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„Novel tungstenocene complexes bearing bioactive chelates and their anticancer activity"

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To my mother, Ani Banc, for her love and support

To Ass.-Prof. Dr. Peter Unfried, the professor who taught me the beauty of chemistry

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ABBREVIATIONS

°C	Celsius degree
Å	Angstrom
A	Ampere
d	doublet (NMR)
DCM	dichloromethane
d_6-DMSO	deuterated dimethyl sulfoxide
E	potential
$E_{1/2}$	redox potential
EtOH	ethanol
ESI-MS	electrospray ionization mass spectrometry
g	gram
h	hour
Hz	Hertz
IC_{50}	half maximal inhibitory concentration
J	coupling constant (NMR)
m	multiplet (NMR)
M	molar
m/z	mass-to-charge ratio
Me	methyl
MeCN	acetonitrile
MeOH	methanol
mg	milligram
mL	milliliter
mM	millimolar
mmol	millimole
mol	mole
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NHE	normal hydrogen electrode
NMR	nuclear magnetic resonance
s	singlet (NMR)
t	triplet (NMR)
UV	ultraviolet

V	volt
Vis	visible
Z	atomic number
δ	chemical shift
v	wavenumber

ABSTRACT

Cancer is one of the deadliest diseases worldwide, occupying second place after the cardiovascular diseases. Therefore, the search for medicines to cure cancer has become a priority in today`s society.

In the 1960s, Barnett Rosenberg discoverd the application of cisplatin as a chemotherapeutic. Nowadays, cisplatin, carboplatin and oxaliplatin are worldwide approved metal-based anticancer drugs. Unfortunately, these compounds display a spectrum of severe side effects (e.g. nephrotoxicity, nausea, and neurotoxicity), intrinsic resistance to some tumors and also acquired resistance during consecutive therapy.

In order to circumvent all of these drawbacks, there have been developed and investigated new anticancer thearapeutics based on metals other than platinum and with different modes of action. Two ruthenium complexes (IT 139 and NAMI-A) and one gallium complex (KP 46) are in clinical trials, showing promising results.

In 1979 Köpf and Köpf-Maier tested a series of metallocenes Cp_2MX_2 (M= Ti, V, Nb, Mo; X = halides and pseudo-halides) on different tumor cells, which showed that they had antitumor activity against them.

Tungsten is the only third row transition metal required by living organisms. It presents an unique chemical versatility and high bioavailability, this metal can be found in different enzymes (e.g. oxidoreductases). On the other hand it shows similarities with other transition metal, molybdenum, another metal of which compounds where investigated as possible antitumor drugs. Surprinsingly, the chemistry of tungstenocene is largely unexplored, the last report in this field being from 2013.

The aim of my master thesis was to obtain new tungstenocene compounds by substituing both chlorido groups in the bis(cyclopentadienyl)tungsten dichloride complex with different bioactive O,O- and O,S- chelating ligands (maltol, allomaltol, ethylmaltol, thiomaltol, thioallomaltol, thioethylmaltol and dithiomaltol) and test their stability in aqueous solution and possible impact on cancer activity. The ligands and the complexes were studied by ^{1}H and ^{13}C-NMR, elemental analysis, mass spectrometry (ESI-MS), UV-VIS spectroscopy, FT-IR spectroscopy and cyclic voltammetry.

ZUSAMMENFASSUNG

Krebs ist eine der tödlichsten Krankheiten weltweit, auf dem zweiten Platz nach den Herz-Kreislauferkrankungen. Aus diesem Grund wurde eine Suche nach Lösungen gestartet um ihn zu besiegen.

1960 entdeckte Barnett Rosenberg die Anwendung von Cisplatin als Chemotherapeutikum. Heutzutage sind Cisplatin, Carboplatin und Oxaliplatin weltweit zugelassene Metall basierte Chemotherapeutika. Leider zeigen diese Verbindungen ein breites Spektrum an gravierenden Nebenwirkungen (zum Beispiel: Nephrotoxizität, Übelkeit, Neurotoxizität), zudem gibt es intrinsische oder während der Therapie erworbene Resistenzen durch manche Tumore.

Um alle diese Nachteile zu reduzieren wurden neue Chemotherapeutika mit anderen Metallzentren und verschiedenen Wirkungsmechanismen entwickelt und untersucht. Zwei Ruthenium-Verbindungen (IT-139 und NAMI-A) und eine Gallium Verbindung sind in klinischen Studien und zeigen vielversprechende Resultate.

1979 haben Köpf und Köpf-Maier die Wirkung einer Serie von Metallocenen Cp_2MX_2 (M= Ti, V, Nb, Mo; X = Halid, pseudo-Halid) an verschiedenen Tumorzelllinien getestet, was gezeigt hat, dass diese Verbindungen eine vielversprechende Antitumor-Aktivität haben.

Wolfram ist das einzige Übergangsmetall aus der dritten Reihe, das von lebenden Organismen benötigt wird. Es besitzt eine einzigartige chemische Vielseitigkeit und eine hohe Bioverfügbarkeit, dieses Metall kann in verschiedenen Enzymen gefunden werden (zum Beispiel: in Oxidoreduktasen). Außerdem, zeigt es Ähnlichkeiten mit einem anderen Übergangsmetall, Molybdän, von dem Verbindungen als mögliche Chemotherapeutika untersucht wurden. Überraschenderweise, ist die Chemie der Tungstenocenen wenig untersucht worden, der letzte Bericht aus diesem Bereich stammt aus 2013.

Das Ziel meiner Masterarbeit war es neue Wolframocene, durch Substitution der zwei Chlorido-Liganden im Bis(cyclopentadienyl)wolframdichlorid-Komplex durch verschiedene bioaktive O,O- und O,S,-Chelatliganden (Maltol, Thiomaltol, Allomaltol, Thioallomaltol, Ethylmaltol, Thioethylmaltol und Dithiomaltol), zu synthetisieren und auch deren Stabilität in wässriger Lösung, Interaktionen mit Biomolekülen und mögliche Zytotoxizität zu untersuchen. Die Liganden und Komplexe wurden durch verschiedenen Methoden untersucht, wie ^{1}H- und ^{13}C-NMR, Elementaranalyse, Mas-

senspektrometrie (ESI-MS), UV-VIS Spektroskopie, FT-IR Spektroskopie und Cyclovoltammetrie.

1. INTRODUCTION

1.1. Cancer – facts and statistics

Cancer is one of the major health problems worldwide, being the second leading cause of death, after heart diseases. According to WHO (World Health Organization) information, in 2015, cancer was responsable for 8.8 milion deaths globally (which means that 1 in 6 deaths were caused by cancer).[1]

In Austria, in 2016, cancer occupied second place in the causes of death with 25% (1 in 4 deaths was caused by cancer), after the cardiovascular diseases with 41%. Other death causes were respiratory diseases, diseases of the digestive system, injuries, poisonings, amongst others.[2]

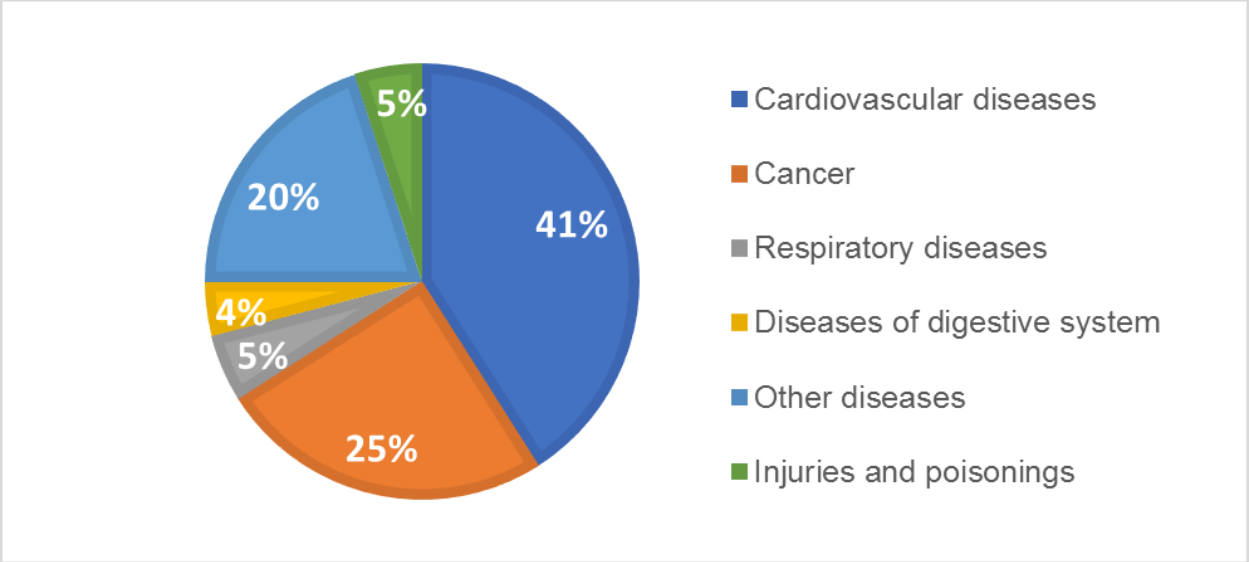


Fig. 1: Causes of death in Austria in 2016 (according to Statistik Austria) [2]

According to the data available from Statistik Austria, in the last 57 years, the deaths caused by heart disease registered a slightly decrease, while the ones caused by cancer remained constant, with about 18.000-20.000 cases every year.[3]

Cancer is a disease caused by malignant tumors (or neoplasms), which are abnormal cell growths beyond their boundaries, that invade the nearby parts of the body and spread to other organs, leading to metastasis. It can affect every tissue or organ of the body.[1]

After the cell type that the tumors cell originated from, cancers can be classified as follows: carcinoma (cancer devellops from epithelial cells; in this group there

are included most of the cancers, such as breast, prostata, lung, pancreas cancer), sarcoma (cancer arises from connective tissue), lyphoma and leukemia (from cells that make blood), germ cell tumor (from pluripotent cells) and blastoma (from embryonic tissue, theses types are more common in children than in adults).

Worldwide the most common is lung cancer, with high rates being observed in North America and Europe (especially Eastern Europe). Unfortunatly this type of cancer hast a low survival rate (ca 8% in Europe). Other frequent types of cancers are stomach, breast, colon and rectal, prostate and liver cancer.[4]

In 2014 in Austria, the most common tumor localisation for men was prostate (22%), followed by lung, bladder, collon and kidney. In the case of women, the localisations were breast (28%), lung, colon, corpus uteri and thyroid.[6] The incidence (number of new cases occurring) and the mortality (number of deaths occurring) do not, however, correlate.[4] Most men died of lung, liver, intestine, pancreas and prostate cancer, while most women died from lung, pancreas, breast and intestine cancer.[5]

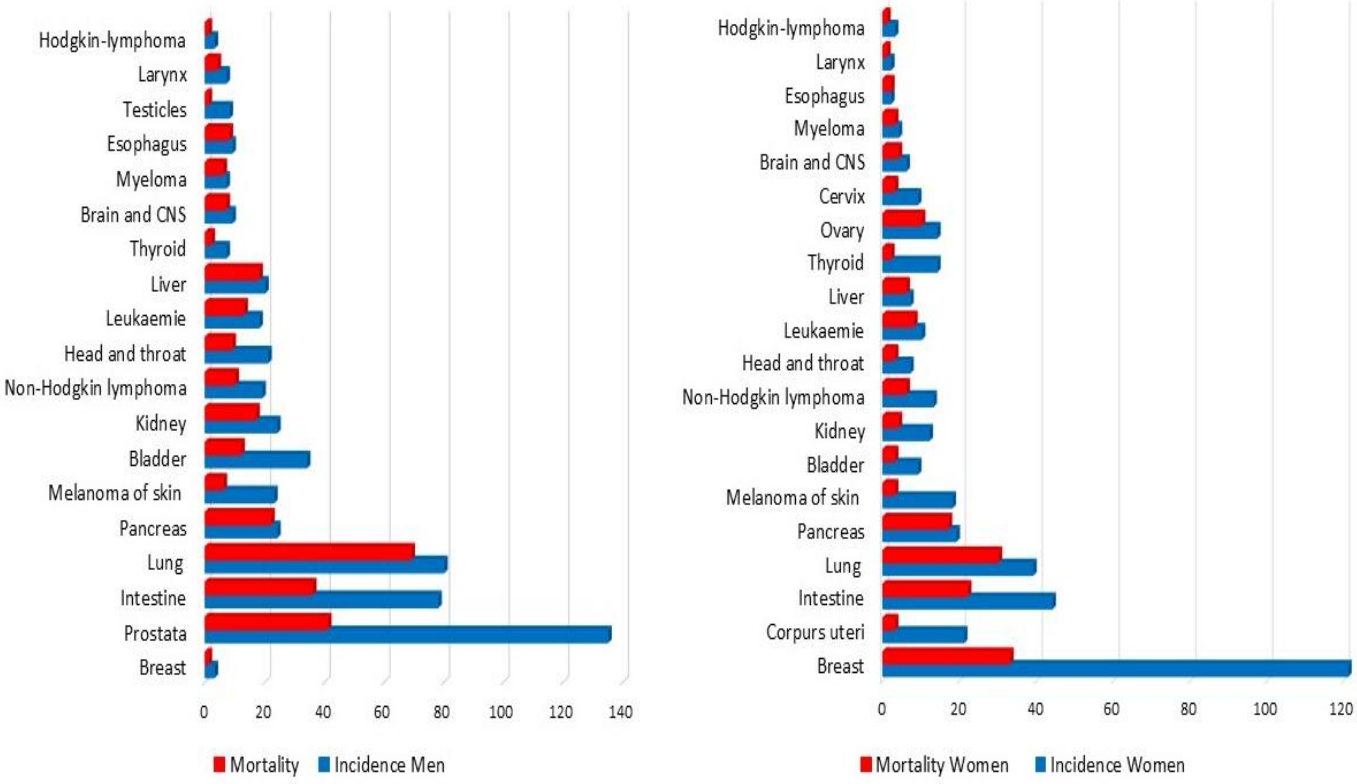


Fig. 2: Cancer incidence and mortality form en and women, diagnosis period 2010-2014 (Source: Statistik Austria, from 1.2.2017). Data is registered per 100.000 people.[5]

Among the factors that cause cancer, are genetics, physical carcinogens (e.g. ultraviolet, ionization radiation), chemical carcinogens (e.g. components of tabacco smoke, asbestos, contaminants of food and drinking water) and biological carcinogens (infections from certain viruses, bacteria or parasites).

Another major factor in the development of cancer is aging, as a person grows older the cellular repair mechanisms tend to be less effective and also the risks for specific cancers tend to accumulate.

Worldwide there are 4 risk factors for cancers: tobacco use (cigarettes and smokeless tabacco), unhealthy diet (low intake of fruit and vegetables, combined with obesity), lack of physical activity and alcohol abuse.[1, 7] The most important of them is tobacco use, which causes about 22% of the cancer-related deaths (in Austria in 2016 about 20% of the deaths related to cancer were neoplasms of the respiratory system).[1, 2, 7, 8]

In low- and middle-income countries there are chronic infections, such as Hepatitis B and C virus (increase the risk of liver cancer), Human papillomavirus (HPV) and HIV (increase the risk of cervical cancer).[1]

Thirty to fifty percent of cancers can be prevented by avoiding the risk factors (abstinence from smoking and drinking alcohol, a healthy lifestyle – including an increased intake of fruits and vegetables and sport), controlling the occupational hazards, reducing the exposure to ultraviolet or ionizing radiation and vaccination against HPV and Hepatitis B viruses (this could prevent ca 1 milion cancer cases each year).[1, 9]

Early detection of cancer is very important for increasing the chances of recovery of the pacient (if detected in time and treated correspondingly, cervical cancer, breast cancer, oral cancer and colorectal cancer have high cure rates). For this reason regular check-ups are highly recommended (especially to those have/had close relatives, such as parents or siblings, suffering from cancer), because many times cancers don't show any specific symptom and cannot be diagnosed before entering to the late stages, when a curative treatment is no longer an option.[1, 5]

1.2. Cancer therapy

For an effective treatement, first of all, a correct cancer diagnosis is required, followed by a specific treatement regimen, which may encompass surgery, chemotherapy, targeted therapy, radiation therapy, hormonal therapy, immuntherapy, or a

combination thereof. The aim of this approaches is to cure cancer or to prolong the life of the patient as much as possible, offering then improved conditions for living.[1]

1) **Surgery** – is the primary method of treatment for solid tumors and it is limited to the ones located in accesible parts of the body. This method is normally followed by radiotherapy or chemotherapy. In this case it is called adjuvant therapy, whose objective is to eradicate the cancer cells who might have been left after surgery. In order to reduce the size of the tumor and so, to facilitate its extraction, there are some therapeutic agents which can be provided before the surgery, this practice being called neoadjuvant therapy.[10]

But not all malignt neoplasms are solid tumors, some of them can be dispersed or extended in the body, like leukemia. In all these cases, the methods of choice are radiotherapy or chemotherapy.[11, 12]

2) **Radiotherapy** – by using radioactive radiation, γ-rays or X-rays, the DNA of the cancer cells can be damaged and so, they can be eradicated. There are two different types of radiation therapies: external (implies a radiation beam which is directed into the tumor) and internal (uses radionucleotides, like ^{131}I, in the treatment of thyroid diseases). Because of the lack of specifity of this method, the non-cancerous cells are also affected and it is accompanied by side effects, like skin alterations, fatigure or loss of appetite. Nonethells, this method covers about 40% of the treatments nowadays.[11, 13]

3) **Immunotherapy** – including cytokines, vaccines, bacillus Calmette-Guerin (BCG) and monoclonal antibodies to stimulate or suppress the immune system, this method helps the body to fight cancer, infections or other diseases. It targets only certain cells of the immune systems.[14]

4) **Targeted therapies** – are small molecules or monoclonal antibodies, which block the growth and the spread of cancer by interfering with targeted molecules (like enzymes or proteins), which are needed in carcinogenesis and tumor growth. Another way of action is the deliverance of toxins directly to the

cancer cell in order to kill them or help the immune system do it. This method has fewer side effects than other treatments.[14]

5) **Hormonal therapy** – slows down and stops the growth of certain types of cancer, by adding, blocking or removing hormones. Synthetic hormones or other drugs inhibiting the natural ones, help adjust the hormonal levels, as well as the surgical removal of the gland which produces certain hormones.[14]

6) **Chemotherapy** – is the application of drugs (natural or synthetic) to kill cancer cells. These substances travel through the bloodstream to the affected cells all over the body.[15] Unfortunately, not only the rapidly dividing cancer cells are targeted, but also the healthy cells (like the ones located in the hair follicles, the bone marrow or the digestive tract), causing the well known severe side effects. Nonetheless, this method there can beused to treat metastasised tumors, non-solid tumors or small tumors escaping detection.

The Anatomical Therapeutic Chemical (ATC) classification system is used by WHOCC (World Health Organization for Collaborating Centre for Drug Statistics Methodology) to classify drugs acccording to the targeted organ and their chemical and therapeutic characteristics. The antineoplatic agents, which are included in the L01 group, contain: alkylating agents, antimetabolites, plant alkaloids, antitumor antibiotics and topoisomerase inhibitiors, among others.[15]

a. Alkylating agents – damage the DNA (bind at the nitrogen in the position 7 of the purine ring of the guanine base) and prevent the cells from reproducing. Not only the cancer cells are targeted by them, but also cells who divide frequently. The alkylating agents include: mustard gas derivatives (mechlorethamine, cyclophosphamide, chlorambucil), ethyleneimines (hexamethylmelanine, thiotepa), alkylsulfonates (busulfan), hydrazines and triazines (procarbazine, dacarbazine), nitrosoureas (lomustine, carmustine, streptozocin) and others. Sometimes also the platinum-based drugs (cisplatin, carboplatin, oxaliplatin) are included in this group, since they bind to the DNA, damaging it and interfering in its repair, leading to apoptosis (programmed cell death).[17, 18, 19]

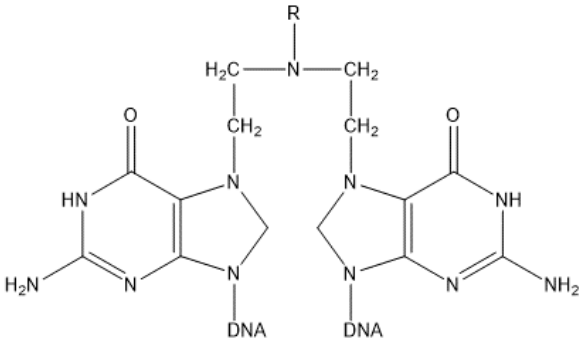


Fig. 3: Two DNA bases that are cross-linked by a nitrogen mustard. [21]

b. Antimetabolites - substitute normal building blocks of DNA and RNA during the S-phase of the cell cycle. They contain analogues of folic acid (methotrexate), purine (6-mercaptopurine, 6-thioguanine), pyrimidine (5-fluoururacil, cytarabine) or adenosine deaminase inhibitor (cladribine, fludarabine, nelarabine and pentostatin).[19, 20]

c. Plant alkaloids – prevent cells from reproducing (are mitotic inhibitors). They include: vinca alkaloids (incristine, vinblastine vinorelbine), taxanes (paclitaxel, docetaxel), podophyllotoxins (etoposide, tenisopide), camptothecan analogues (iIrinotecan, topotecan).[19]

d. Topoisomerase inhibitors – suppress enzymes topoisomerase I and II so that DNA can not unwind during the S-phase of the cell cycle. These inhibitors include the ones who affect the topoisomerase I, like ironotecan or topotecan and the ones who suppress the topoisomerase II, like amsacrine, etoposide, etoposide phosphate, or teniposide.[19]

e. Antitumor antibiotics – inhibit the replication of DNA during various phases of the cell cycle, for example, anthracyclines (oxorubicin, daunorubicin, epirubicin) or chromomycins (dactinomycin, plicamycin).[19]

Beyond all the therapies mentioned before, the accidental discovery of the cytotoxic activity of cis-diamminedichloridoplatinum(II), also known as cisplatin, a platinum based complex, marked a milestone in the fight against cancer and opened the door to the research of various transitional metal complexes, which might be used as chemotherapeutics.[22]

1.3. Metal complexes as anticancer drugs

Before the discovery of cisplatin, several metal-based drugs had been used to treat various diseases, like the ones arsenic-based against syphilis (since 1910) or the lithium-based to treat depression (since 1952).

1.3.1. Platinum-based anticancer drugs

Although it was first synthesized by Michele Peyrone, in 1844, the anti-proliferatives properties of cisplatin, cis-diamminedichloridoplatinum(II), where only in 1965 accidentally discovered by Barnett Rosenberg.[23] In the experiment with Escherichia coli (E.coli), Rosenberg and his co-workers wanted to investigate the effect of an electric field in the mitosis of this bacteria. For this purpose, were employed two platinum electrodes and ammonium chloride as growth medium. He observed that the bacteria grew three hundred times in length and that oxidation of the platinum electrodes to Pt(IV) led to the formed ammoniumhexachloridoplatinate(IV) complex, which was converted by a photocatalytic reaction to cis-diamminetetrachloridoplatinate(IV)-complex, which then was reduced by the environment of the bacteria to cisplatin. So, cisplatin had an influence on the cell growth.[24, 25]

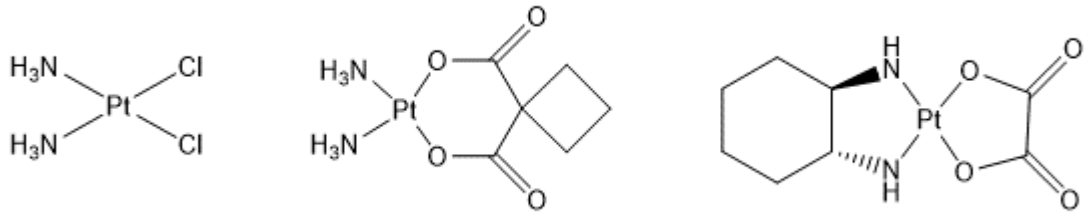


Fig. 4: cisplatin, carboplatin, oxaliplatin

In 1978, cisplatin was succesfully aproved in the cancer therapy worldwide, showing a particulary high effectiveness against testicular cancer, where 80% of the patients treated with it survived.[26] In the case of breast, ovarian, bladder, cervical, prostate, head and neck, lung cancers and refractory non-Hodgkin's lymphomas positive reactions were observed. However, cisplatin is not affective against all types of cancer.[27, 28]

The main target of cisplatin is the DNA. First it is administred intravenously and once in the body it binds to proteins, like HSA (human serum albumin), which

transports it in bloodstream; the uptake into the cell is achieved by active transport or passive diffusion. In order to be able to bind to DNA, cisplatin must first be hydrolysed, the chlorido ligands being replaced by water molecules, forming the single or double aqua species: $[(Pt(NH_3)_2Cl(H_2O)]^+$ and $[(Pt(NH_3)_2(H_2O)_2]^{2+}$.[29, 30] The dissociation of the chlorido ligands is facilitated inside the cell by the much lower chlorido concentration than outside the cell (about 100mM).

The aqua species binds then covalently to the N7 position to the imidazole ring of the purine bases of DNA, predominaly to the guanine (G) base, but also to adenine (A), forming 1,2 or 1,3 intrastrand and interstrand crosslinks.[31, 25] The major adduct formed is the cis 1,2-$[Pt(NH_3)_2]^{2+}$-d(GpG) (about 65%), followed by 1,2-d(ApG) (25 %) and 1,3-d(GpNpG) (5–10 %), as well as interstrand crosslinks (which are less frequently formed). These adducts induce a bend in the DNA and unwind the doble helix, leading in the end to programmed cell death, apoptosis.[28, 32, 33]

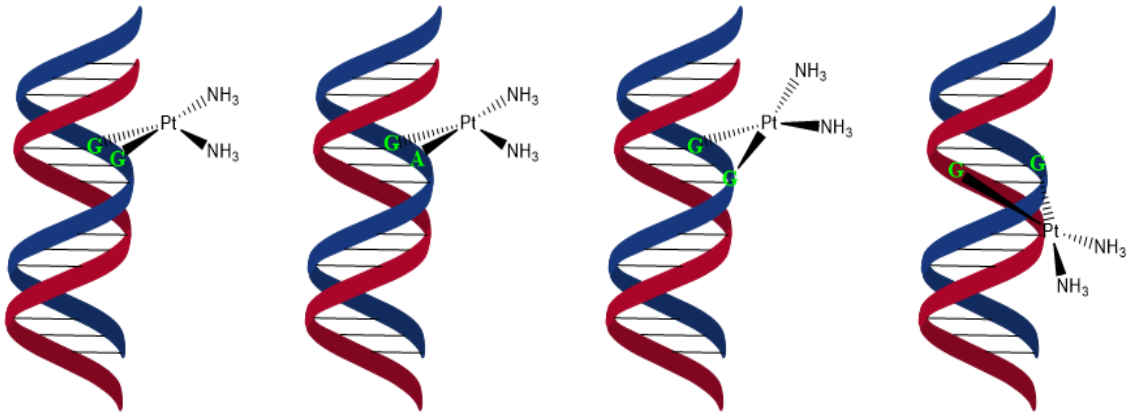


Fig. 5: Binding of cisplatin to DNA: intrastrand (1,2 and 1,3) and interstrand crosslinks

The major drawback of cisplatin is its severe side effects, like kidney toxicity, ototoxicity, nausea, vomiting, decrease in the amount of red and white blood cells, but also hair loss, peripheral neuropathy and loss of appetite.[34] Another disadvantage of the drug is the intrinsic as well as acquired resistance that the cancer cell may develop.[35] So, in order to reduce the side effects, new platinum based drugs have been developed, leading to second and third generation analogues.[36]

In 1972 carboplatin, cis-diammine(1,1-cyclobutanedicarboxylato) platinum(II) was discovered. This second generation platinum compound contains two diamine non-leaving groups like cisplatin and also a bidentate dicarboxylato leaving group, which makes it experience slower ligand exchange kinetics than cisplatin. Because of the

lower activity and the slower binding to DNA, the dosage applied must be almost four times higher than in the case of cisplatin. A major adventage of carboplatin is the fact that it has fewer side effects than cisplatin (neurotoxicity, ototoxicity and gastrointestinal toxicity). It is mainly administred against tumors of the urogenital tract.[36, 33, 37, 38]

In 1976, Oxaliplatin, (1R,2R)-diamminecyclohexaneoxalato platinum(II), the third generation platinum(II)-based drug was approved developed, in order to overcome the limitations of cis- and carboplatin. It contains a chiral (1R,2R)-diaminecyclohexane (DACH) as non-leaving group and a bidendate oxalato ligand as leaving group. Oxaliplatin was found to be active against cis- and carboplatin resistent cell lines and tumors. Its main feature is the activity against metastatic colorectal cancer, in combination with 5-fluorouracil and folinic acid.[33, 39, 40]

Additionaly to the worldwide approved cis-, carbo- and oxaliplatin, there are also some other platinum-based complexes, which are approved regionally, like nedaplatin (in Japan), lobaplatin (China) and heptaplatin (South Korea).[36]

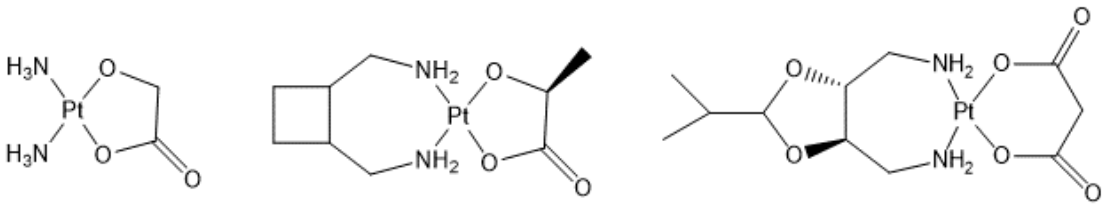


Fig. 6: nedaplatin, lobaplatin, heptaplatin

In order to reduce the side effects of the platinum(II)-based compounds, platinum(IV)-based complexes were developed and investigated. These should be activated in the cell, through reduction to Pt(II). Additonally, their kinetic inertness should enable them to be oral administered, thus improving the bioavailability of the drug.[41] Platinum(IV)-based complexes investigated include satraplatin, tetraplatin, iproplatin, and LA-12, but only the first of them is still in clinical trials.[28, 42]

1.3.2. Ruthenium-based anticancer drugs

The limitations of the platinum-based drugs (side effects, intrinsic and acquired resistance, and the limited range of treatable tumors) stimulated researchers to explore new fields and investigate alternative metal complexes as potential anticancer drugs. Amongst the first ones, there were the platinum group metals, like osmium, iridium, rhodium and ruthenium.

Ruthenium complexes show interesting features, like less toxic side effects and activity in cancer cells that are resistant or unresponsive to cisplatin.[43]

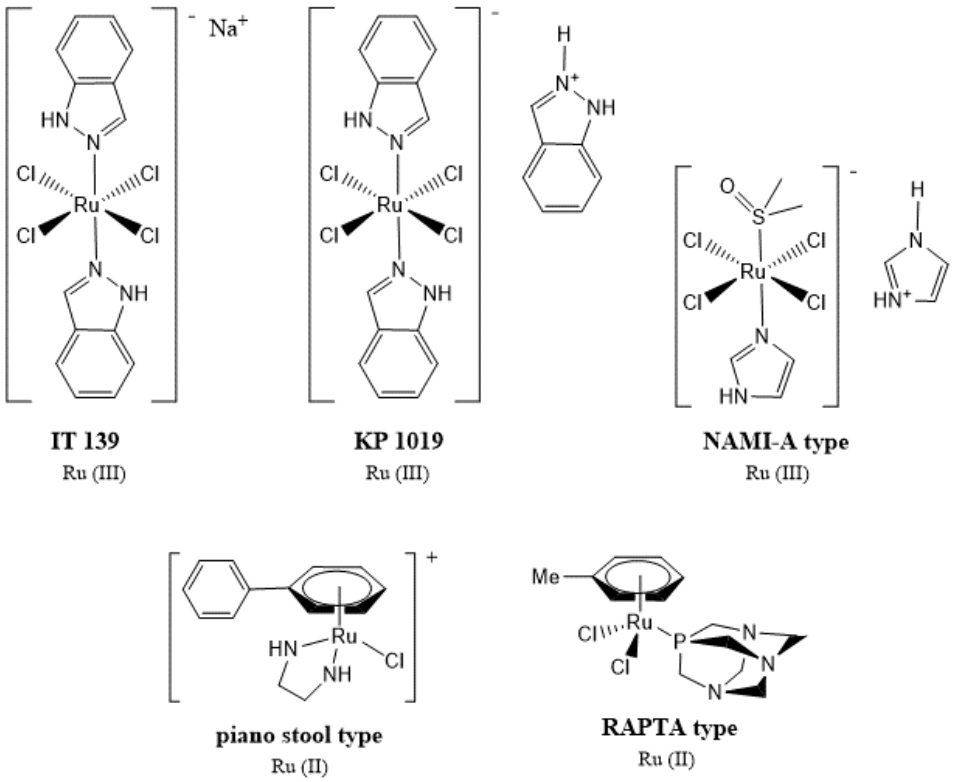


Fig. 7: ruthenium-based complexes

Among the interesting properties of ruthenium are its accesible oxidation states under physiological conditions (+2, +3 and +4), ability to bind with O- and N-donor molecules similar to platinum or the low systemic toxicity (the ruthenium-based complexes can mimic the iron bound to biomolecules).[44, 45, 46]

Two ruthenium compounds are in clinical trials. The first one NAMIA-A, trans-[tetrachloro-S-dimethylsulfoxideimidazole-ruthenate(III)], was moderately tolerated as monotherapy, but exhibted less activity in combination with gemcitabine.[47, 48, 49]

IT-139 (former KP 1339), sodium trans-[tetrachlorobis(1H-indazole) ruthenate(III)], developed by Keppler et al., passed the phase I clinical study, which evalueted the safety, tolerability, MTD (maximum-tolerated dose), PK (pharmacokinetics) and pharmacodynamics in patients with advanced solid tumors. The complex is a modulator of stress-induced GRP78, a protein which supports drug resistence and tumour progression. IT-139 showed a modest anti-tumor activity in the treatment of patients with solid tumours. However, the lack of neurotoxicity and of dose-limiting haematological toxicity, make it a promissing candidate to be used in combinations with other anticancer drugs.[49]

Other important ruthenium-based complexes include the ones bearing biologically active or arene ligands, like the RAPTA-type complexes or the “piano-stool“ type compounds by Dyson and Sadler.[50, 51] The last category of complexes are active against solid tumours (activity is dependent on the aryl unit). Unfortunatly, they are not active against primary tumours.[48]

1.3.3. Other metal-based anticancer drugs

1.3.3.1.Gallium

Due to the similarities (size and charge) to iron(III) and aluminium(III), gallium presents interest in the development of anticancer drugs.[48] It can compete with iron for the enzyme binding sites, for exameple it can bind to transferrin (inferfering in the celular transport of iron) or inactivate ribonucleotide reductase. It contrast to iron (III), gallium is redox inactive under physiological conditions.[52] Gallium salts, like gallium nitrate, presented antitumor activity and so further gallium-based compounds were developed. The second generation gallium complexes included KP 46, tris(8- quinolinolato)gallium(III), which showed promising signs of anticancer activity against renal cancer in phase I clinical trials.[53, 54, 55, 56]

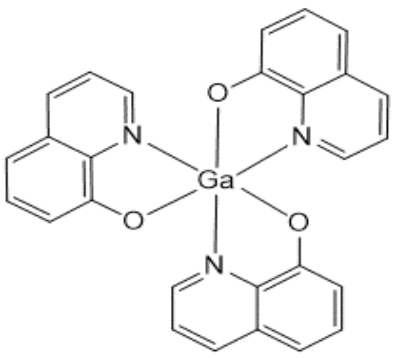


Fig. 8: KP 46 [53]

1.3.3.2. Osmium

In the last years, several osmium analogues of the ruthenium anticancer drugs where synthesized, because of the similar electrochemical behaviour of osmium and ruthenium. For example, the osmium-based derivative of NAMI-A showed in vitro cytotoxic activity.[57, 58]

1.3.4. Metallocenes

Metallocenes contain two cyclopentadienyl (Cp) ligands and a transition metal coordinated in a sandwich structure.[60] Structurally, they can be classified as classical and bent.

The resemblence of the bent metallocenes to cisplatin (they have a cis dihalido motif just like the the platinum-based compounds) encouraged the exploration of their biological activity.[60] Köpf and Köpf-Maier were the first ones who reported the antitumor activity of the titanocene dichloride. Afterwards, they also investigated other biological active metallocenes, with the general formula Cp_2MX_2 (M = Ti, V, Nb, Mo; X = halides and pseudo-halides). They exhibited antitumor activity with fewer secondary effects than cisplatin. Among the tumor cells which were tested were colon 38 carcinoma, B 16 melanoma and Lewis lung carcinoma. Titanocene dichloride (Cp_2TiCl_2) was found to be active against colon, breast and lung cancers.[61]

1.3.4.1. Titanium

Near titanocene dichloride, (dichloridobis(η^5-cyclopentadienyl) titanium), also budotitan, [cis-diethoxybis(1-phenylbutane-1,3-dionato) titanium(IV)], entered into clinical trials. Both compounds showed promising anticancer activity against cisplatin-resistent tumors and fewer secondary effects, but unfortunately failed in phase II clinical trials because of formulations problems.[62] The fact that these two complexes possess two leaving groups which hydrolyze very fast in water, made the researchers focus on novel water-soluble titanium aniticancer drugs.[63]

Second generation titanocene compounds, with aromatic groups at the Cp (Cp = cyclopentadienyl) ligands have been developed in order to overcome the drawbacks of titanocene dichloride, for example, titanocene Y (dichloridobis(η^5-(p-methoxybenzyl)-cyclopentadienyl) titanium). This compound was found to be active in vitro against colon, renal, lung and ovarian cancers.[64]

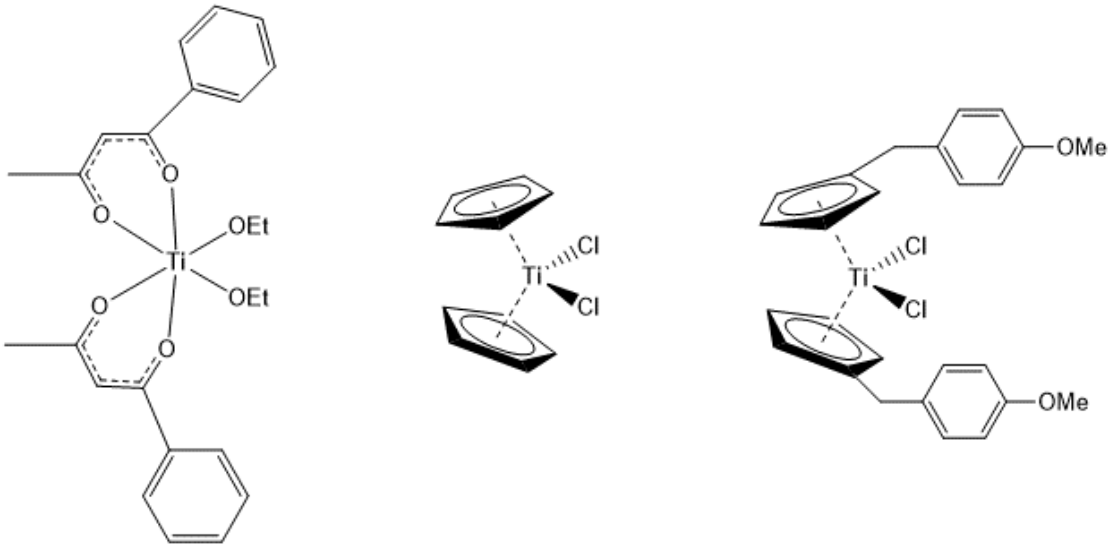


Fig. 9: budotitan, titanocene dichloride and titanocene Y [53]

1.3.4.2. Iron

The first iron complexes to exhibit anticancer activity were the ferrocenium (Fc^+) salts, like ferrocenium tetrafluoroborate.[54, 65] Later, further derivatives of the ferrocenium salts were developed, like decamethylferrocenium tetrafluoroborate ($DEMFc^+Fc$) or ferrocifens (derivatives of tamoxifen, a selective estrogen receptor antagonist), which showed activity against estrogen-dependent and independent breast cancer.[53, 66]

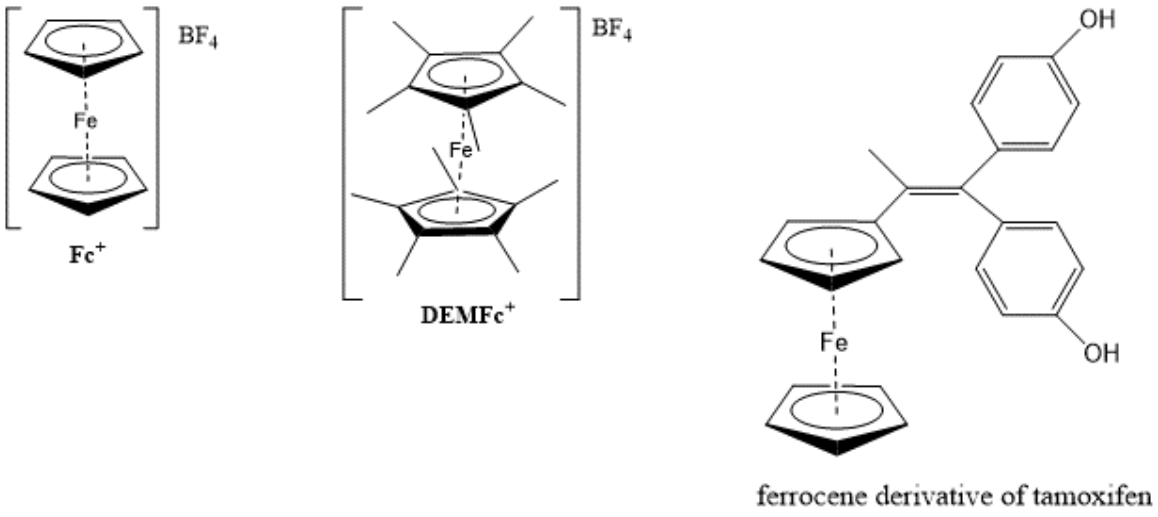


Fig. 10: iron complexes

1.3.4.3. Molybdenum

Additionaly, the anticancer activity of several molybdenum-based compounds has been investigated**.** The research of Köpf and Köpf-Maier revealed the fact that molybdenocene dichloride (Cp_2MoCl_2) was active against a variety of tumors, presenting fewer side effects than cisplatin.[67] Of all the simple metallocenes, this compound showed also the highest aqueous stability at physiological pH. Unlike Cp_2TiCl_2, Cp_2MoCl_2 keeps its two Cp-ligands permanently bounded and exchanges just the two chlorido ligands with aqua ligands.[68] Although the mechanism of action of the molydenocenes is not completely understood, it is believed that it damages the DNA.[69, 70]

In the last years, several approches were made in order to enhance the anticancer activity of the molybdenocenes, like changing the two chlorido ligands with other ligands or functionalizing the cyclopentadienyl rings.[68]

1.4. Tungsten

Tungsten (W) is a chemical element with the atomic number 74. Its name means in swedish „heavy stone“ Its ores are scheelite (calcium tungstate ($CaWO_4$)) and wolframite (iron–manganese tungstate $(Fe,Mn)WO_4$).[71]

It has the highest melting point of all elements and, because of it, one of its applications is as light bulb filament. It exhibits all oxidation states from -2 to +6, the most common of them being +6.[72]

Tungsten is the only third row transitions element to be present in life (in a few species of bacteria and archeea). Because of its presence in the same group with molybdenum, it shares similar properties with it (rich redox chemistry, vast number of oxides), although it has a lower biological importance than molybdenum, which can be found both in pro- and eukaryotes.[73, 74] Tungsten can bind to the same pterin cofactor as in many molydbdenum containing enzymes, which catalyze redox reactions.[75]

In 1980 Köpf and Köpf-Maier investigated the anticancer activity of tungstenocene dichloride (Cp_2WCl_2), which presented a lower cytotoxic activity against colon 38 carcinoma, B 16 melanoma and Lewis lung carcinoma than the titanocenes or the molybdenocenes.[76] A few years ago, Melendez el al. reported an improved in vitro anticancer activity of novel tungstenocenes bearing 3-hydroxy-4-pyrone ligands (synthe-

sized through the exchange of the two chlorido ligands with the bidentate O,O-ligands).[77]

1.5. Bioactive ligand scaffolds

The derivatisation of metal complexes with bidentate biological active ligands is an interesting approach, presenting several advantages like increased solubility and cellular uptake of the bioactive ligands or enhanced stability towards ligand substitution.[78] Using this strategy, several compunds including flavonoids[79], picolinic acid[80], hydroxypyrones[81, 82, 83], hydropyridones[80, 84], quinolones[85, 86] or indoloquinolines[87, 88] as ligands, have been developed.

1.5.1. Pyrone Derivatives

Pyrones are natural products with an interesting toxicity profile. The O,O- chelating ligand, especially the 3-hydroxypyrones gained a special attention due to their affinity to bind to metal ions. The derivatization with these ligands makes the synthesized complexes thermodynamically stabile under physiological pH. This stability can be even enhanced by modifying the ligands through thionization (for example with Lawesson's reagent). The new obtained ligands, with S,O-moieties, have a higher affinity towards the soft metal center (like tungsten or molybdenum) and so, the stability of the complexes is increased.

Undoubtedly, one of the most studied 3-hydroxypyrone is maltol, (3-hydroxy-2-methyl-4(1H)-pyrone), which is well known for its favorable bioavailability and low toxicity. Maltol is utilized as food additive in bread, beer or sweet, in order to obtain the aroma and malty tase and can be gained from pine, larch bark and roasted malt or even synthesized.[89]

In the last years, there have been developed many novel anticancer compounds containing maltol.[84, 89, 90, 91, 92] For example, by changing the two chlorido ligands of cisplatin with maltol, it is possible to increase the aqueous solubility (but cytotoxicity remains the same). [93]

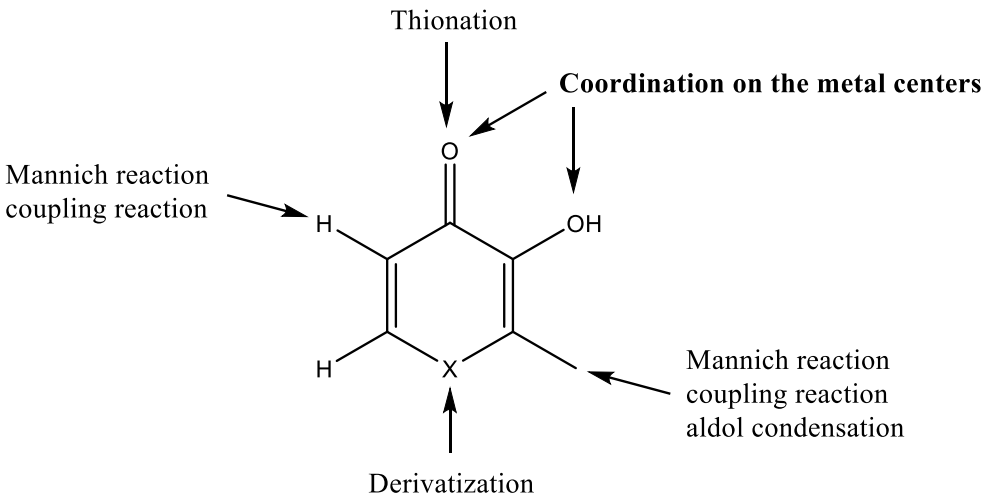


Fig. 11: possible coordination sites of pyrones[89]

2. OBJECTIVE

In 1979 the investigation lead by Köpf and Köpf-Maier on various metallocenes containing tungsten, molybdenum, titanium, vanadium and niobium showed that they had a significant anticancer activity on different cancer cell lines (B 16 melanoma, Lewis lung carcinoma, Ehrlich ascites tumor and colon 38 carcinoma) and that titanocene dichloride had fewer secondary effects than cisplatin.[77, 94-98]

In the past years, various derivatives of the mentioned metallocenes were investigated by different groups, especially the bioorganometallic chemistry of molybdenocenes has been studied. A few years ago, in the Keppler group, new molybdenocenes bearing a bioactive ligand were synthesized and their anticancer activity on 3 types of human cancer cell lines was investigated.[68, 99, 100, 101, 106]

Surpringsingly, the chemistry of tungstenocenes remained less explored, until a few years ago, when the group of Melendez synthesized three new tungsten-based compounds and investigated their antiproliferative activity on **HT-29** colon cancer and **MCF-7** breast cancer cell lines. The study showed that the substitution of the two chlorido ligands by the 3-hydroxy-4-pyronato bidentate ligands enhanced the cytotoxic activity of the compounds towards the cancer cell lines, although, in the case of the **MCF-7** cell line, this effect was less pronounced. Compared with their molybdenum counterparts, the tungstenocene complexes showed an encreased cytotoxicity towards the **HT-29** cell line.[77]

The objective of this master thesis is to develop novel tungsten based anticancer drugs, similar to the molybdenocenes synthesized in the Keppler group, with the general formulation Cp_2WL_2, where L_2 are bioactive O,O- and O,S-ligands, coordinated to the metal center. These ligands include maltol, ethylmaltol, allomaltol and their thionated derivatives. By replacing the two chlorido ligands with a bidentate ligand, the aqueous stability of the tungstenocene complexes are increased.

In order to isolate the desired products, PF_6^- was used as counterion and not Cl^- (chlorido), like in the case of the study of Melendez et al.[77] This enables the improvement of the yield of the resulting compounds and the avoidance of the column chromatography purification step.

All the new compounds were characterized by standard methods ^{1}H- and ^{13}C-NMR, elemental analysis (EA), mass spectrometry (ESI-MS), FT-IR spectrosco-

py and X-Ray diffraction techniques. In order to investigate their stability, electrochemical and aqueous studies over 25 hours via UV-Vis were performed.

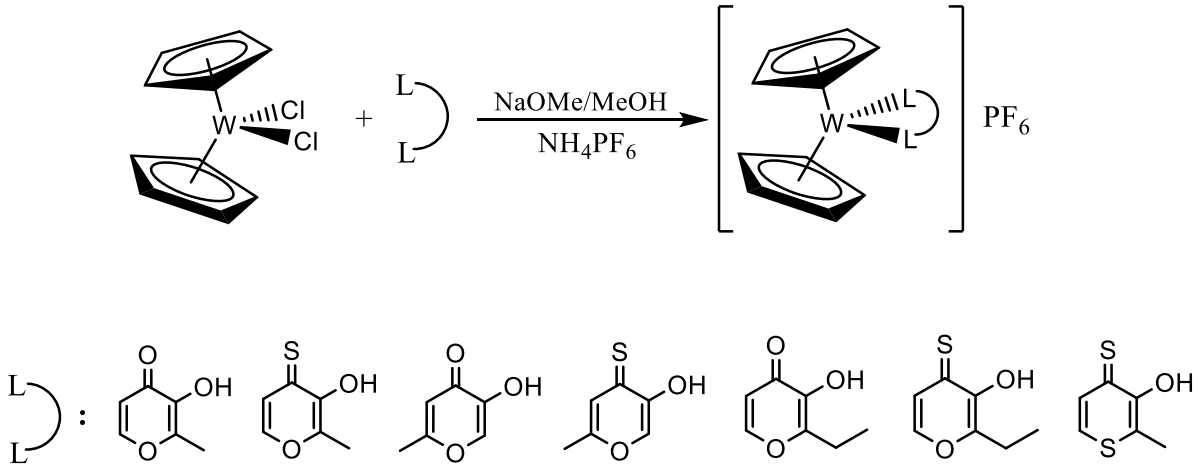


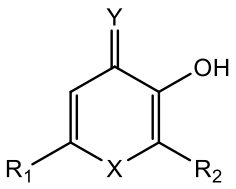
Fig. 12: The synthetic pathway and the bidentate ligands used in this thesis

In vitro-tests on 3 human cancer cell lines: **A549** (non-small cell lung carcinoma), **SW480** (colon carcinoma) and **CH1/PA-1** (ovarian carcinoma) will be performed, in order to investigate their anticancer activity.

3. DISCUSSION AND RESULTS

3.1. Ligands synthesis

The aim of my mastersthesis was to synthesize bidentate ligand scaffolds with O,S-coordination motives, along with the O,O-chelating ligands already available, in order to investigate their influence on the bioactivity of the tungstenocenes. The ligands were characterized by ^{1}H-NMR spectrocopy.



Maltol:	X = O, Y = O, R_1 = H, R_2 = CH_3
Thiomaltol:	X = O, Y = S, R_1 = H, R_2 = CH_3
Allomaltol:	X = O, Y = O, R_1= CH_3, R_2 = H
Thioallomaltol:	X = O, Y = S, R_1= CH_3, R_2 = H
Ethylmaltol:	X = O, Y = O, R_1= H, R_2 = C_2H_5
Thioethylmaltol:	X = O, Y = S, R_1= H, R_2 = C_2H_5
Dithiomaltol:	X = S, Y = S, R_1 = H, R_2 = CH_3

Fig. 13: pyrone ligands used in this master thesis

All O, S - chelating ligands were obtained by thionation with Lawesson's reagent (2,4-Bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide), according to the literature procedure.[102]

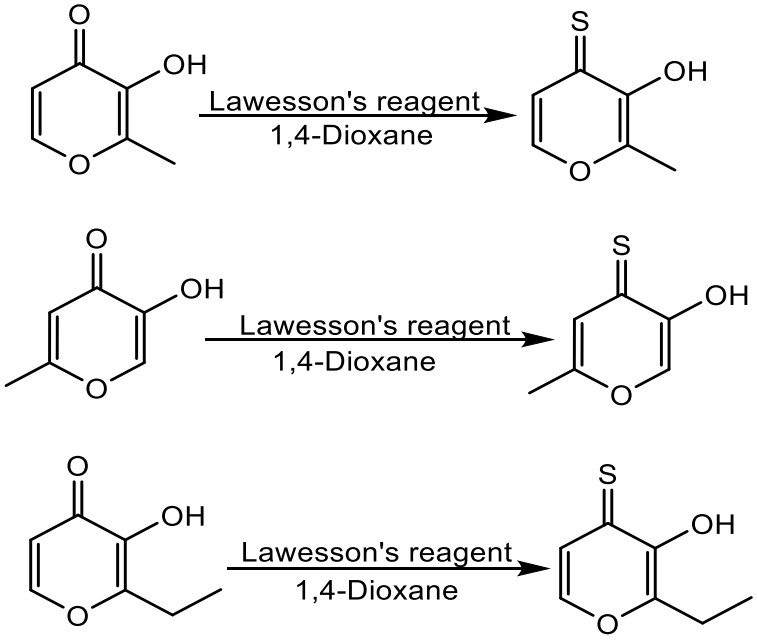


Fig. 14: synthesis of the O,S-chelating ligands

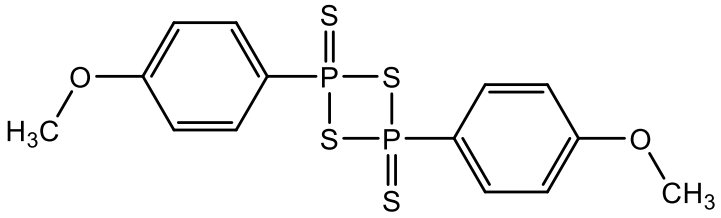


Fig. 15: Lawesson's reagent

The synthesis of dithiomaltol ligand presents a special case. Previously, thiopyran-4-thiones were obtained via thiopyrone intermediates. In 2005, the Farmer group reported that, by thionating maltol with an excess of Lawesson's reagent, the second substitution of oxygen by sulfur takes place at the hetetocyclic ring and not in the α-hydroxyl group, as expected. It has been suggested that the reaction might be initiated by a Michael additon at the 2 – position of the pyrone ring, followed by ring opening and closing steps. Interestingly, this reaction has been observed only in the case of pyrones with proton or aliphatic substituents. The ones with an arene substituent did not undergo this substitution. Thus, in an one-pot reaction, 3-hydroxy-2-methyl-4H-thiopyran-4-thione was obtained and characterized by a variety of methods (^{1}H-NMR, ms and crystalographic analysis).[103]

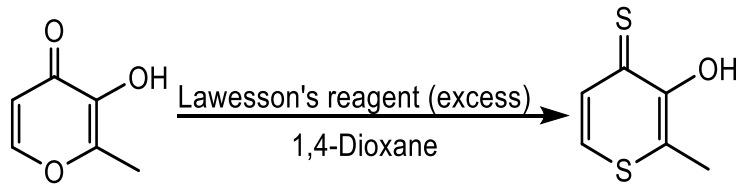


Fig. 16: synthesis of dithiomaltol ligand (3-hydroxy-2-methyl-4H-thiopyran-4-thione)[103]

The ligands were then purified via column chromatography, with hexane/ethyl acetate (10:1) as eluent and characterized via ^{1}H-NMR spectroscopy.

3.2. Tungstenocenes synthesis and characterization

3.2.1. Synthesis

The seven novel tungstenocenes were synthesized using the following procedure: the two chlorido ligands of the tungstenocenes were exchanged with a bidentate ligand (O,O- or O,S-ligand), obtaining a positive charged complex (see Figure

17), which was then precipitated using PF_6^- as counter ion . The O,O-ligands employed were maltol, allomaltol and ethylmaltol. The O,S-ligands were thiomaltol, thioallomaltol, thioethylmaltol and dithiomaltol.

The complexation was carried out under inert atmosphere (argon) and the reagents were previously dried.

In the glovebox there were weighed Cp_2WCl_2, the corresponding ligand and the base NaOMe, then stirred for 72h under argon at room temperature. The solution was then filtered, in order to separate possible unreacted reagents and then the precipitating salt, NH_4PF_6, was added (the anion exchange reacton was carried out at open air). The mixture was stirred for 4h at room temperature and stored for 48h at 4°C. The precipitate was then filtreted and dissolved in DCM (dichloromethane), which was then removed under reduced pressure. The yields were moderate to very good: 31-95 %.

It has been observed, that all the compounds are soluble in chlorinated solvents, but also in acetone, ethanol and ethyl acetate. The compounds are stable in air, however, for longer periods, it is recommended to store them under argon and at 4°C.

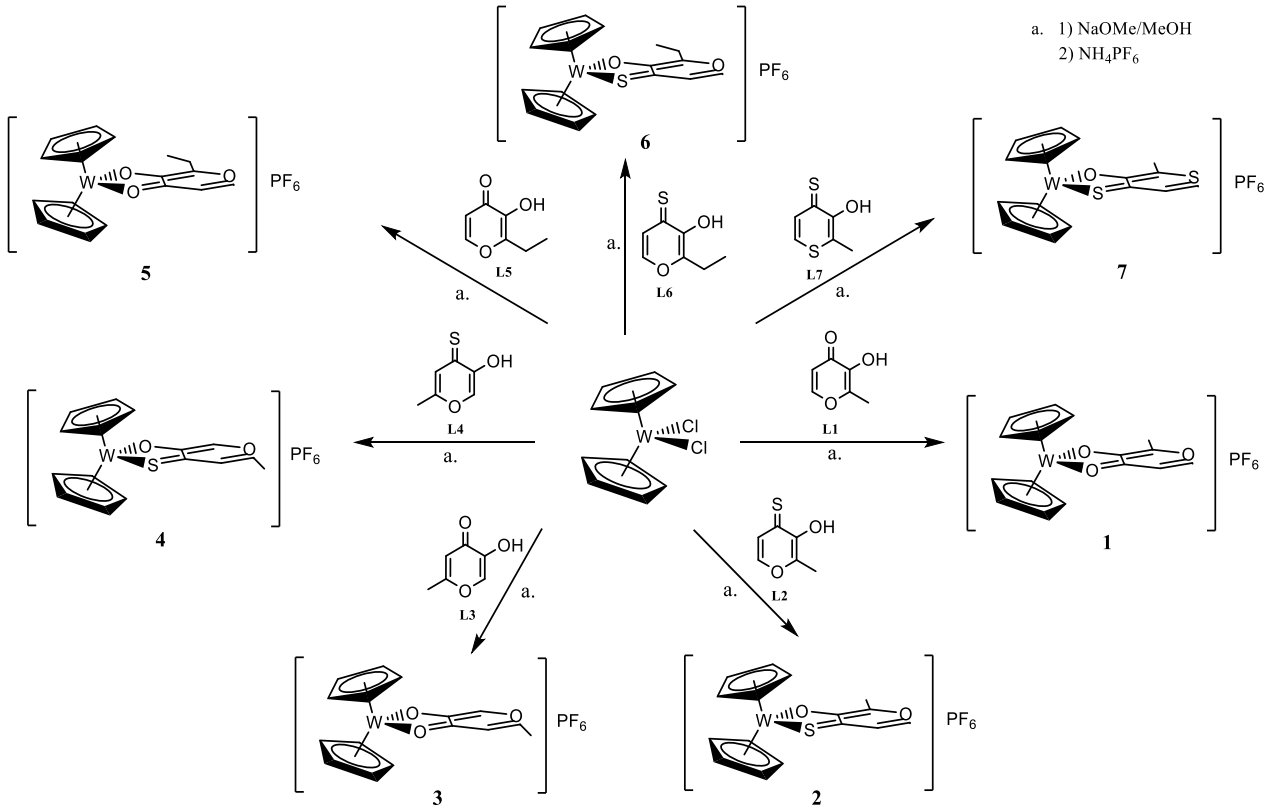


Fig. 17: synthesis of the 7 novel tungstenocenes

3.2.2. NMR-Spectroscopy

All the spectra of both the ligands and the tungstenocenes complexes were taken in d_6-DMSO.

The fact that both Cp-rings are symmetrically coordinated to tungsten in a η^5-manner and the bidentate ligand is in an ancillary position (in the plane bisecting the Cp-W-Cp), has as consequence that there is just one signal seen for both Cp-groups in the ^{1}H-NMR. This signal is observed shifted downfield in the ^{1}H-NMR spectra of all the seven complexes (δ = 5.70-5.91 ppm), than compared to the one in the tungstenocene dichloride spectrum (^{1}H-NMR (d^6-DMSO): δ = 5.63). The full assigment of the ^{1}H- and ^{13}C-NMR signals can be found in the experimental section of this master thesis (chapters 4.2 and 4.3).

3.2.3. IR-Spectroscopy

In the IR spectra of the seven complexes, there was observed the characteristic C-H strech for the Cp (Cp=cyclopentadienyl) moieties at 3115-3128 cm^{-1}. Typical bands for C=O and C=C streches were seen at 1522-1606 cm^{-1} and 1413-1499 cm^{-1}, respectively. In the case of the complexes bearing the thionated ligands, the bands for the C=S streches were observed at lower wavenumbers than the C=O streches of the complexes bearing the corresponding O,O- chelating ligands at 1587-1505 cm^{-1}. The ν_{C-H} bending was found at 815-832 cm^{-1}. In the spectrum of the tungstenocen bearing the dithiomaltol ligand there was also observed a band of C-S strech at 712 cm^{-1}. The values obtained are comparable to the ones found in the literature.[77]

3.2.4. ESI-MS

The structures of the obtained complexes where verified by ESI-MS. This is a soft ionization method with very little fragmentation, where the compounds are converted into ions in the gas phase.

For the analysis of the compounds of this thesis, the complexes where first dissolved in 1% methanol/water and then dispersed by electrospray into a fine aerosol.

The peaks of **$[Cp_2W(ligand)]^+$** where observed (having the characteristic tungsten isotope pattern) and recorded in the following table, as well as the theoretical values:

Detected Ion	m/z theoretical	m/z
$[Cp_2W(L1)]^+$	439.05	439.05
$[Cp_2W(L2)]^+$	455.03	455.17
$[Cp_2W(L3)]^+$	439.03	439.02
$[Cp_2W(L4)]^+$	455.03	454.98
$[Cp_2W(L5)]^+$	453.07	452.97
$[Cp_2W(L6)]^+$	465.05	464.94
$[Cp_2W(L7)]^+$	471.00	470.93

Table 1: experimental and theoretical m/z values of the compounds

3.2.5. X-Ray

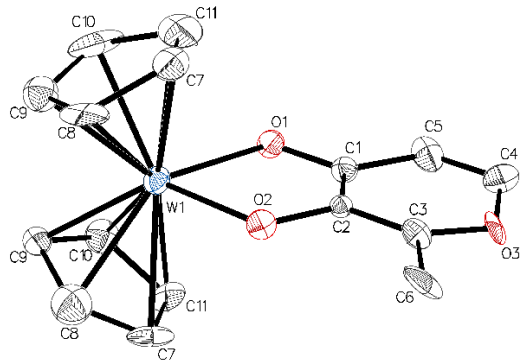
X-Ray suitable crystals (of 6 of the 7 compounds) using the vapor diffusion method, where a volatile solvent **A** slowly diffuses in a less volatile solvent **B**. For the crystallisation of compounds **2** and **3** there was used ethanol and hexane, in case of all the other complexes acetone and hexane.

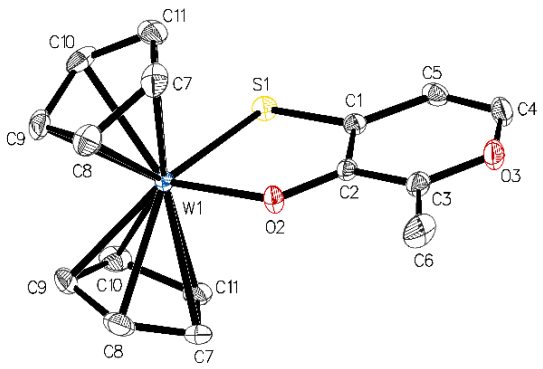
Compounds **2** and **5** crystallized in the orthorhombic *Pnma* space group, compound **3** in the orthorhombic $Pmn2_1$ space group, compound **4** in the $P2_1/c$ space group and compound **6** in the *P-1* triclinic group.

The bond lengths of the coordinating atoms in position 1 (O and S) are, as expected, very distinctive. W-S bonds are the longest, with values of in average 2.45 Å, whereas the W-O bonds are the shortest, collecting values in average of 2.11 Å.

In case of the W-O2 bonds, the observed values are between 2.067 and 2.102 Å. The bond lengths between the W and the Cp-rests are found in all compounds to be the in same range, collecting values from 2.297 to 2.319Å.

This proves that the only variations in the bond lengths oft he compounds are found at the newly formed bond between the central atom (W) and the coordinating ligand.





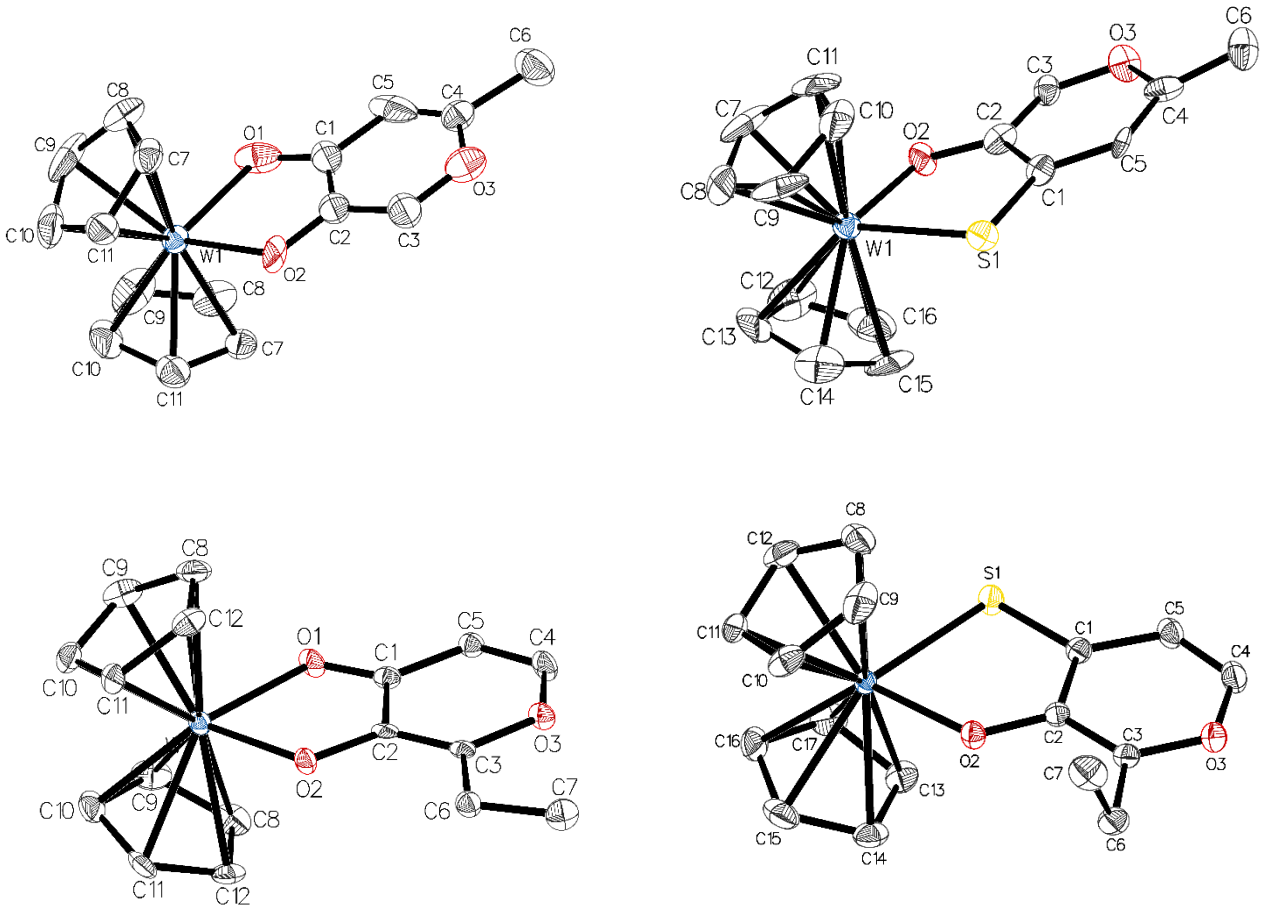


Fig.18: Molecular structure of compounds **2-6** (from left to right) in crystalline state. The hydrogen atoms where omitted for clarity, as well as the PF_6^- rests.

Compound	W-O2	W-O1/S1	W-Cp
2	2.067	2.441	2.319
3	2.102	2.114	2.300
4	2.091	2.454	2.297
5	2.074	2.118	2.309
6	2.082	2.454	2.318

Table 2: relevant bond lengths for compound **2-6**

The compound **7** could not be crystalized. Compound **1** is not listed because of its crystallographic disorder no valuable information could be extracted from it.

Supplimentary crystal data, data collection parameters, and structure refinement details are given in the tables in appendix.

3.2.6. Cyclic voltammetry

The electrochemical behavior of Cp_2WCl_2 and the seven complexes was studied by cyclic voltammetry in MeCN, with a scan rate of 200 mV/s, from -0.5 to +1.6V (in the case of compound **3** from -0.9 to +1.8V).

All measurements were performed in triplicate. MeCN was used in order to reach the desired concentration of 2 mM, because of the low solubility of the compounds in water. All the complexes exhibit a reversible one-electron process W^{IV} to W^{V}.

The stability of the tungstenocene derivatives is higher than the starting materia. For the synthesized complexes, the oxidation from W^{IV} to W^{V} takes place in the potential range 1.019 to 1.080 V vs. NHE measured in acetonitrile, while for the Cp_2WCl_2 it occurs at 0.627 V.

In the cell, the physiological significant potential is in the range -0.320 V ($NADP^+ + H^+ + 2e^- \rightarrow NADPH$) to +0.820 V ($O_2 + 4\ H^+ + 4e^- \rightarrow 2\ H_2O$), region in which the synthesized compounds don't show any redox activity.[104, 105] So, they will not be redox active.

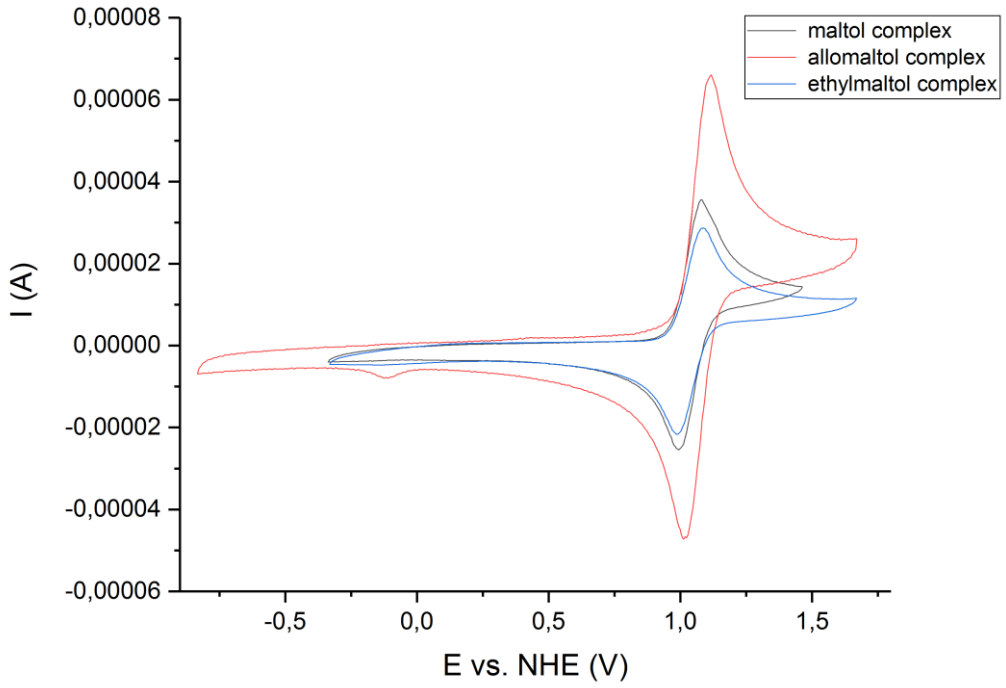


Fig.19: Cyclic Voltammogram of the O,O-chelates, compounds **1**, **3** and **5** in MeCN referenced to the NHE.

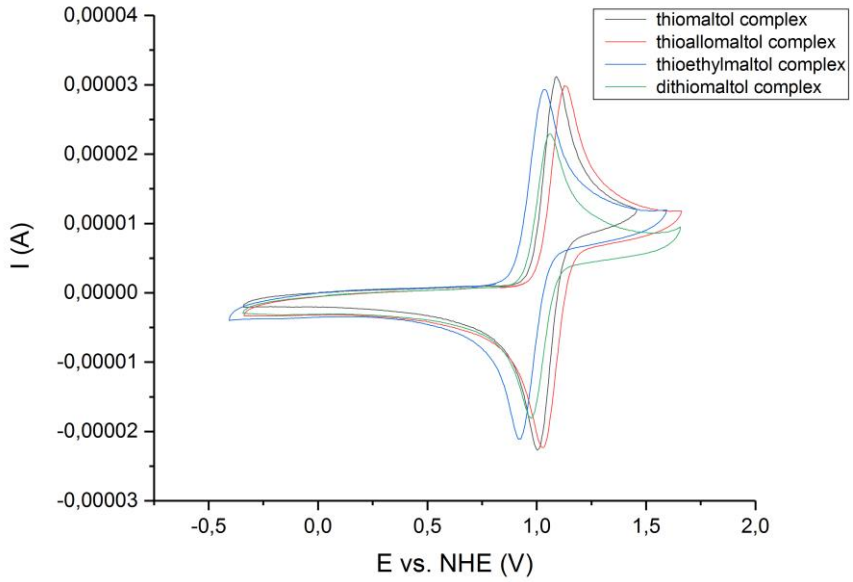


Fig.20: Cyclic Voltammogram of the O,S-chelates, compounds **2**, **4**, **6** and **7** in MeCN referenced to the NHE.

Upon coordination, previous studies have shown a shifting of the redox potential to higher potentials, like in the case of O,O-donor ligand scaffolds, which showed an increase of potential with 0.20-0.26 V.[77]

The compounds **1**, **3** and **5**, bearing O, O-donor atoms, experience the smallest shift to higher potentials when compared with Cp_2WCl_2. Due to the higher affinity of tungsten towards the softer donor S-atom, the compounds **2**, **4**, **6** and **7**, bearing the O,S-ligands show an increase of the redox potential, compared to their O,O-counterparts.

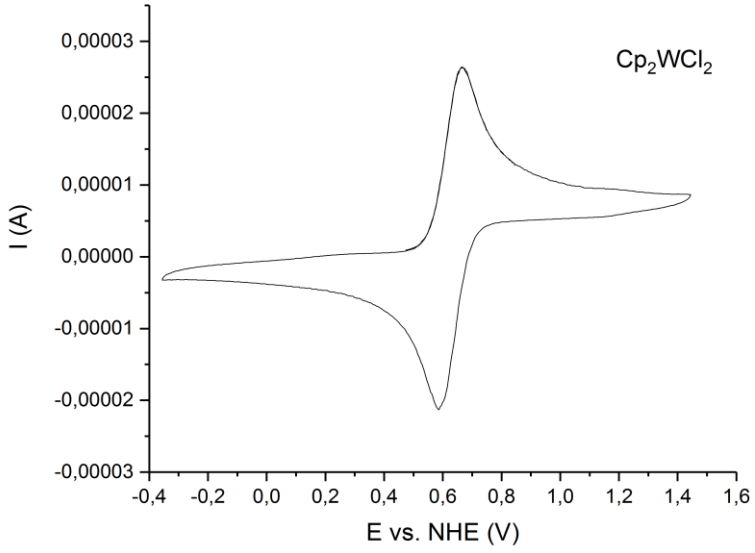


Fig.21: Cyclic Voltammogram of Cp_2WCl_2 in MeCN referenced to the NHE

The compounds **5** and **6**, the ones bearing an ethylmaltol- and thioethyl -maltolscaffold, show the highest shifts in the potential. Interestingly, the lowest redox potential of all the seven complexes, is shown by the one bearing the dithiomaltol-scaffold.

Compound	$E_{1/2}$ (V)
1	1.037
2	1.047
3	1.071
4	1.080
5	1.038
6	1.064
7	1.019
Cp_2WCl_2	0.627

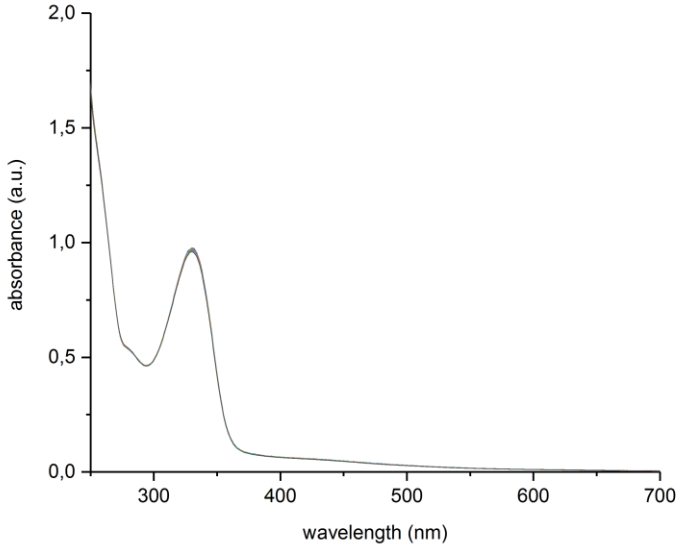
Table 3: Cyclic voltammetry data for compounds **1 - 7** and **Cp_2WCl_2** in MeCN. The redox potentials are reported vs. NHE.

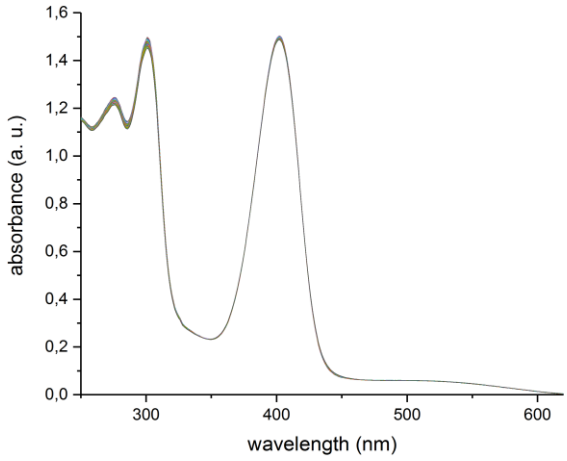
3.2.7. Aqueous solubility and stability measurements

UV/Vis measurements were performed in order to examine the stability of the synthesized compounds in aqueous solution (every hour, during 24 hours, at 293 K).

For this purpose, solutions of the compounds **1-7** in 10% PBS were prepared and to every solution was added 1 vol % of DMSO. PBS was used in order to mimic the physiological pH, which is about 7.4.

Over the 24 hours no shift of the peak maxima (λmax) was observed, this prooves that all complexes are stable in PBS at 25°C. Compounds **3**, **4**, **6** and **7** show a slowly precipitation out of solution over time.





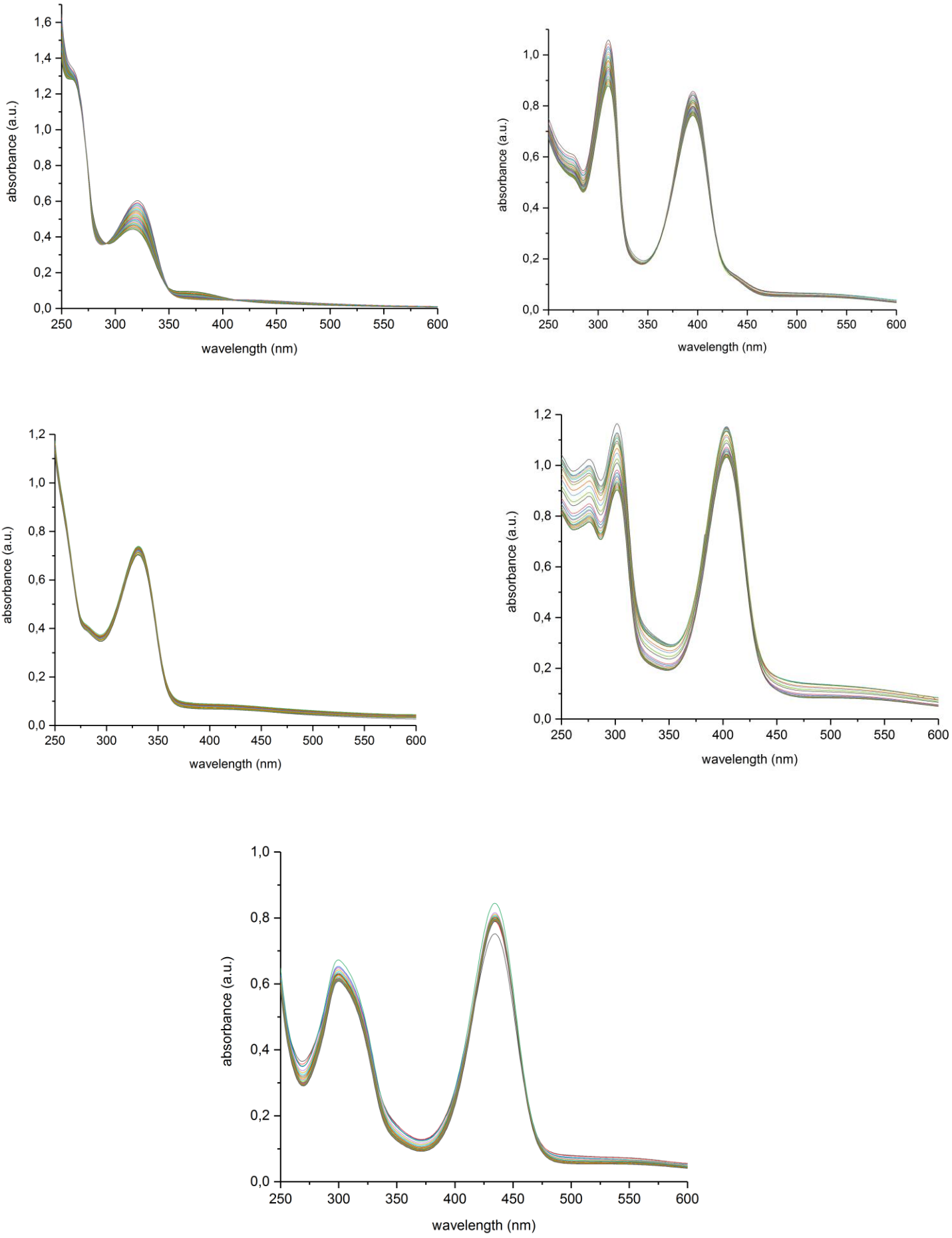


Fig.22: UV/Vis spectra of compound **1-7** (from left to right) over 24 hours in PBS (1% DMSO).

In the table bellow there are listed the wavelength of all the peak maxima and their molar extinction coefficients.

Compound	λmax [nm] (ε [M^{-1} cm^{-1}])
1	330 (39049)
2	402 (120301), 301 (119818), 276 (99628)
3	320 (60272)
4	395 (85700), 310 (105823)
5	331 (73797)
6	403 (115229), 302 (116416), 276 (102373)
7	435 (75152), 299 (62960)

Table 4: Wavelength of peak maxima(s) and molar extinction coefficients (ε) of compounds **1-7** in 10% PBS (1% DMSO)

3.2.8. Cytotoxic activity

The cytotoxic activities in vitro of **2** of the **7** synthesized compounds (at the point of the writing of this thesis) were investigated through the colorimetric MTT assay in 3 human cancer cell lines: A549 (non-small cell lung carcinoma), SW480 (colon carcinoma), and CH1/PA-1 (ovarian carcinoma). Their cytotoxic potential was compared with the one of the starting material, **Cp_2WCl_2**.

Compound	IC_{50} values [μM]		
	A549	**SW 480**	**CH1/PA-1**
1	> 200	> 200	85.21 ± 41.47
2	36.23 ± 5.97	36.41 ± 7.46	24.07 ± 3.07
Cp_2WCl_2	> 200	38.41 ± 21.29	2.41 ± 0.49

Table 5: Inhibition of cancer cell growth (IC_{50}) in three human cancer cell lines; 50 % inhibitory concentrations (mean ± SD), obtained by MTT assay (exposure time: 96 h).

All the tested compounds show the highest activity against the CP1/PA-1 cell line, which is the most sensitive of all 3, whereas against the SW 480 and A549 the activity was moderate and low, respectively.

Compound **2**, bearing the S,O-donor thiomaltol ligand, showed an interesting profile because it was active against all three types of cancer cells, highly active against

CH1/PA-1 and moderately active against SW 480 and A549. In the case of compound **1** no IC_{50} values could be reached for the other two cancer cell lines.

Compound	IC_{50} values [µM]		
	A549	**SW 480**	**CH1/PA-1**
$Cp_2Mo(L1)PF_6$	> 200	> 200	>200
$Cp_2Mo(L2)PF_6$	106.23 ± 15.46	55.42 ± 9.05	28.91 ± 9.53
Cp_2MoCl_2	> 200	>200	>200

Table 6: Inhibition of cancer cell growth (IC_{50}) in three human cancer cell lines; 50 % inhibitory concentrations (mean ± SD), obtained by MTT assay (exposure time: 96 h).

The anticancer activity of the analogous molybdenocene derivatives synthesized and characterized in the Keppler group were summarized in table 6. IC_{50} values for Cp_2Mo(**L1**)PF_6 and for Cp_2MoCl_2 could not be obtained for the three cancer cell lines. Cp_2Mo(**L2**)PF_6 showed cytotoxic activity against all cancer cell lines, but the results of compound **2** were better than the ones of its molybdenum-based counterpart (against A549, the cytotoxic activity was three times higher).[106]

Because of the instability of **Cp_2WCl_2** exposed to air or in physiological envioronment (it decomposes in less then 3h), it cannot be a possible candidate for further investigations (although it shows quite promising values).[77] Actually, the anticancer activity tests performed in vivo by Köpf and Köpf-Maier pointed out that tungstenocene dichloride showed the lowest activity among the other metallocene dichlorides of the neighboring transition metals (Ti, V, Mo).[77]

The high aqueous stability and redox potential of the substitubed tungstenocenes, make them ideal alternatives to **Cp_2WCl_2**. Further in vitro tests on the 3 cancer cell lines will be performed with the other 5 compounds and the results will be reported.

4. EXPERIMENTAL SECTION

4.1. CHEMICALS AND EQUIPMENT

4.1.1. Chemicals

Bis(cyclopentadienlyl)tungsten dichloride (99 %, Abcr), sodium methoxide (~ 95 %, Fluka), ammonium hexafluorophosphate (99 %, Sigma Aldrich), Lawesson's reagent (99 %, Acros Organics), PBS (sterile filtered, Sigma Life Science), maltol (99%, Sigma Aldrich) and ethylmaltol (99%, Sigma Aldrich) were purchased from the respective commercial source and used as obtained. Allomaltol was provided by a former colleague, using the literature available. Thiomaltol, thioallomaltol, thioethylmaltol and dithiomaltol were obtained according to the literature procedures.

All solvents, purchased from commercial sources, were of HPLC grade and used without further purification. Methanol was of HPLC grade and dried over molecular sieves (3 Å) before using it.

4.1.2. Equipment

NMR-Spectra

NMR spectra were recorded with a Bruker FT-NMR Avance IIITM 500 MHz spectrometer at 500.10 (^{1}H), and 125.75 MHz (^{13}C), respectively. 2D-NMR measurements were recorded utilizing standard pulse programs at 500.32 MHz (^{1}H) and 125.81 MHz (^{13}C).

ESI-MS

Electrospray ionization mass spectra were recorded on a Bruker AmaZon SL ion trap mass spectrometer (Bruker Daltonics GmbH). Data were obtained and processed with Compass 1.3 and Data Analysis 4.0 (Bruker Daltonics GmbH).

Elemental Analysis

Elemental analysis was performed by the Microanalytical Laboratory of the University of Vienna on a Perkin Elmer 2400 CHN elemental analyzer or a FisonsEA 1108 CHNS-O Element analyzer.

IR Analysis

Infrared spectra were attained on a Bruker Vertex 70 FT-IR-spectrometer with an ATR-unit (attenuated total reflection unit) in the range of 4000 – 600 cm^{-1}. Intensities of the reported bands are noted with s for strong, m for medium and w for weak; broad signals are additionally specified with the letter b in front of these abbreviations.

X-Ray Analysis

X-ray diffraction analyses were carried out on a Bruker X8 APEX II CCD diffractometer at 100 K.

Cyclic voltammetry

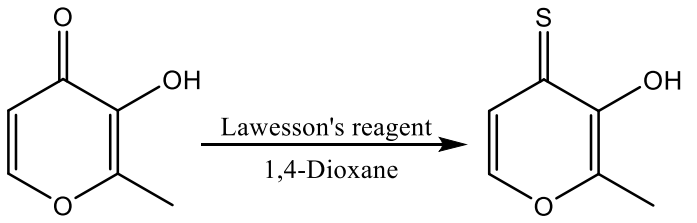
Cyclic voltammograms (CVs) were measured in a three-electrode cell using 2 mm diameter glassy carbon disk working electrode, a platinum auxiliary electrode and a $Ag|Ag^+$ reference electrode containing 0.1 M $AgNO_3$. Measurements were carried out at room temperature using an EG&G PARC potentiostat/galvanostat 273A. Deoxygenation of solutions was performed by purging a stream of argon through the solution for 3 min and the experiment was accomplished under an argon atmosphere. The potentials were measured in a freshly prepared solution of (*n* $Bu_4N)[BF_4]$ (0.1 M) in acetonitrile using ferrocene ($Fe(\eta^5\text{-}C_5H_5)_2$) ($E_{1/2}$ = +0.72 V vs. NHE) as an internal standard and are quoted relative to the normal hydrogen electrode (NHE).

UV/VIS Spectra

UV/Vis data was recorded on a Perkin Elmer Lambda 650 UV/Vis Spectrophotometer with a Peltier element for temperature control.

4.2. SYNTHESIS of O, S – CHELATING LIGANDS

3-Hydroxy-2-methyl-4H-pyran-4-thione



$C_6H_6O_3$
M= 126,11 g/mol

$C_6H_6O_2S$
M= 142,18 g/mol

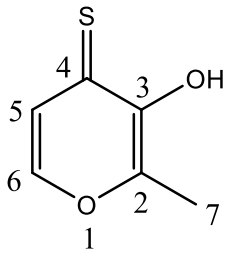
Maltol (1.00 g, 8.2 mmol) and Lawesson's reagent (1.70 g, 4.2 mmol,) were dissolved in 1,4-dioxane (20 mL) and refluxed for 4 hours. The solvent was removed and after column chromatography (n-hexane/ethyl acetate = 10:1) the yellow crystalline product was obtained and dried in vacuo.

Yield: 0.368 mg (32%), yellow crystals

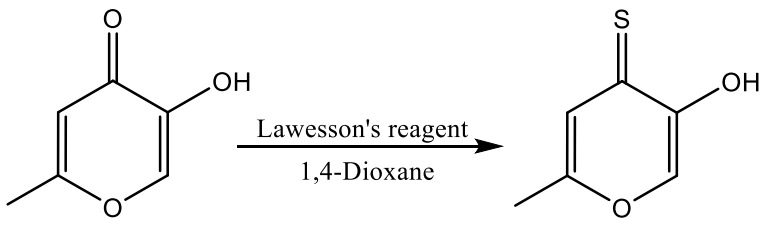
Characterization: ^{1}H-NMR

NMR-Spectroscopy:

^{1}H-NMR (d_6-DMSO): δ = 2.40 (s, 3H, CH_3), 7.35 (d, 1H, ^{3}J(H, H)= 5 Hz, H5), 8.0 (d, 1H, ^{3}J(H, H) = 5 Hz, H6), 8.28 (s, 1H, OH).



5-Hydroxy-2-methyl-4H-pyran-4-thione



$C_6H_6O_3$
M = 126.11 g/mol

$C_6H_6O_2S$
M = 142.18 g/mol

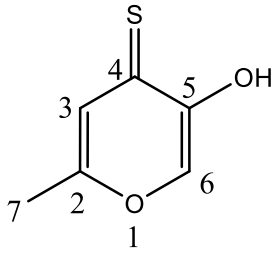
Allomaltol (1.00 g, 8.2 mmol) and Lawesson's reagent (1.70 g, 4.2 mmol,) were dissolved in 1,4-dioxane (20 mL) and refluxed for 4 hours. The solvent was removed and after column chromatography (n-hexane/ethyl acetate = 10:1) the yellow crystalline product was obtained and dried in vacuo.

Yield: 0.210 mg (17%), yellow crystals

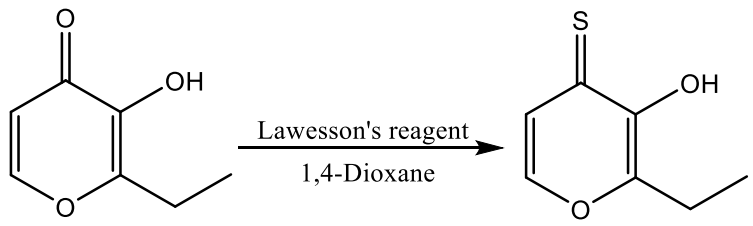
Characterization: ^{1}H-NMR

NMR-Spectroscopy:

^{1}H-NMR (d_6-DMSO): δ = 2.33 (s, 3H, CH_3), 7.35 (s, 1H, H3), 8.27 (s, 1H, H6), 8.42 (s, 1H, OH).



2-Ethyl-3-hydroxy-4H-pyran-4-thione



$C_7H_8O_3$
M = 140.14 g/mol

$C_7H_8O_2S$
M = 156.2 g/mol

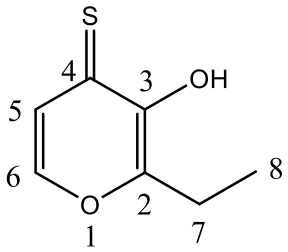
Ethylmaltol (2.00 g, 14.3 mmol) and Lawesson's reagent (1.92 g, 4.8 mmol,) were dissolved in 1,4-dioxane (40 mL) and refluxed for 4 hours. The solvent was removed and after column chromatography (n-hexane/ethyl acetate = 10:1) the yellow crystalline product was obtained and dried in vacuo.

Yield: 1.304g (59%), yellow-orange oil

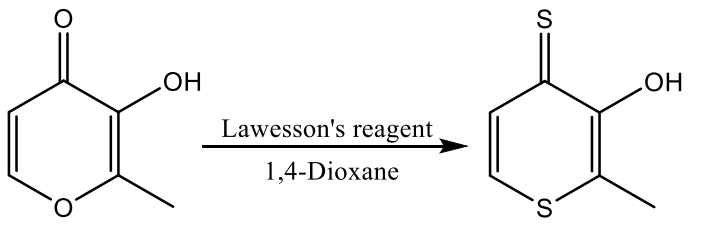
Characterization: ^{1}H NMR

NMR-Spectroscopy:

^{1}H-NMR (d_6-DMSO): δ = 1.22 (t, 3H, ^{3}J(H, H)= 7,6 Hz, CH_3), 2.78 (q, 2H, ^{3}J(H, H)= 7,6 Hz, CH_2), 7.36 (d, 1H, ^{3}J(H, H)= 5 Hz, H5), 8.13 (d, 1H, ^{3}J(H, H)= 4,9 Hz, H6), 8.29 (s, 1H, OH).



2-Methyl-3-hydroxy-4H-thiopyran-4-thione



$C_6H_6O_3$
M = 126.11 g/mol

$C_6H_6OS_2$
M = 158.24 g/mol

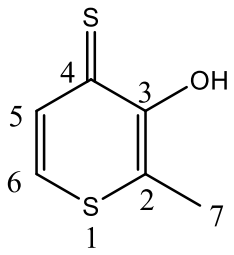
Maltol (2.50 g, 19.83 mmol) and Lawesson's reagent (8.42 g, 20.82 mmol,) were dissolved in 1,4-dioxane (25 mL) and refluxed for 4 hours. The solvent was removed and after column chromatography (n-hexane/ethyl acetate = 10:1) the yellow crystalline product was obtained and dried in vacuo.

Yield: 150 mg (5%), yellow crystals

Characterization: ^{1}H NMR

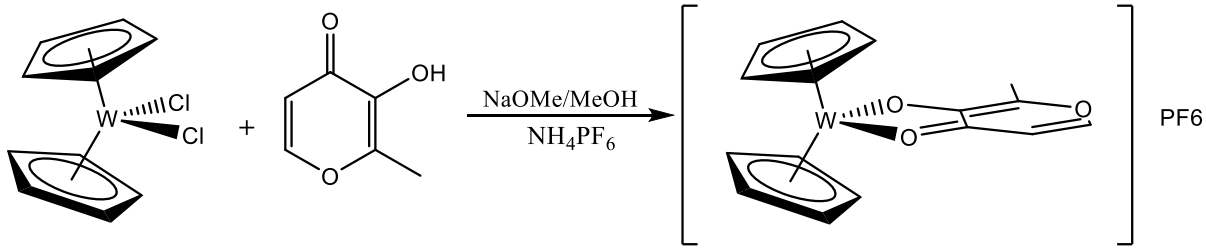
NMR-Spectroscopy:

^{1}H-NMR (d_6-DMSO): δ = 2.47(s, 3H, CH_3), 8.16(d, 1H, ^{3}J(H, H)= 9,4 Hz, H5), 8.21(d, 1H, ^{3}J(H, H)= 9,4 Hz, H6), 9.37(s, 1H, OH).



4.3. SYNTHESIS OF TUNGSTENOCENE COMPLEXES

Bis(η^5-cyclopentadienyl)[2-methyl-3-(oxo-κO)-4-(1*H*)-pyran-4-ato-κO] tungsten(IV) hexafluorophosphate



$C_{10}H_{10}Cl_2W$
M = 384.94 g/mol

$C_6H_6O_3$
M = 126.11 g/mol

$C_{16}H_{15}O_3WPF_6$
M = 584.09 g/mol

Bis(cyclopentadienyl)tungsten dichloride (135 mg, 0.350 mmol), 3-Hydroxy-2-methyl-4*H*-pyran-4-one (48 mg, 0.385 mmol) and sodium methoxide (21 mg, 0.385 mmol) were dissolved in 25 mL methanol (dried over molecular sieves 3Å) and stirred for 16 h at room temperature under an argon atmosphere. Ammonium hexafluorophosphate (63 mg, 0.385 mmol) was added and stirred for 2 hours. The formed precipitate was filtered, washed with MeOH and dried *in vacuo.*

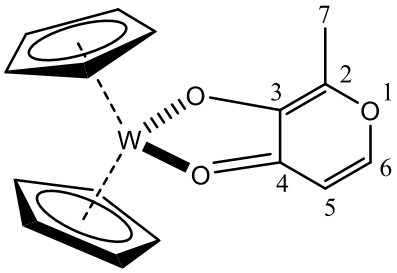
Yield: 119 mg (95 %), brown powder.

Solubility in PBS: 0.23 mg/ml.

Characterization: NMR, MS, EA, IR

^{1}H-NMR (d$_6$-DMSO): δ = 2.35 (s, 3H, H7) 5.90 (s, 10H, H_{Cp}), 7.03 (d, ^3J(H, H) = 5.0 Hz, 1H, H5), 8.34 (d, ^3J(H, H) = 5.0 Hz, 1H, H6) ppm.

^{13}C-NMR (d$_6$-DMSO): δ = 14.5 (C7), 98.1 (Cp), 110.4 (C5), 156.6 (C6), 157.5 (C2), 161.5 (C3), 187.0 (C4) ppm.



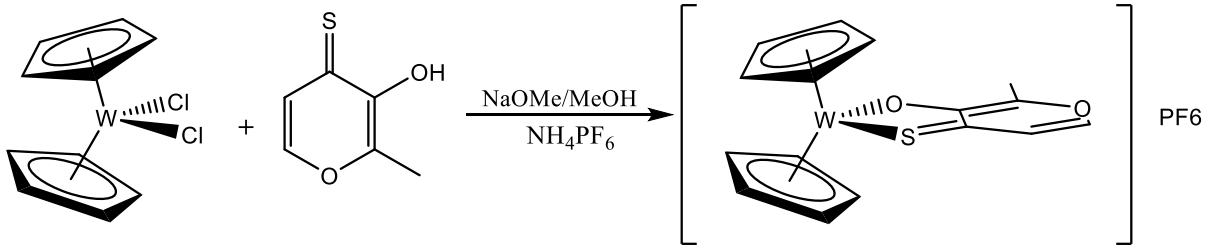
(ESI$^+$) m/z: 439.05 **[Cp_2W(maltolate)]$^+$**

EA: $C_{16}H_{15}O_3WPF_6$

	C	**H**
Calculated (%)	32.90	2.59
Found (%)	32.79	2.63

IR: v = 3128 w (ν_{C-H}, Cp), 1604, 1547 m ($\nu_{C=O}$), 1476, 1429 m ($\nu_{C=C}$), 820 s (ν_{C-H}) cm^{-1}.

Bis(η^5-cyclopentadienyl)[2-methyl-3-(oxo-κO)-pyran-4-(1H)-thionato-κS] tungsten(IV) hexafluorophosphate



$C_{10}H_{10}Cl_2W$
M = 384.94 g/mol

$C_6H_6O_2S$
M = 142.18 g/mol

$C_{16}H_{15}SO_2WPF_6$
M = 600.16 g/mol

Bis(cyclopentadienyl)tungsten dichloride (135 mg, 0.35 mmol), 3-Hydroxy-2-methyl-4*H*-pyran-4-thione (50 mg, 0.35 mmol) and sodium methoxide (19 mg, 0.35 mmol) were dissolved in 20 mL methanol (dried over molecular sieves 3Å) and stirred for 48 h at room temperature under an argon atmosphere. Solid materials were removed by filtration and ammonium hexafluorophosphate (118 mg, 0.7 mmol) was added to the solution and stirred for 3 hours. The formed precipitate was filtered, washed with MeOH and dried *in vacuo.*

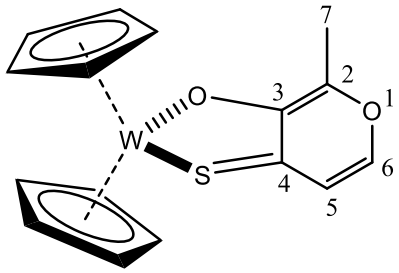
Yield: 108 mg (52 %), red powder.

Solubility in PBS: 0.25 mg/ml.

Characterization: NMR, MS, EA, IR

^{1}H-NMR (d$_6$-DMSO): δ = 2.34 (s, 3H, H7), 5.70 (s, 10H, H_{Cp}), 7.83 (d, ^3J(H, H) = 4.6 Hz, 1H, H5), 8.21 (d, ^3J(H, H) = 4.6 Hz, 1H, H6) ppm.

^{13}C-NMR (d$_6$-DMSO): δ = 15.3 (C7), 96.3 (Cp), 119.4 (C5), 149.7 (C6), 156.7 (C2), 171.4 (C3), 181.5 (C4) ppm.



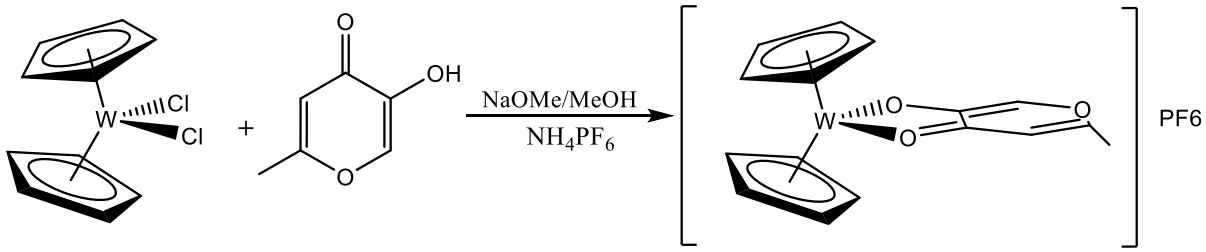
(ESI$^+$) m/z: 455.17 **[Cp_2W(thiomaltolate)]$^+$**

EA: $C_{16}H_{15}O_2SWPF_6$

	C	**H**	**S**
Calculated (%)	32.02	2.59	5.34
Found (%)	32.28	2.45	5.01

IR: v = 3124 m ($\nu_{C–H}$, Cp), 1583, 1505 m ($\nu_{C=S}$), 1470, 1424 m ($\nu_{C=C}$), 822 s (ν_{C-H}) cm^{-1}.

Bis(η^5-cyclopentadienyl)[2-methyl-5-(oxo-κO)-4-(1*H*)-pyran-4-ato-κO] tungsten(IV) hexafluorophosphate



$C_{10}H_{10}Cl_2W$
M = 384.94 g/mol

$C_6H_6O_3$
M = 126.11 g/mol

$C_{16}H_{15}O_3WPF_6$
M = 584.09 g/mol

Bis(cyclopentadienyl)tungsten dichloride (135 mg, 0.350 mmol), 5-Hydroxy-2-methyl-4*H*-pyran-4-one (44 mg, 0.350 mmol) and sodium methoxide (21 mg, 0.385 mmol) were dissolved in 25 mL methanol (dried over molecular sieves 3Å) and stirred for 72 h at room temperature under an argon atmosphere. Ammonium hexafluorophosphate (118 mg, 0.7 mmol) was added and stirred for 4 hours. The formed precipitate was filtered and dried *in vacuo.*

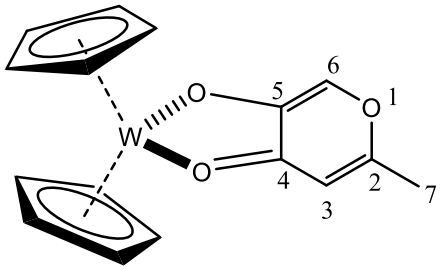
Yield: 78 mg (38 %), brown powder.

Solubility in PBS: 0.35 mg/ml

Characterization: NMR, MS, EA, IR

^{1}H-NMR (d$_6$-DMSO): *δ* = 2.48 (s, 3H, H7), 5.91 (s, 10H, H_{Cp}), 6.98 (s, 1H, H3), 8.31 (s, 1H, H6).

^{13}C-NMR (d$_6$-DMSO): δ = 19.83 (C7), 98.53(Cp), 109.61 (C3), 144.72 (C6), 163.61 (C5), 169.95 (C2), 190.41 (C4).



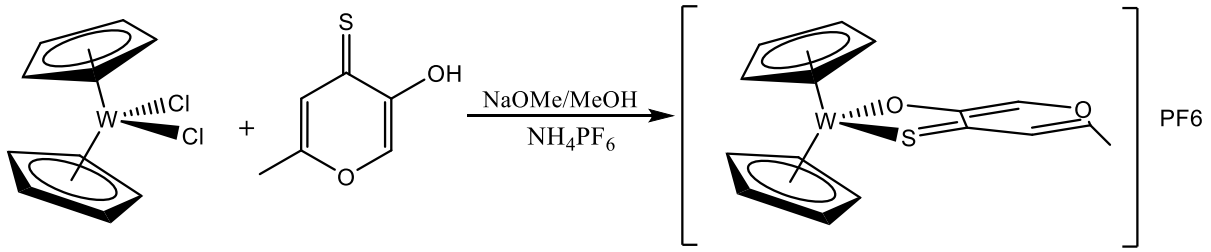
(ESI$^+$) m/z: 439.02 **[Cp_2W(allomaltolate)]$^+$**

EA: $C_{16}H_{15}O_3WPF_6$

	C	**H**
Calculated (%)	32.90	2.59
Found (%)	32.72	2.58

IR: v = 3118 w (ν_{C-H}, Cp), 1606, 1551 m ($\nu_{C=O}$), 1472, 1433 m ($\nu_{C=C}$), 819 s (ν_{C-H}) cm^{-1}.

Bis(η^5-cyclopentadienyl)[2-methyl-5-(oxo-κO)-pyran-4-(1H)-thionato-κS] tungsten(IV) hexafluorophosphate



$C_{10}H_{10}Cl_2W$
M = 384.94 g/mol

$C_6H_6O_2S$
M = 142.18 g/mol

$C_{16}H_{15}O_2SWPF_6$
M = 600.16 g/mol

Bis(cyclopentadienyl)tungsten dichloride (135 mg, 0.35 mmol), 5-Hydroxy-2-methyl-4*H*-pyran-4-thione (50 mg, 0.35 mmol) and sodium methoxide (21 mg, 0.385 mmol) were dissolved in 25 mL methanol (dried over molecular sieves 3Å) and stirred for 72 h at room temperature under an argon atmosphere. Solid materials were removed by filtration and ammonium hexafluorophosphate (114 mg, 0.7 mmol) was added to the solution and stirred for 4 hours. The flask was stored at 4°C till next day. Then the formed precipitate was filtered off and dissolved in DCM (potential salt rests were filtered off). The solvent was removed under reduced pressure and the product was dried *in vacuo.*

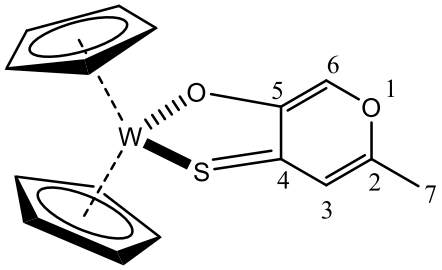
Yield: 88 mg (42 %), red powder.

Solubility in PBS: 0.41 mg/ml

Characterization: NMR, MS, EA, IR

^{1}H-NMR (d$_6$-DMSO): δ = 2.48 (s, 3H, H7), 5.70 (s, 10H, H_{Cp}), 7.86 (s, 1H, H3), 8.15 (s, 1H, H6).

^{13}C-NMR (d$_6$-DMSO): δ = 18.97 (C7), 96.61 (Cp), 119.36 (C3), 142.92 (C6), 162.85 (C5), 172. 97 (C2), 187.96 (C4).



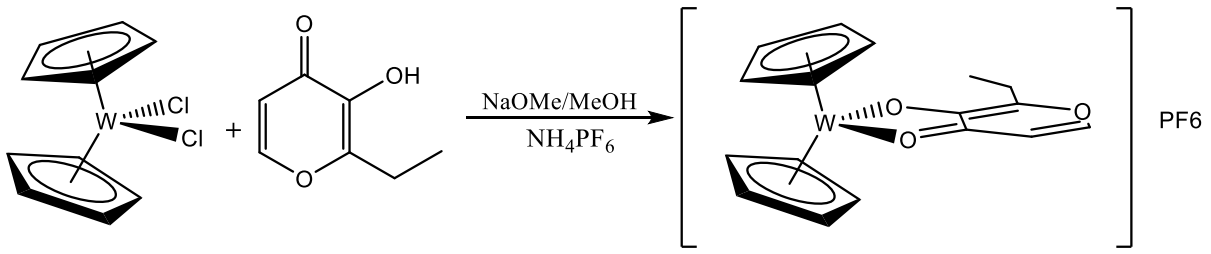
(ESI$^+$) m/z: 454.98 **[Cp$_2$W(thioallomaltolate)]$^+$**

EA: $C_{16}H_{15}O_2SWPF_6$

	C	**H**	**S**
Calculated (%)	32.02	2.59	5.34
Found (%)	31.68	2.48	5.57

IR: v = 3126 m ($\nu_{C–H}$, Cp), 1587, 1510 m ($\nu_{C=S}$), 1439 m ($\nu_{C=C}$), 826 s (ν_{C-H}) cm^{-1}.

Bis(η^5-cyclopentadienyl)[2-ethyl-3-(oxo-κO)-4-(1*H*)-pyran-4-ato-κO] tungsten(IV) hexafluorophosphate



$C_{10}H_{10}Cl_2W$
M = 384.94 g/mol

$C_7H_8O_3$
M = 140.14 g/mol

$C_{17}H_{17}O_3WPF_6$
M = 598.12 g/mol

Bis(cyclopentadienyl)tungsten dichloride (135 mg, 0.35 mmol), 2-Ethyl-3-hydroxy-4H-pyran-4-one (49 mg, 0.35 mmol) and sodium methoxide (21 mg, 0.385 mmol) were dissolved in 25 mL methanol (dried over molecular sieves 3Å) and stirred for 72 h at room temperature under an argon atmosphere. Solid materials were removed by filtration and ammonium hexafluorophosphate (114 mg, 0.7 mmol) was added to the solution and stirred for 4 hours. The flask was stored at 4° for 72h. Then the formed precipitate was filtered off and dissolved in DCM (potential salt rests were filtered off). The solvent was removed under reduced pressure and the product was dried *in vacuo.*

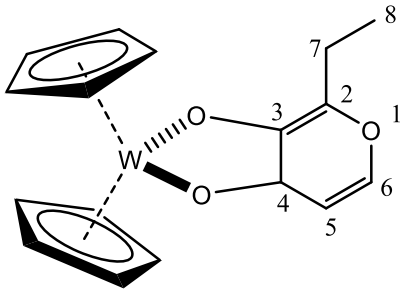
Yield: 65.8 mg (31 %), brown powder.

Solubility in PBS: 0.55 mg/ml

Characterization: NMR, MS, EA, IR

^{1}H-NMR (d$_6$-DMSO): *δ* = 1.14 (t, 3H, ^{3}J (H, H) = 7.6 Hz, H8), 2.72 (q, 2H, ^{3}J (H, H) = 7.6 Hz, H7), 5.91 (s, 10H, H_{Cp}), 7.06 (d, 1H, ^{3}J (H, H) = 5 Hz, H5), 8.39 (d, 1H, ^{3}J (H, H) = 5 Hz, H6).

^{13}C-NMR (d$_6$-DMSO): δ = 11.3 (C8), 21.95 (C7), 98.57 (Cp), 110.88 (C5), 157.16 (C6), 161.3 (C2), 161.9 (C3), 187.74(C4).



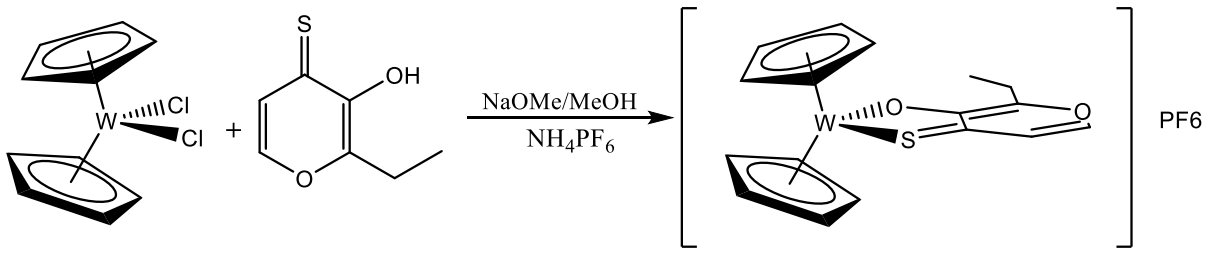
(ESI$^+$) m/z: 452.97 **[Cp_2W(ethylmaltolate)]$^+$**

EA: $C_{17}H_{17}O_3WPF_6$

	C	**H**
Calculated (%)	34.14	2.86
Found (%)	34.67	3.00

IR: v = 3125 w ($\nu_{C–H}$, Cp), 1597, 1522 m ($\nu_{C=O}$), 1475, 1429 m ($\nu_{C=C}$), 998, 945, s (ν_{C-C}), 819 s (ν_{C-H}), 725 s (ν_{-CH2-}) cm^{-1}.

Bis(η^5-cyclopentadienyl)[2-ethyl-3-(oxo-κO)-4-(1*H*)-pyran-4-ato-κS] tungsten(IV) hexafluorophosphate



$C_{10}H_{10}Cl_2W$
M = 384.94 g/mol

$C_7H_8O_2S$
M = 156.2 g/mol

$C_{17}H_{17}O_2SWPF_6$
M = 614.19 g/mol

Bis(cyclopentadienyl)tungsten dichloride (135 mg, 0.35 mmol), 2-Ethyl-3-hydroxy-4H-pyran-4-thione (54 mg, 0.35 mmol) and sodium methoxide (21 mg, 0.385 mmol) were dissolved in 25 mL methanol (dried over molecular sieves 3Å) and stirred for 72 h at room temperature under an argon atmosphere. Solid materials were removed by filtration and ammonium hexafluorophosphate (114 mg, 0.7 mmol) was added to the solution and stirred for 4 hours. The flask was stored at 4° for 72h. Then the formed precipitate was filtered off and dissolved in DCM (potential salt rests were filtred off). The solvent was removed under reduced pressure and the product was dried *in vacuo.*

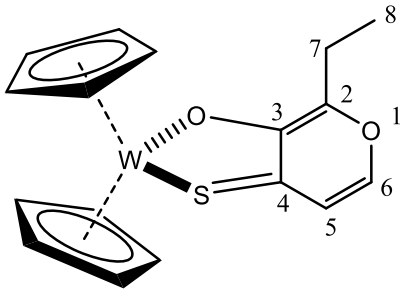
Yield: 104 mg (48 %), brown powder.

Solubility in PBS: 0.32 mg/ml

Characterization: NMR, MS, EA, IR

^{1}H-NMR (d$_6$-DMSO): δ = 1.31 (t, 3H, ^{3}J (H, H) = 7.6 Hz, H8), 2.71 (q, 2H, ^{3}J (H, H) = 7.5 Hz, H7), 5.71 (s, 10H, H_{Cp}), 7.86 (d, 1H, ^{3}J (H, H) = 4.6 Hz, H5), 8.25 (d, 1H, ^{3}J (H, H) = 4.6 Hz, H6).

^{13}C-NMR (d$_6$-DMSO): δ = 10.94 (C8), 22.57 (C7), 96.74 (Cp), 119.89 (C5), 150.25 (C6), 160.77 (C2), 171.30 (C3), 182.38 (C4).



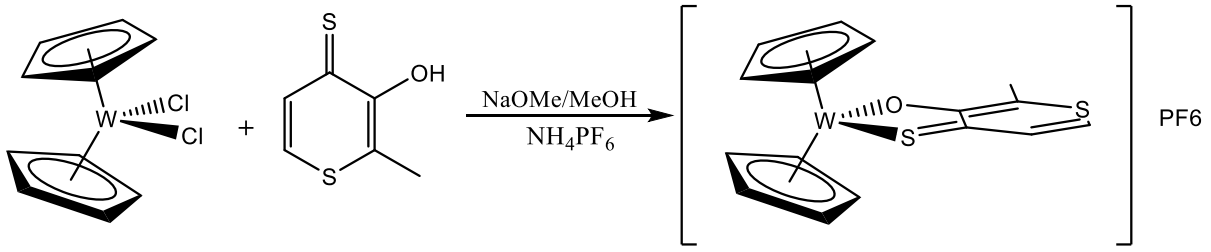
(ESI$^+$) m/z: 468.94 **[Cp_2W(thioethylmaltolate)]$^+$**

EA: $C_{17}H_{17}O_2SWPF_6$

	C	**H**	**S**
Calculated (%)	33.24	2.79	5.22
Found (%)	35.25	3.16	4.95

IR: v = 3126 w (ν_{C-H}, Cp), 1571 m ($\nu_{C=S}$), 1499, 1408 m ($\nu_{C=C}$), 815 s (ν_{C-H}) cm^{-1}.

Bis(η^5-cyclopentadienyl)[2-methyl-3-(oxo-κO)-4-(1*H*)-thiopyran-4-ato-κS] tungsten(IV) hexafluorophosphate



$C_{10}H_{10}Cl_2W$
M = 384.94 g/mol

$C_6H_6OS_2$
M = 158.24 g/mol

$C_{16}H_{15}S_2OWPF_6$
M = 616.23 g/mol

Bis(cyclopentadienyl)tungsten dichloride (135 mg, 0.350 mmol), 3-Hydroxy-2-methyl-4*H*-thiopyran-4-thione (55mg, 0.350 mmol) and sodium methoxide (21 mg, 0.385 mmol) were dissolved in 25 mL methanol (dried over molecular sieves 3Å) and stirred for 72 h at room temperature under an argon atmosphere. Ammonium hexafluorophosphate (114 mg, 0.7 mmol) was added and stirred for 4 hours. The formed precipitate was filtered and dried *in vacuo.*

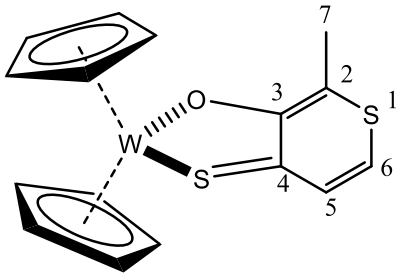
Yield: 76 mg (35 %), brown-green powder.

Solubility in PBS: 0.55 mg/ml

Characterization: NMR, MS, EA, IR

^{1}H-NMR (d$_6$-DMSO): δ = 2.35 (s, 3H, H7) 5.71 (s, 10H, H_{Cp}), 8.32 (d, ^3J(H, H) = 9.0 Hz, 1H, H5), 8.40 (d, ^3J(H, H) = 9.0 Hz, 1H, H6) ppm.

^{13}C-NMR (d$_6$-DMSO): δ = 18.1 (C7), 96.6 (Cp), 134.3 (C5), 135.1 (C6), 138.9 (C2), 178.3 (C3), 178.5 (C4) ppm.



(ESI$^+$) m/z: 470.93 **[Cp$_2$W(dithiomaltolate)]$^+$**

EA: $C_{16}H_{15}OS_2WPF_6$

	C	**H**	**S**
Calculated(%)	31.19	2.45	10.41
Found (%)	25.62	2.15	8.48

IR: v = 3115 m ($\nu_{C–H}$, Cp), 1508 m ($\nu_{C=S}$), 1413 m ($\nu_{C=C}$), 832 s (ν_{C-H}), 712 s (ν_{C-S}) cm^{-1}.

5. CONCLUSIONS AND OUTLOOK

The aim of this master thesis was to synthesize new tungstenocene complexes by changing the two chlorido ligands with different bioactive chelating ligands.

The four O,S-ligands were synthesized through thionization of the O,O-ligands (maltol, allomaltol and ethylmaltol) with Lawesson's reagent and characterized via ^{1}H-NMR spectroscopy.

Seven new tungstenocene compleaxes bearing O,O- and O,S-chelating ligands were synthesized in moderate to very good yields. They had hexafluorophosphate as counter ion and were characterized by ^{1}H- and ^{13}C-spectroscopy, elemental analysis, ESI-MS, FT-IR spectroscopy and by X-Ray diffraction.

The stability in aqueous solution (10% PBS with 1% DMSO) has been investigated by UV-Vis spectroscopy over. All compounds were found to be stable over 24 hours, although compounds **3, 4**, **6** and **7** slowly precipitated out of solution over time.

Cyclic voltammetry studies were also performed, all compounds showed reversible peaks out of the physiological region, which indicates redox stability in biological systems.

The in vitro cytotoxic activity of the first two compounds and of the starting material, Cp_2WCl_2, were investigated in 3 human cancer cell lines, A549 (non-small cell lung carcinoma), SW480 (colon carcinoma) and CH1/PA-1 (ovarian carcinoma). Compound **1** was found to be active only against the CH1/PA-1 cell line, meanwhile compound **2** showed activity against all the three types of cancer cell lines. Besides, the compounds were found to be more active than their molybdenum-based counterparts. The other synthesized compounds will be soon tested on the above mentioned cancer cell lines and the results will be reported.

Further research may include using other bioactive ligands (for example naphthaquinones or oximes) in order to synthesize new tungstenocenes or derivatizing the Cp (Cyclopentadienyl) rings. The information gained via the in vitro tests with the other five compounds will show if further in vivo tests on mice should be performed, in order to obtain a better picture of the effects of these potential anticancer drugs on living organisms.

6. APPENDIX

6.1. X-Ray diffraction data

Bis(η^5-cyclopentadienyl)[2-methyl-3-(oxo-κO)-pyran-4-(1H)-thionato-κS] tungsten(IV) hexafluorophosphate 2

Sample and crystal data – compound **2**

Chemical formula	$C_{16}H_{15}O_2SWPF_6$	**Crystal system**	orthorhombic	
Formula weight [g/mol]	600.16	**Space group**	Pnma	
Temperature [K]	100	**Z**	4	
Measurement method	\Φ and \ω scans	**Volume [Å^3]**	1745.34(7)	
Radiation (Wavelength [Å])	MoKα (λ = 0.71073)	**Uni cell dimensions [Å] and [°]**	16.7909(4)	90
Crystal size/[mm^3]	0.1 × 0.07 × 0.03		8.3122(2)	90
Crystal habit	clear red block		12.5052(3)	90
Density calculated/ [g/cm^3]	2.284	**Absorption coefficient/ [mm^{-1}]**	6.901	
Abs. correction type	multiscan	**F(000) [e$^-$]**	1144.0	

Data collection and structure refinement – compound **2**

Index ranges	-23 ≤ h ≤ 23, -10 ≤ k ≤ 11, -17 ≤ l ≤ 17	**Theta range for data collection [°]**	4.852 to 60.138	
Reflections number	14654	**Data/restraints/ parameters**	2718/0/143	
Refinement method	Least squares	**Final R indices**	[all data]	R_1 = 0.0185, wR_2 = 0.0355
Function minimized	Σ w(F_o^2 - Fc^2)^2		I>=2σ (I)	R1 = 0.0156, wR2 = 0.0347
Goodness-of-fit on F^2	1.065			
Largest diff. peak and hole [e Å^{-3}]	0.46/-1.22			

Bis(η^5-cyclopentadienyl)[2-methyl-5-(oxo-κO)-4-(1*H*)-pyran-4-ato-κO] tungsten(IV) hexafluorophosphate **3**

Sample and crystal data – compound **3**

Chemical formula	$C_{16}H_{15}F_6O_3PW$	**Crystal system**	orthorhombic	
Formula weight [g/mol]	584.10	**Space group**	$Pmn2_1$	
Temperature [K]	100	**Z**	2	
Measurement method	\Φ and \ω scans	**Volume [Å^3]**	854.39(7)	
Radiation (Wavelength [Å])	MoKα (λ = 0.71073)	**Unit cell dimensions [Å] and [°]**	8.5280(4)	90
Crystal size/[mm^3]	0.14 × 0.14 × 0.04		6.8174(3)	90
Crystal habit	Clear brown block		14.6957(6)	90
Density calculated/ [g/cm^3]	2.270	**Absorption coefficient/ [mm^{-1}]**	6.931	
Abs. correction type	multiscan	**F(000) [e$^-$]**	556.0	

Data collection and structure refinement – compound **3**

Index ranges	-10 ≤ h ≤ 10, -8 ≤ k ≤ 8, -17 ≤ l ≤ 17	**Theta range for data collection [°]**	5.522 to 50.692	
Reflections number	6904	**Data/restraints/ parameters**	1666/1/143	
Refinement method	Least squares	**Final R indices**	[all data]	R_1 = 0.0283, wR_2 = 0.0647
Function minimized	Σ w(F_o^2 - Fc^2)^2		I>=2σ (I)	R_1 = 0.0269, wR_2 = 0.0642
Goodness-of-fit on F^2	1.059			
Largest diff. peak and hole [e Å^{-3}]	2.08/-0.65			

Bis(η^5-cyclopentadienyl)[2-methyl-5-(oxo-κO)-pyran-4-(1H)-thionato-κS] tungsten(IV) hexafluorophosphate 4

Sample and crystal data – compound **4**

Chemical formula	$C_{16}H_{15}F_6O_2PSW$	**Crystal system**	monoclinic	
Formula weight [g/mol]	600.16	**Space group**	$P2_1/c$	
Temperature [K]	100	**Z**	4	
Measurement method	\Φ and \ω scans	**Volume [Å^3]**	1730.5(6)	
Radiation (Wavelength [Å])	MoKα (λ =0.71073)	**Unit cell dimensions [Å] and [°]**	7.6463(16)	90
Crystal size/[mm^3]	0.3 × 0.2 × 0.01		20.383(4)	93.003(7)
Crystal habit	clear brown block		11.118(2)	90
Density calculated/ [g/cm^3]	2.304	**Absorption coefficient / [mm^{-1}]**	6.960	
Abs. correction type	multiscan	**F(000) [e$^-$]**	1144.0	

Data collection and structure refinement – compound **4**

Index ranges	-8 ≤ h ≤ 9, -24 ≤ k ≤ 24, -13 ≤ l ≤ 13	**Theta range for data collection [°]**	5.334 to 50.698	
Reflections number	18772	**Data/restraints/ parameters**	3111/60/245	
Refinement method	Least squares	**Final R indices**	[all data]	R_1 = 0.0859, wR_2 = 0.1671
Function minimized	Σ w(F_o^2 - Fc^2)^2		I>=2σ (I)	R_1 = 0.0671, wR_2 = 0.1546
Goodness-of-fit on F^2	1.164			
Largest diff. peak and hole [e Å^{-3}]	2.13/-2.36			

Bis(η^5-cyclopentadienyl)[2-ethyl-3-(oxo-κO)-4-(1*H*)-pyran-4-ato-κO] tungsten(IV) hexafluorophosphate **5**

Sample and crystal data – compound **5**

Chemical formula	$C_{17}H_{17}F_6O_3PW$	**Crystal system**	orthorhombic	
Formula weight [g/mol]	598.12	**Space group**	Pnma	
Temperature [K]	100	**Z**	4	
Measurement method	\Φ and \ω scans	**Volume [Å^3]**	1782.58(12)	
Radiation (Wavelength [Å])	MoKα (λ = 0.71073)	**Unit cell dimensions [Å] and [°]**	13.7943(4)	90
Crystal size/[mm^3]	0.12 × 0.11 × 0.03		8.3019(3)	90
Crystal habit	clear brown block		15.5658(7)	90
Density calculated/ [g/cm^3]	2.229	**Absorption coefficient/ [mm^{-1}]**	6.647	
Abs. correction type	multiscan	**F(000) [e$^-$]**	1144.0	

Data collection and structure refinement – compound **5**

Index ranges	-10 ≤ h ≤ 16, -9 ≤ k ≤ 7, -11 ≤ l ≤ 18	**Theta range for data collection [°]**	5.562 to 50.688	
Reflections number	4136	**Data/restraints/ parameters**	1732/0/149	
Refinement method	Least squares	**Final R indices**	[all data]	R_1 = 0.0339, wR_2 =0.0577
Function minimized	Σ w(F_o^2 - Fc^2)^2		I>=2σ (I)	R_1 =0.0252, wR_2 =0.0548
Goodness-of-fit on F^2	1.048			
Largest diff. peak and hole [e Å^{-3}]	1.10/-1.11			

Bis(η^5-cyclopentadienyl)[2-ethyl-3-(oxo-κO)-4-(1*H*)-pyran-4-ato-κS] tungsten(IV) hexafluorophosphate **6**

Sample and crystal data – compound **6**

Chemical formula	$C_{17}H_{17}F_6O_2PSW$	**Crystal system**	triclinic	
Formula weight [g/mol]	614.19	**Space group**	P-1	
Temperature [K]	100	**Z**	2	
Measurement method	\Φ and \ω scans	**Volume [Å^3]**	905.88(6)	
Radiation (Wavelength [Å])	MoKα (λ = 0.71073)	**Unit cell dimensions [Å] and [°]**	7.6662(3)	82.659(2)
Crystal size/[mm^3]	0.14 × 0.1 × 0.04		10.7376(4)	79.687(2)
Crystal habit	clear brown block		11.3297(4)	83.341(2)
Density calculated/ [g/cm^3]	2.252	**Absorption coefficient/ [mm^{-1}]**	6.650	
Abs. correction type	multiscan	**F(000) [e$^-$]**	588.0	

Data collection and structure refinement – compound **6**

Index ranges	-10 ≤ h ≤ 10, -15 ≤ k ≤ 15, -15 ≤ l ≤ 15	**Theta range for data collection [°]**	5.018 to 60.416	
Reflections number	50145	**Data/restraints/ parameters**	5295/0/254	
Refinement method	Least squares	**Final R indices**	[all data]	R_1 = 0.0181, wR_2 =0.0364
Function minimized	Σ w(F_o^2 - Fc^2)^2		I>=2σ (I)	R_1 =0.0165, wR_2 =0.0357
Goodness-of-fit on F^2	1.031			
Largest diff. peak and hole [e Å^{-3}]	0.91/-0.88			

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