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Bioorganometallic Chemistry of Molybdenocene Dichloride and Its Derivatives

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Abstract

The potential application of metallocene complexes into the cancer research was established by the pioneer work of Köpf-Maeir and Köpf in the late 1970s. The combination of organometallic chemistry and biochemistry created a new research area: bioorganometallic chemistry. Bioorganometallic chemistry has developed rapidly in the last thirty years leading to application of organometallic species into diagnostic, sensors, immunoassays and anticancer research among others. This review focuses on the bioorganometallic chemistry of molybdenocene dichloride and its derivatives as metal-based anticancer drugs. The anticancer properties of molybdenocene dichloride and its derivatives are described as well as the mechanism of action, aqueous and coordination chemistry, and molybdenocene-biomolecule interactions.

Keywords

molybdenocene dichloride; antitumor; mechanism of action; DNA; oligonucleotide; human serum albumin

1. Introduction

Modern organometallic chemistry has been to a great extent influenced by the accidental discovery of bis(cyclopentadienyl)iron(II) (ferrocene) in 1951 [1]. This metallocene revolutionized the ideas about bonding, synthetic methodology and catalytic processes. Afterward, in the last century, metals gained importance in the medicinal chemistry as anticancer, anti-fungus, anti-arthritis, anti-ulcer agents among others [2,3]. Among these metals, molybdenum has attracted the attention of many researchers due to its importance as a cofactor in enzymes (in sulfur containing proteins), as an essential element in human nutrition and as a metal-based drug [2,3]. In this review, we will focus on biologically active molybdenocene complexes, in particular as anticancer drugs.

The research interest in organometallic molybdenum complexes as potential drugs was sparked by the unexpected results of Köpf-Maier and Köpf who discovered that metallocene dihalides and pseudo halides (Cp_2MX_2 , $\text{M} = \text{Ti, V, Nb, Mo}$) were active on a wide variety of murine and human tumors with lower toxicity than cis-platin [4-19]. From their leading experiments, Köpf-Maeir and Köpf determined that titanocene dichloride, Cp_2TiCl_2 , was the

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most active metallocene *in vitro* but its low hydrolytic stability hindered it to be studied thoroughly. In marked difference to titanocene dichloride, $\text{Cp}_2\text{Mo}^{2+}$ possesses good hydrolytic stability at physiological pH while $\text{Cp}_2\text{Ti}^{2+}$ hydrolyzes extensively [20]. Therefore, this stability is an attractive property of Cp_2MoCl_2 and provides an excellent model to study its aqueous chemistry under physiological media and understand which species are prone to reach the target places in the cell. Motivated by this findings, several research groups have been working on organometallic molybdenum complexes, in particular with Mo(IV) species. Molybdenocene dichloride has been investigated extensively in many aspects: mechanistically, its aqueous and coordination chemistry as well as structure modification. Below, we will discuss each of these aspects.

2. Molybdenocene dichloride, Cp_2MoCl_2 : Aqueous stability and coordination chemistry with DNA, oligonucleotides and protein and the implication in the mechanism of action

2.1 Aqueous and coordination chemistry of Cp_2MoCl_2

Bis(cyclopentadienyl)molybdenum dichloride, molybdenocene dichloride, is an organometallic complex containing two planar aromatic five-membered rings and two chlorides coordinated to Mo(IV). It belongs to the sandwich complex category. Molybdenocene dichloride, Cp_2MoCl_2 , was prepared by Cooper and Green and X-ray structural analysis showed two molecules in which one molecule the Mo(IV) is π -bonded to two staggered Cp rings and two chlorides and the second molecule contains two eclipsed Cp rings, both in a pseudo-tetrahedral coordination geometry or better described as an clamshell geometry with two ancillary chloride ligands on the open edge [21,22].

The aqueous chemistry of Cp_2MoCl_2 (hydrolysis of Cl^- , MCp stability and coordination chemistry of " Cp_2M^{2+} " with DNA components) has been initially investigated by Marks and coworkers and later by Meléndez's and Harding's groups. [23-36]. According to Marks and coworkers, the chloride loss from Cp_2MoCl_2 in water (unbuffered) at 37°C occurs very rapid during the complex dissolution. After dissolution, the first chloride is lost followed by the loss of the second chloride within 1 hr (equations 1 and 2). Increasing the ionic strength with KNO_3 to 0.318 M did not affect the chloride hydrolysis [20].

At physiological pH, one of the aqua ligand gets deprotonated and the major species is the monocation, $\text{Cp}_2\text{Mo}(\text{H}_2\text{O})(\text{OH})^+$, which is in equilibrium with the dimeric species $[\text{Cp}_2\text{Mo}(\mu\text{-OH})_2\text{MoCp}_2]^{2+}$ [23].

The coordination chemistry of molybdenocene dichloride with nucleobases and nucleotides was also investigated by Marks and coworkers. In aqueous solution, at physiological pH, for N-methylcytosine molybdenocene dichloride gets engaged in N(3), N(4) coordination forming a four-membered chelate while for N-methyladenine, N(1), N(6) (four-membered chelate) and N(7), NH(6) (five-membered chelate) coordination modes are observed, Figure 1. In the case of the nucleotides, adenosine (5'-dAMP) and guanosine (5'-dGMP), N(7)-

O(phosphate) coordination prevails whereas for cytosine (5'-dCMP) and thymidine (5'-dTTP), N(3), O(phosphate) coordination mode is observed [20].

Binding studies between Cp_2MoCl_2 and calf-thymus DNA and oligonucleotides were pursued by Marks', Harding's and Meléndez's groups. Binding studies, by Harding's group, between Cp_2MoCl_2 and calf-thymus DNA showed that $\text{Cp}_2\text{Mo}^{2+}$ binds the terminal phosphates of DNA using ^{31}P NMR spectroscopy [24]. Arguing that DNA has no terminal phosphates and that such coordination was observed must likely by fragmentation of the DNA after sonication, they carried out a study between Cp_2MoCl_2 and oligonucleotides in saline unbuffered solution at pH 7.0. They reported that Cp_2MoCl_2 does not get involved in any type of bonding with oligonucleotides, disregarding this type of interaction [25].

Marks and coworkers studied the interaction of Cp_2MoCl_2 with self-complementary oligonucleotide d(ApGpGpCpCpT) and the 5'-phosphorylated analog d(pApGpGpCpCpT) by NMR spectroscopy at pH = 7.0 [26]. The interaction of Cp_2MoCl_2 with d(ApGpGpCpCpT) showed minor changes suggesting a weak interaction of $\text{Cp}_2\text{Mo}^{2+}$ to the oligonucleotide, whereas the spectra of d(pApGpGpCpCpT) exhibited significant changes at the 5' end. The upfield shifts for H2 and H8 signals and two nonequivalent Cp rings observed in the ^1H NMR spectrum, the together with the downfield displacement for the 5'-terminal phosphate in the ^{31}P NMR spectrum suggest the coordination of $\text{Cp}_2\text{Mo}^{2+}$ to N(7) and O(phosphate) positions at this terminal [26].

On the other hand, Melendez's group performed several studies in order to elicit mechanistic information about the molybdenocene coordination properties with biologically important molecules. Inductive Coupled Plasma Emission Spectroscopy (ICPES) studies between calf-thymus DNA (pH 7.0 and buffer conditions) and Cp_2MCl_2 (M = Ti, Mo) showed that molybdenocene binds DNA in about 5-10%, while titanocene is bound in 90-95% [27]. Likewise, similar studies were pursued using electrochemistry (cyclic voltammetry) and 1D and 2D NMR spectroscopy [28-31]. In analogous manner as in the ICPES study, cyclic voltammetry experiments confirmed that molybdenocene dichloride and its derivatives bind calf-thymus DNA in 5-12% [28-30]. To elucidate which type of interactions and what nucleobases are involved, Melendez's group pursued molybdenocene-oligonucleotide binding studies at physiological and buffered conditions using 1D and 2D NOESY ^1H NMR spectroscopy and cyclic voltammetry [31]. The self-complementary oligonucleotide CGCATATATGCG with 5'- and 3'-OH terminals was used as a model to establish binding interactions with C_2MoCl_2 . We determined that molybdenocene gets engaged in a weak N(7) coordination with purine bases only and almost negligible phosphoester coordination was observed by ^{31}P NMR spectroscopy [30,31]. Despite the fact that the sensitivity of NMR techniques is much lower than ICPES and electrochemistry, we estimated about a 5-10% of interaction using NMR data [31]. In addition, cyclic voltammetry experiments demonstrated and corroborated that molybdenocene-oligonucleotide interaction is about 5% [31]. These results are somewhat contradictory to Harding's findings [25]. However, our studies were performed at physiological conditions (buffered and pH 7.4) while they used saline solution (NaCl) perhaps inhibiting the chloride hydrolysis of Cp_2MoCl_2 and subsequent interaction with the nucleotides. Additionally, on an independent study, Kuo and collaborators found that Cp_2MoCl_2 promotes phosphoester bond cleavage [32]. Therefore, a weak interaction of Cp_2MoCl_2 with N(7) followed by phosphoester bond cleavage could explain our findings, in particular under the acidic conditions encountered in the cancer cells, but it has not been investigated in further details.

2.2 Mechanism of action of Cp_2MoCl_2

The mechanism of action of Cp_2MoCl_2 at cellular levels is not well understood. The studies on the biochemical aspects of molybdenocene dichloride by several research groups have

provided insights and hypotheses about the possible mechanism of action of molybdenocene dichloride as explained below [26,33-37].

Experiments performed by Marks and coworkers investigating the interaction of Cp_2MCl_2 ($\text{M} = \text{V}, \text{Mo}$) with DNA-processing enzymes revealed that these metallocenes have no effect on the DNA electrophoretic mobility, endonuclease, ligase or polymerase activity [26]. On the other hand Cp_2MoCl_2 demonstrated to inhibit protein kinase C (PKC), an enzyme that regulates cellular proliferation [26]. Thus, this could be one target for Cp_2MoCl_2 to elicit its anticancer activity.

Other hypotheses were tested with regard to the antitumor activity of Cp_2MoCl_2 . For instance, Harding and coworkers have shown that glutathione (GSH), an intracellular tripeptide responsible for reacting with electrophilic cytotoxic drugs, forms stable adducts with molybdenocene dichloride and it is likely that GSH plays an important role in the mechanism of action of Cp_2MoCl_2 [33]. Additionally, glutathione failed to recognize (coordinate to) titanocene and niobocene dichlorides, thus it appears to be specific towards molybdenocene. [33].

Competition experiments with nucleic acid components (dAMP, ribose monophosphate) and aminoacids (histidine, lysine, alanine, cysteine) were performed using ^1H NMR spectroscopy [34]. First, the coordination of lysine and alanine to Cp_2MoCl_2 either by the carboxylate or amino groups was not observed by ^1H NMR spectroscopy. For histidine, coordination of the imidazole ring to molybdenum was observed by the presence of new aromatic signals in the spectrum. The addition of cysteine to this mixture showed the histidine is replaced forming the $\text{Cp}_2\text{Mo}(\text{Cys})_2$. Pivotal experiments were performed with dAMP and ribose monophosphate, see, Figure 2. Addition of cysteine to the dAMP- and ribose monophosphate- molybdenocene complexes yields new NMR signals corresponding to the $\text{Cp}_2\text{Mo}(\text{Cys})_2$. Furthermore, addition of dAMP and ribose monophosphate to the $\text{Cp}_2\text{Mo}(\text{Cys})_2$ solution produced no change in the NMR spectrum, indicating the cysteine cannot be replaced by nucleic acid components. In addition, Cp_2MoCl_2 showed strong coordination to the glutathione. While these experiments suggest that GSH may be involved in the mechanism of action, since GSH coordinates Cp_2MoCl_2 at physiological pH, additional experiments have shown that this interaction leads to significant deactivation of molybdenocene dichloride antitumor activity [34,35]. Therefore, at least for molybdenocene dichloride, glutathione serves as a deactivating molecule rather than metal carrier agent inside the cell [34,35].

In 2005, a distribution study of the transition metal in Chinese hamster lung cells treated with Cp_2MCl_2 ($\text{M} = \text{Ti}, \text{V}, \text{Nb}, \text{Mo}$) was published [36]. Particularly for Cp_2MoCl_2 , molybdenum is dispersed throughout the cell not in a particular organelle, although there is some evidence for accumulation near the center of the cell (in the nuclear region). Since the DNA binding by molybdenocene is very unlikely, according to the authors, it seems that molybdenocene is targeting thiol containing nuclear proteins [36]. With this in mind, binding studies of radioactive (radiotracer) $\text{Cp}_2^{99}\text{MoCl}_2$ with a thiol containing protein, human serum albumin (HAS), were pursued by monitoring ^{99}Mo gamma emission. These studies provided evidence that Cp_2MoCl_2 binds HAS in a ratio of 9.4 to 1 [37].

In 2008, Meléndez and coworkers studied the interaction of Cp_2MoCl_2 with human serum albumin by electrochemical methods [38]. A methodology to determine the Cp_2MoCl_2 -HAS interaction, using the change in current as the molar fraction of HAS increases was developed. Using this method, it was determined that about 30% of the Cp_2MoCl_2 binds HAS, but the interaction is mainly hydrophobic and weak to be measured by spectral changes in UV-Vis and NMR spectroscopy, as observed by other investigators [39-41].

An *in vitro* study using V79 Chinese hamster lung cells was published by Harding and coworkers and provided additional information regarding the mechanism of action of Cp_2MoCl_2 [42]. V79 Chinese hamster lung cells were treated with Cp_2MoCl_2 (ranging from 100 to 400 μM), and studied using micronucleus assay and transmission electron microscopy to elucidate the genotoxic effect induced by the molybdenocene [42]. These studies showed an increase in frequency of micronuclei (chromosome breaks and/or loss) as the dose increases in comparison to the control cells (untreated cells). Additionally, transmission electron microscopy of the treated V79 Chinese hamster lung cells displayed high incidence of polynucleation with high predominance of cells containing three to five nuclei as well as enlargement in the cell diameter by 1-2 μM in contrast to the control cells. Also, the treated cells exhibited increased chromatin condensation and damaged mitochondria in the cytoplasm. These features are different to the untreated control V79 cells, which are characterized by a single commonly well shape nucleus, containing a single, well defined nucleolus and a granular cytoplasm sparingly filled with organelles. With this experimental data the authors proposed that tubulin, a thiol rich protein with 20 cysteine residues positioned in the spindle apparatus, might be involved in coordination of $\text{Cp}_2\text{Mo}^{2+}$ to the Cys residues, deactivating the spindle apparatus and producing the observed micronuclei [42].

With the mechanistic information presented above, it is clear that molybdenocene dichloride could have various targets and its mechanism of action is far from being well understood. The fact that Cp_2MoCl_2 forms stable species at physiological pH, $\text{Cp}_2\text{Mo}(\text{H}_2\text{O})(\text{OH})^+/\text{Cp}_2\text{Mo}(\mu\text{-OH})_2\text{MoCp}_2$, and the possible coordination affinity to sulfur and phosphates (Mo-S and Mo-O(PO_3) ligations) makes it difficult to hypothesize just one mechanism of action. It is very likely that the mechanism of action of Cp_2MoCl_2 is a complex one, involving more than one target place.

3. Molybdenocene Derivatives

After the preliminary studies on molybdenocene dichloride and its binding capability to biomolecules, several research groups have been working in the structure modification of molybdenocene complex to enhance its anticancer activity and to obtain structure-activity relationship (SAR). The main goal is, using structure-activity relationship, the rational design of more effective and potent antineoplastic agents. To accomplish this, two strategies have been pursued: replacement of halides with ancillary ligands to modulate solubility-stability and biological activity in aqueous solution, and functionalization of cyclopentadienyl to improve anticancer activity.

The initial strategy on the structure modification of molybdenocene dichloride was directed to the replacement of the chlorides with ancillary ligands that can lead to more efficient and less toxic drugs [35]. Four molybdenocene derivatives with thiolate ligands (**1-4**, Figure 3) were synthesized varying their size, charge and lipophilicity. In marked difference to Cp_2MoCl_2 , these thiolate derivatives do not undergo ligand hydrolysis at pH 7.0, demonstrating the robustness of these species at neutral pH.

The cytotoxic activities (IC_{50}) of **1-4** were investigated [35]. The IC_{50} is the concentration required to inhibit the 50% of cell growth. The cytotoxic and cellular uptake studies of molybdenum in V79 Chinese hamster lung cells demonstrated that there is no correlation between the cell uptake and cytotoxicity, since the complex that showed the highest intracellular content of Mo is not the most cytotoxic, see Table 1. Furthermore, the thiolate coordination to molybdenocene inactivate the resulting complex due to the inert character of the Mo-S ligation which makes the ligand hydrolysis less accessible to form the vacant coordination site for subsequent reaction with the target biomolecule.

In contrast to the above results, it was found that not always the Mo-S coordination yields inactive molybdenocenes as long as the complex provides a vacant coordination site for further reaction. A series of water soluble molybdenocene thiolate complexes have been synthesized in water at pH 7.0, in presence of thionucleobases and thionucleosides ligands, demonstrating high aqueous stability at physiological pH [43]. The objective of this study was to elucidate the role of the ancillary ligands on molybdenocene and the outcoming anticancer properties. The new water soluble species, $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Mo(L)}]\text{Cl}$ (L= 6-mercaptapurine, 2-amino-6-mercaptapurine, (-)-2-amino-6-mercaptapurine ribose and 6-mercaptapurine ribose), were characterized by spectroscopic methods. NMR spectroscopic data on $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Mo(6-mercaptapurine)}]\text{Cl}$, in D_2O , showed the presence of two coordination isomers, S(6), N(7), **A** and S(6), N(1), **B**, Figure 4. In general, all four complexes exhibited two coordination isomers, S(6), N(7), **A** and S(6), N(1), **B**, in aqueous solution but only $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Mo(6-mercaptapurine ribose)}]\text{Cl}$ showed two coordination modes in DMSO-d_6 [43]. Of these two possible coordination isomers, we believe, based on thermodynamic considerations, that the 5-membered chelate, S(6),N(7) thionucleobase coordination (**A**) is the most stable [43].

The $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Mo(L)}]\text{Cl}$ complexes are stable at pH 7.4 for extended period of time. Based on the aqueous stability at physiological pH of the $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Mo(L)}]\text{Cl}$ complexes, their antiproliferative activities in HT-29 colon cancer and MCF-7 breast cancer cell lines were investigated, see Table 2.

The cytotoxic studies demonstrated that the coordination of the thionucleobases and thionucleosides to $\text{Cp}_2\text{Mo}^{2+}$ apparently improves their antiproliferative activity on HT-29 colon cancer and MCF-7 breast cancer cell lines but, the thiopurine ligands have their own activity. But based on the fact that these thionucleobases/thionucleosides have antiproliferative properties on these cell lines, the cytotoxic data can be explained the other way around. For HT-29 cell line, the free ligands (thionucleobases/thionucleosides) are more cytotoxic than the corresponding complexes. Thus, the inclusion of molybdenocene moiety into the thionucleobases/thionucleosides apparently reduces their intrinsic cytotoxic activities.

The cytotoxic studies of $[\text{Cp}_2\text{Mo(L)}]\text{Cl}$ complexes in MCF-7 breast cancer cell line were more intriguing and different from that of HT-29 cell line, as MCF-7 cell line expresses higher amount of estrogen receptor. Cp_2MoCl_2 possesses proliferative activity on MCF-7 breast cancer cells since it acts as an estrogen-like agonist on the estrogen receptor [44-46]. Additionally, 6-mercaptapurine and 2-amino-6-mercaptapurine have low antiproliferative activity in MCF-7 cell line ($\text{IC}_{50} \gg 100\mu\text{M}$). Interestingly, $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Mo(6-mercaptapurine)}]\text{Cl}$ ($\text{IC}_{50} = 203(\pm 45\mu\text{M})$) and $[(\eta^5\text{-C}_5\text{H}_5)_2\text{Mo(2-amino-6-mercaptapurine)}]\text{Cl}$ ($\text{IC}_{50} = 16(\pm 2\mu\text{M})$) have cytotoxic activities exceeding those of the thionucleobases and Cp_2MoCl_2 . It is very likely that this enhancement in cytotoxicity comes from two sources: the increase in lipophilicity as result of incorporating $\text{Cp}_2\text{Mo}^{2+}$ into the thionucleobase, increasing the permeability across the lipophilic cell membrane, and synergism between the two anticancer moieties [43].

The cytotoxic activity of $[\text{Cp}_2\text{Mo(thionucleobase)}]\text{Cl}$ complexes deserves a more detailed explanation. The cytotoxic activity of $[\text{Cp}_2\text{Mo(thionucleobase)}]\text{Cl}$ complexes differs strikingly from the $\text{Cp}_2\text{Mo(L)}_2$ complexes (L = glutathione, S-4-thio-2,3,4,6-tetrafluorobenzoic acid, S-1-thio- β -D-glucose and S-1-thio-2,3,4,5-tetracetic β -D-glucose) presented above for the following rationale. The $\text{Cp}_2\text{Mo(L)}_2$ complexes have two Mo(IV)-S bonds whereas the $[\text{Cp}_2\text{Mo(thionucleobase)}]\text{Cl}$ complexes contain one Mo(IV)-S and one Mo(IV)-N bonds in the ancillary positions. The Mo(IV)-S bond is more inert than Mo(IV)-N bond [47]. Since in the $\text{Cp}_2\text{Mo(L)}_2$ complexes (L = glutathione, S-4-thio-2,3,4,6-

tetrafluorobenzoic acid, S-1-thio- β -D-glucose and S-1-thio-2,3,4,5-tetracetic β -D-glucose) both ancillary ligands involve thiolate coordination, the availability of vacant sites at this position is non-existent. In contrast, in the $[\text{Cp}_2\text{Mo}(\text{thionucleobase})]\text{Cl}$ complexes, the Mo(IV)-N bond is more labile than Mo(IV)-S bond [47] and the hydrolysis of this bond allow one vacant site on the Mo(IV) center to further react with important biomolecules and express antiproliferative activity.

The role of oxygen containing chelating ligands was also investigated. While based on thermodynamic considerations, the Mo(IV)-O bond strength in $\text{Cp}_2\text{Mo}^{2+}$ is stronger than Mo(IV)-S [48,49], the Mo(IV)-O bond is more labile [47]. Thus, the objective of these two compounds ($\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$) was also to elucidate the role of the oxygen chelating ligands, the aqueous stability and charge on the molybdenocene cytotoxic activity.

The syntheses of water soluble $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ were carried out by reacting Cp_2MoCl_2 with sodium malonate or maltol in a 1:1 ratio in water, Figure 5 [41]. These species were characterized thoroughly by analytical methods. These species are very soluble and stable in aqueous solution at pH of 7.4 for several days without decomposition. These oxygen chelating ligands not only provided solubility to their complexes but also aqueous stability. The NMR data provided evidence that the chelating ligands are located in the horizontal plane, making the structures very symmetric and the Cp rings become magnetically equivalents, as shown in Figure 5.

The electrochemical characterization of $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ was performed using cyclic voltammetry (cv), in order to study the redox behavior of the molybdenocene complexes in aqueous environment. This data is of fundamental importance since there are many redox processes involved in the cell that could result in damages to biological molecules and because these species are known to be genotoxic due to their redox behavior. Moreover, not only Mo(IV) but also Mo(VI) has shown to possess anti-cancer properties [50]. The cyclic voltammetry experiments of the molybdenocene complexes showed that all molybdenum (IV) species exhibit irreversible redox behaviors under physiological conditions. The oxidation potentials for $\text{Cp}_2\text{Mo}(\text{malonate})$, 790 mV and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$, 800 mV, are higher than the oxidation potential of Cp_2MoCl_2 , 700 mV, which indicate higher stability in aqueous solution under physiological conditions than molybdenocene dichloride, as expected due to the stability imparted by a chelating ligand. The irreversible oxidation potentials of Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ have been attributed to the formation of electrode deficient and reactive Mo(V) species, or the formation of a stable Mo(V)-oxo complex, that prevents the reverse reduction. Also, the effect on the charge on the molybdenocene complexes ($\text{Cp}_2\text{Mo}(\text{malonate})$, 790 mV and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$, 800 mV) is negligible based on the oxidation potentials [41].

As mentioned previously, human serum albumin (HSA) has been proposed to be responsible for transport of molybdenocene species into the target places in the cell [37]. Therefore, the binding interactions between Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes and human serum albumin (HSA) under physiological conditions were investigated using cyclic voltammetry, see Table 3 below [41]. Given that the molybdenocene-HSA interaction is mainly hydrophobic and there is no change in coordination environment about the Mo(IV) center, a change in current was monitored in the titration since it could be associated to a change in the diffusion as a result of molybdenocene incorporating in the hydrophobic pocket of HSA. This binding study revealed that Cp_2MoCl_2 has higher affinity to HAS than $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes [41].

In order to investigate the role of the chelating ancillary ligands on molybdenocene complex and correlate it with Mo(IV)-HAS binding and biological activity, cytotoxic studies on HT-29 colon cancer and MCF-7 breast cancer cell lines were pursued. Both species, $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes, are more cytotoxic on HT-29 colon cancer cells than Cp_2MoCl_2 , nevertheless the enhancement is not very significant (Table 3). Particularly important is that the less cytotoxic complex, Cp_2MoCl_2 , is the one with higher HAS binding affinity. Therefore, the authors concluded that there is no correlation between molybdenocene-HSA binding affinity and cytotoxic activity of the complexes in HT-29 cells [41]. In contrast, in hormone dependent MCF-7 breast cancer cell line, none of the molybdenum complexes exhibit antiproliferative effects, instead they showed proliferative effects. In fact, incorporation of maltolate and malonate ligands on molybdenocene yields species with higher proliferative effects on MCF-7 than Cp_2MoCl_2 . Thus, this proliferative activity has been attributed to the estrogen-like effects of the molybdenocene species on hormone dependent cancer cell line with possible coordination interactions with cysteines of the estrogen receptor [44-46].

Functionalization of molybdenocene dichloride by incorporation of pendant groups on the cyclopentadienyl ligands to improve its anticancer activity has been pursued by Tacke and collaborators [51]. Benzyl-substituted molybdenocene dichlorides were prepared by the corresponding methoxy substituted fulvene and LiBEt_3H , forming the lithium salt of the cyclopentadienide and subsequent reaction with MoCl_4 , according to the reaction below, Figure 6. The *in vitro* cytotoxic studies on human renal cell line Caki-1 at 48 h showed that **2** and **3**, both, displayed the same low cytotoxicity IC_{50} values of 290 μM , while substantial improvement was observed for molybdenocene **1**, with IC_{50} of 84 μM . Cp_2MoCl_2 has $\text{IC}_{50} > 290 \mu\text{M}$. In comparison with isostructural metallocenes of Ti and V, (IC_{50} 21 μM and 3.0 μM), molybdenocene **1** does not achieve the same level of cytotoxicity. Nonetheless, this study highlights the importance of the p-methoxyphenyl substituent on the Cp ring since the di- and tri-substituted Cp rings yield molybdenocenes with low cytotoxic activities [51]. This finding provides fertile grounds to further explore structure-activity studies on molybdenocenes and it is clear that it is an area of research largely unexplored.

4. Inclusion complexes

Inclusion complexes of molybdenocene dichloride with cyclodextrins have been reported [52,53]. Cyclodextrins (CDs) are cyclic oligosaccharides that have α -1,4 linked D-glucose units forming a toroid structure and have been investigated as drug delivery systems. In particular β -CD which contains seven glucose units have been used to encapsulate Cp_2MoCl_2 . Cyclodextrins act as molecular hosts to a diversity of guest species: ions, metal complexes, polar and non-polar organic molecules. These inclusion complexes have found applications in the formulation of drugs due to the increased aqueous solubility and stability of the drugs and better oral absorption. Host-guest inclusion complexes between Cp_2MoCl_2 and β -CD, heptakis-2,3,6-tri-*O*-methyl- β -CD (TRIMEB- β -CD) and 2-hydroxypropyl- β -CD (HP- β -CD) (Figure 7) have been characterized structurally and their cytotoxic properties explored [53]. According to the solid state structural characterization, Cp_2MoCl_2 is encapsulated inside the β -CD and HP- β -CD cavities. These adducts exhibit strong host-guest interactions and penetration of one or both Cp rings inside the cavity are possible inclusion geometries. The $\text{Cp}_2\text{MoCl}_2 \cdot \text{TRIMEB-}\beta\text{-CD}$ adduct showed weak host-guest interaction.

The antiproliferative and cytotoxic activity of inclusion species on HeLa cervical cancer and healthy cell lines (BJ fibroblast) were investigated. From this study it was concluded the following. First, cyclodextrins can enhance the antitumor activity of Cp_2MoCl_2 . Second, the $\text{Cp}_2\text{MoCl}_2 \cdot \text{TRIMEB-}\beta\text{-CD}$ showed the highest cytotoxic activity toward HeLa cervical

cancer cell line at 72 h. Third, Cp_2MoCl_2 and $\text{Cp}_2\text{MoCl}_2\cdot\beta\text{-CD}$ have higher toxicity toward healthy cells than $\text{Cp}_2\text{MoCl}_2\cdot\text{TRIMEB-}\beta\text{-CD}$ and $\text{Cp}_2\text{MoCl}_2\cdot\text{HP-}\beta\text{-CD}$. The antiproliferative and cytotoxic effects of $\text{Cp}_2\text{MoCl}_2\cdot\text{TRIMEB-}\beta\text{-CD}$ and $\text{Cp}_2\text{MoCl}_2\cdot\text{HP-}\beta\text{-CD}$ are reversible by 75-80% in HeLa cells and 85-90% for healthy cell line (fibroblast). The $\text{Cp}_2\text{MoCl}_2\cdot\text{TRIMEB-}\beta\text{-CD}$ adduct displayed the highest antitumor activity and the lowest toxicity toward non-neoplastic cells [53].

Another type of macrocyclic molecules studied as possible drug delivery systems are the cucurbit[n]urils, Cucurbit[n]urils (CB[n]s) are pumpkin-like macrocyclic molecules made of glycoluril [=C₄H₂N₄O₂=] monomers linked by methylene groups, Figure 8 [54]. Cucurbit[n]urils have two hydrophilic carbonyls per glycoluril unit lined portals, capping the inner hydrophobic cavity. Their cavity size depends on the number (n) of glycoluril units. CB[7] and CB[8] have portal widths of 5.4 and 6.9 Å and cavity widths of 7.3 and 8.8 Å respectively. Cucurbit[n]urils have very similar host-guest interactions as the cyclodextrins [54].

The inclusion complexes of molybdenocene dichloride with cucurbit[n]urils, n = 7, 8, and their cytotoxic activities on MCF-7 breast cancer and 2008 (human ovary carcinoma) cell lines were investigated [55]. NMR experiments, under rigorous exclusion of oxygen, showed that CB[7] can encapsulate $\text{Cp}_2\text{Mo}(\text{OH})(\text{H}_2\text{O})^+$ at pD 7.0 as well as pD 2.0 forming a 1:1 host-guest complex. Molecular modeling studies for CB[7]- $\text{Cp}_2\text{Mo}(\text{OH})(\text{H}_2\text{O})^+$ suggests that the metallocene is encapsulated deep inside the cavity. In presence of oxygen, the encapsulated Mo species catalyzes the degradation of CB[7]. While encapsulation of $\text{Cp}_2\text{Mo}(\text{OH})(\text{H}_2\text{O})^+$ in CB[8] was not detected, NMR experiments showed that the presence of oxygen in the sample solution promoted CB[8] degradation as well. *In vitro* studies on MCF-7 breast cancer cell and 2008 cell (derived from human ovary carcinoma) lines were performed on CB[7]- $\text{Cp}_2\text{Mo}(\text{OH})(\text{H}_2\text{O})^+$ and showed that the encapsulated species has improved antiproliferative activity on both cell lines than Cp_2MoCl_2 , most likely as a result of the improved solubility and membrane permeability [55].

4. Conclusion

This review article summarizes the development of molybdenocene dichloride and its derivatives as potential anticancer agents. It attests remarkably the development of the bioorganometallic chemistry of molybdenocene in the past two decades. From the mechanistic point of view, inhibition of protein kinase C (PKC), coordination of $\text{Cp}_2\text{Mo}^{2+}$ to the Cys residues in tubulin, the interaction of Cp_2MoCl_2 into the hydrophobic pocket of HAS and weak DNA coordination leading to phosphoester bond cleavage are possible target places to be considered to explain the antineoplastic activity. The replacement of the chlorides by other ancillary ligands has provided insights in terms of aqueous stability and antitumor activity and demonstrated that molybdenocene species needs at least one coordination vacant site to remain active as anticancer agent.

In the future, we expect more synthetic routes to be developed and new functionalized molybdenocenes (on Cp rings and ancillary ligands) will appear to increase/enhance its anticancer activity and perhaps with different mechanism of actions as those proposed currently. The possibilities to modify the molybdenocene structure and fine-tune its biological activity are endless and it is an area of research that remains largely unexplored.

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Highlights

Molybdenocene dichloride anticancer agents with lower toxicity than cis-platin

Human serum albumin as transport protein of molybdenocene dichloride

Structure modification of molybdenocene dichloride yields water soluble complexes with enhanced anticancer activity

Molybdenocene with proliferative activity on hormone dependent breast cancer due to its estrogen-like behavior

Figure 1. Coordination modes between Cp_2MoCl_2 and nucleobases and nucleotide. a) N(7),NH(6) chelation b) N(1), NH(6) chelation, c) N(3), NH(4) chelation d) N(7), O(phosphate) chelation.

Figure 2.
Reactions representing competition experiments.

Figure 3.
Structures of thiol derivatives of molybdenocene dichloride.

Figure 4. Proposed structures of $[\text{Cp}_2\text{Mo}(\text{6-mercaptopurine})]\text{Cl}$. a) S(6), N(7) and b) S(6), N(1) coordination modes.

Figure 5.
Synthesis and structures of molybdenocene derivatives.

Figure 6.
Reaction sequence for the synthesis of benzyl-substituted molybdenocene dichlorides [48].

I, $R = H, R' = H$

II $R = R' = CH_3$, TRIMEB

III $R = H, R' = CH_2CH_2(OH)CH_3$, HP

Figure 7.
Representation of β -CD- Cp_2MoCl_2 inclusion complex .

Figure 8.
General chemical structure of cucurbit[n]uril, where $n = 5, 6, 7, 8, 10$.

Table 1

Cytotoxicities of molybdenocenes in V79 Chinese Hamster Lung cell line using MTT assay and cellular uptake of Mo.

Complex	IC ₅₀ (μM), 24 h	Cell uptake *
Cp ₂ MoCl ₂	820	1.0
1	2230	0.15-0.20
2	2900	
3	>500	3.5-5.0

* Relative values standardized to Cp₂MoCl₂.

Table 2

Cytotoxicities of [Cp₂Mo(L)]Cl complexes studied on HT-29 colon cancer and MCF-7 breast cancer cell lines at 72 h, as determined by MTT assay. IC values are the average of four independent measurements with their standard deviations (). n/a = not active under the concentrations studied.

Complex	IC ₅₀ (μM), HT-29	IC ₅₀ (μM), MCF-7
[Cp ₂ Mo(6-mercaptopurine)]Cl	180(±8)	203(±45)
6-mercaptopurine	10.1(±3)	750(±30)
[Cp ₂ Mo(6-mercaptopurine ribose)]Cl	201(±45)	n/a
6-mercaptopurine ribose	15.7(±8)	100(±7)
[Cp ₂ Mo(2-amino-6-mercaptopurine)]Cl	15(±2)	16 (±2)
2-amino-6-mercaptopurine	7.1(±4)	239(±33)
[Cp ₂ Mo((-)-2-amino-6-mercaptopurine ribose)]Cl	277(±25)	n/a
(-)-2-amino-6-mercaptopurine ribose	31.8(±1)	100(±4)
Cp ₂ MoCl ₂	2600(±300)	Proliferative effect

Table 3

Binding interactions of molybdenocene complexes with HSA in 100 mM Tris/10 mM NaCl buffer solution monitored by cv at a scan rate of 100 mV/s and cytotoxic activity on HT-29 colon cancer cell line at 72 h.

Complex	Mo-HSA interaction % ΔI ($\pm$ sd)	IC ₅₀ (mM) HT-29 ($\pm$ sd)
Cp ₂ MoCl ₂	32(7)	2.6 (0.3)
Cp ₂ Mo(malonate)	21(5)	1.31 (0.02)
[Cp ₂ Mo(maltolato)]Cl	15(4)	1.9 (0.3)