universität wien

MASTERARBEIT

Titel der Masterarbeit

Determination and Correlation of Lipophilicity Parameters of Platinum(IV) Compounds Analyzed by HPLC and ICP-MS

verfasst von

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"Ich bin eine Mischung, die ist ziemlich lecker,

Aus Albert Einstein und Arnold Schwarzenegger.

Soweit so gut, doch das dumme ist nur,

Ich hab Schwarzeneggers Hirn und von Einstein die Figur."

EAV – An Der Copacabana

Abstract

Platinum(IV) complexes are among the most promising metal-based cytostatics and are based on their respective platinum(II) precursors. They are supposed to be as active against tumor cells as their platinum(II) analogs, but are claimed to exhibit less side effects and offer the possibility of oral administration. The axial ligands are the main modification sites for tuning their pharmacological properties, *i.e.* by the addition of relatively long alkyl chains, in order to increase the lipophilicity and enable passive transport of the drug into the tumor cell.

The classical parameter for the estimation of the lipophilicity of a molecule is the $\log P$ value. In the shake flask method, a sample is shaken mechanically in a bi-phasic mixture of water and 1-octanol for a defined time interval. However, there are some drawbacks to this method such as the time consuming sample preparation and the low sample-throughput. An alternative lipophilicity parameter is the $\log k_w$ value, which can be determined by liquid chromatography. The advantage of this method is a very short sample preparation as well as a fully automatic measurement. The third parameter φ_0 is derived from the $\log k_w$ value.

The aim of this master thesis was to develop and evaluate suitable analytical methods for the determination of the lipophilicity of platinum(IV) anticancer drugs using reversed phase-high performance liquid chromatography (RP-HPLC) and inductively coupled plasma-mass spectrometry (ICP-MS). Two dead time markers, uracil and potassium iodide (KI), were evaluated and discussed. The classical shake flask method was used to determine the $\log P$ values by chromatographic and mass spectrometric methods. Moreover, $\log k_w$ and φ_0 were derived from RP-HPLC measurements only and were correlated with the obtained $\log P$ values. For this purpose, 79 platinum(IV) complexes were investigated in terms of their lipophilicity.

Both dead time markers performed well, however, KI is suitable for more hydrophilic compounds compared to uracil and therefore covers a broader range of organic modifier. Furthermore, if the $\log P$ value of a given platinum(IV) complex is within $\log P$ = –1.5 to 1.3 both detection techniques (UV/Vis and MS) can be used. Beyond that range only HPLC shows reasonable results from $\log P$ = –2.0 up to 2.4. Results for $\log k_w$ lay within the range of roughly 0.70 up to 4.0. Whereas for φ_0, the results cover the range of about 20 to 70.

In general the φ_0 lipophilicity parameter might be the most suitable one, since it requires a short sample preparation time and the measurement can be carried out automatically without any further steps.

Zusammenfassung

Platin(IV) Komplexe zählen zu den vielversprechendsten Zytostatika, die strukturell auf Platin(II) Komplexen aufbauen. Platin(IV) Komplexe sollen nach Möglichkeit die gleiche Aktivität gegen Tumorzellen wie ihre Platin(II) Analoga aufweisen, jedoch mit geringeren Nebenwirkungen, besserer Verträglichkeit, sowie einer leichteren Handhabung, da im Gegensatz zu Platin(II) Komplexen, Platin(IV) Verbindungen oral verabreicht werden können. Möglich machen dies die beiden zusätzlichen axialen Positionen, mit denen man gezielt versuchen kann die pharmakologischen Eigenschaften von Platin(IV) Komplexen zu steuern. Stattet man die axialen Positionen mit relativ lipophilen Resten, wie z.B. Alkylketten aus, so erhöht dies den passiven Transport in die Tumorzelle.

Ein klassischer Parameter zur Abschätzung der Lipophilie ist der sogenannte $\log P$ – Wert. Hierbei wird eine Substanz in ein Gemisch aus Wasser und 1-Oktanol eingebracht und ausreichend geschüttelt (Shake Flask Methode), sodass sich die Probe in beiden Phasen verteilen kann. Danach wird die Konzentration der Substanz in den beiden Phasen bestimmt und daraus der $\log P$ – Wert berechnet. Diese Methode hat jedoch Nachteile, wie z.B. der hohe Zeitaufwand der Probenvorbereitung und der daraus resultierende limitierte Probendurchsatz. Ein Messwert zur Abschätzung der Lipophilie ist der $\log k_w$ – Wert, der mit RP-HPLC bestimmt werden kann. Zusätzlich kann aus dieser Größe der sogenannte φ_0 – Wert abgeleitet werden. Die Bestimmung dieser Parameter zeichnen sich durch eine sehr einfache und kurze Probenvorbereitung aus.

Das Ziel dieser Masterarbeit ist es anhand von Platin(IV) Komplexen eine geeignete analytische Methode mit RP-HPLC und ICP-MS zur Bestimmung der Lipophilie von metallhältigen Krebstherapeutika zu entwickeln und zu evaluieren. Hierfür werden zwei Totzeitmarker, Uracil und Kaliumiodid (KI), untersucht. Im Zuge dessen wird die klassische Shake Flask - Methode zur Bestimmung von $\log P$ verwendet und die Detektion mit UV/Vis (HPLC) und ICP-MS verglichen. Des Weiteren werden $\log k_w$ und φ_0 – Werte mittels RP-HPLC bestimmt und mit $\log P$ korreliert. Hierzu werden insgesamt 79 verschiedenartige Platin(IV)-Komplexe bezüglich ihrer Lipophilie untersucht und charakterisiert.

Es konnte aufgezeigt werden, dass die beiden Totzeitmarker, Uracil und KI, sehr gut korrelieren. Jedoch sollte KI gegenüber Uracil bevorzugt werden, da es auch für sehr hydrophile Substanzen geeignet ist. Zusätzlich konnte gezeigt werden, dass die beiden unterschiedlichen Detektionstechniken (UV/Vis und MS) korreliert sind, sofern die Proben sich innerhalb des Bereiches von $\log P = -1.5$ bis 1.3 bewegen. Darüber hinaus liefert die HPLC Methode gute

Ergebnisse in einem Bereich von etwa $\log P$ = –2.0 bis 2.4. Die Ergebnisse von $\log k_w$ sind in einem Bereich von ca. 0.7 bis 4.0, die von φ_0 bei ungefähr 20 bis 70. Als geeigneter Lipophilie-Parameter zeichnet sich der φ_0 – Wert aus. Dieser verspricht eine sehr kurze Probenvorbereitungszeit sowie eine anschließende automatische Probenmessung ohne Zwischenschritte.

Content

Abbreviations:

ACN	Acetonitrile
ADME	Absorption, Distribution, Metabolism and Excretion
AES	Atomic emission spectroscopy
CBDCA	1,1'-cyclobutandicarboxylic acid
CE	Capillary electrophoresis
cha	Cyclohexylamine
DACH	1,2-diaminocyclohexane
DCA	Dichloroacetate
DNA	Deoxyribonucleic acid
en	Ethylenediamine
FAAS	Flame atomic absorption spectroscopy
FDA	U.S. Food and Drug Administration
GST	Glutathione S-transferase
HCC	Hepatocellular carcinoma
(U)HPLC	(Ultra) High Performance Liquid Chromatography
HSAB	Hard and soft acids and bases
i.v.	Intravenous
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry
LC	Liquid Chromatography
M	Mol / L
m/z	Mass to charge (ratio)
MEEKC	Microemulsion electrokinetic chromatography
MeOH	Methanol
MS	Mass spectrometry
NSCLC	Non-small cell lung cancer
SCLC	Small cell lung cancer
TFA	Trifluoroacetic acid
UV/Vis	Ultraviolet / visible spectroscopy
WHO	World Health Organization

1. Introduction

1.1. General Overview

Cancer is one of the leading causes of death worldwide. According to the WHO it is accountable for 7.6 million deaths in 2008 (about 13% of all deaths). In 2004, the WHO published their statistics in which cancer is listed third after cardiovascular and infectious and parasitic diseases. Currently, cancer is ranked second (Figure 1), due to enhanced basic medical care. [1]

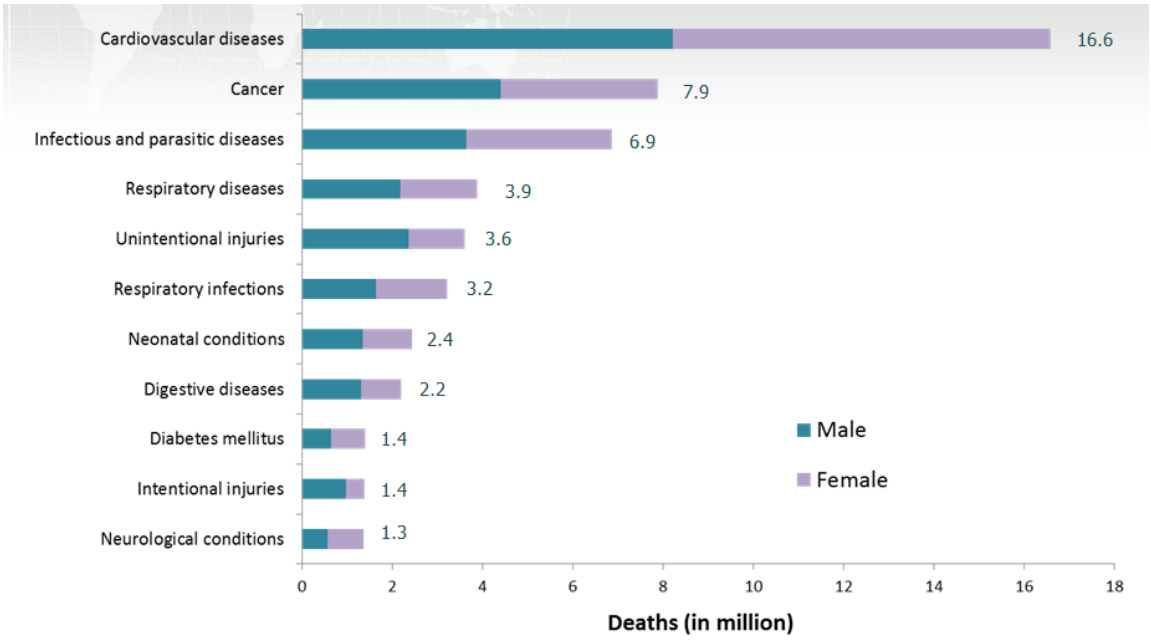


Figure 1: Worldwide leading causes of death by groups and sex in 2011[1]

Each tumour has its own characteristics depending on the cell type it arises from.[2] Men and women differ in cancer incidence, which means that they are affected by different locations of cancer. Moreover, the probability of men to suffer from a tumour is 25% higher than those of women.[3]

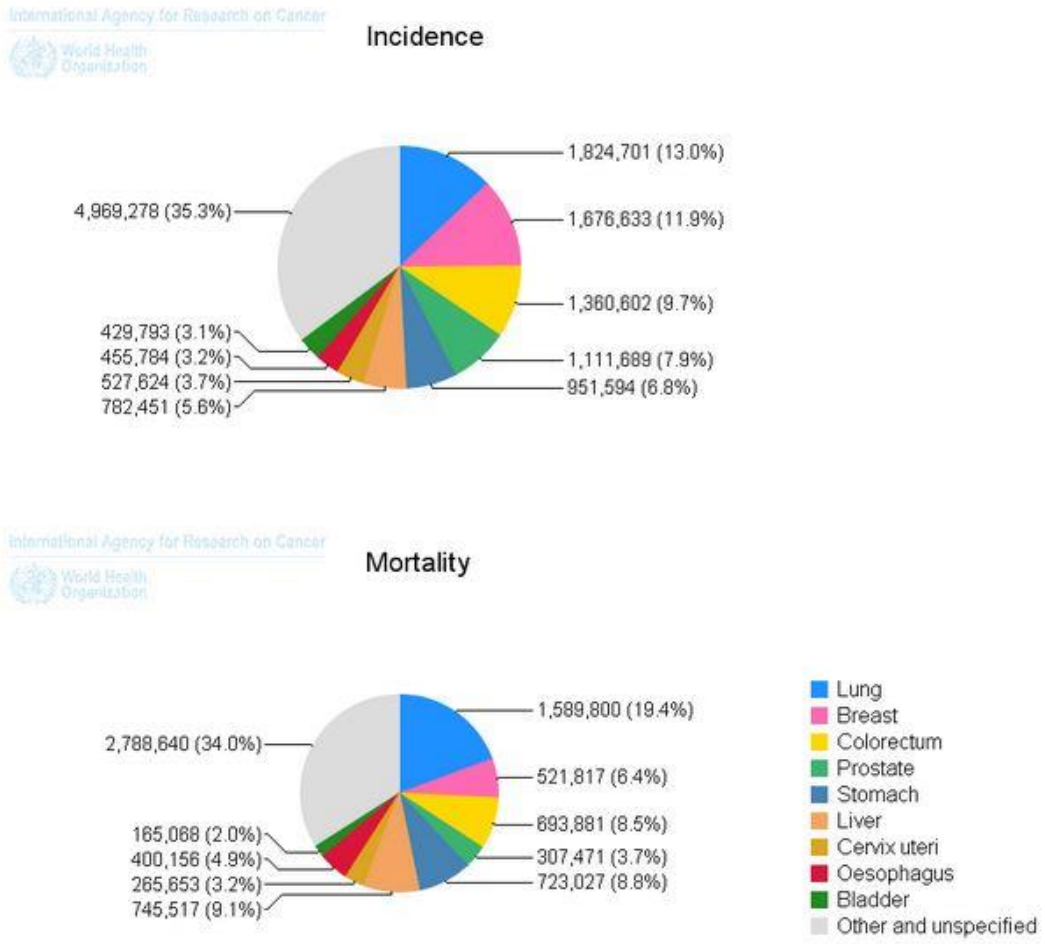


Figure 2: Worldwide cancer incidence and mortality rate 2012[3]

Figure 2 gives an overview about the incidence and mortality rate of various cancer types in the world in 2012. According to the statistic and based on the world population of 7 billion people, 8.2 million died of a cancer disease. Compared to the data of 2008, the absolute deaths which can be attributed to cancer are rising. Combining both sexes, lung cancer is by far the most fatal, with a mortality rate of almost 20%, followed by liver and stomach cancer[3].

In Austria, 20.000 people died of cancer in 2012, which is the second main cause of death in Austria after cardiovascular disease (Figure 3).[4] Figure 3 gives a detailed overview of the overall mortality rate in Austria. The mortality rate has two maxima, one at about 15 years old children, due to juvenile cancer, and the second at the age of around 70.

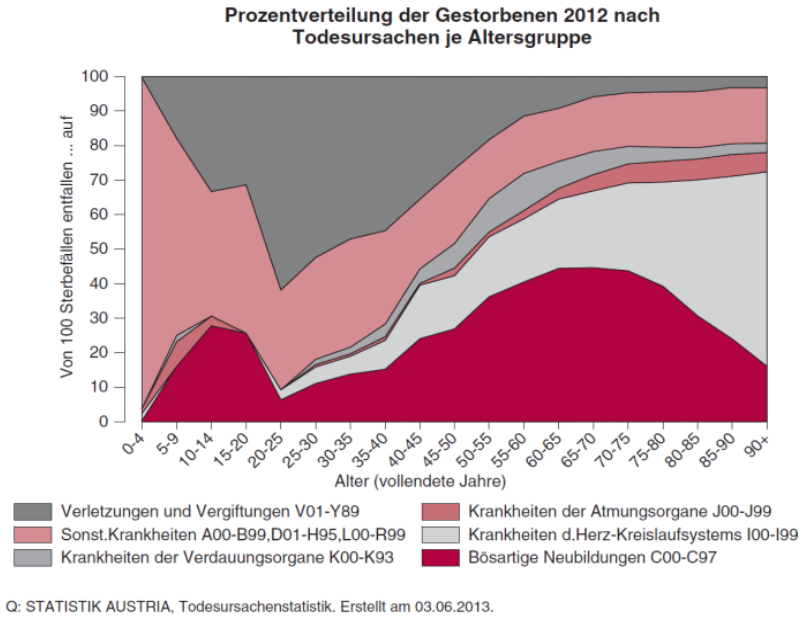


Figure 3: Mortality rate Austria1

While women mostly suffer from breast, colorectal and lung cancer, men are affected by prostate, lung and colorectal cancer.[4] Although cancer treatment and cancer research have been improved during the last decades, Globocan predicted an increase in worldwide cancer deaths up to 13.1 million in 2030.[1,3] However, the WHO predicted that about 30% cancer deaths could be prevented by avoiding risk factors, *i.e.* alcohol, tobacco, high body mass index, low vegetable and fruit intake and lack of physical activity. Importantly, tobacco use has the highest impact of all cancer risks.

Cancer treatment is divided into different approaches:

- Surgery
- Radiotherapy
- Immunotherapy
- Chemotherapy

The choice of therapy depends on the cancer type, location in the body and stage of disease. Since each intervention has its benefits and drawbacks, a combination of methods is often employed .[5]

1.2. Cell cycle and carcinogenesis

Knowledge about the development of cancer is one of the key in order to find appropriate ways to treat cancer. The human body consists of approximately 10^{14} cells, each of them with a specific task.[5] The only way to build up a new cell is *via* duplication of an already existing one. A cell itself does not duplicate constantly, normally it remains in the G_0 phase (G for 'gap') until it is forced to change its status (Figure 4). The cell cycle consists of two different phases: mitotic or M-phase and interphase. Both of them contain several sub conditions. The M phase can be divided into prophase, metaphase, anaphase and telophase, while the interphase consists of G_1, S and G_2. A cell which is undergoing cell division, switches from G_0 phase to G_1 phase. At this stage the cell grows. By switching to S phase ('S' for synthesis) the cell grows further and duplicates its DNA. At the end of the G_2 phase, the cell growing is finished and during the M phase, the cell divides.[6]

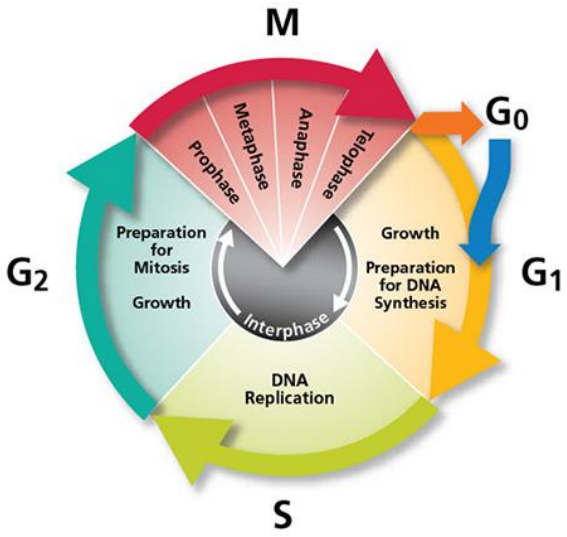


Figure 4: The cell cycle[7]

To enter from one status to another, each cell must pass certain 'checkpoints'. These control mechanisms are able to stop the cell cycle if necessary. If the damage is irreversible, for instance by highly mutated DNA, the cell can commit suicide *via* apoptosis, in order to keep the organism free from severe mutations. Despite these control mechanisms, cancer is able to overcome these checkpoints allowing the cell to duplicate with mutated DNA. Forcing the cell to duplicate with damaged DNA raises the probability of even more mutations. Although the division speed of a cell with mutated DNA is normally decreased, the fact that it can begin a new cycle as soon as the last ended ends up in a very fast cell proliferation.[5]

Cancer originates from one single heritably mutated cell. The process from one abnormal cell to cancer usually requires many years. However, one single mutation in a cell does not result in proliferation without restraint. Several randomly genetic malfunctions have to occur in a lineage

of cells in order for cancer to develop. There are two distinct properties which are inherent to every cancer cell, namely (1) their ability to proliferate without restraint and (2) to invade other cells territory. In combination, these two hallmarks are over time and without treatment, fatal. The tumour state can be described by two patterns. If the tumour is not invasive, its status is referred as benign and the organism can usually be cured *via* surgery. However, if the tumour has acquired the ability to invade surrounding tissues, it is called malignant. The cells are now able to spread around the body forming secondary tumours called metastases *via* the blood and/or lymph vessels (Figure 5). To detect cancer *via* x-ray the tumour has to grow to about 10^8 cells.[5]

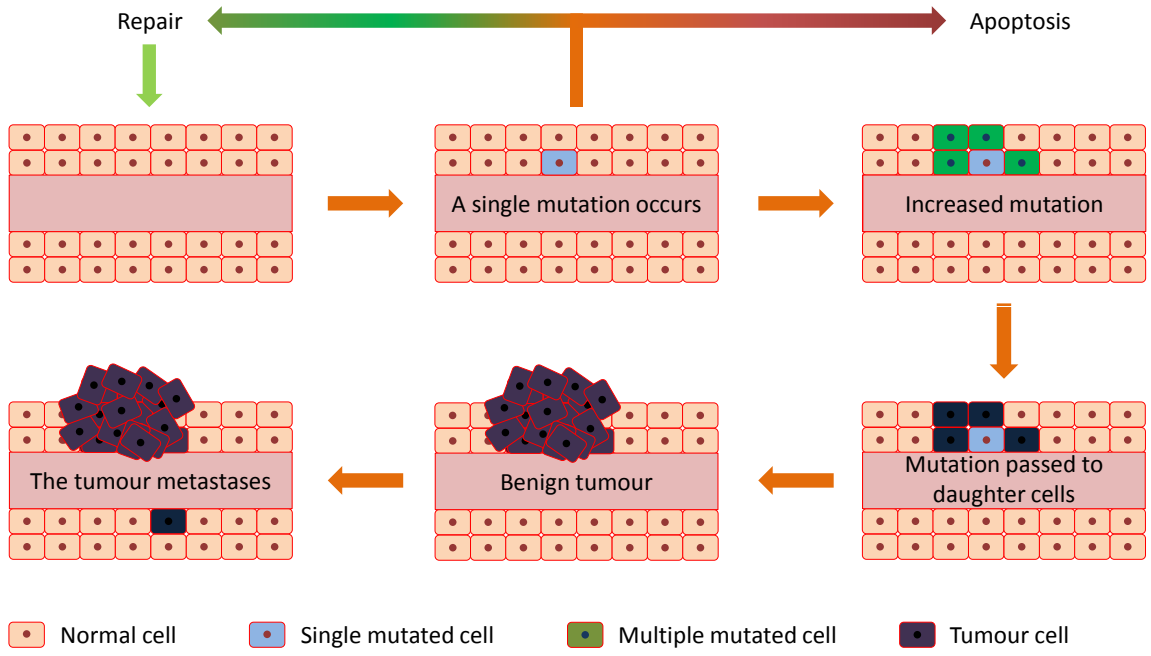


Figure 5: Cancer origin and distribution[5,8]

1.3. Chemotherapy – the discovery of cisplatin

In 1965 Barnett Rosenberg discovered the cell inhibiting properties of cisplatin (cis-diamminedichloroplatinum(II), Figure 7), which is nowadays a leading anticancer agent.[9,10] It is a square planar d^8 complex comprising two ammine and two chlorido ligands, the latter are referred to as leaving groups. Cisplatin is mainly effective against testicular tumours where it shows a cure rate of over 90%. However, it is also used to treat ovarian, bladder, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), lymphomas and myelomas.[11] Cisplatin was approved by the FDA (U.S. Food and Drug Administration) in 1978. The proposed mode of action includes that the chlorido-platinum bond is relatively inert in the blood stream due to the

elevated chloride concentration (100 mM) but may undergo hydrolysis inside the cell because of the lower chloride concentration (4-20 mM) as schematically shown in Figure 6.[11,12] In reality however, cisplatin hydrolyzes already to a significant extent in the blood stream.[12]

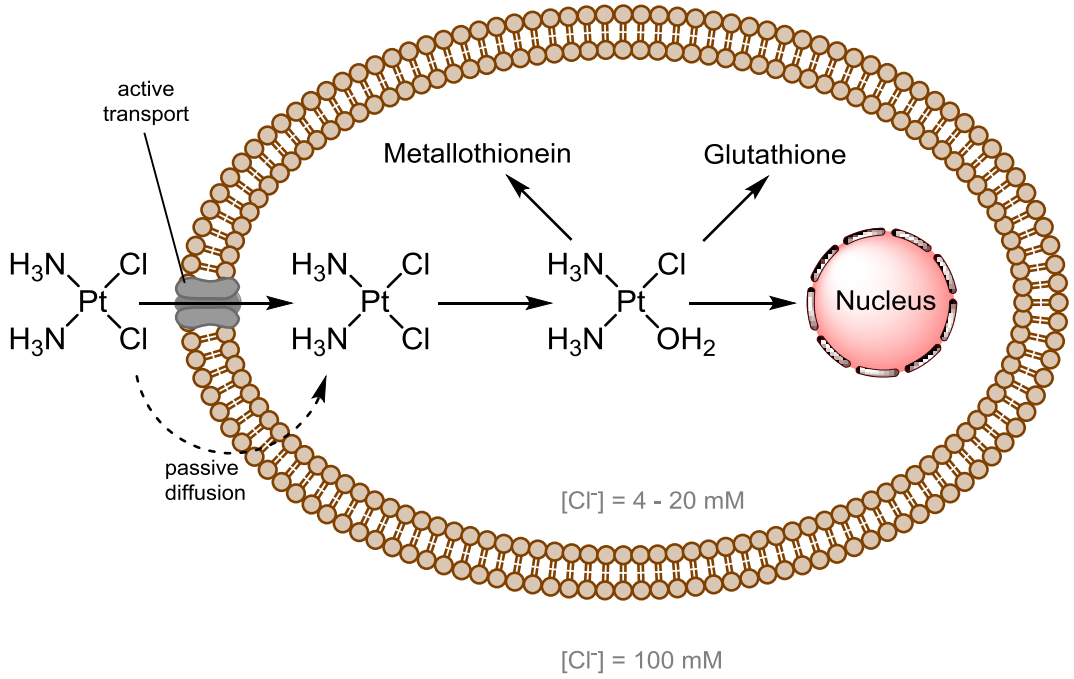


Figure 6: Schematic mode of action of cisplatin inside the cell[13]

Originally, cisplatin was considered to enter tumour cells solely by passive diffusion. Recent data has shown that other transport mechanisms such as active transport through copper transport proteins (hCtr1) might also be involved.[14] Inside the cell, cisplatin undergoes hydrolysis forming the active species of the drug, where one or both chlorido-ligands are replaced by aqua ligands.[13] After aquation cisplatin is able to bind to nucleophiles, but DNA adducts are considered the target adducts. Platinum thereby binds to the N7 position of the imidazole ring of guanine (G) and, to a lesser extent, to adenine (A) (Figure 6). It can form either monofunctional or bifunctional adducts[13,15], which will lead to apoptotic cell death.[16] The most abundant adducts are intrastrand bis-adducts with platinum binding to two adjacent guanines.[15,16]

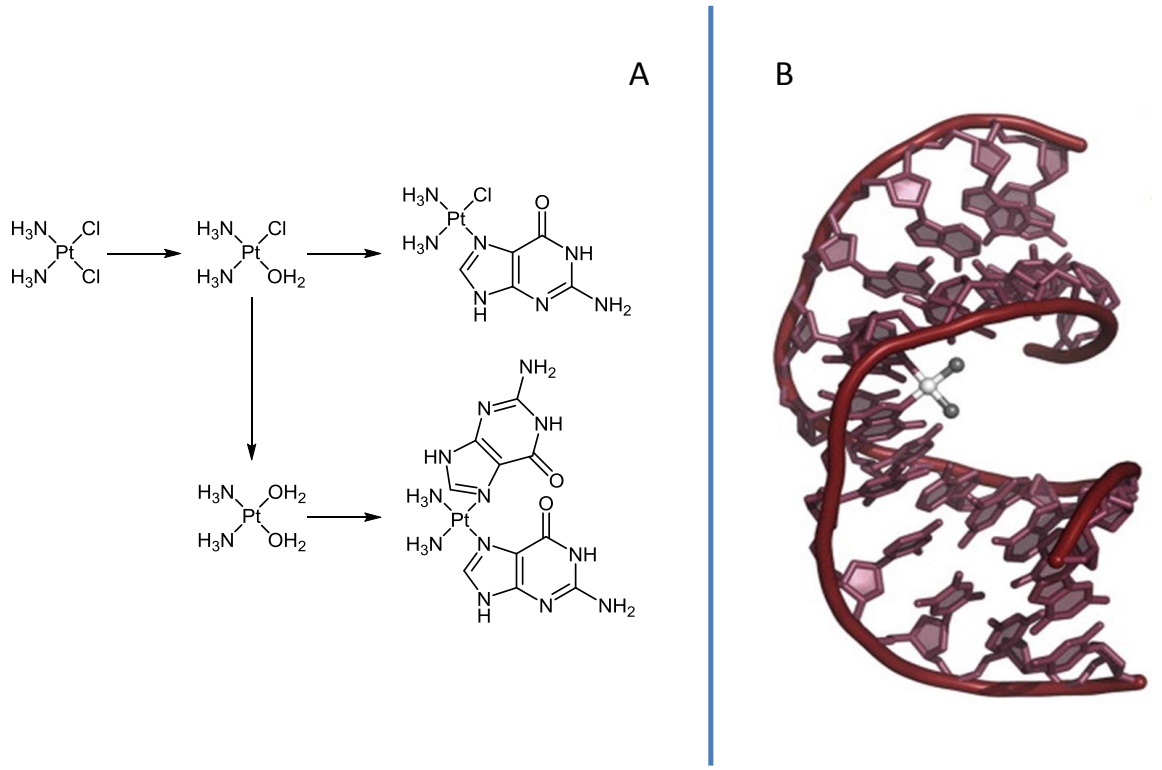


Figure 6: Activation of cisplatin - monofunctional and bifunctional adducts binding to guanine (A).[11,13] Crystal structure of cisplatin cross-linking two guanines in a short double-stranded DNA (B).[17]

Cisplatin shows severe side effects, such as nephrotoxicity, neurotoxicity and myelosuppression, which limit its dose of administration.[11] In addition, cancer cells can acquire resistance to cisplatin by reducing drug uptake, upregulating degradation and deactivation pathways and/or improving tolerance to DNA-cisplatin adducts.[11] Furthermore, cisplatin has to be administered intravenously, which is a practical drawback from a patient's point of view.[12] Once, entering the blood stream cisplatin is capable to bind to any sulphur containing target, especially to amino acids such as methionine and cysteine, fulfilling the HSAB principle. This contrast to the proposed mode of action might be related to the associative binding scheme of platinum(II) compounds. Research has shown that cisplatin binds to serum proteins such as albumin reducing the free drug concentration.[13,18] Whether serum protein adducts serve as a reservoir of platinum anticancer agents or are the cause of side effects is still controversially discussed. Glutathione, a ubiquitous tripeptide is an important detoxifier of cisplatin inside the cell decreasing the amount of molecules interacting with its target, the DNA, even more.

1.3.1. Carboplatin and oxaliplatin

Encouraged from the anticancer properties of cisplatin, scientists have developed other platinum-based metallodrugs aiming to increase the anti-tumour properties and simultaneously to reduce the side effects. Carboplatin (Diammine{1,1-cyclobutanedicarboxylato(2-)-O,O'}platinum(II)) and oxaliplatin ({Oxalate(2-)-O,O'}{1R,2R-cyclohexanediamine-N,N'}platinum(II)) were approved by the FDA in 1989 and 2002, respectively (Figure 7).

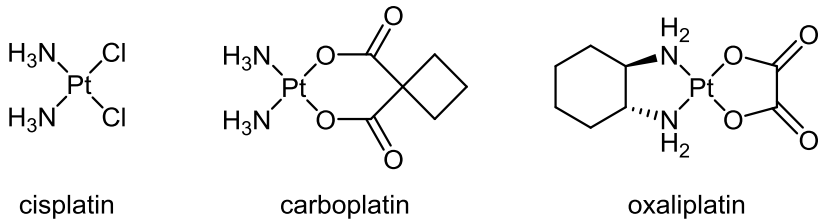


Figure 7: Structure of cisplatin, carboplatin and oxaliplatin

Carboplatin, a second generation drug, shows lower reactivity than cisplatin due to the higher kinetic inertness of the 1,1-cyclobutanedicarboxylate moiety as the leaving group and can therefore be administered at higher doses. Carboplatin exhibits less side effects and is useful for treating the same cancer types as cisplatin, especially ovarian cancer, since it forms the same DNA adducts after aquation.[11] But also carboplatin is not able to overcome tumour resistance similar to cisplatin.[19] The dose limiting toxicity is myelosuppression.[20]

The third generation drug oxaliplatin forms different DNA adducts due to the bulkier 1,2-diaminocyclohexane (DACH) moiety in contrast to cisplatin and carboplatin, and is therefore considered to have a different mode of action. Besides that, oxaliplatin has shown to be effective in cancer cell lines resistant to cisplatin and carboplatin.[19] The uptake into cancer cells has not been fully unravelled yet, and both, uptake *via* passive diffusion[21] as well as *via* active transport mechanisms[22,23] are discussed. Oxaliplatin is currently used to treat metastatic colorectal cancer in combination with 5-fluorouracil.[19] The dose limiting toxicity of oxaliplatin is neuropathy.[20]

1.3.2. Platinum(II) drugs with regional approval

Besides the three worldwide approved platinum(II) anticancer drugs, there are five regionally approved platinum metallodrugs: nedaplatin (Japan), lobaplatin (China), heptaplatin (Korea), miriplatin (Japan) and dicycloplatin (China) (Figure 8). Nedaplatin, a second generation drug, is currently used to treat non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC),

esophageal cancer and head and neck cancer. It is claimed to be more active than carboplatin, but shows similar efficiency like cisplatin.[19,24]

Lobaplatin, a third generation drug is currently used to treat chronic myelogenous leukemia (CML), inoperable metastatic breast cancer and SCLC.[25,26] Heptaplatin is used for treatment of gastric cancer[11,27] and meanwhile undergoing Phase III studies combined with 5-FU regime.[25,28] Miriplatin was approved in Japan in 2009 and is designed for treatment of hepatocellular carcinoma (HCC).[29,30] Dicycloplatin was approved in China in 2012 and is a supramolecule consisting of carboplatin and CBDCA.[31] It was shown to be better soluble than carboplatin and is currently used for treatment of prostate cancer.[32]

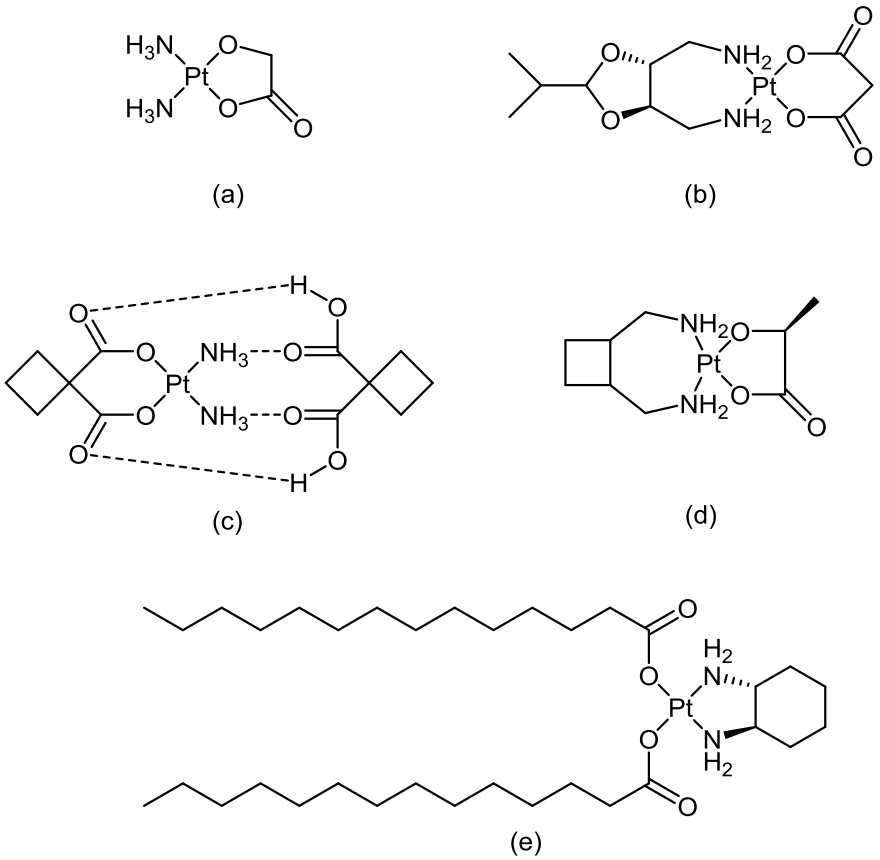


Figure 8: Structures of nedaplatin (a), lobaplatin (b), dicycloplatin (c), heptaplatin (d) and miriplatin (e)

1.4. Platinum(IV) drugs

Although the synthesis of platinum(IV) compounds has already been carried out by Rosenberg in his seminal reports in the 1960s,[9,10] research has mostly been focusing on the development of Platinum(II) drugs until the 1990s. From then on, and probably due to an increased knowledge on platinum chemistry, platinum(IV) complexes were reconsidered to be suitable for metallodrug development.

The general structure of platinum(IV) metallodrugs is mainly based on the platinum(II) precursor scaffolds, *i.e.* cisplatin, carboplatin and oxaliplatin, by oxidation and subsequent addition of two axial ligands (Figure 9). In contrast to platinum(II) complexes they exhibit an octahedral geometry and a low spin d^6 electron configuration.[19] Platinum(IV) complexes show advantageous properties compared to platinum(II) drugs. First, previously unavailable structural modifications were offered due to the octahedral geometry in order to improve their overall pharmacological properties.[19,33] Second, they are considered to be kinetically inert and can thus be administered orally.[11,20] Third, their increased inertness should result in fewer side effects compared to the classical platinum(II) analogues because they pass intact through the bloodstream and cannot react "coordinatively" with biological nucleophiles such as cysteine and methionine. Platinum(IV) anticancer agents are prodrugs. They require reduction and hydrolysis to form the reactive species platinum(II) aqua species (Figure 10).[19,34] Since platinum(IV) compounds may lose their axial ligands upon reduction they are of great importance to modify important pharmacological properties such as pharmacokinetics. Figure 10 highlights the postulated pathway of a platinum(IV) drug in blue.

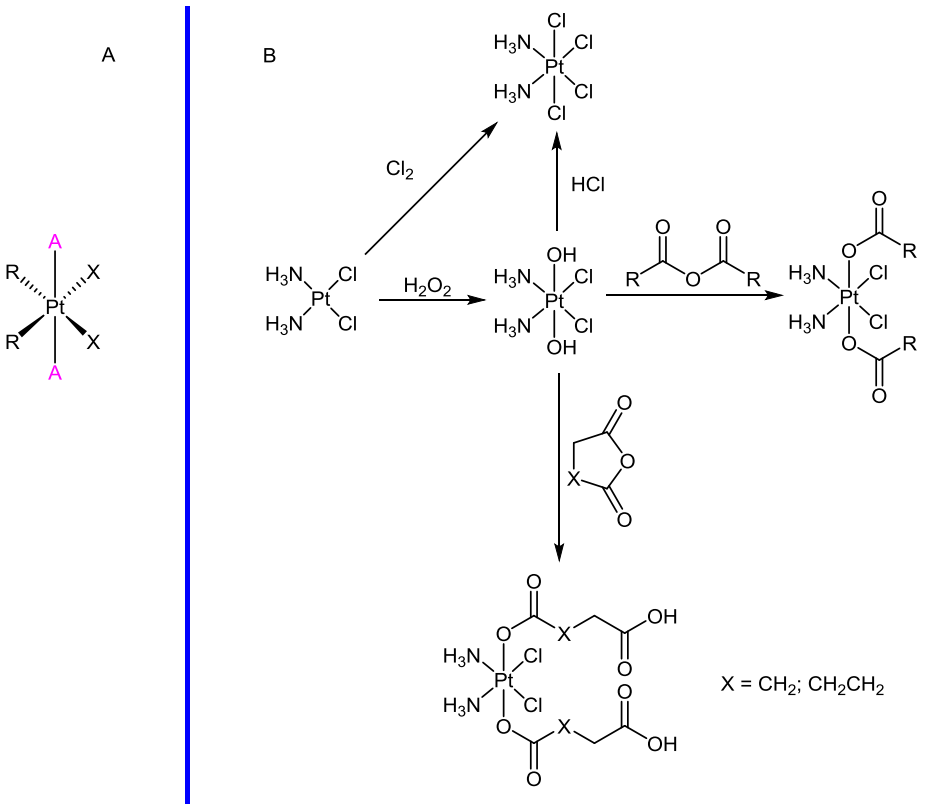


Figure 9: General structure of platinum(IV) compounds (A). Synthetic pathway to symmetric platinum(IV) metallodrugs (B).[35,36]

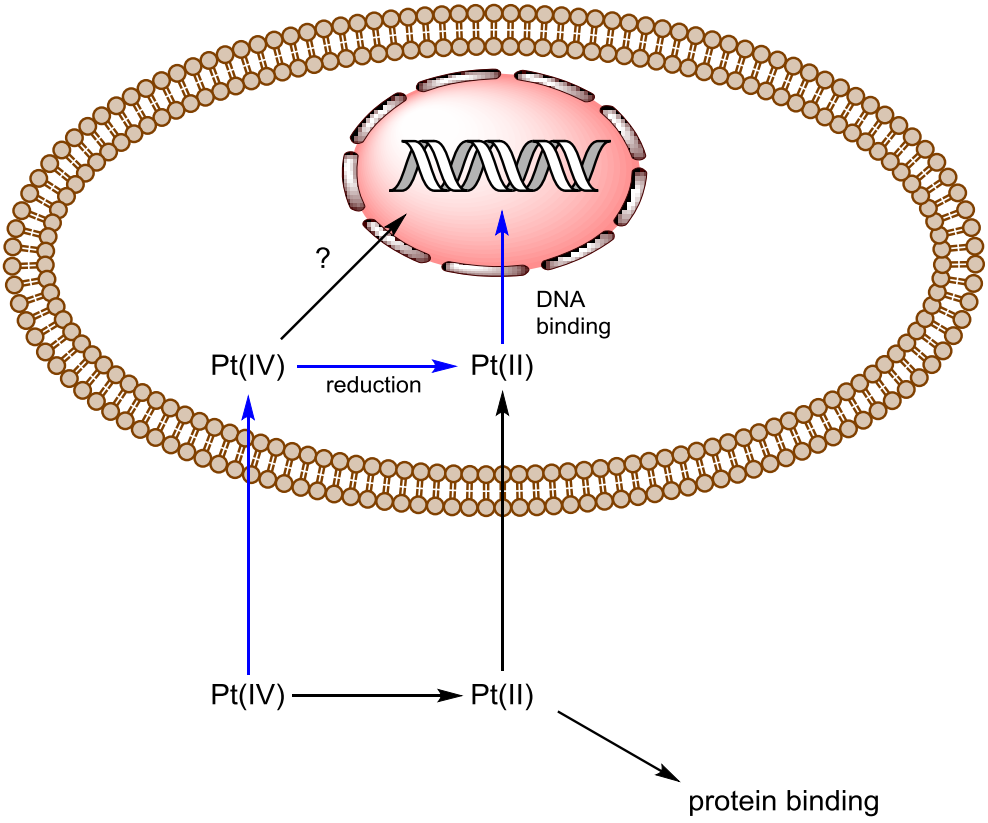


Figure 10: Activation by reduction principle[37]

The first platinum(IV) anticancer agents feature relatively small axial ligands such as chlorides, hydroxides or acetates, *e.g.* tetraplatin (ormaplatin), iproplatin and satraplatin (Figure 11). While tetraplatin and iproplatin were abandoned due to unpredictable neurotoxicity or no benefits compared to cisplatin and carboplatin, satraplatin is currently undergoing clinical trials.[11,19,38,39]

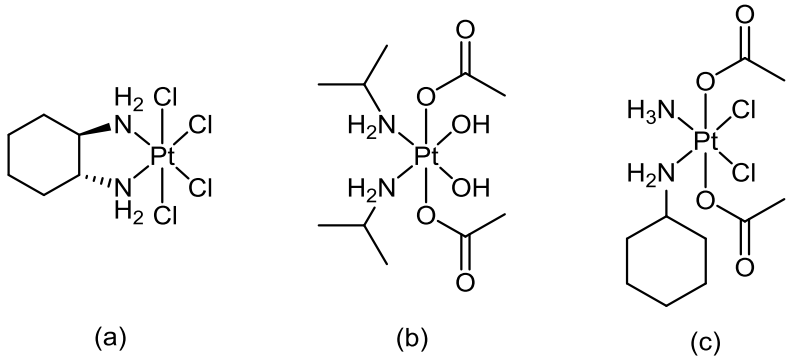


Figure 11: Structures of tetraplatin (a), iproplatin (b) and satraplatin (c)

Platinum(IV) compounds are usually synthesized by oxidation of platinum(II) drugs, either with chlorine or hydrogen peroxide (Figure 9). Based on the axial hydroxide ligands, new compounds have been synthesized by carboxylation or carbamation.[40-42] These compounds are designed with the aim to increase their lipophilicity and/or to couple the axial ligands with bioactive components. In the group of Professor Keppler, platinum(IV) compounds with increased lipophilicity were synthesized resulting in IC_{50} values in the low micromolar range.[43,44] Other examples of rationally designed platinum(IV) pro-drugs are given in Figure 12. Dyson et al. used ethacrynic acid, a cytosolic GST inhibitor to overcome resistance issues.[45] Lippard et al. designed a platinum(IV) compound which releases dichloroacetate (DCA) to modify the mitochondrial membrane potential.[19,46]

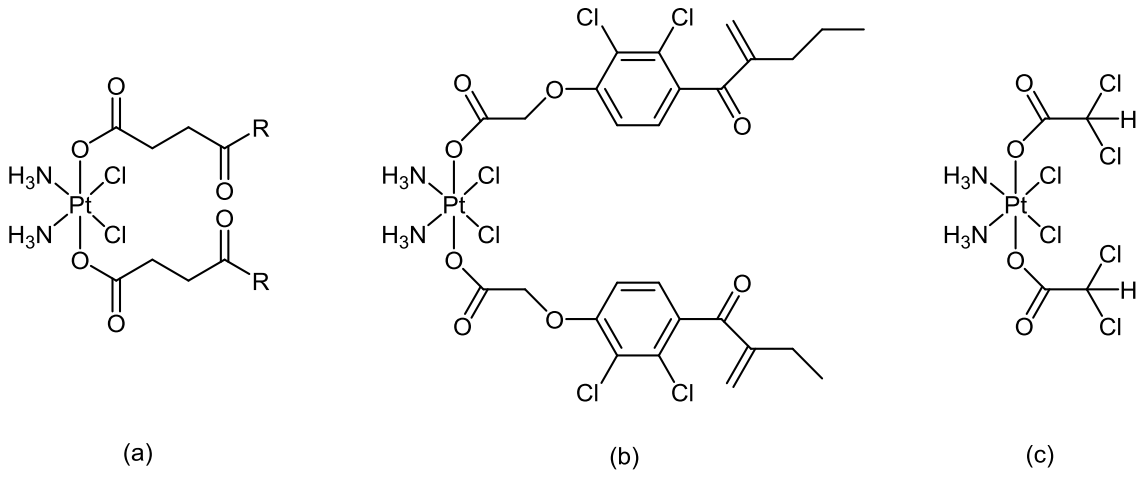


Figure 12: Platinum(IV) drugs can be tuned by axial ligand modifications in terms of lipophilicity (a), as a GST inhibitor (b) or as an agent altering the mitochondrial membrane potential (c).19

1.5. Non-platinum metal-based drugs

Over the past decades, platinum complexes have been established as routinely used anti-cancer agents. Nearly half of the chemotherapic interventions to treat cancer are based on platinum compounds.[12] Beside the classical compounds, therapeutics with alternative metal centres have also been investigated including copper,[47] titanium,[48] gallium,[49] etc. Currently, the most promising drug candidates are ruthenium compounds. Ruthenium exhibits a relatively slow ligand exchange rate. It offers three different oxidation states (II, III and IV) and may mimic iron in biological settings.[50,51] The most promising candidates are the RAPTA-type metallodrugs[52,53] along with NAMI-A[54], RM175[55] and KP1339[56] (Figure 13). While NAMI-A is effective against lung metastases, KP1339 shows anti-tumour properties against various primary tumours, especially slowly growing adenotumors. RAPTA is effective against mammary carcinoma and has anti-angiogenic properties.[52,57,58] RM175 shows activity against platinum-resistant human ovarian cancer cell line.[59]

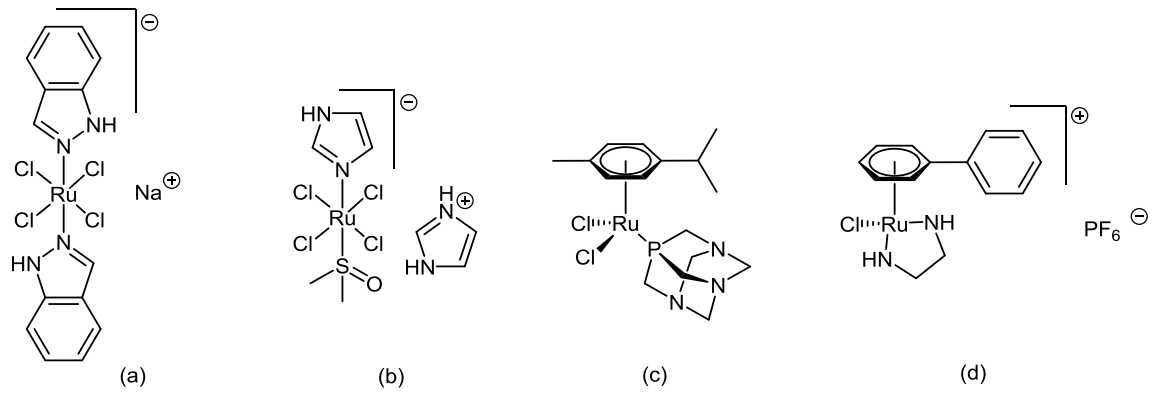


Figure 13: KP1339 (a), NAMI-A (b), RAPTA-C (c) and RM175 (d)

1.6. Pharmacokinetic properties

Solubility and lipophilicity are important parameters in drug design, since they influence pharmacokinetic (ADME) processes. ADME stands for absorption, distribution, metabolism and elimination.[60]

Absorption is defined as "the passage of the drug from its site of administration into the general circulatory system after enteral administration".[61] If a drug is administered intravenously it will directly enter the systemic circulation. All other administration methods (oral, parenteral, buccal, rectal, sublingual *etc.*) are called extravascular routes. The most convenient way for patients is oral administration. After oral administration the drug has to cross several cell membranes and the gut wall and has to enter the tumour tissue in the case of anticancer drugs.[60] After oral administration, the drug encounters drastic pH shifts from the stomach to the intestine, inside a cell and into the blood stream, which may influence the drugs permeability across cell membranes.[61]

The *distribution* process is referred to as the reversible transfer of a drug from one location to another within the body. Once a drug enters the blood stream it can spread throughout the body. The distribution will be most likely non-uniform in tissues.[60]

Metabolism is divided into phase I and phase II. In general, both stages are used to render xenobiotics more hydrophilic in order to enhance their elimination. The typical reactions in phase I are hydroxylation, reduction and epoxidation. In phase II the compound is conjugated with polar biomolecules such as amino acids or functional groups.[60] Before entering the circulatory system, orally administered drugs may be metabolized in the liver already. Therefore, the amount of the drug binding to its target is reduced. This is commonly referred as the first pass effect.[61]

In most cases the *elimination* of drugs is carried out by micturition and, to a much lesser extent, *via* faeces.[60]

On the one hand, a drug with high solubility may be well suited for *i.v.* administration, but for oral administration it will not pass the cell membranes and would require an active transport. On the other hand, a highly lipophilic substance would be able to enter cells *via* passive diffusion, but may be excreted in the first place since it will dissolve in fat globules.[36] Therefore, a reliable estimation of the lipophilic character of a drug is of high importance and three different lipophilicity parameters are assayed and discussed with respect to their suitability for characterizing the lipophilicity of platinum(IV) anticancer agents.

1.7. High Performance Liquid Chromatography – HPLC

HPLC is a chromatographic technique based on the development of column chromatography by Michail Tsvet in 1906. Tsvet was able to separate chlorophyll and xanthophylls, which resulted in different coloured zones on his column inspiring him for the name chromatography.[62] It is currently one of the most popular analytical method.

The basic principle of column chromatography is the distribution of a compound between two immiscible phases, namely the stationary and the mobile phase. The stationary phase forms an immobile layer while the mobile phase exhibits a constant flow in one direction (Figure 14). A mixture of compounds can be separated according to the different extents of interaction of the compounds with both phases. A highly retained substance remains longer on the stationary than a compound which does not interact with the stationary phase at all. In conventional liquid chromatography, the mobile phase moves forward due to gravitation. The stationary phase is nowadays usually made of silica-particles with 150–200 µm diameters.[62,63]

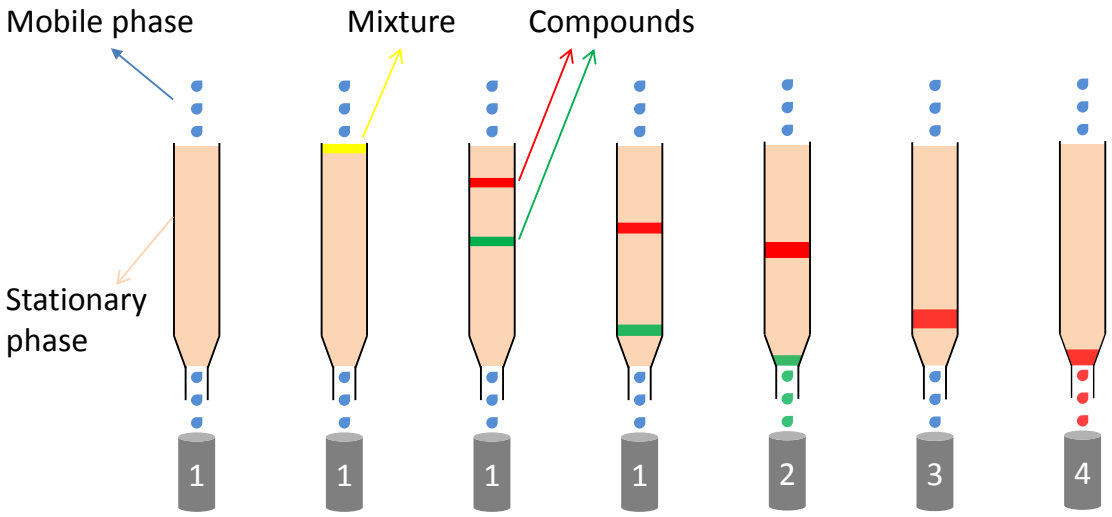


Figure 14: Chromatography scheme[63] – the yellow band is a mixture containing different compounds. Due to the different interactions with the stationary phase the compounds will be separated into two different bands (red and green band)

A further development of the classical column chromatography is the HPLC and was invented in the late 1960s.[63] A modern analytical HPLC system consists of:

- mobile phase reservoir
- pump system
- injection system
- (pre-)column
- detector
- waste reservoir

A *mobile phase reservoir* is necessary for storage of the solvents. It is usually made of glass or, less commonly, steel and normally comprises 250 to 1000 mL. The most frequently used solvents are water, MeOH and ACN, however, the choice of solvent depends on the column type. To pump the mobile phase (eluent) through the system, further purifications steps are necessary in order not to harm the column, the detector or to ensure a good resolution. One of them is the so called degasser, a small item which prevents bubbles to reach the detector in order to maintain good resolution. The degasser can be combined with a filter unit to remove small particles from the eluent. An HPLC system is capable of mixing solvents from different mobile phase reservoirs. This allows various compositions of mobile phases between and even during HPLC runs. If the eluent composition is kept constant during the run, it is referred as an isocratic elution mode, as accordingly a gradient elution mode if the compositions are varied. While isocratic elution is not prone to baseline drifts, a gradient elution typically exhibits a drifting baseline over time. On the other hand, a gradient elution can offer shorter analysis time and sharper peaks.[63]

The *pump system* has to generate pressure up to 400 bars (1200 bars in an UHPLC system). It should be chemically inert and should maintain continuous flow rates from 0.1 mL/min up to 10 mL/min for classical analytical pumps.[63]

The *injection system* should support a broad volumina range from 1 to 100 µL depending on the size of the sample loop. However, this is not an easy task since the pressure within the system should not be influenced by the injection itself. Therefore, a so called sample injection 6-port valve was invented which can bypass the eluent flow from the pump directly on the column. In this position, the sample can be injected into the sample loop. Upon switching the valve into main pass, the sample is flushed on the column. Although every HPLC system has a manual injection system, an autosampler used for routinely analysis is more common.[63]

The *column* is one of the most critical issues of an HPLC system, since its stationary phase is responsible for a chromatographic separation. There are basically two different kinds of columns: ‘normal phase’ and ‘reversed phase’. A normal phase exhibits a polar stationary phase while a reversed phase uses a non-polar column, e.g. alkyl-chains. Obviously, the type of column selected depends on the sample properties. However, a reversed phase column is used in most cases, since the compounds which are separately mostly nowadays such as amino acids, antibiotics or (metallo-) drugs can be separated very well.[63] Commercial columns are available in

various lengths, diameters and particle sizes. The most frequently used columns are 5-25 cm in length, have an inner diameter of 1.8-10 mm and consist of spherical particles of 2 to 10 µm. There is a specific column for almost every application on the market.[63]

Basically, there are two different types of *detectors*. It can either measure an intrinsic physically characteristic of the mobile phase such as refractive index or conductivity, or properties of the analytes, such as UV/Vis or fluorescence. The UV/Vis detector is used the most frequently used detector with an estimated usage of 70%.[62] Since UV detectors are based on absorption, it is also susceptible to interferences. Therefore, a small cell in 'z'-shape is used, which should use as little volume as possible and is normally ranging from 1 to 11 µL at 2 to 10 mm length (Figure 15).[63]

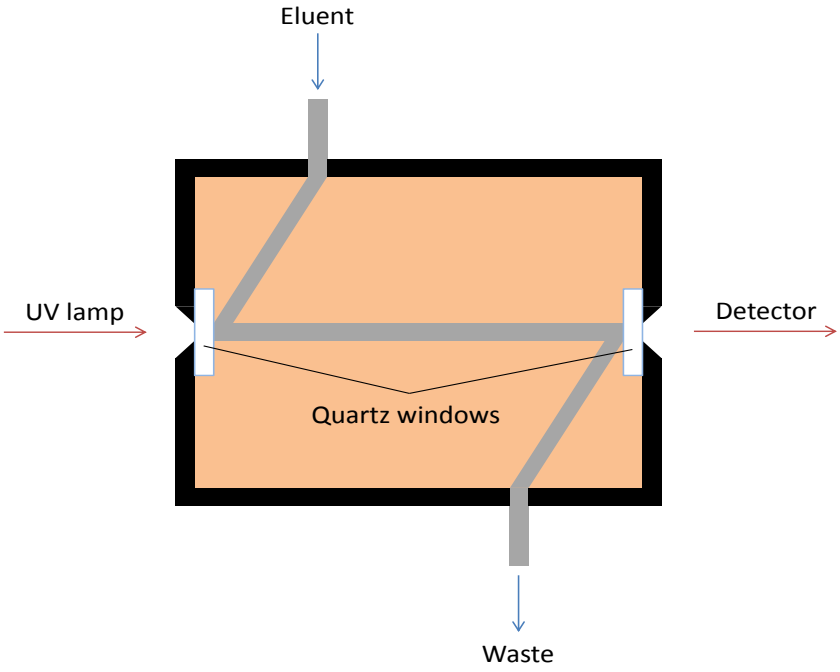


Figure 15: UV detection cell[63]

However, there are some disadvantages/limitations of an HPLC system. For example, the detection limits for UV/Vis, which depend on the molar extinction coefficient, are quite high and are usually in the nanogram range. Peaks can also overlap, which may lead to masking effects. Although HPLC is a 'stand-alone' technique, it can be combined with other analytical instruments such as MS for an enhanced sensitivity and the possibility of gaining additional information based on the m/z ratios of the analytes. In the last years chiral columns have been introduced to facilitate chiral separations on HPLC systems even on semi-preparative scale. Furthermore, UHPLC systems (Ultra High Performance Liquid Chromatography) have been developed. These systems are characterized by small column particles (<2 µm) which enable relatively fast separations, but at the price of higher back pressure compared to conventional HPLC systems.[64,65]

1.8. Inductively Coupled Plasma-Mass Spectrometry – ICP-MS

Inductively coupled plasma - mass spectrometry (ICP-MS) is a powerful technique which was designed in the early 1970s to combine the rapid multi-element capabilities of inductively coupled plasma - optical emission spectrometry (ICP-OES) with the detection limits (ng/g scale) of graphite furnace atomic absorption (GFAA).[66] The benefits are its multi-element analysis capabilities, a very high sensitivity to sub 1 ppb range and on-line sample introduction.[67] ICP-MS has been established as a routine technique for a common field in inorganic chemistry known as metallomics, which can be described as the analysis of metal-containing biomolecules (structure, function *etc.*) within a tissue or cell type.[67] This can be derived from the fact that an ICP-MS does not detect the analyte structure due to the atomization process and therefore, metabolites can be determined by elements of interest *e.g.* platinum, ruthenium or any other metal. Although ICP-MS is a superb stand-alone technique, it can be coupled with analytical separation methods such as LC or capillary electrophoresis (CE), to gain additional information. Especially HPLC systems can be easily hyphenated to ICP-MS, due to the similar flow rate. Furthermore, ICP-MS is able to handle solid samples as well, such as soil- and rock samples *via* a laser ablation (LA) unit hyphenated to ICP-MS (LA-ICP-MS).

The main parts of an ICP-MS system are the introduction system, the ionisation source, the interface region, the ion focusing system, the mass analyser and the detector. Figure 16 illustrates an ICP-MS setup.

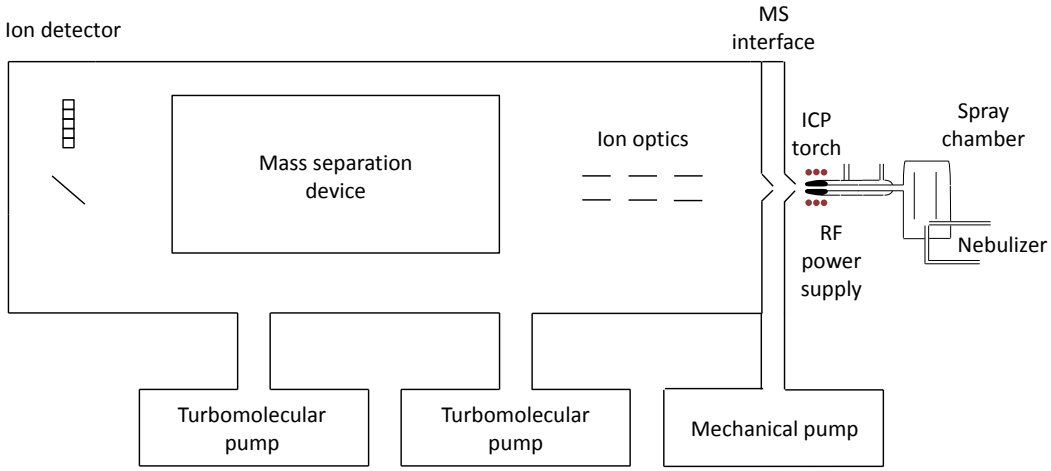


Figure 16: Overview of an ICP-MS system[66]

The first part of an ICP-MS is the sample *introduction system*. It consists of the nebulizer and the spray chamber. The two main tasks of this setup are the generation of an aerosol and droplet selection. It is often defined as the weakest part of an ICP-MS since only 1-2% of the sample will pass through the introduction system.[68] When the sample enters the nebulizer, the liquid is broken up into a fine aerosol by the pneumatic action of the argon gas flow. Since the plasma

discharge is less effective for large droplets, the spray chamber selects the small droplets exclusively and prevents the bigger droplets to enter the plasma.[66]

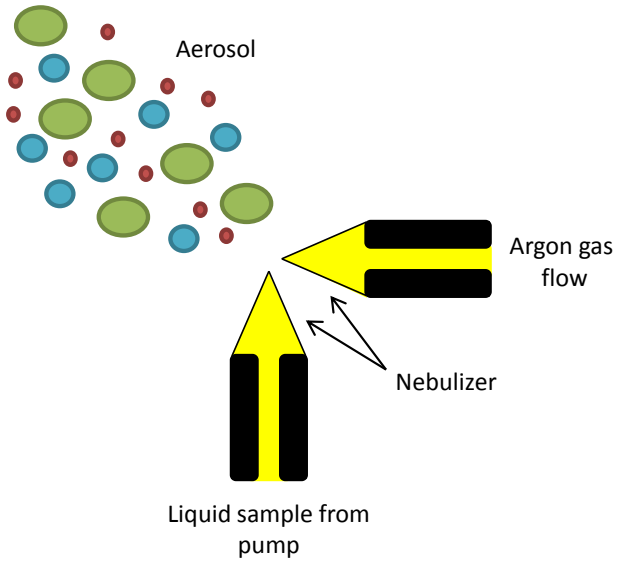


Figure 17: Generation of an aerosol in an ICP-MS spray chamber[66]

The second part of an ICP-MS system is the ionization source. It comprises the plasma torch, a radio frequency (RF) coil and an RF power supply.[66] The plasma torch itself consists of three different tubes, the outer tube, the inner tube and the sampler injector. Between the outer and the middle tube, the plasma gas (usually argon) generates the plasma with a flow rate of 12 – 17 L/min. Furthermore, a second gas flow, the auxiliary gas, between the middle tube and the sample injector is used to modify the position of the plasma relative to the injector. Its flow rate is usually around 1 L/min. The sample aerosol is carried from the nebulizer at a flow rate of typically 1 L/min.

The plasma itself is generated by a radio frequency, which causes an oscillation and therefore an electromagnetic field. The plasma is then ignited with a high voltage spark, withdrawing some electrons of argon atoms. The electrons are then accelerated due to the magnetic field. They collide with other argon atoms, pulling off even more electrons. This chain reaction is known as inductively coupled plasma (ICP) discharge. Inside the plasma torch, the sample coming from the spray chamber has to pass though different heating zones, ranging from 6000 to 10000 K. While the sample travels through the different heating zones, the sample is dried, vaporized, atomized and ionized. These steps are necessary to transform a liquid sample to an ionized gas form with singly positively charged ions.[66]

The third part of an ICP-MS device is the *interface region*. The interface region separates the plasma source working at atmospheric pressure from the mass analyzer, which is under high vacuum (10^{-6} Torr). After ionization in the plasma, the ions pass through two metallic cones,

namely the sample and skimmer cone. The sample cone has an orifice diameter of 0.8-1.2 mm, while the skimmer cone has an orifice diameter of 0.4-0.8 mm. The main function of these cones is to align the ions and they are usually made of nickel or platinum. The interface region consists of a mechanical pump, keeping the pressure at 3-4 Torr.

The main task of the *ion focusing system* is to focus the ions into the mass analyzer, while rejecting non-desired species from reaching the mass analyzer and eventually the detector. These non-desired species can be photons, neutral elements, matrix elements or particles. One approach is to set the ion lens or mass analyzer slightly off-axis. By traveling through the interface region and the ion focusing system, the beam will expand due to the passage from atmospheric pressure to high vacuum. Therefore the beam will be positively charged, since the electrons will diffuse faster than the ions. Furthermore, the positively charged ions will now repel each other. This will result in a higher mass-to-charge ratio in the center of the beam, since the lighter ions will be pushed on the edge of the ion beam. To compensate for this effect, the ion lenses are positively charged and therefore, refocus the lighter ions into the center of the beam. While the positively charged ions are focused by the lens system into the analyzer, the non-desired species will simply clash into a barrier.

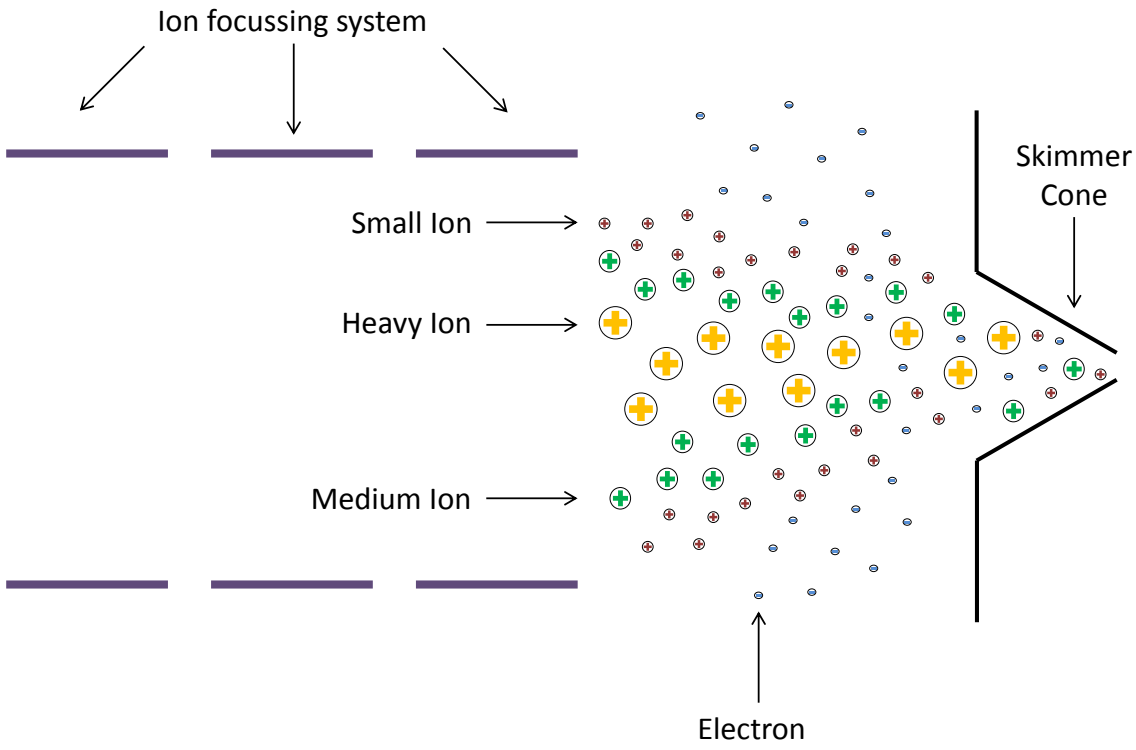


Figure 18: Ion focusing system[66]

Followed by the ion focusing system and before the detector, a *mass analyzer* selects the ions according to their mass-to-charge ratio. Three different mass analyzers can be employed in an ICP-MS device, such as quadrupole, double-focusing magnetic-sector field and time of flight (TOF). According to the literature, about 90% ICP-MS systems use a quadrupole as a mass analyzer. The quadrupole and the double-focusing magnetic-sector screen within a defined m/z ratio and select the desired ions. Double-focusing magnetic-sector mass analyzers offer high resolution, but are more expensive than quadrupole- based ICP-MS. TOFs are designed to detect more than one element simultaneously, which offers a full mass spectrum in short time. Finally, the detector is usually a channel electron multiplier.[66]

The occurrence of mass interferences is one of the challenges of ICP-MS. Depending on the sample and its matrix, there can be interferences caused by atomic or polyatomic species having the same mass to charge ratio as the analyte. These interferences appear mostly on low mass metal ions, due to the formation of oxide, nitrogen, sulfur, phosphor and/or argon species. To overcome this problem either a sector-field high resolution ICP-MS can be used or a reaction and/or collision cell can be placed after the focusing system and before the mass analyzer.

1.9. Determination of Chromatographic Lipophilic Parameters

Lipophilicity is a crucial value for every prospective drug, since a lipophilic drug is able to cross cell membranes *via* passive diffusion more easily compared to hydrophilic analogues. The capacity factor k is a good approximation to obtain an estimation of lipophilicity, since the stationary phase mimics an apolar organic (e.g. C18) and the eluent a polar phase (e.g. water). Furthermore it can be easily determined experimentally. The capacity factor can be derived by the equation (1)

$$k = \left(\frac{t_r - t_0}{t_0}\right) \qquad (1)$$

And therefore:

$$\log k = \log\left(\frac{t_r - t_0}{t_0}\right)$$

t_0 is the retention time of a non-retained standard and t_r is the retention time of the analyte. Various non-retained standards can be found in the literature, *i.e.* uracil, $NaNO_3$, acetone or D_2O. Uracil was found to be the most appropriate one, since acetone is retained to some extent on C18 support, D_2O is not UV- visible and $NaNO_3$ showed Donnan exclusion effects.[69]

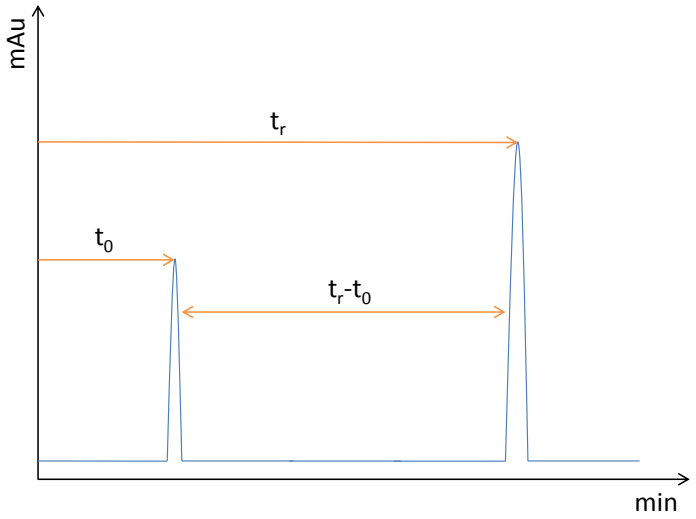


Figure 19: Illustration of retention indices

The advantage of the capacity factor k is its independence of the column size and the flow rate which should therefore offer a good comparability of different instruments.[63] By measuring the k value at different percentages of mobile organic phase modifier and by calculating the $\log k$, a linear slope is obtained. By extrapolating this line to a theoretically zero percentage of organic modifier a new value, named $\log k_w$ can be derived. However, the linearity of the linear slope and therefore the accuracy of this method is only valid between $\log k$: –0.5-1.5 values.[70]

In the early 1990's papers have introduced a new value in order to achieve better correlation of various compounds sets.[71] The chromatographic lipophilicity index φ_0 is calculated from the $\log k_w$ value by the equation

$$\varphi_0 = \frac{-\log k_w}{S} \qquad (3)$$

where 'S' is the slope obtained by plotting $\log k$ values as a function of the percentage of the organic modifier.[34,35] The φ_0 value is defined as the percentage of organic modifier in the mobile phase, where the $\log k$ value is zero for a given compound, or in other words, where the distribution of a compound is equal in the stationary and the mobile phase.[72] The retention time at φ_0 is therefore exactly twice the column dead time. The φ_0 value has been introduced in 1993 to overcome correlation problems of $\log k_w$ with $\log P$ occurring by the usage of structurally different types of compounds.[71,72]

1.10. Determination of the Partition Coefficient

The classical value to describe the lipophilicity of compounds is the so called $\log P$ (sometimes referred as $\log P_{o/w}$ or $\log P_{oct}$) value. The $\log P$ parameter is constituted as the distribution of a neutral compound between 1-octanol and water phases in contact (equation (4).

$$\text{Log P} = \log \frac{c_{\text{octanol}}}{c_{\text{water}}}$$

A positive $\log P$ value means that the 1-octanol phase contains the majority of the compound, which itself is lipophilic. If the value is zero, there is an equal distribution of the substance in both phases. A good log P value for a prospective drug is between $\log P$ = 0.5 to 3.5.[36] There are several ways for the determination of the $\log P$ value.[69,73-75] The classical method is the shake flask method followed by UV/Vis detection. This technique exhibits six orders of magnitude ranging from -2 to $+4$, although a slightly different range from -3 to $+3$ was also reported.[69,73] In this case, the concentration of the analyte is measured in both the aqueous and the 1-octanol phase. Beside UV/Vis several alternative detection methods have been proposed, such as ICP-MS, ICP-AES and FAAS.[76] In such cases, the $\log P$ value can be indirectly derived by measuring the concentration of the analyte in the aqueous stock solution and in the water phase after shaking by the equation 5:

$$\text{Log P} = \log \frac{c_{\text{stock}} - c_{\text{water}}}{c_{\text{water}}} \qquad (5)$$

If a compound's lipophilicity is depending on the pH of the aqueous solution, the $\log P$ value is replaced by the $\log D$ value. The correct notation is: $\log D_{pH}$

Determining the $\log P$ value by the shake flask method shows several drawbacks. First of all, it is a very time consuming method, since after bringing the compound into the system it has to be ensured that the system will be measured at equilibrium. Another point is the obtrusive smell of 1-octanol. Furthermore, the 1-octanol/water layer may not always be well-defined.

1.11. Timeline of log P measurements

Table 1 features a timeline of $\log P$ determinations of platinum(IV) drugs carried out by various techniques.

Table 1: Timeline of log P measurements

Date	Technique	Sample preparation	Sample conditions	Compounds	Output	Reference
2011	ICP-MS (indirect)	Shake Flask		Platinum(IV)	$\log P$ & $\log P$ (*via* $\log k_w$)	Varbanov et al.[43]
2011	ICP-MS (indirect)	Shake Flask	-	Platinum(IV)	$\log P$	Reithofer et al.[77]
2011	RP-HPLC	-	-	Platinum(IV)	$\log P$ (*via* $\log k_w$)	Platts et al.[78]
2011	AAS (indirect)	Shake Flask	-	Platinum(II)	$\log P$	Buß et al.[23]
2008	ICP-AES (direct)	Shake Flask	0.9% (w/v) NaCl	Platinum(II)	$\log P$	Tetko et al.[79]
2006	RP-HPLC	-	-	Platinum(II)	$\log P$ (*via* $\log k_w$)	Platts et al.[75]
2004	ICP-AES (indirect)	Shake Flask	-	Platinum(II)	$\log P$	Kim et al.[80]
2004	GFAAS (direct)	Shake Flask	0.15 M KCl	Platinum(II) and Platinum(IV)	$\log P$	Hall et al.[81]
2002	FASS (direct)	Shake Flask	0.15 M NaCl	Platinum(II)	$\log P$	Robillard et al.[82]
2000	Calculation	-	-	Platinum(II) and Platinum(IV)	$\log P$	Platts et al.[83]
2000	FAAS (direct)	Shake Flask	Saline water	Platinum(IV)	$\log P$	Lee et al.[84]
2000	ICP-MS (direct)	Shake Flask	0.9% (w/v) NaCl	Platinum(II) and Platinum(IV)	$\log P$	Screnci et al.[85]
	HPLC-ICP-MS		0.9% (w/v) NaCl	Platinum(II) and Platinum(IV)	$\log k_w$	
1994	FAAS (direct)	Shake Flask	Saline water	Platinum(II) and Platinum(IV)	$\log P$	Yoshida et al.[86]
1991	AAS (direct)	Shake Flask	0.15 M NaCl	Platinum(II)	$\log P$	Souchard et al.[87]

2. Experimental Part

2.1. Chemicals and Reagents

Milli-Q water (18.2 MΩ cm, Milli-Q Advantage A10, Darmstadt, Germany) was used for all dilutions. Nitric acid (65% p.a.) was purchased from Fluka (Buchs, Switzerland) and further purified before usage in a quartz sub-boiling point distillation unit (Milestone-MLS GmbH, Leutkirch, Germany). Platinum, indium and rhenium standards were obtained from CPI International (Amsterdam, The Netherlands). Uracil (99%) and potassium iodide were bought from Fluka and Sigma-Aldrich, respectively. 1-Octanol (HPLC grade) was purchased from Fluka. Triflouroacetic acid (peptide grade) was obtained from Iris Biotech.

2.2. Log k – sample preparation

The compounds were dissolved in 1 mL methanol : water (1 : 1) yielding a final concentration of 0.5 mmol/L. The samples were vortexed and sonicated for 15 min. Afterwards, the samples were filtered through a 0.45 µm filter (Minisart RC 25, Sartorius AG, Göttingen, Germany) into a 1.5 mL HPLC vial (Schnappringflasche, Markus Bruckner Analysentechnik, Linz, Austria). Uracil and potassium iodide were used in order to evaluate the column dead time. The samples were spiked with 200 µL of a filtered aqueous uracil solution (c = 1 µmol/L). Potassium iodide was used as an additional standard *via* an extra run. The capacity factors ($\log k$) were determined using an UHPLC instrument (UltiMate3000 RS, Dionex, Bremen, Germany) and a Poroshell SB-120 column (150 x 2.1 mm, 2.7 µm particle diameter). The $\log k$ values were derived by the equation 2: $\log k = \log ((t_r - t_0)/t_0)$. For each compound, isocratic methods were used with at least three different mobile phase compositions and each method was run in duplicate, except for reference platinum(II) compounds. Furthermore, $\log k_w$ values were obtained by extrapolating the $\log k$ to 100% aqueous mobile phase. The φ_0 value can be derived from the $\log k_w$ by equation 3. A minimum of $R^2 = 0.98$ was obtained in all $\log k_w$ determinations. The chromatographic and instrumental parameters are listed in Table 2.

Table 2: HPLC – parameters for log k determination

System Manufacturer	Dionex
Model	UltiMate 3000 RS
Column Manufacturer	Agilent
Column Name	Poroshell 120 SB-C18 (2.1 x 150 mm; 2.7 Micron)
Column Type	Reversed Phase
Mobile Phase	MeOH/Water (0.1% TFA)
Mobile Phase Composition Range	5-95% MeOH
Elution Mode	Isocratic
Flow Rate	0.20 [mL/min]
Detector Wavelength	210 [nm]
Auto Sampler Temperature	23 [°C]
Column Temperature	23 [°C]
Collecting Data Rate	5 [Hz]

2.3. Log P – Sample preparation

Solutions of 1-octanol saturated with water and water saturated with 1-octanol were prepared by stirring for one week prior to use. Samples were dissolved in 1.5 mL water saturated with 1-octanol at a final concentration of *approx.* 0.5 mmol/L and the initial and output weights were recorded. The samples were vortexed and subsequently sonicated for 15 min. Afterwards the stock solutions were filtered through a 0.45 µm filter. The filtered stock solution was divided into three parts. One part was used for HPLC measurements, while the second was diluted gravimetrically with 1% HNO_3 for ICP-MS measurements yielding Pt concentrations lower than 20 µg/L. Equal amounts of the third part of the stock solution and 1-octanol (700 µL each) were pipetted into a vial (TwistTop Vials, Sorensen BioSciene Inc., Salt Lake City, USA) and shaken mechanically for 1 h at 60 rpm at RT. Afterwards the vials were centrifuged at 10000 rpm for 10 min. 500 µL of the 1-octanol phase were directly pipetted into an HPLC vial. The 1-octanol phase was carefully removed from the water phase. The water phase was split into two parts. The first was used for HPLC measurements, while the second was again diluted with 1% HNO_3 for Pt determination with ICP-MS in the same manner as described before. A platinum standard stock solution was used to prepare eight calibration standards ranging from 0.05 µg/L to 20 µg/L. Since it is not possible to measure the concentration in the 1-octanol phase with ICP-MS directly, it has to be calculated via the stock solution and the water phase by the equation: $c_{Octanol} = c_{Stock} - c_{water}$. Therefore, the $\log P$ value is calculated by equation 5: $\log P = \log ((c_{Stock} - c_{water})/c_{water}))$.

For HPLC measurements of the stock-solution, the 1-octanol and the water-phase were injected onto the column separately. Although the measurement of the 1-octanol and the water-phase would be sufficient for the $\log P$ calculation, the measurement of the stock solution gives additional certainty, since the summation of the area values of the 1-octanol and the water-phase should result in the area value of the stock solution. When this was not the case the result was rejected. This direct method allows the $\log P$ calculation by the common equation: $\log P = \log\ (c_{octanol}/c_{water})$. For the aqueous solutions an isocratic mode was applied, whereas for the 1-octanol phase a multigradient method was developed to remove all the 1-octanol from the column. The multigradient method starts with an isocratic elution, based on the φ_0-value of a given compound. After the isocratic elution of the compound in the 1-octanol phase the organic modifier was increased to 95% and held for at least 20 min. Finally, the column was flushed for 12 min with the initial MeOH/water ratio to equilibrate the column. All measurements were repeated at least twice. The HPLC parameters were the same as in Table 2, only the collecting rate was increased to 100 Hz in order to increase accurateness of the peak shape.

ICP-MS measurements were carried out using an ICP-MS Agilent 7500ce (Agilent Technologies, Waldbronn, Germany) equipped with a CETAC ASX-520 autosampler (Nebraska, USA) and a MicroMist nebulizer at a sample uptake rate of about 0.25 mL/min. Rhenium served as internal standard for platinum. The Agilent MassHunter software (Workstation Software, Version B.01.01, 2012) was used for data processing. The instrumental ICP-MS parameters are summarized in Table 3. Figure 20 gives an illustration of the sample preparation scheme.

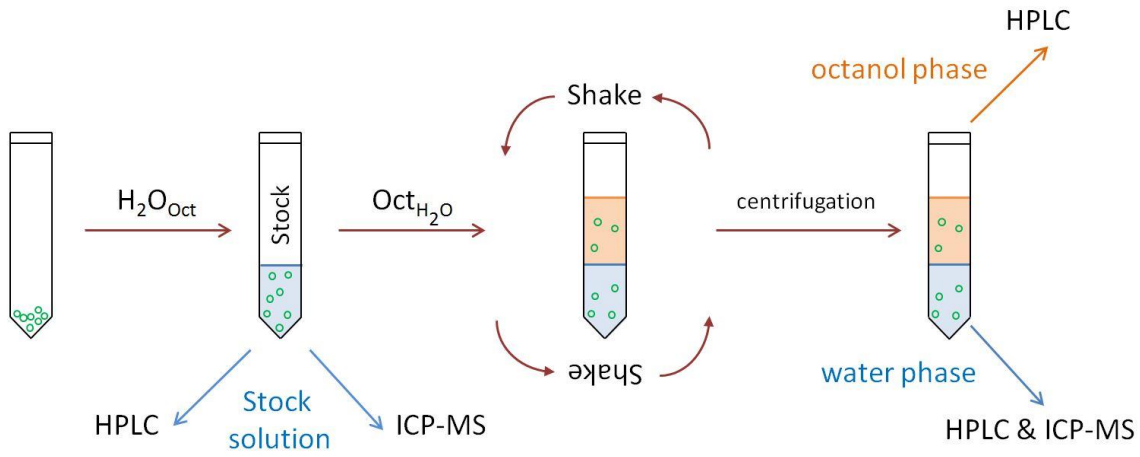


Figure 20: Scheme of Log P sample preparation and determination

Table 3: ICP-MS – parameters for log P determination

RF-Power	1500 [W]
Cone Material	Nickel
Carrier Gas	0.96 (Argon) [L/min]
Make up Gas	0.16 (Argon) [L/min]
Plasma Gas	15 (Argon) [L/min]
Recorded isotopes	^{185}Re, ^{194}Pt, ^{195}Pt
Dwell time	0.1 [s]

2.4. Log P – Buffer experiments

Five compounds containing an acidic moiety were evaluated in dependence of buffer conditions. The buffers were prepared with either phosphate or acetic acid buffer at a final concentration of 10 mmol/L using MQ water. Phosphate buffers were used for pH = 7.4, 6.2, 2.5 and 1.7, while acetic acid buffers were used for pH = 5.6, 4.7, 4.2 and 3.7. All experiments were carried out using HPLC and are therefore directly measured log D values. The sample preparation was identical to chapter 2.3.

3. Results and Discussion

Lipophilicity is a crucial value for every prospective drug, since a lipophilic drug is able to cross cell membranes *via* passive diffusion more easily compared to hydrophilic analogues. The log P value of a prospective metallodrug should be within $\log P$ = 0.5-3.5.[36]

In a first step two dead time markers, uracil and KI, are evaluated and discussed. Then, the lipophilicity parameters $\log k_w$, φ_0 and $\log P$ are discussed. Furthermore five compounds with an acidic moiety were investigated in buffer experiments ($\log D$ values).

In total, the $\log k_w$ of 79 platinum compounds was determined, including 6 platinum(II) and 73 platinum(IV) compounds were analyzed. Furthermore, the $\log P$ of a subseries of 28 compounds was determined. The entire lists of results are shown in Appendix 5.2.

3.1. Determination and correlation of log k_w and φ_0

3.1.1. Comparison of uracil and KI as dead time markers

Uracil and KI are compared and evaluated for their suitability as dead time markers for the determination of $\log k_w$. A complete list of retention times can be found in Appendix Table 10: Retention times of uracil and KI.

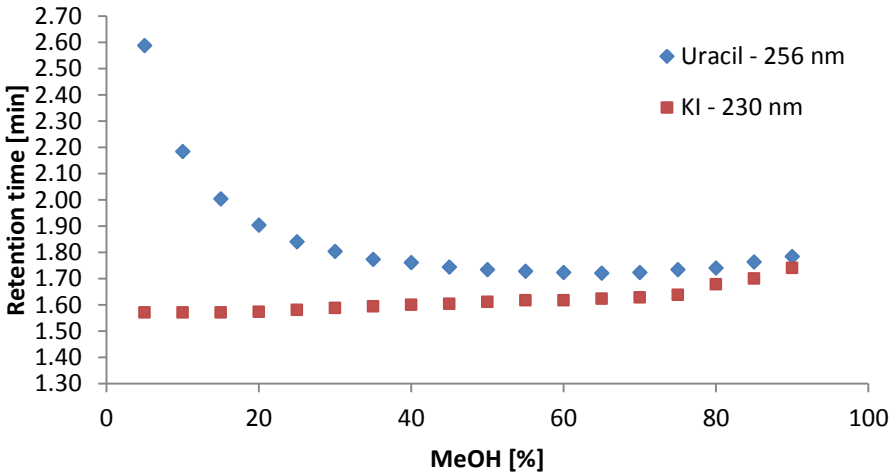


Figure 21: Comparison of the retention times of uracil and KI at different percentages of MeOH in the eluent

Figure 21 shows the two dead time markers, measured at their absorption maxima at 256 nm and 230 nm for uracil and KI, respectively, and at different compositions of the organic modifier in the eluent. The retention times were obtained by isocratic experiments at a fixed percentage of organic modifier. There are three major differences between the two dead time markers.

First, KI displays relatively constant retention times in a broad range of mobile phase compositions. Second, KI elutes generally faster than uracil independent on the methanol concentration. Third, the retention time of uracil increases drastically at lower methanol concentrations by approximately 60 s. KI shows ionic character and will not interact with the highly non-polar C18-column at all. Furthermore, KI is better soluble in water than MeOH and therefore the retention time increases slightly by using more organic modifier in the mobile phase. On the other hand the retention time of uracil increases significantly below 25% organic modifier. This suggests that the distribution equilibrium between the mobile and the stationary phase is shifted in favour of the stationary phase. Overall, the retention time increases slightly above 80% MeOH.

Consequently, KI seems to be the more suitable dead time marker because of its lower retention time compared to uracil. This allows the determination of more hydrophilic compounds. Therefore, while the experimentally determined $\log k$ value using uracil may not be within the linear range of –0.5 to 1.5[70], the calculated $\log k$ value using KI may be. Therefore, KI is expected to display a larger linear spectrum, however, it can potentially coordinate to aquated Pt-complexes, which must be kept in mind during the chromatographic runs. The retention time of KI was measured externally in this study.

3.1.2. Calculation of $\log k_w$ and φ_0

Before the comparison of $\log k_w$ and φ_0 is discussed, the actual calculation of these two characteristics are explained on the basis of KP1916.

Table 4: Retention times of uracil and KP1916 & calculated log k_w values

MeOH [%]	KI [min]	KP1916 [min]	Log k
60	1.727	3.160	–0.081
50	1.697	5.253	0.321
40	1.687	11.817	0.778
30	1.677	35.613	1.30

Table 4 shows the retention times of uracil and KP1916 measured at different mobile phase compositions. According to $\log k = \log((t_r - t_0)/t_0)$ (equation (2)) the $\log k_w$ value of the compound can be observed by plotting the percentage of MeOH vs. the resulting $\log k$ value (Figure 22).

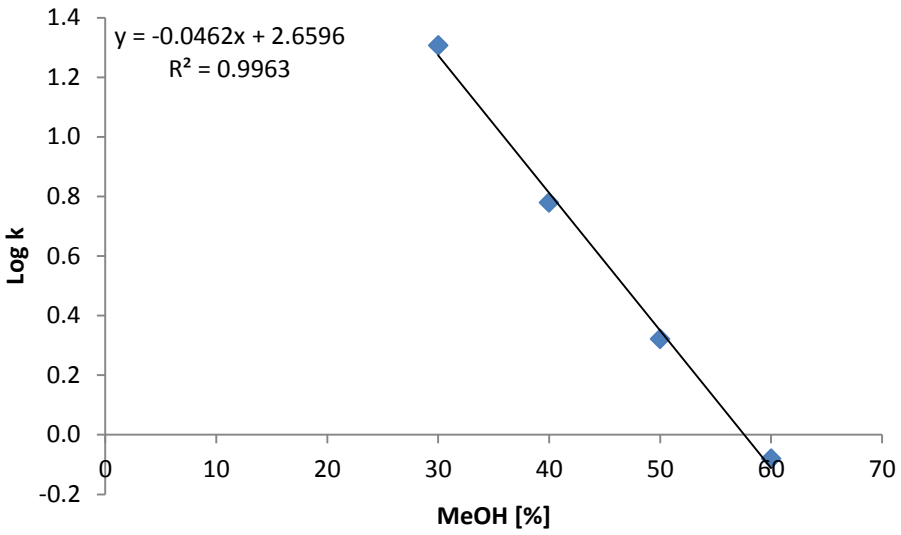


Figure 22: Plot of log k vs. % of MeOH

The resulting $\log k_w$ value is just the intercept of the line with the y-axis in Figure 22, therefore $\log k_w = 2.63$. It can be easily seen that this value is obtained by extrapolation. The calculation of the φ_0 value is according to equation (3) $\varphi_0 = -\log k_w / S$, with $\log k_w = 2.63$ and $S = -0.046$. The resulting φ_0 value is the intercept of the line with the x-axis, $\varphi_0 = 57.10$. It can be seen that the φ_0 value lies within the plotted line. This is the major difference between the extrapolated $\log k_w$ value and the φ_0 value.

3.1.3. Comparison of log k_w and φ_0 determined with uracil and KI

This chapter features the correlation of $\log k_w$ and φ_0 in respective of their different calculation by using uracil or KI as dead time markers.

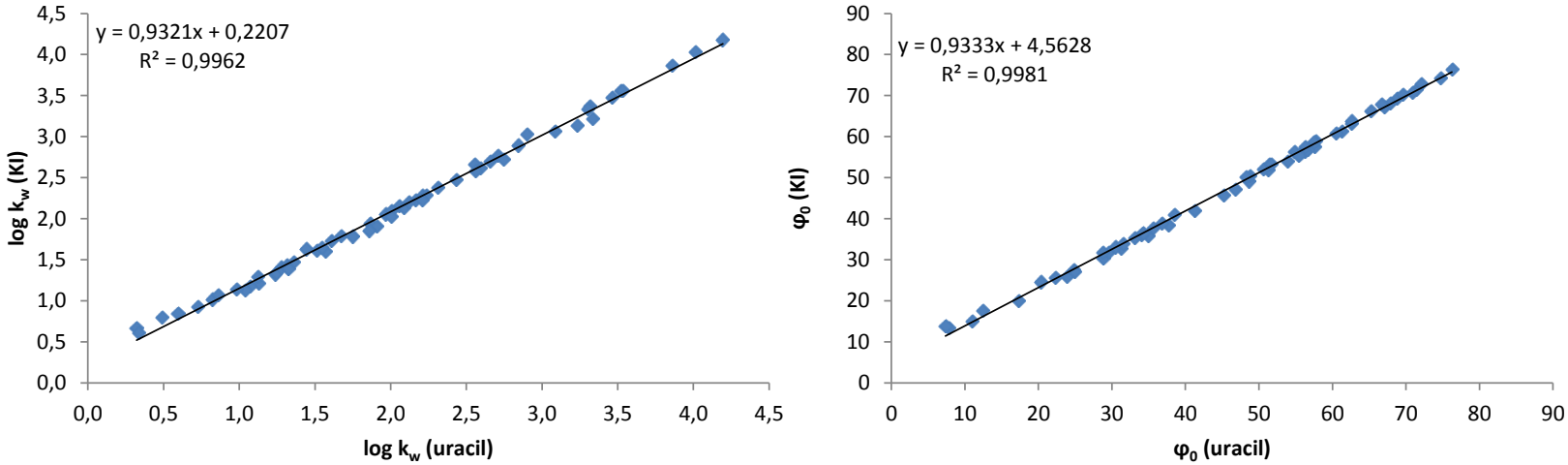


Figure 23: Correlation of log k_w and φ_0 with uracil and KI of 67 platinum(IV) complexes

Figure 24 shows the correlation of $\log k_w$ values (*left*) as well as φ_0 values (*right*) calculated with uracil and KI as dead time marker for 67 platinum(IV) compounds at 210 nm. The φ_0 values were calculated with uracil and KI, which were derived by equation (3): $\varphi_0 = -\log k_w / S$. Platinum(II) compounds and substances with an acid moiety are not included into the correlation. The correlation is linear with an R^2 of 0.9962 for $\log k_w$ and R^2 = 0.9981 for φ_0. A small bend for low $\log k_w$ values, and less distinctive for φ_0 values, can be observed. This can be explained by the previously mentioned tendency of uracil to display increased retention times at low methanol concentration. However, the log-scale has a smoothing effect and therefore, the difference in $\log k_w$ values might not be as pronounced as expected from Figure 21. The regression of the φ_0 values increases slightly, compared to the $\log k_w$ values, to R^2 = 0.9981. The two curves show a slight offset, since they should run through the origin. However, this can as well

be explained by the consideration with uracil above. In practical terms: as long as a $\log k_w$ value (and therefore a φ_0 value) is derivable in its range from –0.5 to 1.5, both dead time markers can be used. However, for more hydrophilic components uracil may be problematic. Therefore, KI is recommended for measurements of $\log k_w$ and φ_0 as the dead time marker.

3.1.4. Comparison of log k_w vs. φ_0

The correlation of $\log k_w$ vs. φ_0 for the same set of compounds appears to be quadratic polynomial, with an acceptable fit of R^2 = 0.968 for uracil and to R^2 = 0,953 for KI (Figure 24). A similar observation was already made by Schoenmakers.[72,88] In a more recent publication, it was noted that the choice of organic modifier has a considerable impact on the $\log k_w$ value of purely organic molecules with respect to the regression model used.[89] MeOH emerged as the favoured organic modifier, which was largely independent of the regression type and was therefore also used in this study. Interestingly, the fit is slightly less accurate for KI as the dead time marker compared to uracil.

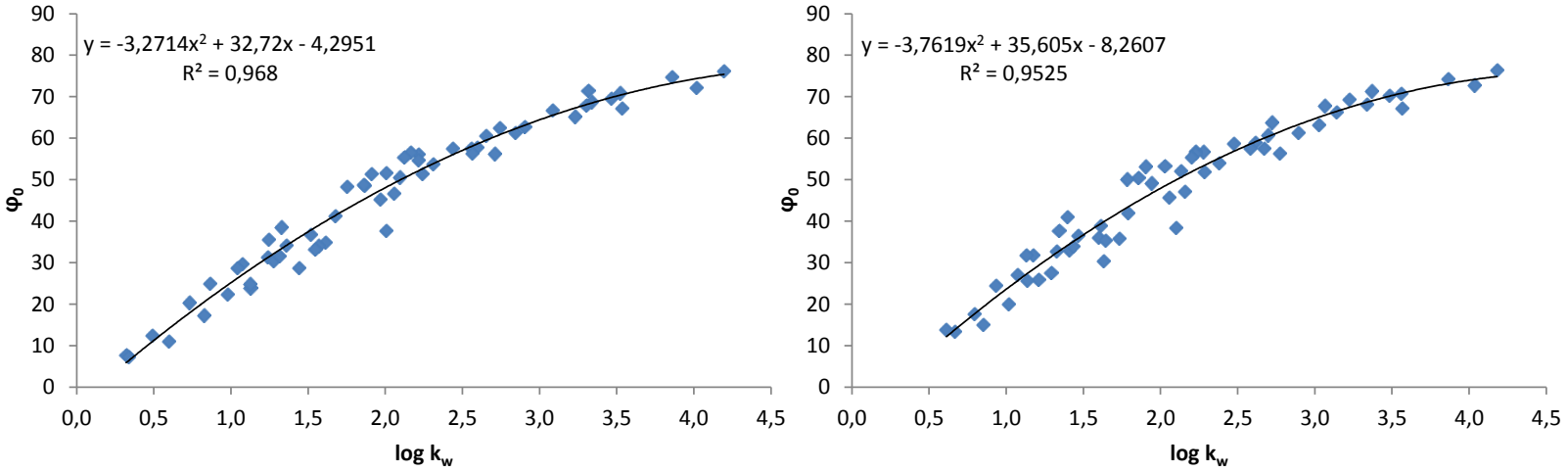


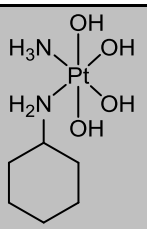
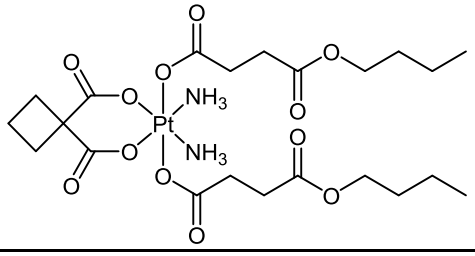
Figure 24: Log k_w vs. φ_0 - uracil (left) and KI (right)

3.2. Determination of log P

The experiments were carried out using the classical shake flask method. Components were dissolved in water which was presaturated with 1-octanol. This stock solution was then measured with HPLC (*via* absorbance area) and ICP-MS (*via* intensity of the platinum mass signal). Equal amounts of the stock solution and 1-octanol were then shaken mechanically for 1 h. After centrifugation, the phases were split into a 1-octanol and a water phase. While the water phase was measured with both techniques the organic phase could only be measured with HPLC. Therefore, the calculation of $\log P$ was performed either directly for HPLC ($\mathrm{Log\,P} = \log c_{octanol}/c_{water}$) or indirectly for ICP-MS ($\mathrm{Log\,P} = \log (c_{stock} - c_{water})/c_{water}$; chapter 1.10). Furthermore, $\log P$ data were also calculated indirectly for HPLC as well. This approach allows validation of (1) the two different detections techniques (UV/Vis and MS) and (2) the two different calculation methods of $\log P$ (direct and indirect).

The $\log P$ value was determined for 19 platinum(IV) anticancer drugs, and additionally for four platinum(II) complexes and five platinum(IV) compounds featuring carboxylic groups. The compounds were chosen according to their $\log k_w$ values, covering the entire lipophilicity range from the most hydrophilic ($Pt(NH_3)cha(OH)_4$) to the most lipophilic (HRVA035) compound (see Table 5). The results are given in Table 8. Furthermore, three reference compounds (carboplatin, satraplatin and HRVA101) with known $\log P$ value were chosen, in order to validate the shaking time of the classical shake flask method. These experiments were run in duplicate.

Table 5: Comparison of the most hydrophilic ($Pt(NH_3)cha(OH)_4$) and lipophilic (HRVA035) compounds investigated

Name/ Acronym	Structure	Log k_w KI	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct
$Pt(NH_3)cha(OH)_4$		0.76	22.52	–1.70 (±0.29)	–1.87 (±0.15)
HRVA035		4.18 (±0.159)	76.45 (±0.652)	1.06 (±0.16)	2.37 (±0.04)

3.2.1. Evaluation of the shaking time

The shaking time was optimized in a first step. This is crucial, since a too short shaking time will result in non-equilibration of the compound between the two phases. On the other hand a too long shaking time may entail partial hydrolysis of the compound. Fortunately, platinum(IV) complexes generally show negligible hydrolysis. Carboplatin was initially employed for this purpose, but turned out to be unsuitable with ICP-MS detection. Being a platinum(II) compound, carboplatin may partly hydrolyze during the shaking time period, which is also indicated by varying $\log P$ values in the literature ranging from –2.3[85] to –1.4[80] (see Appendix Complete Data List 5.2). This observation can be also referred to the fact that carboplatin is too hydrophilic to gain valid results with the indirect method using ICP-MS.

Satraplatin turned out to be the better choice since the platinum(IV) complex is more inert with respect to hydrolysis. The shaking time was evaluated from 1–24 h and measured using both the direct HPLC as well as the indirect ICP-MS methods. HPLC and ICP-MS yielded the same results for satraplatin and $\log P$ values of *approx*. 0.25 were determined. An exception is the ICP-MS measurement after 24 h, in which a $\log P$ of 0.11 was determined. As the $\log P$ values remain constant from 1–4 h, a shaking time of 1 hour seems sufficient and was used for the shake flask method.

In a next step, the compound HRVA101 was used to validate the $\log P$ result of the 1 h shaking time using HPLC and ICP-MS methods with the literature $\log P$ value. Again, both methods performed well and the determined average $\log P$ of –0.33 corresponding to the literature value (Table 6).

Table 6: Shaking Time evaluation using carboplatin, satraplatin and HRVA101

Acronym	Shaking Time [min]	HPLC [log P] (direct)	ICP-MS [log P]	Literature [log P]
Carboplatin	15	–1.87	–1.60	Ranging from –2.3[85] to –1.398[80]
	30	–1.86	–0.90	
	60	–1.88	–2.68	
	120	–1.87	–1.75	
Satraplatin	60	0.24	0.23	–0.16 (±0.16)[85] –0.14[78]
	120	0.26	0.24	
	240	0.25	0.23	
	1440	0.26	0.11	
HRVA 101	60	–0.34 (±0.02)	–0.35 (±0.02)	–0.32 (±0.01)[77]

3.2.2. Log P correlation HPLC vs. ICP-MS

In the following chapter the log P correlation of HPLC and ICP-MS will be discussed. Due to the different detection methods (UV/Vis and MS) a cross validation between these two is possible. Furthermore, since ICP-MS determination requires an indirect calculation of log P, a comparison and correlation of direct and indirect calculation can be performed.

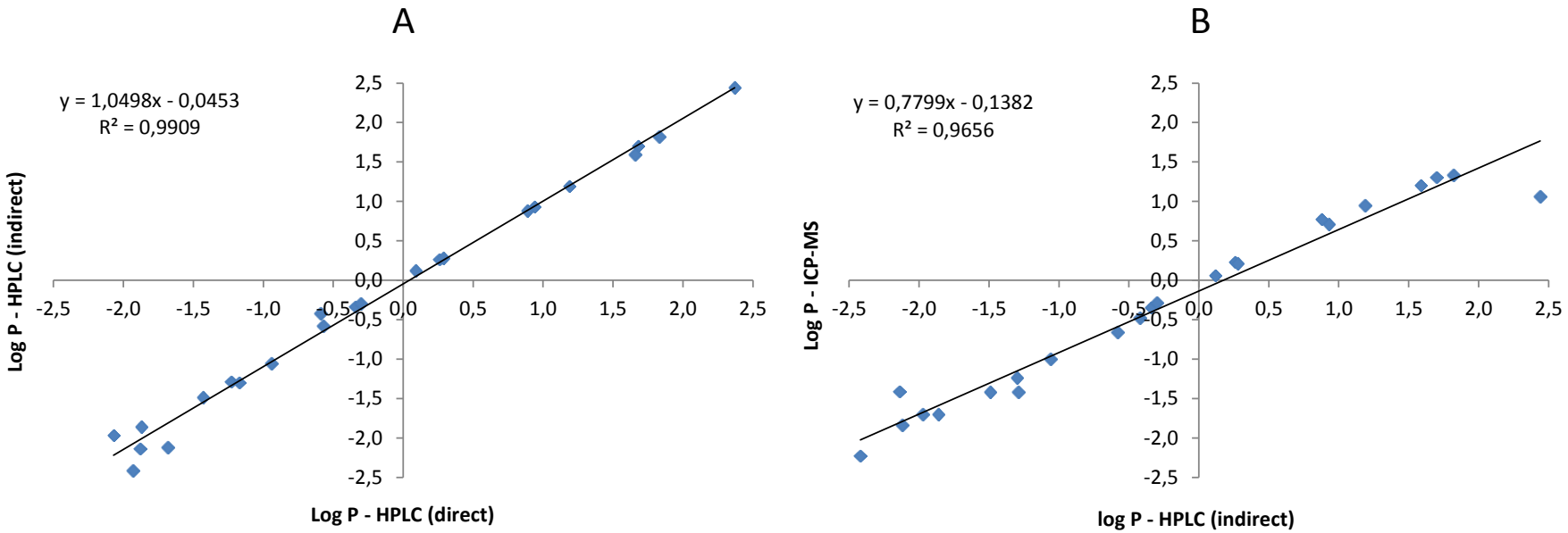


Figure 25: (A) Log P correlation of HPLC (direct) vs. HPLC (indirect) and (B) HPLC (indirect) vs. ICP-MS

Figure 25 (A) shows the correlation of log P values of 19 platinum(IV) compounds measured directly and indirectly by HPLC. The correlation shows a satisfactory $R^2 = 0.991$. Since the slope is nearly 1 and the linear approximation goes almost through the origin the calculation methods lead to basically

the same results. The greatest variances are found for hydrophilic compounds. It seems that the indirect measurement fails to yield accurate results in particular for hydrophilic substances where the c_{stock} and c_{water} approach the same value. For lipophilic substances, however, the chromatographic method remains linear to the most lipophilic substance tested.

Figure 25 (B) shows the correlation of the same compounds for the indirect HPLC and ICP-MS methods. It can be seen that although the correlation is quite high with $R^2 = 0.966$ the results differ from each other quite drastically. A hypothetical $\log P$ of 1.00 for HPLC (indirect) would lead to 0.64 at the ICP-MS. Again, the indirect method is problematic to a certain extent for hydrophilic compounds. In contrast to the comparison between the direct and indirect HPLC methods, which were linear for lipophilic compounds, the ICP-MS method seems to level off.

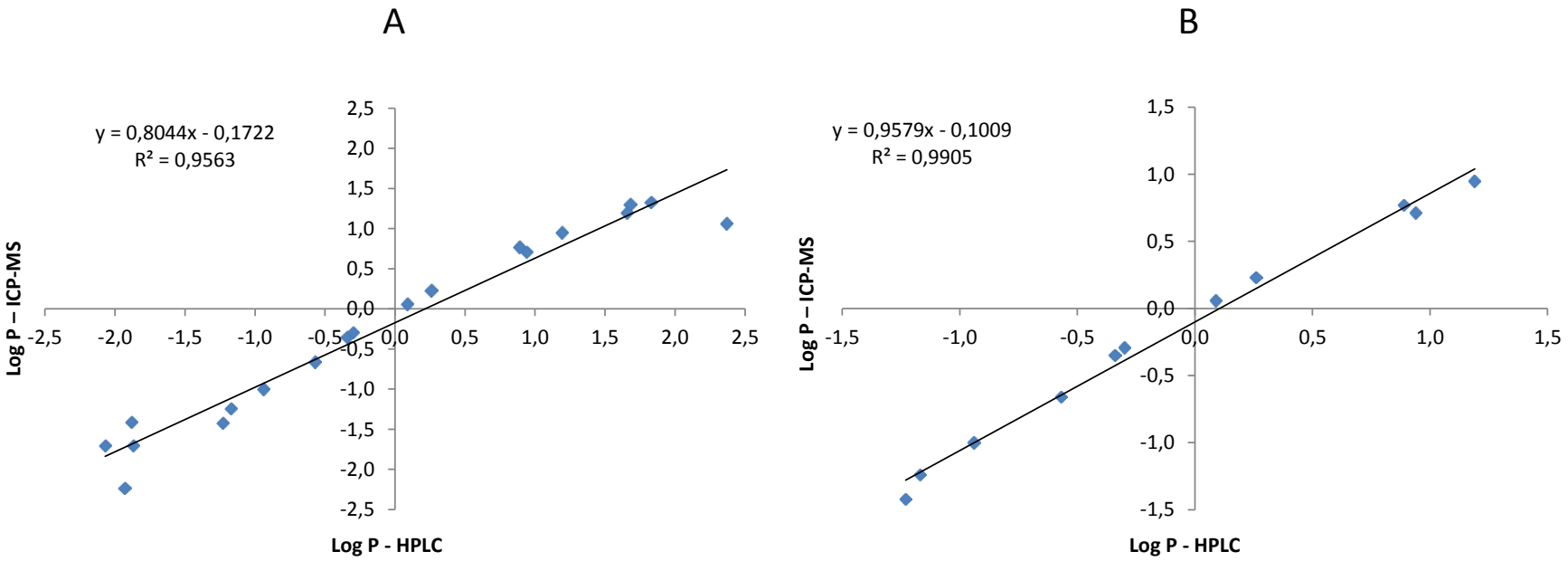


Figure 26: Log P (HPLC, direct) vs. Log P (ICP-MS) correlation. (A) All 19 compounds and (B) compounds within the linear range of –1.5 to 1.3 for both methods (11 substances)

Figure 26 (A) shows the correlation of 19 platinum(IV) complexes, measured with HPLC (direct) and ICP-MS (indirect). The overall correlation is quite high with an $R^2 = 0.956$. However, HPLC and ICP-MS do not lead to the same results, since the slope is 0.8 and the intercept is –0.17. A hypothetical $\log P$ of 1.00 resulting from HPLC would be 0.63 for ICP-MS. It can be clearly seen that at $\log P > 1.3$ and $\log P < -1.5$ the linearity fails between the two methods. As can be seen in Table 8, it is not possible with ICP-MS to determine the $\log P$ value of very hydrophilic and lipophilic compounds properly. For hydrophilic compounds the limitation is due to the indirect measurement. On the other hand, lipophilic compounds such as HRVA035, show unexpected results taking into consideration its $\log k_w$ value. It could be that the lipophilic compounds are adsorbed to the plastic vials, which were used for the ICP-MS autosampler and that adsorption is more pronounced at the high dilutions required for ICP-MS analysis. It is interesting to note that the chromatographic log P determination seems to allow for a broader linear range compared to the ICP-MS in particular for lipophilic compounds.

Because of these restrictions for hydrophilic and lipophilic compounds when correlating the HPLC with ICP-MS methods, it seems necessary to limit the linear ranger to $-1.5 < \log P < 1.3$. Figure 26 (B) shows the correlation of the remaining 11 compounds within this range featuring an increased fit ($R^2 = 0.991$). In this case the hypothetical compound of $\log P = 1.00$ for HPLC would lead to $\log P = 0.86$ for ICP-MS.

Several conclusions can be derived from the correlations of the different detection methods (UV/Vis and ICP-MS). The correlation between the direct and the indirect HPLC methods with the indirect ICP-MS method is satisfactory with $R^2 = 0.95$ (Figure 25 (A)). The difference between the direct and indirect HPLC methods is only marginal. Second, the indirect method for determining $\log P$ values is valid for both techniques if the log P value is above $\log P = -1.5$. At $\log P < -1.5$ the indirect method is not suitable because the differences between the concentration in the stock and water phase becomes as small to approach the method error. However, HPLC is the method of choice for the lipophilic compounds because it seems that above $\log P = 1.3$ the adsorption effects for the ICP-MS measurement become significant.

In practical terms the correlation of both techniques is valid within $\log P = -1.5$ and 1.3. Within this range, the log P of the indirect ICP-MS method can be calculated from the direct HPLC method *via* to following equation:

$$\text{Log P (ICP} - \text{MS)} = 0.958 \cdot \log \text{P (HPLC, direct)} - 0.101,\ R^2 = 0.991$$

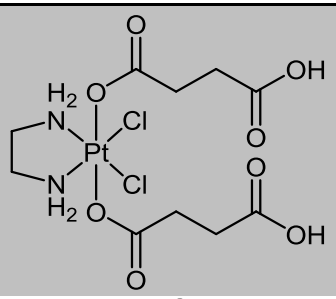
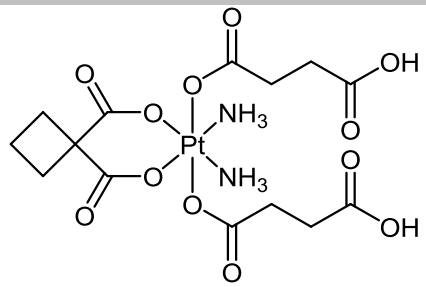
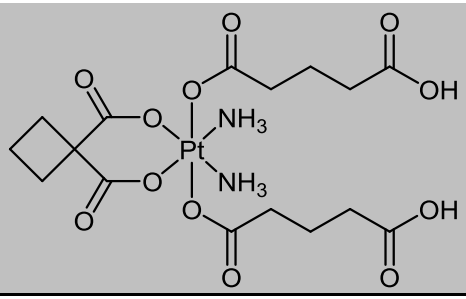
Below values of $\log P = -1.5$, it is not possible to derive $\log P$ values with ICP-MS and at log P < –2.0 both techniques cannot be recommended. For values higher than $\log P = 1.3$, the $\log P$ values determined with ICP-MS seem to reach a plateau, probably due to the fact that lipophilic compounds are adsorbed on the vials. For HPLC even higher $\log P$ values of $\log P = 2.4$ can be determined, which corresponds to the most lipophilic substance tested. In summary, ICP-MS offers a magnitude of roughly 3 units (from –1.5 to 1.3), while HPLC offers a better range of at least 4 magnitudes (from –2.0 to at least +2.4).

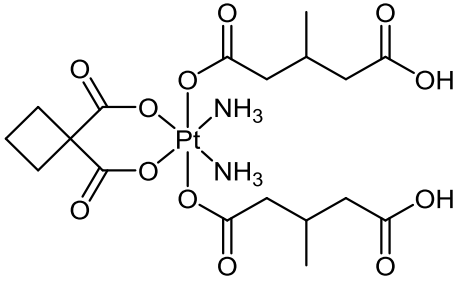
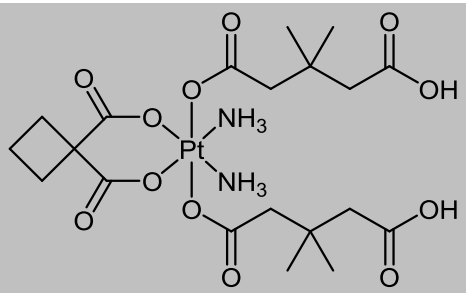
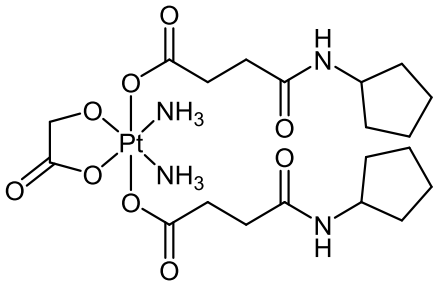
3.2.3. Determination of log D at different pH values – buffer experiments

This subchapter investigates five acid complexes at different pH of the water phase commonly referred as buffer experiments. If a compound's lipophilicity is depending on the pH of the aqueous solution *e.g.* due to the existence of an acidic moiety, the $\log P$ value is replaced by the $\log D$ value. The correct notation is: $\log D_{pH}$. Table 7 shows the complete list of the buffers experiments. All buffers were prepared at a final concentration of 10 mmol/L using either phosphate buffer (pH = 7.4, 6.2, 2.5 and 1.7) or acetic acid buffer (pH = 5.6, 4.7, 4.2 and 3.7). The unbuffered water exhibits a pH of around pH = 6.0, and by dissolving e.g. the component HRVA045B to a final concentration of 0.5 mmol/L the solution has an effective pH of 3.8. Consequently all the $\log D^*$ results should be approximately at the same level as the $\log D_{3.7}$ measurement, which is true for all measured complexes, with slightly deviance for HRVA034 and HRVA026. All experiments were carried out using RP-HPLC and are therefore $\log D$ values measured by the direct method. In comparison, the unbuffered $\log D$ values are given as well and are marked as $\log D^*$, since the exact pH remains unknown but should be around 3.8.

The compound Lust004 was barely detectable without buffer, since it is very hydrophilic with a $\log D^*$ of –1.87. However, in our buffer experiments it was not detectable at all. As reference substance KP2016, which has no acidic group, was used. It resulted throughout the whole measurement at a constant level between a $\log P$ value of –0.30 and –0.25. The measurements were only performed once.

Table 7: Log P – buffer experiments

Name	Structure	Log D*	Log $D_{7.4}$	Log $D_{6.2}$	Log $D_{5.6}$	Log $D_{4.7}$	Log $D_{4.2}$	Log $D_{3.7}$	Log $D_{2.5}$	Log $D_{1.7}$
Lust 004		–1.87	-	-	-	-	-	-	-	-
HRVA026		–1.68	-	-	-	–2.91	–2.16	–1.82	–1.54	–1.56
HRVA045B		–1.43	-	-	-	–2.03	–1.69	–1.42	–1.26	–1.19

Name	Structure	Log D*	Log $D_{7.4}$	Log $D_{6.2}$	Log $D_{5.6}$	Log $D_{4.7}$	Log $D_{4.2}$	Log $D_{3.7}$	Log $D_{2.5}$	Log $D_{1.7}$
DoHo012		–0.59	-	-	–2.73	–1.36	–0.91	–0.56	–0.39	–0.35
HRVA034		0.29	-	–2.17	–1.34	–0.23	0.15	0.37	0.45	0.46
KP2016		–0.30	–0.27	–0.29	–0.27	–0.27	–0.27	–0.24	–0.28	–0.25

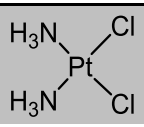
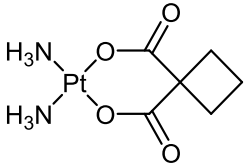
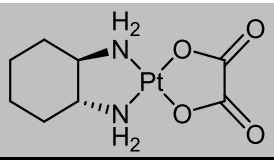
3.3. Evaluation of log k_w, φ_0 and log P with regard to their applicability to characterize the lipophilicity of platinum(IV) anticancer agents

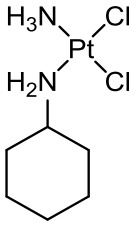
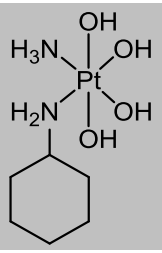
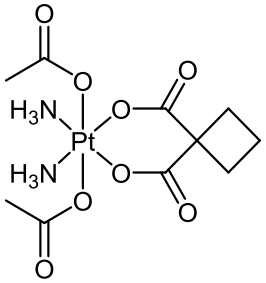
Table 8 features the chromatographic $\log k_w$ and φ_0 values, which are calculated with uracil (U) or KI as dead time markers. Moreover, three different $\log P$ values are shown. The first was determined by ICP-MS using the indirect method. The second and the third $\log P$ was determined by RP-HPLC using the direct and indirect method, respectively. The $\log P$ values of several platinum(II) complexes were determined, *i.e.* cisplatin, carboplatin, oxaliplatin and $Pt(NH_3)_2chaCl_2$. The investigated platinum(II) compounds show a very hydrophilic character and accurate $\log k_w$ values were only obtained for $Pt(NH_3)_2chaCl_2$. The retention times of these compounds were in general very close to the dead time markers. Carboplatin, oxaliplatin and $Pt(NH_3)_2chaCl_2$ showed $\log P$ values in the range of roughly –2.0 to 0 analyzed by HPLC and ICP-MS, while the $\log P$ of cisplatin could not be obtained. It was not possible to determine $\log P$ values below –1.5 by ICP-MS. The only platinum(II) compound which could be measured properly with ICP-MS was $Pt(NH_3)_2chaCl_2$. This may be due to the limitation of the indirect calculation method, which fails to calculate a $\log P$ value if the stock solution is approximately of the same concentration as the water phase. For instance, carboplatin has a very high standard deviation of ±0.65. It is most likely that these compounds lay on the edge or even beyond the method range for both values – $\log k_w$ *via* HPLC and $\log P$ *via* ICP-MS. In general, HPLC shows lower standard deviation of the values compared with ICP-MS. This can be an effect of the indirect method.

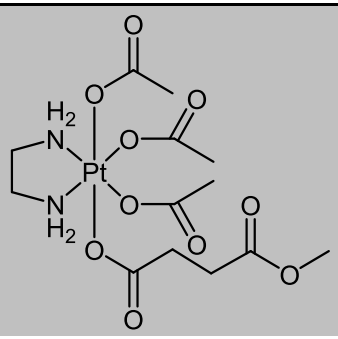
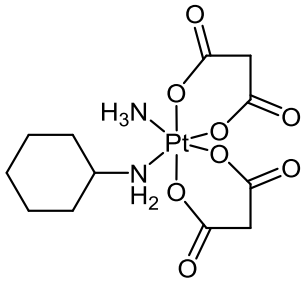
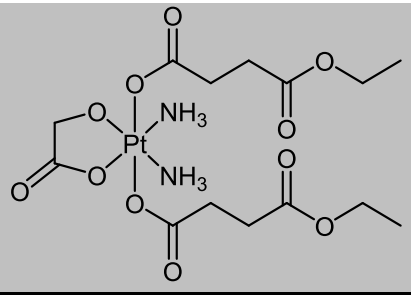
One crucial point for metal based complexes is hydrolysis. Platinum(IV) complexes are claimed to be stable, whereas platinum(II) complexes can undergo rapid loss of their leaving groups followed by formation of the respective aqua complexes. These are usually charged and therefore, more hydrophilic, which results in an enrichment in the water phase and lead to incorrect results. Hence, the measurement will result in a mean $\log P$ value of all generated species. Therefore the values measured for platinum(II) complexes using ICP-MS have to be treated with caution. An indication for this phenomenon is that literature values differ significantly for identical compounds. For example carboplatin shows $\log P$ values from –2.3[85] to –1.398[80]. More literature results are given in Appendix 5.2 Complete Data List.

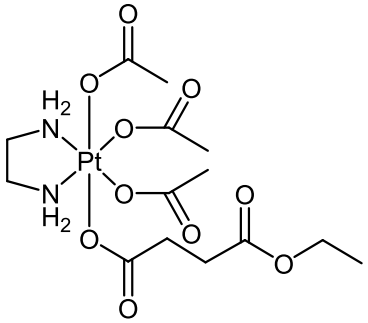
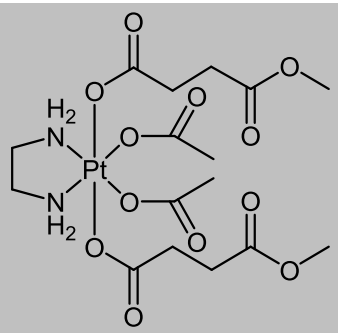
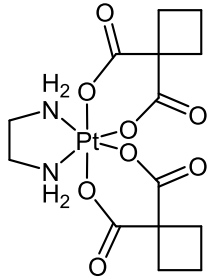
The $\log P$ values of the platinum(IV) complexes cover a broad range. The HPLC results range from < –2.0 up to almost 2.4. Again, the ICP-MS values for hydrophilic compounds could not be determined. As for lipophilic compounds the results of these two methods differ again from each other, it seems that the lipophilic compounds are adsorbed on the plastic vial from the autosampler and therefore lead to false results at the ICP-MS. Three substances (DoHo117, DoHo133 and DoHo102) show lower $\log P$ values than expected from their $\log k_w$ value. This could be related to their structure, since these three compounds exhibit the same platinum(IV) core.

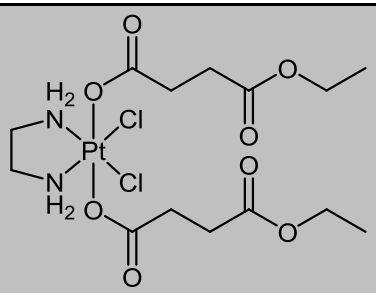
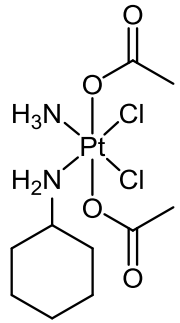
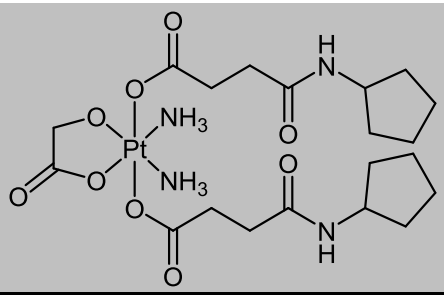
Table 8: Log P determination without buffer. Compounds marked as * are platinum(II) substances

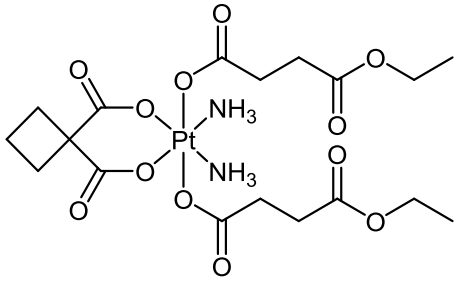
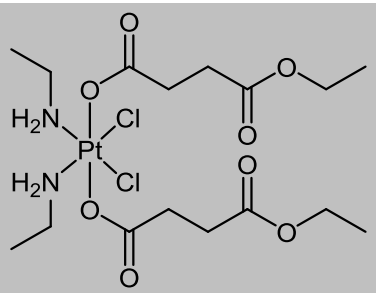
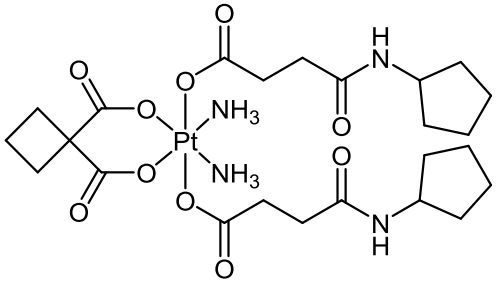
Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
1	Cis-Pt*		-	-	-	-	-		-
2	Carbo-Pt*		-	0.11	-	2.29	–1.66 (±0.65)	–1.98 (±0.15)	-
3	Oxali-Pt*		0.30	0.52	6.05	10.08	–1.37 (±0.15)	–1.88 (±0.17)	–1.45 (±0.31)

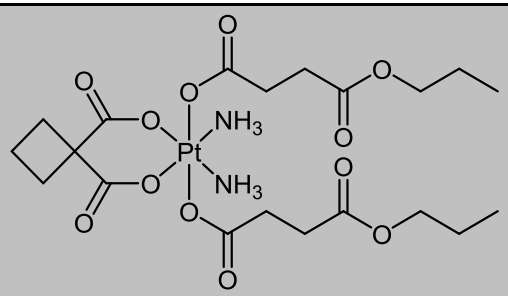
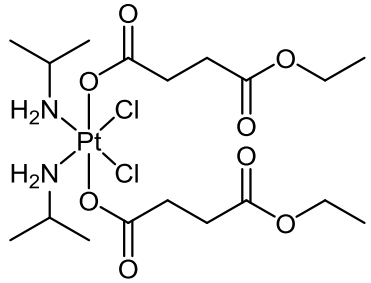
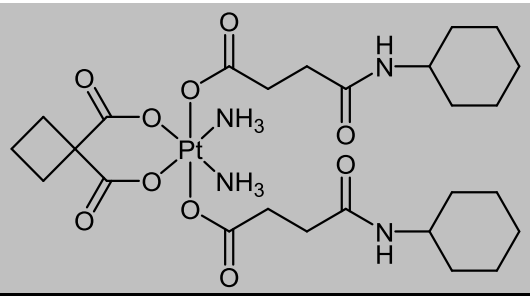
Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
4	$Pt(NH_3)_2chaCl_2$*		1.11	1.31	37.21	38.02	–0.11 (±0.14)	–0.09 (±0.03)	–0.03 (±0.13)
5	$Pt(NH_3)cha(OH)_4$		0.50	0.76	18.15	22.52	–1.70 (±0.29)	–1.87 (±0.15)	–1.86 (±0.10)
6	$Pt(NH_3)_2$ $CDBA(OAc)_2$		0.86	1.07	24.93	27.14	–1.42 (±0.06)	–1.23 (±0.00)	–1.29 (±0.00)

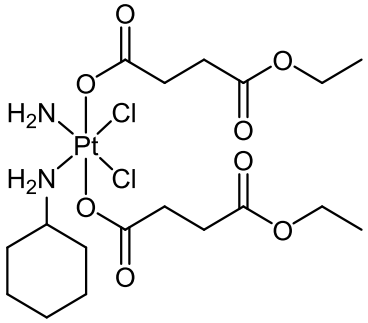
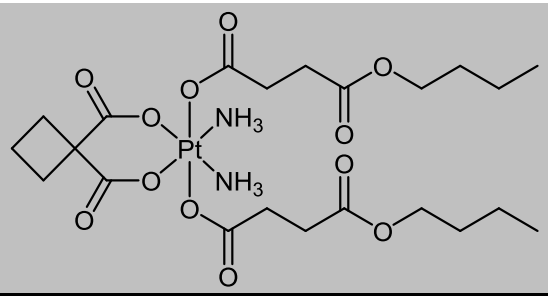
Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
7	DoHo117		0.98 (±0.067)	1.14 (±0.085)	22.34 (±0.127)	25.58 (±0.047)	–1.81 (±0.24)	–2.07 (±0.04)	–1.97
8	HRVA140		1.06 (±0.022)	1.19 (±0.021)	29.98 (±0.525)	32.66 (±1.327)	–1.00 (±0.02)	–0.94 (±0.01)	–1.06 (±0.05)
9	HRVA118		1.32 (±0.096)	1.39 (±0.119)	38.54 (±0.343)	40.99 (±0.102)	–1.24 (±0.07)	–1.17 (±0.01)	–1.30 (±0.10)

Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
10	DoHo133		1.36 (±0.052)	1.47 (±0.072)	34.23 (±0.022)	36.55 (±0.127)	–1.41 (±0.11)	–1.88 (±0.04)	–2.14
11	DoHo102		1.57 (±0.075)	1.60 (±0.004)	34.01 (±0.019)	36.08 (±0.030)	–2.23 (±0.23)	–1.93 (±0.10)	–2.42 (±0.65)
12	HRVA093		1.66 (±0.006)	1.77 (±0.002)	42.88 (±0.075)	44.66 (±0.026)	–0.66 (±0.01)	–0.57 (±0.01)	–0.58 (±0.01)

Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
13	HRVA101		1.86 (±0.006)	1.86 (±0.003)	48.77 (±0.036)	50.52 (±0.020)	–0.35 (±0.02)	–0.34 (±0.02)	–0.34 (±0.01)
14	Satra-Pt		2.12 (±0.027)	2.20 (±0.022)	55.44 (±0.292)	55.43 (±0.362)	0.23 (±0.01)	0.26 (±0.02)	0.26 (±0.05)
15	KP2016		2.21 (±0.025)	2.28 (±0.025)	56.17 (±0.708)	56.72 (±0.182)	–0.39 (±0.12)	–0.30 (±0.09)	–0.30 (±0.06)

Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
16	KP1916		2.56 (±0.099)	2.67 (±0.014)	57.60 (±0.701)	57.48 (±0.098)	0.06 (±0.08)	0.09 (±0.05)	0.12 (±0.04)
17	KP1819		2.90 (±0.098)	3.03 (±0.043)	62.63 (±0.462)	63.21 (±0.418)	0.71	0.94	0.93
18	KP1927		3.23 (±0.090)	3.14 (±0.029)	65.20 (±1.353)	66.27 (±0.085)	0.77 (±0.07)	0.89 (±0.01)	0.88 (±0.01)

Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
19	HRVA048		3.34 (±0.070)	3.26 (±0.099)	68.76 (±0.259)	68.95 (±0.372)	0.95 (±0.06)	1.19 (±0.05)	1.19 (±0.04)
20	HRVA107		3.46 (±0.003)	3.48 (±0.002)	69.61 (±0.048)	70.27 (±0.050)	1.30 (±0.11)	1.68 (±0.01)	1.70 (±0.04)
21	HRVA058		3.86 (±0.044)	3.86 (±0.004)	74.76 (±0.858)	74.24 (±0.028)	1.33 (±0.03)	1.83 (±0.04)	1.82 (±0.04)

Number	Name/ Acronym	Structure	Log k_w U	Log k_w KI	φ_0 U	φ_0 KI	Log P ICP-MS indirect	Log P HPLC – direct	Log P HPLC – indirect
22	HRVA149		4.02 (±0.006)	4.04 (±0.001)	72.154 (±0.011)	72.75 (±0.015)	1.20 (±0.34)	1.66 (±0.12)	1.59 (±0.06)
23	HRVA035		4.19 (±0.114)	4.18 (±0.159)	76.28 (±0.472)	76.45 (±0.652)	1.06 (±0.16)	2.37 (±0.04)	2.44 (±0.13)

3.3.1. Log P – correlation with log k_w and φ_0 – complete list

In this subchapter values of $\log k_w$ and φ_0 were correlated with $\log P$ values measured with HPLC and ICP-MS. Only figures with KI as dead time marker are shown in the figures. However, results for uracil are given as well.

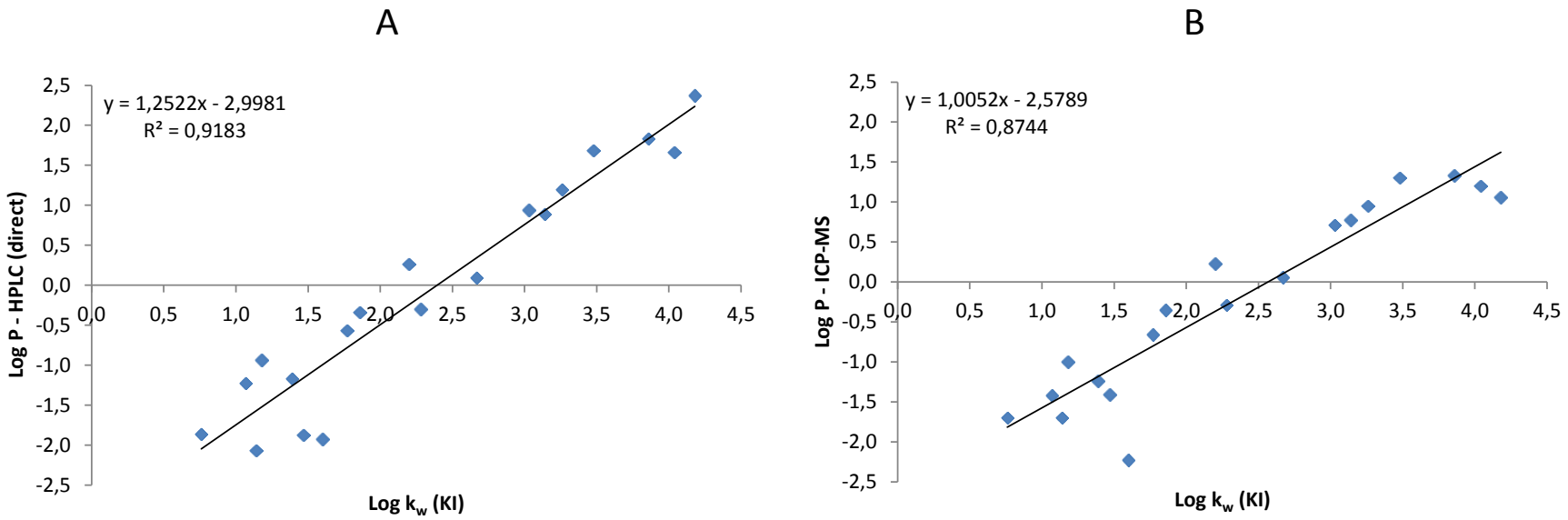


Figure 27: Log k_w (KI) vs. log P – HPLC (A) & ICP-MS (B)

Figure 27 shows the overall correlation of $\log k_w$ (calculated *via* KI) and $\log P$ (for 19 platinum(IV) compounds) measured with HPLC (direct) and ICP-MS. The correlation coefficient is $R^2 = 0.918$ and $R^2 = 0.874$ for HPLC and ICP-MS, respectively. By using uracil instead of KI, the R^2 show similar values of 0.913 and 0.870.

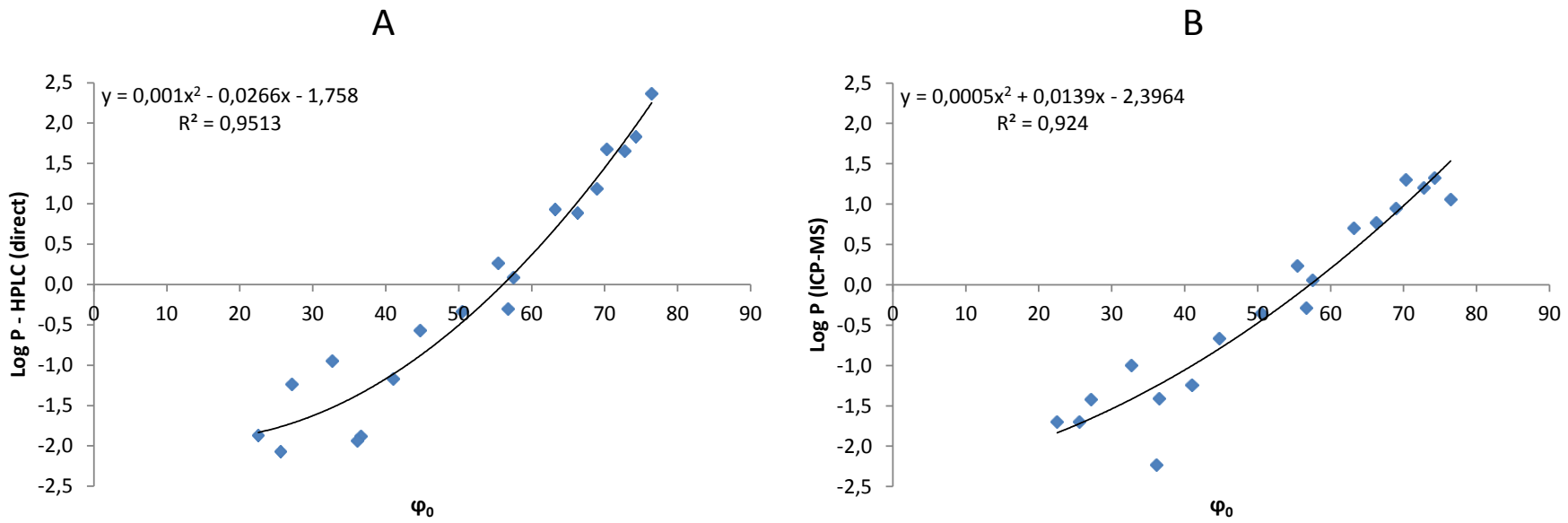


Figure 28: φ_0 (KI) vs. log P – HPLC (A) & ICP-MS (B)

By correlation with φ_0, calculated with KI, the curves exhibit a quadratic polynomial fit (Figure 28). The R^2 increases significantly to $R^2 = 0.951$ and $R^2 = 0.924$. Again, the R^2 remains similar by calculating φ_0 using uracil: $R^2 = 0.949$ for HPLC and $R^2 = 0.923$ for ICP-MS.

As pointed out before the φ_0 value was develloped to increase correlation coefficients for a large data set and/or for structurally various compounds. Figure 27 and Figure 28 illustrate that this assertion can be confirmed. The R^2 increases by using φ_0 instead of $\log k_w$. In general the correlation is higher using the $\log P$ values from the HPLC method compared to the ICP-MS method, which is to be expected because φ_0 is also determined by HPLC.

3.3.2. Log P – correlation with log k_w and φ_0 – adjusted list

Since the log P values of the three most lipophilic compounds seem to reach a plateau with ICP-MS they were excluded from the correlation with log P measured with ICP-MS. Also, the indirect method has difficulties with hydrophilic compounds as pointed out above and consequently, the three most hydrophilic compounds (DoHo102, 117, 133) were excluded for both the HPLC and ICP-MS methods. Therefore, 16 compounds for HPLC and 13 compounds for ICP-MS were correlated. The exclusion of these borderline compounds leads to a significant improvement of the R^2-factor in particular for the ICP-MS method and underlines that the correlation is linear between log k_w and log P. By using uracil as a dead time marker instead of KI the correlation improves further to $R^2 = 0.969$ and $R^2 = 0.963$ for the HPLC and the ICP-MS methods, respectively.

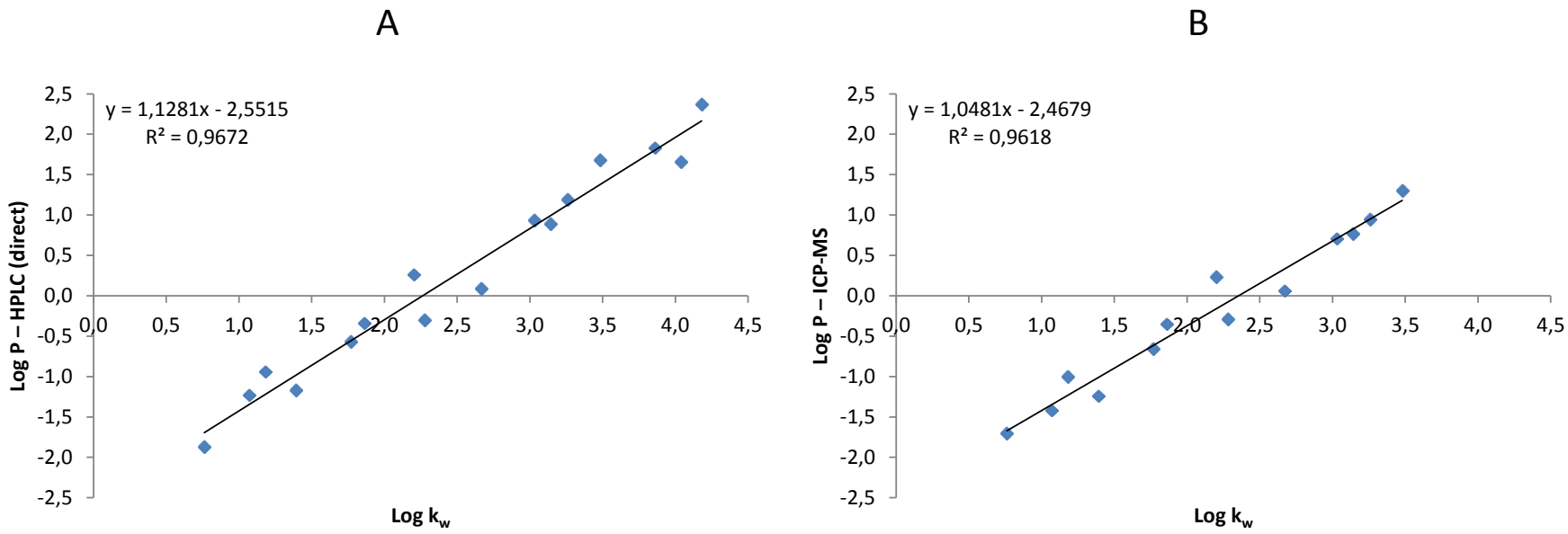


Figure 29: Log k_w (KI) vs. log P – HPLC (A) & ICP-MS (B) – adjusted list

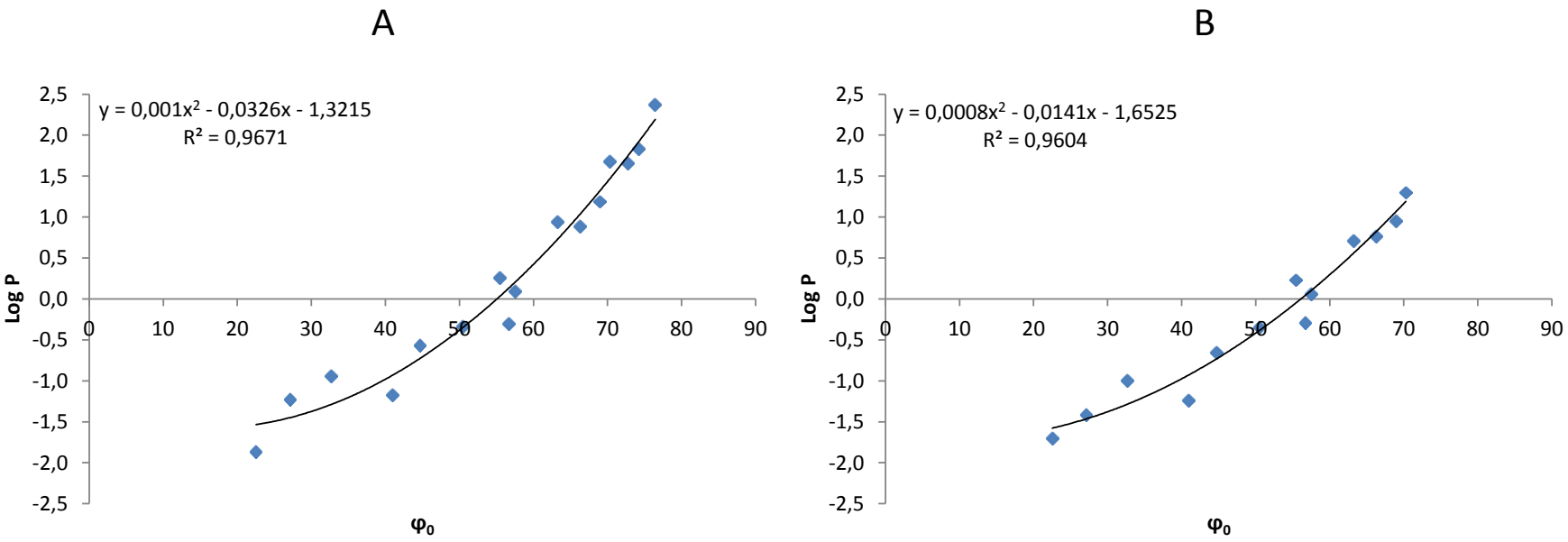


Figure 30: φ_0 (KI) vs. log P – HPLC (A) & ICP-MS (B) – adjusted list

With the correlation between $\log P$ and φ_0 (KI) of the adapted data set does not display a major improvement of R^2 compared to $\log k_w$. The same situation can be observed by using uracil. The R^2 even drops slightly to $R^2 = 0.963$ for HPLC and $R^2 = 0.958$ for ICP-MS.

The dead time marker does not affect the quality of the correlations between the chromatographically obtained $\log k_w$ or φ_0 with the $\log P$ values. However, due to the same reasons as described above (see 3.1.2), KI should be preferred over uracil. While the correlation of $\log P$ with $\log k_w$ results in lower R^2 using large data sets, the φ_0 fits are better. This is a benefit of φ_0 compared to $\log k_w$, since the improvement itself requires just a simple

calculation. Nevertheless the advantage of φ_0 being capable to correlate larger data sets diminishes by using fewer samples or structurally related compounds. The three DoHo (except DoHo102) substances exhibit a complete different core structures compared to all the other compounds. While most compounds feature a carbo-, ipro-, or satraplatin core, the DoHo substances exhibit an ethylenediamine (en) core structure with aceto groups.

Summarized, the "classical" shake flask method exhibits a magnitude of around 6 $\log P$ orders, ranging from –2 up to at least 4, according to the OECD guidelines.[73] However, there are several drawbacks to this method with regard to metal-based anticancer agents. Hydrolysis of the metallodrugs may be the most crucial point to mention because the method requires a shaking time of 1 h. Partial hydrolysis during this time period would amount to an increased concentration in the water phase and therefore a false result. Moreover, the shake-flask method itself is susceptible to impurities of the samples.

In this work the HPLC (UV/Vis) and ICP-MS detection techniques were investigated to determine $\log P$ of 19 platinum(IV) anticancer agents (Table 9). The HPLC method comprises a high dynamic range of $\log P$ from –2.0 up to at least 2.4. It is less prone to impurities, since the calculation is based on the area values of eluting peaks and is therefore recommended. However, since 1-octanol has a higher back pressure and interacts more strongly with the C18 surface a gradient method is required to wash off all 1-octanol remaining in the column after the injection, which is time consuming.

ICP-MS offers a limited dynamic range of $\log P$ from–1.5 up to 1.3. The lower limit is due to the indirect calculation method, and the upper limit is probably caused by adsorption of the lipophilic compounds to the plastic tube during the dilution process. Furthermore, the sample preparation takes more time compared to HPLC, since the dilution was done gravimetrically. On the other hand, once prepared, the ICP-MS measurement requires only 5 min for stock and water solution of one compound.

Table 9: Comparison of HPLC and ICP-MS for log P determination

	HPLC	ICP-MS
Log P minimum	–2.0	–1.5
Log P maximum	2.4	1.3
Approximate log range	~4.4	~2.8
Method calculation	direct	indirect
Calculation based on	Area value	^{194}Pt
Time consumption (measurement only)	~1.2 h (for stock, water and 1-octanol phase)	~5 min (for stock and water phase)
Sample preparation time	~1 h	~1.5 h

A remaining question is, whether $\log k_w$ or φ_0 is the better choice for correlation with $\log P$. As the sample preparation and time is the same, the only difference is the correlation with $\log P$ itself. In this case, the φ_0 value gives better correlation the more data points are correlated and/or, if the components are structurally diverse. Therefore φ_0 is recommended for correlation, which can be converted into $\log P$ and vice versa. The equation to convert φ_0 from the non-adjusted list into the HPLC $\log P$ is:

$$\text{Log P} = 0.001\varphi_0^2 - 0.027\varphi_0 - 1.758, R^2 = 0.951$$

4. Conclusion and Outlook

The aim of this master thesis was the evaluation of analytical methods using RP-HPLC and ICP-MS to determine the lipophilicity of platinum(IV) anticancer drugs. In contrast to platinum(II) cytostatics, platinum(IV) complexes may be administered orally due to their higher inertness of ligand exchange reactions. Oral administration requires the crossing of cell membranes or tight junctions of the drug in order to reach the blood stream. Therefore, the lipophilicity is a crucial property of every platinum(IV) compound and has a direct impact on the pharmacokinetics. The classical way to measure the lipophilicity of compounds is by the 'shake flask' method. This method features a bilayer of 1-octanol and water phases, where the concentration of a substance is measured after reaching an equilibrium distribution. This distribution is reported as the $\log P$ value. However, this method has several drawbacks, for instance the long sample preparation, low sample throughput and most importantly possible hydrolysis of analytes during the shaking time, which alters the lipophilicity of the compounds. In order to avoid these drawbacks two chromatographic parameters to measure the lipophilicity, namely $\log k_w$ and φ_0, have been evaluated as well. Although the $\log k_w$ measurements are equally time consuming compared to $\log P$ experiments, there is no further sample preparation needed and the measurement may run completely automatized.

Log P values derived from HPLC and ICP-MS, show high correlation within a range of $\log P = -1.5$ to 1.3. Within this range, it does not matter if the $\log P$ is calculated *via* the direct ($\text{Log } P = \log c_{octanol}/c_{water}$) or indirect ($\text{Log } P = \log (c_{stock} - c_{water})/c_{water}$ HPLC method. Below values of $\log P = -1.5$ it is not possible to measure log P values with ICP-MS, whereas with HPLC values can be measured down to $\log P = -2.0$. This is mainly due to the indirect method, which becomes problematic as the concentrations in the stock solution and in water approaches each other. For values higher than $\log P = 1.3$, ICP-MS seems to reach a plateau due to adsorption effects. HPLC, however, exhibits linearity at least up to $\log P = 2.4$.

Two substances (uracil and KI) were investigated as dead time markers for the determination of $\log k$ and φ_0. Two major differences could be observed. First, KI features an almost constant retention time at different percentages of the organic modifier, while uracil does not. Second, the retention time of KI is shorter at all mobile phase compositions compared to uracil. Therefore, KI allows the determination of more hydrophilic compounds compared to uracil and KI is generally recommended for future measurements.

$\mathrm{Log\,k_w}$- and φ_0 values were derived by RP-HPLC using KI and uracil as dead time marker. The φ_0 values display a better correlation $\log \mathrm{P}$ than $\log \mathrm{k_w}$. Since φ_0 values ranging from 14 to 76 could be easily derived, the φ_0 values cover a broad lipophilicity range equal to about 4 $\log \mathrm{P}$ orders. Additionally, φ_0 does not feature any disadvantages from indirect measurements or adsorption effects compared to $\log \mathrm{P}$ determination using ICP-MS.

5. Appendix

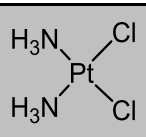
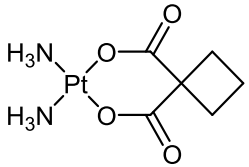
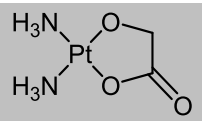
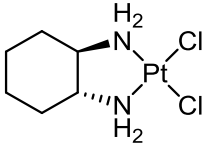
5.1. Retention times of uracil and KI

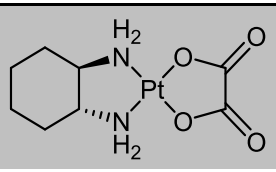
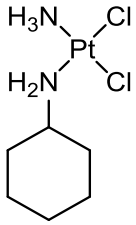
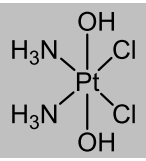
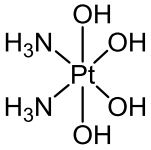
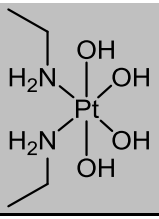
Table 10: Retention times of uracil and KI

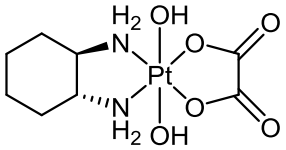
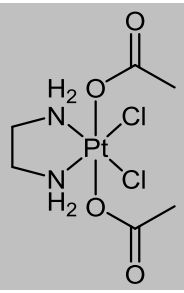
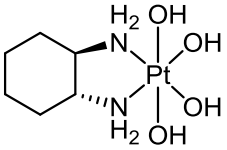
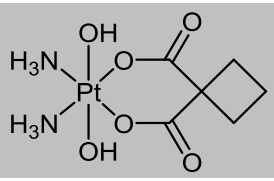
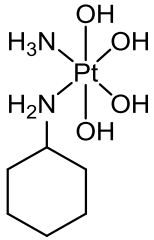
MeOH [%]	Retention time Uracil [min]	Retention time KI [min]
5	2,581	1,570
10	2,183	1,570
15	2,003	1,570
20	1,903	1,573
25	1,840	1,580
30	1,803	1,587
35	1,773	1,593
40	1,760	1,600
45	1,743	1,603
50	1,733	1,610
55	1,727	1,617
60	1,723	1,617
65	1,720	1,623
70	1,723	1,627
75	1,733	1,637
80	1,740	1,677
85	1,763	1,700
90	1,783	1,740

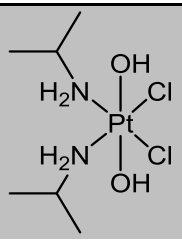
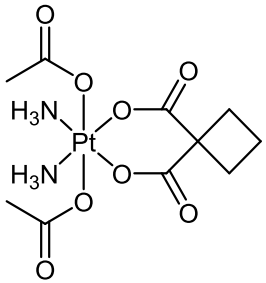
5.2. Complete Data List

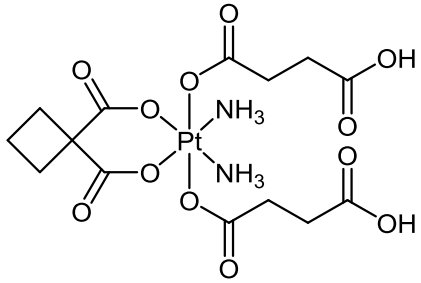
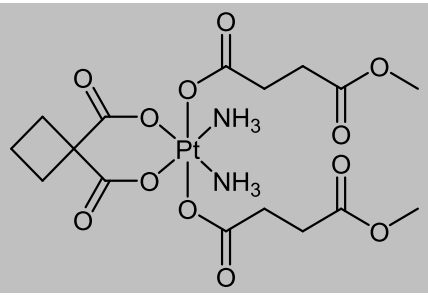
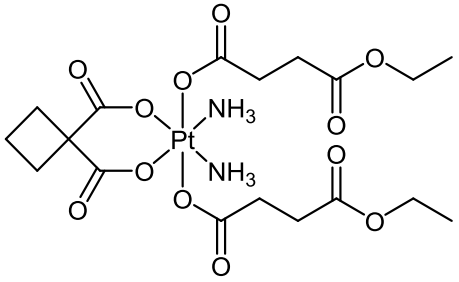
In this chapter the complete data list is shown. Substances marked as "#" are platinum(II) complexes. Since the correlation of $\log k_w$ and φ_0 with $\log P$ includes only platinum(IV) complexes, no $\log P$ values for platinum(II) compounds are given (unless they were measured directly). Substances marked with a "~" are reference compounds for the data set. As for the data set, the complete data set is used (see chapter 3.3.1). In short: All values are given to the base of KI as dead time marker. Complexes marked with "*" feature an acidic moiety. In this case, the unbuffered $\log D^*$ values are given (see chapter 3.2.3). Please keep in mind that acidic compounds were not used for the calibration curve of the extrapolating $\log P$ data set.

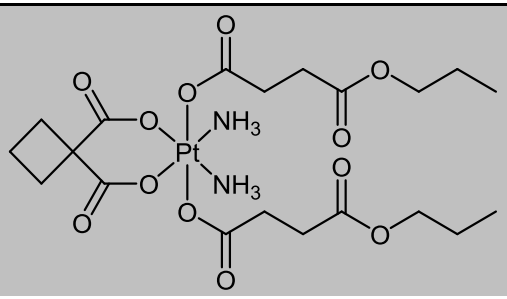
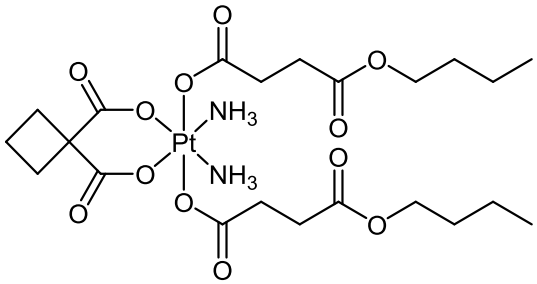
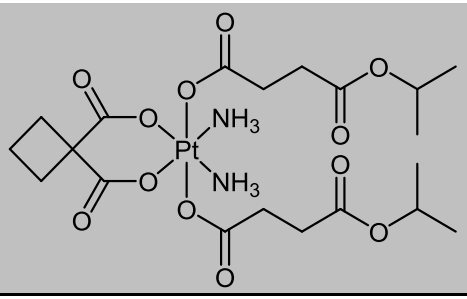
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
1[#]	Cis-Pt		-	-	–2.10	-	–2.53 (±0.28)[85] –2.53[23] –2.19 (±0.06)[87] –1.74[79]
2[#]	Carbo-Pt		KI: 0.11	KI: 2.29	–1.98 (±0.148)	–1.66 (±0.65)	–2.3 (±0.10)[85] –2.30[23] –1.48[79] –1.398[80]
3[#]	Neda-Pt		-	-	-	-	
4[#]	$Pt(DACH)Cl_2$		U: 0.02 KI: 0.40	U: 0.44 KI: 8.51	-	-	

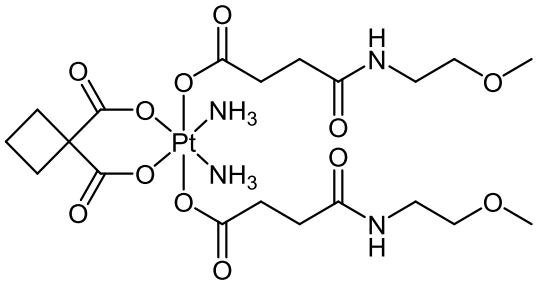
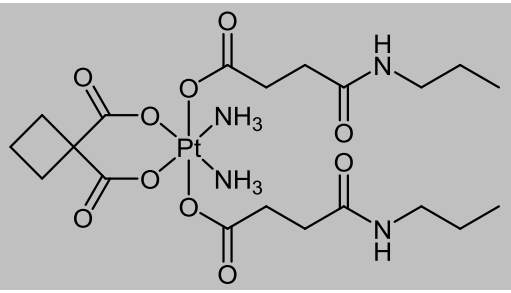
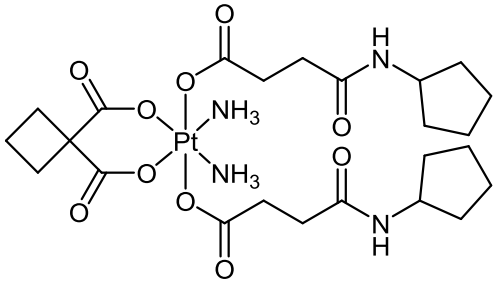
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
5[#]	Oxali-Pt		U: 0.30 KI: 0.52	U: 6.05 KI: 10.08	–1.88 (±0.17)	–1.37 (±0.15)	–1.76[23] –1.65 (±0.21)[85]
6[#]	$Pt(NH_3)chaCl_2$		U: 1.11 (±0.010) KI: 1.31 (±0.039)	U: 37.21 (±0.758) KI: 38.02 (±1.211)	–0.09 (±0.03)	–0.11 (±0.14)	
7	Ox-Cis-Pt		-	-	-	-	
8	$Pt(NH_3)_2(OH)_4$		-	-	-	-	
9	$Pt(EtNH_2)_2(OH)_4$ HRVA 122		-	-	-	-	

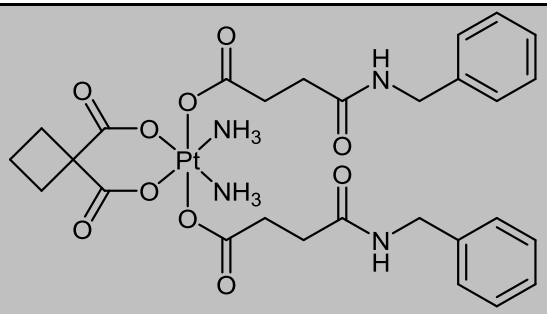
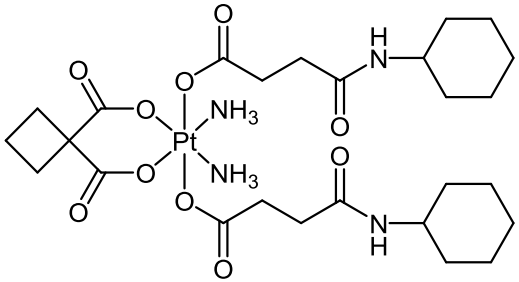
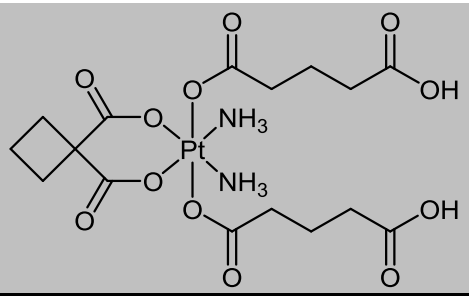
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
10	Ox-Oxali-Pt		KI: 0.08	KI: 1.85	Log k_w: –2.90 φ_0: –1.80	Log k_w: –2.50 φ_0: –2.37	
11	$Pt(en)Cl_2(OAc)_2$		-	-	-	-	
12	$Pt(DACH)(OH)_4$		KI: 0.23	KI: 5.53	Log k_w: –2.71 φ_0: –1.87	Log k_w: –2.35 φ_0: –2.30	
13	Ox-Carbo-Pt		KI: 0.29 (±0.025)	KI: 9.22 (±0.688)	Log k_w: –2.63 φ_0: –1.92	Log k_w: –2.29 φ_0: –2.23	
14~	$Pt(NH_3)cha(OH)_4$		U: 0.50 KI: 0.76	U: 18.15 KI: 22.52	–1.87 (±0.15)	–1.70 (±0.29)	

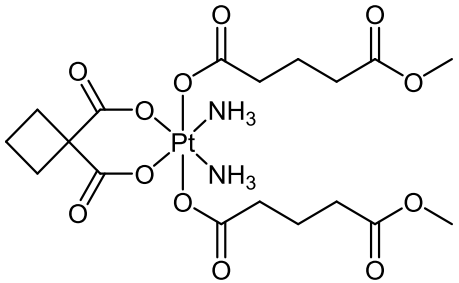
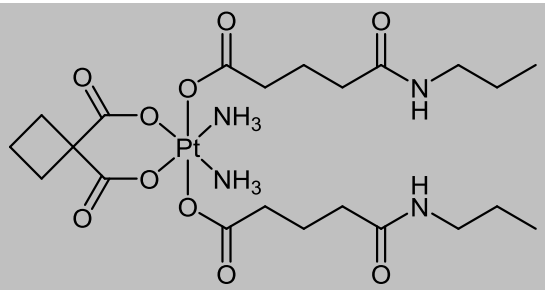
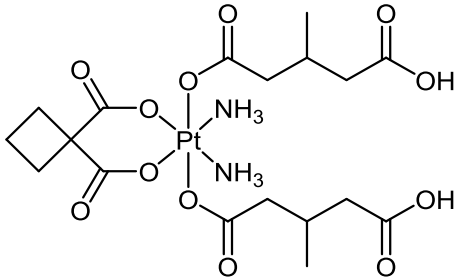
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
15	Ipro-Pt		U: 0.73 (±0.007) KI: 0.95 (±0.023)	U: 20.34 (±0.192) KI: 24.57 (±0.260)	Log k_w: –1.81 φ_0: –1.81	Log k_w: –1.62 φ_0: –1.75	
16~	$Pt(NH_3)_2(CDBA)(OAc)_2$		U: 0.86 (±0.016) KI: 1.07 (±0.039)	U: 24.93 (±0.360) KI: 27.14 (±0.519)	–1.23 (±0.00)	–1.42 (±0.06)	
17~	Satra-Pt		U: 2.12 (±0.027) KI: 2.20 (±0.022)	U: 55.44 (±0.292) KI: 55.43 (±0.362)	0.26 (±0.02)	0.23 (±0.01)	–0.16 (±0.16)[85] –0.14[78] 0.1[84]

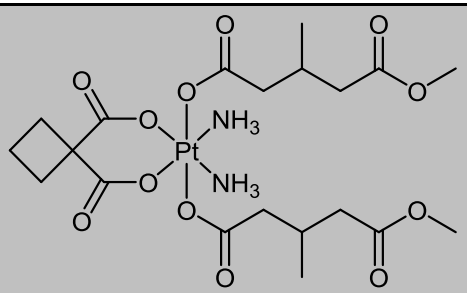
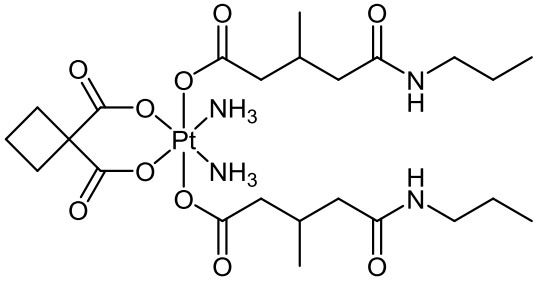
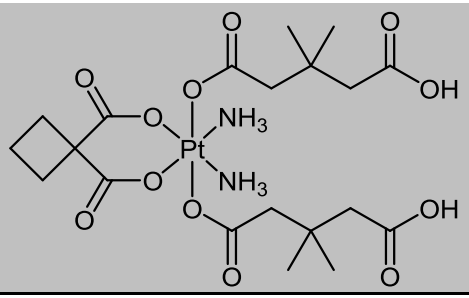
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
18*	HRVA 026		U: 1.28 (±0.045) KI: 1.41 (±0.065)	U: 30.47 (±0.107) KI: 33.10 (±0.101)	–1.68 (±0.16)	–1.84 (±0.65)	
19	HRVA 031		U: 1.97 (±0.013) KI: 2.05 (±0.004)	U: 45.23 (±0.077) KI: 45.76 (±0.054)	Log k_w: –0.43 φ_0: –0.88	Log k_w: –0.52 φ_0: –0.71	
20~	KP 1916		U: 2.56 (±0.099) KI: 2.67 (±0.014)	U: 57.60 (±0.701) KI: 57.48 (±0.098)	0.09 (±0.05)	0.06 (±0.08)	

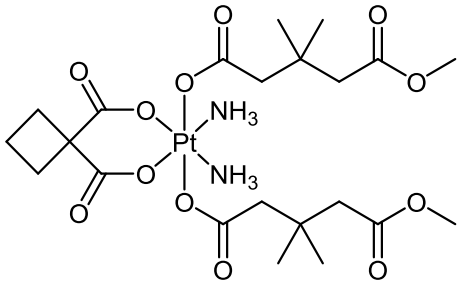
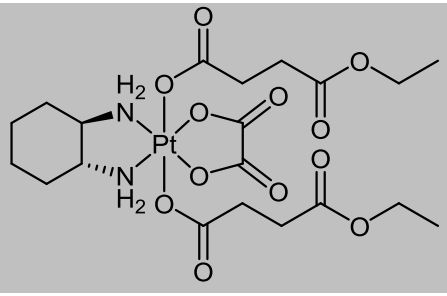
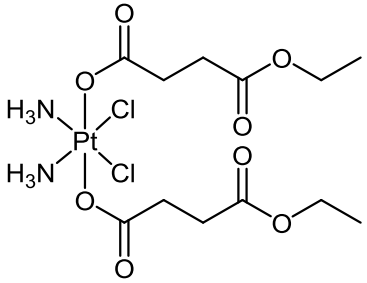
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
21~	HRVA 048		U: 3.34 (±0.070) KI: 3.26 (±0.099)	U: 68.76 (±0.259) KI: 68.95 (±0.372)	1.19 (±0.05)	0.95 (±0.06)	
22~	HRVA 035		U: 4.19 (±0.114) KI: 4.18 (±0.159)	U: 76.28 (±0.472) KI: 76.45 (±0.652)	2.37 (±0.04)	1.06 (±0.16)	
23	HRVA 065		U: 3.09 (±0.003) KI: 3.07 (±0.005)	U: 66.71 (±0.044) KI: 67.79 (±0.003)	Log k_w: 0.85 φ_0: 1.03	Log k_w: 0.51 φ_0: 0.84	

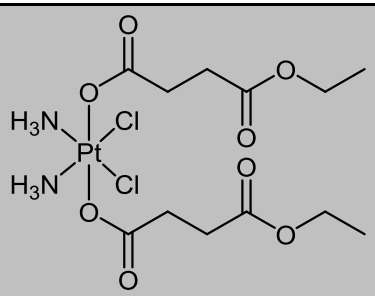
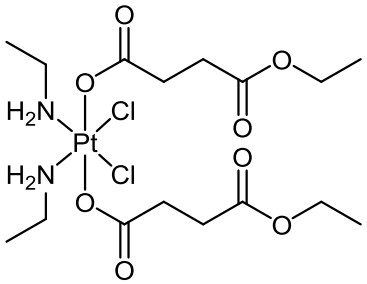
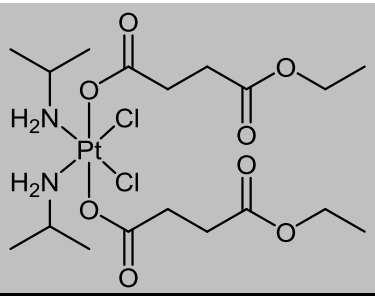
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
24	HRVA 059		U: 2.01 (±0.002) KI: 2.10 (±0.004)	U: 37.70 (±0.010) KI: 38.44 (±0.015)	Log k_w: –0.37 φ_0: –1.30	Log k_w: –0.47 φ_0: –1.12	
25	HRVA 052		U: 2.31 (±0.129) KI: 2.38 (±0.121)	U: 53.87 (±0.006) KI: 54.03 (±0.037)	Log k_w: –0.02 φ_0: –0.28	Log k_w: –0.19 φ_0: –0.19	
26~	KP 1927		U: 3.23 (±0.090) KI: 3.14 (±0.029)	U: 65.20 (±1.353) KI: 66.27 (±0.085)	0.89 (±0.01)	0.77 (±0.07)	

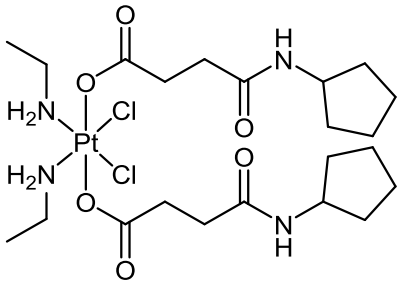
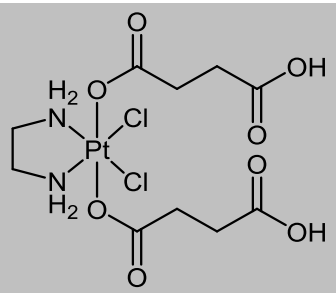
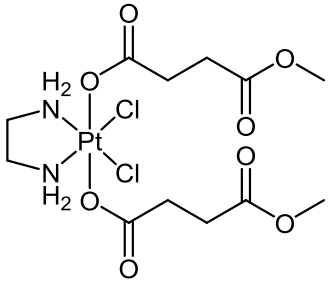
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
27	HRVA 068		U: 3.53 (±0.005) KI: 3.57 (±0.06)	U: 67.11 (±0.004) KI: 67.21 (±0.025)	Log k_w: 1.47 φ_0: 0.97	Log k_w: 1.01 φ_0: 0.80	
28~	HRVA 058		U: 3.86 (±0.044) KI: 3.86 (±0.004)	U: 74.76 (±0.858) KI: 74.24 (±0.028)	1.83 (±0.04)	1.33 (±0.03)	
29*	HRVA 045B		U: 1.61 (±0.077) KI: 1.73 (±0.127)	U: 34.91 (±0.093) KI: 35.85 (±0.252)	–1.43 (±0.04)	–1.42 (±0.03)	

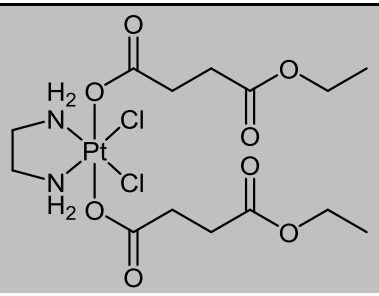
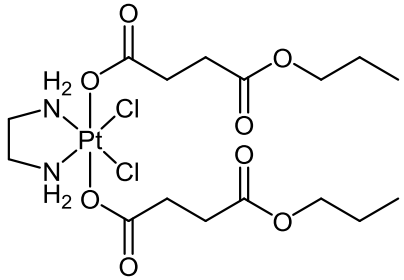
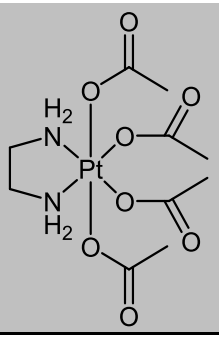
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
30	HRVA 067		U: 2.24 (±0.002) KI: 2.29 (±0.001)	U: 51.31 (±0.039) KI: 51.81 (±0.051)	Log k_w: –0.13 φ_0: –0.45	Log k_w: –0.28 φ_0: –0.33	
31	HRVA 066		U: 2.56 (±0.001) KI: 2.59 (±0.002)	U: 56.31 (±0.020) KI: 57.45 (±0.054)	Log k_w: 0.25 φ_0: 0.01	Log k_w: 0.02 φ_0: 0.05	
32*	DoHo 012		U: 2.06 (±0.225) KI: 2.16 (±0.193)	U: 46.80 (±0.442) KI: 47.20 (±0.305)	–0.59 (±0.10)	–0.48 (±0.07)	

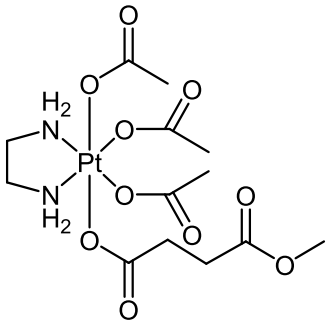
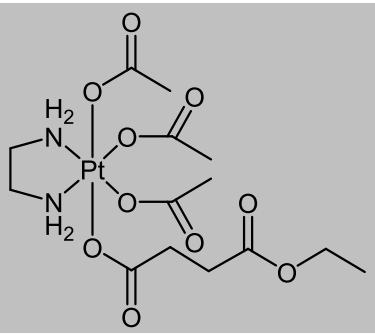
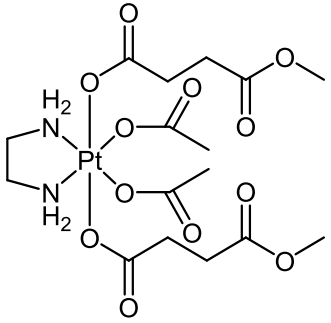
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
33	HRVA 064		U: 2.66 (±0.002) KI: 2.70 (±0.001)	U: 60.54 (±0.061) KI: 60.72 (±0.068)	Log k_w: 0.38 φ_0: 0.31	Log k_w: 0.14 φ_0: 0.29	
34	HRVA 062		U: 2.84 (±0.002) KI: 2.89 (±0.007)	U: 61.28 (±0.045) KI: 61.28 (±0.025)	Log k_w: 0.62 φ_0: 0.37	Log k_w: 0.33 φ_0: 0.33	
35*	HRVA 034		U: 2.71 (±0.003) KI: 2.77 (±0.003)	U: 56.26 (±0.017) KI: 56.33 (±0.013)	0.29 (±0.04)	0.21 (±0.05)	

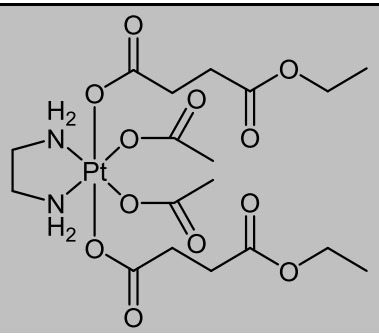
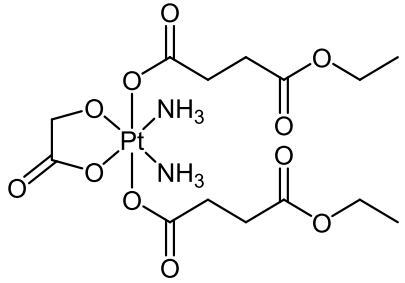
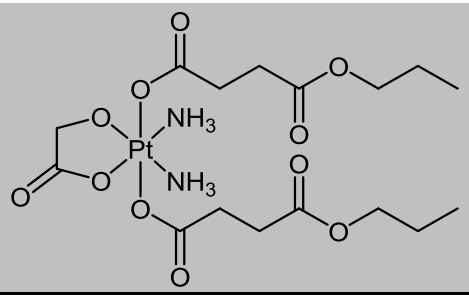
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
36	HRVA 057		U: 3.37 (±0.029) KI: 3.41 (±0.032)	U: 67.76 (±0.185) KI: 67.92 (±0.040)	Log k_w: 1.21 φ_0: 1.05	Log k_w: 0.85 φ_0: 0.85	
37	HRVA 110		U: 2.00 (±0.004) KI: 2.03 (±0.005)	U: 51.71 (±0.017) KI: 53.22 (±0.062)	Log k_w: –0.46 φ_0: –0.34	Log k_w: –0.54 φ_0: –0.24	
38 a	HRVA 114 a		U: 1.87 (±0.104) KI: 1.94 (±0.115)	U: 48.61 (±0.032) KI: 49.14 (±0.090)	Log k_w: –0.57 φ_0: –0.65	Log k_w: –0.63 φ_0: –0.51	

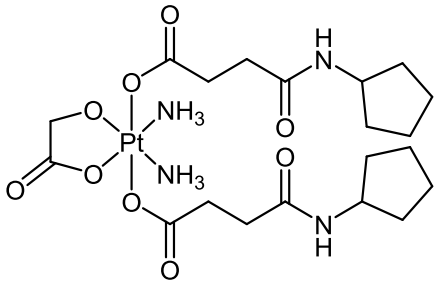
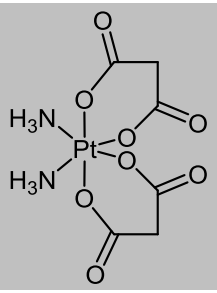
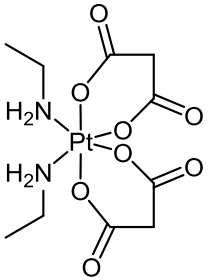
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
38 b	HRVA 114 b		U: 1.74 (±0.083) KI: 1.78 (±0.104)	U: 48.314 (±0.215) KI: 50.11 (±0.147)	Log k_w: –0.77 φ_0: –0.58	Log k_w: –0.79 φ_0: –0.44	
39~	KP 1819		U: 2.90 (±0.098) KI: 3.03 (±0.043)	U: 62.63 (±0.462) KI: 63.21 (±0.418)	0.94	0.71	
40~	HRVA 107		U: 3.46 (±0.003) KI: 3.48 (±0.002)	U: 69.61 (±0.048) KI: 70.27 (±0.050)	1.68 (±0.01)	1.30 (±0.11)	

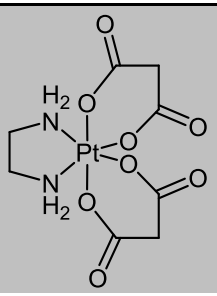
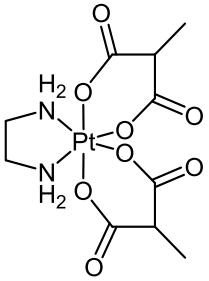
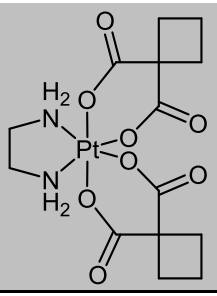
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
41	HRVA 023		U: 3.52 (±0.001) KI: 3.56 (±0.002)	U: 70.89 (±0.07) KI: 70.76 (±0.046)	Log k_w: 1.46 φ_0: 1.37	Log k_w: 1.00 φ_0: 1.09	
42*	Lust 004		U: 0.34 (±0.039) KI: 0.61 (±0.077)	U: 7.36 (±0.431) KI: 13.80 (±0.336)	–1.87	–2.20	
43	HRVA yyy		U: 1.32 (±0.004) KI: 1.44 (±0.005)	U: 31.56 (±0.103) KI: 34.00 (±0.121)	Log k_w: –1.19 φ_0: –1.51	Log k_w: –1.13 φ_0: –1.35	–0.81 (±0.02)[77]

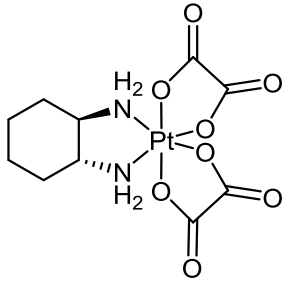
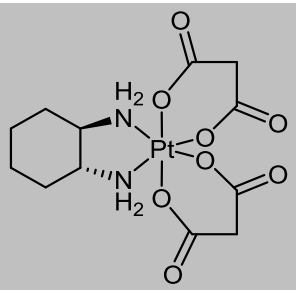
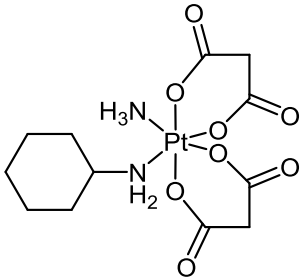
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
44~	HRVA 101		U: 1.86 (±0.006) KI: 1.86 (±0.003)	U: 48.77 (±0.036) KI: 50.52 (±0.020)	–0.34 (±0.02)	–0.35 (±0.02)	–0.32 (±0.01)[77]
45	HRVA 097		U: 2.75 (±0.007) KI: 2.72 (±0.004)	U: 62.59 (±0.049) KI: 63.78 (±0.013)	Log k_w: 0.41 φ_0: 0.61	Log k_w: 0.16 φ_0: 0.52	0.70 (±0.01)[77]
46	DoHo 106		KI: 0.15 (±0.012)	KI: 5.24 (±0.314)	Log k_w: –2.81 φ_0: –1.76	Log k_w: –2.43 φ_0: –2.40	

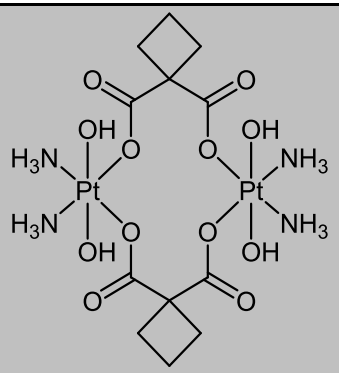
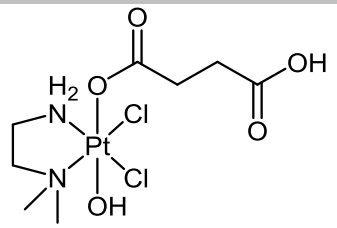
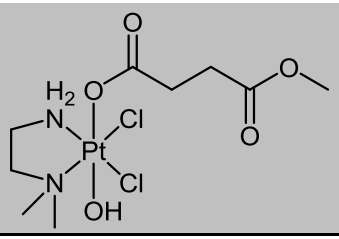
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
47~	DoHo 117		U: 0.98 (±0.067) KI: 1.14 (±0.085)	U: 22.34 (±0.127) KI: 25.58 (±0.047)	–2.07 (±0.04)	–1.81 (±0.24)	
48	DoHo 133		U: 1.36 (±0.052) KI: 1.47 (±0.072)	U: 34.23 (±0.022) KI: 36.55 (±0.127)	–1.88 (±0.04)	–1.41 (±0.11)	
49	DoHo 102		U: 1.57 (±0.075) KI: 1.60 (±0.004)	U: 34.01 (±0.019) KI: 36.08 (±0.030)	–1.93 (±0.10)	–2.23 (±0.23)	

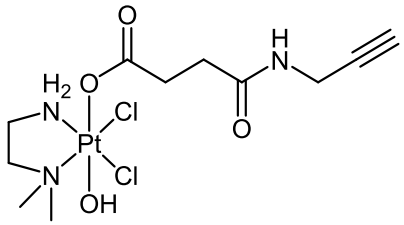
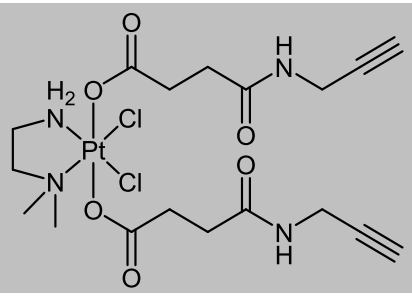
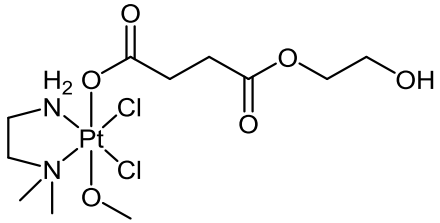
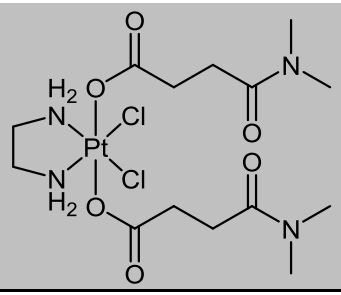
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
50	DoHo 124		U: 2.09 (±0.003) KI: 2.13 (±0.003)	U: 50.60 (±0.023) KI: 52.06 (±0.097)	Log k_w: –0.33 φ_0: –0.43	Log k_w: –0.44 φ_0: –0.32	
51~	HRVA 118		U: 1.32 (±0.096) KI: 1.39 (±0.119)	U: 38.54 (±0.343) KI: 40.99 (±0.102)	–1.17 (±0.01)	–1.24 (±0.07)	
52	HRVA 078		U: 2.16 (±0.108) KI: 2.23 (±0.095)	U: 56.66 (±0.094) KI: 56.77 (±0.168)	Log k_w: –0.21 φ_0: –0.05	Log k_w: –0.34 φ_0: 0.00	

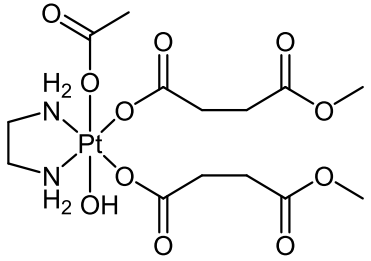
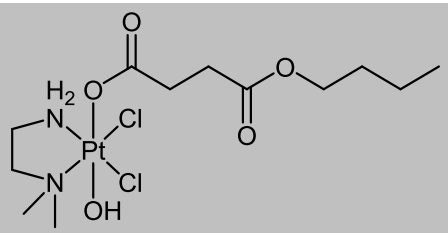
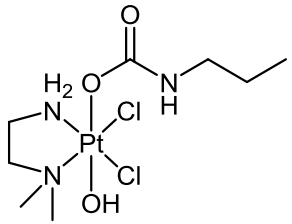
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
53~	KP 2016		U: 2.21 (±0.025) KI: 2.28 (±0.025)	U: 56.17 (±0.708) KI: 56.72 (±0.182)	–0.30 (±0.09)	–0.39 (±0.12)	
54	HRVA 127		-	-	-	-	
55	HRVA 133		-	-	-	-	

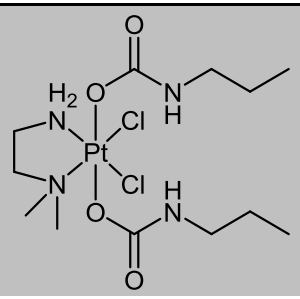
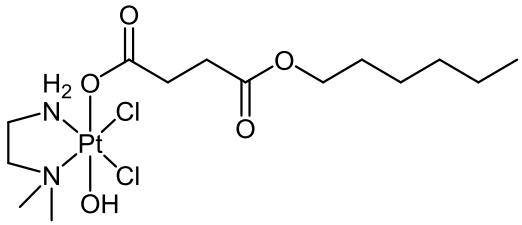
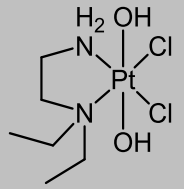
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
56	HRVA 090		-	-	-	-	
57	HRVA 094		U: 0.34 (±0.023) KI: 0.68 (±0.024	U: 8.08 (±0.310) KI: 13.85 (±0.685)	Log k_w: –2.15 φ_0: –1.93	Log k_w: –1.90 φ_0: –2.11	
58~	HRVA 093		U: 1.66 (±0.006) KI: 1.77 (±0.002)	U: 42.88 (±0.075) KI: 44.66 (±0.026)	–0.57 (±0.01)	–0.66 (±0.01)	

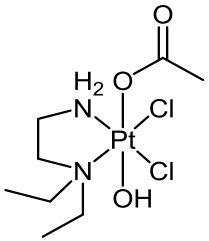
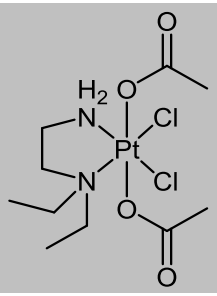
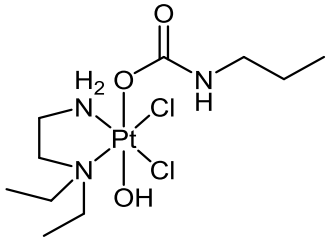
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
59	HRVA 142		-	-	-	-	
60	HRVA 138		-	-	-	-	
61~	HRVA 140		U: 1.06 (±0.022) KI: 1.19 (±0.021)	U: 29.98 (±0.525) KI: 32.66 (±1.327)	–0.94 (±0.01)	–1.00 (±0.02)	

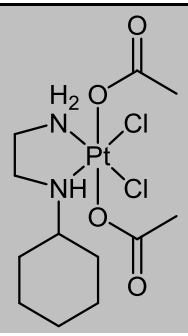
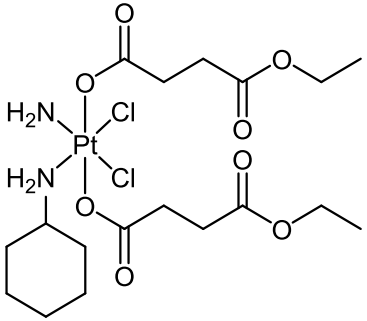
Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
62	HRVA 137		-	-	-	-	
63*	KP 1820		U: 0.60 (±0.021) KI: 0.85 (±0.001)	U: 11.00 (±0.151) KI: 15.12 (±0.015)	Log k_w: –1.93 φ_0: –1.72	Log k_w: –1.93 φ_0: –2.07	
64	KP 1821		U: 1.12 (±0.001) KI: 1.29 (±0.000)	U: 24.86 (±0.031) KI: 27.59 (±0.005)	Log k_w: –1.38 φ_0: –1.73	Log k_w: –1.28 φ_0: –1.63	

Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
65	KP 1941		U: 0.82 (±0.002) KI: 1.02 (±0.001)	U: 17.26 (±0.121) KI: 20.09 (±0.007)	Log k_w: –1.72 φ_0: –1.89	Log k_w: –1.55 φ_0: –1.92	
66	KP 1843		U: 1.13 (±0.159) KI: 1.21 (±0.173)	U: 23.89 (±0.850) KI: 25.84 (±0.844)	Log k_w: –1.40 φ_0: –1.78	Log k_w: –1.29 φ_0: –1.71	
67	KP 1938		U: 1.24 (±0.042) KI: 1.32 (±0.055)	U: 31.24 (±0.110) KI: 32.85 (±0.088)	Log k_w: –1.35 φ_0: –1.55	Log k_w: –1.25 φ_0: –1.40	
68	KP 1822		U: 1.44 (±0.210) KI: 1.63 (±0.102)	U: 28.83 (±0.162) KI: 30.31 (±0.024)	Log k_w: –0.96 φ_0: –1.65	Log k_w: –0.94 φ_0: –1.62	

Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
69	DoHo 140		U: 1.55 (±0.069) KI: 1.64 (±0.089)	U: 33.15 (±0.067) KI: 35.25 (±0.008)	Log k_w: –0.94 φ_0: –1.45	Log k_w: –0.93 φ_0: –1.29	
70	KP 1894		U: 2.44 (±0.004) KI: 2.48 (±0.002)	U: 57.50 (±0.006) KI: 58.77 (±0.042)	Log k_w: 0.11 φ_0: 0.13	Log k_w: –0.09 φ_0: 0.15	
71	KP 2215		U: 1.24 (±0.024) KI: 1.34 (±0.054)	U: 35.61 (±0.033) KI: 38.00 (±0.127)	Log k_w: –1.32 φ_0: –1.32	Log k_w: –1.23 φ_0: –1.15	

Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
72	KP 2214		U: 2.60 (±0.016) KI: 2.62 (±0.021)	U: 57.75 (±0.077) KI: 59.00 (±0.002)	Log k_w: 0.28 φ_0: 0.15	Log k_w: 0.05 φ_0: 0.16	
73	KP 1875		U: 3.32 (±0.003) KI: 3.37 (±0.098)	U: 71.38 (±0.046) KI: 71.36 (±0.098)	Log k_w: 1.22 φ_0: 1.44	Log k_w: 0.81 φ_0: 1.14	
74	KP 1975		U: 0.49 (±0.004) KI: 0.80 (±0.003)	U: 12.49 (±0.015) KI: 17.58 (±0.044)	Log k_w: –2.00 φ_0: –1.92	Log k_w: –1.77 φ_0: –2.00	

Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
75	KP 2212		U: 1.04 (±0.027) KI: 1.13 (±0.060)	U: 28.76 (±0.022) KI: 31.79 (±0.051)	Log k_w: –1.58 φ_0: –1.59	Log k_w: –1.44 φ_0: –1.45	
76	KP 2054		U: 1.52 (±0.029) KI: 1.61 (±0.059)	U: 36.82 (±0.116) KI: 38.78 (±0.170)	Log k_w: –0.98 φ_0: –1.29	Log k_w: –0.96 φ_0: –1.11	
77	KP 2216		U: 1.91 (±0.004) KI: 1.91 (±0.016)	U: 51.37 (±0.120) KI: 53.16 (±0.084)	Log k_w: –0.61 φ_0: –0.35	Log k_w: –0.66 φ_0: –0.24	

Number	Name/Acronym	Structure	Log k_w	φ_0	Log P (HPLC)	Log P (ICP-MS)	Log P Literature
78	KP 2213		U: 2.21 (±0.077) KI: 2.23 (±0.080)	U: 54.82 (±0.112) KI: 56.28 (±0.303)	Log k_w: –0.21 φ_0: –0.09	Log k_w: –0.34 φ_0: –0.03	
79~	HRVA 149		U: 4.02 (±0.006) KI: 4.04 (±0.001)	U: 72.154 (±0.011) KI: 72.75 (±0.015)	1.66 (±0.12)	1.20 (±0.34)	

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9. Curriculum vitae

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Date of Birth:	29.10.1985
Place of Birth:	Mödling, Lower Austria, Austria
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Education

May 2013 – March 2014	Master Thesis at the Institute of Inorganic Chemistry, University of Vienna, Vienna. Master Thesis: Determination and Correlation of Lipophilicity Parameters of Platinum(IV) Compounds Analyzed by HPLC and ICP-MS Supervisor: Univ.-Prof. DDr. Bernhard Keppler
October 2011 – April 2013	Master program: Chemistry, University of Vienna, Vienna
August 2011	Bachelor Thesis: „Amino-Sulfonierung einfacher Alkene. Optimierung der Syntheseschritte und chromatographische Trennung der Enantiomere.“ Supervisor: Univ.-Prof. Dr. Wolfgang Lindner
July 2008 – August 2011	Bachelor program: Chemistry, University of Vienna, Vienna
October 2006 – January 2008	Magister program: Pharmacy, University of Vienna, Vienna
2005 – 2006	Civilian Service at ‘Landespflegeheim Perchtoldsdorf - Beatrixheim’, Perchtoldsdorf
2005	General qualification for university entrance / school leaving examination

Research Interests

Analytical chemistry: drug development and characteristics; metallomics

Method Skills

HPLC, ICP-MS

Personal Skills

Mother tongue:	German
Languages:	German, English
Computer skills:	Word
	Excel
	LaTeX

„Ich habe mich bemüht, sämtliche Inhaber der Bildrechte ausfindig zu machen. Sollte dennoch eine Urheberrechtsverletzung bekannt werden, ersuche ich um Meldung bei mir."
– M.H.M. Klose