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Water-soluble molybdenocene complexes with both proliferative and antiproliferative effects on cancer cell lines and their binding interactions with human serum albumin

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Abstract

Two water-soluble molybdenocene complexes containing oxygen chelating ligands, maltolato and malonate, have been synthesized to elucidate the role of the ancillary ligands in the molybdenocene cytotoxic activity. The structural characterizations of these species by ^1H NMR and IR spectroscopies suggest that both molybdenocene complexes contain the ligands in a bidentate fashion and elemental analysis and mass spectrometry corroborate the proposed formula for the species to be Cp_2Mo (malonate) and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ (Cp is cyclopentadienyl). Metal–albumin binding studies were pursued using UV–vis spectroscopy and cyclic voltammetric techniques. Whereas metal–albumin binding studies using UV–vis spectroscopy did not show any evidence of interaction, cyclic voltammetry experiments showed that molybdenocene complexes may be involved in weak binding interactions with albumin, most likely in hydrophobic interactions. The cytotoxic activities of $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ alone with Cp_2MoCl_2 were investigated in HT-29 colon cancer and MCF-7 breast cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell viability assay. $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ showed slight improvement in terms of cytotoxic activity as compared with Cp_2MoCl_2 in the HT-29 colon cancer cell line, whereas for MCF-7 all the molybdenocene species exhibited a proliferative profile. The molybdenocene-containing chelating ligands showed stronger proliferative effects than Cp_2MoCl_2 . There is no correlation between the binding affinity of molybdenocenes for human serum albumin and cytotoxic activity toward HT-29 and MCF-7 cancer cells.

Keywords

Molybdenocene dichloride; HT-29; MCF-7; Maltolato; Malonate

Introduction

Metallocene dihalides and pseudo halides (Cp_2MX_2 , Cp is cyclopentadienyl, M is Ti, V, Nb, Mo) (Structure I) have biological activity toward a wide variety of murine and human tumors [1-17]. Titanocene dichloride has attracted the attention of many research groups and currently is the subject of extensive investigations either modifying the titanocene structure with functional groups appended to the Cp ring or with new ancillary ligands, to improve its anticancer properties and to obtain structure–activity relationships [18-39].

In contrast to Cp_2TiCl_2 , molybdenocene dichloride has not been extensively investigated. In this regard, it is known that $\text{Cp}_2\text{Mo}^{2+}$ is hydrolytically stable under physiological conditions, whereas $\text{Cp}_2\text{Ti}^{2+}$ hydrolyzes extensively [40,41]. Several thiol derivatives of molybdenocene have been prepared and cell uptake and cytotoxicity studies

were pursued [42]. It was found that the molybdenocenes containing thiols as ancillary ligands are robust toward hydrolysis but yielded noncytotoxic complexes [42]. Strong coordination of molybdenocene by the thiolates apparently hinders the formation of vacant coordination sites on the metal center and the net result is inactivation. In addition, the cytotoxicity results do not correlate with the molybdenum cell uptake or charge of the complexes [42]. It has recently been proposed that albumin is responsible for carrying molybdenocene species into the target places in the cell [43].

In the past, our group reported a series of water-soluble molybdenocenes containing thionucleobases [44]. These complexes are stable under physiological conditions, create vacant sites on the metal center, and showed good anti-proliferative activity toward the HT-29 colon cancer cell line. Now we report the synthesis of water-soluble molybdenocenes with oxygen chelating ligands and the properties of their binding to human serum albumin (HSA) and their cytotoxic activity toward HT-29 colon cancer and MCF-7 breast cancer cell lines.

Results and discussion

Synthesis and characterization

The syntheses of $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ were performed by reacting Cp_2MoCl_2 with sodium malonate or maltol in a 1:1 ratio in water (Fig. 1). For $\text{Cp}_2\text{Mo}(\text{malonate})$, the reaction mixture was kept at a pH of 6, whereas for maltol the pH was adjusted to 10. The objective for these two compounds was to elucidate the effect of the ancillary ligands on the molybdenocene cytotoxic activity. The ligands, malonate and maltolato, provided highly soluble and stable complexes in an aqueous environment. In addition, these species are stable at pH of 7.4 for several days without decomposition. Both complexes were characterized by analytical and spectroscopic methods.

The NMR spectral data showed that there is only one signal for the Cp rings, on both $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes, which suggests the ancillary ligands are symmetrically located on the equatorial plane, as presented in Fig. 1. Table S1 summarizes the most important spectroscopic features. In the IR spectrum, $\text{Cp}_2\text{Mo}(\text{malonate})$ shows the $\nu(\text{C}=\text{O})$ band at $1,605\text{ cm}^{-1}$ as well as the $\nu(\text{Cp})$ stretching band at $3,102\text{ cm}^{-1}$. For $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$, in addition to the $\nu(\text{Cp})$ stretching band at $3,093\text{ cm}^{-1}$, the $\nu(\text{C}=\text{O})$ band is shifted from $1,612$ to $1,597\text{ cm}^{-1}$ as a result of ligand coordination. Attempts to obtain single crystals of these complexes failed and resulted in powdery solids.

Mass-spectrometric characterization by electrospray positive ion mode in the presence of formic acid was performed on the new complexes. The theoretical m/z peaks corresponding to $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes were 331 and 389, respectively. However, the experimental data indicate that the m/z peak was 331 for $\text{Cp}_2\text{Mo}(\text{malonate})$ complex and 353 for $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$. These results suggest that for the $\text{Cp}_2\text{Mo}(\text{malonate})$ complex, the molecular ion ($[\text{Cp}_2\text{Mo}(\text{malonate})]^+$) is detected. In contrast, in the $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complex, the parent peak observed corresponds to $[\text{Cp}_2\text{Mo}(\text{maltolato})-\text{H}]^+$ under electrospray ionization (ESI) conditions. This is expected since the chloride is not covalently bound to molybdenum(IV); it is ionic in nature.

The theoretical mass-spectrometric isotopic distributions for $\text{Cp}_2\text{Mo}(\text{malonate})^+$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})-\text{H}]^+$ ions were calculated using the Molecular Weight Calculator program available at <http://jjorg.chem.unc.edu/personal/monroe> and were compared with the experimental ones, see Table S2. It is evident that the experimental values have a pattern very similar to the predicted theoretical values, supporting the proposed formula for these new species.

An electrochemical characterization was performed, using cyclic voltammetry (CV), to study the redox behavior of the molybdenocene complexes in an aqueous environment. These data are of fundamental importance because there are many oxidation processes that could result in damage to biological molecules and because not only Mo(IV) but also Mo(VI) has been shown to possess anticancer properties [45]. Furthermore, CV has been shown to be a very sensitive analytical technique to determine changes in redox behavior of metallic species in the presence of biologically important molecules [46-51]. The redox behavior of metallic species is very sensitive to the environment surrounding the metal center (solvent, ligand, charge); therefore, metal-based interaction can be detected using this technique.

As can be seen in the voltammograms (Fig. 2), the three Mo(IV) species exhibit irreversible electrochemical behaviors under physiological conditions. According to Rodríguez et al. [51], Cp_2MoCl_2 shows an irreversible oxidation peak at 700 mV, which is attributed to the formation of electrodeficient and reactive Mo(V) species, or the formation of a stable metal-oxo complex, which prevents the reverse reduction. The same irreversible behaviors were observed for the two molybdenocene derivatives. The $\text{Cp}_2\text{Mo}(\text{malonate})$ complex exhibits an

oxidation peak at 790 mV, whereas the $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complex exhibits an oxidation peak at 800 mV. Both complexes showed oxidation potentials higher than the oxidation potential of the parent compound, Cp_2MoCl_2 , which suggests higher stability in aqueous solution under physiological conditions.

Metal–albumin binding studies

HSA has been proposed to be responsible for carrying/ stabilizing molybdenocene species into/ in the target places in the cell [43]. Therefore, we investigated 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 HSA under physiological conditions, monitored by UV–vis spectroscopy and CV. The latter has proved to be a sensitive tool to measure weak metal–DNA interactions [51].

The electronic spectra of Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$, and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes were recorded in 100 mM tris(hydroxymethyl)aminomethane (Tris)–HCl/10 mM NaCl, pH 7.4 as buffer solution, and at room temperature. Under these conditions the molybdenocene dichloride exhibits an absorption band at 385 nm, which is attributed to $d-d$ transitions (since $\epsilon = 96 \text{ M}^{-1} \text{ cm}^{-1}$). For the $\text{Cp}_2\text{Mo}(\text{malonate})$ complex, this band is shifted to a greater wavelength, at 402 nm ($\epsilon = 102 \text{ M}^{-1} \text{ cm}^{-1}$). This shift suggests that the electronic environment around Mo(IV) has changed as a result of malonate coordination. Likewise, for $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ a similar shift is observed in the $d-d$ transition, appearing as a shoulder at 411 nm ($\epsilon = 195 \text{ M}^{-1} \text{ cm}^{-1}$). Furthermore, the band of free maltol at 272 nm is shifted to 330 nm in the complex. All complexes exhibit $\pi-\pi^*(\text{Cp})$ transitions at about 225 nm.

Studies of the binding between HSA and molybdenocene dichloride were performed, in 100 mM Tris–HCl/ 10 mM NaCl, pH 7.4, in which aliquots of the metal solution were added to a HSA solution until a ratio of 1:24 (HSA to molybdenocene) was obtained, and the binding was monitored by UV–vis spectroscopy from 200 to 600 nm. As can be seen in Fig. 3, the spectra did not show new bands corresponding to new species and no isosbestic points were observed in the spectra, which indicates that none of these species, the free HSA and molybdenocene and complex “HSA–molybdenocene”, coexist in equilibrium.

In an analogous manner, studies of binding between HSA and $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes were performed. Neither $\text{Cp}_2\text{Mo}(\text{malonate})$ nor $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ showed any spectral change that could be attributed to a HSA–molybdenocene interaction.

To investigate in more detail the fate of HSA–molybdenocene interactions, we monitored the electrochemical behavior of the molybdenocenes upon addition of HSA (titration) by cyclic voltammetric techniques. Four aliquots of 250 μL of 100 mM Tris/10 mM NaCl buffer (as a blank) or HSA ($2.5 \times 10^{-5} \text{ M}$) were added to the metal solution consecutively and their electrochemical behaviors were compared. Cyclic voltammograms were recorded after each addition, and the oxidation current (I_{pa}) and the oxidation potential (E_{pa}) were obtained. Comparison of the electrochemical behavior of the blanks and the HSA titrations allowed us to estimate binding interactions. In the past, E_{pa} was used to evaluate the percentage of interaction between the metal and DNA, because it is more sensitive to the coordination environment than the current [51]. Table 1 presents the summary of the electrochemical data and Table S3 presents the complete titration data.

Figure 4a shows the Cp_2MoCl_2 voltammograms after the addition of buffer at pH 7.4 and HSA solution. It is evident that the E_{pa} does not change significantly after each addition of buffer. Upon addition of HSA to Cp_2MoCl_2 , the E_{pa} shifts toward higher potentials and the oxidation peak eventually disappears. Using the same method as for DNA [51], the maximum interaction between molybdenocene and HSA is 15%. Although the value appears to be rather small, caution must be taken. The possible interactions between molybdenocene and HSA are mainly

hydrophobic in nature, as suggested by two independent research groups [52, 53]. Therefore, 15% is very significant and strongly suggests that HSA is a potential target or transporter for Cp_2MoCl_2 . Similar titrations with $\text{Cp}_2\text{Mo}(\text{malonate})$ or $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ and HSA showed 3.2 and 0.9%, respectively. Upon examination of these results, we reconsidered these titrations based on the fact that the possible interactions between molybdenocenes and HSA are mainly hydrophobic [52, 53] and since there is no change in coordination environment, the potential is not as sensitive as the current for estimation of binding interactions.

A change in current could be associated with a change in the diffusion as a result of molybdenocene incorporating in the hydrophobic pocket of HSA. In this regard, using the change current, molybdenocene dichloride showed a maximum of 32% of interaction with HSA, whereas $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ showed only 21 and 15%, respectively. This suggests that molybdenocene dichloride has more affinity for HSA than $\text{Cp}_2\text{Mo}(\text{malonate})$ and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$, following the same pattern observed using the oxidation potentials, but we believe that these numbers are more realistic since they show that HSA–molybdenocene interaction could be significant. In fact, there are recent reports with evidence that HSA could be responsible for the transport and delivery of titanocene dichloride into the target place [54,55].

Cytotoxicity studies

To investigate the effect of the ancillary ligands on the molybdenocene complex, we performed cytotoxicity studies on HT-29 colon and MCF-7 breast cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay [56], at 72 h, to determine their biological activity. Figure 5 depicts the dose–response curves for the effects of Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$, and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes on the HT-29 cancer cell line.

The values of the cytotoxicity (IC_{50}) against HT-29 obtained for the Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$, and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes are $2.6 (\pm 0.3)$ mM, $1.31 (\pm 0.02)$ mM, and $1.9 (\pm 0.3)$ mM, respectively. Although they are of the same order of magnitude, it can be seen that the concentration of Cp_2MoCl_2 required to inhibit 50% of cancer cell proliferation is slightly higher (less cytotoxic) than the concentrations of the molybdenocene derivative complexes. The addition of these bidentate chelating ligands (malonate and maltol) to the molybdenum center does not improve significantly the cytotoxic activity of the resulting molybdenocenes. Upon examination of the IC_{50} values of the two modified complexes, one can see that the $\text{Cp}_2\text{Mo}(\text{malonate})$ complex was more cytotoxic than $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$. However, there is no correlation between molybdenocene and HSA binding and cytotoxic activity of the complexes under study since Cp_2MoCl_2 has a higher binding affinity for HSA but it is the least cytotoxic.

To evaluate and compare the cytotoxic activity of Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$, and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ in a cell line other than HT-29, we investigated their response in terms of cell killing on a well-known breast cancer cell line, MCF-7, at 72 h. In contrast to the results obtained for the dose–response of the HT-29 cell line, in the MCF-7 cell line, none of the molybdenum complexes exhibit antiproliferative effects (Fig. 6).

Figure 6 shows that as we increase the complex concentration, the viability increases, demonstrating a proliferative instead of an antiproliferative effect. The complex with the highest proliferative effect is $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$. Similar behaviors, on the MCF-7 cancer cell line, have been reported for Cp_2TiCl_2 and titanocene vectorized with tamoxifen and have been attributed to estrogenic effects of the metallocene on the hormone-dependent cancer cell line [55,57].

Conclusions

Two molybdenocene derivatives containing oxygen chelating ligands were synthesized to investigate the role of the ancillary ligands on the cytotoxic properties of HT-29 and MCF-7 cancer cell lines and the binding capabilities toward HSA. Whereas maltolate and malonate ligands provide water-soluble molybdenocene complexes with higher stability than Cp_2MoCl_2 , the improvement in their cytotoxic activity toward the HT-29 colon cancer cell line is not highly significant. There is no correlation between their cytotoxic activity and the binding affinity for HSA. Cp_2MoCl_2 has a higher binding interaction with HSA than the molybdenocene derivatives but it is less cytotoxic. Thus, whether HSA serves a carrier/stabilizing agent of molybdenocene species is not clear and remains under investigation. In contrast, Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$, and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ showed proliferative effects on hormone-dependent, MCF-7 breast cancer cells. This response has been reported for titanocene dichloride and titanocenyl tamoxifen complexes and has been attributed to the estrogenic effects of the titanocenes on the hormone-dependent cancer cell line. Whether this scenario applies for the molybdenocenes is uncertain at this point and needs to be investigated in more detail. In addition, incorporation of maltolate and malonate ligands on molybdenocene yields complexes with higher proliferative effects on MCF-7 than Cp_2MoCl_2 .

Materials and methods

Materials

Molybdenocene dichloride was handled under dried nitrogen in Schlenk-type anaerobic lines. The Cp_2MoCl_2 complex was obtained from Aldrich and used without further purification. Maltol and sodium malonate were obtained from Sigma-Aldrich and used as received. The purity of molybdenocene dichloride, maltol, and sodium malonate was checked by IR and/or by ^1H NMR spectroscopy. Water was doubly distilled, deionized, and thoroughly saturated with dried nitrogen. All solvents for NMR measurements were 99.9% D purity grade. The MTT was obtained from Sigma and used as purchased.

Physical measurements

The complexes were dissolved in a mixture of water/methanol (1:1) containing 0.2% of formic acid prior to mass-spectral analysis. Solutions of 1 ppm for both complexes were used. A Bruker Daltonics Esquire 6000 instrument was used to record the mass-spectral data. The electrospray positive ion mode was used as the ionization mode during the mass-spectrometry (MS) experiment. The experimental intensities were reported relative to the parent peak in each mass spectrum. To determine the theoretical isotopic distribution pattern of the $[\text{M}]^+ m/z$ peaks, the Molecular Weight Calculator program available at

<http://jjorg.chem.unc.edu/personal/monroe> was used. Since the IR characterizations were performed using reflectance IR, pure complexes do not require any sample preparation before the analysis. The IR spectra were obtained using a Scimitar series Digilab FIS 1000 reflectance IR Fourier transform spectrophotometer, equipped with a Digilab Resolution 4. UV-vis spectra were recorded with a double-beam Lambda Bio 20 PerkinElmer spectrophotometer, equipped with a temperature controller, and quartz cells were used. The system was interfaced with a 586 Nokia computer system and the spectral handling was carried out using WinLab (PerkinElmer). Solutions of molybdenocene dichloride and molybdenocene complexes at 4.0×10^{-4} M were prepared in 0.1 M Tris-HCl/10 mM NaCl at a pH of 7.4. Studies of binding between HSA and molybdenocenes monitored by UV-vis spectroscopy were performed on the same buffer solution.

Electrochemical characterization and titrations were carried out using a BAS CV-50W voltammetric analyzer. The water used in the CV experiments was doubly distilled, deionized,

and thoroughly saturated with dried nitrogen. A three-electrode configuration consisting of a glassy carbon working electrode, a platinum wire counter electrode, and an Ag/AgCl, 3 M NaCl reference electrode in 100 mM Tris/10 mM NaCl buffer solution at a pH of 7.4 was used. The working electrode was polished with 0.05- μ m alumina slurry for 1–2 min, and then rinsed with deionized water. This cleaning process was done before each CV experiment. A sweep between 1,000 and –1,000 mV was performed on the 100 mM Tris/10 mM NaCl buffer solution to verify any possible deposition. The ^1H spectra were recorded using Bruker 500 MHz Avance spectrometers under controlled temperature. 3-(Trimethylsilyl) propane-sulfonic acid was used as the internal reference. Elemental analysis was performed by Atlantic Microlab.

Cytotoxicity studies

MTT assays [56] were performed for two different cell lines, HT-29 and MCF-7, both obtained from American Type Culture Collection (ATCC HTB-38 and ATCC HTB-22). The colon cancer cell line, HT-29, was grown under 95% air/5% CO_2 (USP grade) atmosphere at 37 °C. The growth medium used was McCoy's 5A (ATCC) complete medium adjusted by the supplier to contain 1.5 mM L-glutamine and 2.2 $\text{g}^{-1} \text{L}^{-1}$ sodium bicarbonate. In addition, this medium was supplemented with 10% (v/v) fetal bovine serum (ATCC) and with 1% (v/v) antibiotic–antimycotic (Sigma).

The breast cancer cell line, MCF-7, was grown and maintained, as well as HT-29, at 37 °C and 95% air/5% CO_2 (USP grade). This cell line was grown in Dubelcco's modification of Eagle's medium from Cellgro, which was supplemented by the supplier with L-glutamine, 4.5 $\text{g}^{-1} \text{L}^{-1}$ glucose, and sodium pyruvate. This complete medium was supplemented with 10% (v/v) fetal bovine serum (ATCC) and with 1% (v/v) antibiotic–antimycotic (Sigma).

A 100- μ L suspension with an initial population of 10,000–15,000 (for the HT-29 cell line) cells per well was seeded in a 96-well plate (VWR) and after 24 h of incubation, a dose of the metal complex was added. Complex concentrations were between 8 and 8,470 μM (ten data points evenly distributed, one concentration per column of eight wells) dissolved in 5% dimethyl sulfoxide (Sigma) and 95% medium. Experiments were performed in quadruplicate plates. The plates were left at 37 °C and 95% air/5% CO_2 for 72 h. Two to 4 h before the completion of the 72 h of incubation, a solution of MTT (1.0 $\text{mg}^{-1} \text{mL}^{-1}$) was added and incubated. When the incubation was complete and the purple formazan insoluble product was observed, the cell medium was removed and the plates were washed with cold phosphate buffer solution (PBS). The PBS was prepared with sodium chloride, potassium chloride, sodium phosphate, and potassium phosphate (all from Sigma-Aldrich) dissolved in double-distilled, deionized, and autoclaved water. The PBS was autoclaved and filtered through 0.2- μ m cellulose-acetate filters.

For the MCF-7 cell line, an initial population of cells per well greater than that for the HT-29 was required because MCF-7 cells have a doubling time (ATCC) greater than that of HT-29 cells.

At this point 200 μL per well of a detergent solution, 10% (v/v) Triton X-100 (Sigma) in 2-propanol (Fisher), was added and left at 37 °C to dissolve the formazan product.

The absorbances of the resulting colored solutions were measured at 570 nm in the microplate reader with background subtraction at 630 nm. The instrument used was a 340 ATTC microplate reader from SLT Lab Instruments equipped with a temperature control unit and interfaced with a computer with the WinSeLecT software program. The IC_{50} , a metal complex concentration necessary to inhibit cell proliferation by 50%, was calculated by fitting the data to a four-parameter logistic plot using the SigmaPlot software program from SPSS.

All of the MTT protocol was performed in a dark room. Experiments were designed to contain blank wells (controls), which contained only cells with the medium, control wells, which contained untreated cells but the medium contained 5% dimethyl sulfoxide, and test wells, which contained cells treated with the metal compound at different concentrations.

Synthesis of bis(cyclopentadienyl)molybdenum(IV) malonate

A 50-mL three-neck round-bottom flask was charged under nitrogen with 50 mg (0.168 mmol) of Cp_2MoCl_2 , 27.9 mg (0.168 mmol) of sodium malonate, and 10 mL of deionized water. The resulting pH of the solution was about 6.0 and a dark-green color formed. The reaction mixture was stirred overnight and the final pH was between 3.5 and 4.5. At this point, the reaction mixture volume was reduced to about 3 mL and the product precipitated with addition of a few drops of acetone. The solid isolated was finally purified by column chromatography using lipophilic Sephadex (20–100 μm , from Aldrich) as the stationary phase and methanol as the solvent. A bright-green solid was isolated upon removal of methanol, obtaining the product in about 90% yield. ^1H NMR (D_2O , ambient) δ : 5.85 (s, 5H, Cp), 3.27 (s, 2H, CH_2). Reflectance IR: 3,101(mw), 2,908(w), 1,605(s), 1,366(s), 1,180(w), 1,065(w), 1,018(w), 965(w), 935(w), 833(m), 802(m), 718(m), 648(w), 579(m). ESI-MS (positive mode), m/z (relative intensity): $\text{Cp}_2\text{Mo}(\text{malonate})^+$, 325 (55.4), 327 (41.8), 328 (70.3), 329 (74.1), 330 (51.6), 331 (100), 333 (43.1). Anal. Calc. for $\text{C}_{13}\text{H}_{12}\text{MoO}_4$: C, 47.28; H, 3.66. Found: C, 46.99; H, 3.63.

Synthesis of bis(cyclopentadienyl(maltolate) molybdenum(IV) chloride

In a 50-mL three-neck round-bottom flask, 100 mg (0.337 mmol) of Cp_2MoCl_2 and 42.5 mg (0.337 mmol) of 3-hydroxy-2-methyl-4-pyrone (maltol) were loaded and dissolved in 20 mL of deionized and degassed water. The initial pH was 5.6 and it was adjusted to 10 with 1.0 M NaOH. The initial solution was amber in color and after 24 h the solution turned brown. The solvent was removed in vacuo and the resulting brown complex was purified by column chromatography using lipophilic Sephadex (20–100 μm , from Aldrich) as the stationary phase and methanol as the solvent. The complex was obtained with 84.4% yield. The complex is highly hygroscopic. ^1H NMR (D_2O , ambient) δ : 8.05 (d, 1H, $J = 5$ Hz, H-6), 6.72 (d, 1H, $J = 5$ Hz, H-5), 5.88 (s, 10H, Cp), 2.27 (s, 3H, CH_3). Reflectance IR: 3,086(mw), 3,047(w), 2,970(w), 1,597(m), 1,543(s), 1,473(s), 1,435(m), 1,381(m), 1,265(s), 1,203(s), 1,018(m), 918(w), 841(s), 725(mw), 594(m). ESI-MS (positive mode), m/z (relative intensity): $[\text{Cp}_2\text{Mo}(\text{maltolato})\text{-H}]^+$, 349 (8.6), 350 (32.7), 351 (63.5), 352 (76.9), 353 (46.4), 354 (100), 355 (16.3). Anal. Calc. for $\text{C}_{16}\text{H}_{17}\text{MoO}_4\text{Cl}$ ($[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl} \cdot \text{H}_2\text{O}$): C, 47.49; H, 4.23. Found: C, 47.54; H, 4.27.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Syntheses and structures of molybdenocene derivatives

Fig. 2. Cyclic voltammograms of Cp_2MoCl_2 , $\text{Cp}_2\text{Mo}(\text{malonate})$, and $[\text{Cp}_2\text{Mo}(\text{maltolato})]\text{Cl}$ complexes (Cp is cyclopentadienyl), at a scan rate of 100 mV/s and in 100 mM tris (hydroxymethyl)aminomethane (Tris)/10 mM NaCl, pH 7.4 as the supporting electrolyte. The complex concentration was 5.0×10^{-4} M

UV-Vis spectra for Human Serum Albumin and Cp_2MoCl_2 titration

Fig. 3. UV-vis spectra of human serum albumin (HSA)- Cp_2MoCl_2 titration in 100 mM Tris/10 mM NaCl buffer solution, at pH 7.4

Fig. 4. Cyclic voltammograms for the titration of Cp_2MoCl_2 with **a** 100 mM Tris/10 mM NaCl buffer and **b** HSA in 100 mM Tris/10 mM NaCl buffer, at a scan rate of 100 mV/s. The complex concentration was 5.0×10^{-4} M

Fig. 5. Dose-response curves for Cp₂MoCl₂, Cp₂Mo(malonate), and [Cp₂Mo(maltolato)]Cl against HT-29 at 72 h of drug exposure. The data presented are the mean $\pm$ 1 standard deviation (SD) based on quadruplicate experiments

Fig. 6. Effects of Cp₂MoCl₂, Cp₂Mo(malonate), and [Cp₂Mo(maltolato)]Cl on the MCF-7 cell line after 72 h of drug exposure. The data presented are the mean $\pm$ 1 SD based on quadruplicate experiments

Table 1

Summary of the titration of molybdenocene complexes with human serum albumin (*HSA*) in 100 mM tris (hydroxymethyl)aminomethane/10 mM NaCl buffer solution at a scan rate of 100 mV/s

Complex	Mo-HSA interaction	
	ΔE_{pa} (%) ($\pm SD$)	ΔI (%) ($\pm SD$)
Cp ₂ MoCl ₂	15(2)	32(7)
Cp ₂ Mo(malonate)	3.2(4)	21(5)
[Cp ₂ Mo(maltolato)]Cl	0.9(4)	15(4)

The complex concentration was 5.0×10^{-4} M

Cp cyclopentadienyl, *SD* standard deviation