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Marlene Reikersdorfer BSc

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O. Univ.-Prof. Dr. Dr. Bernhard K. Keppler

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ABSTRACT

Cancer is among the three leading causes of death worldwide next to cardiovascular disease and infectious disease. The discovery of cisplatin by Barnett Rosenberg in the 1960s and its application as a chemotherapeutic gained a lot of attention. Cisplatin, carboplatin and oxaliplatin are worldwide approved metal-based anti-cancer drugs, however, they all display a broad spectrum of severe side effects including nephrotoxicity, nausea, neurotoxicity, etc. Other drawbacks are the intrinsic resistance of some tumours and the acquired resistance during consecutive therapy. In order to reduce the side effects and circumvent these drawbacks complexes with other metal centres have been investigated. At the moment there are two ruthenium compounds (NAMI-A and KP1019) and one gallium complex (KP46) in clinical trials with promising results.

In 1979 Köpf and Köpf-Maier tested a group of metallocenes Cp_2MX_2 ($\text{M} = \text{Ti}, \text{V}, \text{Nb}, \text{Mo}$; $\text{X} = \text{halides and pseudo-halides}$) on a variety of tumour cells and revealed that these compounds exhibit antitumor activity with less toxic effects than cisplatin. Titanocene was found to be the most active representative of the tested series of metallocenes. Titanocene dichloride entered clinical trials but was abandoned due to its low efficacy vs. toxicity. In contrast the Cp rings of molybdenocene dichloride possesses a good hydrolytic stability, but in aqueous solution the labile chloride ligands are rapidly replaced by water molecules. By substituting these chloride ligands with biologically active ligands, favourable effects such as stabilisation of ligand geometries, acquired redox-activity, increased solubility, enhanced cellular uptake, different mode of action and synergistic effects on bioactivity can be observed.

The aim of this master thesis was to substitute chlorido ligands of molybdenocene dichloride with different bioactive *O,O*-; *S,O*-; and *N,O*-chelating ligands and analyse the impact on stability in aqueous solution, interaction with biomolecules and anticancer activity. These ligands include picolinic acid, pyrone, naphthoquinone and flavone derivatives. Characterisation of ligands and complexes were carried out by standard analytical methods using 1D and 2D NMR spectroscopy, ESI-MS, UV/Vis spectroscopy, X-ray diffraction analysis, melting points and cyclic voltammetry. The stability in aqueous solution was investigated using NMR and UV/Vis spectroscopy. The cytotoxicity of the compounds was determined by the use of colorimetric MTT assays in different human cancer cell lines. Furthermore, the interaction with human serum albumin was investigated using fluorescence spectroscopy and ICP-MS measurements.

ZUSAMMENFASSUNG

Krebs ist weltweit eine der drei häufigsten Todesursachen neben Herz-Kreislauf-Erkrankungen und Infektionskrankheiten. Die Entdeckung von Cisplatin durch Barnett Rosenberg in den 1960er Jahren und dessen Anwendung als Chemotherapeutikum erweckte viel aufsehen und neben Cisplatin sind Carboplatin und Oxaliplatin weltweit als metallbasierende Chemotherapeutika zugelassenen. Allerdings weisen diese Verbindungen ein breites Spektrum an schwerwiegenden Nebenwirkungen auf, zum Beispiel Nephrotoxizität, Übelkeit, Neurotoxizität, etc. Weitere Nachteile sind intrinsische oder durch mehrere Therapiezyklen erworbene Resistenzen von Tumoren. Um die Nebenwirkungen zu reduzieren und das Aktivitätsspektrum zu erweitern wurden Komplexe mit andern Metallzentren auf ihre Zytotoxizität untersucht. Derzeit sind zwei Ruthenium Verbindungen (NAMI-A und KP1019) und ein Gallium Komplex (KP46) in klinischen Studien mit sehr vielversprechenden Resultaten. In 1979 untersuchten Köpf und Köpf-Maier eine Gruppe von Metallocenen Cp_2MX_2 ($M = Ti, V, Nb, Mo$; $X = \text{Halid und pseudo-Halid}$) auf ihr zytotoxisches Potential. Diese Verbindungen wurden an verschiedenen Tumorzelllinien getestet und dabei herausgefunden, dass die getesteten Metallocene eine vielversprechende Aktivität mit geringeren toxischen Effekten als Cisplatin besitzen. Titanocendichlorid, die aktivste Verbindung der untersuchten Serie, wurde in klinischen Testphasen untersucht, aber aufgrund der zu geringen Effizienz im Vergleich zur Toxizität wurde die Studie abgebrochen. Im Gegensatz zu Titanocene zeigen die Cp Ringe des analogen Molybdenkomplexes eine gute hydrolytische Stabilität, allerdings werden die zwei labilen Chloridoliganden sehr schnell unter physiologischen Bedingungen durch Wassermoleküle ersetzt. Durch Substitution der beiden Chloridoliganden durch biologisch aktive Moleküle können vorteilhafte Effekte erzielt werden z.B. Stabilisierung der Ligandengeometrie, Redox-Aktivität, erhöhte Löslichkeit, verbesserte Zellaufnahme, unterschiedliche Wirkmechanismen und synergistische Effekte bezüglich der biologischen Aktivität. Das Ziel der Masterarbeit war die Substitution der leicht hydrolysierbaren Chlorliganden von Molybdenocendichlorid mit unterschiedlichen bioaktiven O,O-, S,O-, und N,O-Chelatliganden und deren Einfluss auf die Stabilität in wässriger Lösung, die Interaktion mit Biomolekülen und die Zytotoxizität zu bestimmen. Als Ligandensysteme wurden Picolinsäure, Pyron-, Naphthochinon- und Flavon-Derivate verwendet. Die Charakterisierung der Liganden und Komplexe wurde mittels

verschiedener analytischer Methoden durchgeführt z.B.: 1D und 2D NMR Spektroskopie, ESI-MS, UV/Vis Spektroskopie, Röntgenstrukturanalyse, Schmelzpunktbestimmung und Cyclovoltammetrie. Die Stabilität der Verbindungen in wässriger Lösung wurde mittels NMR- und UV/Vis Spektroskopie analysiert und Voruntersuchungen bezüglich der Zytotoxizität wurden in verschiedenen Zelllinien mittels MTT-Tests durchgeführt. Außerdem wurde die Interaktion mit HSA mittels Fluoreszenzspektroskopie und ICP-MS Messungen untersucht.

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ABBREVIATIONS

1D/2D NMR	one- and/or two-dimensional NMR spectroscopy
A	adenine (nucleobase)
Å	Angstrom
ACN	acetonitrile
APC	Adenomatous-polypsis-coli
BAK	BCL2-antagonist
BAX	BCL2-associated X protein
c	concentration
°C	degree Centigrade
C	cytosine (nucleobase)
cm	centimetre
CNS	central nervous system
Cp	cyclopentadienyl
cps	counts per second
cys	cysteine
δ	chemical shift (NMR)
d	days
d	doublet (NMR)
DMSO- <i>d</i> 6	deuterated dimethyl sulfoxide
DNA	deoxyribonucleic acid
ε	extinction coefficient
<i>e.g.</i>	<i>exempli gratia</i> (for example)
eq	equivalent
ER	estrogen receptor
ESI-MS	electrospray ionization - mass spectrometry
EtOH	ethanol
FDA	food and drug administration
G	guanine (nucleobase)
h	hour
HBV	hepatitis B virus
HPV	human papillomavirus
HSA	human serum albumin
IAP	inhibitor of apoptosis protein
IC ₅₀	drug concentration that causes 50 % cell growth inhibition
ICP-MS	inductively coupled plasma – mass spectrometry
IUPAC	international union of pure and applied chemistry
<i>J</i>	coupling constant (NMR)

K.....	Kelvin
kDa.....	kilo Dalton
λ	wavelength
L.....	litre
logP.....	partition-coefficient
m.....	multiplet (NMR)
(m/ μ /n)M	(milli/micro/nano)molar (mol/L; mmol/L; μ mol/L; nmol/L)
Mcl-1	myeloid cell leukaemia sequence 1 (BCL2-related)
MeOH.....	methanol
(M)Hz	(mega)hertz
min	minute
MTT.....	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MYC	v-myc avian myelocytomatosis viral oncogene homolog
NAD	nicotinamide adenine dinucleotide
NADP ⁺	nicotinamide adenine dinucleotide phosphate
NaOMe.....	sodium methoxide
NHE	normal hydrogen electrode
NMR.....	nuclear magnetic resonance spectroscopy
PBS.....	phosphate buffered saline
PET	positron emission tomography
pH	pondus Hydrogenii (power of hydrogen)
pic	picolinate
Pin1.....	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
ppm.....	parts per million
pRb	retinoblastoma protein
PTA.....	1,3,5-triaza-7-phosphaadamantane
RAPTA.....	ruthenium (II) arene PTA
RAS.....	rat sarcoma
RNA	ribonucleic acid
r.t.....	room temperature
s	singlet (NMR)
SCF.....	Skp, Cullin, F-box containing complex
SPECT	single-photon emission computed tomography
t.....	triplet (NMR)
T.....	thymine (nucleobase)
UV/Vis	ultraviolet/visible
V.....	volt
v/v	volume/volume
WHO	World Health Organization
WNT.....	wingless-type MMTV integration site family

1 INTRODUCTION

1.1 FACTS AND STATISTICS ABOUT CANCER

Malignant neoplasms are one of the three leading causes of death worldwide with the others being cardiovascular and infectious disease. Cancer accounted for 8.2 million deaths in 2012.¹ In more developed regions vaccinations, immunisation and higher hygiene standards led to the decrease of infectious disease. Therefore in these countries non-communicable cardiovascular disease and malignant neoplasms are the leading cause of death. In Austria infectious disease only account for 1 % of the death, while cancer accounts for 26 % and cardiovascular disease for 43 %.² Nevertheless in 2012 57 % of the new cancer cases and 65 % of the cancer deaths occurred in less developed regions (Figure 1).³

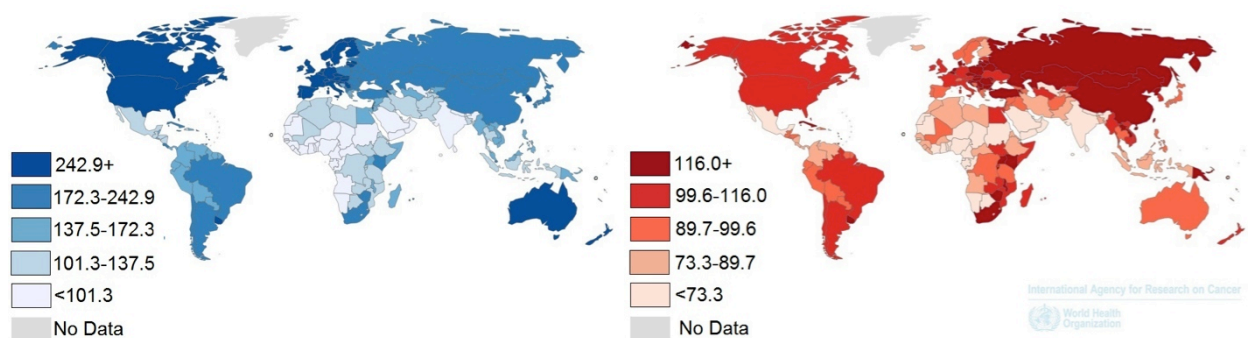


Figure 1: age-standardised rates (ASR) of cancer incidence (left) and mortality (right) worldwide in 2012⁴

Cancer is a class of diseases that can affect every tissue and organ of the body. It is influenced by age, sex, nutrition, ethnic descent, geographic location and personal lifestyle e.g. unhealthy diet, use of tobacco, no physical exercise, being overweight, excessive exposure to sunlight and excessive use of alcohol.⁵ Cancer differs in every patient and progresses diversely in men and women. In 2011, 37067 people in Austria were diagnosed with cancer, slightly more men (19,298) than women (17,769), leading to the death of 10,525 men and 9,371 women. Under consideration of the changing age distribution the incidence and mortality have been decreasing in

¹ http://globocan.iarc.fr/Pages/fact_sheets_population.aspx (accessed on 21/12/2013)

² https://www.statistik.at/web_de/statistiken/gesundheit/todesursachen/todesursachen_ausgewaehlte/index.html (accessed on 21/12/2013)

³ http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx (accessed on 21/12/2013)

⁴ <http://globocan.iarc.fr/Pages/Map.aspx> (accessed on 21/12/2013)

⁵ Umar, A., Dunn, B. K. and Greenwald, P., *Nat Rev Cancer*, **2012**, 12, 835-848.

the long term, whereas the absolute number of cancer incidences increased.⁶ The most common tumour localisation for men in Austria are prostate, lung, colon, bladder and kidney and in women breast, colon, lung, corpus uteri and thyroid. However mortality does not correlate. Most women died from breast, lung, colon, pancreas and ovary cancer, while most men died from lung, colon, prostate, pancreas and liver cancer (Figure 2).

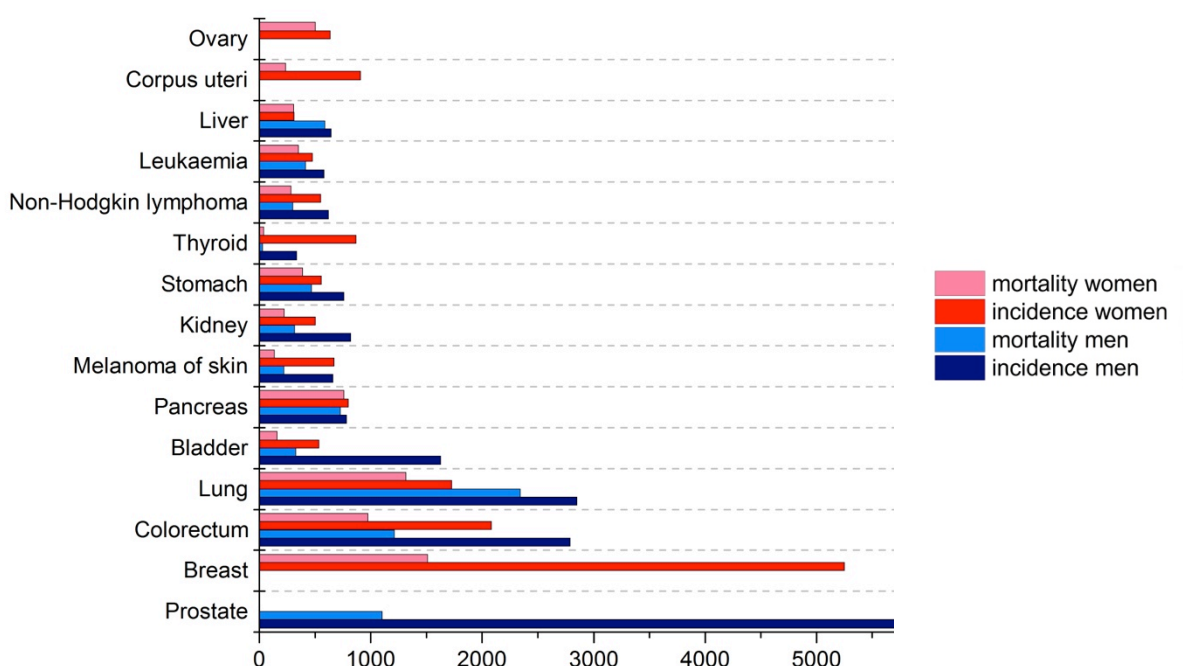


Figure 2: cancer incidence and mortality for men and women in Austria in 2012.¹

Incidence and mortality rates worldwide differ from those in Austria. Worldwide the most common tumour localisations for men are lung, prostate, colon, stomach and liver and for women breast, colon, lung, cervix uteri and stomach. The types of cancer most commonly causing death in men are lung, liver, stomach, colon and prostate and in women breast, lung, colon, cervix uteri and stomach. Due to a higher awareness, more people attending regular check-ups and better diagnostic tools in more developed countries, especially those of breast and prostate cancer, have increased. However the diagnosis is more often made at an early stage thereby increasing the chance of recovery and decreasing the mortality rate of those two types of cancer.⁵

⁶https://www.statistik.at/web_de/statistiken/gesundheit/krebserkrankungen/krebs_im_ueberblick/index.html (accessed on 21/12/2013)

1.2 CELL CYCLE

The most basic function of the cell cycle is to form two genetically identical daughter cells by duplicating the amount of DNA in the chromosomes and segregating the copies precisely. The cell cycle is divided into two major phases: the interphase (G_1 , S, G_2) and the mitotic phase (M). The M phase involves a series of events beginning with mitosis (prophase, metaphase, anaphase, telophase) and followed by cell division. During the S phase or synthesis phase DNA replication occurs. G_1 and G_2 are gap phases. During the gap phases the cell increases in size, monitors the internal and external environment for suitable conditions and checks if preparations are completed. The G_2 phase is between S phase and mitosis and the G_1 phase is between M phase and S phase. If extracellular conditions are unfavourable in the G_1 phase, the cell may enter a resting state (G_0) (Figure 3). In this resting state the cell has stopped dividing. It can remain in this state for days, weeks or years before favourable conditions and signals to grow and divide are present to resume proliferation.⁷

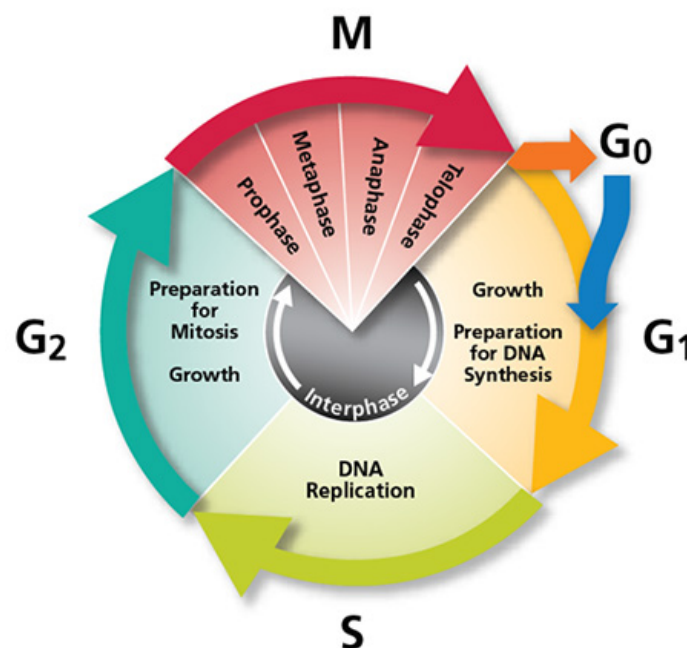


Figure 3: The phases and functions of the cell cycle⁸

⁷ Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*, Garland Science: New York, USA and Abingdon, UK, **2008**, 1053–1059.

⁸ <http://www.bdbiosciences.com/research/apoptosis/analysis/> (accessed on 25/12/2013)

The cell cycle has a complex control system that is responsible for detecting and repairing genetic damage and to prevent uncontrolled cell division. Central parts of the control system are cyclin-dependent kinases (CDK) that are in turn regulated by cyclins. The activity of these cyclin-CDK-complexes oscillates and leads to changes in the phosphorylation of intracellular proteins that regulate or initiate the major events of the cell cycle (DNA replication, mitosis and cytokinesis). There are four classes of cyclin-CDK complexes. G₁/S-cyclins (cyclin E + CDK 2) commit the cell to DNA replication. S-cyclins (cyclin A + CKD2) initiate DNA replication. M-cyclins (cyclin B + CDK1) are essential for the start of mitosis and G₁-cyclins (cyclin D + CDK4/CDK6) help with the transition to a new cell cycle. The activity of cyclin-CDK complexes can be inhibited by phosphorylation or increased by dephosphorylation of amino acids of the active site. It can also be regulated by the binding of CDK inhibitor proteins (CKIs). Inactivating key proteins of the control system by proteolysis and changes in the transcription of genes encoding CDK regulators influence the activity as well. Two enzyme complexes (APC and SCF) are important in the destruction of cyclins and other cell cycle regulators. The APC (anaphase promoting complex) ubiquitylates and carries out proteolysis of M-cyclins. The SCF complex ubiquitylates and destroys G₁/S-cyclins and CKIs that control S-phase initiation.

When extracellular conditions are unfavourable, when events are not completed successfully or when DNA is damaged, inhibitory mechanisms arrest the cell cycle at certain checkpoints to regulate the cell cycle progression correctly. Cells that are no longer needed or are dangerous to the organism, commit suicide. This programmed cell death is also called apoptosis. A cell that undergoes programmed cell death dies without damaging its neighbouring cells. By contrast if a cell dies as a result of an acute injury it swells and bursts, thereby spilling its content over their neighbours and potentially causing a damaging inflammation. This process is called cell necrosis. Apoptosis is mediated by proteolytic enzymes called caspases (cysteine-dependent aspartyl-specific protease). They exist in all cells as procaspases, which are inactive precursors. These procaspases are activated by extracellular or intracellular death signals regulated by the Bcl-2 and IAP protein families. In healthy tissue cell division and cell death balance each other exactly.⁹

⁹ Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*, Garland Science: New York, USA and Abingdon, UK, **2008**, 1060–1112

1.3 CARCINOGENESIS AND HALLMARKS OF CANCER

Carcinogenesis is the emergence of cancer, starting from one aberrant cell. The human body has more than 10^{14} cells. If a cell experiences an initial mutation, this cell must undergo further changes and numerous additional mutations to become cancerous. Cancer cells are neoplastic if they proliferate uncontrollably in disregard of normal control. These benign tumours can ideally be removed by surgery. Malignant tumours are formed if the cells are able to invade surrounding tissues. Secondary tumours, also called metastases, arise by entering the lymphatic system or the blood stream and spread throughout the whole body (Figure 4).

There are three different tumour types classified by the tissue they arose from. Carcinomas are the most common form of cancer originating from epithelial cells. Leukaemia arises from white blood cells and sarcomas from connective tissue.

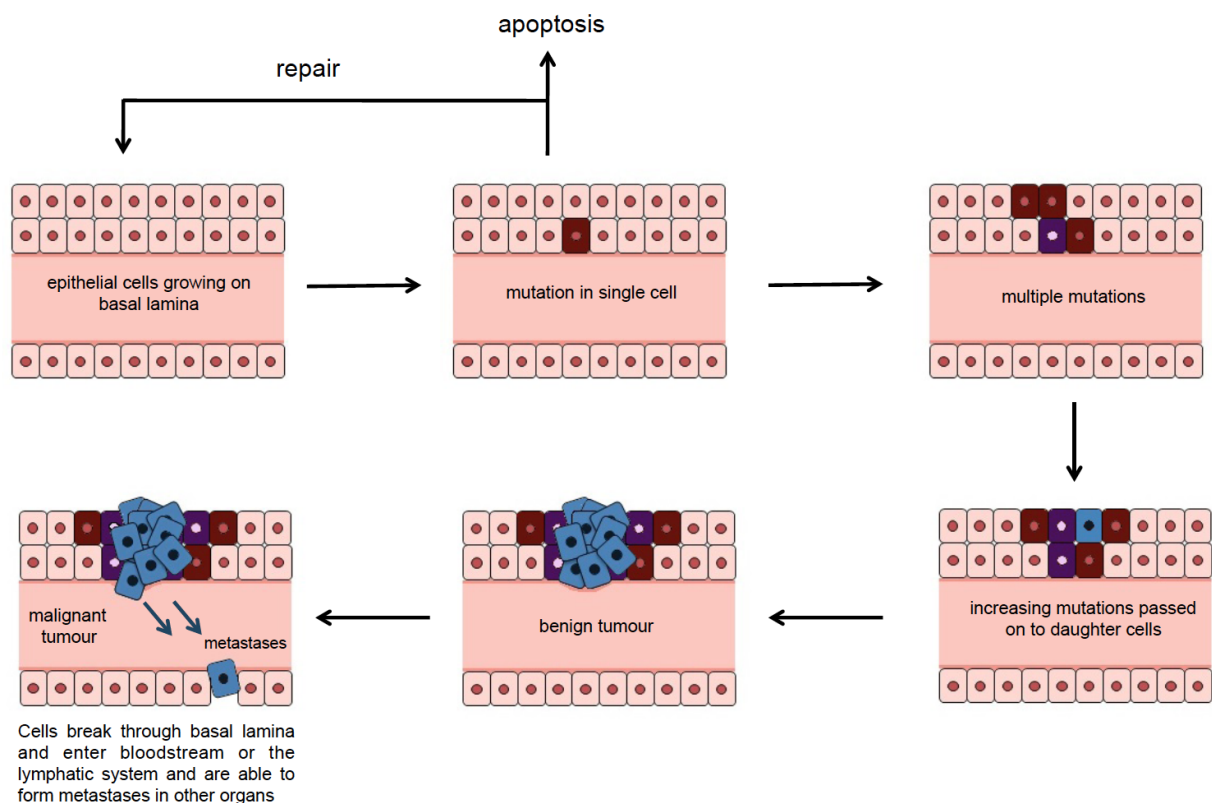


Figure 4: development of cancer (adapted from reference 11)

Tumour progression and evolution is accelerated by tumour initiators called mutagenic agents and by tumour promoters called non-mutagenic agents. These agents affect gene expression, initiate cell proliferation and alter the balance of mutated and non-mutated cells. There are many mutagens, which cause cancer

including viruses, like HBV or HPV¹⁰, chemical carcinogens (unhealthy diet, smoking, polycyclic aromatic hydrocarbons, asbestos, vinyl chloride, etc.) and radiation like UV light and ionizing radiation.

Cancer-critical genes are genes that are repeatedly altered in human cancer and contribute to its emergence. There are two classes of cancer-critical genes.¹¹ If proto-oncogenes are mutated and overexpressed they are called oncogenes. Proto-oncogenes are involved in the regulation of cell growth and differentiation. Examples for proto-oncogenes are RAS, MYC, and WNT proteins.¹² The other class are tumour suppressor genes. They regulate the cell growth and prevent and inhibit the growth of tumours. If these genes are mutated or altered they lose their control ability and allow the formation of cancer cells. Examples for tumour suppressor genes are pRb, p53 and APC.¹³ Oncogenes are dominant in their effects on the cell. There are three ways to convert a proto-oncogene in an overactive oncogene: deletion or point mutation in the coding sequence, gene amplification and chromosome rearrangement. Mutations in tumour suppressor genes are recessive. Both gene copies have to be inactivated to lose control. The first copy may be inactivated by point mutation, chromosomal deletion or epigenetic changes. The second copy may be inactivated by chromosome loss, nondisjunction and duplication or mitotic recombination. The protein p53 regulates the progression through the cell cycle as well as the initiation of apoptosis and the maintenance of genetic stability. If p53 is inactivated or lost, damaged and senescent cells continue replicating DNA thereby escaping apoptosis and increasing the damage by proliferating with a corrupted genome. The loss of p53 also contributes to the genetic instability of cancers allowing further cancer-promoting mutations to accumulate.¹¹

There are more than 100 types and subtypes of cancer that can be found within specific organs and their development is a multistep process. Hanahan and Weinberg suggested that most cancer cell genotypes show six essential alterations in the cell that lead to malignant growth.¹⁴ These six Hallmarks of cancer were later extended with four more potential characteristics. These hallmarks are: sustaining proliferative signalling, evading growth suppressors, activating invasion and

¹⁰ Schiller, J. T. and Lowy, D. R., *Annu Rev Microbiol*, **2010**, 64, 23-41.

¹¹ Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*, Garland Science: New York, USA and Abingdon, UK, **2008**, 1205–1223

¹² Todd, R., Wong, D.T., *Anticancer Res.*, **1999**, 19, 4729-4746.

¹³ Chial, H., *Nature Education*, **2008**, 1, 177

¹⁴ Hanahan, D. and Weinberg, R. A., *Cell (Cambridge, Mass.)*, **2000**, 100, 57-70.

metastasis, enabling replicative immortality, inducing angiogenesis, resisting cell death, avoiding immune destruction, tumour promoting inflammation, genome instability and mutation and deregulating cellular energetics. In addition to these hallmarks, cancer can only be understood by studying the individual specialized cell types within the tumour as well as their microenvironment that they build during carcinogenesis. There are not only new findings in the biology of the tumour but as well new therapeutic ways to target cancer. There are a great amount of drugs being developed categorized by their hallmark target (Figure 5).¹⁵

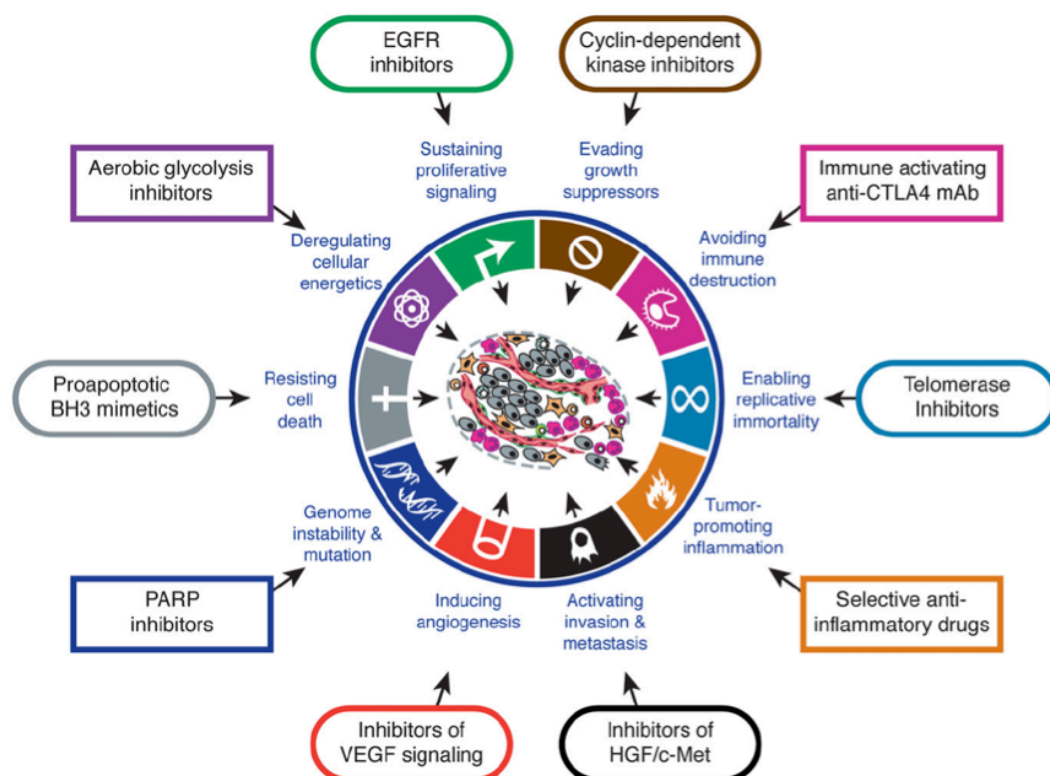


Figure 5: hallmarks of cancer and their hallmarks-targeting cancer drug.¹⁵

1.4 CANCER THERAPY

Depending on the different types of cancer, the grade and location of the tumour, the health state of the patient and the stage of the disease, different types of cancer treatment have to be considered. The most common approaches include surgery, radiation therapy, chemotherapy, targeted therapy, immunotherapy, hormonal therapy or a combination of different therapies.

¹⁵ Hanahan, D. and Weinberg, R. A., *Cell*, **2011**, 144, 646-674.

SURGERY is the oldest form of cancer therapy but limited to solid tumours. The goal is to remove the whole tumour.

RADIATION THERAPY uses ionizing radiation to kill cancer cells by damaging the DNA and to shrink tumours. Radiation therapy may be administered externally or internally from radioactive isotopes placed near the tumour cells or injected into the bloodstream. The radiation damages both normal cells as well as cancerous cells leading to severe side effects. It is used to treat most types of solid tumours, leukaemia and lymphomas. Radiation therapy can be applied alone, before, during or after surgery or in combination with chemotherapy.¹⁶

CHEMOTHERAPY generally refers to the treatment with drugs that kill cancer cells on a molecular level. In contrast to radiation therapy and surgery, chemotherapy is a systemic treatment, meaning that the used substances for treatment travel through the bloodstream, reaching and affecting cells all over the body.¹⁷ Chemotherapeutics target rapidly dividing cells, like cancer cells but also normal cells e.g. in bone marrow, digestive tract and hair follicles, leading to the well-known severe side effects. With chemotherapy non-solid tumours, metastasised tumours and small tumours escaping detection can be treated. The WHO Collaborating Centre for Drug Statistics Methodology (WHOC) classifies drugs according to their target organ or system and their therapeutic and chemical characteristics using the Anatomical Therapeutic Chemical (ATC) classification system. Antineoplastic agents are located in group L01, which contains 5 subgroups.¹⁸

Alkylating agents (L01A) have the ability to alkylate proteins, RNA and DNA. They damage the DNA, directly preventing the cancer cell from reproduction. They work in all phases of the cell cycle and are therefore not phase-specific. This group includes nitrogen mustards, alkyl sulfonates, ethylenimines, epoxides, nitrosoureas and others.

Antimetabolites (L01B) interfere with DNA and RNA growth. They substitute the normal building blocks of RNA and DNA. The damage occurs during S phase of the

¹⁶ <http://www.cancer.gov/cancertopics/factsheet/Therapy/radiation> (accessed on 17/01/2014)

¹⁷ <http://www.cancer.gov/Common/PopUps/popDefinition.aspx?id=CDR0000045922&version=Patient&language=English> (accessed on 17/01/2014)

¹⁸ <http://www.whocc.no/> (accessed on 17/01/2014)

cell cycle. Antimetabolites contain folic acid (methotrexate), purine (mercaptopurine) and pyrimidine (5-fluorouracil) analogues.¹⁹

Plant alkaloids and other natural products (L01C) include vinca alkaloids, podophyllotoxin derivatives, colchine derivatives, texanes and others.

Cytotoxic antibiotics and related substances (L01D) include actinomycines, like dactinomycin, a RNA synthesis inhibitor, anthracyclines such as doxorubicin²⁰, which interacts with DNA by intercalation, and others.

Other antineoplastic agents (L01X) constitute the biggest group with platinum compounds, methylhydrazines, monoclonal antibodies, sensitizers used in photodynamic/radiation therapy, protein kinase inhibitors, combination of antineoplastic agents and others.¹⁸

TARGETED THERAPIES block the growth and spread of cancer by interfering with target molecules (enzymes, proteins, etc.) needed for carcinogenesis and tumour growth. Others deliver toxins directly to the cancer cells and kill them or help the immune system kill cancer cells. Most targeted therapies are small molecules or monoclonal antibodies and may have fewer side effects than other treatments.²¹

IMMUNOTHERPAY uses substances to stimulate or suppress the immune system, thereby helping the body to fight cancer, infections, and other diseases. These substances include cytokines, vaccines, bacillus Calmette-Guerin (BCG) and monoclonal antibodies. Immunotherapy either targets only certain cells of the immune system or targets them in a general way.²¹

HORMONAL THERAPY adds, blocks, or removes hormones thereby slowing down or stopping the growth of certain types of cancer. Hormone levels are adjusted by synthetic hormones, other drugs inhibiting natural hormones or by surgery removing the gland producing certain hormones.²¹

¹⁹ <http://www.cancer.org/treatment/treatmentsandsideeffects/treatmenttypes/chemotherapy/>

²⁰ Fornari, F. A., Randolph, J. K., Yalowich, J. C., Ritke, M. K. and Gewirtz, D. A., *Mol. Pharmacol.*, **1994**, 45, 649-656.

²¹ <http://www.cancer.gov/dictionary> (accessed on 17/01/2014)

1.5 METALL IONS IN BIOLOGY AND MEDICINE

Metal ions are important for critical functions in humans. There are four metal bulk elements (Na, K, Mg, Ca) and several essential metal trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo and Cd). A deficit or an excess of some metal ions can lead to diseases. Iron deficiency can lead to pernicious anaemia, zinc deficiency to growth retardation and copper deficiency to heart disease in infants, but excess in copper can lead to Wilson's disease. So it is important for metal ions to be adequately absorbed, circulated, stored and inserted into the proper environment to carry out their biological functions.²² However, the amount and the type of chemical bond of the metal ion are crucial to its effect in the human body. The concept that the dose determines the toxicity, was first introduced in the 16th century by Paracelsus.²³ In 1912 Bertrand established a relationship between benefit/detriment from a trace metal and its concentration. Any metal ion or complex has an area of optimum physiological response and varies according to the element, oxidation state, biochemistry and speciation. This area can be varied by these features but also by alteration of the delivery of the metal ion to the biological system e.g. design of ligands.²⁴

1.5.1 PLATINUM ANTICANCER DRUGS

The first platinum anticancer drug with anti-proliferative properties, cisplatin, was discovered by Barnett Rosenberg in 1965 by serendipity.²⁵ Cisplatin (*cis*-diamminedichloridoplatinum(II)) was first synthesised by Michele Peyrone in 1844 without knowledge of its anti-proliferative properties.²⁶ Rosenberg and co-workers originally studied the effect of an electric field in mitosis of *Escherichia coli* bacteria. He used ammonium chloride as growth medium and two platinum electrodes. The bacteria showed no cell division but grew three hundred times in length. They later found that the oxidation of the platinum electrodes to Pt(IV) led to the formed ammoniumhexachloridoplatinate(IV)-complex, which was converted by a

²² Bertini, I., Gray, H. B., Valentine, J. S., Lippard, Stephen J., *Bioinorganic Chemistry*, University Science Books, **1994**, 505-583.

²³ Thompson, K. H.; Orvig, C. in *Concepts and Models in Bioinorganic Chemistry*, Wiley-VCH: Weinheim, Germany, **2006**, pp. 25-46.

²⁴ Orvig, C. and Abrams, M. J., *Chemical Reviews*, **1999**, 99, 2201-2204.

²⁵ Rosenberg, B., Vancamp, L. and Krigas, T., *Nature*, **1965**, 205, 698-699.

²⁶ Peyrone, M., *Ann. Chem. Pharm.*, **1844**, 1-29

photocatalytic reaction to the *cis*-diamminetetrachloridoplatinate(IV)-complex. Under the reductive environment of the bacteria the complex was reduced to active cisplatin.²⁷ Shortly thereafter its anti-tumour activity and possibility of medical usage was discovered.²⁸ They started the first clinical phase I study with patients in 1972 and in 1978 the FDA approved cisplatin as the first metal-based anticancer agent.^{29,30} Cisplatin is particularly effective against testicular cancer. Prior to the use of cisplatin testicular cancer had a mortality rate of more than 90 %. The mortality rate nowadays is at about 10 %, mostly due to late diagnosis.³¹ It is furthermore used in combination therapy for tumours of the cervix, ovaries, bladder, lung, head and neck and lymphomas.³² Cisplatin is administered intravenously and binds to a great extent to serum proteins like HSA. The formed adducts are transported in the bloodstream and the uptake is achieved by active transport or passive diffusion.³³ The main target of cisplatin is DNA. In order to bind to DNA cisplatin has to be hydrolysed, whereas the chlorido ligands are replaced by water molecules.³² Inside the cell the chloride concentration is lower than outside the cell thereby facilitating the hydrolysis. The hydrolysed cisplatin preferably interacts with *N*7 of the guanine bases³⁴ leading to interstrand or intrastrand adducts.²⁷ The major adduct is *cis* 1,2-[Pt(NH₃)₂]²⁺-d(GpG) intrastrand crosslink accounting for about 65 % of total products. Other adducts include 1,2-d(ApG) (25 %) and 1,3-d(GpNpG) (5–10 %) intrastrand adducts, as well as a smaller number of interstrand cross-links (ICL) and monodentate adducts. X-ray crystal structure showed that these adducts induce a bend in the DNA and unwind the double helix.³⁵ However, only about 1 % of the administered cisplatin reaches the cellular target. The majority is bound to plasma proteins, which in turn is supposed to inactivate the drug.³⁶ One major drawback of cisplatin is its severe side effects such as oto- and neurotoxicity, nausea and the dose-limiting nephrotoxicity. Another downside is the

²⁷ Galanski, M. and Keppler, B. K., *Pharmazie in unserer Zeit*, **2006**, 35, 118-123.

²⁸ Rosenberg, B., VanCamp, L., Trosko, J. E. and Mansour, V. H., *Nature*, **1969**, 222, 385-386.

²⁹ Ariyoshi, Y. and Ota, K., *Gan To Kagaku Ryoho*, **1989**, 16, 1379-1385.

³⁰ Jakupiec, M. A., Galanski, M., Arion, V. B., Hartinger, C. G. and Keppler, B. K., *Dalton Trans*, **2008**, 183-194.

³¹ Feldman, D. R., Bosl, G. J., Sheinfeld, J. and Motzer, R. J., *JAMA, J. Am. Med. Assoc.*, **2008**, 299, 672-684.

³² Lippert, B., *BioMetals*, **1992**, 5, 195-208.

³³ Ishida, S., Lee, J., Thiele, D. J. and Herskowitz, I., *Proc Natl Acad Sci U S A*, **2002**, 99, 14298-14302.

³⁴ Fichtinger-Schepman, A. M. J., Van, d. V. J. L., Den, H. J. H. J., Lohman, P. H. M. and Reedijk, J., *Biochemistry*, **1985**, 24, 707-713.

³⁵ Todd, R. C. and Lippard, S. J., *Metallomics*, **2009**, 1, 280-291.

³⁶ Graf, N. and Lippard, S. J., *Adv Drug Deliv Rev*, **2012**, 64, 993-1004.

intrinsic resistance of some tumours and the acquired resistance during consecutive therapy.³⁷

Therefore to improve stability and reduce the side effects new platinum drugs have been developed leading to second and third generation analogues. The second generation platinum anti-tumour drug carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) has properties almost identical to those of cisplatin, but an altered toxicological profile with reduced side effects. These side effects are neurotoxicity, gastrointestinal, ototoxicity and the dose-limiting toxicity is myelosuppression.³⁷ The stability of carboplatin was improved towards hydrolysis by exchanging the leaving group. The chlorido ligands were substituted by the chelating cyclobutane-1,1-dicarboxylato ligand.³⁸ Carboplatin is mainly administered against tumours of the urogenital tract.³⁷

In order to overcome the limitations of *cis*- and carboplatin the third generation platinum drug oxaliplatin ((*trans*-*R,R*-cyclohexane-1,2-diamine)-oxalatoplatinum(II)) was developed. It was found to be active in cisplatin and carboplatin resistant cell lines and tumours. Prior untreatable with platinum anticancer drugs, oxaliplatin, in combination with 5-fluorouracil and leucovorin, is active against metastatic colorectal cancer. Side effects of oxaliplatin include gastrointestinal and neurological effects and the dose limiting sensory neuropathy.

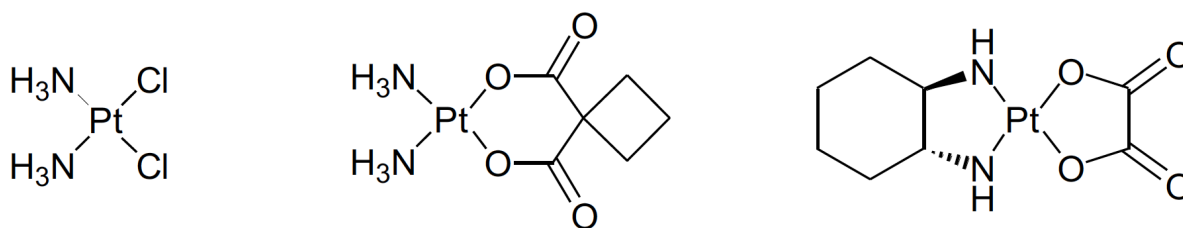


Figure 6: worldwide approved platinum anticancer drugs, namely cisplatin (left), carboplatin (middle) and oxaliplatin (right)

In addition to the worldwide approved platinum anticancer drugs *cis*-, carbo- and oxaliplatin (Figure 6) three other complexes are approved regionally. Nedaplatin is approved in Japan, lobaplatin in China and heptaplatin in South Korea (Figure 7).³⁷

³⁷ Galanski, M. and Keppler, B. K., *Anticancer Agents Med Chem*, **2007**, 7, 55-73.

³⁸ Knox, R. J., Friedlos, F., Lydall, D. A. and Roberts, J. J., *Cancer Res.*, **1986**, 46, 1972-1979.

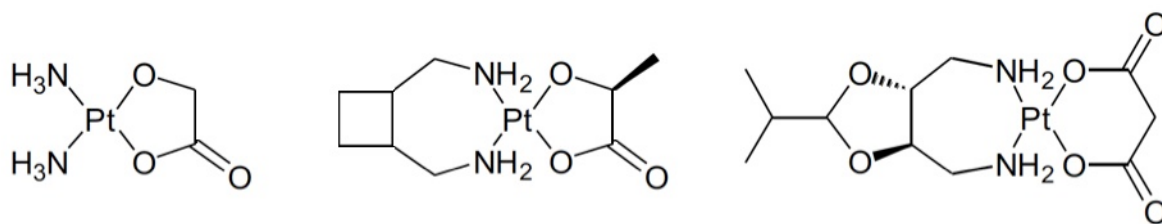


Figure 7: structures of the regionally approved platinum anticancer drugs: nedaplatin (left), lobaplatin (middle, both diastereomers), heptaplatin (right).

In order to overcome side effects, intrinsic and acquired resistance and to broaden the targeted tumours, research focused on different strategies e.g. kinetically inert Pt(IV) compounds. Platinum(IV) compounds act as a prodrug and can be activated by reduction by releasing the axial ligands in the reducing environment of tumour sites. This approach might reduce unwanted reactions with biomolecules thereby minimizing side effects. Due to their stability even in the gastro-intestinal tract Pt(IV)-complexes can be orally administered. The extended coordination sphere with two additional ligands leads to a rich chemistry improving the properties of the platinum compounds e.g. cellular uptake and toxicity.^{36, 37}

About forty platinum-based anticancer drugs have reached clinical studies so far but severe side effects, toxicity and acquired and intrinsic resistance are still major drawbacks for this compound class.^{39, 40} Therefore the studies of metal-based anticancer agents were expanded to other metals.

1.5.2 OTHER METAL BASED ANTICANCER DRUGS

In the last years a lot of research has focused on new metallodrugs with different metal centres (e.g. Ru, Ga, As, Rh, Os, Ir, Fe, Ti, Mo, etc.).

Compared to platinum anticancer drugs, ruthenium complexes show less toxic side effects and exhibit activity in cells that are inactive or resistant to the use of cisplatin.⁴¹ Their low toxicity is partly due to their ability to mimic the binding of iron to biomolecules. Ruthenium has accessible oxidation states of 2+, 3+ and 4+ under physiological conditions.⁴² The redox potential between these oxidation states

³⁹ Rabik, C. A. and Dolan, M. E., *Cancer Treat Rev*, **2007**, 33, 9-23.

⁴⁰ Heffeter, P., Jungwirth, U., Jakupec, M., Hartinger, C., Galanski, M., Elbling, L., Micksche, M., Keppler, B. and Berger, W., *Drug Resist Updat*, **2008**, 11, 1-16.

⁴¹ Reisner, E., Arion, V. B., Keppler, B. K. and Pombeiro, A. J. L., *Inorganica Chimica Acta*, **2008**, 361, 1569-1583.

⁴² Jakupec, M. A., Galanski, M., Arion, V. B., Hartinger, C. G. and Keppler, B. K., *Dalton Trans*, **2008**, 183-194.

enables the body to catalyse oxidation and reduction depending on the physiological environment.⁴³

Two ruthenium anticancer drugs are currently in clinical trials. The first to enter clinical trials was NAMI-A (imidazolium *trans*-[tetrachloro(dimethylsulfoxide) (imidazole)ruthenate(III)]) developed by Alessio and Sava (Figure 8).⁴⁴ The ruthenium(III) complex is lacking in activity against primary tumours. It is however a potent agent against metastasis tumours. The mode of action of NAMI-A seems to be associated with its anti-angiogenic and anti-invasive properties.⁴⁵ The second compound is KP1019⁴⁶ (respectively the better soluble sodium salt KP1339)⁴⁷, *trans*-[tetrachlorobis(1H-indazole)ruthenate(III)] developed by Keppler (Figure 8). In contrast to NAMI-A KP1019/KP1339 is active against primary tumours and is tested for its activity against colorectal cancers. It causes apoptosis via the mitochondrial pathway.

Next to these classical coordination compounds also organometallic compounds with biologically active and arene ligands have been studied. Examples are the RAPTA-type complexes⁴⁸ or the “piano stool” type compounds⁴⁹ by Dyson and Sadler (Figure 8). The “piano stool” type complexes are active against solid tumours and their activity is dependent on their aryl unit. However RAPTA complexes like NAMI-A are inactive against primary tumours but active against metastases.⁴⁵

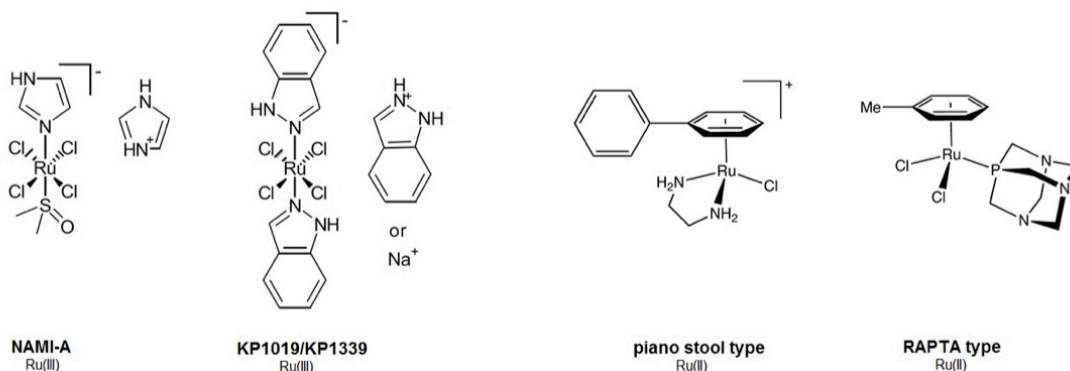


Figure 8: structures of NAMI-A, KP1019/1339, piano stool type and RAPTA type complexes

⁴³ Allardyce, C. S. and Dyson, P. J., *Platinum Met. Rev.*, **2001**, 45, 62-69.

⁴⁴ Alessio, E., Mestroni, G., Bergamo, A. and Sava, G., *Met. Ions Biol. Syst.*, **2004**, 42, 323-351.

⁴⁵ Hannon, M. J., *Pure and Applied Chemistry*, **2007**, 79, 2243-2261.

⁴⁶ Hartinger, C. G., Zorbas-Seifried, S., Jakupec, M. A., Kynast, B., Zorbas, H. and Keppler, B. K., *J Inorg Biochem*, **2006**, 100, 891-904.

⁴⁷ Keppler, B. in *Compositions containing a ruthenium(III) complex and a heterocycle and their screening for cytotoxicity*, Vol. Faustus Forschungs Cie., Germany . **2002**, p. 41 pp.

⁴⁸ Ang, W. H. and Dyson, P. J., *Eur. J. Inorg. Chem.*, **2006**, 4003-4018.

⁴⁹ Yan, Y. K., Melchart, M., Habtemariam, A. and Sadler, P. J., *Chem. Commun. (Cambridge, U. K.)*, **2005**, 4764-4776.

Gallium has similar coordination characteristics to aluminium(III) or in particular iron(III). This similarity seems to be a reason for their inhibition of tumour growth.⁴⁵ Gallium is considered redox-inactive under physiological conditions in contrast to iron(III).⁴² Gallium binds to transferrin thereby interfering with the cellular transport of iron. Another target is the ribonucleotide reductase. By interfering with its action, the synthesis of DNA is inhibited.⁴⁵ To stabilise gallium against hydrolysis, different chelating ligands have been attached to the metal centre and the compound KP46⁵⁰ (tris(8-quinolinolato)gallium(III)) with quinolinolato ligands has entered clinical trials (Figure 9).

The only non-platinum metal compound that has been approved for clinical cancer treatment so far is arsenic trioxide (Figure 9). It is administered against acute promyelocytic leukaemia (APL). At low concentrations arsenic trioxide leads to APL cell differentiation. At higher concentrations oxidative stress and DNA strand breaks induce apoptosis.⁵¹

Though many other metals have been assigned to exhibit anticancer activity, they have not been investigated as intensely.

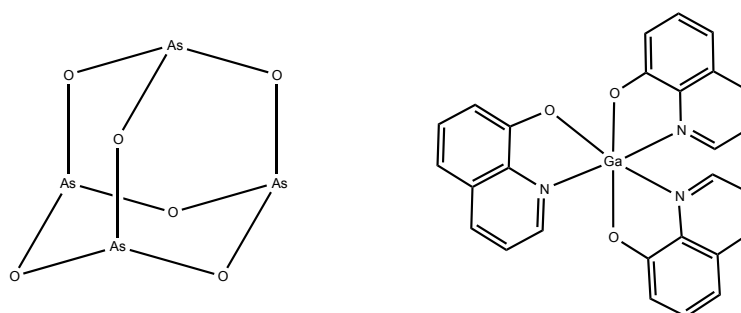


Figure 9: structures of arsenic trioxide and KP46

⁵⁰ Rudnev, A. V., Foteeva, L. S., Kowol, C., Berger, R., Jakupec, M. A., Arion, V. B., Timerbaev, A. R. and Keppler, B. K., *J. Inorg. Biochem.*, **2006**, 100, 1819-1826.

⁵¹ Heffeter, P., Jungwirth, U., Jakupec, M., Hartinger, C., Galanski, M., Elbling, L., Micksche, M., Keppler, B. and Berger, W., *Drug Resist Updat*, **2008**, 11, 1-16.

1.5.3 METALLOCENE ANTICANCER DRUGS

Another group of potential anticancer compounds are metallocenes. According to IUPAC metallocenes contain a transition metal and two cyclopentadienyl ligands coordinated in a sandwich structure.⁵² These sandwich complexes can be classified structurally into the “classical” and the “bent” metallocenes (Figure 10). The research of these compounds started in 1952 with the discovery of ferrocene. Ferrocene is classified as a “classical” metallocene and by itself it is not particularly toxic. Without causing major health problems, it can be taken up orally, inhaled or injected and is degraded in the liver through enzymatic hydroxylation by cytochrome P₄₅₀. The hydroxyferrocene is unstable and decomposes in aqueous solution thereby releasing solvated iron atoms and showing antianemic properties. By testing the toxicity of ferrocene in beagle dogs no acute toxicity was observed but a massive iron overload was diagnosed, which could be reduced afterwards.⁵³ In contrast to the antianemic properties of ferrocene, ferroquine, an organometallic derivative of chloroquine, shows antimalarial properties.⁵⁴ Ferrocene can form a stable ferrocenium ion by a reversible one-electron oxidation. Simple ferrocenium salts were the first iron compounds that showed anti-proliferative effects on certain cancer cell lines. Studies showed that ferrocenium salts might form hydroxyl radicals, damaging DNA in a Fenton-type reaction. Further investigations showed that the cytotoxicity of ferrocenium salts depends on the water solubility and thereby on the nature of the counter ion. Other research on ferrocene focused on its redox activity. Jaouen and co-workers suggested a mechanism whereby redox activation induces anticancer activity in ferrocene derivatives. This work uncovered a group of derivatives of the anticancer drug tamoxifen.⁵³ Tamoxifen is an organic drug used in the treatment of hormone-dependent breast cancers upon its action on the oestrogen receptors. However about 30 % of breast cancers fail to respond to treatment with tamoxifen. There are two different oestrogen receptors, ER α and ER β , and tamoxifen is only effective against ER α (ER α +ve) rich cancers.⁴⁵ In ferrocifen, ferrocene replaces one phenyl ring of tamoxifen (Figure 10).⁵⁵ Other than tamoxifen itself, ferrocifens are

⁵² Gomez-Ruiz, S., Maksimovic-Ivanic, D., Mijatovic, S. and Kaluderovic, G. N., *Bioinorg Chem Appl*, **2012**, 2012, 140284.

⁵³ Gasser, G., Ott, I. and Metzler-Nolte, N., *J Med Chem*, **2011**, 54, 3-25.

⁵⁴ Biot, C., Taramelli, D., Forfar-Bares, I., Maciejewski, L. A., Boyce, M., Nowogrocki, G., Brocard, J. S., Basilico, N., Olliaro, P. and Egan, T. J., *Mol. Pharm.*, **2005**, 2, 185-193.

⁵⁵ Jaouen, G., Top, S., Vessieres, A., Leclercq, G. and McGlinchey, M. J., *Curr. Med. Chem.*, **2004**, 11, 2505-2517.

active against breast cancer cells that are ER α -ve (ER β +ve) and ER α +ve.⁴⁵ By competitive binding to the ER α subtype, tamoxifen represses oestradiol-mediated DNA transcription in the tumour tissue. For ferrocifen a dual mode of action was suggested. In addition to the tamoxifen like mode of action the redox activity of ferrocene is important for the additional biological activity exceeding that of the purely organic compound.

Bent metallocenes have a *cis*-dihalo motif similar to cisplatin. This resemblance encouraged the interest in metallocenes.⁵³ In 1979 Köpf and Köpf-Maier were the first to report anti-tumour properties of titanocene dichloride. They continued their investigations on other biological active metallocenes. The compounds of other metallocenes with the general formulas Cp₂MX₂ (M = Ti, V, Nb, Mo; X = halides and pseudo-halides), Cp₂Fe⁺ and main group (C₅R₅)₂M (M = Sn, Ge; R = H, CH₃) exhibited antitumor activity with less toxic effects than cisplatin. They were tested in a variety of tumour cells including Ehrlich ascites tumours, B16 melanoma, colon 38 carcinoma and Lewis lung carcinoma. Titanocene was the most active species in colon, breast and lung cancers.⁵⁶ As a consequence it was studied in clinical phase I trials in 1993 where a dose limiting nephrotoxicity was observed.⁵² Titanocene was abandoned in phase II due to its low efficacy vs. toxicity. In comparison with vanadocene dichloride it displayed similar activities in Ehrlich ascites tumours *in vivo* but the cytotoxicity of titanocene dichloride *in vitro* was 100 times lower, which was attributed to hydrolytic instability of the compound. Titanocene dichloride forms insoluble aggregates in aqueous solution and at pH 5 titanocene dichloride rapidly loses one chloride ion and the second after about 50 minutes. Both Cp ligands were quantitatively cleaved off after approximately 54 hours.⁵⁷

To improve the two major problems, the poor aqueous solubility and the hydrolytic stability of titanocene, variations of the labile ligands and bis-cyclopentadienyl moiety have been investigated. Examples of these derivatives are benzyl-substituted titanocene, derivatives with amino acids, *ansa*-titanocene, amide functionalised titanocenyl, derivatives with alkylammonium substituents on the Cp rings, steroid-functionalised titanocenes and alkenyl-substituted titanocene or *ansa*-titanocene derivatives.⁵² Substitutions of the chlorido ligands have been explored to achieve a better solubility. Modifications at Cp moiety affect the aqueous solubility and hydrolytic stability, thereby influencing the cytotoxic activity. Titanocene analogues

⁵⁶ Melendez, E., *Inorganica Chim Acta*, **2012**, 393, 36-52.

⁵⁷ Tshuva, E. Y. and Ashenhurst, J. A., *Eur. J. Inorg. Chem.*, **2009**, 2203-2218.

with aromatic groups at the Cp ligands have shown good *in vitro* activity with titanocene Y as most promising candidate (Figure 10).^{58, 53} This complex showed good activity against renal cell cancer.⁵⁷ Substitution of the chlorido ligands by carboxylate resulted in a more favourable pharmacokinetic profile.⁵³

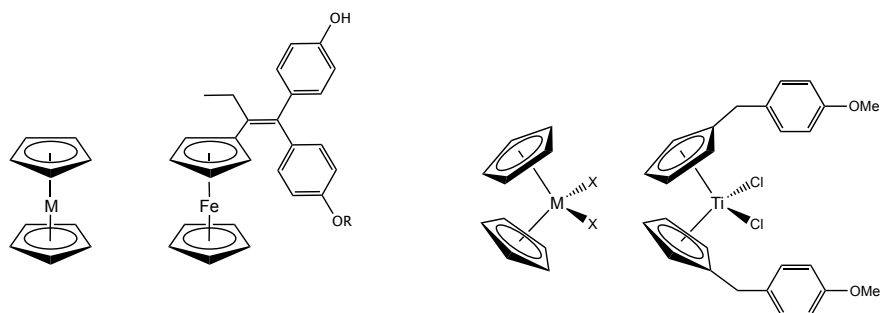


Figure 10: structures of the classic metallocenes, ferrocifen, bent metallocenes and titanocene Y (from left to right)

In recent years the attention of researchers also led to the variation of the central metal of metallocenes and thereby to the variation of properties like hydrolysis, solubility and cytotoxicity. Examples of these metallocenes are vanadocene, zirconocene, niobiocene, hafnocene and molybdenocene derivatives.

1.5.4 MOLYBDENUM

The human body contains about 0.07 mg of molybdenum per kg of weight with a total amount of about 5 mg. The concentration in the serum is 0.0057 mg/L, in blood plasma 5 to 34 µg/L and in the liver 0.48 mg/kg. For humans molybdenum is an essential trace element and the average daily intake should be 2 µg/kg. Significant dietary sources of molybdenum are pork liver (2 mg/kg), beef liver (1.6 mg/kg), beef kidney, rye, soybean, sea fish, eggs and milk. It is passively absorbed in the small intestine but copper inhibits the adsorption. The resorption rate depends on the dietary source and varies from 35 to 90 %. Molybdenum is distributed equally throughout the whole body and is accumulated in the liver and kidney. Excretion occurs through the kidney and to some extent through the bile. A deficiency in molybdenum leads to an increased excretion of xanthine, decreased excretion of uric acid and decrease tolerance of amino acids. An exaggerated supply presumably leads to increased xanthine-oxidase reactivity.⁵⁹

⁵⁸ Kelter, G., Sweeney, N. J., Strohsfeldt, K., Fiebig, H.-H. and Tacke, M., *Anti-Cancer Drugs*, **2005**, 16, 1091-1098.

⁵⁹ W. Ternes, *Biochemie der Elemente: Anorganische Chemie biologischer Prozesse*, Springer Spektrum, **2013**, 88-94.

1.5.5 MOLYBDENOCENE DICHLORIDE

In the experiments of Köpf and Maier-Köpf molybdenocene dichloride was one of the compounds that were active on a variety of tumours with lower toxicity than cisplatin. Though titanocene dichloride is the most active metallocene *in vitro*, molybdenocene dichloride shows better hydrolytic stability at physiological pH. Molybdenocene dichloride rapidly loses its chloride ligands during dissolution but the $\text{Cp}_2\text{Mo}^{2+}$ fragment stays intact. At physiological pH an equilibrium between the monomeric complex $\text{Cp}_2\text{Mo}(\text{H}_2\text{O})(\text{OH})^+$ and the dimeric species $[\text{Cp}_2\text{Mo}(\mu\text{-OH})_2\text{MoCp}_2]^{2+}$ is formed. To investigate the mode of action, several studies have been made with oligonucleotides, DNA and biologically relevant proteins.⁶⁰

Marks and co-workers studied the interaction of Cp_2MoCl_2 with self-complementary oligonucleotides and 5'-phosphorylated analogues. Results suggested a weak interaction with the oligonucleotides and significant changes at the 5' end.⁶¹ Melendez's group found a weak coordination with N(7) of purine bases only and negligible coordination with phosphoesters.⁶² Kuo and collaborators explained this negligible coordination saying that molybdenocene dichloride promotes phosphoester bond cleavage.⁶³

Several studies with DNA showed that Cp_2MoCl_2 binds DNA in about 5-10 %, while titanocene is bound up to 90–95 %. No interaction with DNA could be monitored when inhibiting the chloride hydrolysis with a saline solution.⁶⁰ Other experiments by Marks with DNA-processing enzymes showed no effects of the metallocene on DNA electrophoretic mobility, endonuclease, ligase or polymerase activity but inhibition of protein kinase C (PKC), an enzyme that regulates cellular proliferation.⁶¹

Harding and co-workers showed that molybdenocene forms stable adducts with glutathione (GSH) but these interactions lead to significant deactivation of the anti-tumour activity.⁶⁴ In competition experiments with nucleic acid components a stable complex with cysteine ($\text{Cp}_2\text{Mo}(\text{Cys})_2$) was formed. The cysteine could not be replaced with other nucleic acid components. Thereupon molybdenocene derivatives with thiolate ligands were synthesised. These compounds show hydrolytic stability but their cytotoxic activity demonstrated that thiolate coordination inactivates the complex due to the inertness of the Mo-S bond.

⁶⁰ Melendez, E., *J Organomet Chem*, **2012**, 706-707, 4-12.

⁶¹ Kuo, L. Y., Liu, A. H. and Marks, T. J., *Met. Ions Biol. Syst.*, **1996**, 33, 53-85.

⁶² Vera, J. L., Roman, F. R. and Melendez, E., *Bioorg Med Chem*, **2006**, 14, 8683-8691.

⁶³ Kuo, L. Y., Kuhn, S. and Ly, D., *Inorg. Chem.*, **1995**, 34, 5341-5345.

⁶⁴ Waern, J. B. and Harding, M. M., *Journal of Organometallic Chemistry*, **2004**, 689, 4655-4668.

Due to the affinity of molybdenocene dichloride to thiol containing proteins other targets have been investigated.⁶⁰ Radioactive binding studies with HSA provided evidence for a binding ratio of Cp_2MoCl_2 to HSA of 9.4 to 1.⁶⁵ Melendez stated that these interactions are mainly hydrophobic.⁶⁶

Harding treated V79 Chinese hamster lung cells with molybdenocene dichloride and studied them using micronucleus assays and transmission electron microscopy. The studies showed that with an increase in dose, an increase in frequency of micronuclei (chromosome breaks and/or loss) occurs. Also these cells displayed high incidence of polynucleation with high predominance of cells containing three to five nuclei as well as enlargement of the cell diameter compared to control cells. Further they showed increased chromatin condensation and damaged mitochondria in the cytoplasm. Harding reasoned that tubulin, a thiol rich protein with 20 cysteine residues positioned in the spindle apparatus, might be involved thereby deactivating the spindle apparatus and producing micronuclei. These results implicate that the mode of action is complex and involves multiple targets.⁶⁷

Structure modifications of molybdenocene to improve its anticancer activity have been performed similarly to titanocene and two strategies have been pursued: replacing the chloride ligands and functionalising the cyclopentadienyl rings for better solubility, stability and activity.⁶⁰

Because two thiol ligands inactivate molybdenocene, at least one coordination site has to be occupied by a labile donor molecule. Molybdenocene complexes with thionucleosides or thionucleobases as chelating ligands (S,N) have been reported. These demonstrated high aqueous stability and improved anti-proliferative activity.⁶⁸ Also, derivatives with O,O-chelating ligands showed better solubility and stability. Electrochemical characterisation of these O,O-chelating complexes revealed that they exhibit irreversible redox behaviour under physiological conditions.⁶⁹ Tacke and co-workers showed that benzyl-substitution of the Cp ring improved *in vitro* cytotoxicity compared to Cp_2MoCl_2 .⁷⁰ Another way to increase the anticancer activity is inclusion of the complexes in carrier molecules, where cyclodextrines or

⁶⁵ Campbell, K. S., Dillon, C. T., Smith, S. V. and Harding, M. M., *Polyhedron*, **2007**, 26, 456-459.

⁶⁶ Pavlaki, M., Debeli, K., Triantaphyllidou, I.-E., Klouras, N., Giannopoulou, E. and Aletras, A. J., *JBIC, J. Biol. Inorg. Chem.*, **2009**, 14, 947-957.

⁶⁷ Campbell, K. S., Foster, A. J., Dillon, C. T. and Harding, M. M., *J Inorg Biochem*, **2006**, 100, 1194-1198.

⁶⁸ Acevedo-Acevedo, D., Matta, J. and Melendez, E., *J Organomet Chem*, **2011**, 696, 1032-1037.

⁶⁹ Feliciano, I., Matta, J. and Melendez, E., *J Biol Inorg Chem*, **2009**, 14, 1109-1117.

⁷⁰ Gleeson, B., Claffey, J., Deally, A., Hogan, M., Mendez, L. M. M., Mueller-Bunz, H., Patil, S. and Tacke, M., *Inorg. Chim. Acta*, **2010**, 363, 1831-1836.

cucurbit[n]urils act as a molecular host. The encapsulated species enhanced the anti-proliferative activity due to better solubility and improved membrane permeability.⁶⁰

1.6 BIOLOGICALLY ACTIVE LIGANDS

Derivatisation of metal complexes with biologically active ligands that have antineoplastic effects on their own may have favourable effects. These include: stabilisation of ligand geometries, acquired redox-activity, increased solubility, enhanced cellular uptake, different mode of action and synergistic effect of metal and ligand.³⁰

1.6.1 PYRONE DERIVATIVES

Many pyrones are present in natural products. Thus many derivatives show favourable biocompatibility and toxicity profiles. One group of these derivatives are 3-hydroxypyrones. They are found in plants as natural products but can be synthesised as well and are commercially available. One of the best studied 3-hydroxypyrones is maltol (3-hydroxy-2-methyl-4(1H)-pyrone). It is known for its low toxicity and advantageous bioavailability. It is used as food additive for malty taste and aroma in e.g. bread, beer and cake. Maltol can be obtained from roasted malt, larch bark and pine, and by synthesis. Thionation of hydroxypyrones leads to S,O-chelating ligands (Figure 11). These exhibit a higher affinity towards softer metal ions and thereby increase stability of the formed complexes. Thionation can be achieved with Lawesson's reagent or P_4S_{10} .

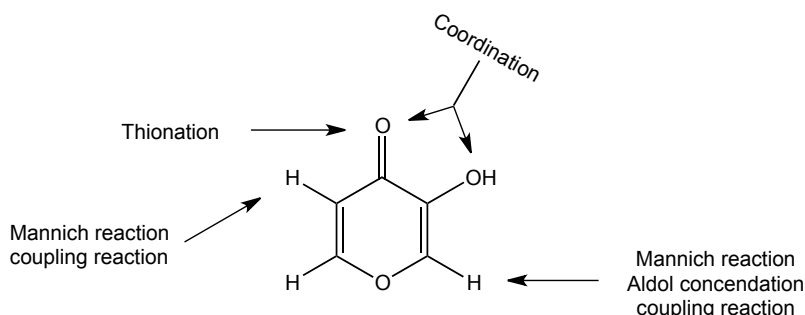


Figure 11: possible derivatisation sites of pyrones

3-Hydroxy-4(1H)-pyrones and their analogues can be used as building blocks for biologically active compounds. Modifications generate the opportunity for alterable biological properties. They are used as chelating ligands due to their high affinity to

metal ions. The chelating ligand forms a five-membered ring with the metal ion that is thermodynamic stable at physiological pH.

Pyrone based complexes have potential for delivery and release of metals. These properties are adjustable by the substitution pattern of the backbone. Upon coordination of maltol to cisplatin instead of the two chloride ligands, the solubility of the platinum-based drug increased, whereas DNA damaging properties did not change. The property of pyrones to release and deliver metal ions could be useful in the treatment of iron deficiency. Compared to iron salts, iron(III) complexes based on maltol have a higher intermediate stability. Thereby immediate hydrolysis and precipitation of iron can be inhibited. Other advantages are a more favourable bioavailability, allowing the administration of lower doses, and the ligands, which are not toxic.⁷¹ [Bis(maltolato)oxovanadium(IV)] (BMOV) increased the uptake of vanadium compared to the inorganic sodium and ammonium vanadate salts. Vanadium compounds have insulin-enhancing properties, which can be used to treat type-2 diabetes.⁷² The gallium salts GaCl₃ and Ga(NO₃)₃ possess antineoplastic properties and gallium nitrate was approved for the treatment of cancer-related hypercalcaemia, demonstrating activity against lymphomas and bladder cancer. However, low bioavailability and severe side effects were reported. To increase the stability of gallium towards hydrolysis and the bioavailability gallium maltolate (tris(maltolato)gallium(III)) was synthesised. This compound can be administered orally, has an increased lipophilicity and therefore higher bioavailability. Even so, the molecular target is still unknown and clinical trials were terminated.^{71,73} Coordination compounds of thiopyrones show potential in SPECT and PET imaging with Ga(III) and In(III).⁷⁴ With Zn(II) and VO²⁺ they exhibit good *in vitro* results as anti-diabetic agents.⁷⁵ Coordination of (MoO₂)²⁺ inhibits xanthine oxidase and due to their ability to chelate zinc ions, thiopyrones may act as inhibitors of matrix metalloproteins.⁷⁶ Sadler and co-workers reported Ru^{II}(arene) complexes with maltol as potential anticancer drugs. The Ru^{II}(arene)(maltolato) complex loses the maltolato ligand in

⁷¹ Kandoller, W., Kurzwehnart, A., Hanif, M., Meier, S. M., Henke, H., Keppler, B. K. and Hartinger, C. G., *J. Organomet. Chem.*, **2011**, 696, 999-1010.

⁷² Thompson, K. H., Lichter, J., LeBel, C., Scaife, M. C., McNeill, J. H. and Orvig, C., *J. Inorg. Biochem.*, **2009**, 103, 554-558.

⁷³ Bernstein, L. R., Tanner, T., Godfrey, C. and Noll, B., *Met.-Based Drugs*, **2000**, 7, 33-47.

⁷⁴ Monga, V., Patrick, B. O. and Orvig, C., *Inorg. Chem.*, **2005**, 44, 2666-2677.

⁷⁵ Katoh, A., Tsukahara, T., Saito, R., Ghosh, K. K., Yoshikawa, Y., Kojima, Y., Tamura, A. and Sakurai, H., *Chem. Lett.*, **2002**, 114-115.

⁷⁶ Chaves, S., Gil, M., Canario, S., Jelic, R., Romao, M. J., Trincao, J., Herdtweck, E., Sousa, J., Diniz, C., Fresco, P. and Santos, M. A., *Dalton Trans*, **2008**, 1773-1782.

aqueous solution and exhibits minor cytotoxicity.⁷⁷ By derivatisation an increase in lipophilicity and thereby increased cellular uptake and cytotoxicity were found. Furthermore, the substitution of carbonyl oxygen by sulphur increased lipophilicity, thus facilitating intracellular accumulation and improved stability by stronger binding. Therefore, these complexes showed improved anticancer activity.^{71, 78}

1.6.2 PICOLINIC ACID

Picolinic acid is a small six-membered ring structure compound and an isomer of nicotinic acid. It has been found in a variety of biological media e.g. cell culture supernatants, blood serum cerebrospinal fluid, human milk, pancreatic juice and intestine homogenates. Picolinic acid is synthesised from L-tryptophan through a side branch of the kynurenine pathway (Figure 12). The primary role of this pathway has not yet been determined but maintenance of cellular NAD concentrations may be a critical function in brain cells.

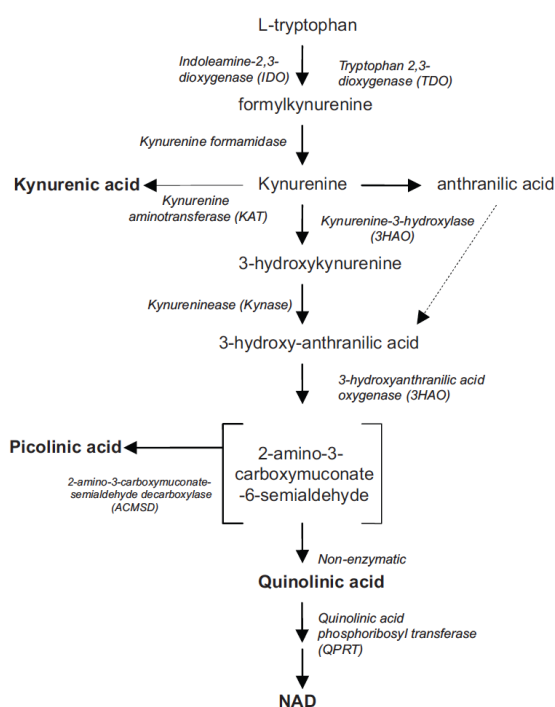


Figure 12: the kynurenine pathway in the CNS.⁷⁹

One of the most researched characteristics of picolinic acid is its property as a chelator. It was first reported in 1879 that picolinic acid efficiently chelates copper

⁷⁷ Peacock, A. F. A., Melchart, M., Deeth, R. J., Habtemariam, A., Parsons, S. and Sadler, P. J., *Chem. - Eur. J.*, **2007**, 13, 2601-2613.

⁷⁸ Kandjoller, W., Hartinger, C. G., Nazarov, A. A., Kuznetsov, M. L., John, R. O., Bartel, C., Jakupec, M. A., Arion, V. B. and Keppler, B. K., *Organometallics*, **2009**, 28, 4249-4251.

⁷⁹ Grant, R. S., Coggan, S. E. and Smythe, G. A., *Int. J. Tryptophan Res.*, **2009**, 2, 71-79.

and iron. It was later shown that it efficiently coordinates a broad range of other metals such as Ni, Zn, Cd, Pb and Cu. Picolinic metal complexes are widely used to introduce bioactive metals into biological systems.⁷⁹ The increased absorption of chromium by dietary supplementation with chromium picolinate [Cr(pic)₃] has been advocated in type 2 diabetes. This supplementation has effects on blood glucose, lipid metabolism and body composition by solubilising the metal through the formation of a chelate complex.⁸⁰ However, picolinic acid has a low solubility in lipophilic medium, reflecting its low partition coefficient (logP = 0.098).

Within the body, picolinic acid seems to have a wide range of effects, including neuroprotective, immunological and anti-proliferative effects.⁷⁹ *In vitro* studies suggested that picolinic acid can enhance macrophage interferon gamma dependant gene expression, and induce expression of macrophage inflammatory proteins (MIP) 1 α and 1 β . Induction of MIP 1 α and 1 β is thought to be through an iron chelation dependant process.^{79, 81} High concentrations of picolinic acid reportedly show selective inhibition on a number of viruses *in vitro* including the Human Immunodeficiency virus (HIV), Herpes Simplex virus (HSV) and Simian virus (SV).^{82, 83} In Addition anti-microbial effects have been observed against *Mycobacterium avium* complex (MAC) with additional enhancement of anti-microbial action of the drugs clarithromycin, rifampin and different fluoroquinolones.^{84, 85} The effect on tumour growth has been shown in *in vivo* studies on mice inoculated with tumour cells. Treatment with picolinic acid in combination with activated macrophages led to an increased lifespan compared to the control.⁸⁶ Furthermore, micromolar concentration of picolinic acid demonstrated a significant decrease in growth in human neuroblastoma cell lines.⁸⁷

⁸⁰ Broadhurst, C. L. and Domenico, P., *Diabetes Technol. Ther.*, **2006**, 8, 677-687.

⁸¹ Bosco, M. C., Rapisarda, A., Massazza, S., Melillo, G., Young, H. and Varesio, L., *J. Immunol.*, **2000**, 164, 3283-3291.

⁸² Fernandez-Pol, J. A. and Johnson, G. S., *Cancer Res.*, **1977**, 37, 4276-4279.

⁸³ Fernandez-Pol, J. A., Klos, D. J. and Hamilton, P. D., *Anticancer Res.*, **2001**, 21, 3773-3776.

⁸⁴ Blasi, E., Radzioch, D. and Varesio, L., *J. Immunol.*, **1988**, 141, 2153-2157.

⁸⁵ Shimizu, T. and Tomioka, H., *Antimicrob. Agents Chemother.*, **2006**, 50, 3186-3188.

⁸⁶ Ruffmann, R., Schlick, R., Chirigos, M. A., Budzynsky, W. and Varesio, L., *Drugs Exp. Clin. Res.*, **1987**, 13, 607-614.

⁸⁷ Coggan, S. E., Smythe, G. A., Bilgin, A. and Grant, R. S., *J Neurochem*, **2009**, 108, 1220-1225.

1.6.3 NAPHTHOQUINONE DERIVATIVES

The National Cancer Institute (NCI) considers the quinone moiety as an important biologically validated scaffold for the development of new bioactive compounds with good levels of cytotoxicity.⁸⁸ Naphthoquinones are derivatives of naphthalene with the three most prominent isomers: 1,2-naphthoquinone, 1,4-naphthoquinone and 2,6-naphthoquinone. Naphthoquinones occur as natural products but also as components of air pollutants from combustion of fossils, diesel fuel and tobacco smoke.⁸⁹ 1,4-Naphthoquinones have diverse pharmacological properties including antibacterial, antifungal, antiviral, anti-inflammatory, antipyretic and anticancer activity.⁹⁰ The most prominent examples of 1,4-naphthoquinones are vitamin K derivatives, which are based on 2-methyl-1,4-naphthoquinones and include two natural vitamins: K₁ and K₂. Vitamin K₃ is a synthetic product also called menadione (Figure 13).

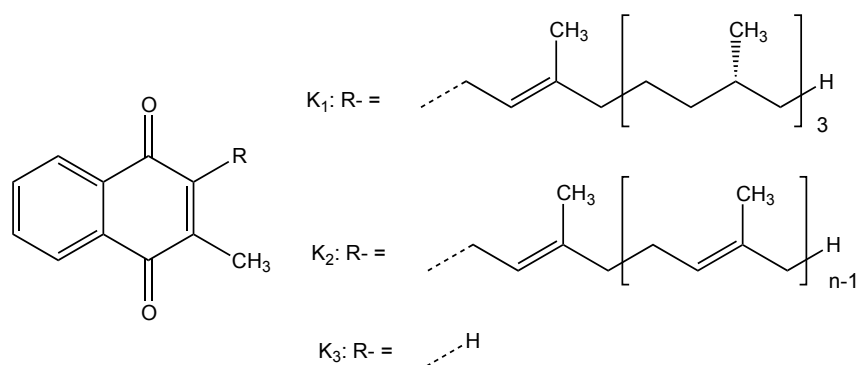


Figure 13: structures of vitamin K₁, vitamin K₂ and vitamin K₃

Vitamin K₂ has a polyisoprenoid side chain and exerts inhibitory effects on cancer cell growth and angiogenesis.⁹¹ Vitamin K₃ has been demonstrated to inhibit cell growth and cell death *in vitro* and *in vivo*. Researchers demonstrated that menadione induced cell death is associated with apoptosis and over expression of the *c-myc* gene.⁹² It has an inhibitory effect on angiogenesis and inhibits DNA polymerase gamma activity.⁹¹ The mode of action of vitamin K₃ is not well understood. One

⁸⁸ Oramas-Royo, S., Torrejon, C., Cuadrado, I., Hernandez-Molina, R., Hortelano, S., Estevez-Braun, A. and de Las Heras, B., *Bioorg Med Chem*, **2013**, 21, 2471-2477.

⁸⁹ Kumagai, Y., Shinkai, Y., Miura, T. and Cho, A. K., *Annu Rev Pharmacol Toxicol*, **2012**, 52, 221-247.

⁹⁰ Kumar, M. R., Aithal, K., Rao, B. N., Udupa, N. and Rao, B. S., *Toxicol In Vitro*, **2009**, 23, 242-250.

⁹¹ Kayashima, T., Mori, M., Yoshida, H., Mizushina, Y. and Matsubara, K., *Cancer Lett*, **2009**, 278, 34-40.

⁹² Chen, C., Liu, Y.-Z., Shia, K.-S. and Tseng, H.-Y., *Bioorg. Med. Chem. Lett.*, **2002**, 12, 2729-2732.

possible mechanism is oxidative stress. Another possibility is covalent binding of thiols by Michael addition reaction, leading to depletion of glutathione and the inhibition of sulfhydryl-dependent proteins.⁹² In general quinones are known for their reactions as prooxidants, reducing oxygen to reactive oxygen species, and as electrophiles, forming covalent bonds with tissue nucleophiles. In biochemical reactions, the quinone part can be reduced to the free radical semiquinone and further to the hydroquinone. The semiquinone and hydroquinone species are potentially toxic to cells. Free radicals can transfer unshared electrons thereby creating other free radicals. If oxygen is reduced, reactive oxygen species (ROS) are formed such as superoxide, hydroxyl radical and hydrogen peroxide. These ROS species can oxidise functional groups on proteins. The prooxidant properties of quinones are dependent on their reduction potential. As electrophiles, quinones form covalent bonds with nucleophilic functions of biological molecules in an arylation reaction. They have been shown to modify cell-signalling pathways by reacting with key regulatory proteins. Numerous naphthoquinone derivatives exhibit properties for a possible use as anticancer agents.⁸⁹

Kayashima and co-workers examined the anticancer and anti-angiogenic effects of 1,4-naphthoquinones. It exerted suppressive effects on growth and angiogenesis on the colon cancer cell HCT116. They also examined the effect of 1,4-naphthoquinone on human umbilical vein endothelial cells (HUVEC). It exerts its anti-angiogenic effects by suppressing endothelial cell tube formation and proliferation.⁹¹

Other examples of naphthoquinones with anti-tumour activity include lawsone, plumbagin, juglone, lapachol, β -lapachone and rhinacanthone (Figure 14).

Growth inhibitory effects of lawsone and juglone are mediated via blocking the S-phase of the cell cycle.⁸⁹ Juglone induces apoptosis and accumulation of cells in S-phase by its action on Pin1 and thereby affecting microtubules and preventing mitotic exit.⁹³ Plumbagin inhibits cell proliferation and induces apoptosis by modulation of different signal transduction pathways.⁹⁴ It has also been reported that plumbagin induces mammalian topoisomerase II mediated DNA cleavage *in vitro*.⁹⁵ The anti-tumour activity of lapachol may be due to its interaction with nucleic acids

⁹³ Fila, C., Metz, C. and van der Sluijs, P., *J. Biol. Chem.*, **2008**, 283, 21714-21724.

⁹⁴ Hsu, Y.-L., Cho, C.-Y., Kuo, P.-L., Huang, Y.-T. and Lin, C.-C., *J. Pharmacol. Exp. Ther.*, **2006**, 318, 484-494.

⁹⁵ Fujii, N., Yamashita, Y., Arima, Y., Nagashima, M. and Nakano, H., *Antimicrob. Agents Chemother.*, **1992**, 36, 2589-2594.

and intercalation of the naphthoquinone moiety with DNA thereby inhibiting DNA replication and RNA synthesis.⁹⁶

Quinones can form complexes with metal ions in three different oxidation states: quinone, semiquinone (one electron reduced form) and catechol (two electron reduced form). Ormay-Royo and co-workers reported metal complexes (Zn, Co, Cu, Ni, Mn) of lawsone with cytotoxicity in human cancer cells. The copper lawsone complex $[\text{Cu}(\text{Lw})_2(\text{H}_2\text{O})_2]$ was the most active and significantly induced apoptosis in HepG2 human cancer cells. The mechanism of action involves caspase activation and modulation of several apoptosis markers.⁸⁸

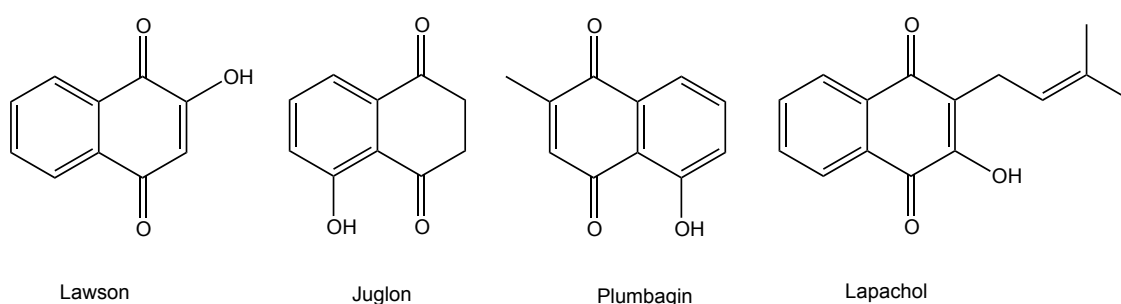


Figure 14: structures of the naphthoquinone derivatives lawson, juglon, plumagin and lapachol

1.6.4 FLAVONOID DERIVATIVES

Flavonoids are polyphenolic compounds and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea and wine. Their functions include pigmentation of flowers, fruits and leaves, pollination, seed dispersal, pollen tube growth, resorption of mineral nutrients, tolerance to abiotic stress and UV protection.⁹⁷ Flavonoids can be divided into different classes based on their structure: flavones, flavonols, flavanones, flavanonols, flavanols, catechins, isoflavones and anthocyanidins (Figure 15). Natural flavonoids occur predominantly glycosylated rather than as an aglycone.⁹⁸ Flavonoids are known for their beneficial effects on health. Their biological activities include: anti-inflammatory, anti-allergic, antiviral, anti-carcinogenic, anti-oxidant and modulation of enzymatic activities.^{98, 99}

⁹⁶ Rodrigues de Almeida, E., *Open Nat. Prod. J.*, **2009**, 2, 42-47.

⁹⁷ Batra, P. and Sharma, A., *3 Biotech*, **2013**, 3, 439-459.

⁹⁸ Nijveldt, R. J., Van Nood, E., Van Hoorn, D. E. C., Boelens, P. G., Van Norren, K. and Van Leeuwen, P. A. M., *Am. J. Clin. Nutr.*, **2001**, 74, 418-425.

⁹⁹ Ren, W., Qiao, Z., Wang, H., Zhu, L. and Zhang, L., *Med Res Rev*, **2003**, 23, 519-534.

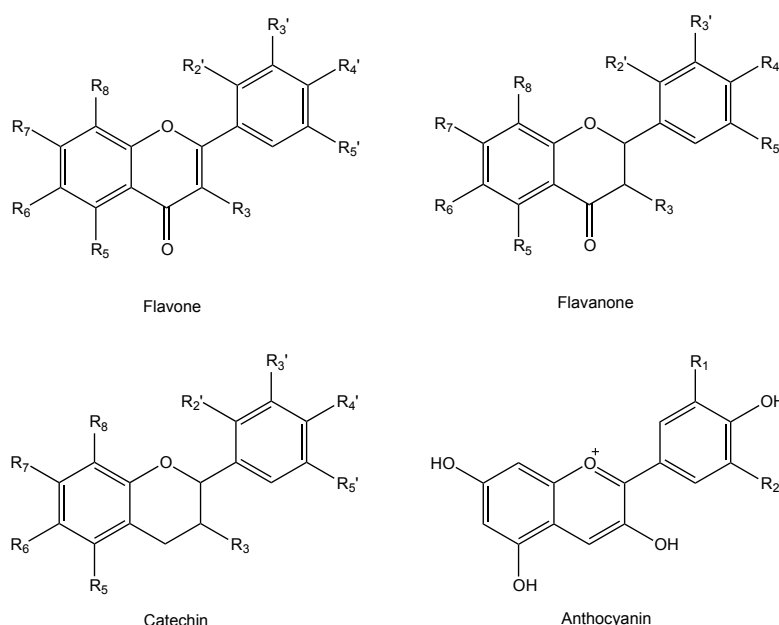


Figure 15: structures of different flavonoid classes

As anti-oxidants they protect the body against reactive oxygen species by acting as a scavenger. Flavonoids are oxidised by radicals thereby resulting in less-reactive, more stable radicals. The high reactivity of the hydroxyl group of the flavonoids makes the radicals inactive. Some flavonoids have been reported to inhibit xanthine oxidase. The xanthine oxidase pathway plays an important role in oxidative injuries to tissues, because xanthine oxidase is a source of oxygen free radicals. By inhibiting xanthine oxidase activity, the oxidative injuries decreased. An anti-atherosclerotic effect was observed by a few clinical studies. These stated that flavonoid intake protects against coronary heart disease. Their anti-inflammatory effects are based on the inhibition of the cyclooxygenase and 5-lipoxygenase pathways thereby reducing the release of arachidonic acid, which is involved in general inflammation response. Lipid peroxidation causes cellular membrane damage. This damage causes a shift in the cell charge and changing the osmotic pressure yielding swelling and eventually cell death. The anti-thrombogenic effect of flavonoids is also based on the inhibition of the activity of cyclooxygenase and lipoxygenase. The antiviral effect of flavonoids is due to interaction with different stages in the replication cycle of viruses, including herpes simplex virus, parainfluenza virus and adenovirus.⁹⁸

The anti-cancer activity of flavonoids has been shown in numerous *in vitro* and *in vivo* studies and some compounds entered clinical trials. Epidemiological studies provided data that associated high dietary uptake of flavonoid compounds with fruits

and vegetables with low cancer prevalence in humans. Several possible mechanisms of action have been reported.

Studies showed that some flavonoids modulate the metabolism and disposition of carcinogens thereby contributing to cancer prevention. For example they demonstrated inhibition of certain phase I enzymes, cytochrome P450 isozymes, thus having a protective role against the induction of cellular damage by the activation of carcinogens. Nonetheless, they can also activate phase II enzymes, thereby increasing detoxification and elimination of carcinogens from the body.

Most flavonoids have been demonstrated to inhibit proliferation in human cancer cell lines, whereas less or non-toxic to normal cells. The mechanism may involve inhibition of the prooxidant process that causes tumour promotion. Flavonoids can also inhibit ornithine decarboxylase induced by tumour promoters thereby inhibiting DNA and protein synthesis. Furthermore, flavonoids can inhibit signal transduction enzymes involved in the regulation of cell proliferation including protein tyrosine kinase, protein kinase C and phosphoinositide 3-kinases.⁹⁹ Various studies with intact cells showed that flavonoids can cause cell arrest in correlation to their ability to inhibit CDKs.⁹⁷

Another significant anticancer property of flavonoids may be due to apoptosis. The mechanisms leading to apoptosis are not yet clarified but may involve DNA topoisomerase I/II activity, regulation of heat shock proteins expression, decrease of ROS, nuclear transcription factor kappaB, modulation of signalling pathways, downregulation of Bcl-2 and Bcl-X(L) expression but promotion of Bax and Bak expression, release of cytochrome c, activation of endonuclease and suppression of Mcl-1 protein.

Several studies found flavones to induce differentiation of cells that may lead to the eventual elimination of tumourigenic cells and rebalance of normal cellular homeostasis.⁹⁹ Flavonoids have been reported as angiogenesis inhibitors and therefore can cause lack of diffusion of nutrients and oxygen to rapidly growing cancerous cells and hence lead to cell death.⁹⁷

Multidrug resistance is a serious impediment to successful chemotherapy. Certain flavonoids possess potent inhibitory activity against multidrug associated proteins. The reversal of multidrug resistance might help protect against multidrug-resistant tumours.⁹⁹

2 OBJECTIVE

Tests by Köpf and Maier-Köpf showed that molybdenocene dichloride is active on a variety of tumours with lower toxicity than cisplatin. The Cp rings of the compound are stable in aqueous solution but molybdenocene rapidly loses its two chloride ligands. The aim of this thesis is to increase the hydrolytic stability by substituting the labile chlorido ligands of molybdenocene dichloride by different bioactive O,O-, O,S- and O,N-chelating scaffolds, yielding organometallics with different coordination motives (Figure 16). The obtained compounds will be characterised by standard analytical methods to confirm the formation and purity of the synthesized complexes. The substituted molybdenocenes will be investigated towards their stability under physiological conditions. Blood proteins are the first potential binding partners after intravenous administration of metallodrugs. Therefore the interaction with HSA will be studied in detail by different approaches. The impact on the anticancer activity compared to molybdenocene dichloride will be discussed.

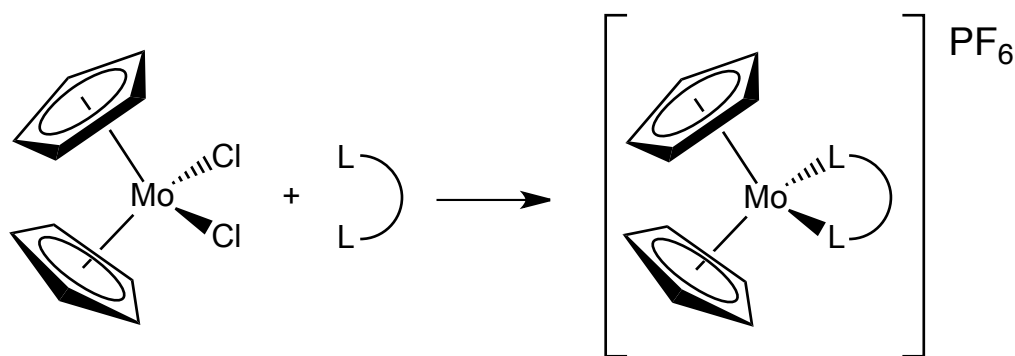


Figure 16: substitution of the two chloride ligands by different bidentate ligands

3 RESULTS AND DISCUSSION

3.1 LIGAND SYNTHESSES AND CHARACTERISATION

3.1.1 LIGAND SYNTHESSES

Aim of the master thesis was to synthesise bidentate ligand scaffolds with different coordination motives to elucidate the influences of O,O-, O,S- and O,N-chelating ligands on resulting molybdenocene-based organometallics. The ligands were characterized by $^1\text{H-NMR}$ spectroscopy.

3.1.1.1 O,O-chelating Ligands

The naphthoquinone-based O,O-chelate was obtained from menadione via epoxidation with hydrogenperoxide under alkaline conditions and subsequent acid catalysed ring opening on silica gel (Figure 17).

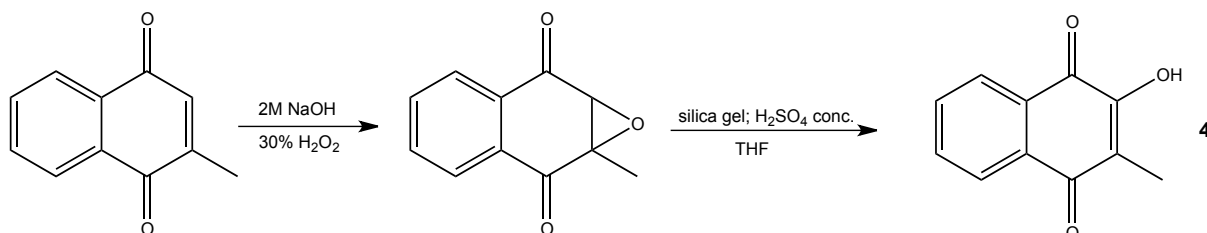


Figure 17: synthesis of **4** starting from menadione (2-methylnaphthalene-1,4-dione)

The O,O-chelating flavone-derived ligand was synthesised via the chalcone intermediate by Claisen-Schmidt condensation and the desired product was prepared by oxidative cyclisation via Algar-Flynn-Oyamada reaction (Figure 18).

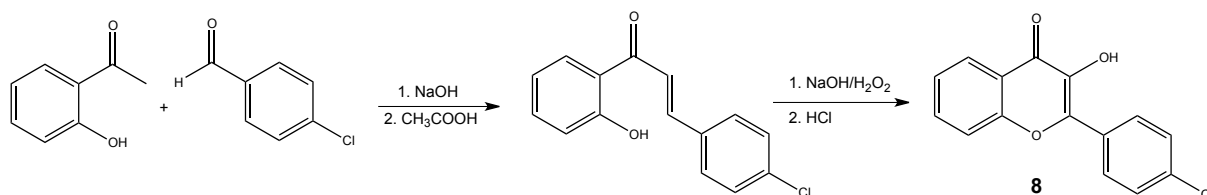


Figure 18: reaction of 2-Hydroxyacetophenone with 4-Chlorobenzaldehyde forming the flavone **8**

Ligands **4**^{100,101} and **8**¹⁰² were synthesised according to literature procedures in fair to good yields (31 – 69 %). If purification was necessary recrystallization in methanol was performed and the obtained ligands were characterized by ¹H NMR spectroscopy.

3.1.1.2 O,S-chelating Ligands

Both O,S-chelating ligands were obtained by thionation with Lawesson's reagent according to literature procedure (Figure 19).¹⁰³

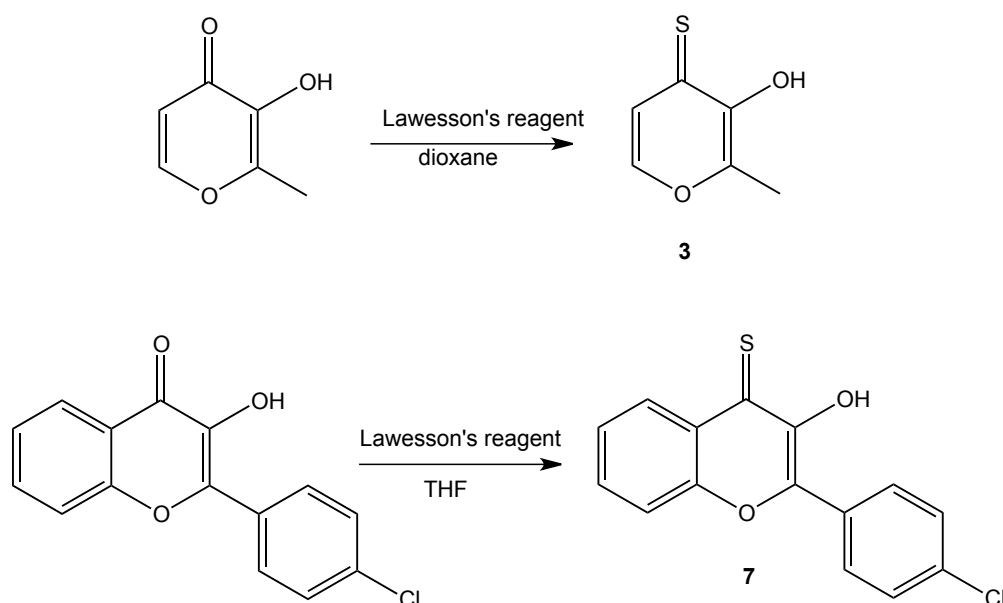


Figure 19: synthesis of **3** by thionation of maltol and **7** by thionation of 2-(4-chlorophenyl)-3-hydroxy-4H-1-benzopyran-4-one

Ligand **3** was purified via column chromatography with hexane/ethyl acetate (10 : 1) as eluent. Ligand **7** was recrystallized from ethanol. The compounds were obtained in good to excellent yields (69 – 89 %) and characterized by ¹H NMR spectroscopy.

¹⁰⁰ Fioroni, G., Fringuelli, F., Pizzo, F. and Vaccaro, L., *Green Chemistry*, **2003**, 5, 425.

¹⁰¹ Hu, Y., Zhu, R., Xing, L., Wang, X., Cheng, C. and Liu, B., *Synlett*, **2007**, 2007, 2267-2271.

¹⁰² Qin, C. X., Chen, X., Hughes, R. A., Williams, S. J. and Woodman, O. L., *J. Med. Chem.*, **2008**, 51, 1874-1884.

¹⁰³ Chaves, S., Gil, M., Canario, S., Jelic, R., Romao, M. J., Trincao, J., Herdtweck, E., Sousa, J., Diniz, C., Fresco, P. and Santos, M. A., *Dalton Trans*, **2008**, 1773-1782.

3.1.1.3 O,N-chelating Ligands

The respective oxime was synthesised by condensation of the naphthoquinone oxo-group with hydroxylamine (Figure 20).¹⁰⁴

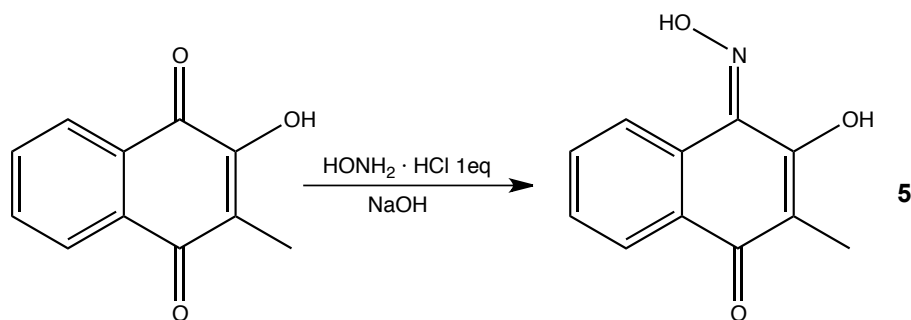


Figure 20: reaction of **4** with hydroxylamine to the oxime **5**

The compound was obtained in good yield (85 %) without any further purification and characterized by ¹H NMR spectroscopy. The spectrum showed two sets of signals in a ratio of 5:1 implicating the formation of E/Z isomers (Figure 21).

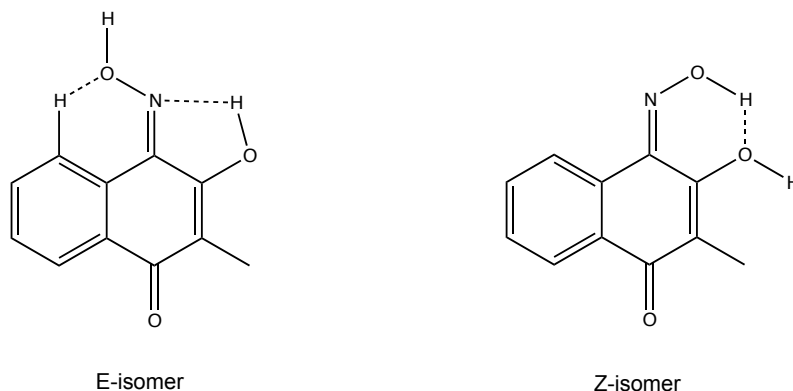


Figure 21: E and Z isomers of 1,4-Naphthalenedione, 2-hydroxy-3-methyl-, 1-oxime

The ratio of the isomers can be determined via ¹H NMR spectroscopy by integrating the different sets of signals. The shift to a lower field of the aromatic proton H8 by approximately 1 ppm may occur due to an intramolecular hydrogen bond with the oxygen of the oxime (Figure 22).

¹⁰⁴ Jagtap, S. B., Joshi, S. G., Litake, G. M., Ghole, V. S. and Kulkarni, B. A., *Met.-Based Drugs*, **2000**, 7, 147-150.

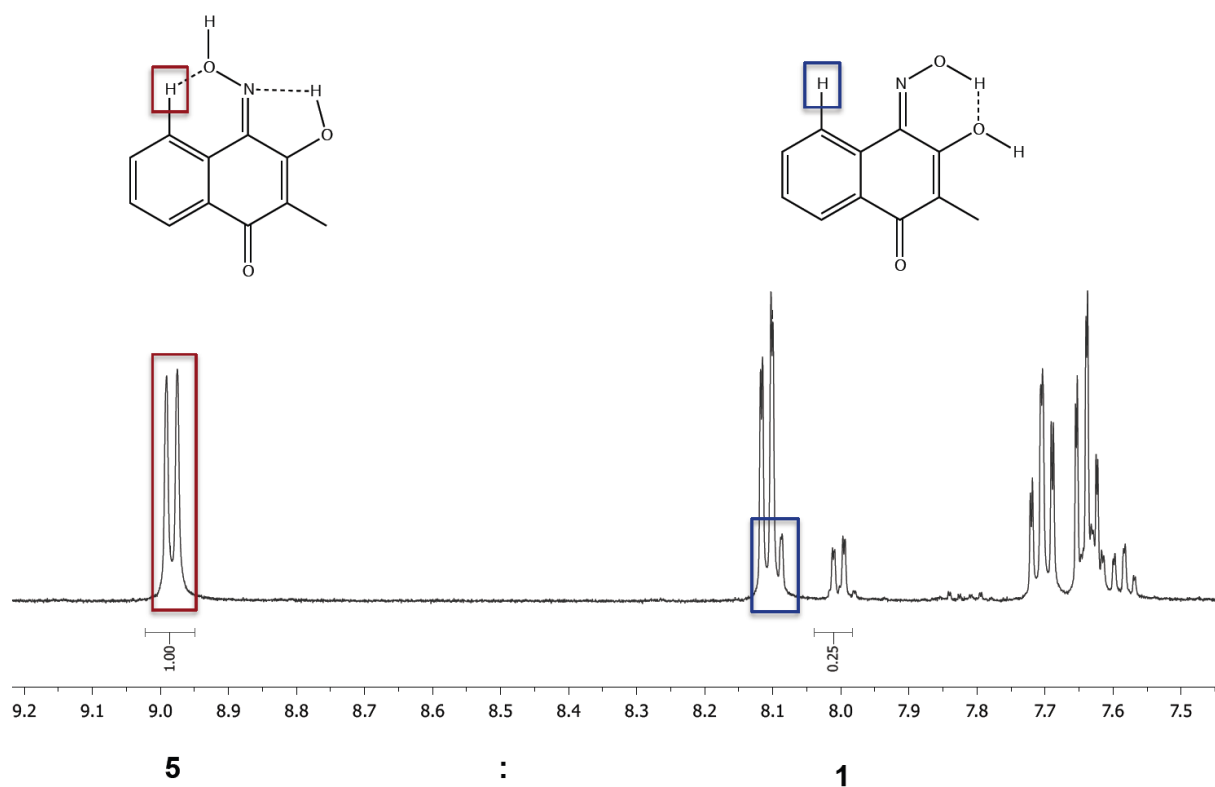


Figure 22: ^1H NMR spectrum of **5** showing two sets of signals of the E/Z isomers in the ratio 5:1

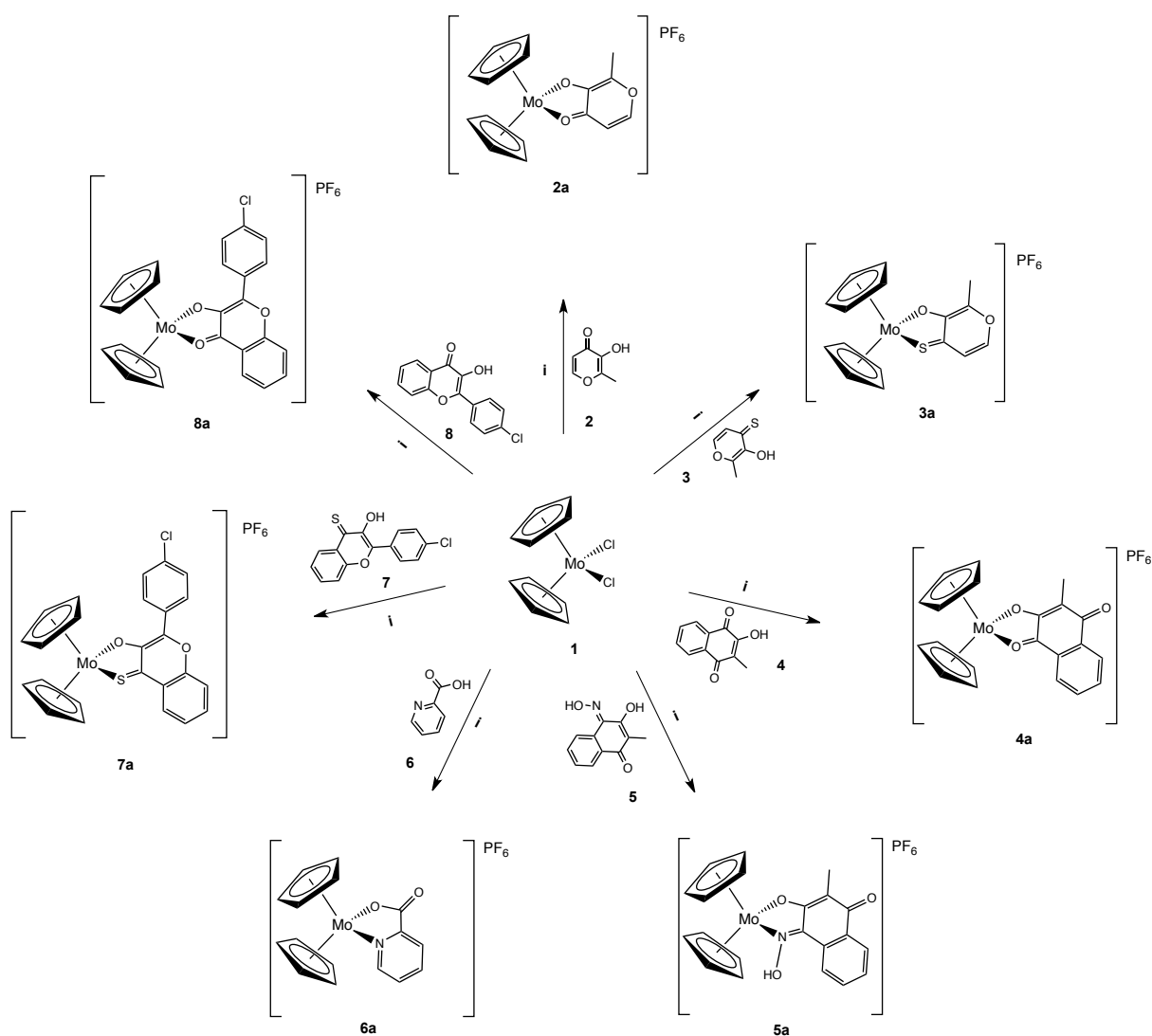
3.2 COMPLEXATION WITH MOLYBDENOCENE DICHLORIDE AND CHARACTERIZATION

The complexation of the O,O-, O,S- and O,N-chelating ligands with molybdenocene dichloride yielded in positively charged complexes (Scheme 1). All complexes were characterised by ^1H , ^{13}C , 2D, ^{19}F and ^{31}P NMR-spectroscopy, elemental analysis, ESI-MS and melting points. The crystal structure of **1** and **7a** could be obtained by X-ray diffraction analysis. Their stability was examined using NMR and UV/Vis spectroscopy and their redox properties were investigated by cyclic voltammetry. Binding studies with the serum protein HSA were carried out using ICP-MS and fluorescence spectroscopy.

It was tried first to synthesize the molybdenocene-derived complexes with chloride as a counter ion but purification of the desired complexes from obtained compound mixture was not successful. The next attempt was to exchange the counter ion of the charged complexes by addition of either sodium or ammonium hexafluorophosphate. The exchange of the counter ion resulted in a decreased aqueous solubility of the compounds. However, the complexes could be obtained by precipitation in high

purity and sufficient yield (**2a**, **3a**, **6a** and **8a**). The remaining compounds were isolated by different purification protocols, like various solvents (MeOH, (dry) MeOH, THF, H₂O, solvent mixtures (MeOH/DMF, MeOH/THF), different bases (NaOMe, NaOH, diethylamine, triethylamine) or without a base, temperature (room temperature – up to 50 °C), strict argon atmosphere and complexation by microwave heating.

All compounds except **7a** and **8a**, due to their limited solubility, could be obtained by microwave-assisted synthesis. Especially complex **4a** could only be obtained by under these conditions. Compound **7a** was synthesised under a strict argon atmosphere and the reaction mixture had to be concentrated in order for the complex to precipitate.



Scheme 1: synthetic scheme of all prepared molybdenocene complexes; *i* = MeOH, 1.1 eq NaOMe, NaPF₆/NH₄PF₆

The general procedure for the compounds **2a** – **8a** consists in the deprotonation of ligands **2** – **8** with sodium methoxide in methanol, addition of molybdenocene dichloride and reaction time of 5 – 48 hours. After full conversion, the products were precipitated after addition of either sodium or ammonium hexafluorophosphate. The compounds were obtained in poor to moderate yields: standard conditions: 15 - 47 %, microwave conditions: 12 – 64 %.

The ^1H NMR spectra of each complex showed only one signal for the two cyclopentadienyl rings concluding that the ligands are coordinated symmetrically to molybdenum. Comparison of the cyclopentadienyl shifts in the ^1H and ^{13}C spectra of the series revealed that the *O,O*-chelated complexes are shifted to the lowest field and *O,S*-complexes to the highest field compared to the *O,N*-complexes (Figure 23, Table 1). The Cp signals of molybdenum dichloride were detected significantly in higher fields (^1H NMR (d^6 -DMSO): $\delta = 5.65$).

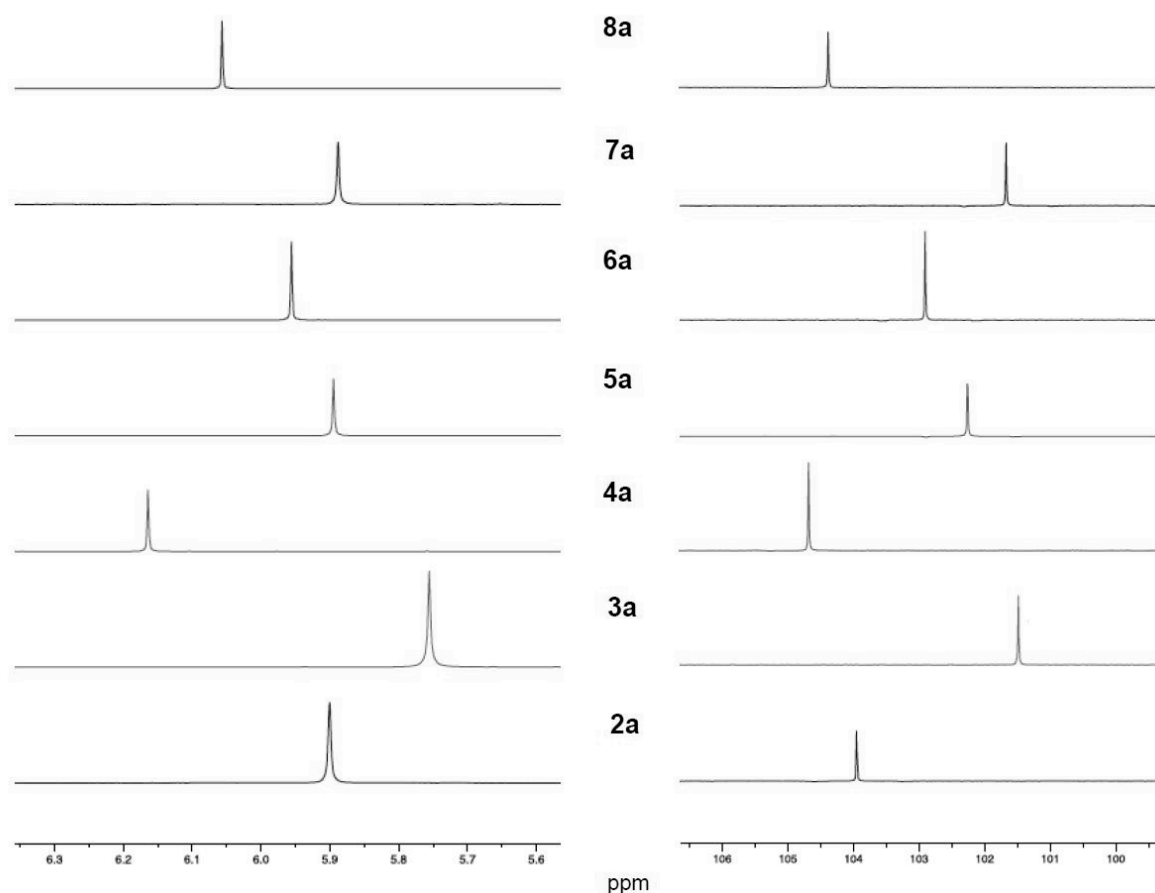


Figure 23: comparison of the ^1H NMR (left) and the ^{13}C NMR shifts (right) of the Cp signal from compound **2a-8a**

Table 1: ^1H and ^{13}C NMR shifts of the Cp signal from compound **2a-8a** in ppm

compound	^1H [ppm]	^{13}C [ppm]
1	5.65	-
2a	5.90	103.9
3a	5.76	101.5
4a	6.16	104.7
5a	5.90	102.2
6a	5.96	102.9
7a	5.89	101.7
8a	6.06	104.4

To confirm the formation of the respective complex with hexafluorophosphate as counter ion, ^{19}F and ^{31}P NMR spectra were recorded (Figure 24). ^{19}F and ^{31}P both have a nuclear spin of $\frac{1}{2}$, like the ^1H nucleus, leading to the coupling of the two nuclei. The ^{19}F signal is split to a doublet and the ^{31}P signal to a septet.

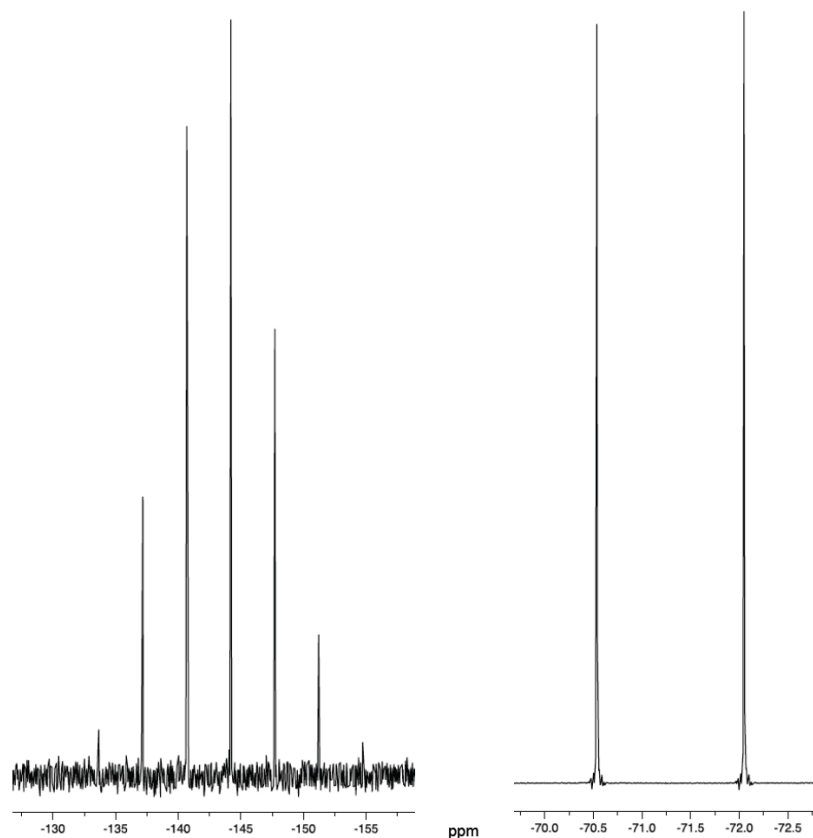


Figure 24: ^{31}P NMR spectrum (left) of the counter ion hexafluorophosphate of **2a-8a** showing a septet and the ^{19}F spectrum (right) showing a doublet.

3.3 CRYSTALLOGRAPHIC STRUCTURE DETERMINATION

The single crystals of **1** and **7a** suitable for X-ray diffraction analysis were obtained from methanol (Figure 25). Compound **1** crystallized in the monoclinic space group $P2_1/c$ and **7a** in the triclinic space group $P-1$. The attachment of the bidentate flavone-based O,S-chelating ligand led to the formation of a non-planar (torsion angle $O2-Mo-S-C3 / C2-S-Mo-O2$ unequal zero) ring. Compared to the dichlorido precursor **1**, the bond length from molybdenum to the centroid of the Cp rings of compound **7a** is only marginally longer (1.9750 [Å] for **1**, 1.9865 [Å] for **7a**). The Cp rings, measured from the centroid to molybdenum, are bent in a similar angle (132.91 [°] for **1**, 134.11 [°] for **7a**). The distances between Mo-Cl and Mo-S were found in the same range (2.4827 [Å] for **1**, 2.4486 [Å] for **7a**). Due to the electron withdrawing effect of the transition metal centre the C=S bond in complex **7a** (1.7099 [Å]) is significantly longer than the usual thiocarbonyl bond length of 1.60 Å. Selected bond lengths and angles, crystal data and details of data collection are listed in Table 2.

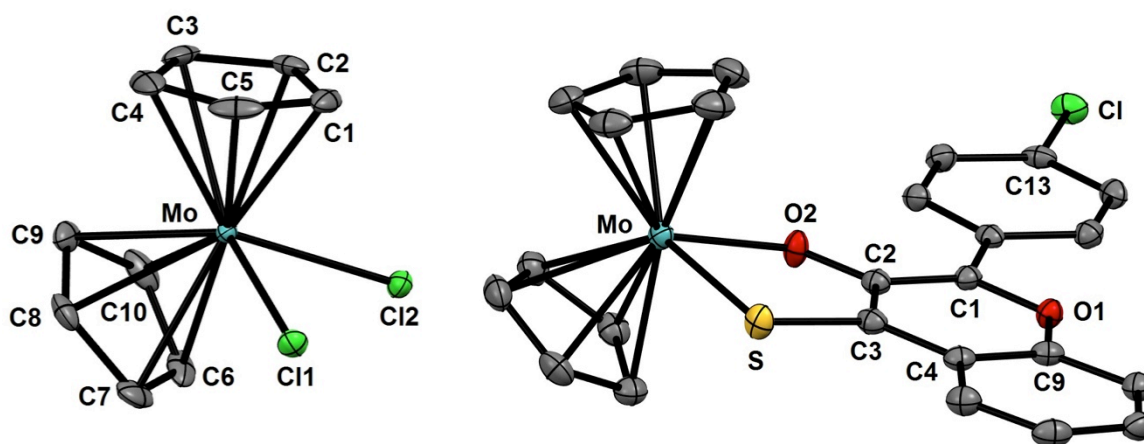


Figure 25: molecular structure of complexes **1** and **7a**. The hydrogen atoms were omitted for clarity.

Table 2: selected bond lengths and angels, crystal data and details of data collection for **1** and **7a**

	1	7a
Chemical formula	C ₁₀ H ₁₀ Cl ₂ Mo	C ₂₅ H ₁₈ ClF ₆ MoO ₂ SP
M [g/mol]	297.04	658.83
Temperature [K]	150	150
Crystal colour	grey, block	pink, block
Crystal system	monoclinic	triclinic
Space group	P2 ₁ /c	P-1
a [Å]	13.1871 (6)	8.4503 (3)
b [Å]	13.0595 (5)	10.6345 (5)
c [Å]	11.7986 (4)	13.3110 (7)
α [°]	90	97.0672 (15)
β [°]	105.5250 (11)	93.1636 (16)
γ [°]	90	93.8587 (16)
V [Å ³]	1957.78	1181.97
Z	4	2
Mo-Cl1 [Å]	2.4826	-
Mo-Cl2 [Å]	2.4828	-
Mo-O2 [Å]	-	2.0829
Mo-S [Å]	-	2.4486
Mo-Cp*1 [Å]	1.9750	1.9860
Mo-Cp2 [Å]	1.9750	1.9870
Cp1-Mo-Cp2 [°]	132.91	134.11
Cl1-Mo-Cl2 [°]	81.52	-
O2-Mo-S	-	78.89
Mo-S-C3-C4 [°]	-	175.98
Mo-O2-C2-C1 [°]	-	-171.92

*centroid of Cp ring

3.4 ESI-MS STUDIES

The structures of all compounds were verified by ESI-MS. It is a soft ionization method and converts the desired compounds into gas phase with little fragmentation. The compounds were dissolved in methanol and dispersed by electrospray into a fine aerosol. The found and calculated m/z values are listed in Table 3.

Table 3: experimental and theoretical m/z values of compounds **2a-8a**

Detected ion	m/z	m_{theor}
[2a -PF ₆] ⁺	351.2	351.2
[3a -PF ₆] ⁺	367.3	367.3
[4a -PF ₆] ⁺	413.1	413.3
[5a -PF ₆] ⁺	428.3	428.2
[6a -PF ₆] ⁺	348.4	348.2
[7a -PF ₆] ⁺	513.4	513.9
[8a -PF ₆] ⁺	497.0	497.8

3.5 STABILITY IN AQUEOUS SOLUTION

To study the stability of the compounds in aqueous solution NMR- and UV/Vis spectra were recorded over the course of several days. The limiting factor to determine the stability with NMR-spectroscopy was the poor solubility of the compounds **4a – 8a**.

3.5.1 NMR-SPECTROSCOPY

The compounds **1**, **2a** and **3a** were dissolved in D₂O and measured with ¹H NMR spectroscopy to determine their stability in aqueous solution. The compounds **4a**, **5a**, **6a**, **7a** and **8a** were insufficient soluble under these conditions to obtain reliable data. **1** was measured after 5 minutes, 1 hour, 24 hours and once every day up to 7 days. For **2a** and **3a** a spectrum was recorded after 5 minutes, 1 hour, 4 hours, 6 hours, 24 hours and up to 12 days (Figure 26).

Compound **2a** and **3a** did not show any hydrolysis of the ligands and were stable in aqueous solution for 12 days.

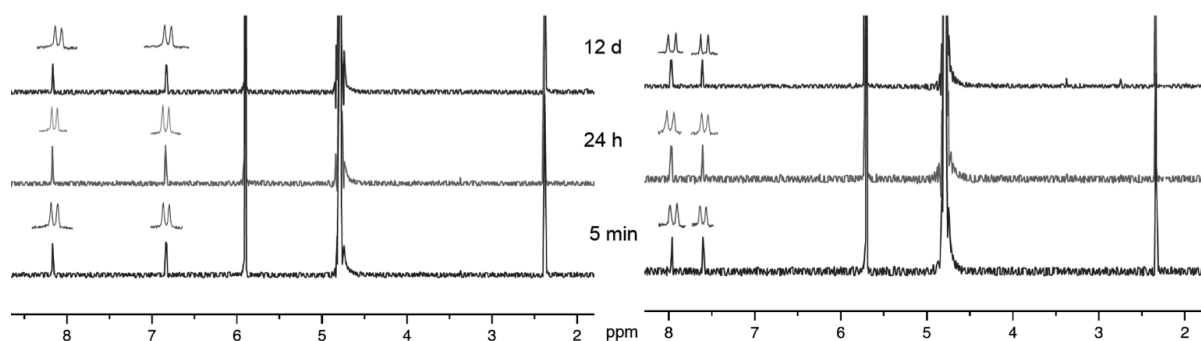


Figure 26: ^1H NMR spectra of compounds **2a** (left) and **3a** (right) in D_2O after 5 minutes, 24 hours and 12 days

In contrast, molybdenocene dichloride rapidly loses both chlorido ligands in a stepwise process (Figure 27). At physiological pH one aqua ligand gets deprotonated and an equilibrium is formed between $\text{Cp}_2\text{Mo}(\text{H}_2\text{O}(\text{OH}))^+$ and the dimeric species $[\text{Cp}_2\text{Mo}(\mu\text{-OH})_2\text{MoCp}_2]^{2+}$ (Figure 28). The cyclopentadienyl ligands of Molybdenocene are not prone to substitution reactions in contrast to other metallocenes ($\text{M} = \text{V}$, Nb , Ti).¹⁰⁵

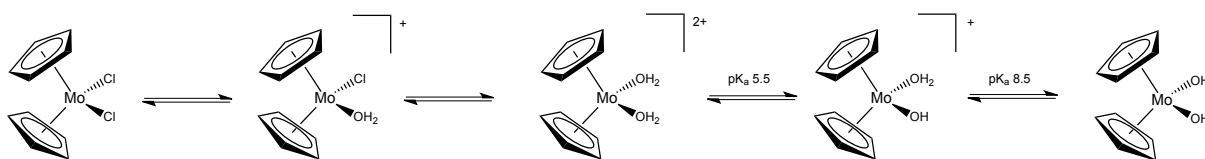


Figure 27: hydrolysis of molybdenocene dichloride

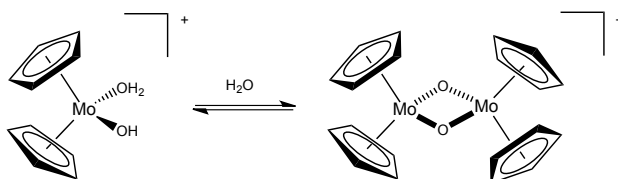


Figure 28: equilibrium between monomeric and dimeric species

The ^1H -NMR spectrum after five minutes showed two peaks in the ratio of 2:1 leading to the conclusion that after a few minutes both chloride ligands are already cleaved off (Figure 29). The activated molybdenocene undergoes dimerization leading to the formation of an oxo-bridged dimer in a ratio of 1:4. The observed equilibrium did not change over a period of 7 days.

¹⁰⁵ Waern, J. B. and Harding, M. M., *Journal of Organometallic Chemistry*, **2004**, 689, 4655-4668.

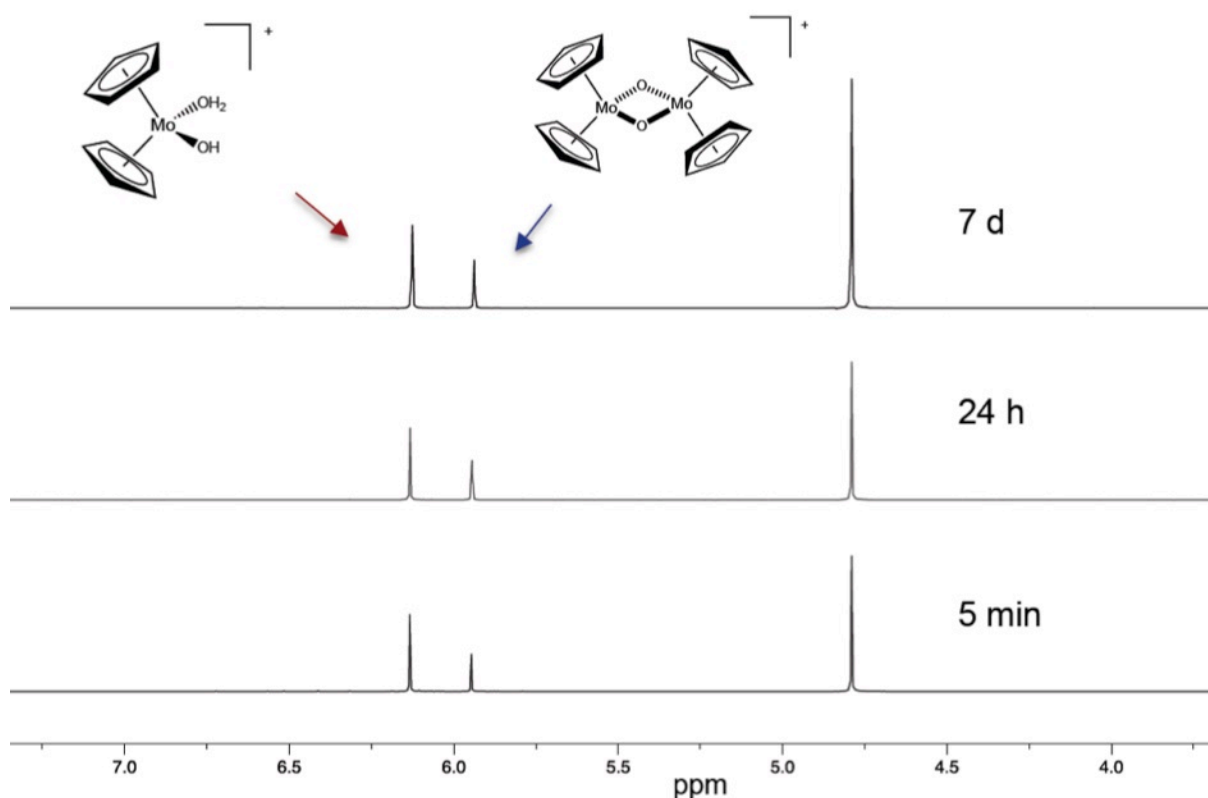


Figure 29: ^1H NMR spectra of **1** in D_2O after 5 minutes, 24 hours and 7 days showing the dimer and the monomer formed in the equilibrium

3.5.2 UV/VIS SPECTROSCOPY

In addition to NMR experiments, the stability of the molybdenocene complexes **2a** - **8a** was studied in 10 % v/v DMSO/ H_2O and 10 % v/v DMSO/PBS at 293 K for 7 days by UV/Vis spectroscopy (Figures 30-36). Prior to the investigations in DMSO/ H_2O or DMSO/PBS the stability of all complexes in DMSO was determined by NMR spectroscopy. No formation of new signal sets was detected over 24 hours. The UV/Vis experiments showed that all complexes are stable in aqueous solution over 7 days and do not undergo hydrolysis. Due to their poor solubility, precipitation (especially compound **4a** and **7a**) occurred but despite the decreasing intensity their peak maxima did not shift. The wavelength of the peak maxima and molar extinction coefficients are listed in Table 4.

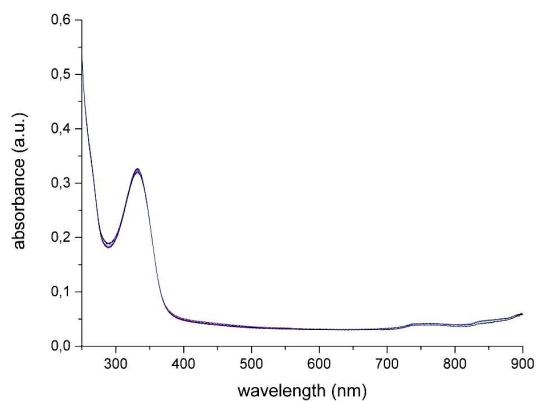
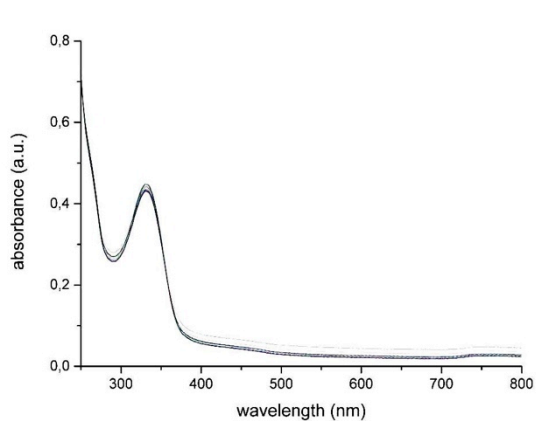


Figure 30: UV/Vis spectra of complex **2a** in 10 % v/v DMSO/H₂O (left) and 10 % v/v DMSO/PBS (right)

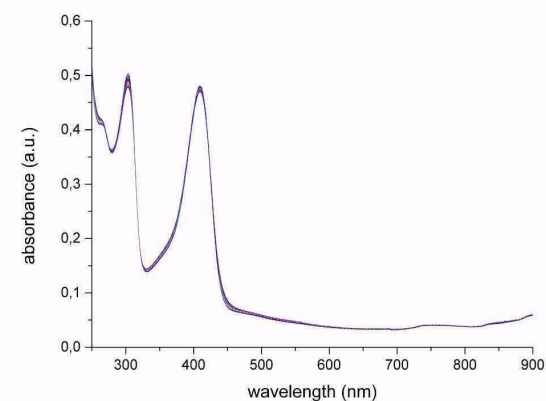
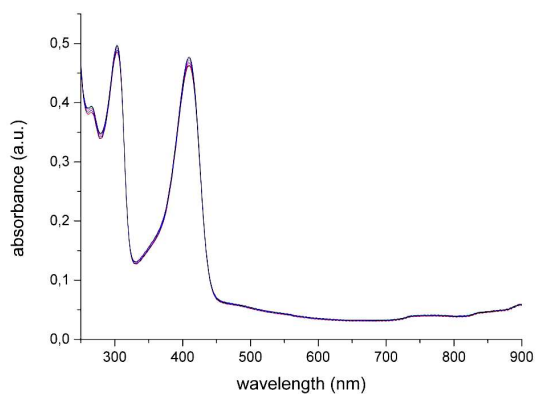


Figure 31: UV/Vis spectra of complex **3a** in 10 % v/v DMSO/H₂O (left) and 10 % v/v DMSO/PBS (right)

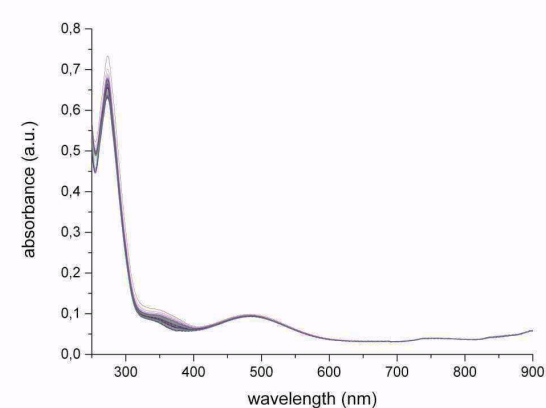
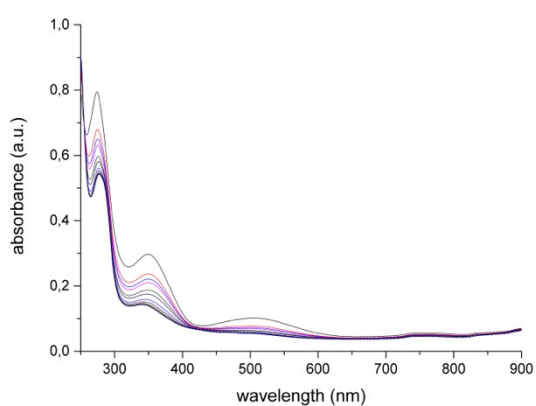


Figure 32: UV/Vis spectra of complex **4a** in 10 % v/v DMSO/H₂O (left) and 10 % v/v DMSO/PBS (right)

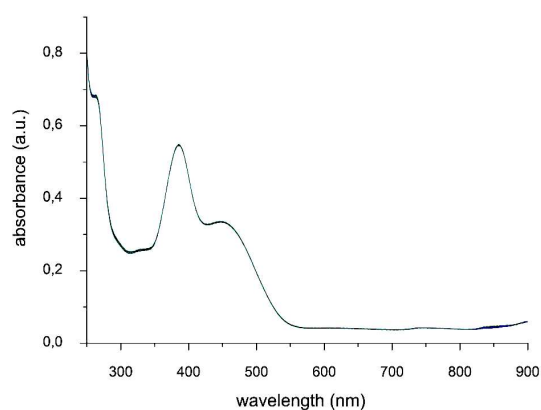
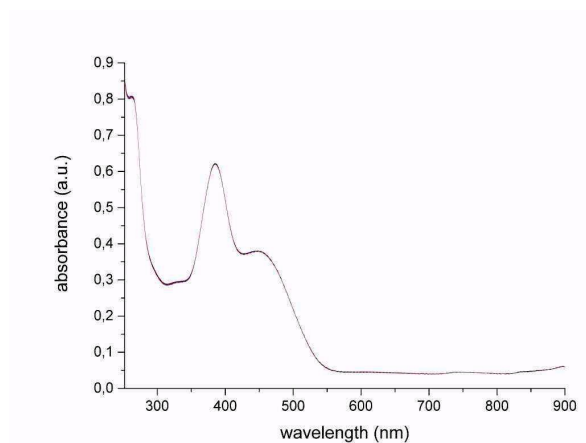


Figure 33: UV/Vis spectra of complex **5a** in 10 % v/v DMSO/H₂O (left) and 10 % v/v DMSO/PBS (right)

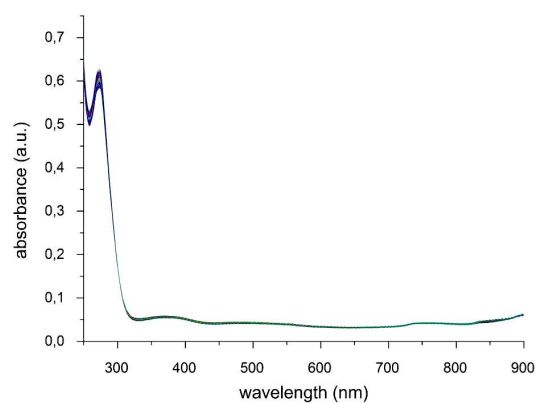
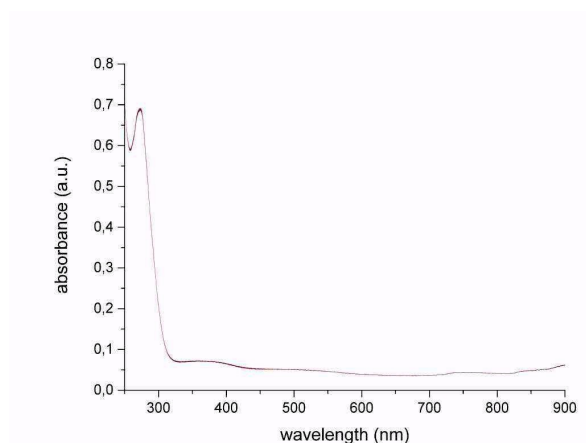


Figure 34: UV/Vis spectra of complex **6a** in 10 % v/v DMSO/H₂O (left) and 10 % v/v DMSO/PBS (right)

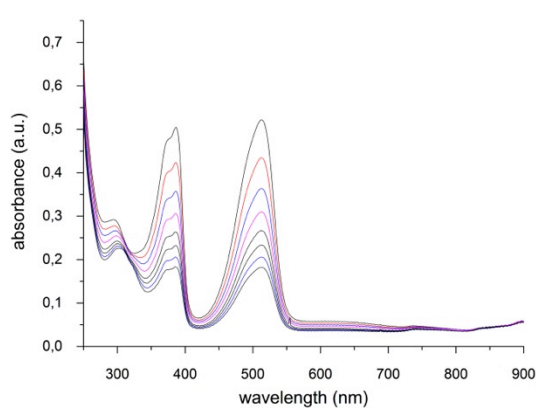
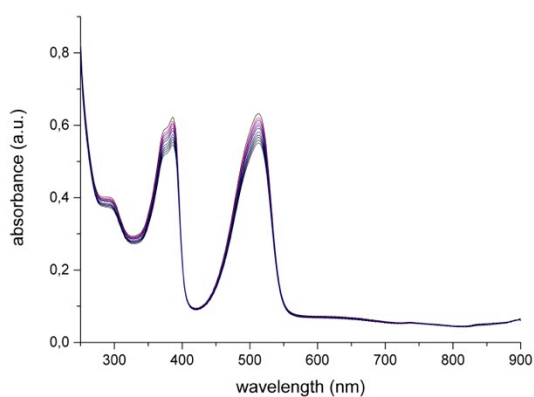


Figure 35: UV/Vis spectra of complex **7a** in 10 % v/v DMSO/H₂O (left) and 10 % v/v DMSO/PBS (right)

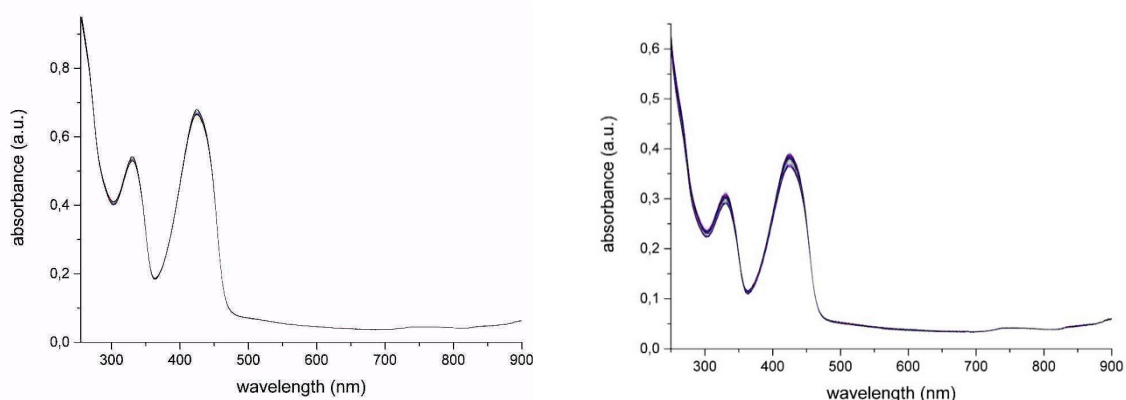


Figure 36: UV/Vis spectra of complex **8a** in 10 % v/v DMSO/H₂O (left) and 10 % v/v DMSO/PBS (right)

Table 4: wavelength of the peak maxima and molar extinction coefficients of compounds **2a – 8a** in 10 % v/v DMSO/H₂O or 10 % v/v DMSO/PBS

compound	λ_{max} [nm] (ϵ [$\text{M}^{-1} \text{cm}^{-1}$]) in 10 % v/v DMSO/H ₂ O	λ_{max} [nm] (ϵ [$\text{M}^{-1} \text{cm}^{-1}$]) in 10 % v/v DMSO/PBS
2a	331 (10837)	331 (7419)
3a	303 (12475), 409 (11866)	303 (12907), 409 (12130)
4a	273 (22129), 350 (8293), 508 (2859)	273 (20485), 350 (3041), 508 (2561)
5a	384 (16016), 448 (9845)	384 (16000), 448 (9832)
6a	271 (8418)	271 (7080)
7a	294 (19800), 386 (30751), 512 (31229)	294 (14797), 386 (25560), 512 (26456)
8a	330 (16998), 425 (21362)	330 (10698), 425 (13403)

3.6 CYCLIC VOLTAMMETRY

Cyclic voltammetry experiments were carried out on a Princeton applied research Potentiostat/Galvanostat Model 273A. The samples (**1**, **2a – 8a**) were dissolved in DMF (final concentration 1 mM) with 0.1 M tetrabutylammoniumtetrafluoroborate as supporting electrolyte. A platinum wire was used as auxiliary electrode, Ag/Ag⁺ (0.1 M AgNO₃ in ACN) as reference electrode and a glassy carbon as working electrode. An argon stream was passed through all solutions for 5 minutes prior to the measurements. The sweep was performed between either -1.8 V or -1.5 V to +1.0 V or +1.2 V with a scan rate of 200 mV/s. For referencing to the normal hydrogen electrode, ferrocene ($E^\circ = +0.72$ V vs. NHE in DMF) was added after each sample. Each compound was measured in triplicate (Figures 37-39).

The physiological relevant potential in cells is limited on one side by NADP⁺ at -0.320 V ($\text{NADP}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADPH}$) and on the other by oxygen at +0.820 V ($\text{O}_2 + 4\text{H}^+ + 4\text{e}^- \rightarrow 2\text{H}_2\text{O}$).

3.6.1 O,O-CHELATING LIGANDS

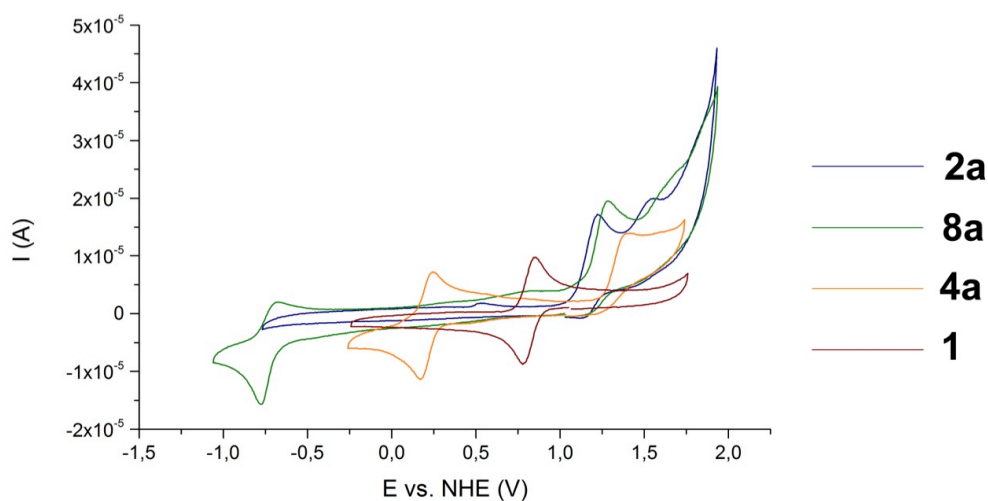


Figure 37: cyclic voltammogram of **1** and O,O-chelating ligands in DMF referenced to NHE

The oxidation of Mo^{IV} to Mo^V of compound **2a** and **8a** is similar, compound **4a** is shifted to a higher potential. But compared to **1**, all are shifted to higher potentials and irreversible.

3.6.2 O,S-CHELATING LIGANDS

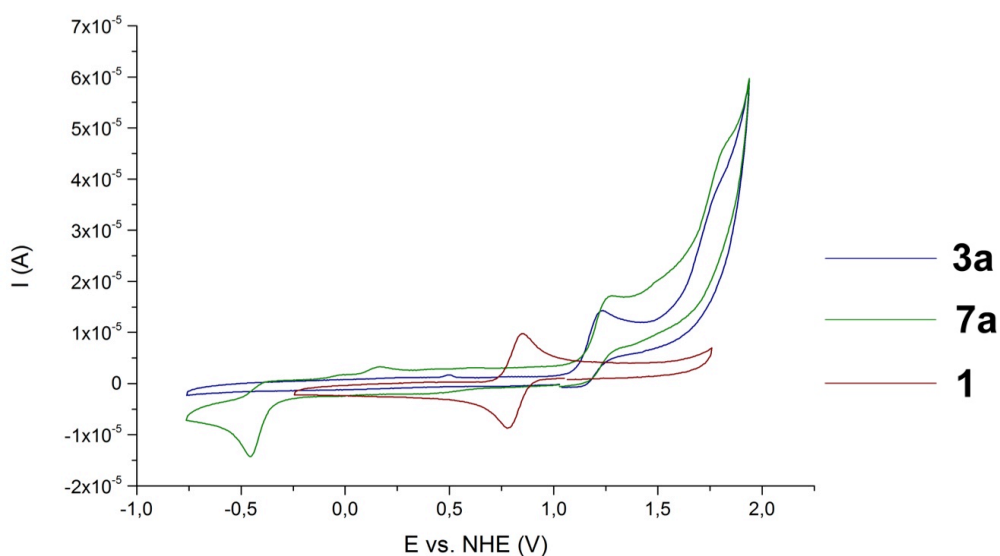


Figure 38: cyclic voltammogram of **1** and O,S-chelating ligands in DMF referenced to NHE

Like compound **2a** and **8a** their O,S-chelating derivatives have a similar oxidation peak that is shifted to a higher potential than compound **1**.

3.6.3 O,N-CHELATING LIGANDS

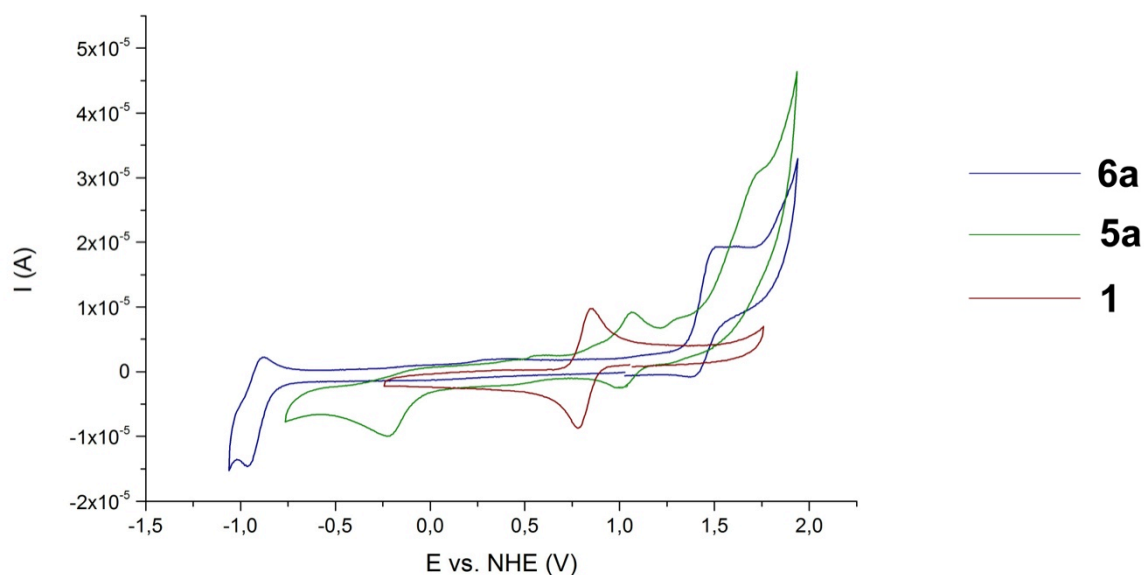


Figure 39: cyclic voltammogram of **1** and O,N-chelating ligands in DMF referenced to NHE

The oxidation potentials of the O,N-chelating ligands are far apart ($\pm 0,5$ V) with compound **5a** showing the lowest potential and **6a** the highest out of the 7 measured molybdenocene complexes. All oxidation potentials of Mo^{IV} to Mo^{V} for compounds **2a–8a** are listed in Table 5.

Table 5: oxidation potentials of Mo^{IV} to Mo^{V} for compounds **2a – 8a**

compound	$E_{\text{p}}\text{Mo}^{\text{IV/V}}[\text{V}]$
2a	+1,221
3a	+1,226
4a	+1,411
5a	+1,062
6a	+1,503
7a	+1,277
8a	+1,284

All oxidation peaks from Mo^{IV} to Mo^V are irreversible and are outside of the physiological region, as are most of the reductions and oxidations of the organic ligands. The only reaction inside the physiological area is the redox process of the naphthoquinone based ligand of compound **4a** with $E_{1/2} = +0.206$ V. The reaction of compound **1** in DMF seems to be reversible or quasi-reversible $E_{1/2} = +0.815$ V. This result differs to its reaction in aqueous solution where compound **1** exhibits irreversible electrochemical behaviour.¹⁰⁶ All samples were measured in DMF and not under physiological conditions due to their poor solubility.

3.7 HSA-BINDING STUDIES

In order to investigate the anticancer drug affinity for serum proteins, the compounds **1** and **2a – 8a**, were incubated with human serum albumin. After incubation the samples were measured with fluorescence spectroscopy or further treated with either centrifugal filtration or size exclusion chromatography and the molybdenum content detected with ICP-MS.

3.7.1 CENTRIFUGAL FILTRATION

The incubation with HSA was carried out at biologically relevant ratios (1:5, $c(\text{complex}) = 2 \mu\text{M}$, $c(\text{HSA}) = 10 \mu\text{M}$) in phosphate buffered saline (PBS). As reference, the molybdenocene complexes ($c = 2 \mu\text{M}$) were incubated in PBS.

After 24 hours of incubation at 37°C, the mixtures were transferred into the centrifugal filter devices that cut off a molecular weight greater than 3 kDa (Figure 40). The centrifugation was performed for 30 minutes at 14 000 rpm. The higher molecular fraction containing free HSA and complex bound to HSA was separated through centrifugal forces from the lower molecular fraction (<3 kDa, free complex). The fraction >3 kDa remained in the filter device and the low molecular fraction was filtrated. The obtained filtrate was diluted to 10 ppb of Molybdenum-species with 1 % HNO₃. The molybdenum content was determined via ICP-MS.

¹⁰⁶ Feliciano, I., Matta, J. and Melendez, E., *J Biol Inorg Chem*, **2009**, 14, 1109-1117.

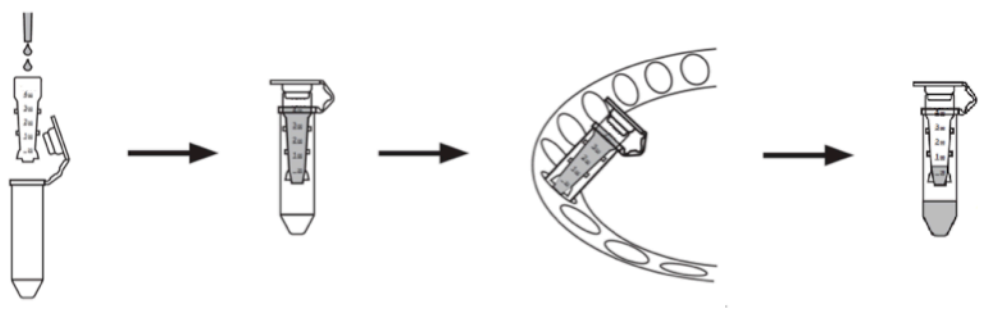


Figure 40: user manual for centrifugal filter devices by millipore ¹⁰⁷

The compounds **1** and **2a – 8a** were quantified in the filtrate of both incubation mixtures with HSA and with PBS as negative control and each sample was run in triplicate. The quantity found in the filtrate of the buffered mixture represents 100 % molybdenum content. The samples incubated with HSA showed the same result as the negative control, leading to the conclusion that molybdenocene does not bind to HSA (Table 6). In the case of albumin binding, the molybdenum content in low molecular fraction would be <100 %

Table 6: Molybdenum content in low molecular fraction

Sample	Molybdenum (%)	Sample	Molybdenum (%)
1 + PBS	100	1 + HSA	100
2a + PBS	100	2a + HSA	100
3a + PBS	100	3a + HSA	100
4a + PBS	100	4a + HSA	100
5a + PBS	100	5a + HSA	100
6a + PBS	100	6a + HSA	100
7a + PBS	100	7a + HSA	100
8a + PBS	100	8a + HSA	100

The method was validated with cisplatin. The platinum content in low molecular fraction after HSA-incubation was <100 %.

In order to confirm the data obtained via centrifugal filtration compounds **1** and **2a** were investigated via size exclusion chromatography monitoring the molybdenum signal.

¹⁰⁷ <http://www.millipore.com/userguides/tech1/pr03711> (accessed on 10/12/2013)

3.7.2 SIZE EXCLUSION CHROMATOGRAPHY

The incubation with HSA was carried out at biologically relevant ratios (1:5, $c(\text{complex}) = 40 \mu\text{M}$, $c(\text{HSA}) = 200 \mu\text{M}$) in phosphate buffered saline (PBS). As reference, the molybdenocene complexes ($c = 40 \mu\text{M}$) were incubated in PBS.

After 24 hours incubation at 37°C , the incubated mixtures were diluted in PBS in order to obtain the optimal molybdenum concentration ($1 \mu\text{M}$) for the subsequent size exclusion separation and molybdenum detection via ICP-MS.

The size exclusion column was calibrated with proteins of known molecular mass. Under these conditions, albumin showed a retention time of around 20 minutes.

The method was again validated with cisplatin. The platinum signal that confirmed the binding of albumin was observed at approximately 20 minutes.

However no molybdenum signal was observed around 20 minutes retention time but at 29 and 30 minutes like the negative control, incubated with PBS instead of HSA.

Analogously, the molybdenocene complexes were incubated in FBS (fetal bovine serum) at 37°C for 24 hours, in order to investigate the drug binding to albumin in biological samples. After the incubation, the mixtures were diluted and the separation via size exclusion was carried out. The molybdenum signal was detected via ICP-MS. Both methods reach the result that the molybdenocene compounds show no covalent binding with HSA.

3.7.3 FLUORESCENCE SPECTROSCOPY

To investigate molecular interactions of compounds **1** and **2a – 8a** with HSA the intrinsic fluorescence of the single tryptophan (Trp214) was measured. When HSA is excited at 295 nm it fluoresces at around 350 nm. A decrease in intensity can be attributed to a change in the microenvironment of tryptophan e.g. by a binding event.

The compounds **1** and **2a – 8a** were prepared as DMSO stock-solution due to their poor solubility. To avoid structural transformations of HSA, the concentration of DMSO was less than 10 % during the incubation¹⁰⁸. The samples were diluted in phosphate buffered saline and incubated for 24 hours at 37°C in the ratios 5:1 ($c(\text{HSA}) = 50 \mu\text{M}$, $c(\text{complex}) = 10 \mu\text{M}$), 1:1 ($c(\text{HSA}) = 50 \mu\text{M}$, $c(\text{complex}) = 50 \mu\text{M}$), and 1:5 ($c(\text{HSA}) = 50 \mu\text{M}$, $c(\text{complex}) = 250 \mu\text{M}$). HSA and the compounds **1** and **2a – 8a** were incubated with only PBS as reference. After incubation the samples were further diluted with PBS 10:1. Thus reaching final concentrations for

¹⁰⁸ Pabbathi, A., Patra, S. and Samanta, A., *Chemphyschem*, **2013**, 14, 2441-2449.

5:1 ($c(\text{HSA}) = 5 \mu\text{M}$, $c(\text{complex}) = 1 \mu\text{M}$), 1:1 ($c(\text{HSA}) = 5 \mu\text{M}$, $c(\text{complex}) = 5 \mu\text{M}$), and 1:5 ($c(\text{HSA}) = 5 \mu\text{M}$, $c(\text{complex}) = 25 \mu\text{M}$).

The fluorescence spectra were recorded at room temperature in a 1-cm quartz cell using an excitation and emission slit width of 3 nm. The excitation wavelength was 295 nm and the emission spectra were recorded in the range of 300 to 450 nm.

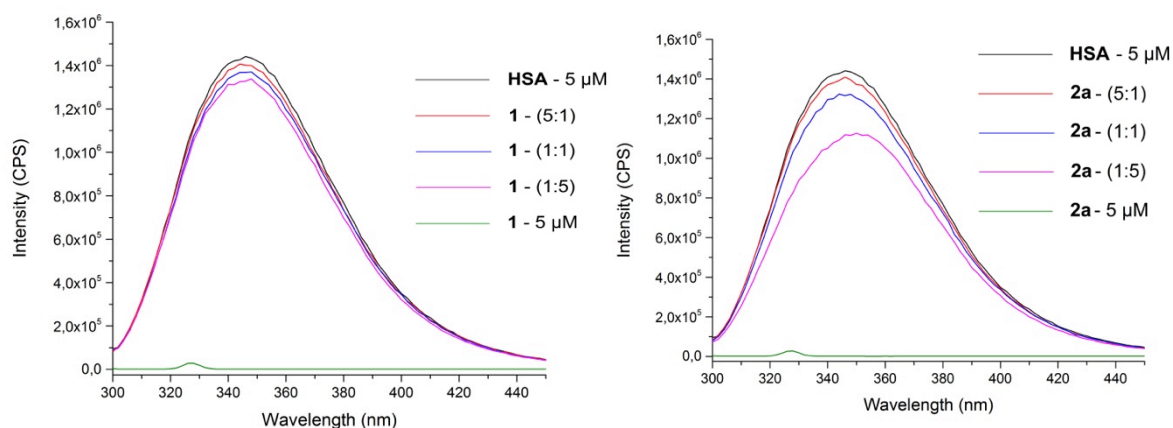


Figure 41: fluorescence spectral changes of HSA incubated with **1** (left) and **2a** (right) at various molar ratios: 1:0, 5:1, 1:1, 1:5, 0:1.

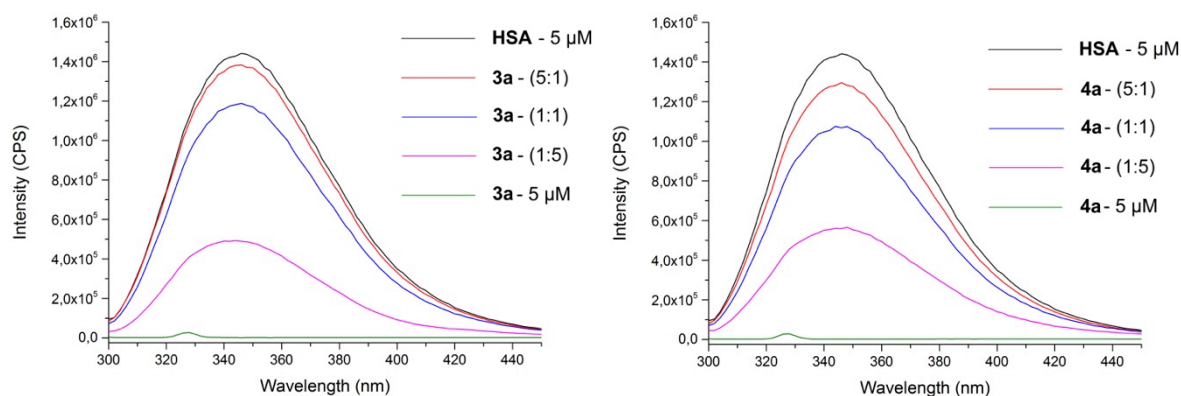


Figure 42: fluorescence spectral changes of HSA incubated with **3a** (left) and **4a** (right) at various molar ratios: 1:0, 5:1, 1:1, 1:5, 0:1.

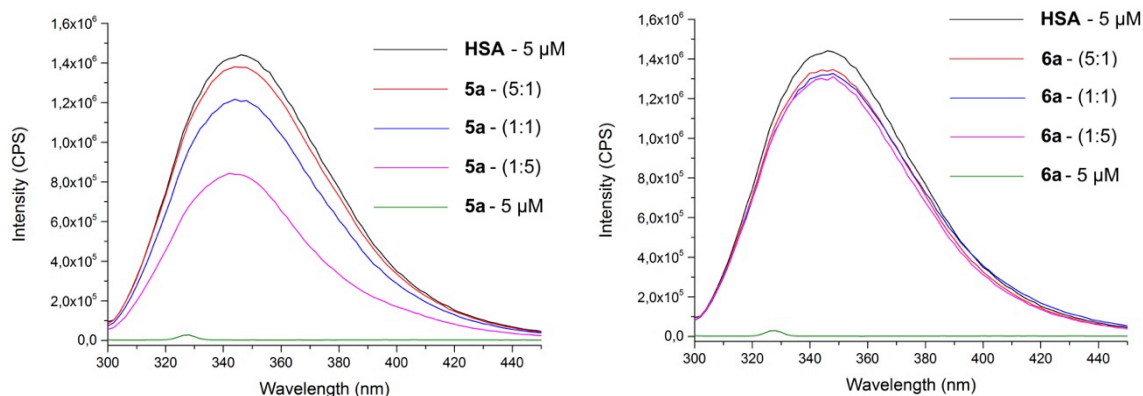


Figure 43: fluorescence spectral changes of HSA incubated with **5a** (left) and **6a** (right) at various molar ratios: 1:0, 5:1, 1:1, 1:5, 0:1.

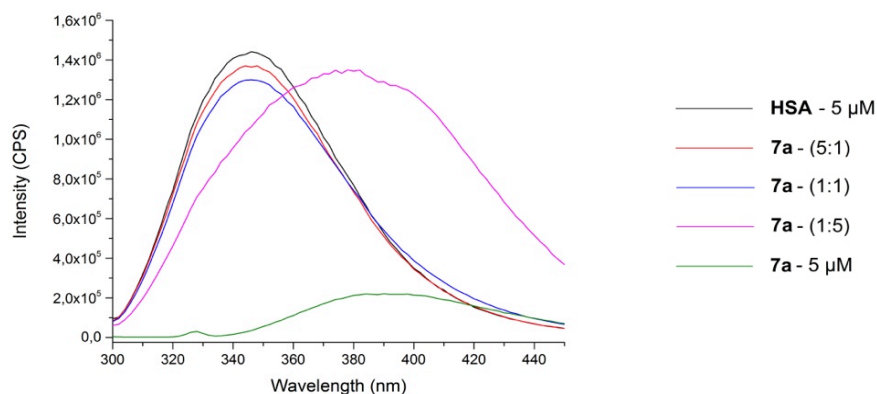


Figure 44: fluorescence spectral changes of HSA incubated with **7a** at various molar ratios: 1:0, 5:1, 1:1, 1:5, 0:1.

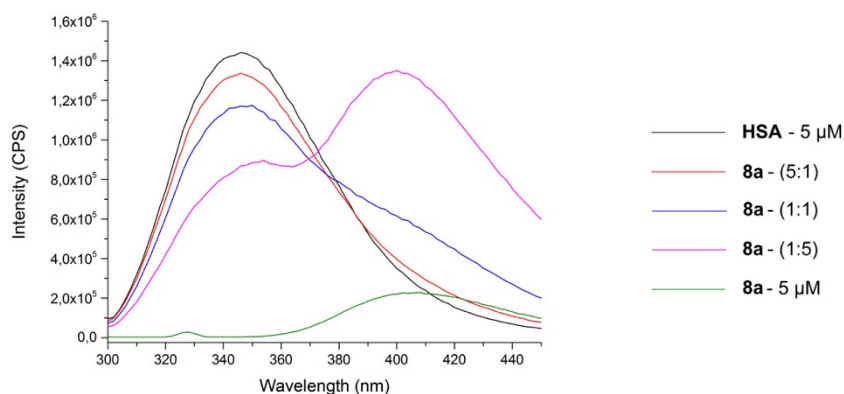


Figure 45: fluorescence spectral changes of HSA incubated with **8a** at various molar ratios: 1:0, 5:1, 1:1, 1:5, 0:1.

Figures 41 to 45 show the spectral changes of HSA incubated with compounds **1** and **2a – 8a**. The spectra of **1** and **2a – 6a** (5 μ M) without HSA show hardly any emission, whereas **7a** and **8a** show significant fluorescence emission when excited at 295 nm

in the recorded range leading to difficulties interpreting the quenching by the compounds.

The complexes **1** and **6a** show only little decrease in fluorescence intensity indicating that there are no or few interactions with HSA confirming the ICP-MS experiments. Compound **2a** shows hardly any quenching in the molar ratios of 5:1 and 1:1. With an excess of **2a** (1:5) the intensity decreases indicating some kind of interaction with HSA. **3a** and **5a** show no interaction with an excess of HSA, but a decrease in intensity at the ratios 1:1 and 1:5. Compounds **4a** and **8a** gradually decrease the fluorescence intensity of HSA showing interactions even at the ratio of 5:1. **7a** shows a slight decrease in intensity at 5:1 at 1:1; at 1:5 the fluorescence intensity of **7a** is higher than that of HSA making it unable to interpret a decrease in HSA intensity. Though the ICP-MS experiments rule out a covalent binding of compounds **1** and **2a – 8a** with HSA the fluorescence spectra clearly indicate some kind of interaction. To elaborate the kind of interaction that led to the quenching of HSA further experiments would have to be carried out. Nonetheless, the interactions are only significant in non-physiological relevant ratios ruling HSA out as possible binding partner.

3.8 CYTOTOXICITY PRE-SCREEN IN CANCER CELL LINES

To pre-screen the cytotoxicity of the complexes **1**, **2a – 4a** and **6a – 8a**, by means of the colorimetric MTT assay was carried out in the human cancer cell lines A549 (non-small cell lung carcinoma), CH1 (ovarian carcinoma) and SW480 (colon carcinoma). The cytotoxic potential was compared with the unsubstituted molybdenocene dichloride, compound **1**, and it was found that ligands maltol and picolinic acid of the complexes **2a** and **6a** don't seem to have a strong influence on the cytotoxicity. The coordination of naphthoquinone ligands only has a minor impact on the cytotoxicity, whereas the replacement of the carbonyl oxygen by a sulphur atom in the case of the thiomaltolato complex **3a** resulted in an increased activity. The compounds with the highest cytotoxicity are **7a** and **8a** bearing flavonoids as chelates. However, the exact IC₅₀ values have to be determined to confirm the observed cytotoxic tendencies in the pre-screen experiments and are currently performed.

4 EXPERIMENTAL PART

4.1 EQUIPMENT, MATERIALS AND METHODS

4.1.1 CHEMICALS

Solvents:

Methanol (HPLC grade) was purchased from Fisher Scientific and THF from Acros Organics. Both were dried over molecular sieves (3 Å) prior use. Ethanol (96 %) was purchased from Brenntag AG and was used without any further purification.

For ligand synthesis:

2-Hydroxyacetophenone (99 %, Acros Organics), 4-chlorobenzaldehyde (98.5 %, Acros Organics), Lawesson's reagent (99%, Acros Organics), sodium hydroxide (≥ 98 %, Sigma Aldrich), hydrogen peroxide (30 %, Sigma Aldrich), 3-hydroxy-2-methyl-4H-pyran-4-one (99+ %, Sigma Aldrich), pyridine-2-carboxylic acid (99 %, Arcos Organics), 2-methyl-1,4-naphthoquinone (98 %, AcrosOrganics), sulphuric acid (98 %, Sigma Aldrich), Hydroxylamine hydrochloride (≥ 99 %, Sigma Aldrich), hydrochloric acid (30 - 33 %, Donauchem)

For complexation:

Molybdenocene dichloride (99 %, Strem chemicals), sodium methoxide (~ 95 %, Fluka), ammonium hexafluorophosphate (95+ %, Sigma Aldrich), sodium hexafluorophosphate (98 %, Sigma Aldrich)

For HSA binding studies:

PBS (sterile filtered, Sigma Life Science), HSA (≥ 98 %, Sigma Aldrich), FBS (sterile filtered, Sigma Aldrich), centrifugal filter units (Amicon[®] Ultra – 0.5 mL 3K, Millipore)

All chemicals were used as obtained from commercial sources.

4.1.2 EQUIPMENT

NMR spectra

NMR spectra were recorded with a Bruker FT-NMR Avance IIITM 500 MHz spectrometer at 500.10 (¹H), 125.75 (¹³C), 470.53 (¹⁹F) and 202.53 MHz (³¹P). 2D NMR measurements were recorded using standard pulse programs at 500.32 MHz (¹H) and 125.81 MHz (¹³C).

Elemental analyses

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

X-ray structures

X-ray diffraction analyses were performed on a Bruker X8 APEX II CCD diffractometer at 150 K.

Melting points

Melting points were determined using a Büchi Melting Point M-560.

Solubility

The solubility was determined by either dissolving the compound by dropwise addition of PBS or dissolving the compound in DMSO and dilution with PBS to obtain a final concentration of 1 % DMSO/PBS. The highest concentrated dilution resulted in the determined solubility.

Mass Spectrometry

Electrospray ionization mass spectra (ESI-MS) were recorded on a Bruker Esquire 3000 ion trap spectrometer. The samples were dissolved in methanol and determined in the positive and negative mode.

UV/Vis spectra

UV/Vis data was recorded on a Perkin Elmer Lambda 650 UV/Vis Spectrophotometer with a Peltier element for temperature control. The samples were dissolved in 10 % v/v DMSO/H₂O and 10 % v/v DMSO/PBS.

Cyclic voltammetry

Cyclic voltammetry data was recorded on a Princeton applied research Potentiostat/Galvanostat Model 273A. The samples were dissolved in DMF with 0.1 M tetrabutylammoniumtetrafluoroborate.

ICP-MS

ICP-MS measurements were carried out on Agilent 7500ce ICP-MS (Waldbronn, Germany). The samples were dissolved in PBS and incubated with HSA for 24 hours at 37 °C and diluted with 1 % HNO₃.

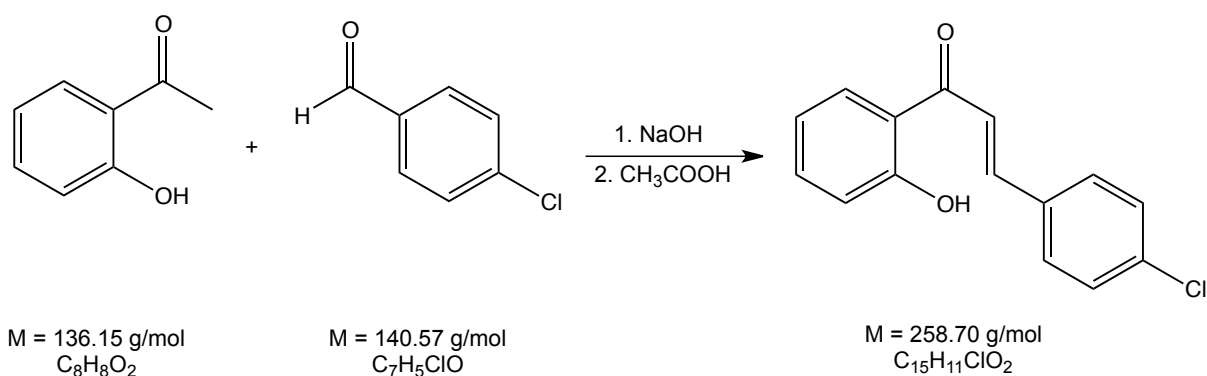
Fluorescence spectroscopy

Fluorescence data was recorded with a Horiba Scientific Fluoromax-4 Spectrofluorometer. The samples were dissolved in PBS and incubated with HSA for 24 hours and diluted with PBS.

4.2 SYNTHESIS OF THE LIGANDS

4.2.1 O,O-CHELATING LIGANDS

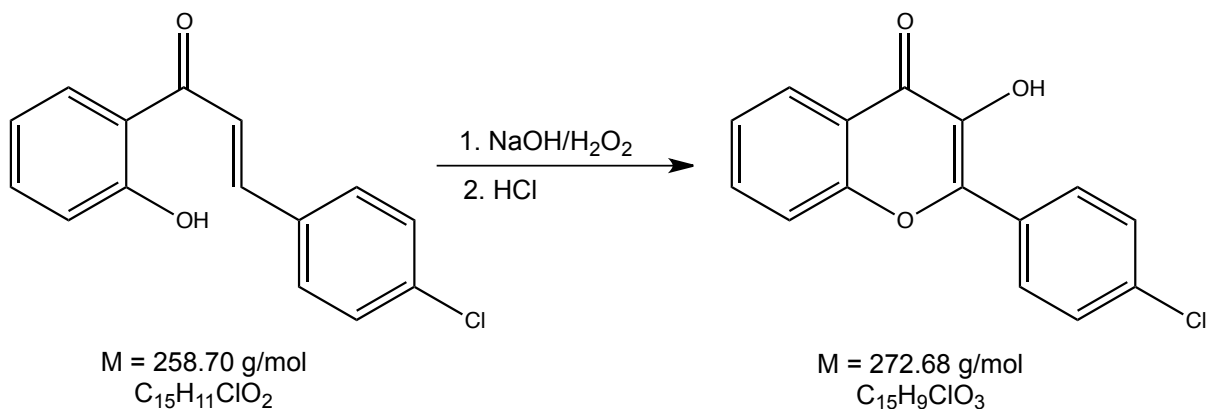
4.2.1.1 3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one



Synthesis:

2-Hydroxyacetophenone (2.00 g, 14.7 mmol, 1 eq) and 4-Chlorobenzaldehyde (2.07 g, 14.7 mmol, 1 eq) were dissolved in ethanol (90 mL). Sodium hydroxide (12.6 mL, 5M) was added and the mixture was stirred at room temperature overnight. The mixture was acidified to pH 6 with acetic acid (30 %). The product was filtrated and washed with distilled water. The product was used without any further purification for the synthesis of 2-(4-chlorophenyl)-3-hydroxy-4*H*-1-benzopyran-4-one.

4.2.1.2 2-(4-Chlorophenyl)-3-hydroxy-4*H*-1-benzopyran-4-one



Synthesis:

3-(4-Chlorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (3.80 g, 14.7 mmol, 1 eq) was suspended in ethanol (120 mL) and sodium hydroxide (5 M, 4.5 mL, 22.5 mmol, 1.5 eq) was added. The mixture was cooled to 4 °C. Hydrogen peroxide (30 %, 2.8 mL, 28.0 mmol, 1.9 eq) was slowly added and the orange suspension was stirred overnight. The suspension was acidified with hydrochloric acid (2 M) to pH 1, suspended in distilled water (400 mL) and cooled in the fridge for 30 minutes. The product was filtrated, recrystallized in methanol and dried *in vacuo*.

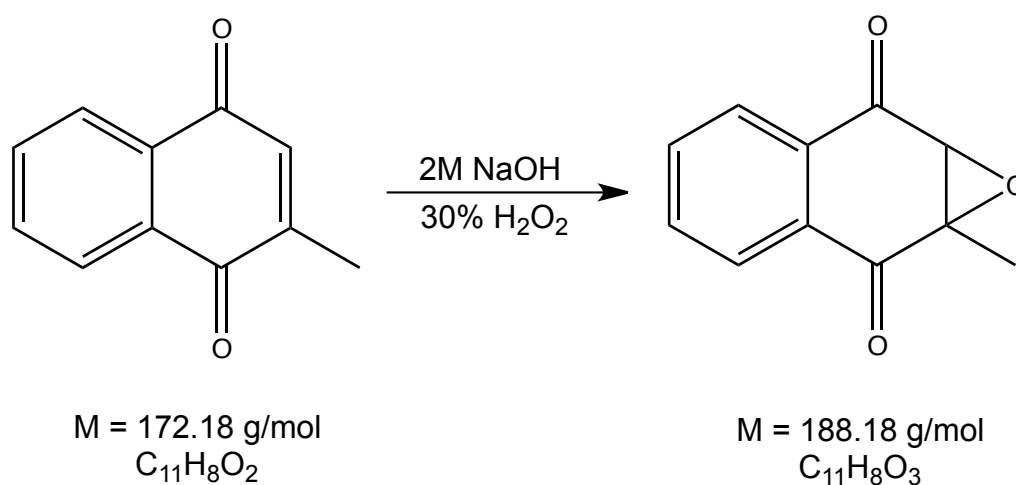
Yield: 1.81 g (45 %), yellow powder

Melting point: 198 – 203°C

NMR-spectroscopy:

¹H NMR (d⁶-DMSO): δ = 7.48-7.51 (m, H_{Ar}), 7.65–7.68 (m, 2H, H_{Ar}), 7.79 (dd, 1H, ⁴J(H, H) = 1 Hz, ³J(H, H) = 9 Hz, H_{Ar}), 7.82-7.85 (m, H_{Ar}), 8.14 (dd, 1H, ⁴J(H, H) = 2 Hz, ³J(H, H) = 8 Hz, H_{Ar}), 8.26–8.29 (m, 2H, H_{Ar}), 9.84 (s, 1H, OH) ppm.

4.2.1.3 Naphtho[2,3-b]oxirene-2,7(1aH,7aH)-dione



Synthesis:

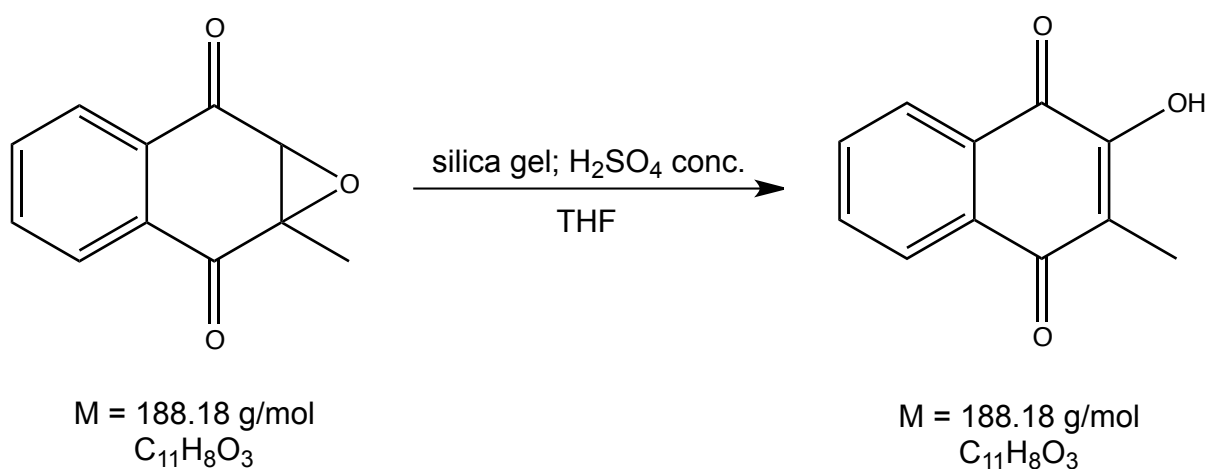
2-Methyl-1,4-naphthoquinone (1.00 g, 5.8 mmol, 1 eq) was dissolved in a mixture of methanol (160 mL) and water (40 mL). The mixture was cooled to 0 °C and NaOH (2 M, 1.5 mL, 3.0 mmol, 0.5 eq) and H_2O_2 (30 %, 0.9 mL, 8.8 mmol, 1.5 eq) were slowly added. The suspension was stirred for 10 minutes at 0 °C and 2 hours at room temperature. The solvent was concentrated under reduced pressure and the yielded suspension was stored at 4 °C overnight. The formed precipitate was filtrated, washed with H_2O and dried *in vacuo*.

Yield: 1.09 g (69 %), salmon-coloured powder

NMR-spectroscopy:

^1H NMR (d^6 -DMSO): $\delta = 1.63$ (s, 3H, H_{CH_3}), 4.12 (s, 1H), 7.86–7.92 (m, 3H, H_{Ar}), 7.95–7.98 (m, 1H, H_{Ar}) ppm.

4.2.1.4 2-Hydroxy-3-methyl-1,4-naphthoquinone



Synthesis:

To a mixture of naphtho[2,3-b]oxirene-2,7(1aH,7aH)-dione (856 mg, 4.55 mmol, 1 eq) and silica gel (7.5 g), H_2SO_4 conc. (1.77 mL, 31.88 mmol, 7 eq) in THF (16 mL) was added and evaporated very carefully at 70 °C with a pressure of 500 mbar. During removal of the solvent the colour slowly changed from red to yellow and the powder was evaporated for further 10 minutes. The silica gel was washed several times with CH_2Cl_2 and the liquid decanted until the extracted solvent was uncoloured. The combined organic fractions were washed with a saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 , filtrated, concentrated to dryness and further dried *in vacuo*.

Yield: 805 mg (94 %), yellow needles

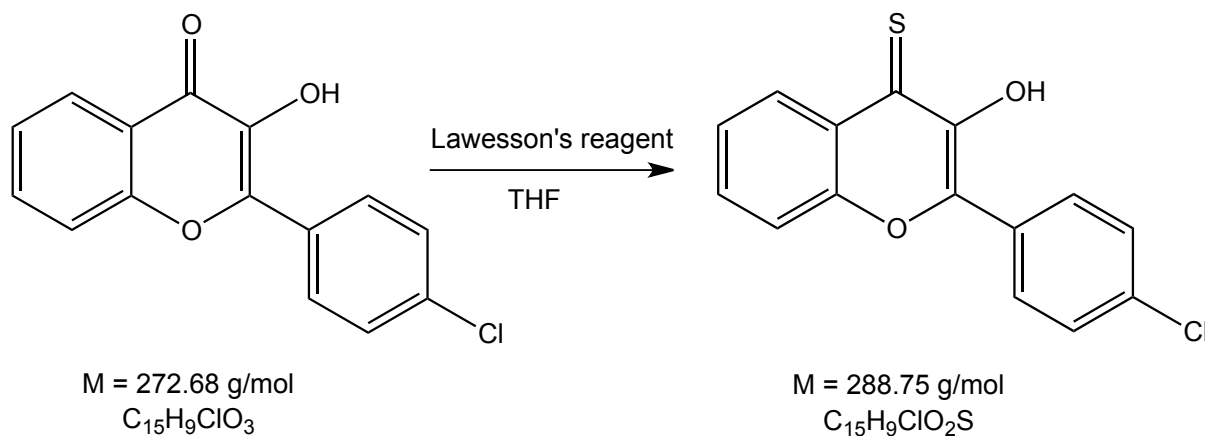
Melting point: 164 – 168°C

NMR-spectroscopy:

^1H NMR (d^6 -DMSO): $\delta = 1.96$ (s, 3H, H_{CH_3}), 7.79 (td, 1H, $^4J(H, H) = 2 \text{ Hz}$, $^3J(H, H) = 8 \text{ Hz}$, H_{Ar}), 7.84 (td, 1H, $^4J(H, H) = 2 \text{ Hz}$, $^3J(H, H) = 8 \text{ Hz}$, H_{Ar}), 7.97–8.02 (m, 2H, H_{Ar}), 10.95 (s, 1H, OH) ppm.

4.2.2 O,S- CHELATING LIGANDS

3.2.2.1 2-(4-Chlorophenyl)-3-hydroxy-4H-1-benzopyran-4-thione



Synthesis:

2-(4-Chlorophenyl)-3-hydroxy-4H-1-benzopyran-4-one (750 mg, 2.75 mmol, 1 eq) and Lawesson's reagent (885 mg, 2.19 mmol, 0.8 eq) were suspended in THF (27.5 mL, dried over molecular sieve 3Å) and refluxed for 5 hours under an argon atmosphere. The solvent was removed and the residue recrystallized in ethanol. The solution was stored at 4°C for crystallisation, the formed crystals separated by filtration, washed with cold ethanol and dried *in vacuo*.

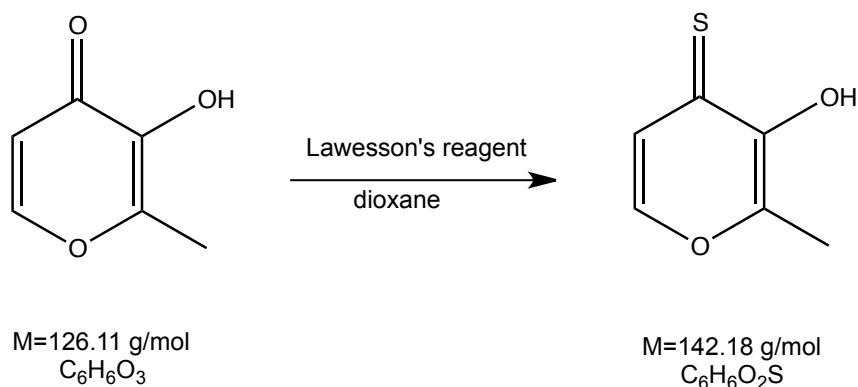
Yield: 705 mg (89 %), orange powder

Melting point: 179 – 182 °C

NMR-spectroscopy:

¹H NMR (d⁶-DMSO): δ = 7.60-7.64 (m, H_{Ar}), 7.72–7.75 (m, 2H, H_{Ar}), 7.90–7.96 (m, 2H, H_{Ar}), 8.39–8.42 (m, 2H, H_{Ar}), 8.47 (d, 1H, ³J(H, H) = 8 Hz, H_{Ar}), 9.11 (s, 1H, OH) ppm.

4.2.2.2 2-Methyl-3-hydroxy-4H-pyran-4-thione



Synthesis:

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*.

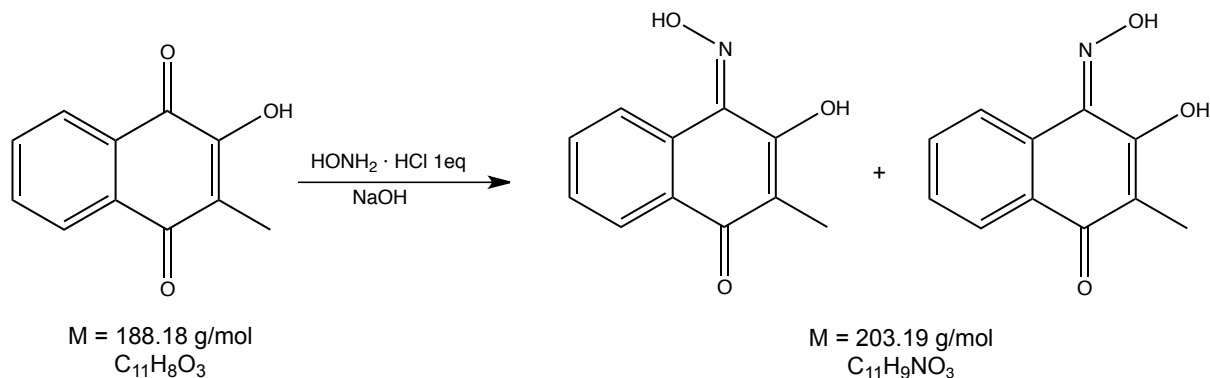
Yield: 0.802 mg (69 %), yellow crystals

NMR-spectroscopy:

^1H NMR (d^6 -DMSO): δ = 2.39 (s, 3H, H1'), 7.35 (d, 1H, $^3J(\text{H}, \text{H})$ = 5 Hz, H6), 8.10 (d, 1H, $^3J(\text{H}, \text{H})$ = 5 Hz, H5), 8.27 (s, 1H, OH) ppm.

4.2.3 O,N- CHELATING LIGANDS

4.2.3.1 3-Hydroxy-4-(hydroxyimino)-2-methylnaphthalen-1(4H)-one, *E/Z* mixture



Synthesis:

2-Hydroxy-3-methyl-1,4-naphthoquinone (900 mg, 4.78 mmol, 1 eq) was dissolved in sodium hydroxide solution (1 M, 90 mL). Hydroxylamine hydrochloride (332 mg, 4.78 mmol, 1 eq) was added and the solution was stirred for 2 hours at room temperature, acidified with hydrochloric acid to pH 2 and stored at 4°C. The formed precipitate was filtrated, washed with distilled water and diethyl ether and dried *in vacuo*.

Yield: 795 mg (82 %), yellow-ochre powder

Melting point: 172 – 174 °C

NMR-spectroscopy:

E/Z isomers (5:1)

^1H NMR (d^6 -DMSO): *E* isomer δ = 1.96 (s, 3H, H_{CH_3}), 7.64 (td, 1H, $^4\text{J}(\text{H}, \text{H}) = 1 \text{ Hz}$, $^3\text{J}(\text{H}, \text{H}) = 8 \text{ Hz}$, H_{Ar}), 7.71 (td, 1H, $^4\text{J}(\text{H}, \text{H}) = 2 \text{ Hz}$, $^3\text{J}(\text{H}, \text{H}) = 8 \text{ Hz}$, H_{Ar}), 8.11 (d, 1H, $^3\text{J}(\text{H}, \text{H}) = 8 \text{ Hz}$, H_{Ar}), 8.98 (d, 1H, $^3\text{J}(\text{H}, \text{H}) = 8 \text{ Hz}$, H_{Ar}), 9.87 (s, 1H, OH), 13.61 (s, 1H, N-OH) ppm.

Z isomer δ = 1.92 (s, 3H, H_{CH_3}), 7.56–7.60 (m, 1H, H_{Ar}), 7.61–7.65 (m, 1H, H_{Ar}), 8.00 (d, 1H, $^3\text{J}(\text{H}, \text{H}) = 7.7 \text{ Hz}$, H_{Ar}), 8.08–8.11 (m, 1H, H_{Ar}), 9.87 (s, 1H, OH), 13.61 (s, 1H, N-OH) ppm.

4.3 GENERAL COMPLEXATION PROCEDURE

4.3.1 STANDARD COMPLEXATION

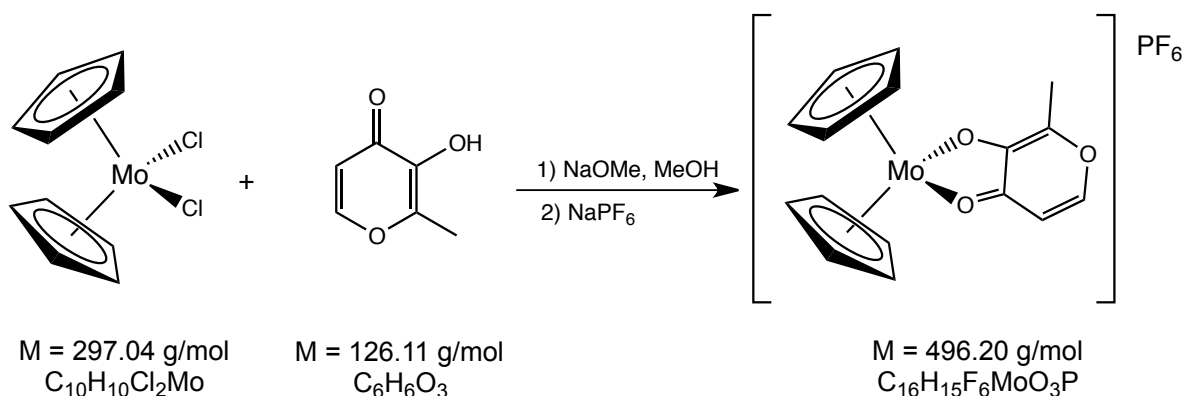
Ligand (1 eq) and sodium methoxide (1.1 eq) were suspended in methanol (dried over molecular sieve 3Å) and stirred for 15 minutes at room temperature under an argon atmosphere. Molybdenocene dichloride (1 eq) was added and the reaction mixture was stirred for 5 – 48 hours. The remaining solids were separated by filtration. Sodium or ammonium hexafluorophosphate (1– 5 eq) was added to the filtrate and stirred for 2 hours. The mixture was stored at 4 °C for crystallisation. The formed precipitate was filtrated and dried *in vacuo*.

4.3.2 COMPLEXATION BY MICROWAVE REACTION

The respective ligand (1 eq) and sodium methoxide (1.1 eq) were suspended in methanol (dried over molecular sieve 3Å) and stirred for 15 minutes at room temperature. Molybdenocene dichloride (1 eq) was added and irradiated for 3 minutes at 40°C with a maximum of 10 Watt. Methanol was added to the mixture and stirred for additional 2 hours. The unreacted solids were filtrated, sodium or ammonium hexafluorophosphate (1 – 2 eq) was added and the reaction mixture was stirred for further 2 hours and stored at 4 °C for crystallisation. The formed precipitate was filtrated and dried *in vacuo*.

4.3.3 SYNTHESIS OF MOLYBDENOCENE COMPLEXES

4.3.3.1 Bis(η^5 -cyclopentadienyl)[2-methyl-3-(oxo- κ O)-4-(1H)-pyronato- κ O4]molybdenum(IV) hexafluorophosphate



Synthesis:

The synthesis was performed according to the standard complexation procedure (see 4.3.1) using 3-hydroxy-2-methyl-4H-pyran-4-one (43 mg, 337 μmol), molybdenocene dichloride (100 mg, 337 μmol), sodium methoxide (20 mg, 370 μmol) and sodium hexafluorophosphate (113 mg, 673 μmol , 2 eq) with a reaction time of 24 hours.

The product was also obtained using the complexation procedure by microwave heating (see 4.3.2).

Yield: 75 mg (45 %), brown powder

Melting point: >240 °C (decomposition)

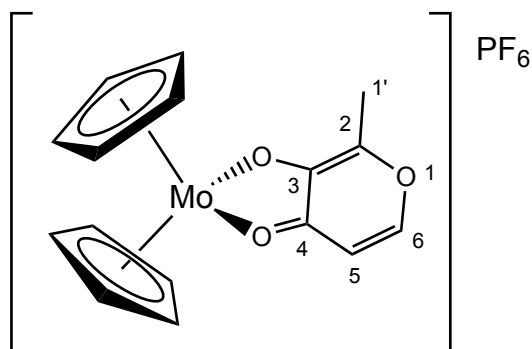
Solubility: 0.50 mg/mL $\equiv$ 1.01 mM in 1% DMSO/PBS

Elemental analysis: for C₁₆H₁₅F₆MoO₃P

	C [%]	H [%]
calculated	38.73	3.05
found	38.65	2.95
Δ	0.08	0.10

(ESI⁺) *m/z*: 351.2 [M – PF₆]⁺

NMR-spectroscopy:



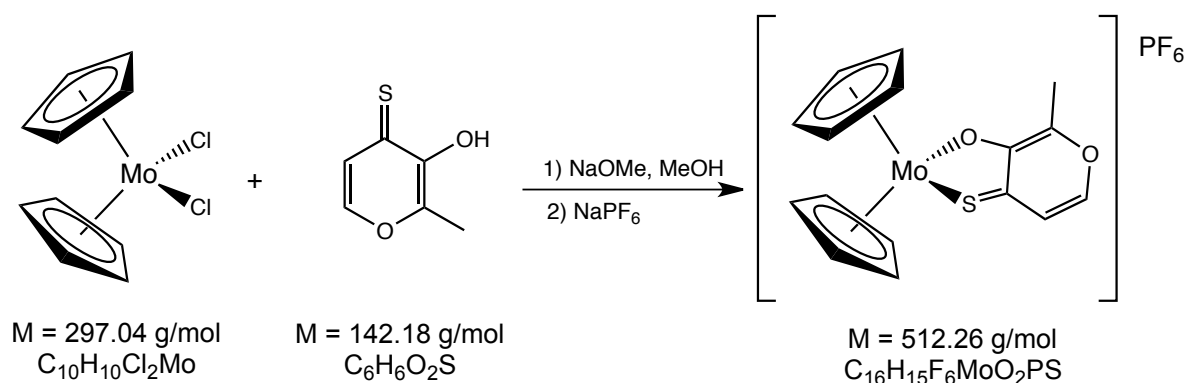
¹H NMR (*d*⁶-DMSO): δ = 2.30 (s, 3H, H1'), 5.90 (s, 10H, H_{Cp}), 6.91 (d, ³J(H, H) = 5 Hz, 1H, H6), 8.37 (d, ³J(H, H) = 5 Hz, 1H, H5) ppm.

¹³C NMR (*d*⁶-DMSO): δ = 15.0 (C1'), 103.9 (C_{Cp}), 111.5 (C5), 156.3 (C6), 157.2 (C2), 158.6 (C3), 184.1 (C4) ppm.

¹⁹F NMR (*d*⁶-DMSO): δ = -71.33 (d, ¹J(F, P) = 711 Hz) ppm.

³¹P NMR (*d*⁶-DMSO): δ = -144.20 (sept, ¹J(P, F) = 711 Hz) ppm.

4.3.3.2 Bis(η^5 -cyclopentadienyl)[2-methyl-3-(oxo- κ O)-pyran-4(1H)-thionato- κ S]molybdenum(IV) hexafluorophosphate



Synthesis:

The synthesis was performed according to the complexation procedure by microwave heating (see 4.3.2) using 2-methyl-3-hydroxy-4H-pyran-4-thione (48 mg, 337 μ mol), molybdenocene dichloride (100 mg, 337 μ mol), sodium methoxide (20 mg, 370 μ mol) and sodium hexafluorophosphate (113 mg, 673 μ mol, 2 eq).

The product was also obtained using the standard complexation procedure (see 4.3.1) with a reaction time of 6 hours.

Yield: 111 mg (64 %), dark brown powder

Melting point: >230 °C (decomposition)

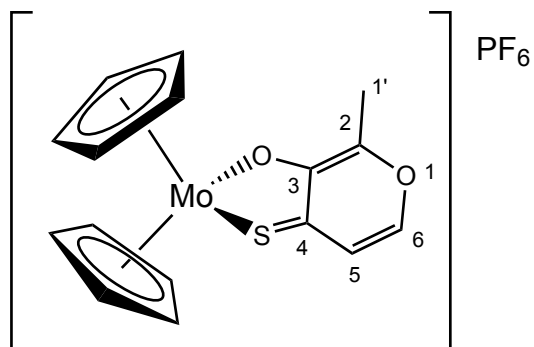
Solubility: 0.50 mg/mL $\equiv$ 0.98mM in 1% DMSO/PBS

Elemental analysis: for $C_{16}H_{15}F_6MoO_2PS$

	C [%]	H [%]	S [%]
calculated	37.51	2.95	6.26
found	37.30	2.92	6.20
Δ	0.21	0.03	0.06

(ESI⁺) *m/z*: 367.3 [M – PF₆]⁺

NMR-Spectroscopy:



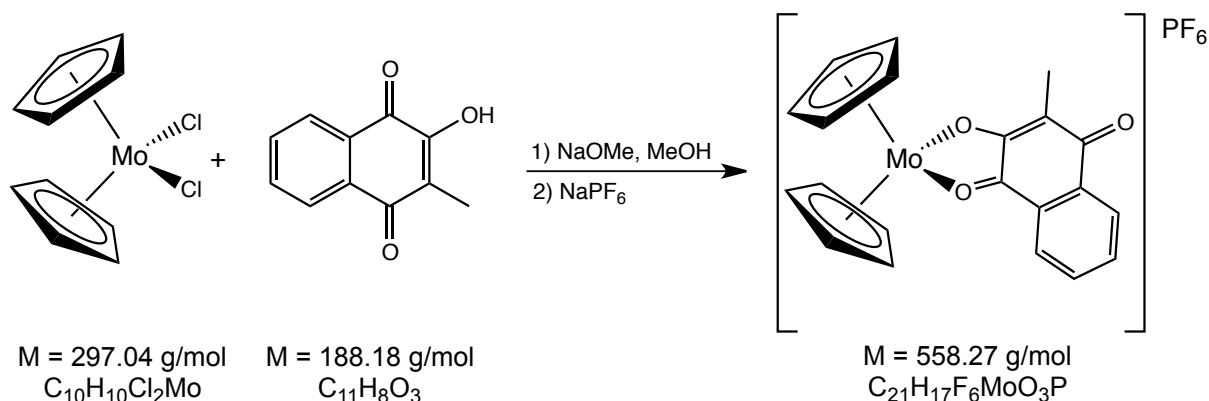
¹H NMR (*d*⁶-DMSO): δ = 2.29 (s, 3H, H1'), 5.76 (s, 10H, H_{Cp}), 7.73 (d, ³J(H, H) = 5 Hz, 1H, H6), 8.27 (d, ³J(H, H) = 5 Hz, 1H, H5) ppm.

¹³C NMR (*d*⁶-DMSO): δ = 15.7 (C1'), 101.7 (C_{Cp}), 120.4 (C5), 149.2 (C6), 157.0 (C2), 168.0 (C3), 177.9 (C4) ppm.

¹⁹F NMR (*d*⁶-DMSO): δ = -71.30 (d, ¹J(F, P) = 712 Hz) ppm.

³¹P NMR (*d*⁶-DMSO): δ = -144.19 (sept, ¹J(P, F) = 712 Hz) ppm.

4.3.3.3 Bis(η^5 -cyclopentadienyl)[3-methyl-(2-oxo- κ O)-[1,4]-naphthoquinonato- κ O]molybdenum(IV) hexafluorophosphate



Synthesis:

The synthesis was performed according to the complexation procedure by microwave heating (see 4.3.2) using 2-hydroxy-3-methyl-1,4-naphthoquinone (63 mg, 337 μ mol), molybdenocene dichloride (337 μ mol, 100 mg), sodium methoxide (370 μ mol, 20 mg) and sodium hexafluorophosphate (673 μ mol, 113 mg, 2 eq).

Yield: 34 mg (18 %), black powder

Melting point: >230 $^{\circ}C$ (decomposition)

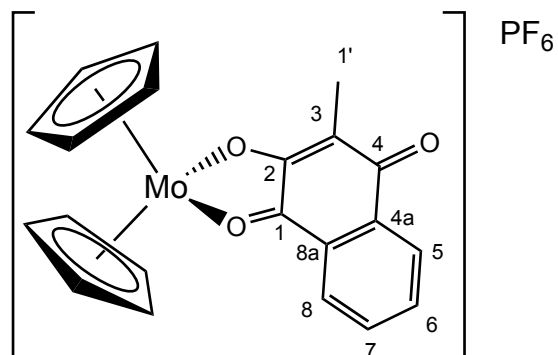
Solubility: 0.50 mg/mL $\equiv$ 0.90 mM in 1% DMSO/PBS

Elemental analysis: for $C_{21}H_{17}F_6MoO_3P$

	C [%]	H [%]
calculated	45.18	3.07
found	45.30	3.13
Δ	0.12	0.06

(ESI⁺) m/z : 413.1 $[M - PF_6]^+$

NMR-spectroscopy:



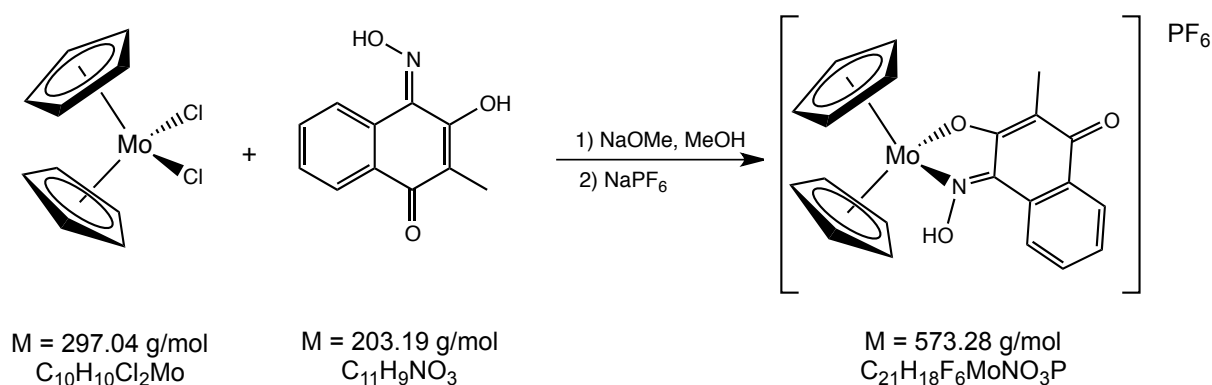
^1H NMR (d^6 -DMSO): δ = 1.84 (s, 3H, H1'), 6.16 (s, 10H, H_{Cp}), 7.74–7.79 (m, 1H, H6), 7.85 (d, $^3J(\text{H}, \text{H})$ = 8 Hz, 1H, H8), 7.91–7.97 (m, 2H, H7/H5) ppm.

^{13}C NMR (d^6 -DMSO): δ = 9.1 (C1'), 104.7 (C_{Cp}), 124.1 (C3), 126.5 (C5), 127.6 (C8), 128.2 (4a), 131.8 (8a), 133.0 (C6), 137.7 (C7), 171.1 (C2), 184.3 (C4), 201.2 (C1) ppm.

^{19}F NMR (d^6 -DMSO): δ = -71.29 (d, $^1J(\text{F}, \text{P})$ = 712 Hz) ppm.

^{31}P NMR (d^6 -DMSO): δ = -144.20 (sept, $^1J(\text{P}, \text{F})$ = 712 Hz) ppm.

4.3.3.4 Bis(η^5 -cyclopentadienyl)[3-methyl-(2-oxo- κ O)-[1,4]-naphthoquinone-1-oximato- κ N]molybdenum(IV) hexafluorophosphate



Synthesis:

The synthesis was performed according to the standard complexation procedure (see 4.3.1) using the E/Z mixture of 3-hydroxy-4-(hydroxyimino)-2-methylnaphthalen-1(4H)-one (34 mg, 168 μ mol), molybdenocene dichloride (50 mg, 168 μ mol), sodium methoxide (10 mg, 185 μ mol,) and ammonium hexafluorophosphate (137 mg, 840 μ mol, 5 eq) with a reaction time of 8 hours.

The product was also obtained using the complexation procedure by microwave heating (see 4.3.2).

Yield: 15 mg (16 %), black powder

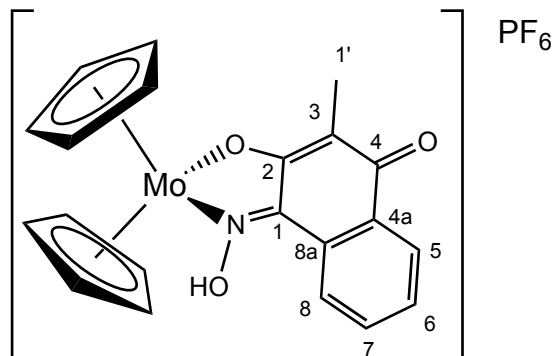
Melting point: >260 °C (decomposition)

Elemental analysis: for $C_{21}H_{18}F_6MoNO_3P$

	C [%]	H [%]	N [%]
calculated	44.00	3.16	2.44
found	43.97	3.23	2.50
Δ	0.03	0.07	0.06

(ESI⁺) *m/z*: 428.3 [M – PF₆]⁺

NMR-Spectroscopy:



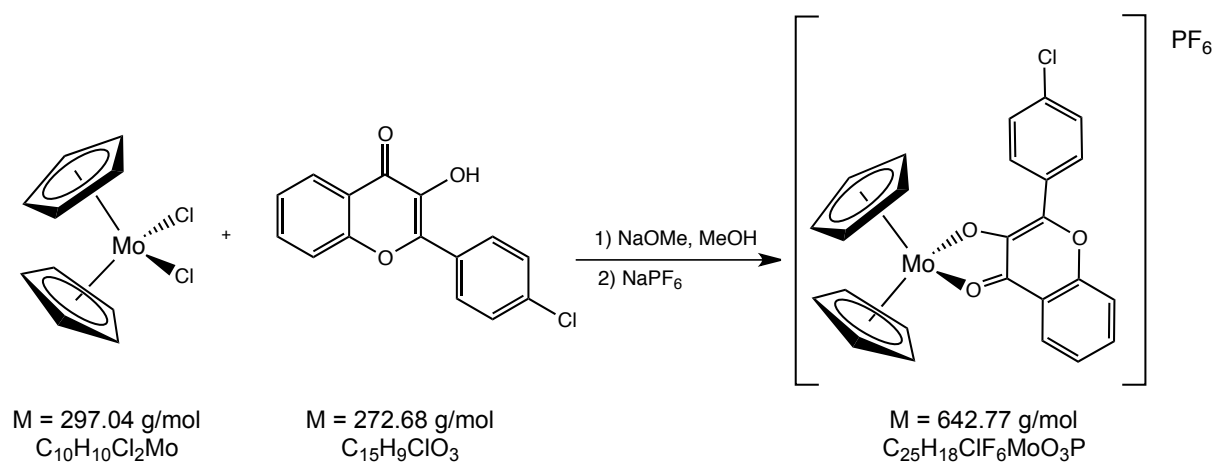
¹H NMR (*d*⁶-DMSO): δ = 1.88 (s, 3H, H1'), 5.89 (s, 10H, H_{Cp}), 7.60 (td, ³J(H, H) = 8 Hz, ⁴J(H, H) = 1 Hz, 1H, H7), 7.65 (td, ³J(H, H) = 8 Hz, ⁴J(H, H) = 1 Hz, 1H, H6), 8.09 (d, ³J(H, H) = 8 Hz, 1H, H5), 9.10 (d, ³J(H, H) = 8 Hz, 1H, H8) ppm.

¹³C NMR (*d*⁶-DMSO): δ = 9.0 (C1'), 102.3 (C_{Cp}), 114.0 (C3), 124.4 (C4a), 125.3 (C5), 125.7 (C8), 126.5 (C8a), 130.1 (C7), 132.6 (C6), 154.0 (C1), 173.9 (C2), 187.6 (C4) ppm.

¹⁹F NMR (*d*⁶-DMSO): δ = -71.29 (d, ¹J(F, P) = 711 Hz) ppm.

³¹P NMR (*d*⁶-DMSO): δ = -144.20 (sept, ¹J(P, F) = 711 Hz) ppm.

4.3.3.5 Bis(η^5 -cyclopentadienyl)[3-(oxo- κ O)-2-(4-chlorophenyl)-chromen-4-onato- κ O]molybdenum(IV) hexafluorophosphate



Synthesis:

The synthesis was performed according to the standard complexation procedure (see 4.3.1) using 2-(4-chlorophenyl)-3-hydroxy-4*H*-1-benzopyran-4-one (138 mg, 505 μ mol), molybdenocene dichloride (150 mg, 505 μ mol), sodium methoxide (30 mg, 555 μ mol) and sodium hexafluorophosphate (170 mg, 1.01 mmol, 2 eq) with a reaction time of 48 hours.

Yield: 117 mg (36 %), black powder

Melting point: >255 °C (decomposition)

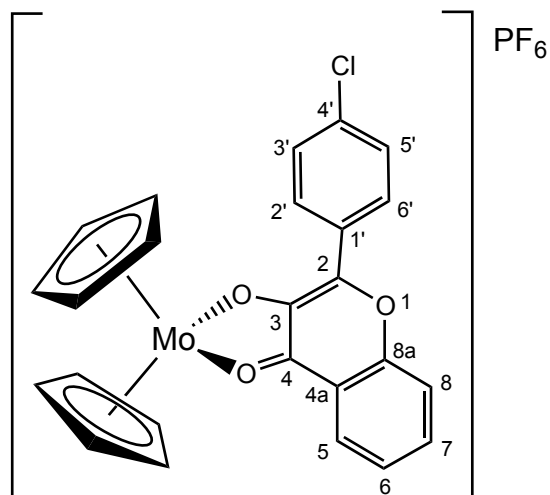
Solubility: 0.15 mg/mL $\equiv$ 0.23 mM in 1% DMSO/PBS

Elemental analysis: for $C_{25}H_{18}ClF_6MoO_3P$

	C [%]	H [%]
calculated	46.71	2.82
found	46.64	2.80
Δ	0.07	0.02

(ESI⁺) *m/z*: 497.0 [M – PF₆]⁺

NMR-spectroscopy:



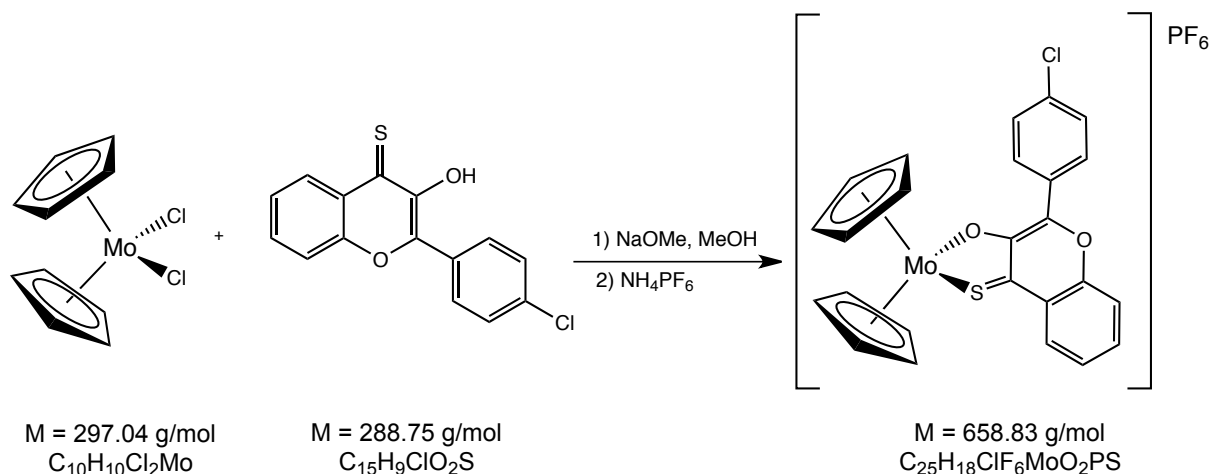
¹H NMR (*d*⁶-DMSO): δ = 6.06 (s, 10H, H_{Cp}), 7.58–7.61 (m, 1H, H7), 7.62–7.66 (m, 2H, H3'/H5'), 7.69–7.98 (m, 2H, H5/H6), 8.03 (d, ³J(H, H) = 9 Hz, 1H, H8), 8.31–8.35 (m, 2H, H2'/H6') ppm.

¹³C NMR (*d*⁶-DMSO): δ = 104.4 (C_{Cp}), 119.0 (C8), 199.2 (C8a), 124.4 (C5), 126.3 (C7), 129.4 (C3'/C5'), 129.6 (C2'/C6'), 130.1 (C4'), 135.4 (C6), 136.0 (C1'), 150.6 (C2), 154.4 (C4a), 155.5 (C3), 185.6 (C4) ppm.

¹⁹F NMR (*d*⁶-DMSO): δ = -71.30 (d, ¹J(F, P) = 712 Hz) ppm.

³¹P NMR (*d*⁶-DMSO): δ = -144.19 (sept, ¹J(P, F) = 712 Hz) ppm.

4.3.3.6 Bis(η^5 -cyclopentadienyl)[3-(oxo- κ O)-2-(4-chlorophenyl)-chromen-4-thionato- κ S]molybdenum(IV) hexafluorophosphate



Synthesis:

The synthesis was performed according to the standard complexation procedure (see 4.3.1) using 2-(4-chlorophenyl)-3-hydroxy-4*H*-1-benzopyran-4-thione (138 mg, 505 μ mol), molybdenocene dichloride (150 mg, 505 μ mol), sodium methoxide (555 μ mol, 30 mg) and sodium hexafluorophosphate (170 mg, 1.01 mmol, 2 eq) with a reaction time of 6 hours.

Yield: 45 mg (27 %), metallic green crystals

Melting point: >265 °C (decomposition)

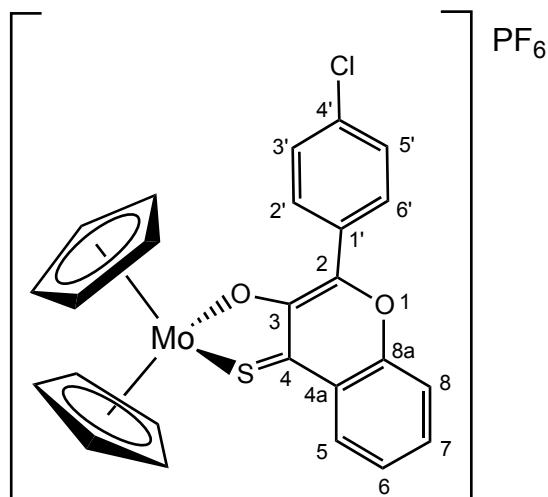
Solubility: 0.11 mg/mL $\equiv$ 0.17 mM in 1% DMSO/PBS

Elemental analysis: for $C_{25}H_{18}ClF_6MoO_2PS$

	C [%]	H [%]	S [%]
calculated	45.58	2.75	4.87
found	45.30	2.59	5.10
Δ	0.28	0.16	0.23

(ESI⁺) *m/z*: 513.4 [M – PF₆]⁺

NMR-Spectroscopy:



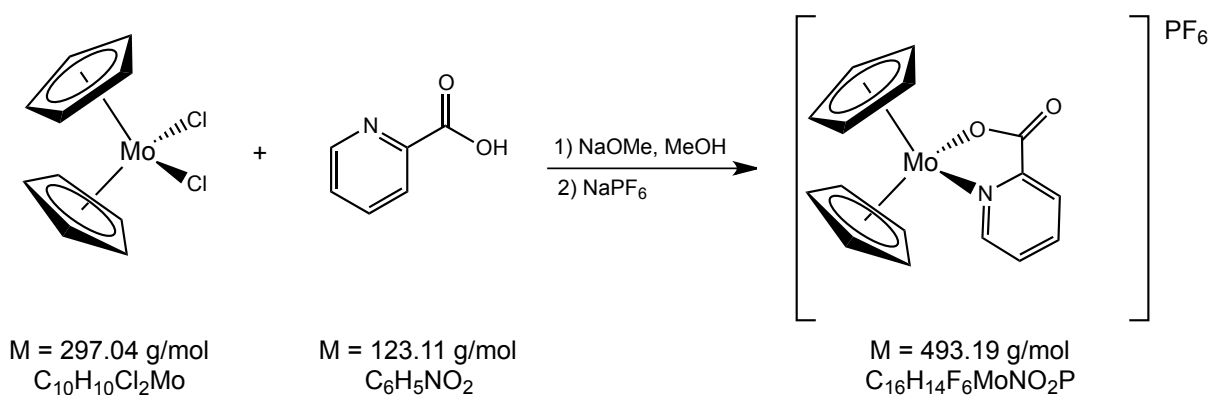
¹H NMR (*d*⁶-DMSO): δ = 5.89 (s, 10H, H_{Cp}), 7.62–7.68 (m, 2H, H3'/H5'), 7.69–7.74 (m, 1H, H7), 7.92–7.97 (m, 1H, H6), 8.10 (d, ³J(H, H) = 9 Hz, 1H, H8), 8.31 (d, ³J(H, H) = 8 Hz, 1H, H5), 8.36–8.41 (m, 2H, H2'/H6') ppm.

¹³C NMR (*d*⁶-DMSO): δ = 101.7 (C_{Cp}), 119.3 (C8), 125.7 (C8a), 126.1 (C5), 127.8 (C7), 129.5 (C3'/C5'), 129.8 (C4'), 130.9 (C6'/C2'), 134.5 (C6), 136.9 (C1'), 148.6 (C2), 149.4 (C4a), 166.1 (C3), 182.9 (C4) ppm.

¹⁹F NMR (*d*⁶-DMSO): δ = -71.31 (d, ¹J(F, P) = 711 Hz) ppm.

³¹P NMR (*d*⁶-DMSO): δ = -144.19 (sept, ¹J(P, F) = 711 Hz) ppm.

4.3.3.7 Bis(η^5 -cyclopentadienyl)[pyridine- κ N-2-carboxylato- κ O]molybdenum(IV) hexafluorophosphate



Synthesis:

The synthesis was performed according to the complexation procedure by microwave heating (see 4.3.2) using pyridine-2-carboxylic acid (41 mg, 337 μ mol), molybdenocene dichloride (100 mg, 337 μ mol), sodium methoxide (370 μ mol, 20 mg) and sodium hexafluorophosphate (67 mg, 337 μ mol, 1eq).

The product was also obtained using the standard complexation procedure (see 4.3.2) with a reaction time of 5 hours.

Yield: 64 mg (39 %), metallic purple powder

Melting point: >265 °C (decomposition)

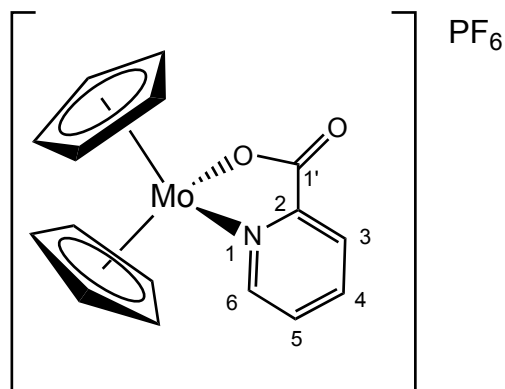
Solubility: 1.00 mg/mL $\equiv$ 2.02mM in 1% DMSO/PBS

Elemental analysis: for $C_{16}H_{14}F_6MoNO_2P \cdot 0.1H_2O$

	C [%]	H [%]	N [%]
calculated	38.96	2.86	2.84
found	38.48	2.71	2.74
Δ	0.34	0.18	0.09

(ESI⁺) *m/z*: 348.4 [**M** – PF₆]⁺

NMR-Spectroscopy:



¹H NMR (*d*⁶-DMSO): δ = 5.97 (s, 10H, H_{Cp}), 7.75–7.78 (m, 1H, H₅), 8.07 (d, ³J(H, H) = 8 Hz, 1H, H₃), 8.35 (td, ³J(H, H) = 8 Hz, ⁴J(H, H) = 1 Hz, 1H, H₄), 8.53 (d, ³J(H, H) = 5 Hz, 1H, H₆) ppm.

¹³C NMR (*d*⁶-DMSO): δ = 102.9 (C_{Cp}), 127.4 (C₅), 129.3 (C₆), 143.2 (C₄), 150.8 (C₂), 157.9 (C₃), 174.8 (C_{1'}) ppm.

¹⁹F NMR (*d*⁶-DMSO): δ = -71.29 (d, ¹J(F, P) = 711 Hz) ppm.

³¹P NMR (*d*⁶-DMSO): δ = -144.19 (sept, ¹J(P, F) = 711 Hz) ppm.

5 CONCLUSION AND OUTLOOK

The aim of this master thesis was the synthesis of molybdenocene complexes where the two labile chlorido ligands were replaced by biologically active chelating ligands.

The different *O,O*-, *O,S*- and *O,N*- ligands were synthesized in good to excellent yields and characterised via ^1H NMR spectroscopy. Seven molybdenocene complexes bearing the prepared bioactive ligands were synthesised in poor to moderate yields. All complexes were obtained with hexafluorophosphate as counter ion and were characterised by ^1H , ^{13}C , 2D, ^{19}F and ^{31}P NMR-spectroscopy, elemental analysis, ESI-MS, melting points and one complex by X-ray diffraction analysis. Their stability in aqueous solution was examined in water or PBS by UV/Vis and ^1H -NMR spectroscopy. All Mo(IV) complexes exhibited low solubility in aqueous media, due to the hexafluorophosphate counter ion. However, no hydrolysis or decomposition was observed for more than seven days. The solubility might be improved by exchanging the counter ion to chloride. Their redox properties exhibit irreversible oxidation peaks outside the physiological region. For investigations of the mode of action, binding studies with the serum protein HSA were carried out. ICP-MS measurements showed no covalent binding of the molybdenocene compounds to HSA. Fluorescence spectroscopy revealed non-covalent interactions of some molybdenocenes by increasing the complex/HSA ratio to a non-physiological level (5:1). However, to determine the type of interaction, fluorescence measurements at different temperatures have to be carried out.

Compared to molybdenocene dichloride most of the synthesized compounds improved their cytotoxic activity upon coordination of bioactive ligand scaffolds in pre-screen experiments. Some of the molybdenocene compounds show anti-proliferative activity even in the low μM range in human tumour cell lines, indicating a synergistic effect of molybdenocene and the bioactive ligand. However, to confirm the observed anticancer activity the respective IC_{50} values will be determined in the near future. To examine a correlation between the activity and the lipophilicity, the partition coefficient will be determined by the shake flask method. Further improvement of the cytotoxic activity could be achieved by modification of the cyclopentadienyl rings.

CURRICULUM VITAE

Marlene Reikersdorfer

Personal Data

Date of birth July 8th, 1988

Nationality Austria

Language skills

German Mother tongue

English Excellent knowledge

French Good knowledge

Education

09/2011 – 03/2014 University of Vienna Studies of Chemistry
Graduate degree: Master of Science (M.Sc.)

10/2012 – 06/2013 Tutor at the Institute of Organic Chemistry,
University of Vienna

3/2008 – 09/2011 University of Vienna Studies of Chemistry
Graduate degree: Bachelor of Science (B.Sc.)

10/2007 – 02/2008 Construction engineering, Technical University of
Vienna, Austria

09/1998 – 06/2006 Bundesgymnasium Bregenz Blumenstraße
Allgemeine Hochschulreife (Matura)

09/1994 – 07/1998 Volksschule Lochau

Research Experience

04/2013-03/2014 Master thesis at the Institute of Bioinorganic
Chemistry University of Vienna

Other Work Experience

10/2006 – 08/2007	Soziales Jahr at Schülertagesbetreuung Rohrbach, Dornbirn
08/2010 and 07/2011	Internship at the laboratory of Rupp Käsle, Hörbranz

Scholarship

2013	merit scholarship Chemistry, University of Vienna
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Computer Skills

Microsoft Office	Word, Excel, Powerpoint
Chemical software	ChemDraw, MestRenova, Mercury, Origin
Graphical software	Photoshop, GIMP etc.