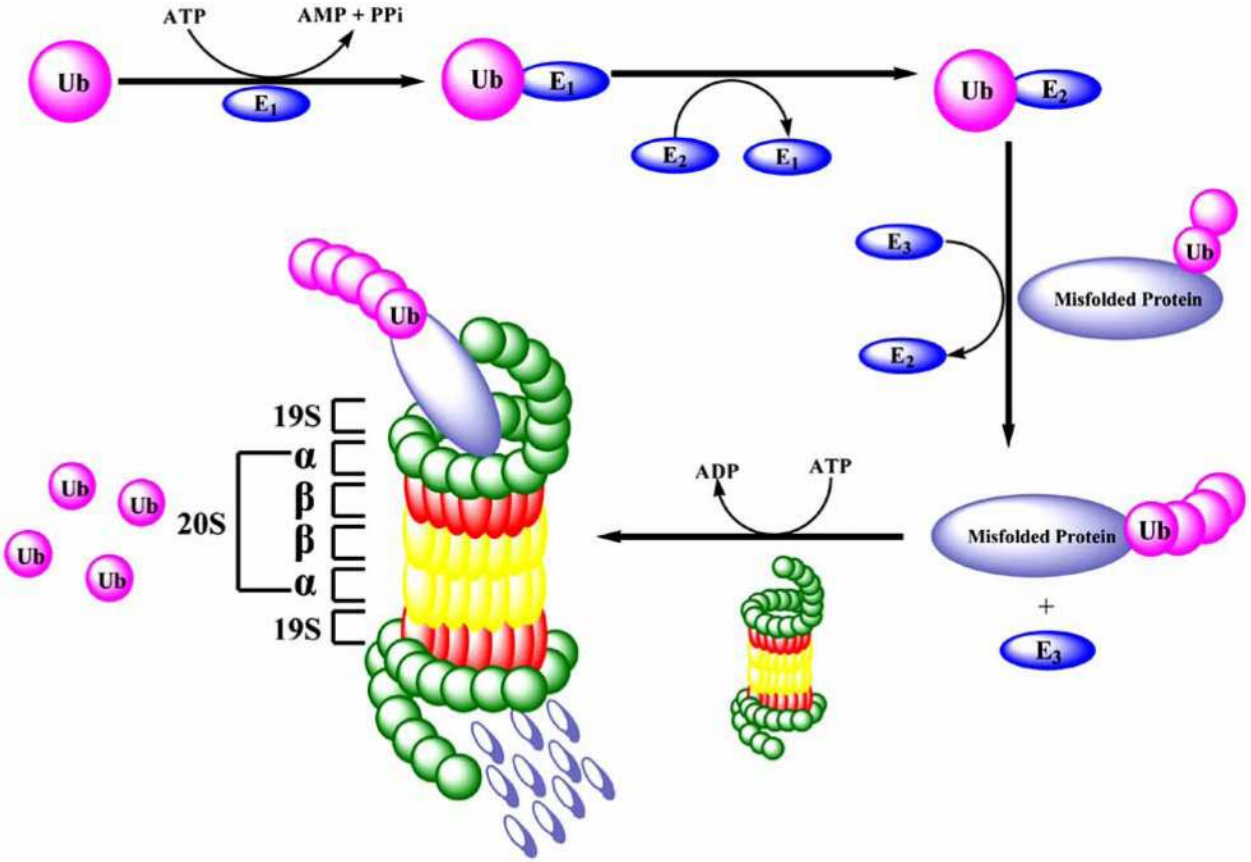

Fig. (2).

The ubiquitin-proteasome pathway plays a crucial role in maintaining cellular homeostatic function by selectively degrading various proteins including those involved in critical cellular functions. Proteins destined for degradation are first tagged with a chain of ubiquitin molecules by a multi-enzymatic system consisting of Ub-activating (E1), Ub-conjugating (E2), and Ub-ligating (E3) enzymes. The ubiquitin tagged protein is then translocated to the 26S proteasome where it undergoes degradation and the ubiquitin molecules are subsequently recycled. The 20S proteasome constitutes the proteolytic core and consists of a series of outer α-subunits and inner β-subunits mediated by atleast three distinct enzymatic activities. These include the chymotrypsin-like, trypsin-like, and peptidylglutamyl peptide hydrolyzing-like/PGPH.

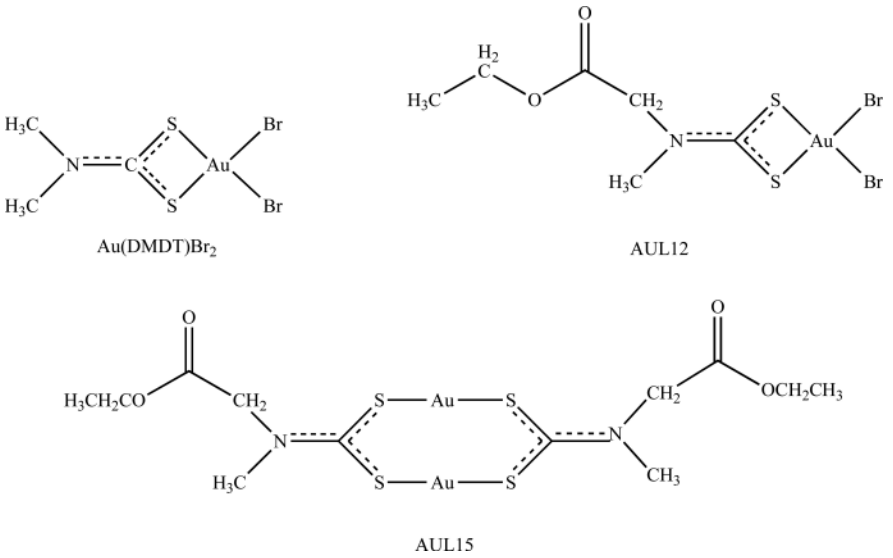

Fig. (3).
Chemical structures of synthetic gold complexes as proteasome inhibitors: Au(DMDT)Br_2, AUL12, and AUL15. Both Au(DMDT)Br_2 and AUL12 have a trivalent oxidation state, whereas AUL15 is monovalent.

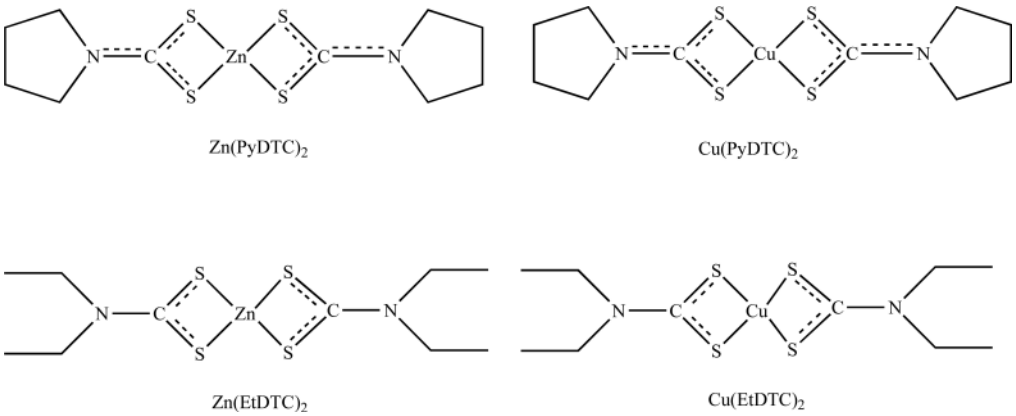

Fig. (4).
Chemical structures of synthesized Zinc and Copper-containing complexes as proteasome inhibitors from the dithiocarbamate family, (PyDTC) and (EtDTC).

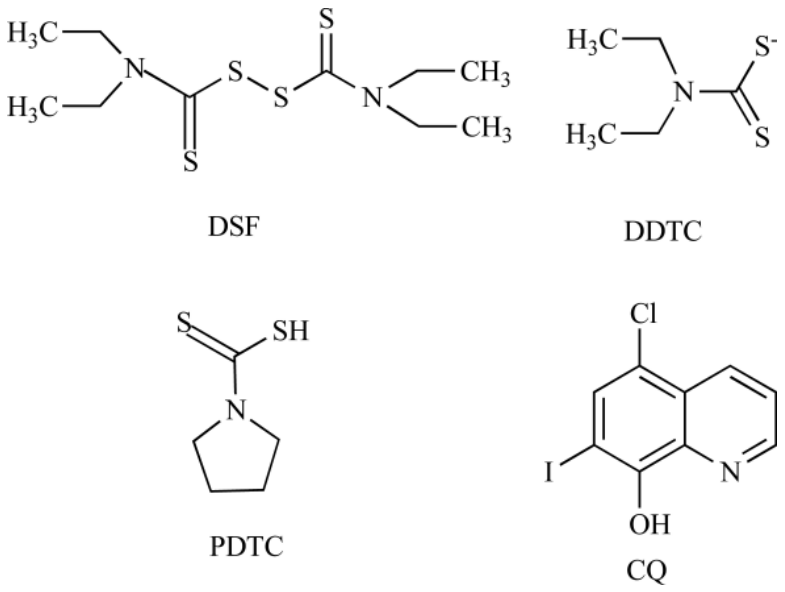

Fig. (5).
Chemical structures of copper-chelating ligands DSF, DDTC PDTC, and CQ.

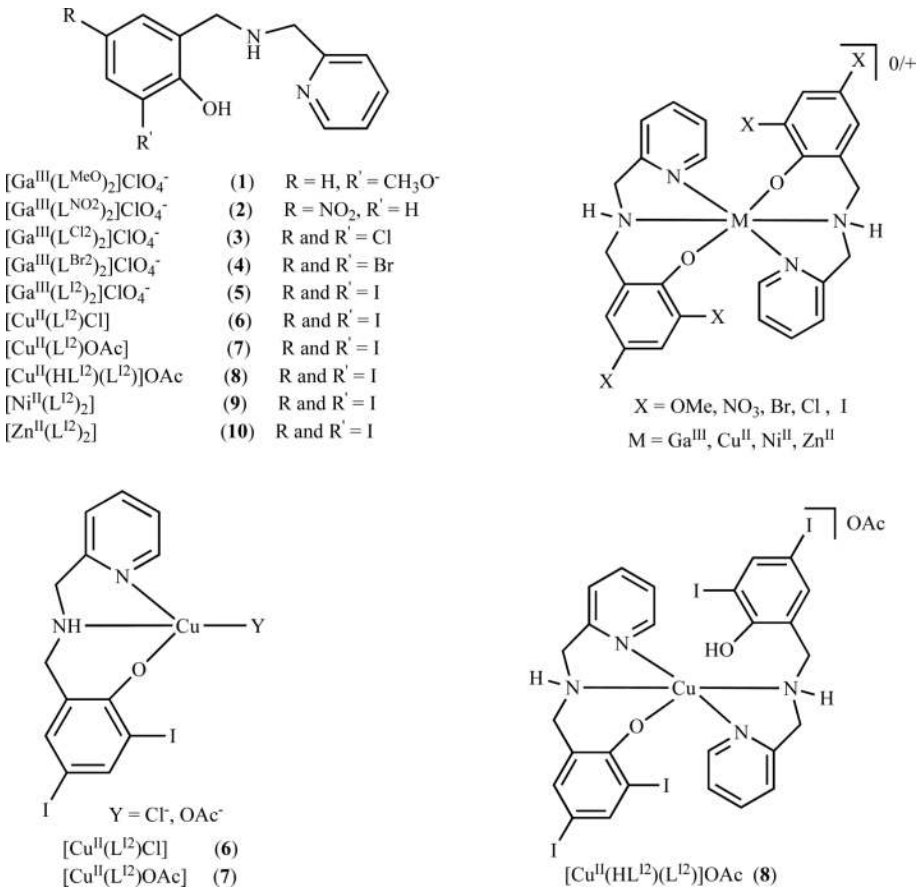

Fig. (6).

Asymmetric [NN'O]-containing metal complexes as proteasome inhibitors. R represents different groups at the 4th and 6th positions. M represents the metal ion, in this case, gallium(III), copper(II), nickel(II), and zinc(II) were used. Complexes were synthesized at a two ligand to one metal ratio.