Cytotoxicity Studies of Water Soluble Coordination Compounds with a $[Mo_2O_2S_2]^{2+}$ Core.

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Abstract: Selected molybdenum sulfur compounds with the formulas (M)$[Mo_2O_2S_4L]$ where $(Et_4N)_2$(**1**), L = S_4^{2-}, (Et_4N)(**2**), L = Cp, (**3**), L = DMF, K(**5**), L = serine, and $[Mo_2O_2S_2L_2]$ where Na_2(**4**), L = cysteine, and (**6**), L = threonine, were prepared and subjected to cytotoxicity studies *in vitro*. The results were analyzed to rank the compounds according to their relative cytotoxicity and to correlate the observed toxicity to specific composition. The results guide future efforts to synthesize highly water soluble, non-toxic, compounds. Strong correlation was observed between toxicity and cation selection, as well as selection of biocompatible ligands combined with alkali metal salts. The most toxic compound analyzed showed about 50 times less cytotoxicity than the cisplatin reference compound in HT-29 cells. Preliminary results from *in vivo* data agree with the ranking obtained *in vitro*.

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1. Introduction

Cyanide is formed as an endogenous molecule that participates in nerve regeneration *in vivo*.[1] Although it is metabolized by the rhodanase enzyme it is highly toxic at elevated concentrations due to it's function as a cytochrome c oxidase inhibitor. HCN also forms in incomplete combustion of nitrogen containing materials and cyanide poisoning has received increasing attention as a toxin in inhalation injuries from fire smoke in recent years.[2] A general review of currently available cyanide poisoning treatments shows a gap in available emergency treatments.[3] The need for a novel, easily handled, and rapidly administratable cyanide poisoning treatment calls for a highly efficient approach due to the acute toxicity of cyanide. Molybdenum's role in biology as the active metal center in xanthine oxidase as an oxo-transfer agent, and in sulfite oxidase where it catalyzes the transformation of sulfite to sulfate, is well documented.[4,5,6] Model studies of xanthine oxidase resulted in many interesting molecules that may have alternate function as catalysts.[7,8,9] Catalytic activity of many molybdenum sulfur compounds has been demonstrated for sulfur removal in hydrodesulfurization processes.[10]

The catalytic sites of molybdenum enzymes possess molybdenum oxo and sulfido moieties. Related compounds are potentially non-toxic and may have biomedical applications. Examples of nanomaterials under recent study include covalently bonded extended solids such as MoO_3, and MoS_2. Nanoplates of molybdenum trioxide, MoO_3 were shown to be non-toxic to HaCaT cells, and to exhibit cytotoxicity towards iMCF-7 cancer cells appearing promising for future cancer therapies.[11] Molybdenum disulfide, MoS_2 was explored as a photocatalyst and as an antibacterial agent.[12] The

reductive ability of MoS_2 was shown to cause increased formation of reactive oxygen species (ROS) in the mechanism of action related to its antibacterial activity. Its cytotoxicity was explored towards epithelial cells in the context of future applications in consumer products.[13]

Tetrathiomolybdate, MoS_4^{2-}, is an effective therapeutic agent aginst Wilson´s disease.[14] The compound is remarkably well tolerated by humans showing very low toxicity.[15] As the ammonium salt this coordination compound is highly soluble in water. It is air sensitive and breaks down *in vivo* to form molybdenum oxo, and sulfido compounds.[7] It was shown that although molybdenum supplements may cause copper deficiency in animals,[16] tetrathiomolybdate prevents this condition.[17]

The $Mo(IV)S_2$ metal chalcogenide is employed as dispersion due to its low aqueous solubility. It's biological activity stems from formation of different oxo/sulfido compounds *in vivo* where the MoS_2 is oxidized.[7] Conversely, $Mo(VI)O_3$ has high oxidation state and its reaction chemistry *in vivo* and in water is characterized by hydrolysis and reduction of the molybdenum.[18] Redox chemistry of $MoOSL_n$ mononuclear compounds is deactivated by dimerization to form Mo(V) dimers[18] such as presented here with a very robust "$Mo_2O_2S_2$" core structure resistant to redox chemistry at acidic and neutral pH.[19]

Organometallic anticancer drugs and development of catalytic drugs place cytotoxicity and physical properties such as water solubility of the potential drugs at the forefront of challenges to overcome.[20,21] The idea of catalysis *in vivo* is very attractive since it implies less quantity of a drug is required and that the drug is specific.[22] The reaction of thiosulfate and cyanide is one of

the current cyanide poisoning treatments where thiosulfate acts as a sulfur donor to the rhodanase enzyme *in vivo*.[23] Water soluble molybdenum sulfur complexes have potential application as catalysts for the reaction of thiosulfate and cyanide to form thiocyanate and sulfite *in vitro*.[2] Dimeric molybdenyl complexes with bridging sulfur atoms are air stable and the $[Mo_2O_2S_2]^{2+}$ (Figure 1) aqua ion is stable, even under acidic conditions.[19]

We synthesized novel complexes with the $[Mo_2O_2S_2]^{2+}$ core, and biocompatible ligands. Compounds that represent bidentate monoanionic, tridentate dianionic, organometallic, and neutral monodentate ligands were synthesized, and the known complexes (**1**-**4**) were included. The water solubility of the compounds was obtained colorimetrically, the cytotoxicty in three different cell lines was studied, and IC_{50} values were calculated based on cell viability data for the compounds in MCF-7 (breast cancer) cells, PT45 (pancreatic cancer) cells, and HT29 (colon cancer) cells. Preliminary toxicity of three of these compounds *in vivo* is discussed in context of the results obtained *in vitro*.

2. Experimental Section

2.1. Reagents. Ammonium molybdate, sodium molybdate, ammonium sulfide, sulfur, sodium sulfide nonahydrate, cysteine hydrochloride, DL-serine, DL-threonine, dicyclopentadiene, sodium lump in oil, and iodine were purchased from Aldrich or Acros and used as received. DMF, acetone, acetonitrile, ether, isopropanol and methanol were purchased from Acros and used as received, or purified using standard methods. Deionized water with

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conductivity of 18 $m\Omega^{-1}cm^{-1}$ or less was used without further treatment. The complexes, $[Mo_2O_2S_2(DMF)_6](I)_2$, [24] $(Et_4N)_2[Mo_2O_2S_8]$; $(Et_4N)_2$(**1**), [25] $(Et_4N)[CpMo_2O_2S_4]$; (Et_4N)(**2**), [24] $[Mo_2O_2S_4(DMF)_3]$; (**3**),[25] and $Na_2[Mo_2O_2S_2(C_3H_7NO_2S)_2].2H_2O$, Na_2(**4**),[26] were synthesized according to published procedures.

2.2. Physical Measurements: UV-Visible spectra and solubility measurements were recorded with Varian Cary 100 Bio spectrophotometer and Perkin Elmer Lambda 25 UV/Vis spectrophotometer at 22°C. Infrared spectra were recorded using a Smart Omni-Transmission Nicolet iS10 at 21°C. ^1H and ^{13}C NMR spectra were recorded using a Bruker 400 Ultrashield NMR spectrometer. Mass spectra were recorded using a Bruker micrOTOF (Bruker autoflex smartbeam) mass spectrometer. Cell counting of cell suspensions was performed with a Bio-rad TC10^{TM} Automated Cell Counter.

2.3. Syntheses.

$K[Mo_2O_2S_4(C_3H_6NO_3)]$, K(**5**) To an acetone (50 mL) solution of (**3**) (0.50 g, 0.87 mmol) was added a solution of ^tBuOK (97 mg, 0.86 mmol) and serine (91 mg, 0.89 mmol) in EtOH (20 mL). The red solution was stirred 30 minutes. The solvent was removed under reduced pressure and the residue treated with diethyl ether. An orange solid was collected by filtration and air-dried. Yield: 0.28 g, 65%.

IR(KBr, cm^{-1}): 3417(m), 3216(m), 3126(m), 2945(w), 2891(w), 2853(w), 1628 (vs), 1401(ms), 1344(m), 1105(m), 1023(m), 997(w), 935(s), 517(m), 464(m). UV/visible (H_2O): 282, 310(sh), 358(sh), 460(sh). ^1H NMR (D_2O): δ, ppm; 3.87(m, 1H), 3.99(m, 2H). ^{13}C NMR{^1H decoupled} (D_2O): δ =

56.35, 60.13, 172.28. MS (ESI): M ($KMo_2O_5S_4NC_3H_6$) 495.34 g/mol; *-m/z* found (calc.) = 459.7275(459.7242) $[M\text{-}K^+]^-$.

$[Mo_2O_2S_2(C_4H_8NO_3)_2]$, (**6**) To an acetone (100 mL) solution of $[Mo_2O_2S_2(DMF)_6](I)_2$ (0.50 g, 0.87 mmol) was added a solution of $Na_2CO_3 \cdot 10H_2O$ (0.24 g, 0.84 mmol) and threonine (0.21 g, 1.7 mmol) in water (20 mL). The red-orange solution was stirred 30 minutes. The solvent was removed under reduced pressure. The residue treated with methylene chloride and ether, collected by filtration, and recrystallized from DMF/ether. A red orange solid was collected. Yield: 0.32 g, 62%.

IR(KBr, cm^{-1}): 3408(m), 3222(m), 3101(m), 2973(w), 2929(w), 1652 (vs), 1366(ms), 1337(ms), 1136(m), 1105(m), 1001(w), 945(ms), 546(w), 465(w). UV/visible (H_2O): 281, 302(sh), 362(sh), 447(sh). ^1H NMR (D_2O): d, ppm; 1.62(m, 3H), 3.85(m, 1H), 4.41(m, 1H). ^{13}C NMR{^1H decoupled} (D_2O): δ = 19.20, 64.76, 72.77, 159.02. MS (ESI): M ($Mo_2O_8S_2N_2C_8H_{16}$) 524.26 g/mol; *-m/z* found (calc.) = 526.8383(526.8387) $[M\text{-}H^+]^-$.

2.4. Water Solubility. The solubility of the compounds was determined by preparing a saturated solution of the respective compound in water, centrifuging and pipetting 100 μL of the solution and diluting to 5 or 10 mL and measuring the absorbance of the compound at its charge transfer peak maximum wavelength, taking care the absorbance is less than 1.0. The previously determined molar absorptivity constant, ε, was used to calculate the solution molar concentration M. The molecular weight was used to convert to g/L. The results are shown in table 2.

2.5. Cell Division. Cell lines used were; MCF-7 (breast cancer), PT45 (pancreatic cancer), HT29 (colon cancer). The MTT assay was used to

measure cell viability.[27] The medium used was DMEM (1x) (Dulbecco's Modified Eagle Medium with [+] 4.5 g/L D-Glucose, [+] L-Glutamine, [-] Pyruvate in 10% Fetal Calf Serum).

Three cell culture dishes (Thermo nuclon and Sarstedt) were incubated in a humidified system (37°C, 10% CO_2); each containing one cell line along with medium. The medium was removed and the cells washed with a DPBS (1x) salt solution (Dulbecco's Phosphate Buffered Saline). TE solution (Trypsin/EDTA, 2 mL) was added to each cell culture dish and the dishes incubated in a humidified system (37°C, 10% CO_2) until the cells were loose from the surface. DMEM (8 mL) was added to each plate and mixed well with the cells. The resulting cell suspensions were transferred to 50 mL reaction tubes. The samples were centrifuged (2 min, 300G) forming cell sediment in each tube. The supernatant was removed from the reaction tubes and fresh medium (10 mL) was subsequently added to each tube, and the cells re-suspended and thoroughly mixed. The cell density was obtained as cells/mL. After preparing all dilutions accordingly, four consecutive columns per compound were plated with 6000 cells/100 μL per well in a 96-well plate.

2.6. Treatment of Cells and Cell Viability Determination. Seven compounds were tested for toxicity on the three different cell lines. Stock solutions of each compound (10 mM) were prepared in either water or DMSO. The stock solutions were further diluted to working solutions with eight different concentrations for each compound. Cell viability was obtained for each compound after 24h, 48h and 72h. The 96-well plates were removed from the incubator and 50 μL of MTT solution (2.5 mg/mL MTT in DPBS (1x), sterile

filtered) were added to each well. The plates were then further incubated for 2h during which the metabolized MTT formed purple formazan crystals proportional to cell viability per well. The supernatant was removed promptly and the crystals dissolved in 200 μL of DMSO. The absorption of each well was measured at 550 nm (background at 620 nm) and used to calculate IC_{50} values of each compound. The IC_{50} values were only determined for the 72h time intervals, because decrease in cell viability was not observed until after 72h. The data was worked up and IC_{50} values determined using GraphPad Prism. The results are presented in Table 1.

2.7. Safe Dose Study in vivo. Male mice (C57bl/6, 8 week old, Taconic) were housed for one week prior to conducting the study. Compounds $(Et_4N)_2$(**1**), (**3**) and Na_2(**4**) were dissolved in Dulbecco's phosphate buffered saline at a dose concentrations of 0.5 mg/mL, 1.0 mg/mL, and 3.5 mg/mL respectively. Each compound was administered to three animals in 7.5 μL/Kg dose. Each animal was given four doses at one-hour intervals. Their well-being and weight was monitored for six days after. The data is presented in Table 3.

3. Results and Discussion

3.1. Synthesis, Characterization and Water Solubility. The molybdenum sulfur complexes, **1- 6**, are based on the molybdenum core structure shown in Figure 1. The core structure is a dianion that is capable of coordinating two to three ligands to each molybdenum center. The compounds were selected to represent bidentate monoanionic, tridentate dianionic, organometallic, and neutral monodentate ligands. Synthesis of these compounds was performed. The novel compounds K(**5)** and (**6)** were synthesized with amino acid ligands with the goal to increase the compound biocompatibility and to reduce

toxicity of the compounds *in vivo* as well as increase aqueous solubility as is desirable for catalytic drug development. Known compounds were included as they are well suited to demonstrate solubility and toxicity of alkali metal salts and for the purpose of analyzing properties for further studies.

The synthesis of the known compounds (**1**-**4**) was performed according to published procedures.[24,25,26] The novel compound K(**5**) was synthesized from (**3**) by displacement of the DMF ligands with deprotonated serine in acetone/ethanol mixture, and isolation of the compound as a red orange solid. Compound (**6**) was synthesized starting with the $[Mo_2O_2S_2(DMF)_6](I)_2$ complex and displacing all six DMF ligands with deprotonated threonine in acetone/water solvent mixture. The progress of the reactions was monitored using electronic spectroscopy. The reaction took place in aqueous solution according to the electronic spectroscopy, but eventually the synthesis was performed in organic solvents because initial efforts employing aqueous synthesis led to low isolated yields when precipitating out of water due to aqueous solubility of complexes K(**5**) and (**6**). Switching to organic solvents achieved 65% and 62% yields of the isolated K(**5**) and (**6**). The compounds are orange colored solids, and air stable. They were characterized using IR (infrared), NMR and mass spectroscopy. Infrared spectra of $(Et_4N)_2$(**1**), K(**5**), and (**6**) as KBr pellets are provided in the supplementary material, as well as UV-visible spectra of (**3**) and K(**5**) in water. The structures of the six compounds are shown in Figure 2.

The compounds (**1**-**6**) all have the common core $[Mo_2O_2S_2]^{2+}$, but contain different ligands. They exhibit several characteristic features in the infrared spectra. The molybdenyl stretch is observed as a single stretch in the

symmetric compounds Na_2(**4**) and (**6**) at 945 cm^{-1}. This stretching frequency is similar as is observed for the molybdenyl in the $Na_2[Mo_2O_4(cys)_2]$ compound [28] The carboxylate in Na_2(**4**) is observed at unusually low wavenumber of 1590 cm^{-1} normally assigned to ionic non-coordinated cysteine.[29] The cysteine coordination is tridentate, and the carboxylate is coordinated to the Mo-O(carboxylate) through an unusually long Mo-O bond, or 230 pm compared to 217 pm in the oxalato complex, $Na[Mo_2O_4(C_2O_4)_2].2H_2O$ where the carboxylate oxygen is also *trans* to the molybdenyl moiety.[30,31] The coordination of the cysteine carboxylate to Mo is therefore weaker than in the oxalato compound and slightly sterically hindered. Threonine, as a monoanionic bidentate ligand in (**6**) shows this carboxylate stretch at higher wavenumber of 1626 cm^{-1} that is comparable to carboxylate stretches observed for molybdenum bound oxalates.[32] In the asymmetric compounds (**1**, **2**, **3**, and **5**), two molybdenyl stretches are observed with the second band at 935 cm^{-1}. The spectroscopy of the known compounds has been discussed in detail along with their crystal structures elsewhere.[24] The bridging sulfur is observed in all of the complexes (**1**-**6**) at 474 cm^{-1}. The terminal disulfide is characteristically observed in range of 517 cm^{-1} to 521 cm^{-1}.

The electronic spectra exhibit an intense charge transfer band that peaks at around 300 nm (**1**-**6**). For the compounds with disulfide stretch, an additional weak transition is observed at 460 nm (**1**, **2**, **3**, **5**). The mass spectra were obtained using electron ionization that provides the molecular ion minus a proton for the neutral complexes (**3**) and (**6**) while the molecular anion was obtained for the monoanions Et_4N(**2**) and K(**5**). The dianions $(Et_4N)_2$(**1**) and

Na_2(**4**) retain one cation and are observed as the complex anion plus one cation to form an overall monoanion.

The compounds used for the study were selected based on three parameters: water solubility, estimated toxicity as high or low, and finally structure. Table 2 shows the water solubility for the least soluble compound, $(Et_4N)_2$(**1**), for (**3**), and for the most soluble compound Na_2(**4**). The structures present variations in the choice of a cation and in ligand selection. For example, compound K(**5**) is highly soluble in water as the potassium salt, but is less soluble in water as the tetraethylammonium salt.[3]

Tetraalkylammonium salts are considerably less soluble in water than the neutral compounds, while $(Et_4N)_2$(**1**) only has water solubility of 1 g/L, and Na_2(**4**) is very soluble with 136 g/L compared to NaCl solubility of 359 g/L in water.[33] Solubility was observed as qualitatively larger for the alkali metal salts than for the neutral compounds. The monopotassium salt K(**5**) with serine ligand is less water soluble than the neutral *bis*-threonine complex (**6**), contrary to expectation. This lack of solubility may be explained by interaction of the hydroxylgroup of serine with neighboring molybdenum atoms. This hydroxylgroup is quite accessible since it does not serve as a donor group but it may interact with molybdenum in a second molecule and form extended structures. Such behavior is known for coordinated amino acids, where the amine group and C=O of the carboxylate of methionine from polymeric structures with first row transition metal ions with unsaturated coordination geometry.[34]

[3] Infrared spectra of Et_4N(**5**) is identical to K(**5**) except for vibrations originating from the Et_4N^+ cation.

Compounds (**3**) and (**6**) are both neutral but with very different ligand set. Interestingly, the polar DMF ligands in (**3**) are not displaced by water in aqueous solution, and the compound is quite stable at neutral pH. The combination of DMF and a disulfide ligand in (**3**) shows less solubility in water than (**6**) with two threonine ligands. Accordingly, K(**5**) is less soluble than compounds Na_2(**4**) and (**6**), but more soluble than (**3**). The compounds were ranked according to aqueous solubility as Na_2(**4**) > (**6**) > K(**5**) > (**3**) > (Et_4N)(**2**) > $(Et_4N)_2$(**1**). These results are promising for continuing studies of how to increase solubility of the compounds further providing a foundation for future studies of the effects of a single anion with various cations.

3.2. Cytotoxicity Studies. Ranking of compounds according to relative toxicity using *in vitro* approach has great value for guiding synthetic efforts for novel compounds with limited reported literature data. Cisplatin was used as a reference compound and, as is known, it is fairly toxic. Cancer cells were deemed suitable since they are already sensitized and therefore expected to show maximum sensitivity allowing us to rank the compounds according to their relative toxicity. The calculated IC_{50} values are shown in Table 1.

The cytotoxicity assays were performed for three different cell lines. Cell viability was measured at 72h with the MTT method. MTT was shown recently to give false positive values for cytotoxicity values in molybdenum, and tungsten dichalcogenides, MoS_2, WS_2, and WSe_2 due to reduction of MTT in the presence of the dichalcogenide.[13] The dichalcogenides exhibit very low cytotoxicity in A549 normal epithelial cells.[12] MTT background for the purpose of ranking our compounds according to relative cytotoxicity was not collected.

IC_{50} values were calculated using nonlinear regression and plotting the data as log(inhibitor) vs. response (three parameters). These values were used to rank the compounds according to relevant toxicity. As can be seen in Table 1, compounds (**1** - **6**) are not appreciably toxic compared to cisplatin. The data analysis method employs "% cell viability" as a function of log([c]) and prefers cell viability measurements close to the actual IC_{50} value to calculate accurate values. Since none of the compounds (**1** – **6**) showed less than 50% cell viability in the cell lines after 72h, it was necessary to employ an endpoint at zero viability and at 10 M concentration to sufficiently extrapolate the curve to correctly predict the IC_{50} values. Figure 3 shows the calculated IC_{50} values for the three cell lines together. The supplementary material has additionally Figures SI-3 - 5 that show graphically the calculated IC_{50} values for the different cell lines.

Analysis of data obtained for (**6**) shows that without employing an endpoint of zero viability at 10 M concentration, the data is greatly misinterpreted. Data analysis of the cisplatin reference compound, where data is available well past the 50% cell viability point, shows that by using the zero viability at 10 M end-point, the IC_{50} values calculated with this endpoint provide reasonable agreement.

Tetraethylammonium salts were expected to show increased toxicity compared to alkali metal salts. Results and ranking of the compounds agrees with that expectation. Complexes $(Et_4N)_2$(**1**) and Et_4N(**2**) are tetraethylammonium salts and clearly show higher cytotoxicity compared to the other complexes. Complexes Na_2(**4**) and K(**5**) show least cytotoxicity comparatively, and complexes (**3**) and (**6**) are in between. Complex (**3**) has

three DMF ligands that are known to increase toxicity. The expected order of toxicity was $(Et_4N)_2$(**1**) >Et_4N(**2**) > (**3**) > K(**5**) > Na_2(**4**) = (**6**). The *bis*-cysteine complex Na_2(**4**) showed the least toxicity at a level that caused difficulty to determine an experimental end-point for the MCF-7 cell line and it was not possible for the HT29 cell line. The three cell lines showed preference towards different compounds and the ranking is therefore not identical for the three cell lines. Figure 4 shows the ranking of the compounds for PT45 cell line since it is representative for the compounds. Surprisingly, (**6**) exhibits more toxicity than expected and is comparable to compounds (**3**) and (**5**). The actual ranking is therefore: $(Et_4N)_2$(**1**) >Et_4N(**2**) > (**3**) = K(**5**) = (**6**) > Na_2(**4**). Preliminary studies using dose escalation method *in vivo* agree with the ranking observed for compounds (**1**), (**3**), and (**4**).

3.3. In vivo Toxicity. Table 3 shows results from a preliminary safe dose study *in vivo*. $(Et_4N)_2$(**1**), (**3**), and Na_2(**4**) were administered four times at one hour interval and the well-being of the animal monitored. None of the animals showed any sign of discomfort during the study. Their weight was monitored for a week afterwards and the maximum weight changes observed are reported with the dose information in table 3. The least soluble and the most soluble compounds were used for this study. They also represent the observed range of cytotoxicity and give an idea of expected future safe dose concentrations.

Low solubility of $(Et_4N)_2$(**1**) prevented administration of sufficiently large dose to reach toxic levels. Compound (**3**) did show progressive weight loss for four days but the animals regained their initial weight after one week and did not show any discomfort. The solubility of compound (**3**) also prevented

administration of sufficiently large dose to induce toxicity at a dose of 7.5 mg/Kg. Compound Na_2(**4**) is highly soluble and we were not able to induce toxic effects *in vivo* in this study. The animals did not show discomfort after four doses and showed very little weight fluctuations in the course of one week.

Each dose of 26.3 mg/Kg administered of Na_2(**4**) is much larger than an estimated dose of 30 mg/70 Kg person for an emergency treatment.[4] This compound appears safe at much larger concentrations than is therapeutically expected and may have a large therapeutic index, currently a consideration for future studies. In comparison, tetrathiomolybdate also has a high therapeutic index where the effective dose of the compound is 25 mg/day for a 70 Kg person with a safe dose reported up to 1400 mg/day.[35] Na_2(**4**) safe dose appears well above potentially effective dose in our initial studies although more detailed studies are necessary to conclude any therapeutic indices for the compounds.

4. Conclusion.

The cytotoxicity of the six compounds in three cancer cell lines was employed to analyze structure-activity correlation of the effects of counter cation and ligands on cytotoxicity and aqueous solubility of the compounds. Our findings show the counter cation plays an important role in both solubility and cytotoxicity. The compounds were expected to be much less cytotoxic than cisplatin and promising for therapeutic applications requiring non-toxic compounds. The calculated IC_{50} values of compounds **1**-**6** are much higher than that of cisplatin as expected for non-toxic compounds. Our

[4] Calculated based on unpublished data on catalytic activity of the compounds.

effort to increase water solubility and reduce cytotoxicity in parallel was successful. We showed the solubility improves both as a function of the counter cation selection as well as the ligands. Structure-activity correlation results are in good agreement with expected order of compound activity based on structure and salt selection. The core "$Mo_2O_2S_2$" exhibits reduced cytotoxicity when combined with biocompatible ligands coordinated to the molybdenum atoms. Exchanging organic cations for alkali metal ions, and organosoluble ligands for amino acids both greatly reduce cytotoxicity and increase water solubility. The results for compounds **4** – **6** are encouraging for continuing studies of similar compounds, including further biological studies of these compounds.

Associated Content

Supporting information. Graphs of data analysis for cisplatin and compound **6**. Graphs of results for calculated IC_{50} values for each cell line. Infrared spectra of (**1**), (**5**) and (**6**). UV-visible spectra of (**3**) and K(**5**). This material is available free of charge.

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Abbreviations

DMF – dimethylformamide; Cp = cyclopentadienyl; HaCaT cells - aneuploid immortal keratinocyte cell line from adult human skin; iMCF-7 – invasive breast cancer cells; MCF-7 – breast cancer cells; PT45 – pancreatic cancer cells; HT29 – colon cancer cells.

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Table 1. The table shows IC_{50} values (μM) obtained for complexes **1**-**6**, and for cisplatin in three different cell lines after treating the cells for 72h and assayed with the MTT method.

	Cell Line, IC_{50} values		
Compound no.	**MCF-7**	**PT45**	**HT29**
1	106 ± 32	34 ± 7	50 ± 3
2	214 ± 35	64 ± 7	780 ± 259
3	383 ± 45	118 ± 17	344 ± 10
4	868 ± 319	189 ± 34	-*
5	882 ± 350	117 ± 15	635 ± 98
6	301 ± 126	95 ± 5	429 ± 219
cisplatin	19.2 ± 1.93	5.73 ± 1.03	10.6 ± 2.28

* Values for each measurement in the table are averages for four repeats.
**It was not possible to determine an end point for this compound in this cell line.

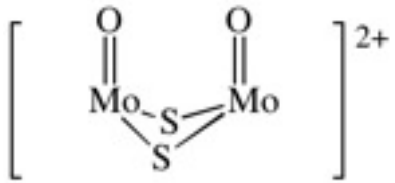
Table 2. The table shows obtained water solubility in g/L for selected compounds.

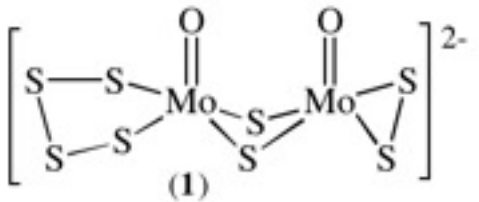
Compound	Water Solubility, g/L
$(Et_4N)_2$(**1**)	1.0
(**3**)	3.5
Na_2(**4**)	136.0

Table 3. The table shows the dose administered per compound, number of administrations and the maximum weight fluctuations recorded.

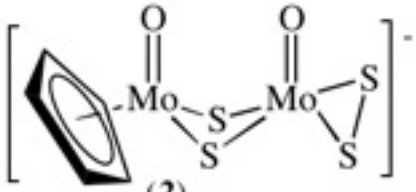
Compound	Dose, mg/Kg	Number of doses	WL, %*						
			day 1	day 2	day 3	day 4	day 5	day 6	day 7
(Et4N)2(1)	3.8	4	0	2	3	1	-8	2	0
[Mo2O2S4(DMF)3]	7.5	4	-7	-10	-12	-16	-12	-9	-4
Na2(4)	26.3	4	1	1	3	0	-1	-2	-3

* maximum weight loss reported compared to weight on day of treatment

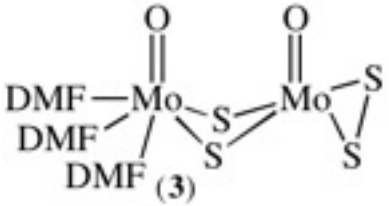

O
O
Mo
S
Mo
S
2+



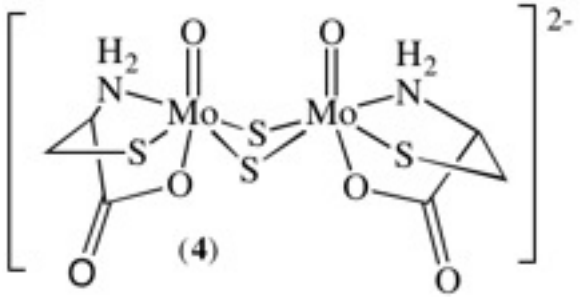
(1)



(2)



(3)



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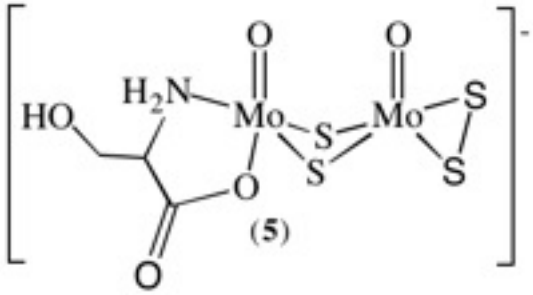

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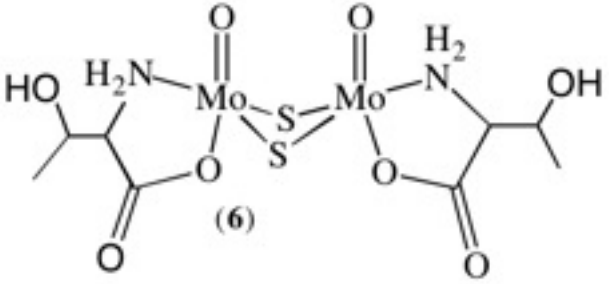
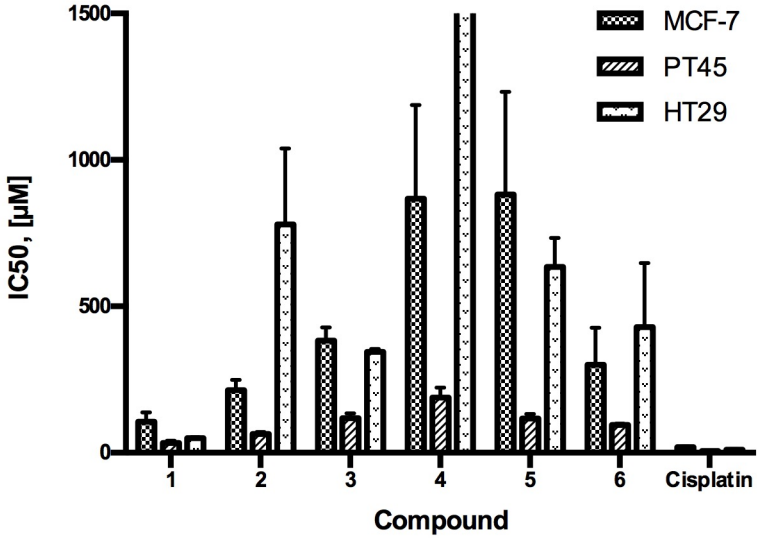
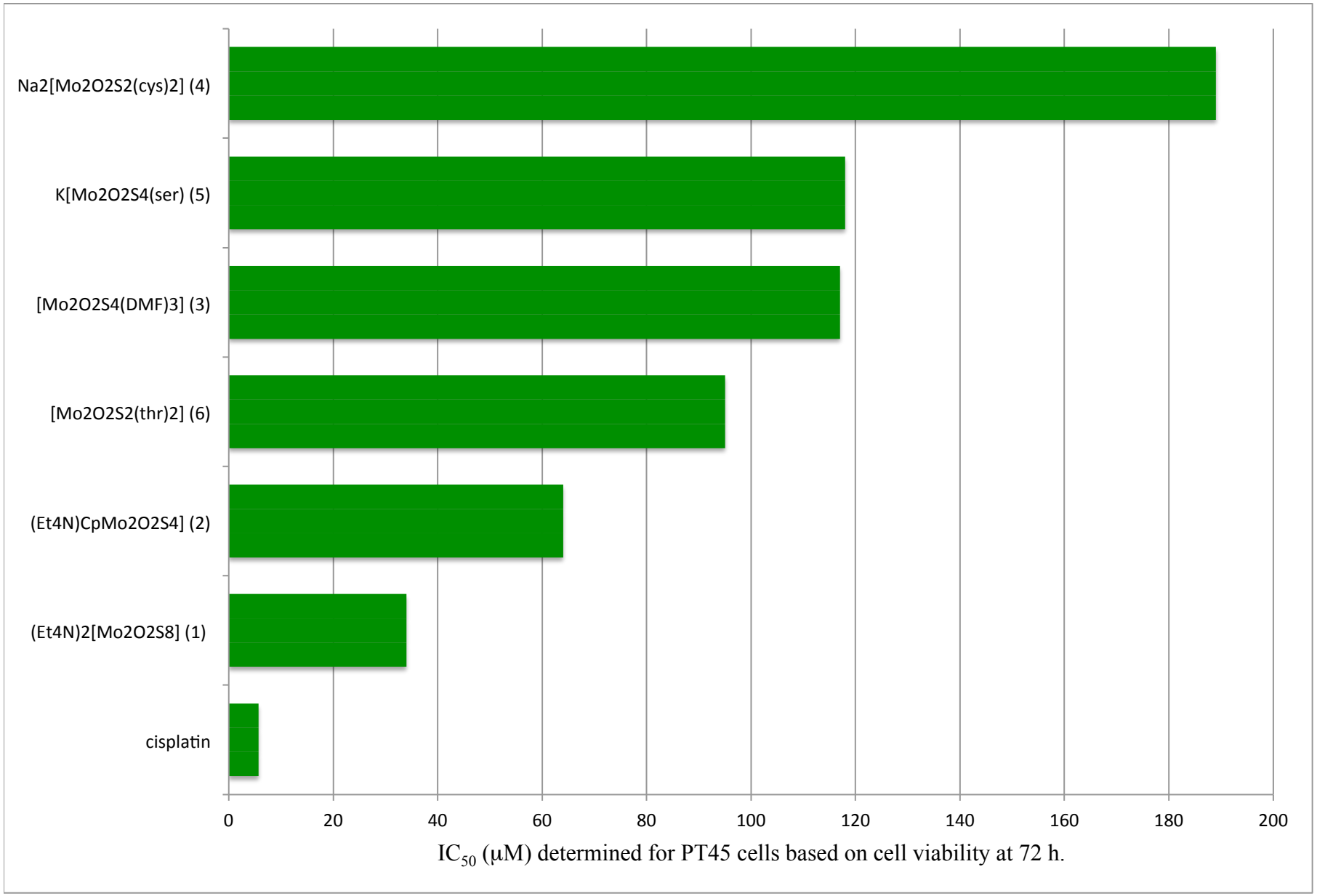


(6)


1500
1000
500
0
IC50, [µM]
MCF-7
PT45
HT29
1
2
3
4
5
6
Cisplatin
Compound


Na2[Mo2O2S2(cys)2] (4)
K[Mo2O2S4(ser) (5)
[Mo2O2S4(DMF)3] (3)
[Mo2O2S2(thr)2] (6)
(Et4N)CpMo2O2S4] (2)
(Et4N)2[Mo2O2S8] (1)
cisplatin
0
20
40
60
80
100
120
140
160
180
200
IC50 (μM) determined for PT45 cells based on cell viability at 72 h.

Supplementary Information to the article

Cytotoxicity Studies of Water Soluble Coordination Compounds with a $[Mo_2O_2S_2]^{2+}$ Core.

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Content:

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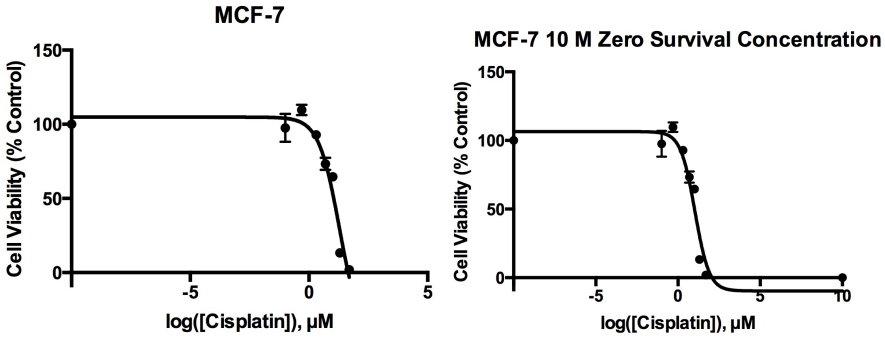
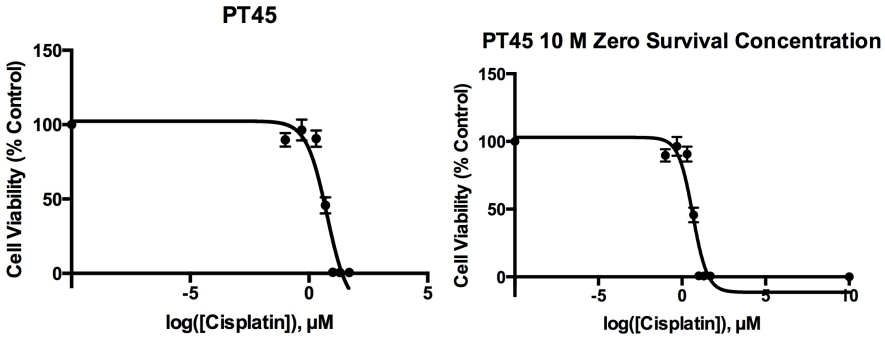
Figure SI 1. Cisplatin in the three cell lines, data analysis using actual vs. using zero cell viability at 10 M concentration.

MCF-7



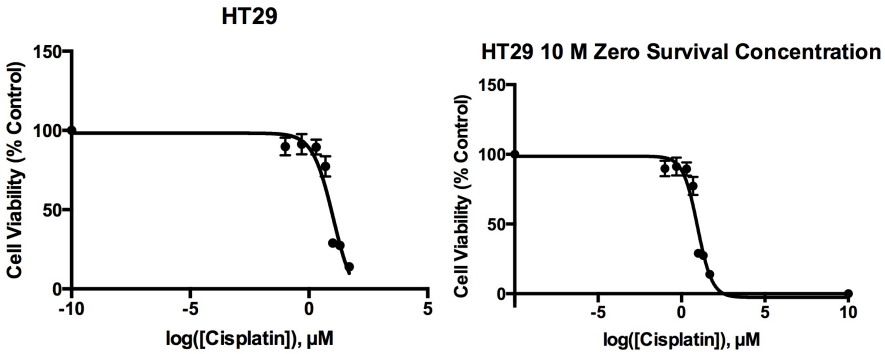
MCF-7 (IC50 = 19.2 ± 1.93)
MCF-7 10 M zero cell viability concentration (IC50 = 10.7 ± 0.57)

PT45



PT45 (IC50 = 5.73 ± 1.03)
PT45 10 M zero cell viability concentration (IC50 = 4.42 ± 0.66)

HT29



HT29 (IC50 = 10.6 ± 2.28)
HT29 10 M zero cell viability concentration (IC50 = 9.18 ± 1.71)

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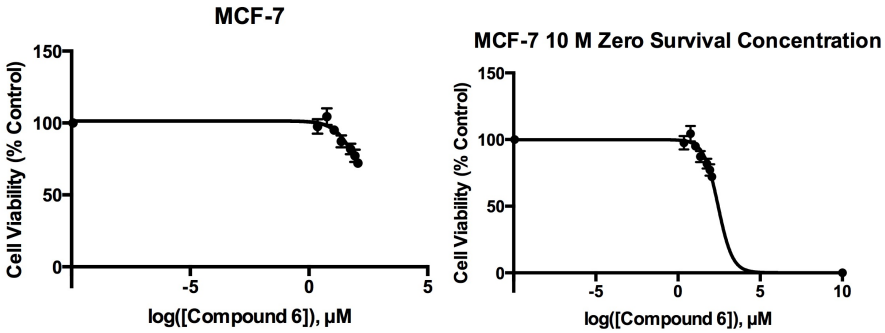
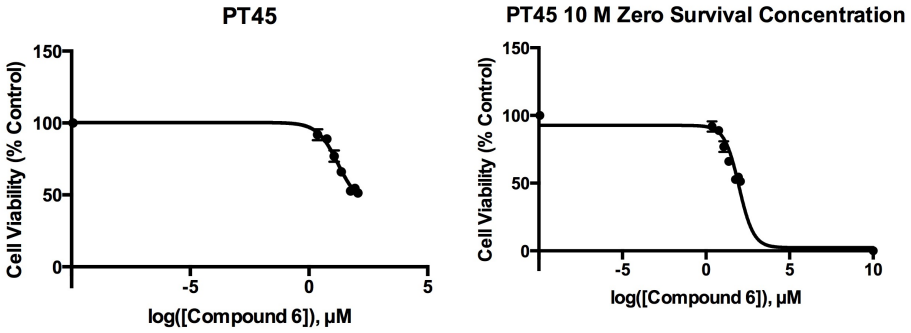
Figure SI 2. Compound 6 in the three cell lines, data analysis using actual vs. using zero cell viability at 10 M concentration.

MCF-7



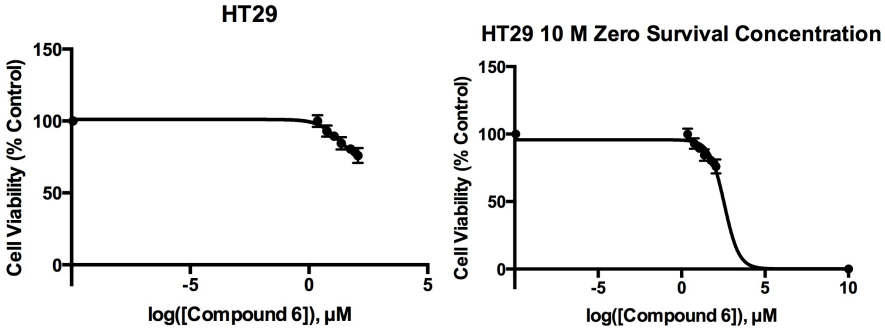
MCF-7 (IC_{50} = 80 ± 67)
MCF-7 10 M zero cell viability concentration (IC_{50} = 301 ± 126)

PT45



PT45 (IC_{50} = 17 ± 4)
PT45 10 M zero cell viability concentration (IC_{50} = 95 ± 5)

HT29



HT29 (IC_{50} = 23 ± 12)
HT29 10 M zero cell viability concentration (IC_{50} = 429 ± 219)

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Figure SI_3. The figure shows the calculated IC_{50} values for the compounds **1** - **6** and for cisplatin in the MCF-7 cell line.

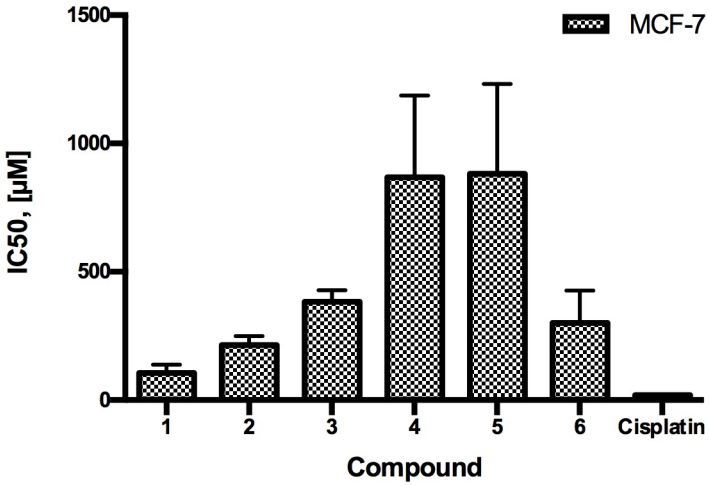

Figure SI_4. The figure shows the calculated IC_{50} values for the compounds **1**- **6** and for cisplatin in the PT45 cell line.

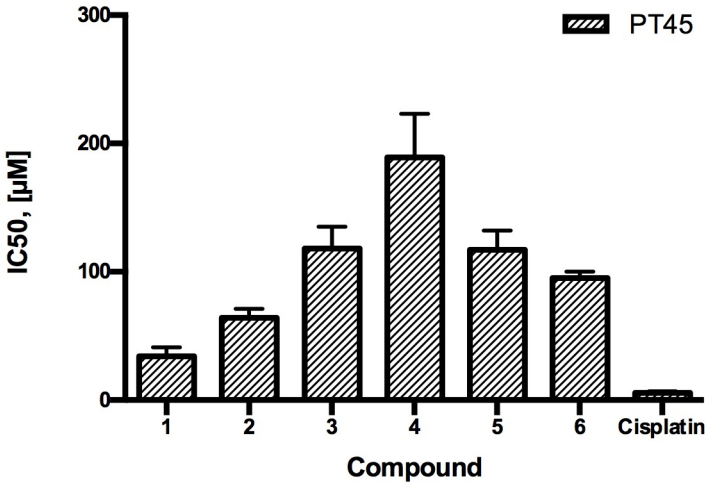

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Figure SI_5. The figure shows the calculated IC_{50} values for the compounds **1** -**6** and for cisplatin in the HT29 cell line. It was not possible to determine the end-point for compound 4 due to high % cell viability at 72 hours.

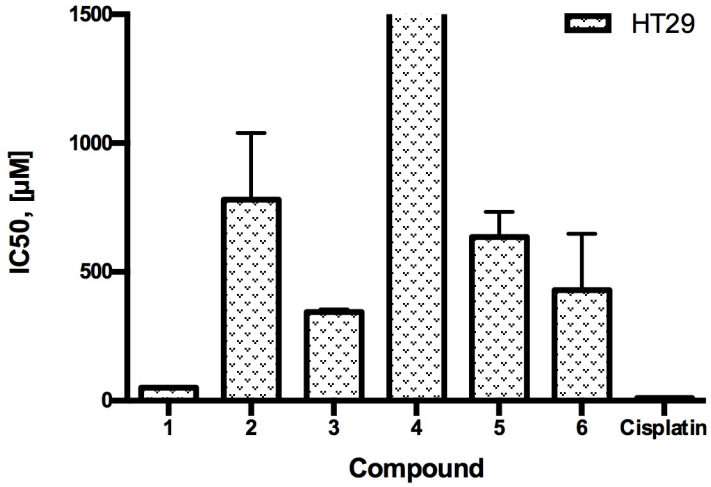

Figure SI_6. Infrared spectrum of $(Et_4N)_2$(**1**)

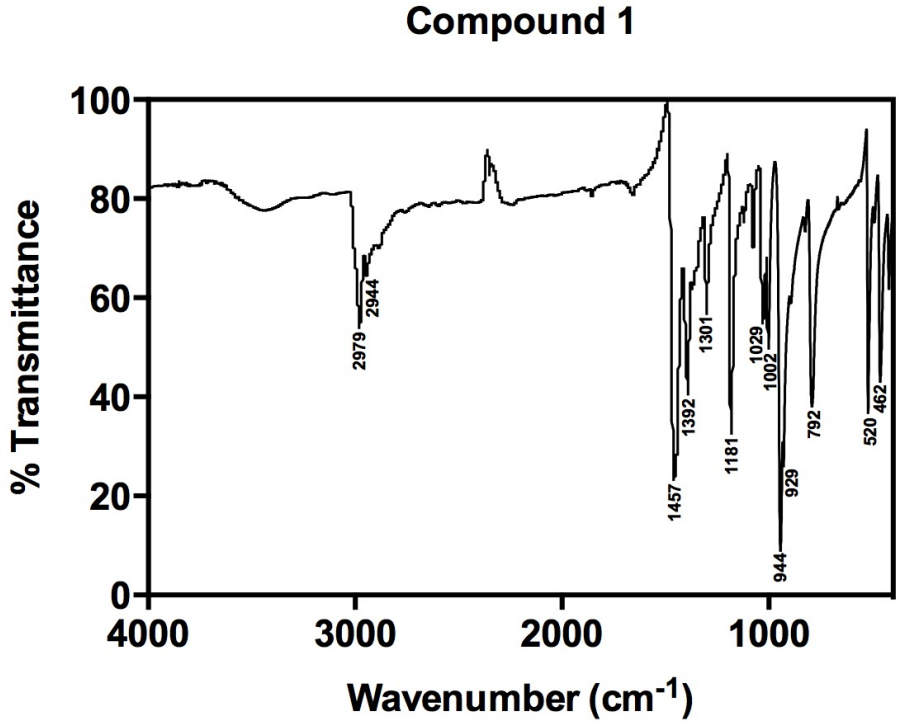

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Figure SI_7. Infrared spectrum of K(**5**)

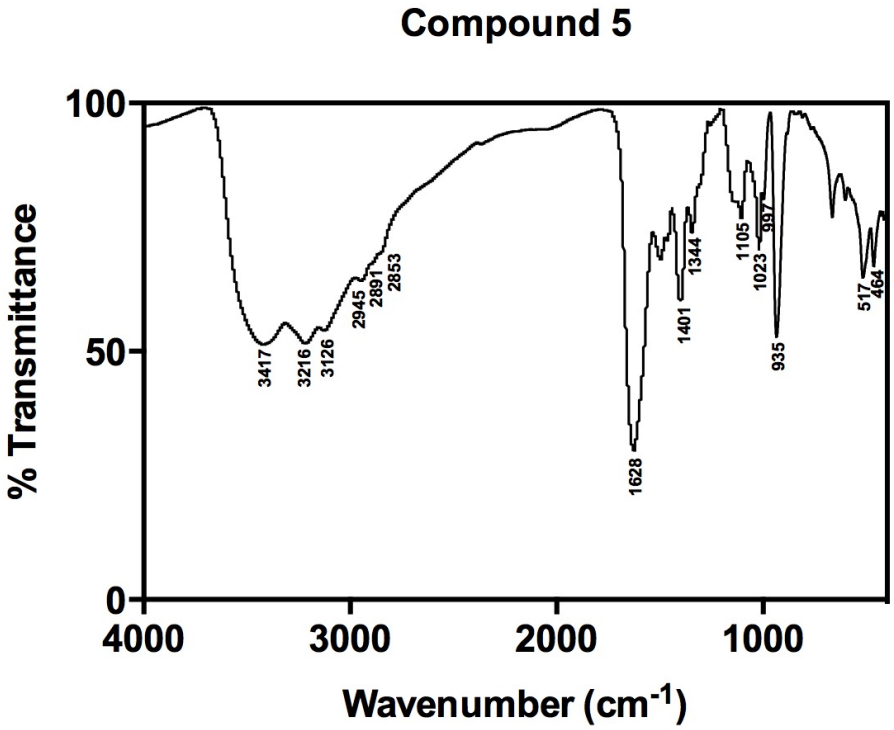

Figure SI_8. Infrared spectrum of (**6**)

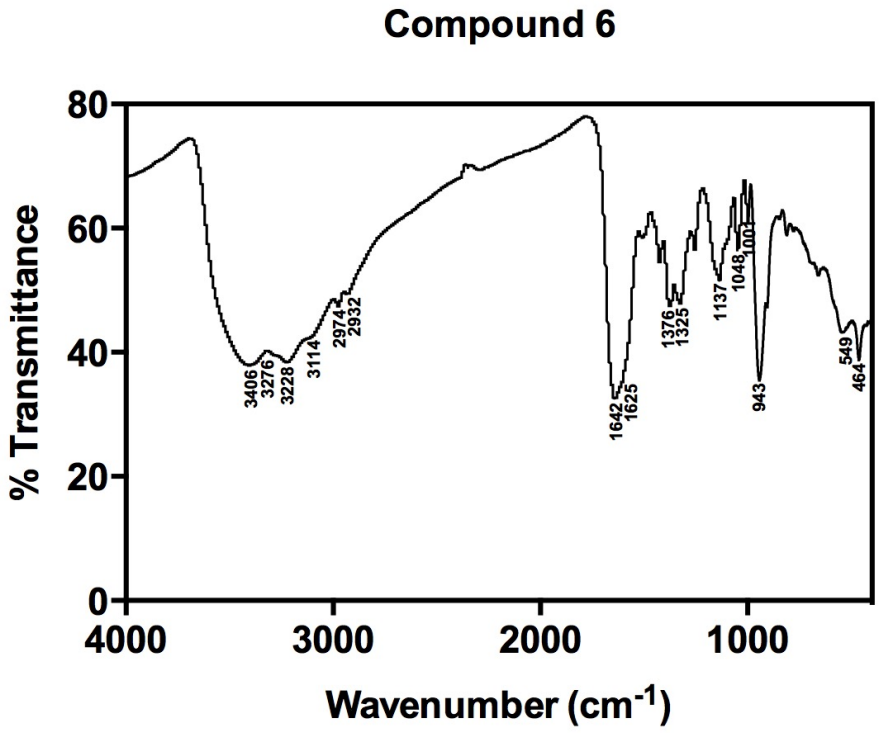

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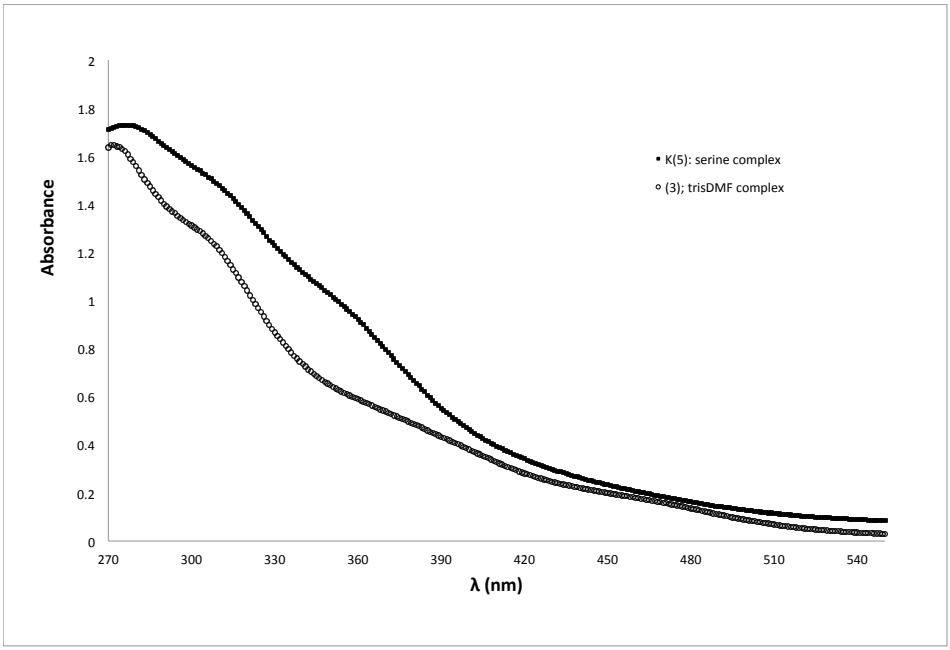
Figure SI_9. UV visible spectra of (**3**) (circles), and K(**5**) (diamonds).



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