universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Development of Novel Molybdenum(IV) Anti-cancer Agents"

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfillment of the requirements for the degree of

Master of Science (MSc)

Wien, 2018 / Vienna, 2018

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 862
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Masterstudium Chemie
Betreut von / Supervisor:	o. Univ.-Prof. Dr. Dr. Bernhard Keppler
Mitbetreut von / Co-Supervisor:	Michael Malarek, PhD

Acknowledgements

First and foremost, I would like to thank **O. Univ.-Prof. Dr. Dr. Bernhard K. Keppler** for the opportunity to work in his group on this fascinating topic.

My appreciation goes to my supervisors **Michael Malarek, PhD** and **DI Dr. Wolfgang Kandioller** for their guidance and dedicated involvement in every step of my project, giving me the best advice in the synthesis of the chemical compounds.

I would like to show gratitude to the NMR service team (**Ao. Univ.-Prof. Dr. Markus Galanski** and his team) for their great support in taking the needed NMR spectra, and also to the **mass spectrometry centre** for their vital involvement in measuring and evaluating the mass spectra of my compounds.

At this point, I would like to thank the following people:

Ing. Alexander Roller for X-ray data collection and structure refinement;

Iker Berasaluce, MSc for his encouragement during my master thesis;

Alessio Terenzi, PhD for his help with the UV-Vis measurements;

Mag. Johannes Theiner and his **team** for elemental analysis measurements;

Mag. Elfriede Limberger for her administrative work;

all members of the group for creating a nice atmosphere during my practical work in the department.

Last but not least, I would like to thank my **family and friends** for their encouragement throughout these years.

Abstract

Since the discovery and clinical success of Pt(II) complexes, such as cis-diamminedichlorido-platinum(II), metallodrugs have been widely investigated as anti-cancer agents. A variety of Pt(II) and Pt(IV) complexes exhibited good antitumour activity; however, their limited scope of activity and severe side effects are major drawbacks. Thus, new chemotherapeutic agents containing non-platinum centers have been investigated in order to reduce the acute adverse effects. Indeed, successful studies on Ru(III) complexes have shown promise in clinical trials in regards to their tumour-inhibiting properties along with reduced side effects.

Köpf und Köpf-Maier investigated molybdenocene, Cp_2MoCl_2, for its anti-cancer properties in the 1980's. The in vivo tests revealed that this Mo(IV) compound possesses promising antineoplastic properties presenting good anti-cancer activity. However, the chlorido ligands of molybdenocene are prone to hydrolysis yielding unreactive dimeric species in aqueous systems within minutes. An exchange of these labile groups by biologically active compounds is a promising approach for further improvements in the anti-cancer activity and their specificity, as well as less toxic side effects. Additional research conducted in this group provided seven molybdenocene complexes chelated with selected bioactive ligands. These compounds have been proven stable to hydrolysis over several days, with some of them exhibiting improved cytotoxic and anti-proliferative activity.

Moreover, work by Keppler et al. analyzing the anti-cancer activity of dihalidobis(β-diketonate) complexes of Mo(IV), such as $Mo(bzac)_2Cl_2$ (also named KP129), against colorectal tumours in rats showed an pronounced impact on the growth of these well differentiated adenocarcinomas. The chemoresistance of the AMMN-induced tumours provide for information similar to the human model and new compounds are needed to enhance the chemotherapy against slowly growing tumours, like colorectal cancer. However, similar to molybdenocene, the two chlorido ligands can be easily aquated. There is currently no research regarding the anti-cancer activity of the aquated complexes. Research by Keppler's group [1] showed that hydrolyzed Pt[II]compelxes are not less active against tumours, but the hydrolysis of the labile groups play a major role for the galenic formulation in the clinical trials. Furthermore, replacing the labile ligands with planar aromatic ring systems was shown to be advantageous for increasing the anti-tumor activity. Work carried out for this thesis was based on this acknowledgements, hence the hydrolysed $Mo(bzac)_2(OH_2)_2)$ was not investigated and the aim of this thesis is to replace the labile chlorido ligands of the Mo(IV) complex with biologically active O,O- and O,S- chelates to increase stability of the coordination compounds and improve anti-cancer activity. The investigated Mo(IV) complexes can be easily obtained by established literature

procedures, carrying out a simple solid state reaction and straightforward work-up. The impact of the applied ligand scaffold on the aqueous stability will be elucidated.

Kurzfassung

Seit der Entdeckung und dem klinischen Erfolg von Pt (II) -Komplexen, wie cis-Diammin-dichlorido-Platin(II), wurden metallhaltige Arzneimittel umfangreich als Antikrebsmittel untersucht. Eine Vielzahl von Pt(II)- und Pt(IV)- Komplexen zeigte eine gute Antitumouraktivität; jedoch ihr begrenzter Umfang an Aktivität und schwere Nebenwirkungen sind wesentliche Nachteile. Daher, um die akuten Nebenwirkungen zu reduzieren, wurden neue Chemotherapeutika, die Nicht-Platin-Zentren enthaltenuntersucht erforscht. Tatsächlich, erfolgreiche Studien an Ru(III) -Komplexen haben sich in klinischen Studien hinsichtlich ihrer tumourhemmenden Eigenschaften als vielversprechend erwiesen.

Köpf und Köpf-Maier untersuchten in den 1980er Jahren Molybdänocen, Cp_2MoCl_2, wegen seiner Antikrebseigenschaften. Die in-vivo-Tests zeigten, dass diese Mo(IV) -Verbindung vielversprechende antineoplastische Eigenschaften und gute Antikrebsaktivität besitzt. Allerdings neigen die Chloridoliganden von Molybdänocen zu Hydrolyse, was in wässrigen Systemen innerhalb von Minuten zu unreaktiven dimeren Spezies führt. Ein Austausch dieser labilen Gruppen durch biologisch aktive Verbindungen ist ein aussichtsvoller Ansatz für weitere Verbesserungen der Antikrebsaktivität und der Spezifizität, sowie weniger toxische Nebenwirkungen. Zusätzliche Forschung hat sieben Molybdänocenkomplexe, die mit ausgewählten bioaktiven Liganden chelatiert sind, ergeben. Diese Verbindungen sind für mehreren Tagen stabil gegen Hydrolyse, und Manche haben zytotoxische und antiproliferativerAktivität erwiesen.

Außerdem, weitere Forschung von Keppler et al. bezüglich der Analyse der Antikrebsaktivität von Dihalidobis(β-diketonat)-Komplexen von Mo(IV), wie $Mo(bzac)_2Cl_2$ (auch KP129 benannt), gegen kolorektale Tumouren bei Ratten, zeigte eine ausgeprägte Wirkung auf das Wachstum differenzierter Adenokarzinome. Die Chemoresistenz der AMMN-induzierten Tumouren sorgt für Information ähnlich zu dem menschlichen Modell und neue Verbindungen sind benötigt um die Chemotherapie gegen langsam wachsende Tumoure, wie Darmkrebs, zu fördern. Allerdings, ähnlich zu Molybdänocen, können die beiden Chlorido-liganden leicht hydrolysiert warden. Derzeit gibt es keine Forschung, die die Antikrebsaktivität von hydroylsierte Mo-Komplexe untersucht. Recherche in Kepplers Gruppe [1] weiste darauf auf, dass hydrolysierte Pt[II]-Komplexe nicht weniger aktive gegen Tomouren wirken, sondern die Hydrolyse der labilen Gruppen ist von wichtiger Bedeutung für die galenische Formulierung in den klinischen Studien. Außerdem wurde es gezeigt, dass der Austausch der labilen Liganden mit planeren aromatischen Ringsysteme sich auf die Antitumouraktivität vorteilhaft auswirkt. Die Forschung geleistet im Rahmen dieser Masterarbeit beruht auf diesen

Feststellungen, weshalb das hydrolysierte Komplex $Mo(bzac)_2(OH_2)_2$) nicht untersucht wurde und Ziel dieser Arbeit ist es, die labilen Chloridoliganden des Mo(IV) -Komplexes durch biologisch aktive O,O- und O,S- Chelate zu ersetzen, um die Stabilität der koordinierten Verbindungen zu erhöhen und die Antikrebsaktivität zu verbessern. Die untersuchten Mo (IV) -Komplexe können leicht mit etablierten Literatur-verfahren synthetisiert werden, durch eine einfache Festkörperreaktion und mittels einer unkomplizierten Aufarbeitung. Der Einfluss des aufgebrachten Ligandengerüsts auf die wässrige Stabilität wird noch aufgeklärt.

Table of Contents

Abbreviations

1D NMR	one-dimensional NMR spectroscopy
Å	Angstrom
ACN	acetonitrile
bzac/$(bazac)_2$	benzoylacetone
c	concentration
°C	degree dentigrade
cm	centimetre
Cp	cyclopentadienyl
δ	chemical shift (NMR)
d	doublet (NMR)
DCM	dichloromethane
DMSO-*d6*	deuterated dimethyl sulfoxide
DNA	deoxyribonucleic acid
ε	extinction coefficient
e.g.	*exempli gratia* (for example)
eq	equivalent
ESI-MS	electrospray ionization - mass spectrometry
EtOH	ethanol
FDA	food and drug administration
g	gram
h	hour
IC_{50}	drug concentration that causes 50 % cell growth inhibition
IUPAC	international union of pure and applied chemistry
J	coupling constant (NMR)
K	Kelvin
kDa	kilo Dalton
λ	wavelength
L	litre
logP	partition-coefficient
m	multiplet (NMR)
M	molar
(m/µ/n)M	(milli/micro/nano)molar (mol/L; mmol/L; µmol/L; nmol/L)
MeOH	methanol
(M)Hz	(mega)hertz
mol	mole
min	minute
Mr	molecular weight

m/z	mass-to-charge ratio
NaOMe	sodium methoxide
NMR	nuclear magnetic resonance spectroscopy
ppm	parts per million
RNA	ribonucleic acid
r.t.	room temperature
s	singlet (NMR)
t	triplet (NMR)
UV/Vis	ultraviolet/visible
v/v	volume/volume
v	wavenumber
WHOCC	World Health Organization for Collaborating Centre for Drug Statistics Methodology

1. Introduction

1.1. General information about cancer

In recent times, more and more deaths are caused by cancer, making it the second major health problem alongside with heart diseases, such as stroke and ischaemic heart disease (Fig 1) [2]. To put it into numbers, according to novel information by WHO (World Health Organization), a total of 9.6 million people (1 in 8) are predicted to die of cancer globally in 2018 alone, accounting for 22% of the total deaths cause by non-communicable diseases, such as cardiovascular, respiratory diseases and diabetes, while sadly 30-50% of the incidences could have been prevented. [3][4][5] One of the non-communicable diseases is cancer, which is a summary of various illnesses, caused by malignant tumours, also named neoplasms, affecting any tissue or organ of the body. [3] [6]

More specifically, in Austria in 2014, cardiovascular diseases were the main cause of death with 43%, while malignant neoplasms (cancers) accounted for 27% (which means 1 in 4 deaths were caused by cancer). [3]

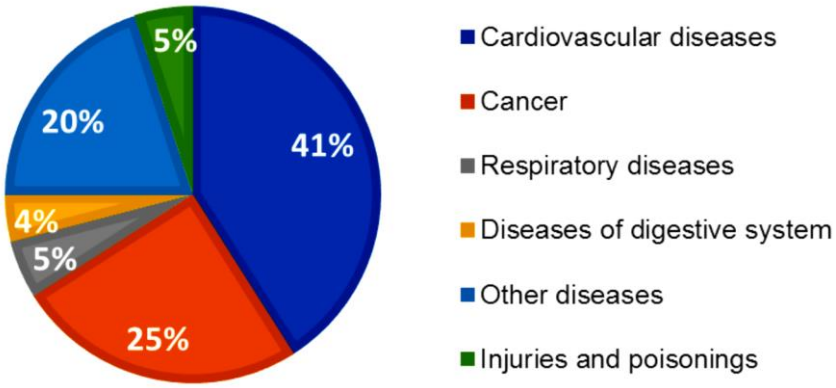


Fig. 1: Causes of death in Austria in 2016 (according to Statistik Austria) [2]

In upper-middle-income countries, the occurrence of cancer can be prevented due to available medical care as well as various awareness programs. A decrease of infectious diseases, poisonings, and diseases of the digestive system was observed after the implementation of vaccines, immunisation techniques and generally higher hygiene standards of the population. [2]

Several factors have a major impact on the development of cancer; these include a person's age, sex, nutrition, ethnic descent, geographic location and personal lifestyle. [7] Also, most relevant aspects that lead to cancer are the contact with various carcinogens, such as physical – UV radiation, chemical – contaminants of food and drinking water and last but not least, biological – infections. [2][3][8][9][10]

Looking at the information available on the GLOBOCAN Database [11], in 2018 more than half of the novel cancer incidences and 71% of the cancer caused deaths took place in low-income regions (Fig. 2). [11] Interestingly, the malignant neoplasms develop and affect men and women differently. [7]

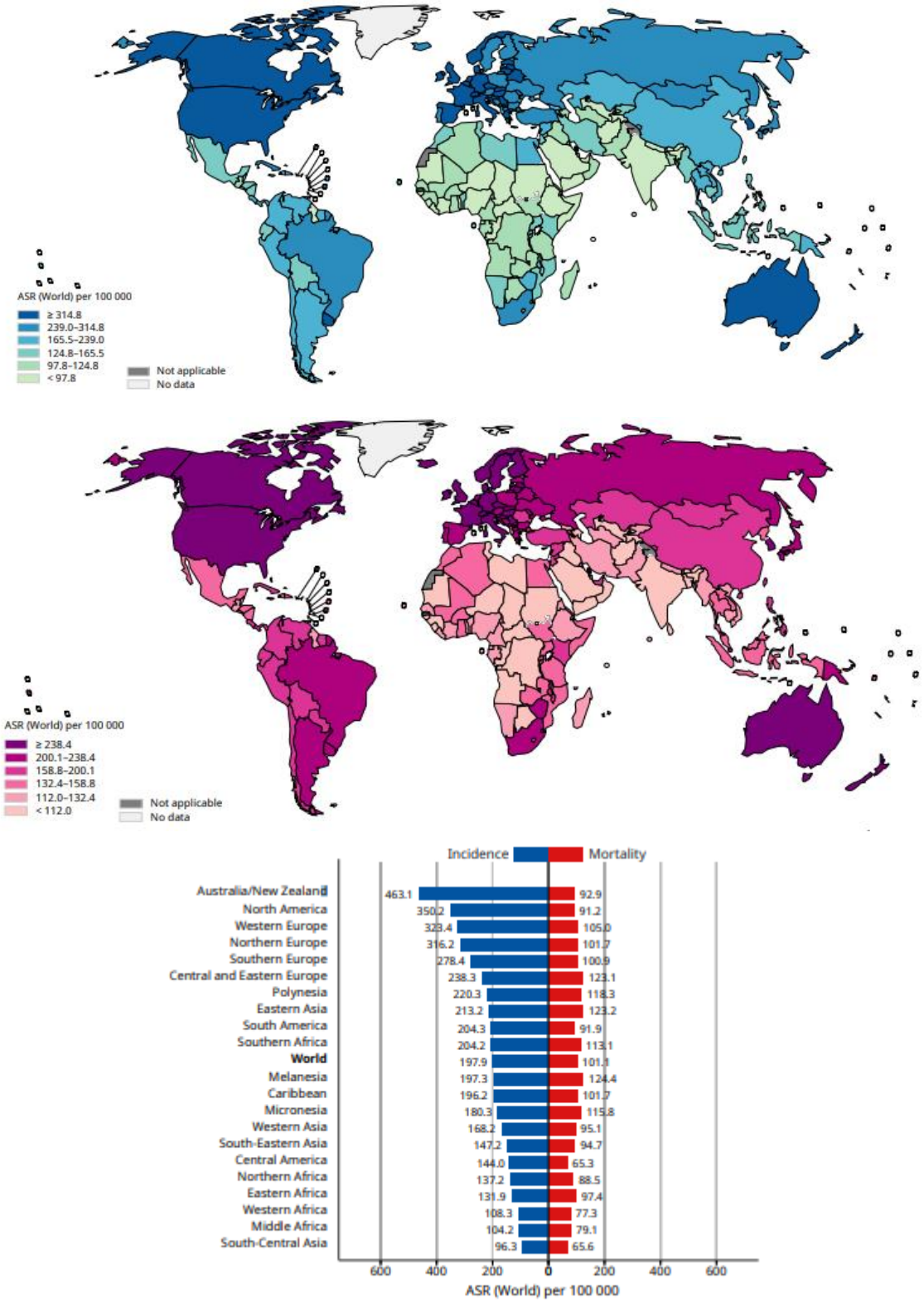


Fig. 2: Overview on the age-standardised rates (ASR) of cancer incidence in men (blue) and in women (purple), as well as the ASR for incidence and mortality worldwide in 2018. All three pictures taken from reference [11]

To have a better understanding on how cancer occurs, a definition of tumours is given according to WHO, as being abnormal growing cells which can invade neighbouring parts of the body and then spread to other organs by the formation of metastases. [3]

To fully understand how tumours occur, the most basic function of the cell cycle will be elucidated. Fundamentally, the cell cycle creates two genetically identical daughter cells by duplicating the amount of DNA in the chromosomes and separating the replicates accurately. [12][13] After the final cell division, the cell goes into the so-called resting state of the cycle where it can remain "inactive" for an undefined period of time. When the appropriate signals to re-start or continue the growth and division occur, the cell exits the resting phase. [14] In order to stop further unrestrained cell division, the cell cycle has a complex control system that is in charge for detecting and repairing genetic damage. [14] This prevention mechanism suspends any given cell cycle step in order to regulate its correct progression in case of sudden unfavourable intracellular conditions, such as impaired DNA; or sets up cells that are no longer required or are unsafe to the organism are to undergo apoptosis. [15] D. Green defines apoptosis as the programmed death of a cell, caused by a cascade of biological events, occurring in such manner to not damage the adjacent cells. [16] Otherwise, they undergo necrosis – which is defined as the sudden death of a cell, that takes effect by swelling and erupting, and by doing so, invading and contaminating the neighbouring tissue (Fig 3a). [15][17]

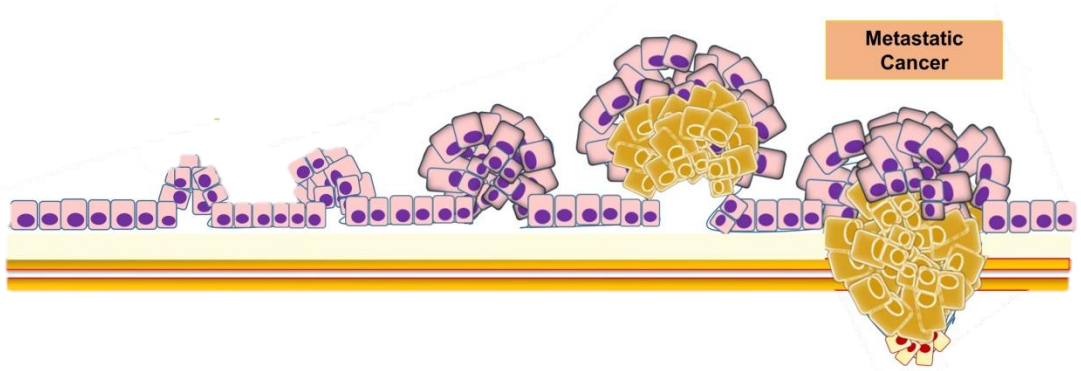


Fig. 3b: Incidence of cancer [17]

One aberrant cell can emerge into cancer by carcinogenesis. A cell becomes cancerous if, after an initial mutation it also undergoes further abnormal mutations and develops into a tumour. Benign tumours are neoplastic cancer cells that can be isolated and removed by surgery. These cells have the ability to proliferate uncontrollably in disregard of normal control. Nevertheless, if the cells manage to invade the surrounding tissue, malignant tumours take shape. If these are able to enter the lymphatic system or the blood stream and spread throughout the whole body, metastases are formed (Fig. 3b).

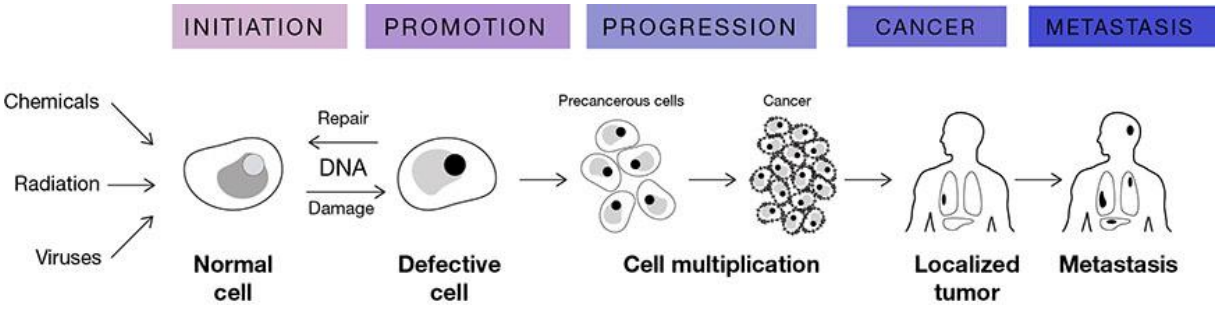


Fig. 3b: Development of cancer [17]

Three major cancers can be classified based on the tissue type that the tumour cells arose from: carcinomas, sarcomas and leukaemia. Carcinomas develop out of epithelial cells and are the most common form of cancer, such as breast, prostate, lung and pancreatic cancer. Sarcomas originate from mesenchymal (connective) tissue, and lead to bone, cartilage, fat, muscle and vascular cancer. Leukaemia and lymphoma arise from white blood cells. There are more than one hundred types and subtypes of cancer that can be found within specific organs, as for example germ cell tumour (from pluripotent cells) and blastomas (from embryonic tissue, rather more common in children than in adults). Studies showed that nearly all cancerous cell genotypes exhibit six fundamental modifications in the cell that lead to malignant growth. [18] Further studies by R. Weinberg and D. Hanahan increased the number of the previously established six biological capabilities with four additional characteristics. These were organised as the principle "hallmarks of cancer" [19] as follows: deregulating cellular energy metabolism, resisting apoptosis and/or necrosis, escaping immune destruction, inducing angiogenesis, avoiding growth suppressors, promoting proliferative signalling, generate invasion and hence induce metastasis, tumour enhancing inflammation, and creating genome instability and/or mutation (Fig. 4). [18][19] During carcinogenesis, individual specialised cell types create a favourable neighbouring genotype and consequently various malignant tumours are unique, depending on the cells within. Therefore, to understand how a type of cancer occurred or could be cured, the neoplasms need to be studied individually. [19]

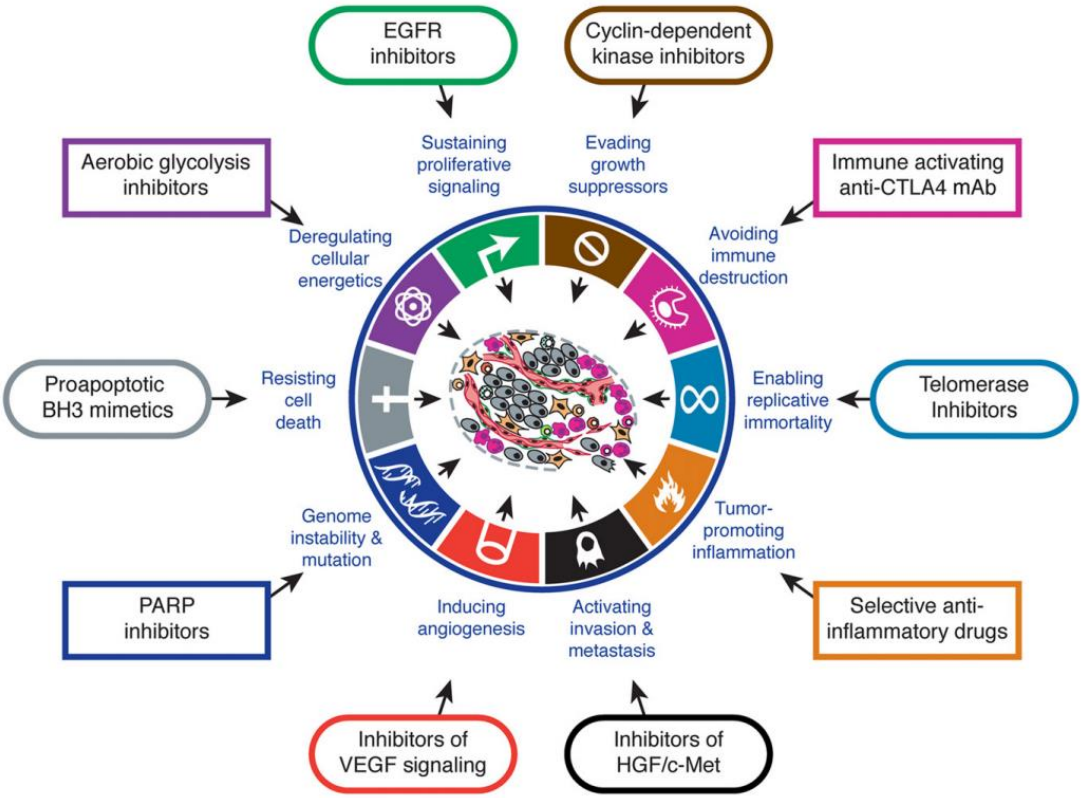


Fig. 4: Overview of the milestones of cancer and their targeting anticancer agents. [19]

The four major risk factors that can cause cancer worldwide are tobacco consumption, alcohol abuse, an unhealthy diet (low intake of fruit and vegetables, combined with obesity) and lack of physical activity. [3][9] Nonetheless, the main cause of the occurrence of cancer is aging. The older a person gets, the lesser operative the cellular repair mechanisms become, and also the mutagen factors that may cause cancer, accumulate in the body. Globally, the rates of cancer incidence and mortality are different from those in European countries. In low- and middle-income countries, the incidence and mortality of cancer is higher than in well-developed countries, e.g. Austria in this case. For example, tobacco use caused about 22% of the cancer-related deaths globally in 2016 although it is the most preventable cause of cancer. Furthermore, another factor that leads to cancer incidence in low- and middle-income regions is due to lack of vaccination against cancer-causing infections, such as HPV. Summarized research on cancer worldwide by GLOBOCAN displayed that the top three localisations of malignant neoplasms in men are lung, prostate and colorectum, while for women in breast, colorectum and lung (Fig 5b). [10][11] In 2015 in Austria, on the other hand, the most frequent tumour prevalence for men was as follows: prostate (23%), lung (14%) and colorectum (12%), and for women: breast (29%), lung (10%) and colorectum (10%) (Fig 5a). [2] Generally, the most deadly (survival rate ca. 8% in Europe) cancer occurrence for men is in the lung, with high incidence rates being observed in North America and Europe (especially Eastern Europe), and breast cancer for

women. [11] Other common cancers worldwide are of the stomach, bladder, oesophagus, and in less-developed and middle- income countries preponderating the liver cancer, caused by diseases of chronic infection origins, such as Hepatitis B or C viruses, and cervical cancer induced by the Human papillomavirus (HPV) and AIDS developed from HIV. [3][11][20][21] However, research showed that the number of freshly arising cases of cancer does not equate to the number of deaths happening as an outcome of cancer, as, summarized for 2018 in Fig. 5b (right), most people died of lung, breast, colorectum, liver and stomach cancer. [7][11][20]

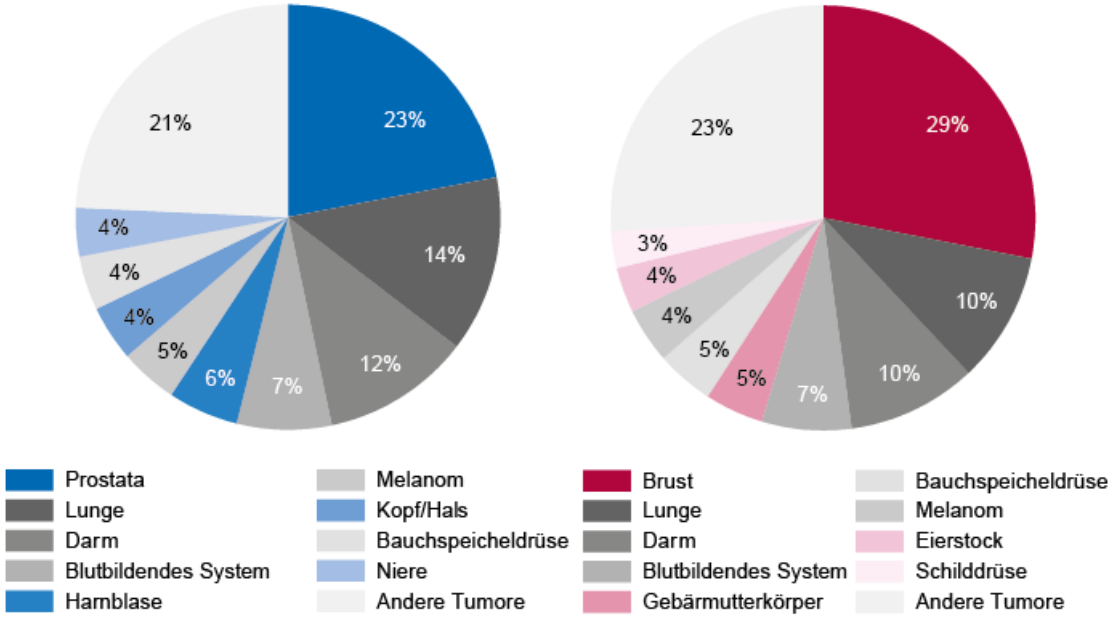


Fig. 5a: Most abundant tumour localisation in about 21.000 men (left) and about 19.000 women (right) in Austria in 2015. Graphic created on 15.12.2017 by Statistik Austria [2]

By avoiding the previously mentioned risk factors by abstaining from drinking alcohol and smoking, keeping a healthy diet and practicing sports, immunisation and vaccination (for example against HPV and Hepatitis B viruses) and reducing the exposure to UV or ionizing radiation, could prevent as many as 30% -50% of cancers. [3] When looking at the incidence and mortality data gathered over a longer period of time, an increase in the absolute number of cancer occurrences was observed, also considering the varied age segregation. [2]

The importance of an early diagnosis of the cancer is vital; the sooner a tumour is discovered, the better recovery chances of a patient. Hence, if the diagnosis of cancer is appointed at an early stage, a corresponding treatment method can be chosen and the mortality rate is decreased. [2][7] In recent times, an enhancement in the recovery rate was recognized in high-income regions as a result of higher risk awareness of the population and improvement in the medical care system (its broader availability and better diagnostic tools). Unfortunately, many times it is too late for a curative treatment, because very often a cancer diagnosis is made just before entering its late metastasis stages, as it doesn't display specific symptom manifestations.[3][7]

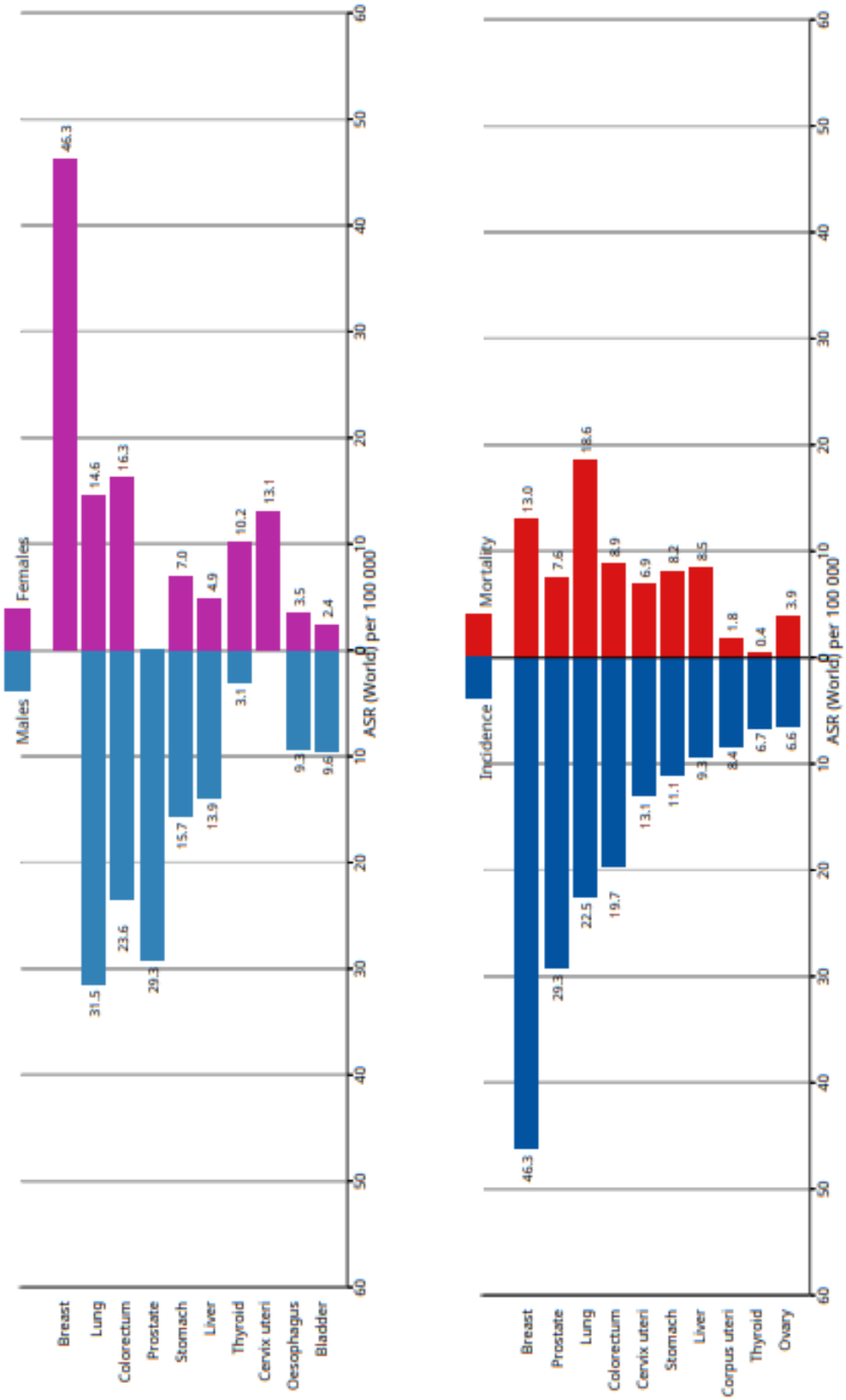


Fig. 5b: Top 10 cancers incidences per sex (left) & incidence and mortality rates (right), globally per 100.000 people 2018 [11]

1.2. Cancer therapy

There are many procedures to treat cancer, but the choice of the most effective curative method requires a correct disease diagnosis, which in turn relies upon the recognition of the type and stage of cancer, localisation of the tumour, the grade of the malignant neoplasm and the general health state of the patient. Therefore, either one of the following therapeutic strategies, or a combination thereof, will be taken into consideration, according to the type and progression of cancer. The choice of a specific therapy approach is aimed to heal the patient of cancer, or at least to enhance the patient's living conditions and extend his or her life as much as possible. [3] The most common types of cancer treatment are summarized as follows:

1. **Surgery:** is the oldest approach of cancer therapy, applied for solid tumours. Unfortunately, this method is limited to accessible located solid tumours. The purpose is to remove the whole tumour from a part of the body with adjuvant therapy, which means combining this method with radio- or chemotherapy in order to eradicate cancerous cells, which might have been left after the surgery itself. [22] Unluckily, some of the tumours can be spread throughout the body, since not all malignant tumours are in solid state (e.g. leukaemia), thence radiotherapy and chemotherapy are more suitable choices in this case. [23][24]
2. **Radiotherapy:** this method uses γ- or X-rays to destroy the DNA of the cells within the tumours and hence shrink or eradicate the tumours by apoptosis. There are two ways of administrating this treatment method; namely externally (implies directing a radiation beam straight into the tumour) and internally (uses radio-nucleotides, like ^{131}I in the treatment of thyroid diseases). The downside of this method is the lack of specificity, meaning that healthy cells are also affected, which leads to severe side effects, like skin alterations, fatigue or loss of appetite. Nonetheless, therapy using radiation is applied against lymphomas, various solid tumours and leukaemia, providing for about 40% of the therapy plans nowadays. Moreover, for an enhanced therapeutic effect, this method can also be combined with chemotherapy or be used before, during or after surgery. [6][23][25]
3. **Hormonal therapy:** its aim is to slow down and even stop the growth of certain types of cancer, by adding, blocking or removing hormones. This method is also referred to as endocrine therapy. The paradoxical usage of hormones to treat cancer lies within every particular disease; supplying hormones to adjust their low levels helps with diabetes, while too high levels of hormones can cause breast or prostate cancer to sprout. In some cases, the gland that produces a certain hormone needs to be removed by surgery, while in other cases, drugs or synthetic hormones can be administrated to inhibit the naturally produced

ones in order to adjust the body's hormone levels to inhibit or stop the further development of cancer. [6]

4. **Targeted therapy:** the goal is to stop the development and progression of malignant neoplasms by inducing monoclonal antibodies or other small substances to interfere with targeted molecules (e.g. proteins or enzymes), which are required in carcinogenesis and metastasis. On the other hand, another targeted therapy strategy is to deliver toxins either straight in the tumours in order to destroy them or in the immune system to stimulate it to identify and kill the cancer cells. This kind of therapy has fewer side effects due to the specific mode of action of specifically chosen drugs. [6]
5. **Immunotherapy:** this widely applied method alleviates the body not only of cancerous cells, but also of other diseases and infections, either by targeted or general stimulation or restraining of the immune system via the use of substances such as monoclonal antibodies, vaccines and cytokines. [6]
6. **Chemotherapy:** involves the administration of natural or synthetic drugs to eradicate rapidly dividing cancer cells on a molecular level. Similarly to the targeted and hormonal therapy, this is also a systemic treatment method, meaning that the employed substances or drugs are carried through the bloodstream until they reach the damaged cells anywhere in the body. [6] Nevertheless, normal healthy cells are also affected by the given aggressive pharmaceuticals, causing the notorious severe side effects (hair loss, anaemia, nausea immune-suppression) due to lack of selectivity. However, chemotherapy is commonly implemented against metastasised tumours, small tumours which escaped previous detection and even non-solid tumours. The WHOCC classifies the administered pharmaceutical anticancer agents according to their therapeutic and chemical characteristics, and the targeted tissue or organ: [26]

I. **Alkylating agents:** are compounds that have the ability to transfer an alkyl group to DNA, RNA and proteins, consequently damaging them and thereby inhibiting further reproduction of the cells. The disadvantage of using these agents is their effectiveness in all cell cycle phases, meaning that regularly dividing cells are also targeted additionally to the cancer cells. Within this group the following substances are encompassed: epoxides (hydrazines and triazines), nitrogen mustard derivatives, nitrosoureas, alkyl sulfonates, ethylenimines and others, such as some Pt-based compounds (cisplatin, carboplatin, oxaliplatin) that also have the ability to bind and damage the DNA by interfering in its repair mechanism. [24][27][28][29][30]

II. **Antimetabolites:** are active during the S-phase of the cell cycle and replace normal building blocks of DNA and RNA, interfering with their growth. They include analogues of purines and pyrimidines, folic acid or adenosine deaminase inhibitors (e.g. pentostatin). [24][30]

III. **Plant alkaloids**: are mitotic inhibitors, so they prevent cells from reproducing. Plant alkaloids and other natural products include colchine derivatives, vinca alkaloids, taxanes, camptothecan analogues and podophyllotoxin derivatives. [30]

IV. **Topoisomerase inhibitors:** interfere with DNA replication by retraining the enzymes topoisomerase I and II, which promote unwinding of the DNA strand during the S-phase of the cell cycle. [30]

V. **Antitumour antibiotics:** such as chromomycins (plicamycin, dactinomycin) or anthracyclines (epirubicin, daunorubicin, oxorubicin) inhibit the replication of DNA or RNA synthesis during different phases of the cell cycle. [30]

In addition to the previously mentioned therapies, there are several antineoplastic compounds with cytotoxic activity. These include various platinum-based drugs, monoclonal antibodies, methylhydrazines, [27] protein kinase inhibitors, sensitizers used in radiotherapy, or combination of thereof. [26]

Since the beginning of the 20th century, numerous diseases were treated with metal-based compounds, as in the case of depression with lithium-based substances or syphilis with arsenic-containing drugs. Nevertheless, the accidental discovery of the cytotoxic activity of the Pt-complex cisplatin (cis-diamminedichlorido-platinum(II)) resulted in a breakthrough in the treatment of cancer and marked the turning point in the field of cancer research to study individual transitional metal complexes that potentially could be active as anti-cancer or antineoplastic agents. [31]

1.3. Metal ions in medicine

Metal ions such as Na, Mg, K, Ca – main group elements, and numerous essential transition metals, Fe, Cu, Zn, V, Cr, Ti, Mo, etc. play an important role for vital functions in the human body. A disease can arise if in the human body an excess or a deficit of some metal ion exists. For instance, a delay in growth can be induced by zinc deficiency, a scarcity in iron can lead to pernicious anaemia and most interestingly, an insufficiency in copper can lead to cardiac diseases in infants but an excess of it can spawn into Wilson's disease - a rare inherited disorder caused by the accumulation of Cu in vital organs, with symptoms like fatigue, lack of appetite, jaundice and yellowing of the skin, fluid build-up, issues with physical coordination. So, both a deficiency and an excess of an essential element can be harmful to the body. Thus, sufficient adsorption, distribution and storage of the metal ions are crucial. Their delivery into the target environment in order to carry out their intended biological functions is essential, as they are important for structural functions (cell wall integrity, skeletal support), muscle contraction, ion pump and enzyme activity, catalysis, charge carriers and many more. [32]

Already known in the 16th century, Paracelsus asserted, that a substance can become harmful to humans, with its toxicity determined by the dose (e.g. concentration). [33] In this regard, to assert the effect of metal ions in the human body, the type of chemical bond and the amount absorbed are of vital importance. There are several factors that have an impact on the area of optimum physiological response of a metal ion or complex, and these are oxidation state of the element, speciation, biochemistry and the structure of the ligand – has an effect on how the metal ion is delivered to the biological system. [34]

Nonetheless, one of the most important milestones was set by the discovery of platinum-based cisplatin in the 19th century. Moreover, a variety of metal-based complexes are presently undergoing clinical trials for their potency as cytotoxic agents.

1.3.1. Platinum-based anti-cancer drugs

Without the knowledge of the anti-proliferative properties of cisplatin (cis-diamminedichlorido-platinum(II)), Michele Peyrone was the first to synthesise this complex in 1844 [35], but only in 1965 Barnett Rosenberg discovered by chance that cisplatin tends to suppress or inhibit cell division. [36] Initially, Rosenberg et al. carried out some experiments in *E.coli* cultures, using two electrodes made of Pt and NH_4Cl as growth medium, to study the effect of an electric field on the mitosis of these bacteria. The observed results were, on the one hand, that the bacteria performed no cell division, interestingly increased their length by 300-fold, and on the other, that the employed platinum

electrodes were oxidised to Pt(IV), which in turn reacted to the ammoniumhexachoridoplatinate(IV) complex. This was then photocatalytically converted to cis-diamminetetrachloridoplatinate(IV)-complex and finally, this complex was reduced by the reductive moiety of the bacteria and led to the formation of the active cisplatin. So it could be concluded that cisplatin had an influence on the cell growth. [36][37] Soon, its anti-proliferative activity and possibility of medical usage as an anti-tumour agent was discovered. [38] The first clinical phase I study was commenced with patients in 1972, and 1978 cisplatin was successfully approved by the FDA as the first metal-based anti-cancer agent to be used in anti-cancer therapy worldwide. [39][40] The study showed a notably high effectiveness against testicular cancer, where 80% of the treated patients survived. [41] For a better understanding, before the discovery of cisplatin's potency, the mortality rate of testicular cancer was over 90%, while at present day the mortality rate of about 10% is mainly because of late diagnosis. [42] In addition, positive results have been observed when used in combination therapy for tumours of the lymphomas, cervix, lung, ovaries, neck, head and bladder. [43] According to previous research, cisplatin is administered intravenously and once in the body it adsorbs mainly to serum proteins like HSA (human serum albumin). The formed adducts are carried in the bloodstream to be taken up by active transport or passive diffusion. [44][45] Unfortunately, cisplatin is not potent against all types of cancer. [46][47]

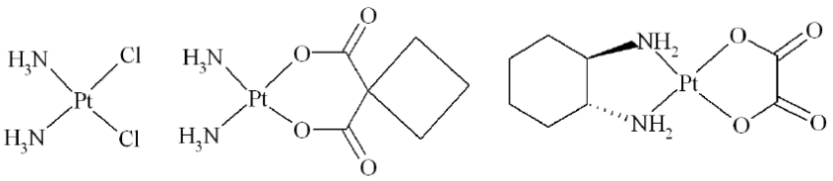


Fig. 6: Structures of the approved platinum anti-cancer drugs: cisplatin, carboplatin, oxaliplatin (left to right)

For cisplatin to be able to bind to DNA, its main target, the complex must undergo hydrolysis, whereby the chlorido ligands will be replaced by water molecules, hence leading to the formation of the single or double aqua species: $[(Pt(NH_3)_2Cl(H_2O)]^+$ and $[(Pt(NH_3)_2(H_2O)_2]^{2+}$. [43][48] The intracellular chloride concentration is lower thereby facilitating the dissociation of the chlorido ligands. The hydrolysed cisplatin binds then covalently to the N7 position of the purine bases of DNA (guanine (G) or adenine (A)), yielding 1,2 or 1,3 intrastrand and interstrand structures (Fig. 7). [37][49] The most significant adduct (~65% of total products) produced is the cis-1,2-$[Pt(NH_3)_2]^{2+}$-d(GpG) intrastrand crosslink, followed by 1,2-d(ApG) (25 %) and 1,3-d(GpNpG) (5–10 %) intrastand products, along with less frequently formed interstrand crosslinks and monodentate compounds. It has been showed by X-ray crystallography that these adducts bend DNA and unwind the double helix, leading ultimately to programmed cell death. [47][50][51] Still, only about 1 % of the provided cisplatin reaches the cellular target, while the majority is inactivated by staying bound to plasma proteins. [52]

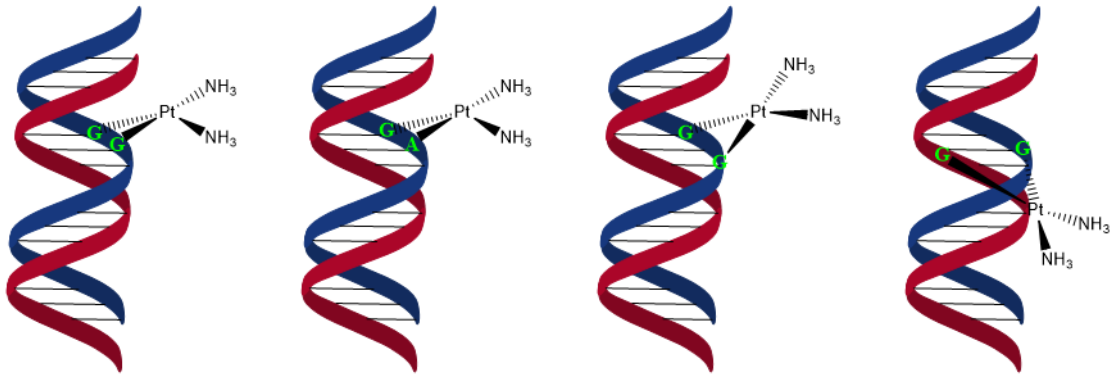


Fig. 7: Exemplified binding mode of cisplatin to DNA

The most significant shortcoming of cisplatin is its harsh side effects, such as the dose-limiting kidney toxicity, neuro- and oto-toxicity, decline in the amount of red and white blood cells, peripheral neuropathy, hair loss, loss of appetite, vomiting and nausea, . [53] Moreover, another critical drawback is the intrinsic resistance of some tumours and also the resistance some cancer cells may develop during consecutive therapy. [54][55] Therefore, the need for new platinum-based anticancer agents was encouraged by the desire to develop new and better drugs, with regard of their stability and reducing the side effects, and so, second and third generation analogues were discovered through further research. [55]

The second generation of platinum-based anti-cancer drugs was marked by the discovery of carboplatin (cis-diammine(1,1- cyclobutanedicarboxylato)platinum(II)) in 1972. This complex holds properties very alike to those of cisplatin, with the most important asset of a modified toxicological profile being fewer side effects, meaning that oto-toxicity, gastrointestinal and neurotoxicity are diminished, and myelosuppresion set the dose-limiting toxicity. [55] Carboplatin contains two ammine ligands, similarly to cisplatin, but additionally also a bidentate dicarboxylato leaving group, which leads to slower ligand exchange kinetics than cisplatin. Thus, by substituting the chlorido ligands with the cyclobutane-1,1-dicarboxylato chelate, the stability of carboplatin was improved towards hydrolysis. [56] One of the major drawbacks of carboplatin is the need of administrating a 4x higher dose than in the case of cisplatin due to its lower reactivity and the slower binding to DNA. [51][55][57][58] The main use of carboplatin is the administration against tumours of the urogenital tract. [55]

In 1976 a third generation of platinum(II)-based drugs was developed. Oxaliplatin, ((*trans*-R,R-cyclohexane-1,2-diamine)-oxalatoplatinum(II)) was approved on account of its prevailed advantages over the limitations of cis- and carboplatin. In contrast to carboplatin, oxaliplatin contains the chiral (1R,2R)-diaminecyclo-hexane (DACH) as non-leaving and a bidendate oxalato ligand as leaving group. Its main significance is due to the activity in cell lines and tumours that are resistant to cis- and

carboplatin. A second major aspect is the activity against metastatic colorectal cancer in combination with 5-fluorouracil and folic acid, which was thought to be untreatable up to now. [51][59][60] Few of the side effects of oxaliplatin include gastrointestinal and neurological effects and the dose limiting sensory neuropathy.

Furthermore, besides the globally approved cis-, carbo- and oxaliplatin as anti-cancer drugs, there are three other regionally approved platinum-complexes namely heptaplatin in South Korea, nedaplatin approved in Japan and lobaplatin in China (Fig. 8). [55]

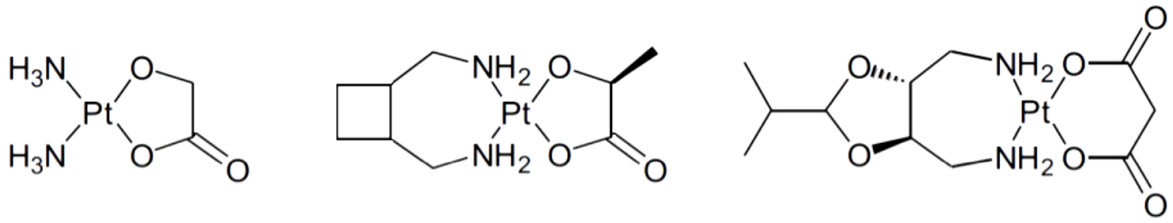


Fig. 8: Regionally approved platinum anti-cancer agents: nedaplatin, lobaplatin (both diastereomeres), heptaplatin (left to right) [55]

In order to overcome the side effects of the platinum(II)-based compounds, as well as the intrinsic and acquired resistance, research focused on different strategies, like the development and investigation of kinetically inert platinum(IV)-based complexes. These behave as a pro-drug and can be activated in the cell as follows: in the reducing moiety of the tumour cells, the axial ligands of the complex are released, hence reducing Pt(IV) to Pt(II). This mode of action leads to a significant reduction in side effects due to fewer undesired reactions with biomolecules. Additionally, as a result of their kinetic inertness and stability even in the gastro-intestinal tract, Pt(IV)-drugs can be administered orally, thus improving the bioavailability of the drug. [61] Cellular uptake and toxicity of these new platinum compounds are improved by the extended coordination sphere with two additional ligands. [52][55] Some of the platinum(IV)-based complexes investigated in clinical trials include LA-12 [62], satraplatin [47][63], iproplatin [64] and tetraplatin[65]. The studies were stopped mainly due to the severe side effects or lack of improvement in patients.

In spite of the high toxicity, severe side effects and acquired and intrinsic resistance being still the main limitations of this drug class, as many as 40 platinum-based anti-cancer compounds with cytotoxic and antiproliferative potency have entered clinical studies hitherto. [66][67]

Nonetheless, other metals have been investigated for their anti-cancer potential.

1.3.2. Ruthenium-based anti-cancer drugs

Over the past years, a lot of research has focused on new fields and investigating alternative metallodrugs with different metal centres (e.g. Ru, Ga, As, Rh, Os, Ir, Fe, Ti, Mo, etc) as potential anti-cancer drugs, on account of the limitations of the platinum-based drugs (severe side effects, intrinsic and acquired resistance, and the limited range of treatable tumours). The platinum group metals were the first elements to be considered for further research: osmium, iridium, rhodium and ruthenium. Compared to platinum anti-cancer drugs, ruthenium complexes exhibit activity in cells that are inactive or resistant to the use of cisplatin. [68]

Sava et al [69] very clearly summarised the appealing properties of Ru-based complexes, such as its ability to bind with O- and N-donor molecules similarly to platinum and furthermore to interact with nucleic acids due to their octahedral geometry, the drugs based on the rather small ruthenium(+2, +3 and +4)-ions can mimic the iron bound to biomolecules, as well as its accessible oxidation states under physiological conditions. The redox potential between these oxidation states enables oxidation and reduction depending on the physiological environment.[69][70][71][72] Furthermore, of the interesting properties of Ru-based drugs is also the possibility to obtain low reactive drug precursors of Ru (III) that can be reduced in the low oxygen milieu and then selectively activated in the targeted tumours. This, and also the transport into the targeted area via transferrin [73], lead to a low systemic toxicity, meaning the reduced harmful effects of these Ru-complexes on the whole body and not-targeted organs, is one of their most valuable properties. This hypothesis is detailed in the more in depth work by Clarke and Srivastava, which proves the ability of ruthenium drugs to distinguish between healthy and malignant cells, on account of the reduction of the prodrug into the active cytotoxic complex in the tumour tissue and of the larger amount carried there by transferrin. [74][75][76] It can be concluded that Ru-based anticancer drugs can selectively invade mainly the tumour cells hence endorsing lower systemic toxicity than previously studied Pt drugs. Toxicity tests showed minor effects on the kidneys, alterations of the mitochondrial membranes and an increased spleen volume. [69]

Furthermore, two ruthenium anti-cancer compounds were investigated in clinical trials. Alessio and Sava developed the compound NAMI-A (trans-[tetrachlorido-S-dimethylsulfoxide(imidazole)-ruthenate(III)]) which was moderately tolerated as monotherapy, but exhibited less activity in combination with gemcitabine, and the first one to enter clinical trials (Fig. 9). [69][77][78][79][80]

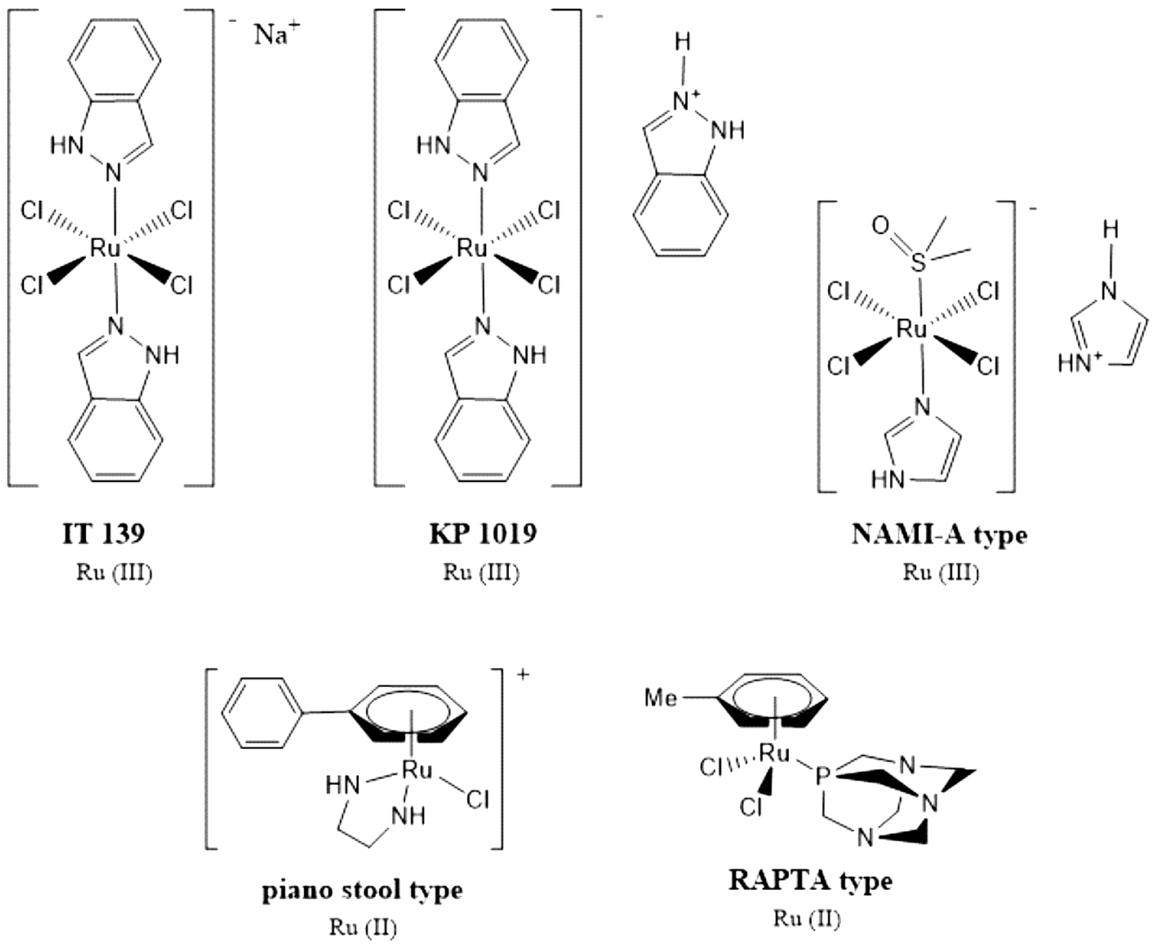


Fig. 9: Structures of ruthenium-based complexes

This ruthenium(III) complex is lacking in activity against primary tumours; but exhibits potency against metastasis. NAMI-A's mode of action correlates with its anti-invasive and anti-angiogenic properties. Unfortunately, due to lack of activity against primary tumours and against cancer cell lines, the clinical trials were then stopped. [79]

The second compound that is currently in clinical trials, is KP1019 [80] (respectively its better soluble sodium salt IT-139 – formerly known as KP1339) [81], trans-[tetrachloridobis(1H-indazole)-ruthenate(III)]) developed by Keppler et al. (Fig. 9). This complex was assessed during phase 1 clinical trials on tolerability and maximum-tolerated dose, pharmacodynamics, pharmacokinetics and safety in patients with late stage carcinomas, and as it turned out it is active against primary tumours, as opposed to NAMI-A. Furthermore, its mode of action via the mitochondrial pathway causes apoptosis. KP1019/IT-139 was also tested for its activity against colorectal cancers. Generally, tumour progression and drug resistance are promoted by a stress-induced protein (GRP78), and interestingly it can be regulated by IT-139. The lack of neurotoxicity and of dose-limiting haematological toxicity, and the ease of use in combinations with other anti-cancer drugs, designates

it as a suitable candidate in the treatment of solid tumours, in spite of its average anti-neoplastic activity. [82]

Further studies researched other significant ruthenium-based complexes including organometallic compounds bearing biologically active and arene ligands, such as work by Dyson and Sadler, who for example, studied the RAPTA(**r**uthenium **a**rene **pta**)-type complexes [83] or the "piano-stool" type [84] (Fig. 9). Regarding their anti-cancer activity, "piano-stool" type complexes are potent against solid tumours - depending on the aryl group, while RAPTA type complexes (e.g. NAMI-A) are more active against metastases rather than primary tumours. [79]

1.3.3. Other metal-based anti-cancer drugs

Another metal of interest in the development of anti-cancer drugs is gallium, due to its analogy in size and charge to iron(III) and aluminium(III), as well for its coordination chemistry. This resemblance appears to be a justification for their inhibition of tumour growth. [79] In contrast to iron(III), gallium is considered to be redox inactive under physiological conditions and can compete with the former for the enzyme binding sites. For example, gallium can also bind to transferrin and thus interfering with the cellular transport of iron, or it can inactivate ribonucleotide reductase and therefore inhibiting the synthesis of DNA. [71][79]

A second generation gallium complexes were investigated to achieve a higher stability against hydrolysis. This includes gallium salts (e.g. nitrates), that presented antitumour activity. In order to accomplish this, different chelating ligands have been attached to the metal centre. One hallmark of this group that has entered clinical trials is the compound KP46 (tris(8-quinolinolato)gallium(III)) with quinolinolato ligands [85] (Fig. 10). KP 46 showed promising signs of anti-cancer activity against renal cancer in phase I clinical trials. [86][87][88][89]

Only one non-platinum metal complex has been approved for clinical administration in cancer therapy, against acute promyelocytic leukaemia (APL), and this is arsenic trioxide (Fig. 10). Interestingly, APL cell differentiation is promoted by low concentrations of arsenic trioxide, while a higher dosage leads to oxidative stress and then apoptosis is induced by DNA strand breaks. [67]

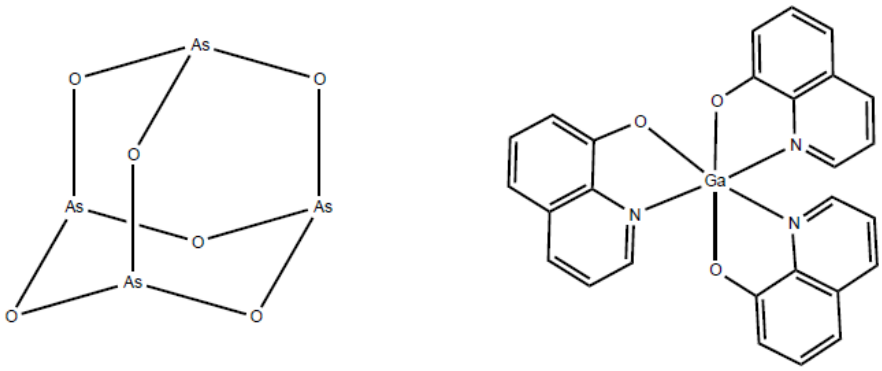


Fig. 10: Structures of arsenic trioxide and KP46 [67] [86]

Due to the analogous electrochemical behaviour of Ru and Os, recent studies focused on developing various structurally similar osmium anti-cancer compounds. Keppler and Cebrian-Losantos prepared several osmium-based NAMI-A derivatives, who exhibited *in vitro* antiproliferative potency and displayed a better kinetic stability in aqueous milieu and resistance to hydrolysis than NAMI-A. [90][91] Further in-depth research on other metals, which have been proven to show anti-neoplastic or cytotoxic activity, is still outstanding.

1.4. Metallocenes

Another type of complexes, metallocenes, also exhibited cytotoxic properties and could potentially be employed as anti-cancer drugs. They consist of a transition metal coordinated to two cyclopentadienyl (Cp) ligands in a "sandwich" structure that can be further classified in either "bent" or "classical" type (Fig. 11). These complexes can be summarized with the general formulas is Cp_2MX_2 (M = Ti, V, Nb, Mo; X = halides and pseudo-halides). [92][93] Bent metallocenes show a cis-dihalido motif and thus displaying a keen resemblance to cisplatin. This conformity encouraged the interest in metallocenes and further exploration of their biological activity. [93]

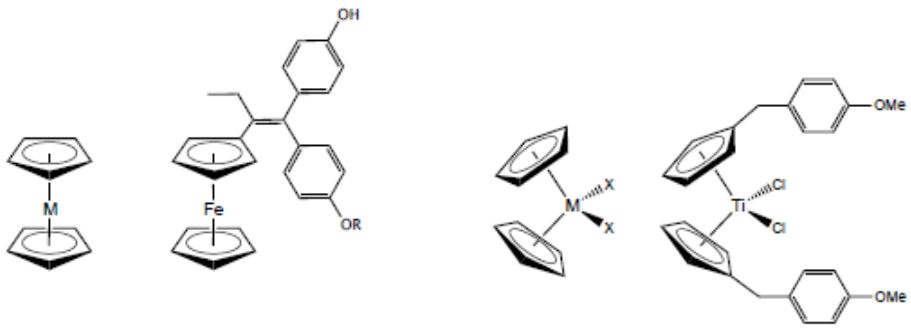


Fig. 11: Structures of the classic metallocenes, ferrocifen, bent metallocenes and titanocene Y (left to right)

The identification of anti-tumour properties of titanocene dichloride was initially published by Köpf and Köpf-Maier, who then pursued to research different biological active metallocenes. Surprisingly, other metallocenes, such as Cp_2Fe^+ and the main group $(C_5R_5)_2M$, where M = Sn, Ge and R = H, CH_3;

showed anti-neoplastic or anti-carcinogenic properties with less severe side effects than cisplatin. The variety of cancerous cells on which they were tested includes: Ehrlich ascites tumours, colon 38, carcinoma, B16 melanoma and Lewis lung carcinoma. The discovery that titanocene dichloride (Cp_2TiCl_2) exhibited anticancer activity against lung, colon and breast cancers set a milestone in this research field. [94] Consequently, it was adopted in clinical phase I trials in 1993. Nevertheless, severe toxicity affecting the liver, kidneys, gastrointestinal and neurological systems was observed in correlation with the administrated dosage [92][95], which lead to its abdication in clinical phase II trials due to its low efficacy in comparison to it high toxicity.

1.4.1. Iron

Generally, the study of metallocenes started in 1952 with the discovery of ferrocene, which is classified as a "classical" metallocene. Ferrocenium tetrafluoroborate, a ferrocenium (Fc^+) salt, was the first Fe-containing complex to display anticancer activity. [87][96] Furthermore, a variety of the ferrocenium salt derivatives were developed, e.g. ferrocifens (tamoxifen derivatives) or decamethylferrocenium tetrafluoroborate ($DEMFc^+Fc$), which were proven to be potent against breast cancer. [86][97] One of the advantages of ferrocene is its minor toxicity, as a result of its degradation in the liver, and thus not leading to major health problems. A second advantage is the administration mode: via injection, inhalation or it can be taken up orally. While the toxicity tests of ferrocene in beagle dogs showed no acute toxicity, a substantial iron excess was diagnosed. Fortunately, this could be reduced afterwards. [93] A stable ferrocenium ion (Fc^+) can be formed by a reversible one-electron oxidation. Other compounds, like hydroxyferrocene, are unstable in aqueous solution and decompose, thereby releasing solvated Fe atoms and thus showing anti-anaemic properties. Moreover, an organometallic derivative of chloroquine, called ferroquine, shows anti-malarial activity, opposed to the anti-anaemic properties of ferrocene. [98]

Anti-proliferative effects on certain cancer cell lines were first observed when simple ferrocenium salts were investigated. These could lead to the development of hydroxyl radicals, damaging DNA in a Fenton-type reaction. The cytotoxicity of ferrocenium salts was proved to be dependent on the water solubility and hence on the nature of the counter ion.

Jaouen et al. focused their work on the redox activity of ferrocene. Their proposed mechanism states that the anti-cancer activity in ferrocene derivatives is produced through redox activation. Several derivatives of the anticarcinogenic tamoxifen were reported by Jaouen's research. Tamoxifen is an organic drug administered in the endocrine therapy of hormone-dependent breast cancers due to its interaction with the oestrogen receptors (with about 70% cure success). [99] Furthermore, the redox

activity of ferrocene is responsible for its increased biological activity, in addition to its implied tamoxifen analogous mode of action, while for ferrocifen a dual mode of action was proposed. [79][93]

1.4.2. Titanium

To enhance the two major drawbacks, the deficient aqueous solubility and the hydrolytic stability of titanocene, and thereby influencing the cytotoxic activity, new studies focused on the substitution of the labile chlorido ligands and modifications of the bis-cyclopentadienyl moiety, leading to complexes with amino acids, benzyl-substituted titanocene derivatives, amide functionalised titanocenyl, alkenyl-substituted titanocenes, ansa-titanocene derivatives, compounds with alkylammonium substituents on the Cp rings and also steroid-functionalised titanocenes. [92]

Apart from titanocene dichloride, budotitan, [cis-diethoxybis(1-phenylbutane-1,3-dionato)Ti(IV)], was also investigated in clinical studies. Due to unsolved formulation problems, it failed in phase II clinical trials although both compounds showed promising anti-cancer activity against cisplatin-resistant tumours and less severe side effects. [100] The crucial property which determined researchers to set their mind on novel water-soluble titanium anti-cancer agents is the fact that these two complexes possess two leaving groups, which hydrolyse very fast in water. [101]

A second generation of titanocene compounds, which includes complexes with aromatic groups at the Cp-ligands, has been worked on in order to surpass the impediments of titanocene dichloride. One candidate of this group is titanocene Y (dichloridobis(η^5-(p-methoxybenzyl)-cyclopentadienyl) titanium), which exhibited favourable in vitro anti-cancer activity against lung, colon, renal and ovarian tumours (Fig. 12). [93][102][103][104] A more convenient pharmacokinetic profile was achieved by substitution of the chlorido ligands with carboxylate. [93]

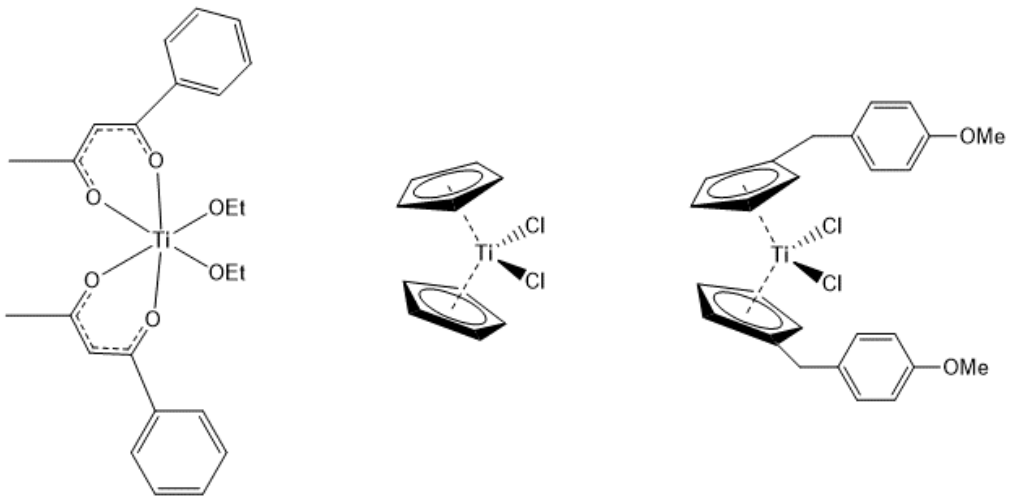


Fig. 12: budotitan, titanocene dichloride and titanocene Y [86]

The anti-cancer potency of titanocene dichloride was proven to be 100x lower than the cytotoxic activity of vanadocene dichloride, when it was tested on Ehrlich ascites tumours *in vivo*, although they initially displayed similar activities in this cell line. This discrepancy was associated with the hydrolytic instability of the compound.

Henceforth, researchers' interest was set to alternate the central metal ion of various metallocenes, such as molybdenocene, niobiocene, vanadocene, hafnocene and zirconocene derivatives, so that an improvement in cytotoxicity, solubility and hydrolysis could be achieved.

1.4.3. Molybdenum

Molybdenum is an essential trace element for the human body with 0,07 mg Mo/ kg body weight (~5mg in total), and about 2 µg/kg should be absorbed daily. The intake occurs passively over the small intestine, but unfortunately its adequate adsorption is inhibited by copper. Depending on the supplying dietary source, the resorption rate varies from 35-90%. Several aliments, such as milk, rye, eggs, pork and beef liver, beef kidney, sea fish and soybean, are of great importance for human consumption, as they provide the crucial dietary sources of molybdenum. It is mainly accumulated in the kidneys and liver, but nevertheless it is dispersed throughout the whole body. An insufficiency in molybdenum induces less tolerance of amino acids, an enhanced excretion of xanthine and diminished excretion of uric acid. It is assumed, that a surplus of molybdenum leads to an increased xanthine-oxidase reactivity. [105]

Further research by Köpf and Köpf-Maier explored the anti-cancer activity of several molybdenum-based compounds. Their work revealed the anti-neoplastic potency of molybdenocene dichloride (Cp_2MoCl_2) against diverse tumours, parading less severe side effects than cisplatin. [106] Furthermore, this compound exhibited the best aqueous stability at physiological pH. In contrast to titanocene dichloride (Cp_2TiCl_2), its molybdenum analogue Cp_2MoCl_2 never loses the two Cp-ligands but it can exchange the two chlorido ligands with aqua ligands. [107] Generally, the mode of action of molybdenocenes is not yet fully discerned, but it is presumed to interfere and thus, damage the DNA. [108][109]

Recently, novel approaches were made in favour of enhancement of the anti-cancer activity of molybdenocenes, such as exchanging the two chlorido ligands with other ligands or functionalizing the cyclopentadienyl rings. [107]

1.5. Molybdenocene Dichloride

As referenced before, pioneering work by Köpf and Köpf-Maier showed that molybdenocene dichloride was, amongst others, active on a various tumours, displaying reduced toxicity than cisplatin. [106] Studies showed that both chloride ligands of molybdenocene dichloride can be exchanged easily, while the Cp_2Mo^{2+} fragment remains undamaged. Moreover, the monomeric compound $Cp_2Mo(H_2O)(OH)^+$ forms an equilibrium with the dimeric complex $[Cp_2Mo(\mu\text{-}OH)_2MoCp_2]^{2+}$ at phyisiological pH, but nonetheless, although this complex exhibited higher hydrolytic stability at physiological pH, titanocene dichloride is however the most potent metallocene *in vitro* experiments. [107] To gain more insight on its mode of action, several studies have been conducted using biologically relevant proteins, oligonucleotides and DNA. [107][159-163]

Numerous experiments with DNA revealed that titanocene interacts with it in about 90–95%, while molybdenocene can be bound only up to about 5-10%. Moreover, if the exchange of chlorido-ligands via hydrolysis is inhibited by saline solution, no interaction with DNA can be observed. [107] In further experiments by Kuo and co-workers with DNA-processing enzymes, the investigated molybdenocene and vanadocene were able to inhibit the protein kinase C (PKC – which regulates cell proliferation), but displayed no effects on the polymerase, ligase or endonuclease activities, nor on DNA electrophoretic mobility. [110] His group also investigated the effect of molybdenocene with 5'-phosphorylated analogues and self-complementary oligonucleotides and reported the resulting substantial changes at the 5'-end but poor interaction with oligonucleotides. [110] Furthermore, research by Melendez's and co. detected only a weak coordination with N7 of purine bases and insignificant coordination with phosphoesters.[111] This negligible coordination is due to molybdenocene dichloride's promotion of the phosphoester bond cleavage, according to Kuo et al. [112]

Studies by Harding and her group proved that Cp_2MoCl_2 produces stable adducts with glutathione (GSH) with the disadvantage of critical deactivation of the anti-tumour activity. [113] Interestingly, experiments with nucleic acid components led to the formation of a stable species with cysteine – $Cp_2Mo(Cys)_2$. Experiments proved that the exchange of cysteine with other nucleic acids was not possible and opened doors to new research upon the synthesis of molybdenocene complexes bearing thiolate ligands. Although their cytotoxic activity showed that thiolate coordination inactivates the complex due to the inertness of the Mo-S bond, these compounds displayed impressive hydrolytic stability.

After the discovery of molybdenocene dichloride`s affinity to thiol-containing proteins, focus was laid on the investigation of other targets. [107] For example, in a study on the interactions of

molybdenocene with HSA, a binding ratio of 9.4 to 1 between Cp_2MoCl_2 and HSA was observed. [114] According to Melendez, these interactions are primarily hydrophobic. [115]

Chinese hamster lung cells were treated in Harding's group with molybdenocene dichloride and were then investigated by micronucleus assays and TEM (transmission electron microscopy). The observations taken from their experiments are with regard to the administered dosage; the higher the given concentration, the more chromosomes break or chromosomal loss occurs. Furthermore, in comparison to control cells, these tested cells showed significant prevalence of polynucleation with high preponderance of cells containing 3 to 5 nuclei, enhanced chromatin condensation, as well as increase of the cell diameter and damaged mitochondria in the cytoplasm. The summarised results from Harding's study suggest that the mode of action is complex and implies multiple targets. [116]

To boost molybdenocene's anti-cancer activity, two strategies to modify its structure similarly to titanocene, have been investigated: functionalising the Cp-rings and the exchange of the chloride ligands to achieve better solubility, stability and activity. [107] Studies showed that Cp_2MoCl_2 is inactivated by two thiol ligands; hence at least one coordination site has to be engaged by a labile donor molecule. The synthesis of molybdenocene complexes with S,N-chelating ligands, such as thio-nucleobases or thio-nucleosides, which exhibited increased anti-proliferative activity and high aqueous stability, have been published. [117] However, derivatives bearing O,O-chelating ligands demonstrated an even better improvement in stability and solubility, as a result of their irreversible redox behaviour under physiological conditions. [118]

Recent work by Tacke's group found that the *in vitro* cytotoxicity of molybdenocene can be improved by substituting the Cp-ring with a benzyl group. [119] Interestingly, by inclusion of the complexes in carrier molecules, where cyclodextrines act as a molecular host, the anti-cancer activity could be increased. The anti-proliferative activity is enhanced via the enclosed species due to improved solubility and better membrane permeability. [107]

1.6. Biologically active ligands

Some groups have worked on the derivatisation of metal complexes with bidentate biologically active ligands that have anti-tumour properties on their own, and they were able to reveal several interesting advantages like increased solubility and cellular uptake of the bioactive ligands or improved stability towards ligand substitution, acquired redox-activity, different mode of action and synergistic effect of metal and ligand. [71][120] Using this strategy, numerous molecules including hydroxypyrones [121][122][123], flavonoids [124], hydroxypyridones [125] [126], picolinic acid [125], quinolones [127] [128] or indoloquinolines [129] [130] as ligands, have been exploited.

Pyrone Derivatives

Pyrones, such as 2-pyrone, 4-pyrone or 3-hydroxypyrones, are natural occurring products with an interesting biocompatibility and toxicity profile. Especially the 3-hydroxypyrones gained increased attention due to their affinity to bind to metal ions. They are found in plants as natural products, can be easily synthesised and some are also commercially available.

Malleable biological properties can be achieved by inducing modifications to 3-Hydroxy-4(1H)-pyrones and their analogues. These compounds can be used as building blocks for biologically active compounds, as well as chelating agents considering their high affinity to selected metal ions. The chelating ligand forms a five-membered coordination ring with a properly chosen metal ion – hard metals (e.g. Ti, V) being favoured by the O,O-chelating derivatives and softer metals (e.g. Ru, Rh, Pt, Os) by the thionated derivatives – that is thermodynamically stable at physiological pH.

One of the straightforward modifications of hydroxypyrones is thionation – in this thesis carried out with Lawesson's reagent. The novel formed ligands with S,O-moieties (Fig. 13) possess a higher affinity towards the soft metal centre (e.g. Ru, Rh, Pt) and the borderline metals (e.g. Fe, Mo, W). The derivatisation with O,O-chelating ligands leads to more thermodynamically stable metal complexes under physiological pH, and this stability can, all the more, be enhanced by substituting these ligands with S,O-chelates. [131]

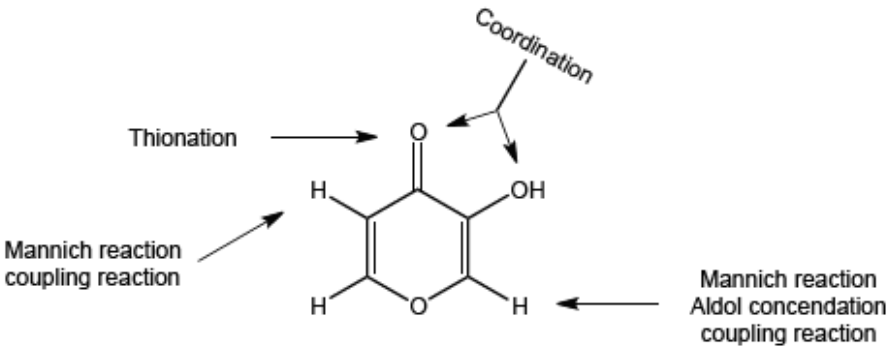


Fig. 13: Possible coordination sites of pyrones [131]

One of the most investigated 3-hydroxypyrones is maltol (3-hydroxy-2-methyl-4(1H)-pyrone). This compound is of great interest because of its poor toxicity and advantageous bioavailability. Maltol can be extracted from roasted malt, larch bark and pine, or synthesised and is used as food additive in cakes, bread or beer, in order to attain the aroma and malty taste. [131]

The properties of pyrone-based complexes, such as the delivery and release of metals, are adjustable by the substitution motif of the backbone. Recent studies focused on developing innovative anti-cancer complexes containing pyrone derivatives. [126][131][132][133][134] Research showed that it is possible

to improve the aqueous solubility of cisplatin by replacing the two chlorido ligands with maltol. Nonetheless, the cytotoxicity of the platinum-based compound remains unchanged. [135] Another advantage of the pyrones' property to release and deliver metal ions is the beneficial use in the treatment of iron deficiency [123], and the increased (in comparison to the inorganic sodium and ammonium vanadate salts) uptake of vanadium out of the compound [Bis(maltolato)oxovanadium(IV)] (BMOV). It has been shown, that vanadium-based complexes possess insulin-enhancing abilities, which can be employed in type-2 diabetes treatment. [136] Known gallium compounds account with poor bio-availability and high toxicity. However, the later synthesised Ga(III)-maltolate complex enhanced the bioavailability and stability of gallium against hydrolysis. This drug can be administered orally, has an improved lipophilicity and thereby enhanced bioavailability, but unfortunately its molecular target remains undiscovered and so the clinical phase trials were discontinued. [123] [137] Moreover, studies showed that thiopyrones may suppress matrix metalloproteins, as a result of the restriction of xanthine oxidase through the coordination of $(MoO_2)^{2+}$ and their property to chelate Zn ions. [138]

Another metal-based complex with anti-cancer properties is RuII(arene) complexes with maltol and has been investigated thoroughly by Sadler and co-workers. The maltolato ligand of the RuII(arene)(maltolato) complex can be substituted in aqueous solution and displays minor DNA damaging effects. [139] Furthermore, enhanced lipophilicity and thereby increased cellular uptake and cytotoxicity were observed after derivatisation. Also, improved lipophilicity, and thus facilitating intracellular accumulation as well as better stability by stronger binding, was proven by the substitution of carbonyl oxygen by sulphur. Therefore, these complexes showed striking anti-carcinogenic potency. [123][131]

1.7. Dihalobis(ß-diketonate) complexes – Introduction to KP129

In the 70s and 80s, dihalobis(ß-diketonate) complexes with the formula $M(acac)_2X_2$ where M = Ti, Zr or Hf and X=Cl, have been studied intensively for anticancer activity (Fig. 14). [140] Several studies have shown the anticancer effect of dihalobis(ß-diketonate) Ti complexes, such as for example diethoxybis-(1-phenyl-1,3-butanedionato)titanium (IV), $(Ti(bzac)_2(OEt)_2)$ on colorectal cancer. [141][142] Colorectal cancer is amongst the most frequent types of cancer with increasing incidence and mortality rates both in men and women and few potent agents suitable for treatment. Furthermore, colorectal adenocarcinomas exhibit a chemoresistance thus making chemotherapy unsuccessful. [143] The most significant side effect of these Ti-complexes is the liver toxicity. [144] In 1971, Doyle [149] was the first to report the synthesis of dihalobis(ß-diketonate) complexes of Mo(IV). Larson and Moore's research [145] showed that $MoCl_4$ can react with acetylacetone creating a red-purple complex of Mo in

the 4+ oxidation state. Nonetheless, several complexes of Mo or W in both higher and lower oxidation states are well studied, e.g. acetylacetonate complexes of oxymolybdenum species in the 5+ oxidation state have been well described. Furthermore, they noticed that prolonged heating of $MoCl_4$ with various ß-diketones give analogous products with those gained from $MoCl_5$ and diketones, thus the ß-diketone must act as a reducing agent for Mo(V). They also showed that even relatively inert chemicals (e.g. benzene) can reduce Mo(V) to Mo(IV), hence this ability of diketones is not unexpected. [145]

Keppler et al focused on developing new anticancer agents against colorectal tumors using analogous metals, guided by the potency of previously studied Ti-compounds, such as dichloro- and diethoxybis-(1-phenylbutane-1,3-dionato)Ti(IV) in the treatment of experimental tumors induced in rats. One of the complexes successfully synthesised by Keppler's group was isostructural dichlorobis-(1-phenylbutane-1,3-dionato)Mo(IV), ($Mo(bzac)_2Cl_2$), also named KP129. [142][143]

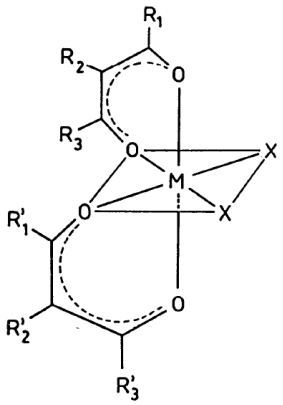
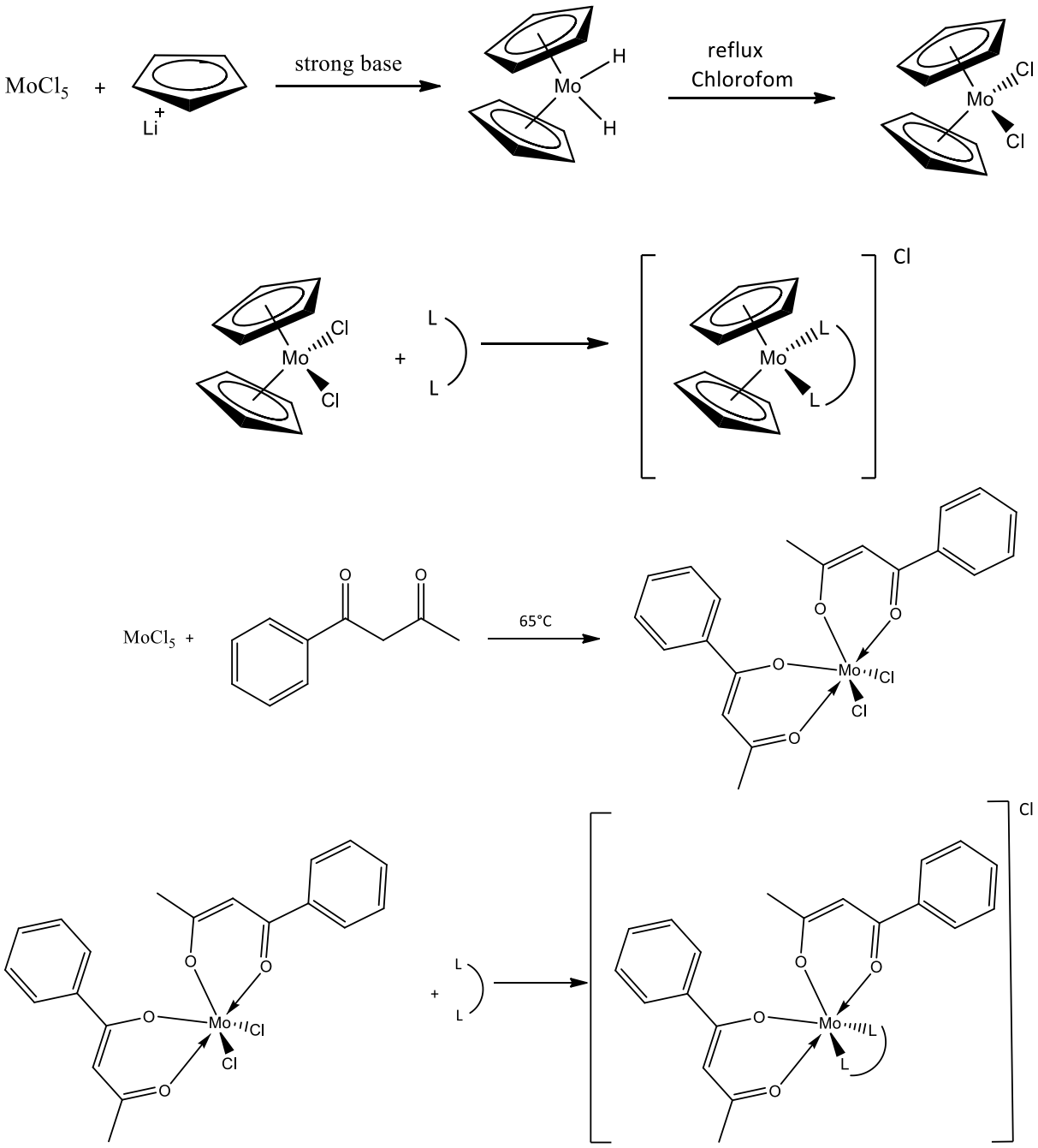


Fig. 14: The general chemical structure of M(diket)2X2 where M = molybdenum and X = Cl [143]

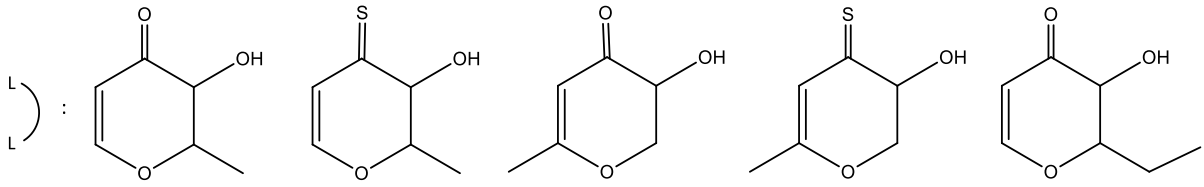
A paradoxical effect of $Mo(bzac)_2Cl_2$ (KP129) on the growth of induced colorectal adenocarcinomas was observed when the complex's anticancer activity was evaluated in treated SD rats. An increase of tumors' volume was observed after treatment with fresh solution of $Mo(bzac)_2Cl_2$ diluted in 0.9% saline and a diminishment when using higher doses. These results are perplexing since treatment with akin dosages of the analogous Ti complexes (e.g. $Ti(bzac)_2OEt)_2$) displayed compelling anticancer activity (inhibitory effect) in the same colorectal cancer model. This controversial results (high vs. low dosage effect) of KP129 are fascinating and the mode of action of these compounds still need to be assessed. [142][143]

2. Project Aim

The aim of this master thesis is to develop novel molybdenum based anti-cancer drugs with the general formula Cp_2MoL_2 or $(bzac)_2MoL_2$, where L_2 are bioactive O,O- and O,S-ligands, coordinated to the metal core (Mo) (Scheme 1a and 1b). The purpose is to increase the hydrolytic stability by replacing the labile chlorido ligands of molybdenocene dichloride or KP129 by different bioactive bidentate ligands such as maltol, ethylmaltol, allomaltol and their thionated analogues.



Scheme 1a: Synthesis of the Mo-complexes and substitution of the two chlorido ligands



Scheme 1b: Substitution of the two chlorido ligands with L, where L is maltol, thiomaltol, allomaltol, thioallomaltol and ethylmaltol (from left to right)

To confirm the formation and purity of the synthesised structures, standard analytical methods, such as ^{1}H-NMR, elemental analysis (EA), mass spectrometry (ESI-MS) and X-Ray diffraction techniques, will be used to characterise the newly obtained complexes. Furthermore, UV-VIS spectrometry was performed over a defined period of time to investigate their aqueous stability.

3. Results and Discussion

All reagents and solvents were dried either by reflux under argon or by the freeze - pump – thaw method. The light and air sensitive reagents were weighed in a glove-box and the reaction set up was protected from sun light.

3.1. Ligand synthesis

The aim of my master thesis was to synthesise, on the one hand Cp-analogues but also bidentate chelating ligand scaffolds with O,O- and O,S-coordination motives, in order to study their influence on the resulting molybdenocene-type complexes. Commercially available kojic acid and maltol were used as the starting material for the synthesis of the other O,O- (allomaltol) and O,S-chelating ligands (thiomaltol and thioallomaltol). The ligands were characterised by ^{1}H-NMR spectroscopy.

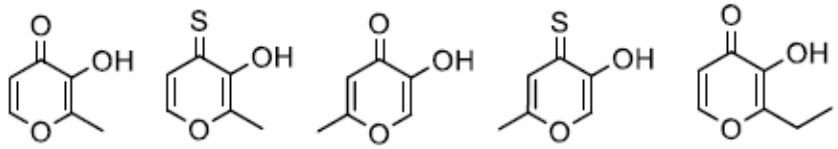
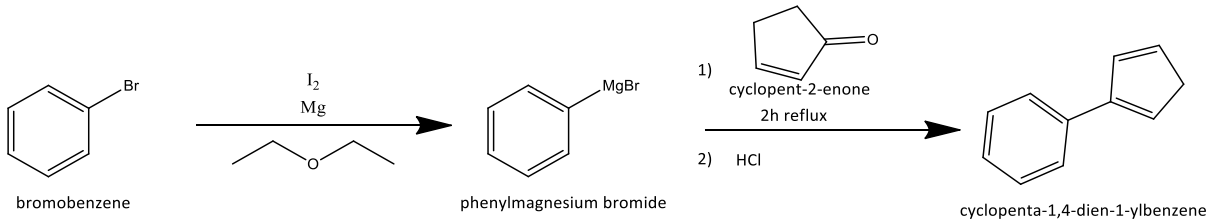


Fig. 15: pyrone ligands used for complexation in this master thesis

3.1.1. Grignard Reaction

Relaying on work by Gleeson and Tacke, who successfully synthesised benzyl-substituted molybdenocene [119], as well as on experiments by Philips and Oku, who prepared substituted cyclopentadienyl-compounds via Diels-Alder reactions [146], and finally on studies by Paradies and co-workers, who synthesised titanocene-analogues with substituted Cp-rings [147], a first approach in this thesis was to prepare a Cp-derivative.

The respective Cp derivatives can be obtained either by a Diels-Alder or Grignard reaction, followed by a deprotonation with BuLi. In this thesis, focus was laid on the classical Grignard Reaction, using an aryl-MgX as the nucleophile and a carbonyl as the attacked electrophile group. The final step is the hydrolysis and elimination with HCl.



Scheme 2: classical Grignard reaction showing the reagents used in this thesis

Several trials have been carried out under similar conditions with minor changes to improve yield or purity of the formed compound. All reactions have been carried out under inert atmosphere and in dry solvents. All reactions have been set up similarly as follows: Mg flakes and a couple of I_2 crystals were covered with dried diethyl ether. The reaction was started by heating with a heat gun. Bromobenzene was mixed with dried diethylether and added dropwise to the stirring reaction mixture. At the point where the reaction started, a colour change to a red-dark brown was observed. The mixture was refluxed for about 45 min. At the end of the reaction, not all Mg was consumed but we proceeded to the next step nonetheless. For the next step, cyclopentenone diluted in dried diethylether was slowly added dropwise to the reaction flask. The solution instantly changed colour from dark brown to green. After this addition, the reaction was allowed to reflux for another 2h. For the hydrolysis and elimination step, ice-cold half concentrated HCl was carefully added. The solution changed colour to bright yellow and formed an orange precipitate. Enough HCl was added in order to dissolve all the remaining Mg flakes, as well as the produced orange precipitate.

The ether phase was separated and enclosed traces of HCl were neutralised with $NaHSO_3$ (colour change to orange), then with K_2CO_3 (colour change to yellow) and finally washed with distilled H_2O. When drying the org. phase over $MgSO_4$ a reaction was observed, possibly a reation with the formed HCO_3^- and the escaping of the formed gases CO_2 and SO_2. Nonetheless, the solution was then concentrated *in vacuo* to give a brown oil (yield 62%).

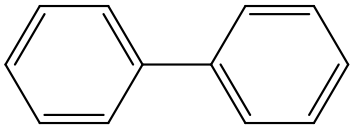
In order to purify this obtained oil, an adequate solvent mixture for the column chromatography was needed. Since the product should be non-polar, and the silica gel is polar, a rather non-polar solvent mixture was needed. Various trials on thin-layer chromatography plates showed that 100% hexane seemed suitable for eluting the product. The obtained reaction product was concentrated under vacuum to give white crystals and an oil. Following this observation, a sublimation of the reaction mixture was undertaken in order to obtain the pure product as crystals. Unfortunately, the gathered crystals weighed only 0.9% of the theoretical yield. ^{1}H-NMR in $CDCl_3$ showed the formation of biphenyl-derivate and traces of the desired compound (Fig. 16). The remaining oil was washed with diethylether and concentrated again to be analysed by NMR as well (Fig. 17).

In another trial, 1/20 of the bromobenzene/DEE mixture was added without stirring right after the Mg – I_2 reaction was started. A colour change to milky white proved the reaction was on-going. After 1 hour of refluxing, the whole amount of Mg flakes was dissolved. The following steps were performed exactly as previously described. For the neutralization step, K_2CO_3 was exchanged with $NaHCO_3$, since it was assumed that K_2CO_3 was too alkaline for this reaction. The organic phase was orange and the aqueous phase was yellow. After separation, the organic phase was dried over

Na_2SO_4 and concentrated in *vacuo* to give a green powder. This powder was then washed with water and changed colour from green to brown. The crude product was then washed with hexane to give white crystals in poor yield. These were then analysed by NMR but as in the case of the previous reaction, only the secondary product, biphenyl, was detected.

For a last trial, the amount of diethylether was significantly increased, since previous trials showed that more diethylether improves the reaction. During the addition of cyclopentenone, the reaction colour changed from white to yellow after quenching with HCl. After final elimination and neutralisation steps, the organic phase was dark orange and the aqueous phase green-yellow. After removing the solvent from the organic phase, a brown oil with white precipitate was obtained. A recrystallisation step out of toluene was conducted and the white powder was analysed by NMR spectroscopy but did not display the formation of the product.

Unfortunately, none of the experiments were successful; ^{1}H-NMR analysis showed the formation of the undesired side product 1,1‘-biphenyl accompanied by traces of the desired product. The latter couldn’t be isolated in sufficient purity.



1,1'-biphenyl

Fig. 16: Side product formed in the attempted Grignard reactions

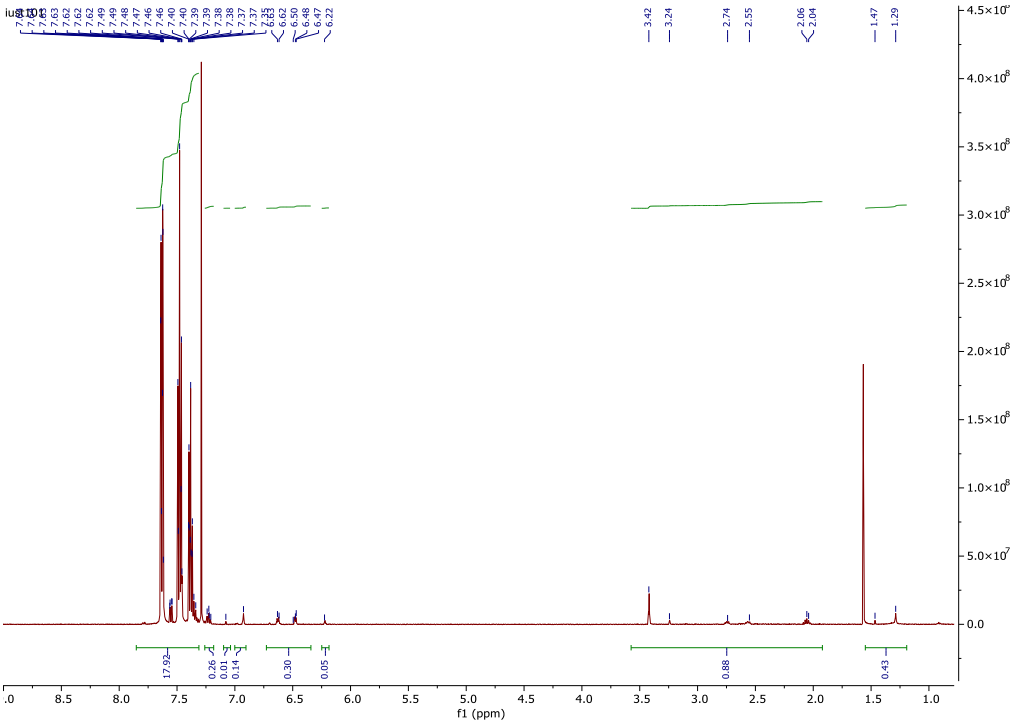


Fig. 17a: Traces of the desired reaction product but mainly secondary product (1,1’-biphenyl)

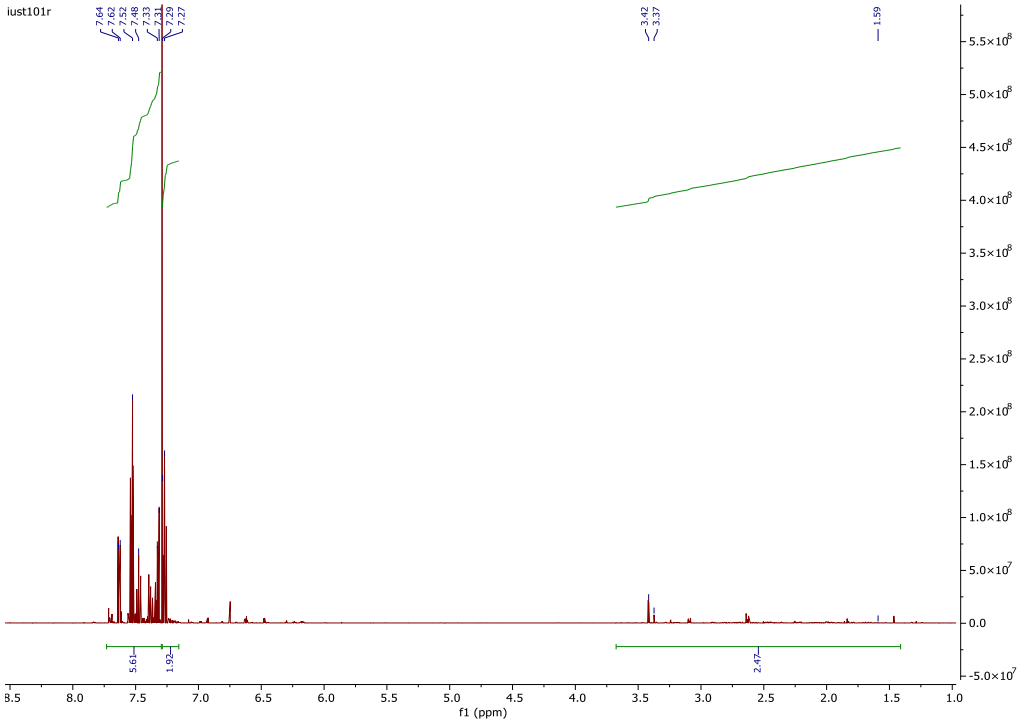


Fig. 17b: Traces of the desired reaction product but mainly secondary product (1,1'-biphenyl)

Since considerable trials in obtaining the desired compound by Grignard reaction did not succeed, further planned experiments to obtain (2-thionylmethyl)cyclopentadiene and (2-phenylethyl)-cyclopentadiene were discarded, and another strategy was approached.

3.1.2. O,O- chelating ligands

Allomaltol was obtained according to literature over two steps. The first step is a chlorination of kojic acid. The reaction set up needs to include a gas deviating glass duct and be cooled in an ice bath, since the conversion occurs with a strong exothermic reaction. The second step is a dehalogenation with Zn yielding the desired O,O-chelating agent allomaltol.

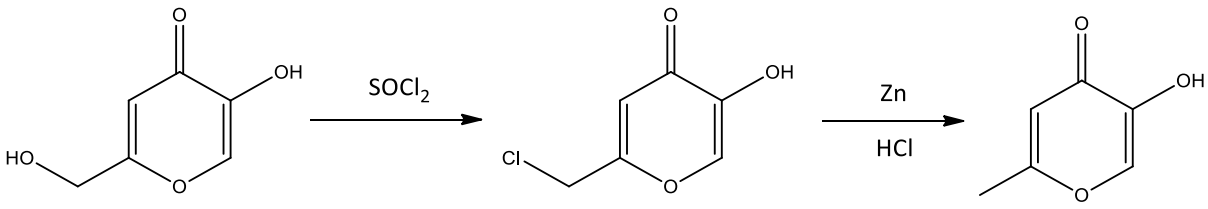


Fig. 18: synthesis of allomaltol starting from kojic acid

The product was purified by recrystallization from isopropanol and was isolated in 33% yield. ^{1}H NMR and mass spectroscopy confirmed the formation of the desired product.

3.1.3. O,S-chelating ligands

Both O,S-chelating ligands, thiomaltol and thioallomaltol, were obtained by thionation with Lawesson's reagent (2,4-bis(4-methoxy-phenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide, Fig. 20) under inert conditions and using dried THF, according to literature procedure (Fig. 19).[138]

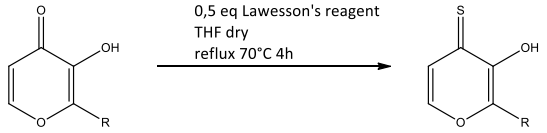


Fig. 19: General synthesis of thio-4-pyrone by thionation of a commercially available 4-pyrone

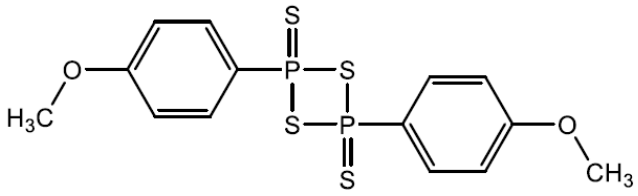


Fig. 20: Lawesson's reagent

The ligands were purified via column chromatography using hexane/ethyl acetate (10:1) as eluent and characterised via ^{1}H-NMR and mass spectroscopy.

3.2. Monomerisation and Deprotonation of the Cp-ring

Commercially available dimeric cyclopentadiene can be easily monomerised by distillation at 170°C under argon atmosphere.

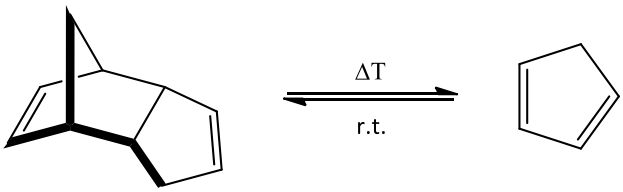


Fig.21: Monomerization/Dimerization of cyclopentadiene

Deprotonation of the cyclopentadiene was realised by a strong base such as superhydride ($LiEt_3BH$) or n-buthyllithium (BuLi) in THF and -78°C, as shown by Al-Dulayymi and Gleeson/Tackes' groups. [119][148]

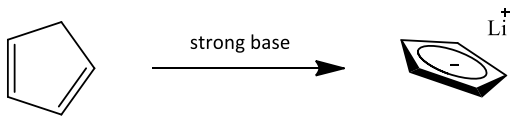


Fig. 22: Trivial representation of the deprotonation of cyclopentadiene

The first experiment was performed following the literature procedure [119] using Superhydride:

	Amount	Mr [g/mol]	n [mmol]	eq
Cp monomer	1.1 mL	66.05	13	1
(1M, THF) Superhydride	15 mL	105.94	15	1.15

For this reaction the monomerised Cp was mixed with dry diethylether and the superhydride was added dropwise via syringe. The reaction was stirred over-night at room temperature. The white precipitate was filtered, dried and isolated in very good yield.

It has been observed that the product turns brown after a couple of days, possibly due to remaining traces of Superhydride that are very sensitive to air or to rehydrolisation of the deprotonated Cp. Hence, to avoid degradation of the product, further experiments have been carried out using n-BuLi instead of Superhydride. It has been observed that the reaction time using Bu-Li is shorter than the reaction time with Superhydride:

	Amount	Mr [g/mol]	n [mmol]	eq
Cp monomer	0.825 mL	66.05	10	1
(2,5M hexane) n-BuLi	4 mL	64.05	10	1

A first trial proved that using a strong base in excess is not beneficial, since it makes the isolation of the product more difficult. Slow addition of a solution containing monomerised Cp in 20 mL dry DEE under inert conditions to a solution of BuLi in 60 ml dry DEE via syringe proved to be the best way to obtain a pure and stable product. The reaction mixture was stirred over-night at room temperature yielding a white powder. The reaction was quenched with NH_4Cl (colour changed to yellow). The product was extracted in good yield and analysed by ^{1}H-NMR spectroscopy.

The deprotonated Cp is a white powder, stable under argon in the freezer until it shows first signs of degradation, like fluid appearance or darkening in colour.

3.3. Synthesis of the Mo-Complexes

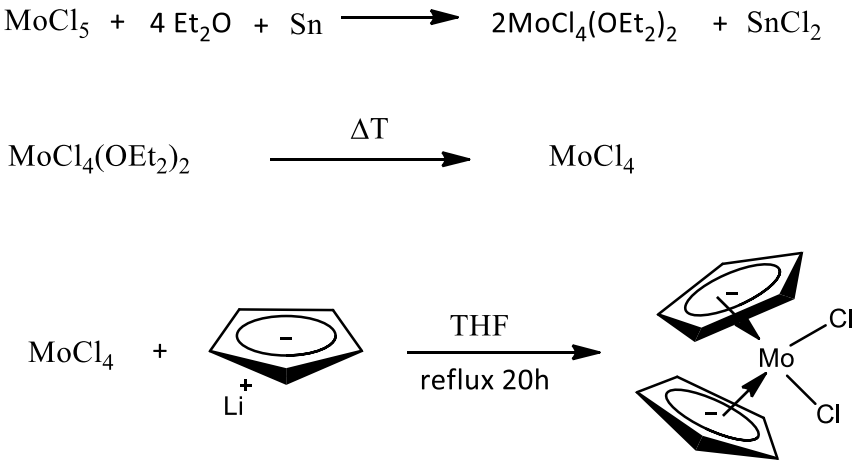
3.3.1. Synthesis of Molybdenocene

Different strategies were approached to synthesise molybdenocene. One strategy consisted of mixing commercially available $MoCl_4$ with the synthesised deprotonated Cp, following the procedure by Gleeson and Tacke [119]. The dissolved solution of deprotonated Cp in dry THF was transferred via syringe to a $MoCl_4$ solution in THF and the reaction mixture refluxed at 70°C for 20h.

	Amount	Mr [g/mol]	n [mmol]	eq
Cp^-Li^+	137 mg	72.03	1.6	1.9
$MoCl_4$	200 mg	237.78	0.84	1

THF was removed, and the residue was washed with hexane to remove all Cp-rests. The obtained compound was dissolved in DCM and filtered in order to remove LiCl salt, and the solvent removed to yield a dark violet powder. This compound is not soluble in $CHCl_3$, acetone, ethanol, and methanol; but partially soluble in DMSO and DCM, and soluble in water. ^{1}H-NMR analysis showed no formation of the desired compound but only traces of Cp in solvent. This reaction was repeated several times, varying the amounts of the starting material (200 mg to 1,5 g $MoCl_4$) and of the deprotonated cyclopentadienyl Cp^-Li^+ (2 eq to 3 eq).

A second approach relied on the procedure by Stoffelbach [149] where $[MoCl_4[Et_2O]_2]$ was synthesised from 1 eq $MoCl_5$ and 2 eq Sn. The following reaction to synthesise molybdenocene did not succeed due to moisture in the reaction set up (scheme 3).

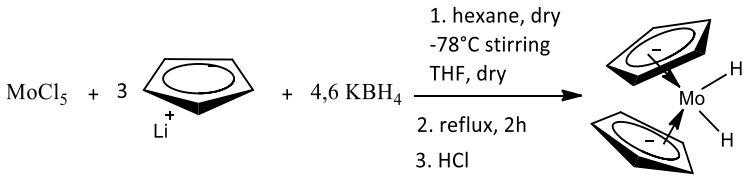


Scheme 3: Synthesis of molybdenocene following Stoffelbach procedure

This reaction was repeated twice, varying the amount of starting material $MoCl_5$ (200 mg to 1.3 g) but unfortunately, this method requires very strict anaerobic and light protected set up, which lead to a

very difficult isolation of the pure product in the lab. Furthermore, the obtained product was not soluble in any solvent, hence ^{1}H-NMR-analysis showed no peaks corresponding to the desired complex. To facilitate the purification and isolation of molybdenocene, a new strategy was attempted.

A third approach involved a one-pot reaction [150], where 1 eq $MoCl_5$, 3.15 eq $Cp^{-}Li^{+}$ and 4.67 eq KBH_4 were dissolved in dry hexane. [151][152] The reaction mixture was cooled in an acetone/N_2 and dry THF was added dropwise under stirring. The reaction colour changed to dark red. Next, the reaction mixture was refluxed for 2h, and finally the solvent was removed *in vacuo*. HCl was then added dropwise until all solids were dissolved. After neutralisation with NaOH, the black powder was extracted with benzene and methanol.



Scheme 4: Reaction to Cp_2MoH_2

The product was analysed by MS and ^{1}H-NMR, but no peak assignable to hydride was detected. This reaction was carried out five times, varying the amount of starting material (250 mg or 300mg), (3 eq to 4 eq) $Cp^{-}Li^{+}$, (4 eq to 6 eq) KBH_4, and the solvent, using toluene or benzene instead of hexane, and adding $CHCl_3$ as a chloride source for final substitution to molybdenocene dichloride.

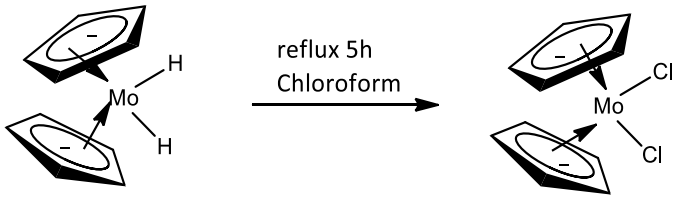


Fig. 23: Molybdenocene

The final reaction step was performed *in situ*; hoping that some intermediate product Cp_2MoH_2 has formed, it was proceeded with the addition of $CHCl_3$ to the crude and refluxed for about 5h, whereby the solution turned green. After work-up, green and white precipitates are formed. [153] The crude product was suspended/dissolved in methanol and submitted for MS analysis. This showed the potential formation of the molybdenocene, according to the MS found values of m/z 339.24 $(M + K)^{+}$ and 323.22 $(M + Na)^{+}$, but the formation of the dichloride in the spectrometer cannot be ruled out (Fig. 24). This step was repeated several times varying the reflux temperature and reaction time but

^{1}H-NMR spectroscopy didn't show any peaks in the expected regions, mainly because the obtained compounds are not soluble in any available solvent (Table 1).

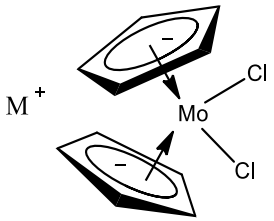


Fig. 24: potentially the desired molybdenocene, where M = Na, K

The obtained compound was tested for solubility and the results are summarised in the following table:

Solvent	Solublility Cp_2MoCl_2
diethyl ether	no
acetone	no
THF	no
DMF	no
methanol	no
ethanol	no
water	Yes – labile ligands*
hexane	no
dichloromethane	no
chloroform	no
toluene	partially
ethyl acetate	no
DMSO	little

Table 1: Molybdenocene solubility in various solvents

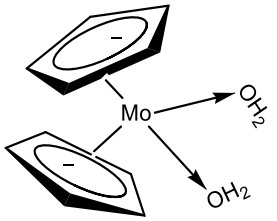


Fig. 25: * Austausch der labilen Liganden mit Aqua-Liganden

The work-up of the crude mixture was complex, since as well the intermediates as the desired compound are known to be light and air sensitive. The formation of salt hindered the purification to obtain a pure product. Characterisation of the crude mixture was difficult, which is expected for the intermediate oxidation state of molybdenum complexes. The behaviour in various solvents is depicted in **Fig. 26**:

Fig. 26: various coloured solutions or precipitates after solubilisation in organic solvents

3.3.2. Synthesis of KP129

The first general strategy, the synthesis of Cp-derivatives and on the other hand to establish molybdenocene following various literature procedures, did not succeed under the available conditions, in spite of performing numerous trials varying the amounts, solvents, reaction temperature and time. Hence, focus was shifted in a brand new direction, namely to synthesise KP129 [140][142][143][144], and finally creating Mo(IV)-complexes bearing the prepared biologically active ligands. The reaction of Mo(V) with ß-diketons develops according to the general equilibrium $MoCl_5$ + mH(diket) $\longleftrightarrow$ $MoCl_4(diket)_m$ + mHCl while a reduction of the metal occurs and was performed ten times for this thesis. This solid state complexation reaction has been carried out following a procedure by G. Doyle [140], where both solids – benzoylacetoacetone and $MoCl_5$, were mixed under an inert argon atmosphere, and finally extracted with hot hexane. In the original procedure, the reaction mixture was heated up to 120°C. When carrying out this reaction at this temperature, the formation of a black insoluble powder has been observed, so further experiments have been conducted at various temperatures, e.g. heating at 85°C, 75°C, 70°C, 65°C and 60°C. The black side-product ceased to form when the temperature was decreased to 65°C. Moreover, thermo-gravimetrical analysis proved compound decomposition when the temperature was increased over 65°C. Finally, to obtain the desired product, the reaction slurry was extracted with hot hexane and heptane to give the red-purple KP129 in very high yield. In some cases, the product was washed additionally with chloroform with the hope to give an even cleaner product, but unfortunately this step only lead to compound decomposition, proved by the change in colour (from red purple to dark green or even blue). The maximum isolated yield was 98%. Furthermore, its solubility in various solvents was tested and summarised in the following table:

Solvent	Solublility KP129
diethyl ether	no
acetone	warm yes, cold no
THF	no
DMF	yes
methanol	no
ethanol	no
water	no
hexane	no
dichloromethane	moderately
chloroform	moderately
toluene	no
ethyl acetate	moderately
DMSO	yes

Table 2: KP129 solubility in various solvents

The following observations have been noted: Mo (V) reacts vigorously with benzoylacetone; when the compound is dissolved/ suspended in chloroform or dichloromethane it turns green after about one hour. When suspended in methanol or diethyl ether, a dark precipitate formed and the green solution turned orange after 2h. When dissolved/suspended in toluene, a dark precipitate formed and the yellow-orange liquid phase turned green. After several experiments were conducted, it was concluded that, if the dark-red product was isolated in high purity, it could be recrystallised out of acetone in the freezer, but if traces of the benzoylacetone were still present, the acetone phase would turn yellow green.

Solubility tests were tried out in PVP and water. KP129 was mixed with PVP to give a stock solution of different concentrations, ranging from 1.65 mg/ml to 13.33 mg/ml. The solvents used were DCM, solutol, DMF and DMSO. Since the complex isn't soluble in these solvents, destilled H_2O was added to help dissolve the complex. Out of the several experiments, only the following two, depicted in the table below, proved to be soluble:

Stock solution					**Solubility experiments**			
KP129 [mg]	PVP [mg]	solvent [ml]	solvent	H_2O [ml]	ß[mg/ml]	µl stock	µl solvent	µl H_2O
6.1	6.1	0.101	DMF	1	0.01 (2.2 µM)	0.4	9.6 DMF	990
8.5	8.5	0.248	DMSO	1	0.01	0.3	9.7 DMSO	990

Table 3: Solubility of KP129 in saline solution

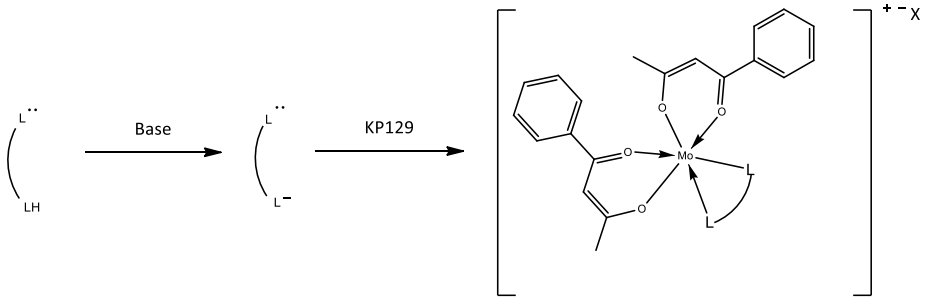
These experiments were extended and conducted also with following amounts: 3mg KP129 + 30mg PVP, 2ml DMSO/ 3ml H_2O/ 2ml DMF but didn't display any solubility. Furthermore, the complex solubility was also tested in 0.9% saline solution. 10 mg KP129 were mixed with 1-4 ml saline solution, as well as saturated saline and 9% saline solution, but didn't display solvation.

It is already well researched [140] that this type of complexes are insoluble in moderately polar organic solvents, although the reason therefore is not yet clarified. Doyle [140] noticed an improvement in the solubility when the size and number of organic substituents on the diketonate ligands was increased.

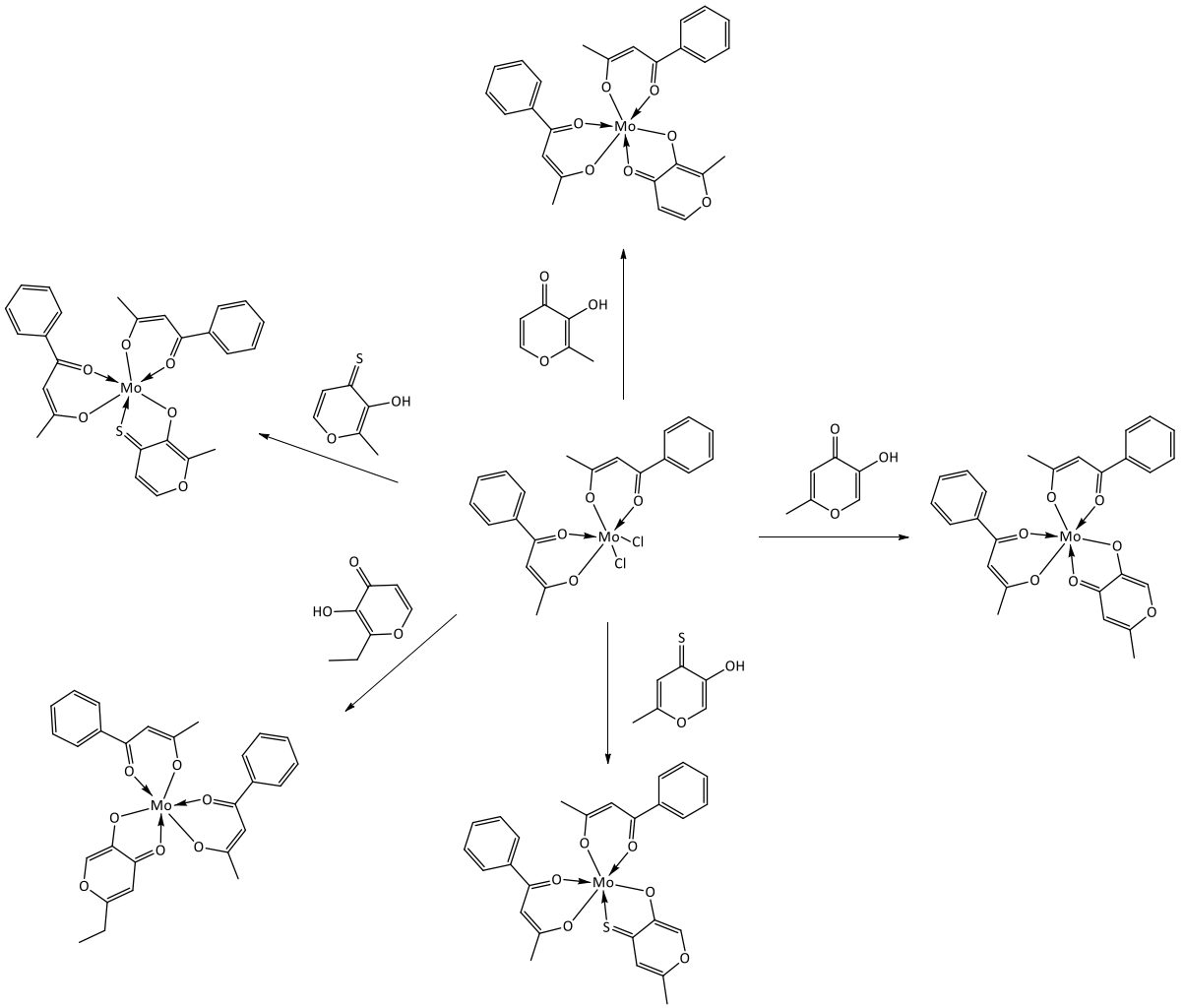
3.3.3. Synthesis of the KP129-type complexes

The general procedure for the synthesis of the four Mo(IV) complexes (Scheme 5 and 6), consists in the deprotonation of the O,O- (maltol, allomaltol) and O,S-chelating ligands (thiomaltol, thioallomaltol) with a strong base (sodium methoxide or triethylamine) in different dry solvents, such as chloroform, DCM and methanol. KP129 was then added and the complexation reaction was allowed to stir. The reaction of KP129 with maltol was performed 40 times, 8 times with thiomaltol,

twice with allomaltol and thioallomaltol respectively and once with ethylmaltol within the scope of this thesis. First, the reaction times and temperature were varied, followed by exchanging the solvents and base (NaOMe or Et_3N). The complexation of the O,O- and O,S- chelating ligands with KP129 should yield positively charged complexes with the respective counter ion X (X= Cl or PF_6 when using the precipitating salt NH_4PF_6) (Scheme 5).



Scheme 5: simplified scheme depicting the synthesis of the Mo(IV) complex, where L ist maltol, allomaltol, ethylmaltol, thiomaltol and thioallomaltol



Scheme 6: Reaction paths using KP129 with the different pyrone ligands

The aim was to synthesise four different complexes of the derivatised KP129 with maltol, allomaltol, thiomaltol and thioallomaltol. The synthesised complexes were characterised by ^{1}H-NMR-spectroscopy, elemental analysis and ESI-MS The stability of the maltolato-complex was examined using NMR kinetic and UV/Vis spectroscopy, while X-ray diffraction analysis was performed in an attempt to determine the crystal structure of this compound.

Initially the experiments were carried out yielding Mo(IV)-complexes with chloride as a counter ion but purification of the desired products out of the obtained crude mixture was not successful. The reactions using the O,S-chelating ligands thiomaltol and thioallomaltol did not produce any precipitate. The experiments were conducted using different bases, solvents and reaction conditions but eventually ^{1}H-NMR and MS analysis did not show the formation of the product; moreover the viscous consistency and black colour proved the obtained product was metal and air sensitive. Although varying the reaction conditions and set up, the solubility of the obtained maltolato-complex was not affected and can be summed up in the following table:

solvent	soluble
ethyacetate	no
hexane	no
ethanol	no
methanol	yes, red solution
water	no
toluene	no
DCM	yes, red solution
chloroform	moderate: green solution +brown dust + white precipitate
acetone	yes, orange solution
DEE	no
THF	yellow solution + white precipitate
DMF	partially, brown
DMSO	yes, dark orange solution

Table 4: solubility of maltolato-KP129-complex

For most suspensions and solutions a change in colour was observed after 20h. The yellow or red mixtures degraded in contact with moisture, turning green or blue. ^{1}H-NMR spectroscopy of the maltolato-KP129-complex using DMSO-d_6 as solvent didn't show any degradation after 6 days in solution, as depicted below:

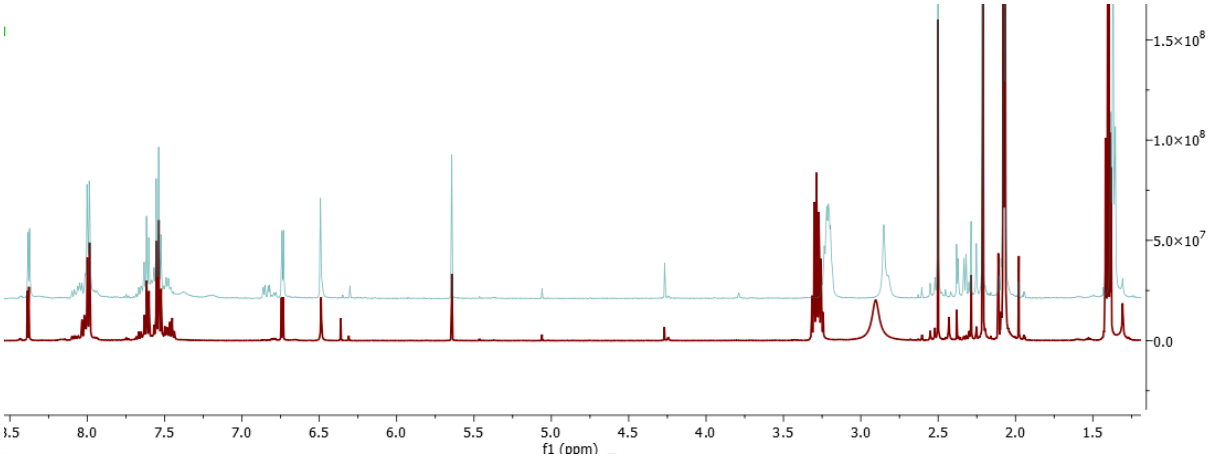


Fig. 27: blue: maltolato-KP129 and in red after 6 days

In an effort to purify the obtained compounds, crystallisation experiments of the maltolato-KP129-complex were attempted as summarised in the next table, the mixture methanol/DEE turned yellow while ethyl acetate/DEE turned red.

	Et_2O	PE
acetone	x	x
methanol	yellow	x
DCM	x	x
EtOAc	red	

Table 5: crystallisation experiments

Interestingly, one product of the synthesis of the maltolato-KP129 complex using 2.5 eq of Et_3N, displayed fluorescence when mixed with DCM.

The next attempt was to carry out a microwave-assisted synthesis, since all compounds possess limited solubility. Although working under a strict argon atmosphere, the desired compounds could not be obtained by this strategy.

Furthermore, the last trial was exchanging the counter ion Cl^- of the charged complexes with PF_6^- by addition of either sodium or ammonium hexafluorophosphate. Nonetheless, the obtained compounds are not better soluble than the previous ones and precipitate instantly in aqueous milieu. This precipitate is insoluble and wasn't analysed by any spectroscopy method.

The various reactions are detailed in the Chapter 4, Experimental Procedures.

3.4. Characterisation

3.4.1. NMR-Spectroscopy

In the ^{1}H-NMR spectrum of commercially available molybdenocene dichloride there is only one signal for both Cp-rings (^{1}H-NMR ($CDCl_3$): δ = 5.63 ppm). This occurs because both Cp-groups are symmetrically coordinated to molybdenum in a η^5-manner and the two dichloride ions are in an ancillary position (in the plane bisecting the Cp-Mo-Cp). This signal is observed shifted downfield in the ^{1}H-NMR spectra of the prepared compounds (δ = 6.48 ppm), concluding that the ligands must be coordinated symmetrically to molybdenum. The full assignment of the ^{1}H-NMR signals can be found in the experimental section of this master thesis (chapter 4).

3.4.2. ESI-MS

The structures of the obtained ligands and complexes where verified by ESI-MS, which is a soft ionization method with very little fragmentation occurring. The compounds are converted into ions in the gas phase. For the analysis, the compounds where first dissolved in 1% methanol/water or just methanol and then dispersed by electrospray into a fine aerosol.

Molybdenum has a characteristic isotope pattern, which was displayed in the spectrum.

KP129 was also analysed by high resolution time-of-flight MS (HR TOF-MS), a method in which the ions are accelerated by an electric field of known strength and their m/z value is determined by measuring the time of flight.

The experimental mass-to-charge ratios of the synthesised compounds are found in the experimental section of this thesis (chapter 4).

3.4.3. Elemental Analysis

The elemental composition of the submitted compounds was determined via the CHNS method by combustion analysis.

Elemental Analysis was performed on KP129 and the results are summarised in the table:

	theoretical	found	
w%C	49,10	50,00	49,33
w%H	3,71	3,91	3,82
w%O	13,08	14,30	14,02

3.4.4. X-ray diffraction

For one of the reaction compounds (Fig. 28), X-ray suitable crystals of the obtained product were grown. The crystals were grown using the slow diffusion method, where a volatile solvent slowly diffuses in a less volatile solvent. The reaction product was crystallised by slow diffusion method from ethylacetate / diethylether.

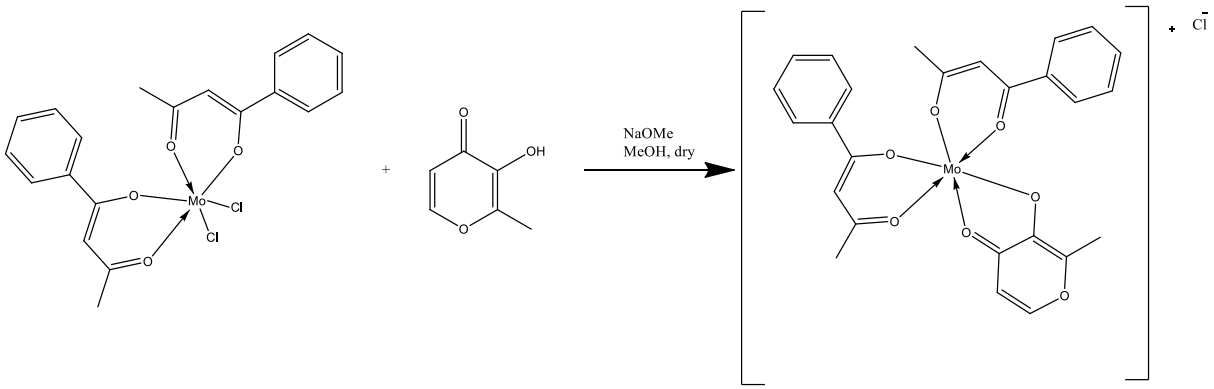
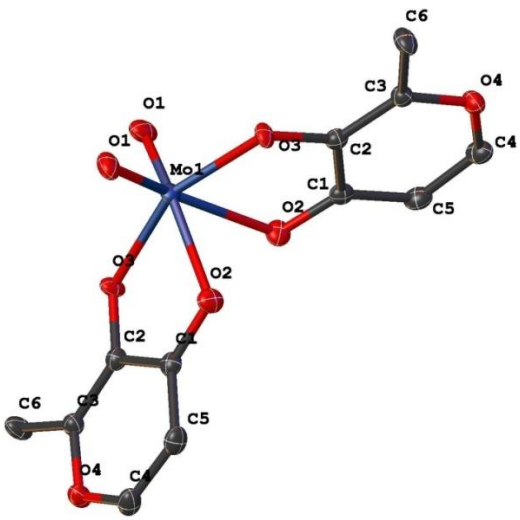


Fig. 28: KP129 + maltol

The obtained crystal structure is depicted below in Fig. 29. Unfortunately, as the crystallization experiments described in 3.3.3. Table 5 showed, the desired compound could not be crystallised and the obtained structure by X-Ray analysis proves that bzac is cleaved off and the molybdenum centre was oxidised. As to be interpreted from the Fig. 29, there are two maltol ligands and two oxo bonds to the Mo core. This dioxo-Mo(VI)- complex is an previously researched compound. [154] The obtained compound crystalised in the monoclinic system, space group C2c. Mo-O2 bonds are the longest, with values of 2.23 Å, whereas the Mo-O1 bonds are the shortest with a value of 1.70 Å.



Atom – Atom	Length [Å]
Mo – O1	1.7040
Mo – O2	2.2342
Mo – O3	2.0002

Fig. 29: Molecular structure of compounds in crystalline state. The hydrogen atoms where omitted for clarity, as well as the Cl- rests.

3.4.5. Solubility assessment and stability in aqueous solution via UV-Vis

UV/Vis measurements were performed in order to examine the stability of the synthesised compounds in aqueous solution (every hour, during 24 hours, at 298 K). In order to do so, the maltolato-complex and KP129 both were dissolved in DCM.

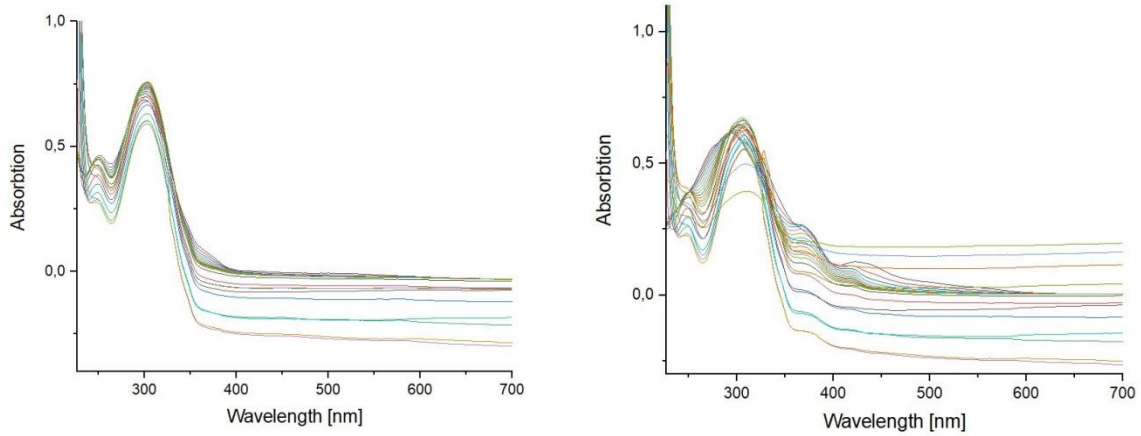


Fig. 30: Maltolato-complex (left) and KP129 (right) in DCM over 24h.

For the maltolato-complex, there is no shift in the peak maxima (λmax), but the absorption decreased over time, which proves that the complex is slowly precipitating out of DCM over time. For KP129, there is an obvious peak at λ=250 nm which emerges besides the main peak (λmax=300 nm) over time, and also two peaks arising at higher wavelengths. This spectra overlay proves the compound is not stable in DCM accompanied by colour change of the solution.

4. Experimental Procedures

4.1. Equipment and Chemicals

NMR spectra were recorded with a Bruker FT-NMR Avance IIITM 500 MHz spectrometer at 500.10 MHz and $CDCl_3$, DMSO-d_6, acetone, MeOD or benzene-d_6 as solvents.

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

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

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

High-resolution mass spectra (HRMS) were acquired on a Bruker MicroTOF instrument with an ESI mass selective detector in positive ion mode.

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 100% DCM.

Methanol (HPLC grade) was purchased from Fisher Scientific and THF from Acros Organics. Both were dried over molecular sieves (3Å) prior use. Ethanol (96%) was purchased from Brenntag AG and was used without any further purification. All other solvents, purchased from commercial sources, were of HPLC grade, dried in an adequate manner and used without further purification.
Following reagents and chemicals were purchased from commercial sources and were used as supplied:

- 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)
- PBS (sterile filtered, Sigma Life Science)
- 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)
- molybdenocene dichloride (99 %, Strem chemicals)
- sodium methoxide (~ 95 %,Fluka)
- ammonium hexafluorophosphate (95+ %, Sigma Aldrich)
- sodium hexafluorophosphate (98 %, Sigma Aldrich)
- maltol (99%, Sig-ma Aldrich) and ethylmaltol (99%, Sigma Aldrich).
- allomaltol, thiomaltol, thioallomaltol, were obtained according to the literature procedures.[138]

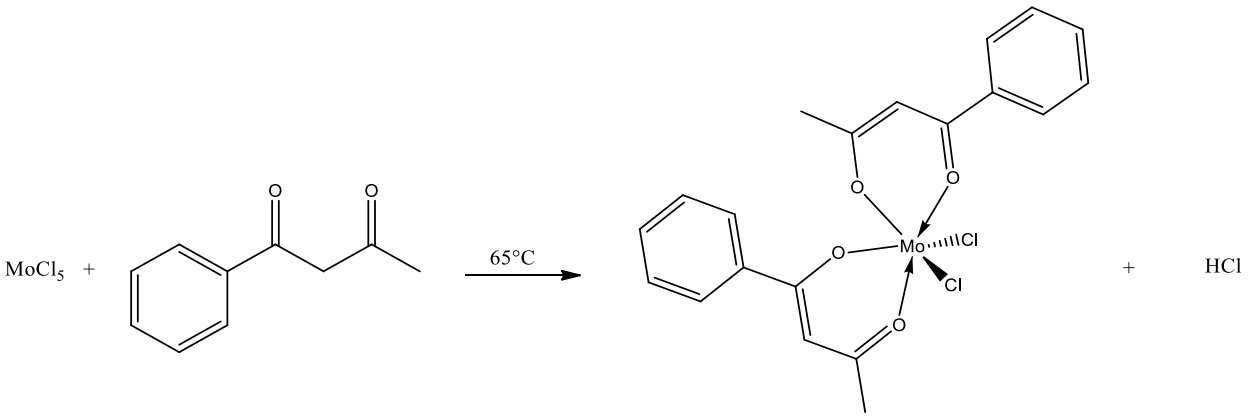
For purification, flash chromatography was carried out using Merck silica gel 60 H (230-400 mesh).

The reagents and products are light and air sensitive, hence all reactions have been carried out under inert atmosphere. Some of the used educts and all of the products seem to be highly toxic and carcinogenic, so it is advised to handle them with care and precautions need to be taken before starting the reactions.

4.2. Experimental Details

All reagents were dried under vacuum before use and all reactions have been carried out under inert atmosphere. Solvents were distilled and stored over molecular sieves. NaOH, HCl solutions and dest. H_2O were degassed using the freeze-pump-thaw method three times each.

4.2.1. Synthesis of KP129



	Amount	Mr [g/mol]	n [mmol]	eq
$MoCl_5$	540 mg	273.21	2	1
Benzoylacetone	1.135 g	162,19	7	3,5

The two reagents were mixed in a Schlenk tube and heated to 65°C under vigorous stirring until gaseous HCl stopped evolving. The reaction mixture was left to cool at r.t. and finally the desired product was extracted with hot hexane. The product was then washed with little hot hexane, heptane and Chloroform to give the pure purple-red compound.

This product was dried under vacuum and recrystallised out of acetone.

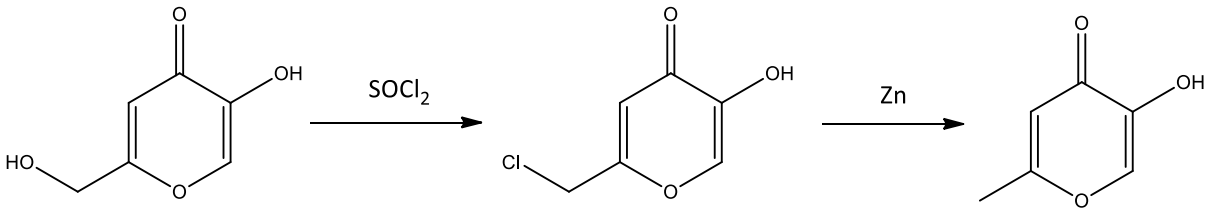
This solid state reaction gives 100% yield, but the last purification step by washing the product with hot hexane also dissolves it. Hence the experimental yield ranges from 80%-98% depending on how much hexane is used for washing.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2,20 (s, 6H, CH_3, bzac), 4,28 (s, 1/2H, CH_3, acac), 6,57 (d, ^3J(H,H) = 21 Hz, 2H, CH, acac), 7,53 (t, 4H, CH, arom), 7,61 (t, 3H, CH, arom), 7,95 (d, ^3J(H,H) = 25 Hz, 4H, CH, arom) ppm

MS (ESI pos. mode): *m/z* $C_{20}H_{18}MoCl_2O_4$ theoretical = 489,9636; found = 489,9664
Elemental analysis: calcd for $C_{20}H_{18}MoCl_2O_4$ C: 49,10%; H: 3,71%; O: 13,08%; found: C 49,67%; H 3,87%; O: 14,16%

4.2.2. Synthesis of the bioactive ligands

4.2.2.1. O,O-chelating ligands: Allomaltol



	Amount	Mr [g/mol]	n [mol]	eq
Kojic Acid	10,07 g	142,11	0,071	1
Thionyl chloride	40 ml	118,97	0,551	8

The thionyl chloride was slowly added to kojic acid under vigorous stirring and ice cooling. The reaction progresses under evolution of gas and formation of a pale yellow crystalline mass. After one hour, the intermediate product was filtered, washed with petrol ether and dried *in vacuo*.

Theoretical yield: 11,37 g
Experimental yield: 11,26 g (99%)

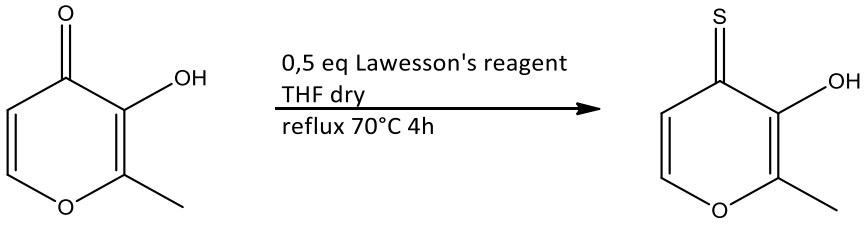
	Amount	Mr [g/mol]	n [mol]	eq
2-Chloromethylmaltol	9.06 g	160.54	0.056	1
Zn	9.41 g	65.38	0.144	2,5

The intermediate product was suspended in H_2O and heated at 50°C under stirring. Zn powder was then added and the reaction mixture was heated to 75°C. The next step was addition of conc. HCl. The reaction slurry was the refluxed for 3h at 75°C. Finally, the excess Zn was separated by hot filtration and the product was extracted with 3x50 mL DCM. The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed in reduced pressure. The product was recrystallised from isopropanol. The desired product was obtained as white needles.

Theoretical yield: 7 g
Experimental yield: 2,3 g (33%)
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2.30 (s, 3H, CH_3), 6.27 (s, 1H, CH), 6.77 (s, 1H, CH), 7.77 (s, 1H, OH) ppm
^{1}H-NMR (500 MHz, d_6-DMSO): δ = 2.23 (s, 3H, CH_3), 6.22 (s, 1H, CH), 7.97 (s, 1H, CH), 8.95 (s, 1H, OH) ppm

MS (ESI pos. mode): *m/z* $C_6H_5O_3Na$ theoretical = 149,0170; found = 149,02

4.2.2.2. O,S-chelating ligands: Thiomaltol



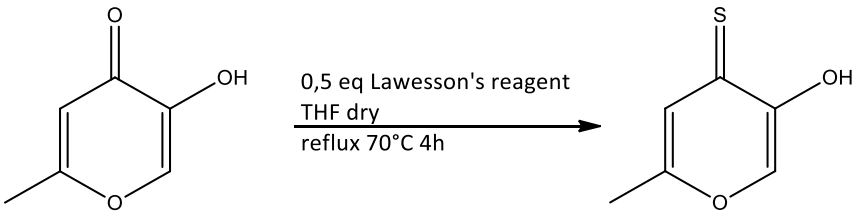
	Amount	Mr [g/mol]	n [mmol]	eq
Maltol	1 g	126,11	8	1
Lawesson's R.	1,604 g	404,47	4	0,5
THF	20 ml			

Maltol and the Lawesson's reagent were mixed in a 100 mL round-bottom flask, dissolved in dry THF and refluxed for 4h at 70°C under argon atmosphere; meanwhile the solution turned from bright orange to red and finally brown. After cooling down to room temperature, the solvent was removed under reduced pressure and an oily product was obtained.

Column chromatography was used for purification, with hexane/EtOAc 10:1 as solvent mixture. The yellow fractions were collected, concentrated under reduced pressure to isolate the desired thiomaltol as a yellow crystals and the product was dried *in vacuo*.

Theoretical yield: 1,137 g
Experimental yield: 0,897 g (79%)
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2.45 (s, 3H, CH_3), 7.3 1 (d, 1H, ^{3}J(H, H)= 5 Hz, CH), 7.57 (d, 1H, ^{3}J(H, H)= 5 Hz, CH), 7.77 (s, 1H, OH) ppm
^{1}H-NMR (500 MHz, d_6-DMSO): δ =2.40 (s, 3H, CH_3), 7.35 (d, 1H, ^{3}J(H, H)= 5 Hz, CH), 8.0 (d, 1H, ^{3}J(H, H) = 5 Hz, CH), 8.28 (s, 1H, OH) ppm
MS (ESI pos. mode): *m/z* $C_6H_6O_2S$ theoretical = 143,0122; found = 143,02

4.2.2.3. O,S-chelating ligands: Thioallomaltol



	Amount	Mr [g/mol]	n [mmol]	eq
Allomaltol	1 g	126,11	8	1
Lawesson's R.	1,604 g	404,47	4	0,5
THF	20 ml			

Allomaltol and the Lawesson's reagent were mixed in a 100 mL round-bottom flask, dissolved in dry THF and refluxed for 4h at 70°C under argon atmosphere; meanwhile the solution turned from bright orange to red and finally brown. After cooling down to room temperature, the solvent was removed under reduced pressure and an oily product was obtained.

Column chromatography was used for purification, with hexane/EtOAc 10:1 as solvent mixture. The yellow fractions were collected, concentrated under reduced pressure to isolate the desired thiomaltol as a yellow crystals and the product was dried *in vacuo*.

Theoretical yield: 1,137 g
Experimental yield: 0,826 g (73%)
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 2.64 (s, 3H, CH_3), 3.49 (s, 1H), 7.48 (s, 1H), 8.21 (s, 1H, OH) ppm
^{1}H-NMR (500 MHz, d_6-DMSO): δ = 2.33 (s, 3H, CH_3), 7.35 (s, 1H, CH), 8.27 (s, 1H, CH), 8.42 (s, 1H, OH) ppm
MS (ESI pos. mode): *m/z* $C_6H_6O_2S$ theoretical = 143,0122; found = 143,02

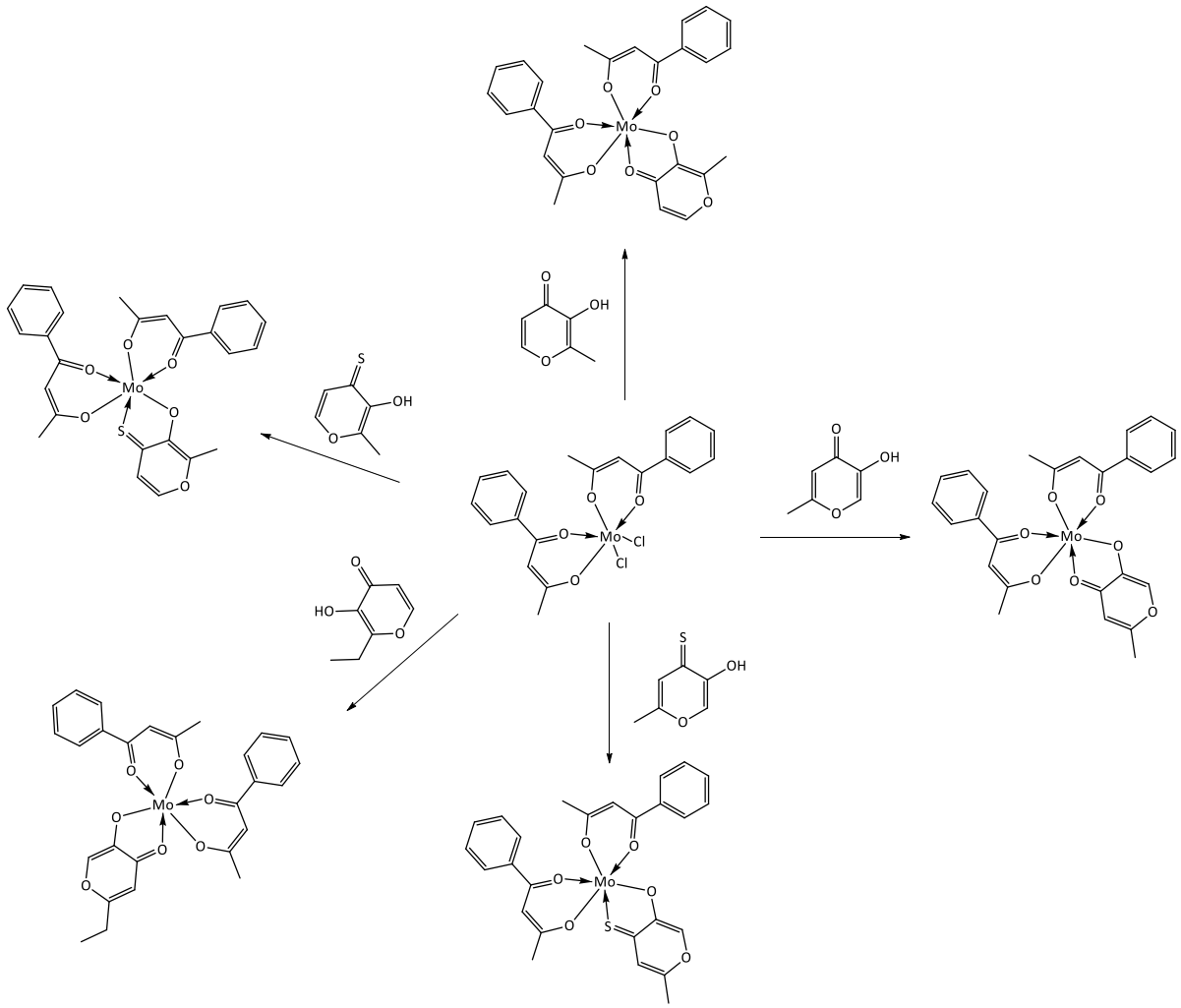
4.2.3. Synthesis of the Mo(IV)-complexes

Different approaches have been adopted in trying to synthesise the desired complexes. Each of the ligands (1 eq), either the commercially available maltol and ethylmaltol, or the synthesised compounds allomaltol, thiomaltol and thioallomaltol, was mixed with different strong bases (various amounts) in a dry solvent (methanol or dichloromethane) and stirred at r.t. under an argon atmosphere. After approx. 15min, KP129 (1 eq) was given to the reaction and this crude was left to stir (Scheme 7). Most experiments were carried out using maltol and at this point the temperature and reaction time were varied (Table 6).

[eq] Ligand	Base	Solvent	[eq] KP129	Reaction
1	1.1 eq NaOMe	MeOH	1	r.t. 1h
1	1.1 eq NaOMe	MeOH	1	r.t. 18h
1	2.5 eq Et_3N	MeOH	1	r.t. 18h
1	1.1 eq NaOMe	DCM	1	r.t. 5h
1	1.1 eq NaOMe	DCM	1	30min, one-pot
1	1.1 eq NaOMe	DCM	1	4h, one-pot
1	1.1 eq NaOMe	MeOH	1	Small scale, one-pot
1	2.5 eq Et_3N	DCM	1	r.t. 3h
1	2.5 eq Et_3N	DCM	1	Ice bath 3h
1	2.5 eq Et_3N	DCM	1	Ice bath 1h
1	2.5 eq Et_3N	DCM	1	Ice bath 4,5h
1.5	2.5 eq Et_3N	DCM	1	Ice bath 4,5h
1	-	DCM	1	Ice bath
1	2.5 eq Et_3N	DCM	1	15°C 3h
1	2.5 eq Et_3N	DCM	1	15°C 1h
1	2.5 eq Et_3N	DMF	1	Ice bath 6h
1	1 eq NaOMe/MeOH	DCM	1	Ice bath 3h
1	1 eq NaOMe/MeOH	DCM	1	r.t. 3h
1	2 eq NaOMe/MeOH	DCM	1	Ice bath 3h
1	1.65 eq NaOMe/MeOH	DCM	1	r.t. 2h
1	2 eq NaOMe/MeOH	-	1	r.t. 2h
1	2.5 eq NaOMe/MeOH	-	1	r.t. 3,5h
1	2.5 eq NaOMe/MeOH	DCM	1	r.t. 2h
1	-	DCM	1	Microwave*
1	2.5 eq Et_3N	DCM	1	Microwave*
1	1.3 eq NaOMe	MeOH	1	r.t. 3h
1.3	1.2 eq NaOMe	MeOH	1	r.t. 18h

Table 6: Different reactions/strategies adopted for the synthesis of the Mo(IV) complex, using maltol

Elemental Analysis was performed on six different reaction products, showing that the reaction with 1.1 eq NaOMe in methanol most probably did not succeed, thus the found values were far from the expected theoretical value



Scheme 7: Reaction paths using KP129 with the different pyrone ligands

5. Conclusion and Outlook

The aim of this master thesis was the synthesis of molybdenocene and its analogous complexes by substituting the two labile chlorido ligands with various biologically active chelating ligands.

The deprotonation of the freshly monomerized Cp was first carried out several times using Superhydride according to literature [119][154] but further experimental work showed that the reaction can be improved by using nBuLi instead.

According to available literature [150/153][155], the preparation of molybdenocene from $MoCl_5$ and freshly cracked cyclopentadienyl is not suitable for small-scale/gram scale reaction. Furthermore, the light and air sensitivity of the starting material, $MoCl_5$, the intermediate dihydride or the desired product, molybdenocene [150/153],[119],[156] challenged the adequate synthesis in the lab with the available setup at that time.

If interest persists in obtaining molybdenocene in the future, the synthesis path via PPh_3 should be more appropriate according to Silavwe [150/153].

KP129 raised our interest based on previous research which showed proliferative properties, but also displayed uwith many many different conditions, is this not true?e maltolato for that. the sensitive Mo(IV) compoundsivatives and coordinnexplainable tumour growth in in vivo tests. [143] Unfortunately, more into depth work on this topic proved this compound cannot be employed in clinical uses since it isn't soluble enough for administration, and showed unwanted effects on the induced tumours in rats. [143] Furthermore, also considering the reaction results, it can be concluded that KP129 is not a good precursor for the studied anti-cancer Mo(IV) complexes, as the isolation of the pure synthesised maltolato-complex is difficult to achieve .

The starting complex KP129 and the different applied O,O- (allomaltol) and O,S- chelates (thiomaltol, thioallomaltol) were synthesised in good yields and characterised via ^{1}H NMR and mass spectroscopy.

Furthermore, an improved protocol for the synthesis of KP129 could be established by decreasing the reaction temperature to 60-65°C, instead of 120°C and also using hot hexane and heptane for work up instead of the toxic CCl_4.

Nevertheless, decomposition of the KP129 was observed during the drying process, where benzoylacetoacetone sublimated out of the solid complex.

If further research will be done on this type of complexes, fluorescence analysis should also be performed, since the synthesis of the maltolato-KP129 complex using 2.5 eq of Et_3N, displayed fluorescence when mixed with DCM. Moreover, since the synthesised compounds show limited solubility in common solvents and solvent mixtures, solid-state NMR would be a better choice of characterisation method. Also, CIDNP (chemically induced dynamic nuclear polarization) NMR-analysis could be performed to detect the formation of radicals and EPR analysis since Mo(IV) is paramagnetic [140].

The experimental work proved that all Mo-containing compounds are extremely air and light sensitive, while elemental analysis showed hygroscopic properties of the prepared compounds. For this thesis, there weren't sufficient means available to achieve a high quality inert reaction set up and work-up; nonetheless future work on molybdenum should be conducted under stricter inert conditions.

Kinetic measurements to determine their stability were carried out in DCM due to solubility issues by UV/Vis and in acetone/water for ^{1}H-NMR spectroscopy. All Mo(IV) complexes exhibited low solubility in aqueous media and in common organic solvents. Furthermore, decomposition was observed when benzoylacetone sublimates out of solid state, and also by the colour change occurring overnight in solution. The solubility wasn't affected by exchanging the counter ion to PF_6^-. [157]

Since the compounds are scarcely soluble in 0.9% saline solution, nor in PVP/DMSO/H_2O, the cytotoxicity could not be tested *in vitro* on cell lines and also binding studies with the HSA to investigate their mode of action were omitted.

For future research, the use of other biologically active ligands, such as naphthoquinone and flavonoid derivatives may be taken into account. As considered by the National Cancer Institute (NCI), the quinone moiety was endorsed as a biological scaffold for the development of new bioactive compounds with good cytotoxicity. [158]

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