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DISSERTATION

Titel der Dissertation

NOVEL ANTINEOPLASTIC PLATINUM(IV) COMPLEXES: SYNTHESIS, CHARACTERIZATION, BIOLOGICAL INVESTIGATIONS AND STRUCTURE-ACTIVITY RELATIONSHIPS

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Hristo Varbanov

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Written by

Mag. Hristo P. Varbanov

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This PhD thesis is based on the following papers, which are presented in the original format:

Synthesis and characterization of novel bis(carboxylato)dichloridobis(ethylamine) platinum(IV) complexes with higher cytotoxicity than cisplatin.
H. Varbanov, S.M. Valiahdi, A.A. Legin, M.A. Jakupec, A. Roller, M. Galanski, B.K. Keppler, *Eur. J. Med. Chem.*, **2011,** 46, 5456-5464.

Novel tetracarboxylatoplatinum(IV) complexes as carboplatin prodrugs.
H.P. Varbanov, S.M. Valiahdi, C.R. Kowol, M.A. Jakupec, M. Galanski, B.K. Keppler, *Dalton Trans.*, **2012**, 41, 14404-14415.

Theoretical Investigations and Density Functional Theory Based Quantitative Structure–Activity Relationships Model for Novel Cytotoxic Platinum(IV) Complexes.
H.P. Varbanov, M.A. Jakupec, A. Roller, F. Jensen, M. Galanski, B.K. Keppler, *J. Med. Chem.*, **2013**, 56, 330-344**.**

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ABSTRACT

Platinum(II) complexes represent one of the most widely used classes of cytostatics in anticancer chemotherapy. Their clinical effectiveness is accompanied by severe dose-limiting side effects, intrinsic and/or acquired tumor resistance and the inconvenient and cost intensive way of intravenous administration. Platinum(IV) complexes also possess antitumor activity and their physicochemical and chemical properties could be utilized in order to overcome the main drawbacks of platinum(II)-based drugs. The successful design of platinum(IV) chemotherapeutics requires a careful examination of their pharmacology and toxicology, the formulation of structure-activity relationships and the development of new synthetic approaches.

Within this PhD work, novel bis-, tris- and tetrakis(carboxylato)platinum(IV) complexes, designed as prodrugs for *cis*-[$Pt(EtNH_2)_2Cl_2$] (cisplatin analogue with higher lipophilicity), carboplatin and nedaplatin were synthesized. For this purpose, the respective platinum(II) complexes were oxidized with H_2O_2 in aqueous media and further carboxylated using different cyclic anhydrides (succinic, glutaric, 3-methylglutaric and 3,3-dimethylglutaric anhydride). The resulting compounds were subsequently derivatized by activation of their free carboxylic groups with CDI (1,1'-carbonyldiimidazol), followed by reaction with various amines or alcohols, yielding the desired amides and esters, respectively. All complexes were fully characterized, using multinuclear (^{1}H, ^{13}C, ^{15}N and ^{195}Pt) 1D and 2D NMR spectroscopy, elemental analysis, ESI-MS, ATR-FTIR, HPLC and exemplarily X-ray diffraction for some of the compounds. *In vitro* cytotoxicity of the novel complexes was examined in four human tumor cell lines originating from ovarian carcinoma (CH1 and SK-OV-3), colon carcinoma (SW480) and non-small cell lung cancer (A549) by means of the MTT colorimetrical assay.

Comparative analysis of the lipophilicity, electrochemistry and rate of reduction by ascorbic acid of the new complexes was conducted in order to better understand their pharmacological behavior. Finally, computational studies with respect to the electronic structure and redox properties of the investigated compounds, using DFT methods were performed. Furthermore, QSAR models with good explanatory and predictive properties for the cytotoxicity in the cisplatin sensitive cell line CH1 and the intrinsically cisplatin resistant cell line SW480 were developed.

ZUSAMMENFASSUNG

Zytotoxische Platin(II) Komplexe gehören zu den meist eingesetzten Substanzklassen in der Chemotherapie. Ihre klinische Effizienz geht jedoch mit schweren dosislimitierenden Nebenwirkungen, intrinsischer und/oder erworbener Tumorresistenz und der unbequemen und kostspieligen Art der intravenösen Verabreichung einher. Platin(IV) Komplexe besitzen ebenfalls tumorhemmendes Potenzial und ihre physikochemischen und chemischen Eigenschaften könnten daher zur Überwindung der Nachteile der Platin(II) Wirkstoffe ausgenutzt werden. Für die erfolgreiche Entwicklung von Platinchemotherapeutika in der Oxidationsstufe +4, bedarf es einer sorgfältigen Untersuchung ihrer Pharmakologie und Toxikologie, der Erarbeitung von Struktur-Aktivitätsbeziehungen und der Entwicklung neuer Synthesestrategien.

Im Rahmen dieser Doktorarbeit wurden neuartige bis-, tris- und tetrakis(carboxylato)platin(IV) Komplexe, die als Prodrugs für *cis*-[$Pt(EtNH_2)_2Cl_2$] (ein Analogon zu Cisplatin, jedoch mit größerer Lipophilie), Carboplatin und Nedaplatin konzipiert wurden, synthetisiert. Zu diesem Zweck wurden die entsprechenden Platin(II) Komplexe mit H_2O_2 in wässriger Lösung oxidiert und dann mit unterschiedlichen zyklischen Anhydriden (Bernstein-, Glutar-, 3-Methylglutar- und 3,3-Dimethylglutaranhydrid) carboxyliert. Die erhaltenen Verbindungen wurden anschließend durch die Aktivierung der freien Carboxylgruppen mit CDI (1,1'-Carbonyldiimidazol) derivatisiert, gefolgt von einer Reaktion mit verschiedenen Aminen oder Alkoholen, die die gewünschten Amide und Ester ergaben. Alle Komplexe wurden durch multinukleare (^{1}H, ^{13}C, ^{15}N und ^{195}Pt) 1D und 2D NMR-Spektroskopie, Elementaranalyse, ESI-MS, ATR-FTIR, HPLC und exemplarisch an einigen Verbindungen durch Röntgendiffraktometrie vollständig charakterisiert. Die *in vitro*

Zytotoxizität der neuen Komplexe wurde mit Hilfe des MTT kolorimetrischen Assays in vier menschlichen Tumorzelllinien, die von Ovarialkarzinom (CH1 und SK-OV-3), Dickdarmkarzinom (SW480) und nichtkleinzelligen Lungenkarzinom (A549) herrühren, untersucht. Zur besseren Einschätzung des pharmakologischen Verhaltens der neuen Komplexe, wurde eine vergleichende Analyse in Bezug auf ihre Lipophilie, Elektrochemie und der Geschwindigkeit der Reduktion mit Ascorbinsäure vorgenommen.

Schließlich wurden Computerstudien, basierend auf DFT-Methoden bezüglich der Elektronenstruktur und der Redoxeigenschaften der untersuchten Verbindungen durchgeführt. Darüberhinaus wurden QSAR-Modelle mit guter Aussagekraft und Prädiktivität hinsichtlich der Zytotoxizität in der Cisplatin sensitiven Zelllinie CH1 und der intrinsisch Cisplatin resistenten Zelllinie SW480 entwickelt.

ABBREVIATIONS

Ac – acetate
BSO - buthionine sulphoximine
CBDCA - 1,1'-cyclobutandicarboxyllic acid
CE - capillary electrophoresis
CML – chronic myelogenous leukaemia
DACH – 1,2-diaminocyclohexane
DFT - density functional theory
DLT - dose-limiting toxicity
DMF – dimethylformamide
DMSO – dimethylsulfoxide
DNA - deoxyribonucleic acid
DTD - drug targeting and delivery
EDDA - 1,2-ethylendiaminediacetic acid
en – ethylenediamine
EPR - enhanced permeability and retention
ESI-MS – electrospray ionisation mass spectrometry
FDA – food and drug administration
GIT - gastro-intestinal tract
GSH – glutathione
GST - glutathione-S-transferase
HCC - hepatocellular carcinoma
HSA – human serum albumin
ICP-MS - inductively coupled plasma mass spectrometry
IR – infrared (spectroscopy)
i.v. – intravenous
LUMO – lowest unoccupied molecular orbital
MEEKC – microemulsion electrokinetic capillary chromatography
MIF - molecular interactions field
MMR - mismatch repair
MW – molecular weight

NADH – nicotineamide adenine dinucleotide

NCI – national cancer institute

NER - nucleotide-excision repair

NMR – nuclear magnetic resonance

NSCLC – non- small cell lung cancer

OCT – organic cation transporter

p.o. – per os

PSA - polar surface area

QM - quantum mechanics

QSAR – quantitative structure activity relationships

QSPR - quantitative structure-properties relationships

ROS – reactive oxygen species

RP-HPLC – reverse phase high-performance liquid chromatography

RT – room temperature

SAR – structure-activity relationships

SCLC – small cell lung cancer

SEC – size exclusion chromatography

STAT - signal transducer and activator of transcription

TS – transition state

XANES - X-ray absorption near edge spectroscopy

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I. INTRODUCTION

1. Cancer, definitions and social significance

Cancer is one of the most spread and life-costing diseases in the modern society. Based on the GLOBOCAN 2008 estimates, about 12.7 million cancer cases and 7.6 million cancer deaths are anticipated to have occurred in 2008.[1] With more than 3 million new cases and 1.7 million deaths (20% of the mortality in Europe) each year, cancer is the most prominent cause of death and morbidity in Europe after cardiovascular diseases.[2]

Cancer is a term for a group of diseases, characterized by uncontrolled division of abnormal ('immortal') cells, which have lost their ability for differentiation and are able to invade other tissues. Localized tumors, which do not spread to other parts of the body, are called benign, while these which are able to invade nearby tissues and to set up second type of tumors (forming metastasis), malignant. There are more than 100 cancer types (named on the base of the organ or type of tissue in which they occur), which could be grouped in categories[3], such as carcinoma (origins from the epithelial tissue), sarcoma (origins from the connective tissue), leukemia (origins in blood-forming tissue such as the bone marrow and causes large numbers of blood cells to be produced and enter the bloodstream), lymphoma and myeloma (origins in the cells of the immune system), central nervous system cancers (begin in the tissues of the brain and spinal cord).

The most frequently diagnosed neoplasm among females is breast cancer, while prostate cancer is the most common in males. Generally, the leading cause of cancer death for both genders in Europe is lung cancer (20%), followed by colorectal cancer (12%), illustrated in **Fig. 1**.

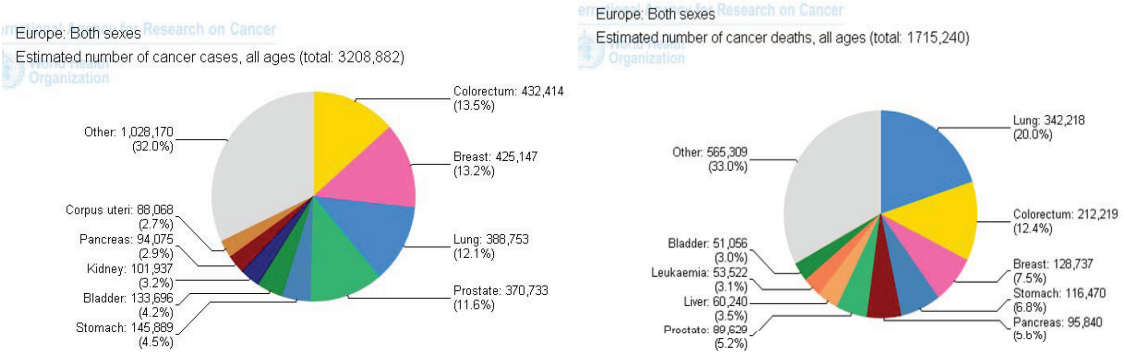

Figure 1. Estimated number and percent of cancer morbidity (left) and mortality (right) in Europe, both sexes, all ages, according to GLOBOCAN 2008[1]

1.1. Treatment of cancer

The classical tool for treatment of cancer is based on a combination between surgery, radiotherapy and chemotherapy, approaches complementary to each other. Other methods for cancer treatment are bone marrow and peripheral blood stem cells transplantation, the use of angiogenic inhibitors, biological therapy (immunomodulators or targeted therapy; cancer vaccines and gene therapies are in clinical studies) and photodynamic therapy.[4] The described approaches, a step to personalized medicine, are characterized with higher selectivity compared to classical radio- and chemotherapy, but are usually used in a combination with them.

The development of successful treatment and early diagnosis has increased dramatically the overall survival rate of cancer patients.[5] (see **Fig. 2**)

1.1.1. Anticancer chemotherapeutic agents

There are more than 70 clinically applied antitumor agents, characterized with relatively low selectivity, reflecting in high general toxicity and side effects. Except the toxicity to non-malignant cells, a main problem of anticancer chemotherapy is intrinsic and/or

acquired resistance of the tumor to cytotoxic agents. As the clinically applied cytotostatics have different cellular targets and mechanisms of action, they are used in combined therapy regimes, rather than as single agents. Anticancer chemotherapy typically implies a combination of drugs from different classes which have dissimilar pharmacology and toxicology. In this manner, the cure effectiveness is increased and the toxic effects are decreased.[6]

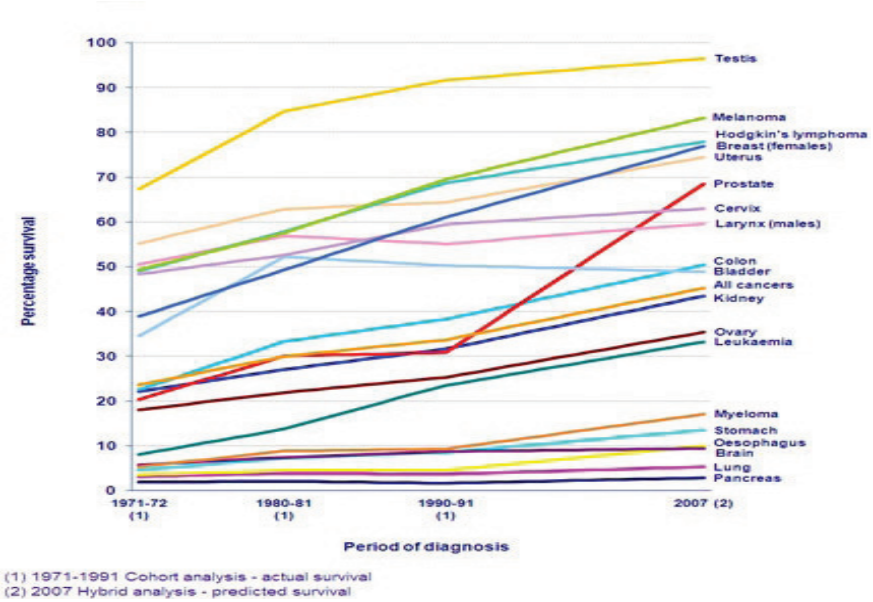

Figure 2. Ten years relative survival (%), adults (15-99 years), selected cancers, England and Wales: survival trends for selected cancers 1971-2007[7]

There are different ways of grouping anticancer drugs, based on their chemical structure, origin, mechanism of action, cell cycle phase specificity, etc. A short classification with examples, based on a mixed principle is shown below:[6, 8]

- Alkylating and metallating agents – react directly with nucleophilic groups of DNA, cell cycle nonspecific agents, poor selectivity:
  - nitrogen mustards (melphalane, chlorambucil, ifosfamide)
  - nitrosoureas (dacarbazine, carmustine)
  - other alkylating agents (busulfan)

- platinum complexes (cisplatin, carboplatin, oxaliplatin)

- Antitumor antibiotics and derivatives – DNA intercalators, generation of reactive oxygen species (ROS), topoisomerase II poisons:

- antracyclins (dactinomycin, doxorubicin)
- synthetic analogues (mitoxantrone)
- glycopeptides (bleomycin)

- Antimetabolites – inhibit the enzymes, involved in DNA synthesis; S-phase specific agents:

- dihydrofolate reductase inhibitors (methotrexate)
- ribonucleotide reductase inhibitor (hydroxycarbamide)
- pyrimidine antagonists (gemcitabine, 5-fluorouracil)
- purine antagonists (6-mercaptopurine, 6-thioguanine)

- Antitumor agents from plant origin and derivatives (cell cycle specific agents):

- topoisomerase inhibitors – podophyllotoxins and camptothecins
- tubulin polymerization inhibitors (Vinca alcaloids, cryptophycins)
- tubulin depolymerization inhibitors – taxanes

- Hormone-based therapies – used for hormone dependent cancers:

- glucocorticoids, estrogens, progestins and androgens (fosfestrol)
- antiandrogens (flutamide)
- antiestrogens (tamoxifen)
- aromatase inhibitors (anastrazole, letrozole)

- Inhibitors of signaling pathways - protein kinase inhibitors and others

- Other cytotoxic agents (Asparaginase, As_2O_3, etc.)

2. Platinum-based therapy

The beginning of platinum-based therapy has its origins in Rosenberg' serendipitous discovery of cell division inhibition in *E.coli* cultures, caused by the platinum species, formed as electrolysis products during the experiments.[9] Nowadays, 34 years after clinical approval of the first metal-based cytostatic, cisplatin (**Fig. 3**, **(1)**), platinum compounds have profound effect in anti-cancer treatment. They are part of first line chemotherapy in twelve neoplasms (testicular cancer, ovarian cancer, bladder cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), head and neck cancer, esophageal cancer, thymoma, osteogenic sarcoma, cervical cancer and colorectal cancer).[10] Moreover, platinum drugs show synergism with a wide range of cytostatics and can be successfully combined with radiotherapy or targeted agents.

Three Pt(II) complexes have been introduced in clinics worldwide (cisplatin, carboplatin, oxaliplatin, **Fig. 3**) and another five have regional approval (nedaplatin, lobaplatin, heptaplatin, miriplatin and dicycloplatin, **Fig. 4**).[11-13]

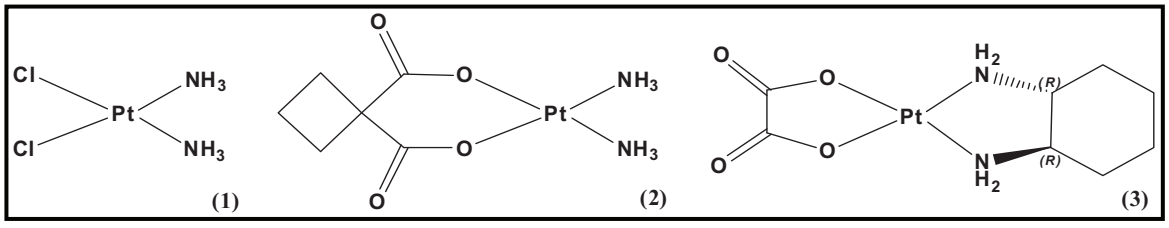

Figure 3. Platinum complexes with worldwide clinical approval: cisplatin **(1)**, carboplatin **(2)**, oxaliplatin **(3)**.

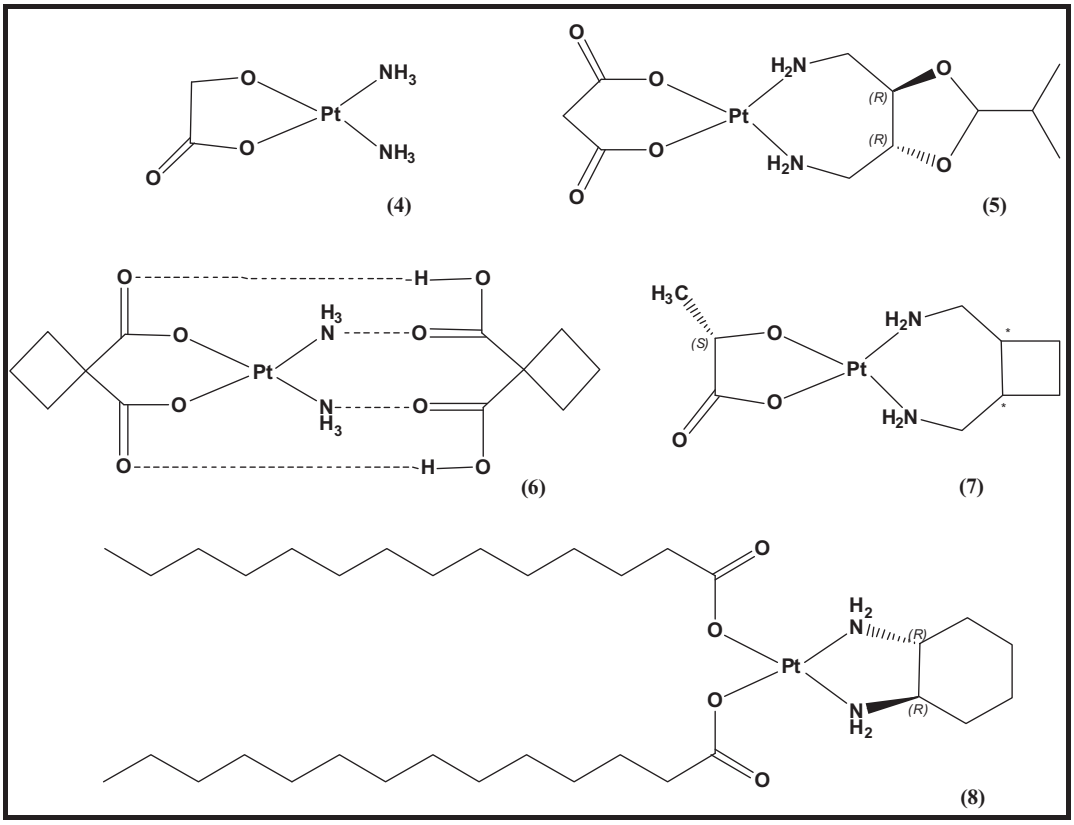

Figure 4. Platinum complexes with regional-limited clinical application: nedaplatin **(4)**, heptaplatin **(5)**, dicycloplatin **(6),** structure taken from ref. 25, lobaplatin **(7)** and miriplatin **(8).**

2.1. Cisplatin, (SP-4-2)-diamminedichloridoplatinum(II)

Cisplatin is the first (FDA approval in 1978) and the most used platinum-based cytostatic; it is applied in 32 of 78 anticancer treatment regimes as a single agents or in a combination with a wide range of other drugs.[11] It has revolutionized therapy of testicular cancer, increasing the cure rate from 5-10 % to over 90%.[10] (see **Fig. 5**)

Treatment with cisplatin is accompanied by severe side effects such as nephrotoxicity (dose-limiting toxicity (DLT)), neuropathy, ototoxicity, myelosuppression (reduction in bone marrow activity), nausea and vomiting (cisplatin is the most emetogenic cytostatic). In order to reduce the main side effects, saline hyperhydration before and after treatment, together with diuretics (manitol) and antiemetic drugs (usually 5-HT_3 antagonists) are

used.[14] Cisplatin is administered *i.v.* as bolus injections or infusions in NaCl containing solutions in order to suppress aquation. It binds covalently and irreversibly to plasma proteins (more than 90%).[15] Cisplatin is distributed in different organs and tissues with highest concentrations in liver, prostate, kidney, ovaries; concentrations in tumors are generally lower, than in the organ where the tumor is located. The major route of elimination is renal excretion (first as a native product, then as metabolites). However, platinum can be found in tissues more than 180 days after administration.[16]

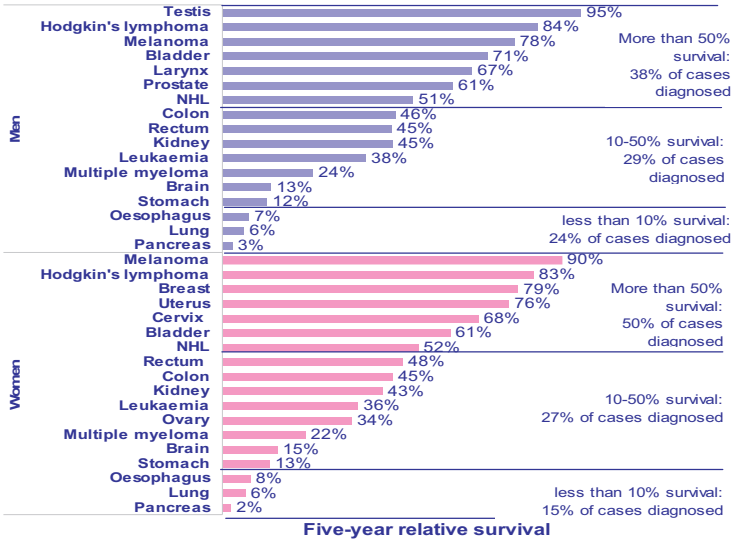

Figure 5. Relative five-year survival estimates based on survival probabilities observed during 2000-2001, by sex and site, England and Wales.[7]

2.2. Carboplatin, (SP-4-2)-diammine(1,1-cyclobutanedicarboxylato)platinum(II)

Carboplatin is the second platinum-based cytostatic with international marketing approval. It shows the same spectrum of activity as cisplatin, but exhibits lower toxicity and therefore higher dosage regimes are possible. Carboplatin is a drug of choice for treatment of advanced ovarian carcinoma.[17] It is usually administered as a rapid intravenous injection (in chloride-free solutions) or as infusion. Carboplatin has much milder side effects in comparison with cisplatin with myelosuppresion as DLT.[14]

Similarly to cisplatin, it binds covalently and irreversibly to plasma proteins, but to a much lower extent (under 30%). The major route of elimination is renal excretion.[18]

2.3. Oxaliplatin, (*SP*-4-2)-((1R,2R)-1,2-diaminocyclohexane)oxalato)platinum(II)

Oxaliplatin, the last platinum-based cytostatic approved for worldwide use, is the first one showing activity against cisplatin-resistant tumors.[11] It is used in adjuvant and especially palliative therapy of metastatic colorectal cancer in combination with 5-fluorouracil and folinic acid. Oxaliplatin is administered i.v. as short infusions. It is less nephrotoxic and emetic than cisplatin and myelosuppression is not common; neuropathy is the dose-limiting toxicity.[14]

2.4. Platinum-based drugs with regional approval

- **Nedaplatin**, (SP-4-3)-diammine(glycolato)platinum(II), **Fig. 4**, **(4)** was approved for clinical use in Japan in 2005.[19] Since then, it has been used in therapies of head and neck cancer, NSCLC and SCLC. Nedaplatin is a second generation platinum-based anticancer drug, analogue of carboplatin, whereas the CBDCA (1,1'-cyclobutandicarboxyllic acid) ligand is exchanged with glycolate. It has the same spectrum of activity as cisplatin and carboplatin. Nedaplatin has shown anticancer activity comparable with that of cisplatin, but with lower nephro- and gastrointestinal toxicity than both cisplatin and carboplatin. Analogously to carboplatin, myelosuppression is the DLT, expressed as thrombocytopenia and neutropenia.[11, 19]
- **Lobaplatin**, (SP-4-3)-(1,2-cyclobutanedimethanamine)(L-lactato)platinum(II), **Fig.4, (7)** is a third generation platinum complex which was approved in China for treatment of chronic myelogenous leukaemia (CML) and inoperable, metastatic breast

and small cell lung cancer.[20] It has shown less and milder side effects than cisplatin with thrombocytopenia as DLT. Lobaplatin is a 50%/50% mixture of two diasteromers, featuring S,S and R,R configuration of the carrier ligand.[21]

- **Heptaplatin,** (SP-4-2)-(malonato)((4R,5R)-2-(1-methylethyl)-1,3-dioxolane-4,5-dimethanamine)platinum(II), **Fig. 4, (5)** was approved in South Korea for treatment of gastric cancer, being their first domestically developed drug.[22] It shows high stability in solution, lower and milder adverse effects and cause less emesis compared with cisplatin. DLTs are nephrotoxicity, hepatotoxicity and myelosuppression.[11]

- **Miriplatin**, (*SP*-4-2)-((1*R*,2*R*)-cyclohexane-1,2-diamine)bis(tetradecanoato)-platinum(II), **Fig. 4, (8)** is a new lipophilic platinum-based anticancer agent, designed specially for the intra-arterial treatment of hepatocellular carcinoma (HCC). It was approved in Japan in 2009 for lipiodolization of HCC and it has been marketed since 2010.[12, 23] The drug is administered as an iodinated poppy seed oil suspension through a catheter inserted into the hepatic artery. [24]

- **Dicycloplatin,** (**Fig. 4, (6)**) is a supramolecule, composed of carboplatin and CBDCA, which are linked via strong hydrogen bonds.[25] It was designed in order to overcome the solubility and stability problems of classical platinum-based therapeutics. Dicycloplatin was approved by the Chinese FDA in 2012 for prostate cancer chemotherapy. Preclinical and clinical studies have shown superior activity and lower adverse effects of dicycloplatin, in comparison to carboplatin.[13, 26]

2.5. Platinum-based cytostatics - pharmacology and structure-activity relationships (SAR)

All platinum compounds in clinical use (**Fig. 3** and **Fig. 4**) are neutral square-planar platinum(II) complexes with *cis* geometry in accordance to the first established SAR[27] after Rosenberg's identification of cisplatin as lead structure.[28] Their ligand sphere is represented by two (or one bidentate) primary am(m)ines, called carrier groups and two chlorides or a chelating carboxylate, called leaving groups. The generally accepted mechanism of action includes intracellular activation by aquation (displacment of the leaving group(s) by water) and subsequent covalent binding to DNA, forming DNA adducts.[29]

The first step in platinum-based drug research after cisplatin's clinical approval in 1978 was the development of less toxic analogues that retained antitumor activity. This effect was achieved by replacement of the two chloride leaving groups in cisplatin with more stable chelating carboxylates (second generation of platinum drugs). Exchange of the carrier ligands (NH_3) with bidentate amineligands, resulted in the third generation of platinum-based medicines, showing activity against tumors, resistant to cisplatin (see **Table 1**).

In general, the leaving groups of platinum-based drugs are responsible for their pharmacokinetics, while the carrier ligands (which do not exchange during aquation) determine the pharmacodynamic characteristics. Which physicochemical properties are relevant for the pharmacology of a platinum-based cytostatic and how they are defined from the ligands is schematized in **Fig. 6**.

Table 1. Generations of platinum-based drugs

generation	carrier ligand(s)	leaving group(s)	examples
I	NH_3	Cl	cisplatin
II	NH_3	CBDCA, glycolate	carboplatin, nedaplatin
III	*1R*,*2R*-DACH, 1,2-cyclobutanedimethanamine, *4R,5R*-2-(1-methylethyl)-1,3-dioxolane-4,5-dimethanamine	oxalate, L-lactate, malonate	oxaliplatin, lobaplatin, heptaplatin

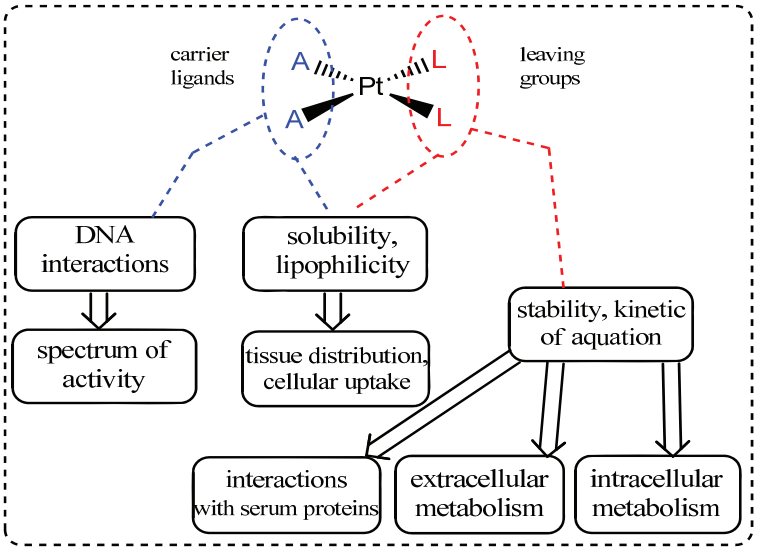

Figure 6. Relation between the ligands, physicochemical, pharmacokinetic and pharmacodynamic properties.

The way from the administration of a platinum drug to its final target (DNA) is schematized in **Fig. 7.**

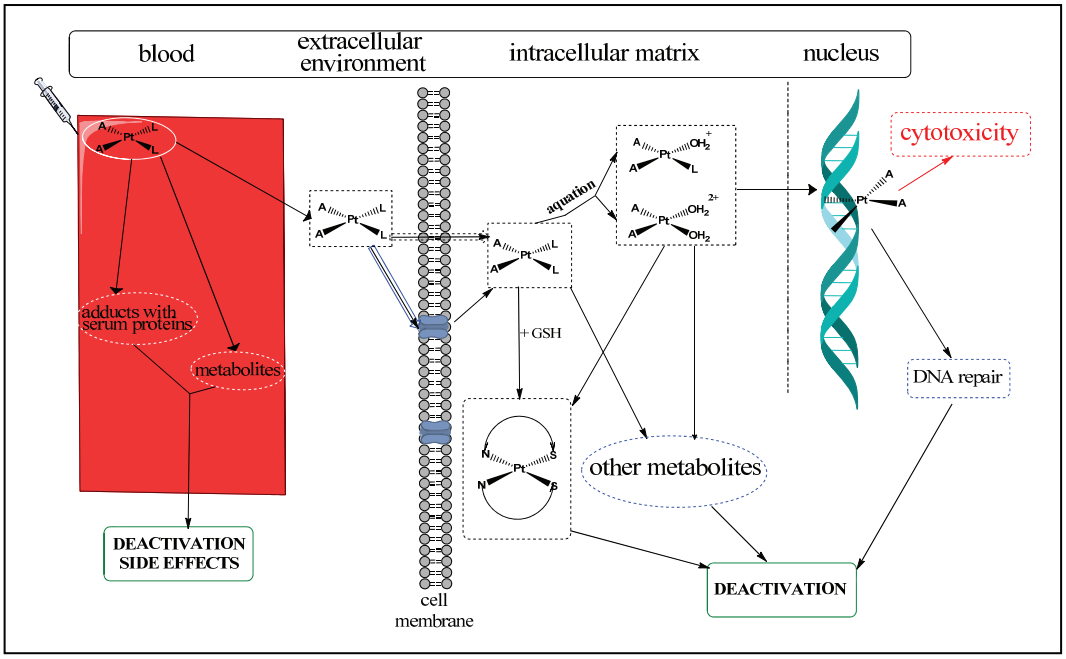

Figure 7. Scheme of the way of a Pt(II) drug from the administration to its target

2.5.1 Administration and fate in the blood circulation

All Pt(II) complexes in clinical use are administered parenterally, because of their poor oral bioavailability and instability in the gastro-intestinal tract (GIT).[14]

Important physicochemical properties on this stage are the solubility (higher water solubility is advantageous due to *i.v.* administration) and stability (pharmaceutical and in the blood circulation). Increasing water solubility and stability in aqueous solution and blood serum is commonly achieved by replacing the chlorido leaving groups with chelating carboxylates[30] (II and III generation complexes). In the case of miriplatin, monodentate coordinated long chained carboxylic acids are used as leaving groups, because of the specific way of administration, where not water solubility but high hydrophobicity is required.[12] More rarely, modification of the carrier ligands could be also used as an approach for increasing water solubility (the development of heptaplatin).[31] Another technique was applied in the development of dicycloplatin, where increased solubility and stability in aqueous solution of carboplatin was achieved by

using the supramolecular concept.[25] Recently, self-association of carboplatin in water which stabilizes its solutions has also been reported.[32]

In the blood circulation platinum complexes bind covalently and irreversibly to plasma proteins, but in different extent (e.g. cisplatin more than 90%, carboplatin less than 30%, clinical data).[15] Their reactivity in plasma is in general related with the strength of leaving groups coordination. Complexes with labile leaving groups (such as H_2O) are highly toxic (high reactivity in the blood, leading to deactivation and high general toxicity); contrary very stable complexes with inert leaving groups (SCN^-, CN^-) are not active (inert intracellularly).[30] Carboxylates and the more reactive chlorides have intermediate binding strength to platinum and represent the usual leaving groups in platinum(II)-based cytostatics. In the last decades, a couple of studies dealing with interactions between clinical used platinum complexes and serum proteins were performed using several analytical approaches (SEC-ICP-MS, RP-HPLC-ICP-MS, RP-HPLC, ESI-MS, CE, NMR).[33] In general, cisplatin and oxaliplatin have shown higher affinity and faster rate of binding to the investigated plasma proteins in comparison to carboplatin.[34] However, conflicting data can be found, concerning the kinetic of binding and the preferential binding sites.[35]

Despite that, the protein-free form of platinum complexes represents their active concentration in the blood stream, protein binding could also be a tool for their selective accumulation in tumor cells. Human serum albumin (HSA) can serve as a drug carrier (due to the EPR (enhanced permeability and retention) effect) for different anticancer drugs, including platinum complexes.[36, 37]

2.5.2 Cellular uptake

Cellular uptake of platinum drugs can be realized by passive, active and facilitated transport mechanisms. For many years passive diffusion was supposed to be the main cell entry pathway for platinum complexes.[38] This consideration was supported by the early observed linear dependence between cellular accumulation and the concentration gradient of the applied platinum drug up to 1 mM concentrations. In addition, no competitive inhibition between cisplatin and structural analogues was detected. As the clinically applied platinum drugs are relatively stable in the bloodstream, they can go through cell membranes as neutral molecules with relatively low molecular weight, despite their hydrophilic character.[39] Nevertheless, exponential relationship between platinum uptake and lipophilicity (as the meaning of log $P_{o/w}$) of platinum complexes has been found.[40]

Later experiments indicated that active transport mechanisms, using carriers/channels as well as various endocytic routes can also play important role in platinum cell uptake. Copper transporters were demonstrated to be involved in the influx, intracellular transport and efflux of platinum drugs. The significance of copper uptake transporter (CTR1) for the cellular accumulation of cisplatin and its analogues can be seen from the observed correlation between platinum drugs resistance and low expression of CTR1 in different cancer cell lines. Furthermore, its significance was confirmed by treating yeast and mammalian cells, in which the CTR1 gene was knocked-out, with platinum-based cytostatics.[41] Moreover, other experiments demonstrated that CTR1 is structurally discriminative and its role in cisplatin and carboplatin accumulation differs from that of oxaliplatin and satraplatin.[39]

The possibility to sensitize resistant tumors to platinum-based therapy by copper-lowering agents such as D-penicillamide and trientine (expression of hCTR1 is up-regulated under copper depleted conditions) was recently shown *in vitro* and *in vivo* in animal tumor models. The last findings encouraged a phase I clinical trial using trientine in sensitizing carboplatin treatment.[42, 43]

2.5.3 Intracellular activation

Due to the significant difference in Cl^- concentrations extra- (~100 mM) and intracellularly (3-20 mM), cisplatin undergoes activation via aquation in the cytosol, while one or both of the chloride ions are exchanged with water molecules.[38] Extracellularly, the aquation process is suppressed due to the high concentration of Cl^-; calculations showed that in such conditions 68% of the complex remains in its original form while the rest is represented by neutral chloridohydroxido species.[44] In the case of carboplatin, which stability against hydrolysis is higher due to coordinated chelating CBDCA and self-association processes in solution, intracellular activation differs from that of cisplatin. Reaction with carbonates and other nucleophiles most likely play a role for its activation in the cell, while chlorides and phosphates seem to have minor importance.[32] Theoretical and experimental studies demonstrated that the rate limiting step in the activation of carboplatin, nedaplatin and oxaliplatin is the first aquation process (accompanied by chelate ring opening), contrary to cisplatin where the second hydration is rate determining.[45] Consequently, the fully hydrolyzed forms of second and third generation platinum drugs and the monoaquated form of cisplatin are suggested to be the main products reacting with DNA. The calculations have also indicated, that in

acidic conditions carboplatin, nedaplatin and oxaliplatin are supposed to reach DNA in their monoaquated form.[46, 47]

Another study showed that the leaving group of oxaliplatin (oxalate) is less stable coordinated than that of carboplatin (CBDCA) and faster activation/deactivation reactions could be expected. [48]

Possible intracellular metabolites of cisplatin and carboplatin formed during the activation processes are shown in **Fig. 8**.

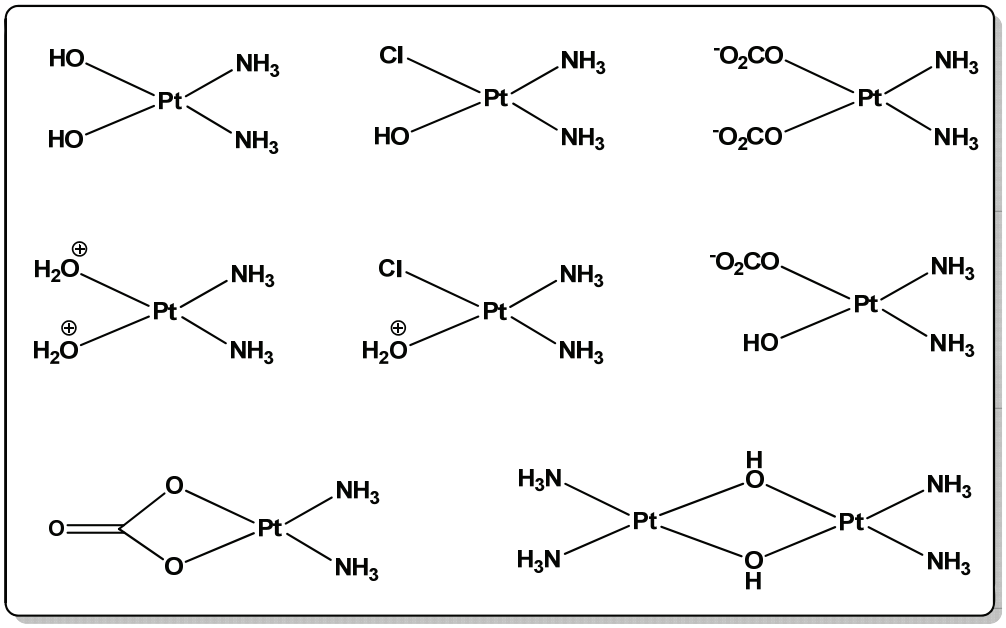

Figure 8. Main products of intracellular activation of first and second generation platinum drugs.

Further steps after the activation are interactions with reactive cellular components such as proteins, membrane phospholipids, RNA, DNA, glutathione, etc., responsible for the cytotoxicity and/or for the inactivation of platinum complexes. [38]

2.5.4 Interactions with DNA

In order to reach their primary target (DNA), activated forms of platinum complexes should pass through various cytosolic components and enter the nucleus, where they react mainly with DNA, due to its high concentration there.[38]

There are different hypotheses, explaining why platinum drugs bind to nucleotide bases (N-donor ligands) in the presence of cellular components like gluthatione and methionine (S-donor ligands), with which they can form more stable platinum adducts. One of them postulated kinetic control, a consequence of slow ligand exchange at platinum.[38] Others proposed the involvement of metallochaperons such as ATOX1 capable of delivering platinum drugs to the nucleus.[41] Nevertheless it was estimated that not more than 1% of administered platinum ends up to its final target, DNA.[49]

DNA platination occurs mainly through binding to the N7 atom of guanine or adenine bases. Platinum(II) complexes with *cis* configuration (all drugs in clinical use) form predominantly bifunctional intrastrand cross-links. Nevertheless, interstrand and monofunctional adducts were also found in small percentage. The major adducts found for platinum-based drugs are: 65% intrastrand GG, 25% intrastrand AG, 5-10% intrastrand GNG and 1-3% interstrand adduct formations.[38] However, significant conformational differences of the GG adducts formed with cisplatin and carboplatin and that with oxaliplatin were observed.[50] In addition, *Brabec et al.* showed that cisplatin and oxaliplatin form different interstrand cross-link adducts with DNA. These differences, caused by the exchange of ammonia with the bidentato DACH ligand, are one of the supposed reasons for the different spectrum of activity of oxaliplatin and II generation platinum drugs. Furthermore, the stereochemistry of the carrier ligand has a significant influence on the character of DNA interactions and the respective cytotoxic activity.[51]

There is no clear agreement in the field which platinum-DNA adducts have the most significant biological effect. The formed DNA adducts provoke distortions in DNA, including unwinding and bending, which are recognized by several proteins. As a consequence, cellular processes like replication and transcription can be inhibited, DNA-repair mechanisms will be activated, signal-transduction pathways, controlling growth, differentiation and stress responses will be affected, etc. The final result will be either cell death (most likely apoptotic) or cell survival, when the DNA lesions were successfully repaired.[29] What proteins are involved in the effects caused by platinum-DNA interactions is an object of continuous investigations.[38, 52]

2.5.5 Deactivation of Pt(II) drugs. Resistance

Inactivation of platinum complexes, a main reason for platinum-drug resistance, can occur during different stages from the time the drug is administered to the moment it interacts with DNA. First, deactivation occurs in the bloodstream where platinum complexes can bind irreversibly to biomacromolecules and consequently their active concentration can be decreased. However, this problem can be controlled by appropriate choice of the leaving groups. Once entering the cell, platinum complexes undergo aquation and the formed reactive species can be inactivated by binding to various intracellular nucleophiles and/or by increased efflux. The major cellular mechanisms proposed for platinum drugs resistance can be summed up to reduced cellular accumulation, cytosolic detoxification and enhanced DNA repair and tolerance.[49]

Gluthatione (GSH) is the main cytosolic component, claimed to be responsible for platinum drugs deactivation. It can form different adducts with platinum complexes,

including total exchange of the ligands and coordination in a bidentate chelate fashion [$Pt(GS)_2$]. About 60% of the intracellular cisplatin was reported to react with GSH. The observed good correlation in several cell lines between intracellular levels of gluthatione and sensitivity to platinum drugs suggested GSH as a major reason for tumor resistance to platinum complexes. [29,49] Recently, Gibson showed, using NMR experiments with cell lysates from different tumors, that gluthatione may not be the major cellular target of cisplatin.[53] Furthermore, he placed the need of reconsidering the main postulates for mechanisms of action of platinum complexes and the models we use to establish them.[54]

Cellular acumulation can be reduced due to decreased influx and/or increased efflux of the drug. Efflux proteins such as copper transporters ATP7A and ATP7B have shown effect on platinum complexes activity as involved in their export from the cells.[41] Another main reason for platinum-drug resistance is the insufficient cellular uptake, due to low expression of CTR1 (discussed in 2.5.2).

A further major mechanism of deactivation of platinum drugs and respective tumor drug resistance is the DNA repair/removal of platinum adducts. The nucleotide-excision repair (NER) is the main pathway known to remove platinum drugs lesions from DNA. Another mechanism, the mismatch repair (MMR) showed importance in recognizing and repairing Pt-DNA adducts, caused by cisplatin and carboplatin, but not by oxaliplatin.[29]

As the mentioned influx/efflux transporters, cellular GSH levels and DNA repair mechanisms capacity are different in various cancer types, the different respond to platinum based therapy of the latter is not surprising.

2.6 Drawbacks of existing platinum-based therapy. Perspectives

The main drawbacks of platinum-based cytostatics, which restrict their usage can be summarized as severe dose-limiting side effects, intrinsic or/and acquired resistance and the inconvenient and cost intensive way of administration (*i.v.*). In principle, these are the main problems, associated with cisplatin, which could not find solution in the next generations of platinum complexes in clinical use, developed during the last 30 years. Toxic effects were decreased, by optimizing the stability/reactivity of the compounds through changing the chloride leaving groups for chelating carboxylato ligands. The replacement of the ammonia carrier ligands with cyclic diamines, forming five, six and seven membered chelate rings, was a base for the development of anti-cancer agents, showing activity in some cisplatin-resistant tumors.[31, 55, 56] However, the potential for further improvements is rather exhausted and other strategies should be employed. Possible approaches for overcoming the main drawbacks of platinum-based therapy are presented below:

- **Combinations** of platinum drugs and new molecular targeting agents, phytochemicals or therapeutic agents, which are able to sensitize the tumor cells to the platinum drugs, are in different stages of preclinical and clinical trials.[57, 58]

- **Drug targeting and delivery (DTD)** methods have the aim to decrease the side effects by selective accumulation of the cytostatics in the tumor tissue. Active DTD is based on specific interactions between the drug and elements from the tumor cells/tissues (e.g. transporter-, antigen or receptor-based drugs). Platinum complexes, attached to estrogens, antiestrogens, bile acids, antimetabolites, nutrients as sugars and amino acids, peptides as well as bisphosphonates have been developed as a trial for active targeting [58, 59]. Passive DTD is based on the enhanced uptake and longer retention of drug-

macromolecule conjugates in tumors in comparison with normal tissues, known as enhanced permeability and retention (EPR) effect. The used macromolecules can be modified plasma proteins, peptides, polysaccharides, dendrimers, artificial polymers, monoclonal antibodies, nanoparticles, etc.[60]

The targeting unit (biovector or macromolecule) is usually bound to the active drug via a spacer and a linker. In the case of Pt(II) complexes, both the leaving groups and the carrier ligands can be used for its attachment and the choice should be made based on considerations, concerning the stability of the linker and the character of the target.[59] Finally, different formulations, including lipid, nanoparticle and liposomal versions have also been explored. Lipoplatin and aroplatin (liposomal formulations of cisplatin and the bis(neodecanoato) analogue of oxaliplatin, resp.) are currently in clinical trials (III and II phase, resp.).[11]

- **Non-classical platinum(II) complexes**. The design of platinum compounds, which do not follow the general formula *cis*-[$Pt(NHR)_2L_2$] (where NHR or $(NHR)_2$ is primary or secondary mono or bischelating diamine and L or L_2 is chloride or (chelating biscarboxylate) determined from Cleare and Hoechele SAR[27] can be summarized in the following approaches:

- **trans complexes**

The inactivity of platinum(II) complexes with trans geometry were postulated in the general SAR drawn by Cleare and Hoeschele[27], mainly based on comparisons between transplatin and cisplatin. The controversial biological effects of the two geometric isomers *in vitro* and *in vivo* and the inactivity of transplatin were observed and reported by Rosenberg in parallel with the discovery of the anticancer activity of cisplatin.[28] Transplatin is much more reactive than its *cis* analogue and therefore it is involved in

higher extent in side reactions with extra- and intracellular components, leading to its deactivation and a lower amount of the complex reaching DNA. Furthermore, due to its geometry, transplatin is not able to form 1,2-intrastrand cross links with DNA, which are considered to be determinative for cisplatin's anticancer activity.

In the last two decades trans complexes, named as rule-breakers, have shown significant cytotoxic activity in different tumor cell lines, including such resistant to cisplatin. The breakthrough was achieved by replacing one or two of the ammonia ligands in transplatin with sterically demanding groups, which decreases the deactivation of the resulting compound. The development of active *trans* complexes is of great interest because of their potential to overcome resistance towards classical platinum-based cytostatics due to their capability of forming completely different Pt-DNA adducts as compared to cisplatin, resulting in a new spectrum of activity. However, there is still no mononuclear platinum(II) complex with trans geometry in clinical trials. The most successful trans complexes developed up to now can be classified in the following groups: with planar heterocyclic ligands, with nonplanar heterocyclic ligands, with asymmetric aliphatic amines, with iminoethers and with oximes.[61-63]

- **ammine(amine) and ammine(N-heterocycle) platinum(II) complexes**

Cisplatin analogues in which one of the ammonia ligands is substituted by a bulky cyclic amine or pyridine derivative showed enhanced activity in cisplatin-resistant cell lines. Introduction of a sterically demanding N-containing ligand hinders the axial attack to the platinum center of intracellular detoxifying agents such as GSH which obstruct the deactivation of the complex. Furthermore, asymmetric compounds can form Pt-DNA adducts different from cisplatin, which are more difficult to be recognized from the DNA repair systems. Picoplatin, (SP-4-3)-amminedichlorido(2-methylpyridine)platinum(II)

(**Fig. 9**, (**9)**) is the most successful example applying that strategy, where the position of the coordinated methylpyridine provides a steric hindrance with regard to the attack of the drug by intacellular thiols. It has demonstrated anticancer activity in cisplatin, carboplatin and oxaliplatin resistant cell lines and tumor models *in vivo*.[14] Furthermore, picoplatin is the first platinum(II) complex showing oral activity. In the moment, it is in various phase II clinical trials.[11] Picoplatin has been granted orphan drug designation (EU/3/07/502, EMEA/OD/055/07) for the treatment of small cell lung cancer.[64] Another strategy based on co-administration of unsymmetric ammine(amine) complexes and BSO (buthionine sulphoximine, an inhibitor of GSH biosynthesis) was recently patented.[65]

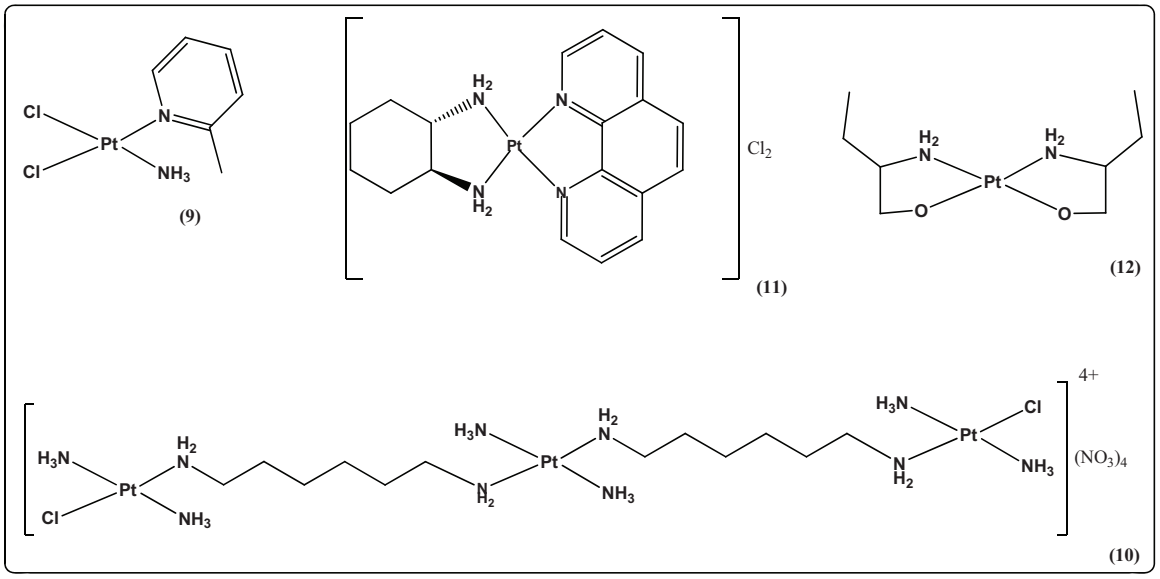

Figure 9. Representative examples of non-classical platinum(II) complexes with antitumor activity: picoplatin **(9)**, BBR3464 **(10)**, PHENSS **(11)**, pH sensitive complex with aminoalcoholate **(12)**.

- **positively charged multinuclear platinum complexes and platinum intercalator**s

Cationic platinum(II) compounds can enter the cell using organic cation transportes (OCT). Multinuclear platinum complexes have shown increased DNA binding affinity

and lack of cross-resistance with cisplatin. BBR3464 (**Fig. 9**, (**10)**) is a trinuclear, positively charged (+4) platinum(II) drug, which demonstrated impressive preclinical *in vitro* and *in vivo* results with superior activity than the clinically applied platinum complexes in both cisplatin sensitive and resistant tumors.[66] However phase II clinical trials have shown only partial responses of the treated cancers and poor maximum tolerated dose of the drug.[11]

By chelate coordination of planar heterocyclic ligands (e.g. phenantroline derivatives) to square planar platinum(II) complexes, compounds with DNA intercalating properties can be obtained (**Fig. 9**, (**11)**). This type of complexes inhibit DNA replication not through forming Pt-DNA adducts, but via reversible insertion of the molecule between the bases. Despite the promising *in vitro/in vivo* activity and lack of cross resistance with cisplatin no compound of this class is close to clinical development.[60]

- **platinum prodrugs with an acidic pH optimum**

Based on acidic conditions, presented in mainly solid tumors (pH=5-6), non-cytotoxic compounds at physiological pH, which can be selectively activated in the tumor acidic media have been designed. Example of complexes displaying antitumor potency with an acidic pH optimum containg bischelating *O*–alkyldithiocarbonato, aminoalcoholato ligands (**Fig. 9**, (**12)**) or monochelating 1,3-dixydroyacetonoxime have been reported in literature.[58, 67]

- **Platinum(IV) complexes** offer a variety of advantages in comparison to their platinum(II) counterparts. In principle, the already mentioned main drawbacks of platinum-based chemotherapy could find a solution by switching to Pt(IV) and choosing an appropriate coordination sphere. In addition the DTD strategy could be applied easier using platinum(IV) compounds (discussed in details in chapter 3).

- **Nonplatinum metal complexes** have also been of great interest during the last decades as they are generally less toxic than platinum compounds and exert anticancer activity without mimicking cisplatin‘s mode of action.[68] The most promising candidates for new metal-based drugs are complexes of Ru(III), Ga(III), Ru(II), Au(III) and Au(I)[69-71] (see **Fig. 10**)

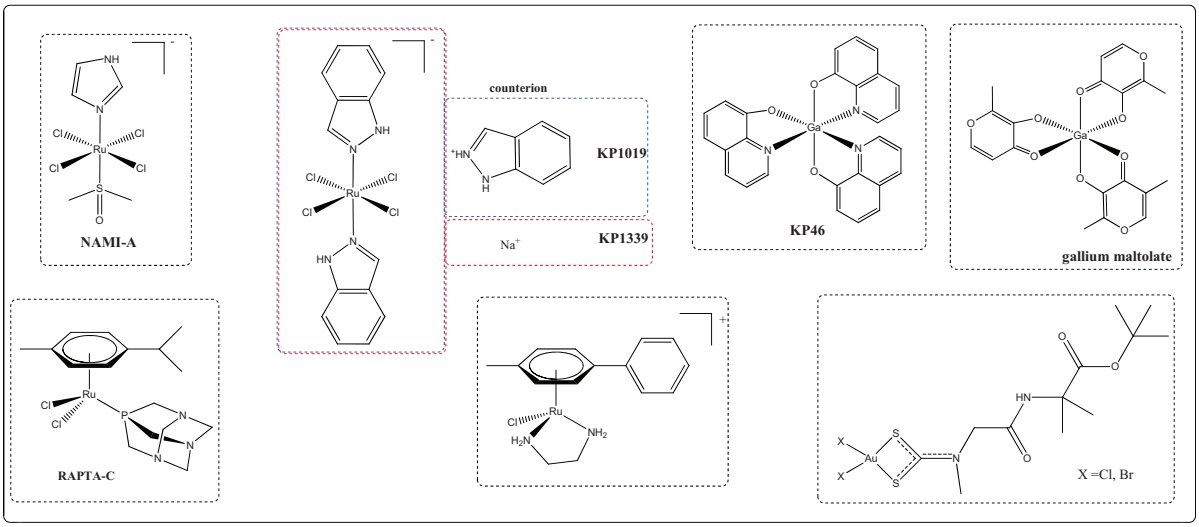

Figure 10. Metal-based cytostatics which were/are in clinical trials (upper row) and such in preclinical development (bottom).

3. Platinum(IV) complexes as an alternative

It is worth mentioning, that prior to identification of cisplatin as major cytotoxic agent, the effects of its Pt(IV) analogue ((OC-6-22)-diamminetetrachloridoplatinum(IV)) were noticed.[72, 73] Platinum(IV) complexes possess octahedral geometry with six coordinated atoms and exhibit higher kinetic inertness in comparison with their square-planar Pt(II) counterparts. Irreversible, two-electron reduction, accompanied by loss of two axial ligands is more common than ligand-exchange reactions. **(Table 2)**

Table 2. General comparison between Pt(II) and Pt(IV) complexes.

oxidation state	+2	+4
electronic configuration	$[Xe]4f^{14}5d^{8}$	$[Xe]4f^{14}5d^{6}$
geometry of the complexes	square planar	octahedral
coordination number	4	6
preferred reactions	ligand-exchange	reduction

Platinum(IV) complexes' physicochemical and chemical properties could be used in overcoming main problems of platinum-based therapy in the following manner:

a) decreasing general toxicity and side effects by using the prodrug concept, selective tumor accumulation and/or selective activation in the tumor

It is generally accepted, that Pt(IV) complexes have to be reduced *in vivo* to their active Pt(II) counterparts in order to exhibit their cytotoxic activity. In the ideal case, the compound is stable in the bloodstream and is reduced intracellularly (Pt(IV) complexes are considered as cellular prodrugs of Pt(II) compounds).[58] It can be speculated, that

Pt(IV) compounds would be predominantly activated (reduced) in the tumor environment due to its hypoxic character.

DTD strategies can be successfully applied for platinum(IV) complexes. Their kinetic inertness and the reduction with expected loss of the axial ligands give a plethora of possibilities for attaching different biovectors or macromolecules to one or both of the axial ligands. Advantageously, the use of special bio-degradatable spacer between the complex and the targeting agent is not required in this case, as this role is carried out by the axial ligands themselves. As an example, Lippard and coworkers have successfully conjugated carbon nanotubes and gold nanoparticles to monocarboxylato Pt(IV) complexes as prodrugs for cisplatin.[74, 75]

Finally, inert and non toxic platinum(IV) prodrugs which can be photoactivated in and around the tumor are in current development. Good examples in this direction are photoactive complexes, featuring equatorial iodido or azido ligands and hydroxide or acetate as axial ligands (see **Fig. 11** for examples).[70, 76-78]

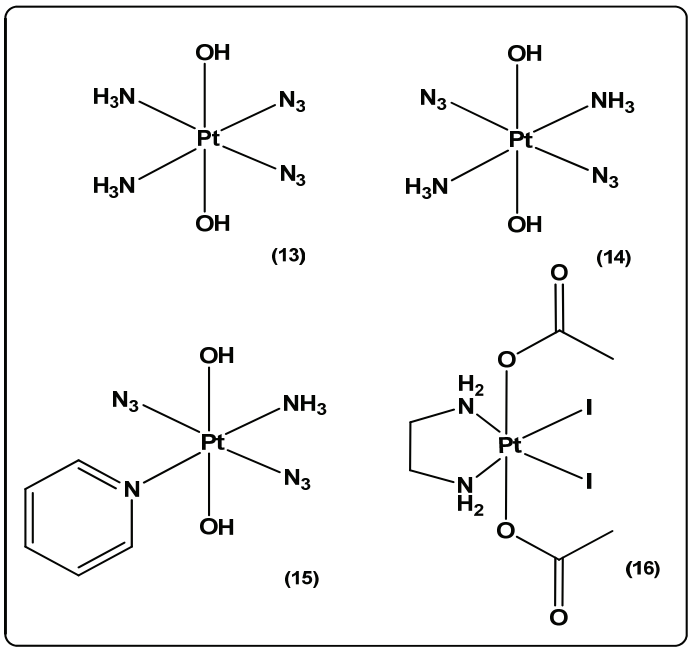

Figure 11. Platinum(IV) prodrugs, inactive prior photo activation

b) switching to oral administration

The higher kinetic stability of Pt(IV) complexes (they can survive in the gastro-intestinal tract, GIT) and the possibilities for easy tuning of their lipophilicity, open a way for the design of compounds with good oral bioavilability.[58] Consequently, satraplatin, employed as hard gelatin capsules was tested in clinical trials, being the first oral platinum-based cytostatic.[14]

c) overcoming platinum drug resistance

The increased number of ligands and synthetical approaches give broader possibilities for the design of cytostatics, able to overcome some of the factors, determining the lack of activity of platinum(II) complexes in various cancers. Additionally, therapeutically active molecules can be coupled to the axial ligands; after reduction in the hypoxic tumor tissue, the reactive platinum(II) species and compounds with their own anticancer activity or/and capable of sensitizing the tumor cells to the reduced platinum complex will be released. Exploring this strategy, Lippard *et al.* have attached cell-sensitizing estradiol units to platinum(IV) compounds as prodrugs of cisplatin (**Fig. 12**, (**17)**).[60, 65] The same group has developed Mitaplatin (**Fig. 12**, (**18)**), a prodrug, designed to release cisplatin and the orphan drug dichloroacetate after *in vivo* reduction. This approach offers a dual-killing mode that can only be effective in cancer cells.[79] Another example is the nitroplatinum(IV) complexes, designed to inhibit STAT (signal transducer and activator of transcription) protein functions (**Fig. 12**, (**20)**).[65] Dyson *et al.* have developed Pt(IV) derivatives of cisplatin, featuring the cytosolic glutathione-S-transferase (GST) inhibitor, etacrynic acid. The approach aimed to overcome GST related Pt-drug resistance (**Fig. 12**, (**19)**).[80]

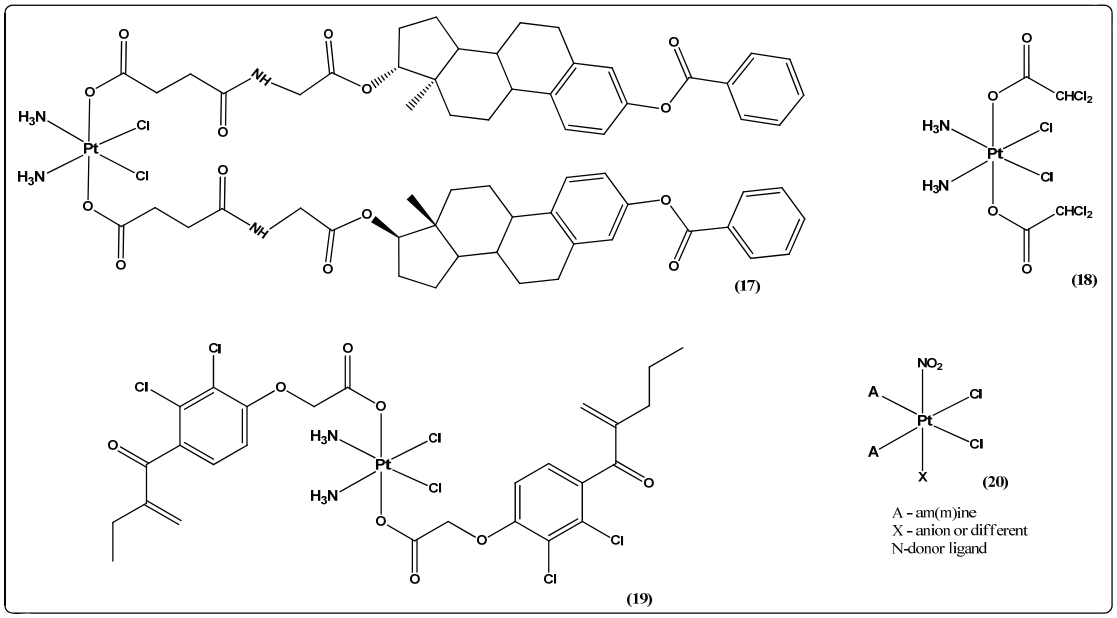

Figure 12. Examples of Pt(IV) complexes, bearing bioactive molecules, able to sensitize tumor cells to platinum therapy.

3.1 Pt(IV) complexes in clinical trials

3.1.1 Ormaplatin (Tetraplatin)

(OC-6-22)-tetrachlorido(*trans*-1,2-cyclohexanediamine)platinum(IV) forms similar active species compared to oxaliplatin after *in vivo* reduction and following aquation. Therefore, it has shown activity in some cisplatin-resistant tumors. Tetraplatin has been tested in six different phase I clinical trials, sponsored by NCI (USA) and cumulative severe neurotoxicity was observed as dose limiting toxicity.[11] No further clinical trials have been reported in literature, probably due to the development of oxaliplatin, a Pt(II) analogue with better solubility and toxicological profile.

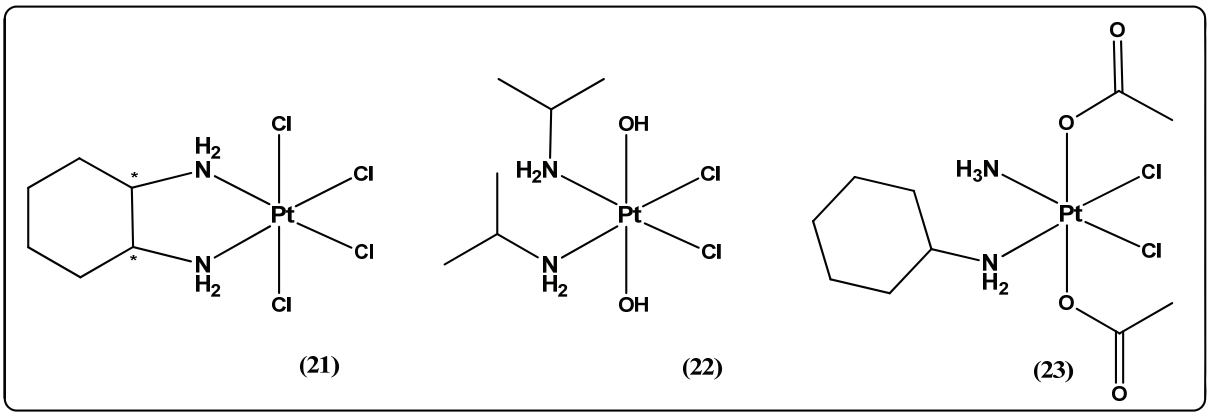

Figure 13. Platinum(IV) complexes, which have been/are investigated in clinical trials: tetraplatin **(21)**, iproplatin **(22)** and satraplatin **(23)**.

3.1.2 Iproplatin

(OC-6-33)-dichloridodihydroxidobis(isopropylamine)platinum(IV) is the most studied platinum-based drug in clinics, which has not gained approval so far. More than 1000 patients participated in clinical trials, including five phase I, 22 Phase II and a single Phase III trial. Iproplatin showed a mild toxicological profile with myelosuppression as DLT and lack of activity in the majority of cancer types tested.[11] As a final outcome of the clinical trials, iproplatin has demonstrated no advantages in comparison to carboplatin (which has gained its approval at that time) and therefore further development was discontinued.[81]

3.1.3 Satraplatin

(OC-6-43)-bis(acetato)amminedichlorido(cyclohexylamine)platinum(IV) is the first oral administered platinum-based cytostatic tested in clinical trials. It has shown activity in both cisplatin sensitive and resistant tumor models *in vitro* and *in vivo*. In addition, *in vivo* experiments with mice demonstrated no reduction of satraplatin's therapeutic index when given orally (in comparison with *i.p.* administration).[14] Preclinical and phase I

clinical trials showed that satraplatin has non-linear pharmacokinetics and improved absorption and tolerability can be achieved by daily x 5 schedule administration. Leukopenia and thrombocytopenia were found as DLT. Satraplatin's absorption is rapid and a high amount of the complex is irreversibly bound to plasma proteins. Biotransformation is fast and more than six new species can be found in patient's plasma ultrafiltrates 15 min after application, while the parent drug cannot be detected anymore. The products of biotransformation are platinum(IV) and platinum(II) complexes, with (SP-4-3)-amminedichlorido(cyclohexylamine)platinum(II) detected as main metabolite.[82] The outcome of different phase II studies conducted, suggested a phase III clinical trial to be performed in patients with refractory prostate cancer. The so-called SPARC study (satraplatin and prednisone against refractory cancer *versus* placebo plus prednisone) was performed in 950 patients with hormone refractory prostate cancer who had progressed after initial chemotherapy. Despite the positive outcome of the trial, expressed as reduced risk of cancer progression and increased progression free survival rate, the FDA has rejected satraplatin on the basis of its non-convincing benefits in terms of overall survival. Currently satraplatin is in various phase I, II and III clinical trials in combination with a range of anticancer drugs.[11, 14]

LA-12, a satraplatin analogue featuring bulky adamantylamine instead of cyclohexylamine, has shown promising results in preclinical studies[83]. A phase I clinical trial has been conducted, but results have not been published yet.

3.2 Proposed mechanism of action and SAR

As platinum(IV) complexes are considered to be prodrugs of platinum(II) compounds, their intracellular pharmacology includes activation and following interaction with the

main target, DNA.[58] The main differences in both species could be found in their pharmacokinetics.

A platinum(IV) complex, which possesses optimal anticancer drug properties, should:

- have adequate solubility, sufficient stability in the GIT and optimal lipophilicity (log $P_{o/w}$ 0.5-3.5) in order to be absorbed from the intestinal lumen into the bloodstream (when applied orally);
- be relatively stable in the bloodstream and accumulate predominantly in the tumor;
- be reduced fast enough intracellularly to the desired platinum(II) complex.

As a consequence of the activity of ((OC-6-22)-diamminetetrachloridoplatinum(IV)) and the first postulated SAR for platinum-based drugs[84], most investigated platinum(IV) complexes have been designed as prodrugs for platinum(II) compounds in clinical use and their analogues (e.g. oxoplatin, tetraplatin, iproplatin). They can be presented by the following general formula: *cis,cis,trans*-$[PtA_2L_2X_2]$, where A is the carrier group, usually am(m)ine or N-containing heterocycle, L is regularly chloride, but can also be iodide, azide or carboxylate and X can be Cl, OH or different functionalized carboxylates. In analogy to platinum(II) complexes, the carrier groups, which stay on the complex after activation (reduction and aquation), are in charge for the character of the DNA adducts formed. The other four ligands (two axial and two equatorial) are usually released after activation with biological reducing agents (e.g. gluthatione, ascorbate, methionine) and following ligand-exchange reactions and are therefore responsible for the pharmacokinetics of the drug. It has been shown, that for complexes of the type $Pt(am(m)ine)_2Cl_2X_2$, X=Cl leads to fast reduction and high systemic toxicity, X=OH reflects in very slow kinetic of activation and consequent low activity, while X=OAc ensures compounds with optimal redox properties.[58] Nevertheless, during the last years,

it was demonstrated, that the redox behavior and the corresponding toxicity of platinum(IV) complexes depend on both the axial and equatorial ligands (L and X) and that reconsidering of classical concepts is required[85] (discussed in detail in chapter 3.3.2). It should be mentioned, that in principle platinum(IV) complexes are able to form adducts with DNA bases.[86] However this process is without clinical relevance due to its very slow kinetic.[87]

3.2.1 *all trans* platinum(IV) complexes

Platinum(IV) complexes in which not only the axial, but also the equatorial ligands are in trans configuration are considered to have *all trans* geometry. JM335 ((OC-6-12)-ammine(cyclohexylamine)dichloridodihydroxido)platinum(IV)) was the first compound from this type, demonstrating *in vitro* and *in vivo* antitumor activity and showing a different cross-resistance pattern to that of its *cis*-isomer.[88] Investigation of series of compounds, following the general formula *all trans*-amine(ammine)dichloridodihydroxidoplatinum(IV) confirmed the results obtained with JM335. Nevertheless, the observed activity against human ovarian carcinoma xenografts was lower than that of cisplatin.[89] Quiroga and coworkers have recently showed, that complexes of the type *trans,trans,trans*-[Pt(amine)(amine')$Cl_2(OH)_2$] demonstrate slightly higher cytotoxicity *in vitro* in comparison to their Pt(II) counterparts.[90] However, when analyzing these results, it should be taken in account that in *all trans*-[$PtA_2Cl_2(OH)_2$], both chlorides and hydroxides can be considered as axial ligands and therefore easier and faster reduction in comparison to their *cis,cis,trans*- analogues could be expected.

Martinez *et al.* have reported a comparison between the biological properties of *trans*-[$PtCl_2(NH_3)$(4-hydroxymethylpyridine)] and *all trans*-[$PtCl_4(NH_3)$(4-hydroxymethyl-pyridine)], showing four times lower activity of the Pt(IV) analogue, but its ability for an easy activation from the reducing agents in the cell.[91] Finally, *all trans*-platinum(IV) complexes have shown to be promising phototherapeutics, when featuring azides and hydroxides as leaving groups[76] (see above, **Fig. 11**, (**14)** and (**15)**).

3.3 Physicochemical properties of interest and their control

3.3.1 Solubility and lipophilicity

Solubility and lipophilicity are important physicochemical parameters for every drug-like molecule playing a crucial role for the way of administration and the following pharmacokinetic processes of absorption, distribution and excretion. Orally administered platinum-based drugs should pass different cell membranes, getting through the gut wall in the blood supply and after distribution to the tumor tissue, entering into the cancer cells. Too polar molecules will not pass through the fatty cell membranes of the intestinal lumen, while too hydrophobic will dissolve in the fat globules and will be excreted with feces. Optimal lipophilicity, which usually corresponds to log $P_{o/w}$ in the region of + 0.5 to 3.5, is a main requirement for good oral bioavailability. However, drug molecules should also possess chemical and metabolic stability in order to survive the digestive enzymes in the GIT as well as liver metabolic enzymes.[8] Although aqueous solubility is less critical for oral administration in comparison to *i.v.*, it plays a role for the drug absorption efficiency, as only the dissolved fraction can be absorbed in the digestive tract.

Lipophilicity and water solubility are both dependent on all six ligands from the platinum(IV) coordination sphere. Nevertheless, these physicochemical parameters can be most conveniently tuned by modification of the axial ligands. At first, Kelland *et al.* have reported that increasing the size of the axial chains (resp. the lipophilicity) in *cis,trans,cis*-(alkylamine)(ammine)bis(carboxylato)dichloridoplatinum(IV) complexes leads to increased cytotoxicity, most likely due to enhanced cellular accumulation.[92] Augmented cytotoxic activity was also observed by increasing the size/lipohilicity of the alkylamine ligands. However, changing the carrier groups can also alter the character of the final Pt-DNA adducts formed and therefore is not a preferable approach for adjusting pharmacokinetic properties of a platinum(IV) complex.

In order to judge the lipophilicity of a compound and to compare platinum complexes with different set of ligands, better parameter than number of carbon atoms in the axial chain or the molecular weight of a complex is required. In medicinal chemistry, the partition coefficient between water and n-octanol, log $P_{o/w}$ as well as the RP-HPLC derived retention index log k_w are widely used and can give a relevant quantitative estimation of the lipophilicity of a metal-based drug. Therefore, various methods for an experimental determination of log $P_{o/w}$ of platinum complexes, beyond the classical shake-flask method, including HPLC and MEEKC have been developed.[93, 94] The isocratic and extrapolated retention factors (log k_w and log k_{30}), obtained by RP-HPLC have also proved to be informative while studying the influence of lipophilicity in series of cytotoxic platinum(IV) complexes.[95-97] Recently, QSPR (quantitative structure-properties relationships) models able to predict lipophilicity (as log $P_{o/w}$ or log k_w) of Pt(IV) complexes, using QM (quantum mechanics) or MIF (molecular interactions field) descriptors have been developed in Osella's group.[95, 98]

A series of compounds with the general formula [Pt(en)Cl_2($OCO(CH_2)nCOOR)_2$] and [Pt(en)Cl_2($OCO(CH_2)nCONHR)_2$] have been synthesized and investigated in our group.[99-101] A good correspondence between lipophilicity, platinum accumulation and cytotoxicity was observed by increasing the size of terminal ester chains, while amide derivatives showed lower activity than expected from their log $P_{o/w}$ values.[102] As a next step, changing the carrier amine and tuning the lipophilicity by suitable derivatization of the axial ligands, yielded complexes with up to 17 times higher *in vitro* cytotoxicity, compared to cisplatin.[103] Applying the same approach for the development of highly active tetracarboxylatoplatinum(IV) complexes as prodrugs for carboplatin was not successful. Remarkably, increasing the lipophilicity by variation of the axial ligands resulted not in a promising cytotoxicity, namely higher than that of the precursor, carboplatin.[96]

Despite, platinum(IV) complexes' activity showed to be dependent on their lipophilicity, their cytotoxic potential cannot be assumed only from this single parameter.

It should be also mentioned that the frequently observed linear relationship between *in vitro* determined cytotoxicity and lipophilicity in a series of analogues is often not valid when the compounds are tested *in vivo*. The main reason for that discrepancy in the case of oral administration is the low bioavailability of very lipophilic compounds due to their dissolution in the fat globules in the intestine. Moreover, high lipophilicity usually leads to low water solubility and a compromise between these two parameters should be found, as demonstrated for series of analogues with the general formula [Pt(DACH)$(OCOR)_4$].[104] Finally, compounds with very high *in vitro* cytotoxicity sometimes have a small therapeutic index *in vivo*, due to a high general toxicity and the low lethal dose.

3.3.2 Redox behavior

How easy, how fast and to which products platinum(IV) complexes will be reduced in the body, are main issues influencing their activity and toxicity. It is crucial to understand how to control these parameters in order to design a successful platinum(IV) drug which will stay in its native form until the moment it will enter the tumor cell where it will be reduced fast enough to develop its cytotoxic effects. Primary, the electrochemical derived redox potentials have been suggested as indicators for the redox activity of platinum(IV) complexes. Hambley *et al.* have reported, that for compounds with the general formula $[Pt(en)Cl_2X_2]$, the highest redox potential (-224 mV) is observed for X=Cl, the lowest (-884 mV) for X=OH, while for X=OAc, propionate or butyrate, intermediate values between -490 and -520 mV were measured. The determined cathodic reduction potentials showed good correlation with the DNA binding activity of the complexes (the more readily reduced complexes bind to DNA in higher extent).[105] However, redox potentials are thermodynamic data and parameters, corresponding to the kinetic of reduction are required for better estimation of platinum(IV) complexes' biological activity. Furthermore, as $Pt^{IV} \rightarrow Pt^{II}$ reduction is an irreversible process, due to the loss of two ligands, cyclic voltammetric measurements often suffer from good reproducibility and big sd values are reported in experimental data. Platinum(IV) drugs can be reduced by both extracellular and intracellular reducing agents such as gluthatione, ascorbic acid, methionine and others. Kinetic rate constants for the reduction by ascorbic acid were reported as estimates for the redox behavior of iproplatin and tetraplatin *in vivo*. Choi *et al.* described for the first time the relation between cathodic redox potentials, rate constants of reduction and cytotoxicity of a group of platinum(IV) complexes, featuring equatorial chlorido ligands. A rough correlation between thermodynamic and kinetic

redox parameters have been demonstrated in the study. The authors showed, that reduction rates and redox potentials are mainly dependent on the axial ligands and change in the order: OH < $OCOCH_2R$ < Cl < $OCOCF_3$. To a lower extent, but also meaningful, reduction was dependent on the bulkiness of both equatorial and axial ligands; easier and faster reduction occurred when a bulkier ligand, destabilizing the six-coordinated sphere, was present. Good correlation between cytotoxicity and rate of reduction was only observed in homologous series (where only one ligand position is variable).[106] The last finding is understandable, as changing the equatorial ligands can affect not only the redox behavior, but also the lipophilicity and the pharmacodynamic characteristics of the complex.

Kratochwil and Bednarski have compared complexes of the type $[Pt(en)Cl_2X_2]$ and $[Pt(en)I_2X_2]$, where X=Cl, OH, OAc, $OCOCF_3$ and OSO_2CH_3 and demonstrated higher redox potentials, reduction rates and faster cancer cell growth inhibition for diiodido derivatives compared to their dichlorido analogues. Moreover, the authors prposed that reduction of the diiodido derivatives results in other products than $[Pt(en)I_2]$.[107]

Recently, Osella and coworkers have developed a QSPR model able to predict redox potentials of platinum(IV) complexes from their polar surface area (PSA), total area, energy of the LUMO and dipole moment.[98]

Using XANES (X-ray absorption near edge spectroscopy), Hambley and coworkers have investigated the extent of cellular reduction of complexes of the type *cis,cis,trans*-$[Pt(NH_3)_2Cl_2X_2]$. The percentage of reduced platinum(IV) in ovarian cancer cells after 2 h of incubation increased in the following manner: X= OH<OAc<Cl, corresponding to the trend found from the redox potential measurements. However, after 24 h of incubation all three complexes were fully reduced.[108]

The outcomes of the clinical trials of tetraplatin, iproplatin and satraplatin have encouraged the belief that chloride ions as axial ligands lead to very fast *in vivo* reduction and high general toxicity, while complexes featuring OH groups are inactive. The intermediate redox potential, measured for complexes bearing carboxylates as axial ligands promised optimal prodrug properties.[58, 109]

All correlations between thermodynamic redox potentials and rates of reduction observed for platinum(IV) complexes (prodrugs of diam(m)inedichloridoplatinum(II) compounds) showed not to be valid for diaminetetracarboxylatoplatinum(IV) complexes. Recently, Gibson and Hambley reported that bis(acetato)platinum(IV) analogues of oxaliplatin are reduced by ascorbic acid much more slowly, than their dihydroxido analogues, despite possessing higher cathodic reduction potential (see **Fig. 14**).[110] Meanwhile, we have reported on a tremendous difference in the rate of reduction by ascorbic acid of tetrakis(carboxylato)diammineplatinum(IV) complexes, prodrugs of carboplatin, and bis(carboxylato)dichloridodiamineplatinum(IV) complexes. The divergence found in redox kinetics correlates well with the observed considerable variance of cytotoxicity between the series, but not with their close redox potentials.[96] In order to find an explanation for these phenomena, a careful look into the mechanism of reduction of platinum(IV) complexes is required.

There are different mechanisms for the reductive elimination of two ligands from the Pt(IV) coordination sphere, suggested on the base of kinetic measurements, using UV or NMR spectroscopy and few theoretical studies.[111-115] Reduction, mediated by the faster inner sphere electron transfer is likely to occur only when a bridge can be formed between the reducing agent and platinum through a polarized ligand. Such role can be carried out by chloride or hydroxide, but not by am(m)ine or carboxylate (see **Fig. 14**).

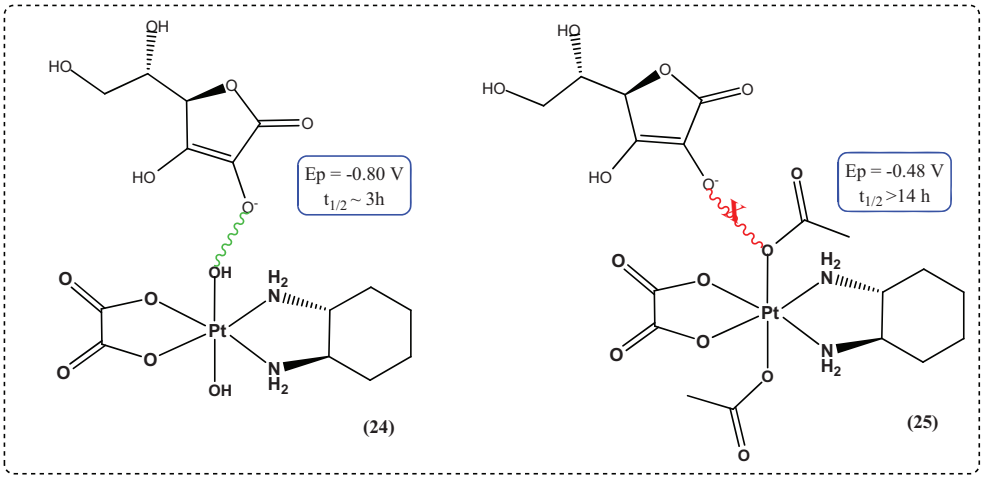

Figure 14. Inner sphere transition state (TS), formed between ascorbate and dihydroxidoplatinum(IV) derivative of oxaliplatin, which facilitates the electron transfer and results in a fast reduction **(24)** and the inability of the diacetato analogue to form such a TS, resulting in a slow reduction through the outer sphere pathway **(25)**; adopted from ref. 129.

Therefore, reduction of diam(m)inotetracarboxylatoplatinum(IV) complexes, which possess neither chloride nor hydroxide as ligands, proceeds slowly through an outer sphere mechanism. In such cases, the rate limiting factor seems to be the electron transfer to the platinum, not the breaking of Pt-L bonds. Such assumptions could explain the observed lack of correlation between kinetics and thermodynamics as in electrochemical experiments the electron transfer is rapid and the measured potentials are dependent mainly on the bond energies.[110]

As the rate of reduction of platinum(IV) complexes strongly depends not only on the complex, but also on the reducing agent and the pH, the possible bioreductants should be cautiously examined. Ascorbate (two-electron reducing agent) and GSH (one-electron reducing agent) have been considered as the main intracellular species, responsible for reduction of platinum(IV) complexes, as they are both present in relatively high concentration in cells. Nevertheless, there are many other biomolecules in the cell,

capable of reducing Pt(IV) complexes.[85] Recently, Gibson and coauthors have monitored the reduction of satraplatin and analogous complexes in aqueous extracts of cancer cells, using [^{1}H,^{15}N] HSQC experiments. They divided the cell extracts into high and low molecular weight (MW) fractions and showed that the low MW fraction (which contains both ascorbate and GSH) are quite inefficient at reducing the tested complexes. Furthermore the observed rate of reduction, by the high MW fraction was similar to that of the whole non-fractioned extracts.[116] These findings proposed high molecular mass biomolecules as main reducing agents of platinum(IV) prodrugs, rather than ascorbic acid and GSH.

Finally, the question which products are formed after reduction of platinum(IV) complexes in the cell arises. It is generally accepted, that reduction is accompanied by the loss of two axial ligands.[109, 111] Recently, Gibson has shown using [^{1}H,^{15}N] HSQC NMR spectroscopy that four different products of the reduction of *trans,cis,cis*-bis(acetato)diam(m)inedichloridoplatinum(IV) complexes by sodium ascorbate were obtained.[117] Consistently, Guo *et al.* have reported different products of reduction of organoamidobis(pyridine)platinum(IV) complexes, featuring chlorides or hydroxides as axial ligands.[118] Osella and coworkers have recently shown, that the reduction by GSH of bis(carboxylato)platinum(IV) analogues of picoplatin also yields more products, than the expected picoplatin.[119] Recently, Natile and coworkers showed that the mechanism of reduction of platinum(IV) compounds and the formed products depend on the configuration of the complex, the bulk of the carrier ligands and the nature of the reducing agent.[120]

A successful design of platinum(IV) prodrugs requires knowledge not only of their intracellular redox behavior, but also of the amount of complex able to reach the tumor

cell in its intact form and the nature of the formed metabolites. Tetraplatin, ([Pt(DACH)Cl_4], **(21)**, **Fig. 13**) showed very rapid reduction in undiluted plasma under physiological temperature with $t_{1/2}$~3s and therefore no parent drug should be expected to reach the cell.[121] On the other site, iproplatin ([Pt(IPA)$_2$$Cl_2(OH)_2$], **(22)**, **Fig. 13**) is stable in plasma for at least 48 h and reduces intracellularly.[122, 123] Nevertheless, due to the slow overall process of intracellular activation (reduction and following aquation of the formed Pt(II) species), iproplatin did not show advantages in comparison with carboplatin and was not approved for clinical use.[11, 81] In the case of satraplatin (bis(acetato)diam(m)inedichlorido platinum(IV) complex, **(23)**, **Fig. 13**), an intermediate rate of extra- and intracellular reduction could be expected. Sufficient amount of the parent drug is estimated to reach the tumor cells and to be reduced there with satisfactory rate. Contrary, no satraplatin in its native form was detectable in patients' blood 15 minutes after oral administration. Furthermore, different Pt(II) metabolites were observed, not only its precursor as could be expected.[82] These results are consistent with later observations for more than one intracellular product of reduction of satraplatin's analogues and the involvement of high MW agents as main reducing agents *in vivo*.[116, 117] *Carr et al.* have shown, that haemoglobin, cytochrom C and liver microsomes can reduce satraplatin in the presence of NADH. Based on its relatively high physiological abundance and fast reaction with satraplatin (half-life time of 35.8 min), haemoglobin is suggested as one of the key reducing agents for platinum(IV) prodrugs in the blood.[124]

For a better estimation of the *in vivo* anticancer activity and toxicity of Pt(IV) complexes their *in vitro* cytotoxicity, together with their redox behavior in the blood and the extracellular environment as well as interactions with proteins should be taken into account. QSAR models for predicting the *in vitro* cytotoxic activity on the basis of redox

potentials, log $P_{o/w}$ values and constitutional and DFT derived descriptors were developed in Osella's group and by us as a first step in rationalizing platinum(IV)-based drugs design. [125, 126]

3.3.2.1 Participation of platinum(IV) complexes in non-redox reactions, which might be of clinical relevance

Despite the relative inertness of platinum(IV) complexes to chemical reactions prior to their reduction to the oxidation state +II, there are some cases of reactivity which can have clinical relevance. Oxoplatin and JM149 (the dihydroxido analogue of satraplatin) transform to their more reactive tetrachloridoplatinum(IV) analogues after short incubation with 0.1 M HCl (mimicking the low pH of gastric acid), while this process has an insignificant rate in the case of satraplatin.[127] The last findings should be taken into account when formulations containing dihydroxidoplatinum(IV) complexes are developed for oral administration.

Hambley et al. have shown, that *cis*-$[Pt(NH_3)_2Cl_4]$ undergoes aquation while both axial and equatorial chloride ligands can be exchanged. Substitution at the axial position was observed prior to that of equatorial chlorides and a catalytic amount of cisplatin showed to accelerate the process significantly. The dihydroxido and diacetato analogues of *cis*-$[Pt(NH_3)_2Cl_4]$ showed a considerably slower rate of aquation without significance for the stability of the complexes in aqueous media.[128]

Recently, Gibson has reported a fast hydrolysis of complexes, bearing axial trifluoroacetate or dichloroacetate ligands, which form subsequently the respective monohydroxido (fast) and dihydroxido (slow) species. As a consequence, complexes like mitaplatin will release their active axial ligands (dichloroacetate) before reaching the cell. Therefore, a more complicated metabolism is expected as not only the redox behavior of

the parent compound but also of its singly and doubly hydrolyzed forms and the rates of hydrolysis have to be considered. On the other hand, bis(acetato)- and bis(monochloroacetato)platinum(IV) complexes showed stability against hydrolysis.[129]

3.4 Synthetic approaches in Pt(IV)-based medicinal chemistry

3.4.1 Using simple Pt(IV) compounds as starting materials

Hazarika and Bora have reported the preparation of complexes of the type *cis*-$[PtL_2Cl_4]$ (in yields around 70%), whereas L is represented by different substituted imidazoles, via mixing aqueous solutions of $PtCl_4$ and 2 eq of the ligand.[130] Formation of trans- isomers of the type $[PtL_2Cl_4]$ is favored by the reaction between $H_2[PtCl_6]$ and triazolopyrimidines in water-ethanol solutions.[131]

However, more convenient methods, providing further possibilities for derivatizations are based on the synthesis of a platinum(II) complex with the desired coordination sphere, its oxidation to Pt(IV) and following reactions, depending on the availability of functional groups of the ligands.

3.4.2 Oxidation with Cl_2

The preparation of platinum(IV) complexes featuring chlorides as axial ligands, by oxidizing their Pt(II) counterparts with chlorine gas was described by Kauffman.[132] The process, when performed in aqueous solution proceeds via fast formation of a *trans*-chloridoaquaplatinum(IV) intermediate and a free chloride ion; consequently, Cl^- attacks the intermediate and the *trans*-dichloridoplatinum(IV) complex is formed as a final product (see **Fig. 15**). Recently, Margiotta *et al.* have confirmed this mechanism, when studying the oxidative addition of chlorine to $[Pt(1,4\text{-DACH})Cl_2]$ in different solvents by

means of ^{1}H NMR. All intermediates formed in water, DMSO, DMF and acetone have been detected and in the case of water and DMF, [Pt(1,4-DACH)Cl_3(OH)] and [Pt(1,4-DACH)Cl_3(O-DMF)]Cl were isolated as pure solids and fully characterized by elemental analysis, ESI-MS, NMR and IR measurements.[133]

Liu and Chen have demonstrated an alternative convenient method for the preparation of *trans*-chlorido(hydroxido)platinum(IV) complexes, using water solution of NaOCl as oxidizing and chlorinating agent.[134]

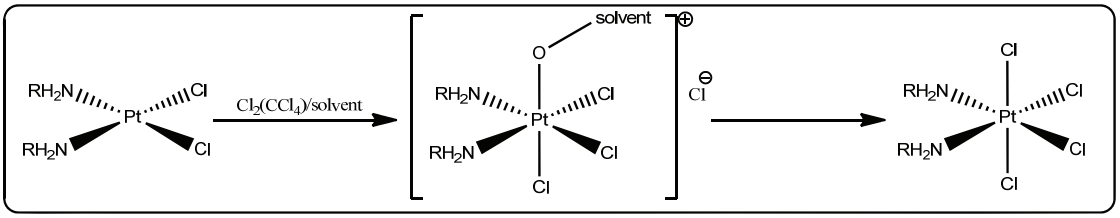

Figure 15. Mechanism of oxidative addition of chlorine to platinum(II) complexes

3.4.3 Oxidation with other halogens

Oxidation of cisplatin with an aqueous solution of bromine has been reported to produce *cis,trans,cis*-[Pt$(NH_3)_2Br_2Cl_2$] in a yield over 60%.[135] The successful oxidative addition of bromine and iodine to platinum(II) complexes with bidentatly coordinated benzoylthiourea derivatives in halogenated organic solvents have also been achieved.[136] Complexes of the type [$Pt^{IV}AI_4$] can be prepared by oxidizing [$Pt^{II}AI_2$] with iodine in solution. The choice of the solvent(s) for the reaction and the further crystallization of the products are based on their solubility.[137]

3.4.4 Oxidation with peroxides

One of the most common ways for the preparation of platinum(IV) complexes is the oxidation of their platinum(II) counterparts with H_2O_2. The most probable reaction

mechanism includes an outer-sphere electron transfer and the formation of a tetragonally distorted square-pyramidal intermediate between H_2O_2, the complex and a sixth coordination site, associated with a molecule from the solvent. The final product is a *trans*-hydroxido(solvent)platinum(IV) complex, where the OH group originates from hydrogen peroxide and the other axial ligand (second OH, alcoholate or carboxylate) from the solvent (see **Fig. 16**)[138, 139]

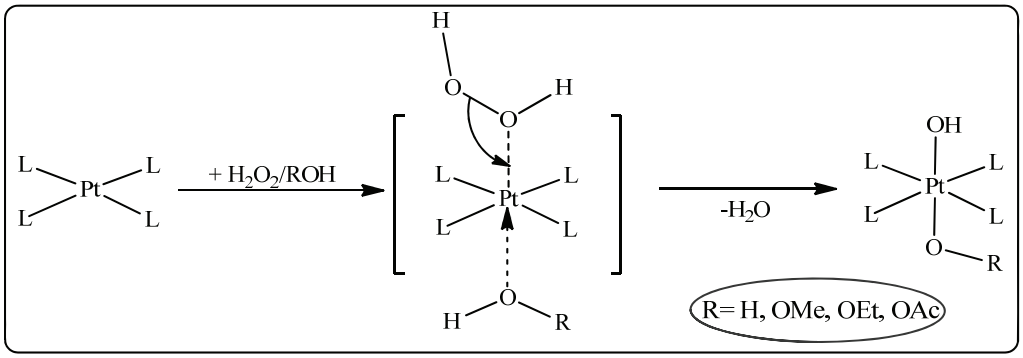

Figure 16. Mechanism of oxidation of Pt(II) complexes with hydrogen peroxide in water, alcohols or acetic acid, based on ref. 139.

When the reaction is performed in aqueous media, *trans*-dihydroxido complexes are formed**.**[140-142] However, depending on the equatorial ligands, also other products can be obtained. The oxidation of $[Pt^{II}(EDDA)Cl_2]$ (where EDDA is 1,2-ethylendiaminediacetic acid) in aqueous H_2O_2, for example, leads to intramolecular esterification and formation of $[Pt^{IV}(EDDA)Cl_2]$, where EDDA is six-fold coordinated[143] (see **Fig. 17**).

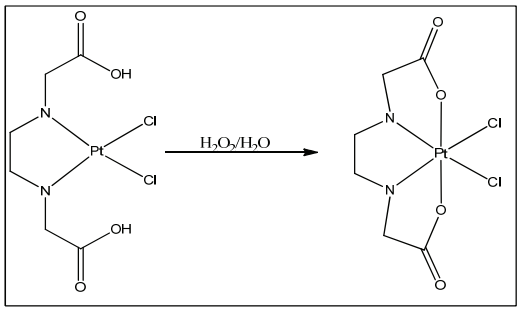

Figure 17. Oxidation of platinum(II) complexes containing ethylenediamine-derived ligands having carboxylic acid substituents.

Lee *et al.* have shown that hydrogen peroxide oxidation of Pt(II) complexes in alcohols or liquid carboxylic acids (such as acetic and propionic acid) leads to the formation of unsymmetric Pt(IV) species, bearing one hydroxide and one alcoholate or carboxylate as axial ligands.[144, 145] Gibson and Hambley have recently reported the generation of monocarboxylato platinum(IV) complexes, using H_2O_2 or *tert*-butyl hydrogen peroxide as oxidizing agent and oily or solid acids, solubilized in acetonitrile or THF.[146] The formation of platinum(IV) compounds, featuring only one free OH group of an axial position broadens the possibility for synthesis of complexes with mixed axial ligands and facilitates the coupling of large biomolecules or nanoparticles. For instance, soluble single-walled carbon nanotubes as delivery systems were coupled via a succinic acid spacer to *cis,cis,trans*-[$Pt(NH_3)_2Cl_2OH(OEt)$] in the group of Lippard.[75]

3.4.5 Oxidation with other oxidizing agents

Oxidation of diam(m)inedichloridoplatinum(II) complexes with NO_2 in the presence of air and an anion, being capable to coordinate to the platinum centre during the oxidation process was used for the synthesis of novel nitroplatinum(IV) complexes, designed to inhibit STAT protein functions (see **Fig. 12**, **(20)**).[65]

The oxidation of Pt(II) compounds with (diacetoxyiodo)benzene in CH_2Cl_2, results in bis(acetato)platinum(IV) compounds, where the two acetato groups are in *cis* position, due to rearrangement of the intermediate.[147]

3.4.6 Further derivatization of *trans*-chlorido and *trans*-hydroxidoplatinum(IV) complexes

a) exchange reactions, using silver salts

Kizu et al. have synthesized orally active platinum(IV) prodrugs of oxaliplatin, featuring one chlorido and one carboxylato ligand, while treating [Pt(DACH)(ox)Cl_2] with silver salts of lipophilic monocarboxylic acids.[148]

b) electrophilic substitution

Platinum coordinated hydroxide(s) in axial position as nucleophiles can participate in electrophilic substitution reactions with classical organic reagents such as anhydrides and acyl chlorides. Most commonly, anhydrides of monocarboxylic acids in excess are utilized as reactant and solvent (or by using CH_2Cl_2 as solvent) in order to synthesize the corresponding carboxylato complexes.[149, 150] An alternative approach, using acyl chlorides in the presence of pyridine in acetone was reported by Galanski and Keppler.[151] Recently, Wilson and Lippard have explored the reaction of oxoplatin ((OC-6-33)-diamminedichloridodihydroxidoplatinum(IV)) with different alkyl and aryl isocyanates in DMF at RT. As a result, eight novel platinum(IV) complexes, featuring axial carbamato ligands and cytotoxicities which were similar or superior than that of cisplatin, have been synthesized.[152]

The main disadvantage of the mentioned methods is the impossibility for further derivatizations. The latter would be possible when the coupled axial ligands possess free carboxylato, aldehydo, amino or hydroxyl group. The carboxylation of platinum(IV) complexes, featuring free OH groups in axial position, using cyclic anhydrides (succinic, maleic and glutaric) was reported for the first time by Navarro-Ranninger and coworkers. The generated bis(carboxylato)bis(diamine) complexes feature free carboxylic acids at the end of the axial ligands; however, their further derivatizations have not been reported.[153] Lippard and coworkers performed the carboxylation of oxoplatin with succinic anhydride in DMSO at 70°C with moderate yields. Amine-modified estrogens

have been attached to the obtained bis(succinato)platinum(IV) compounds by the use of diisopropylcarbodiimide-4-(dimethylamino)pyridine, a common coupling reagent, employed in peptide chemistry.[154] An improved method for carboxylation of platinum(IV) dixydoxido complexes, using cyclic anhydrides in DMF as a solvent was developed in our group. The uncoordinated COOH groups of the prepared complexes were further reacted with simple amines or alcoholates in the presence of 1,1'-carbonyldiimidazole (CDI), forming the corresponding amides or esters (see **Fig. 18**).[101] Using this approach, a plethora of novel cytotoxic bis-, tris- and tetrakis(carboxylato)platinum(IV) complexes have been synthesized.[96, 99, 100, 103, 126, 155]

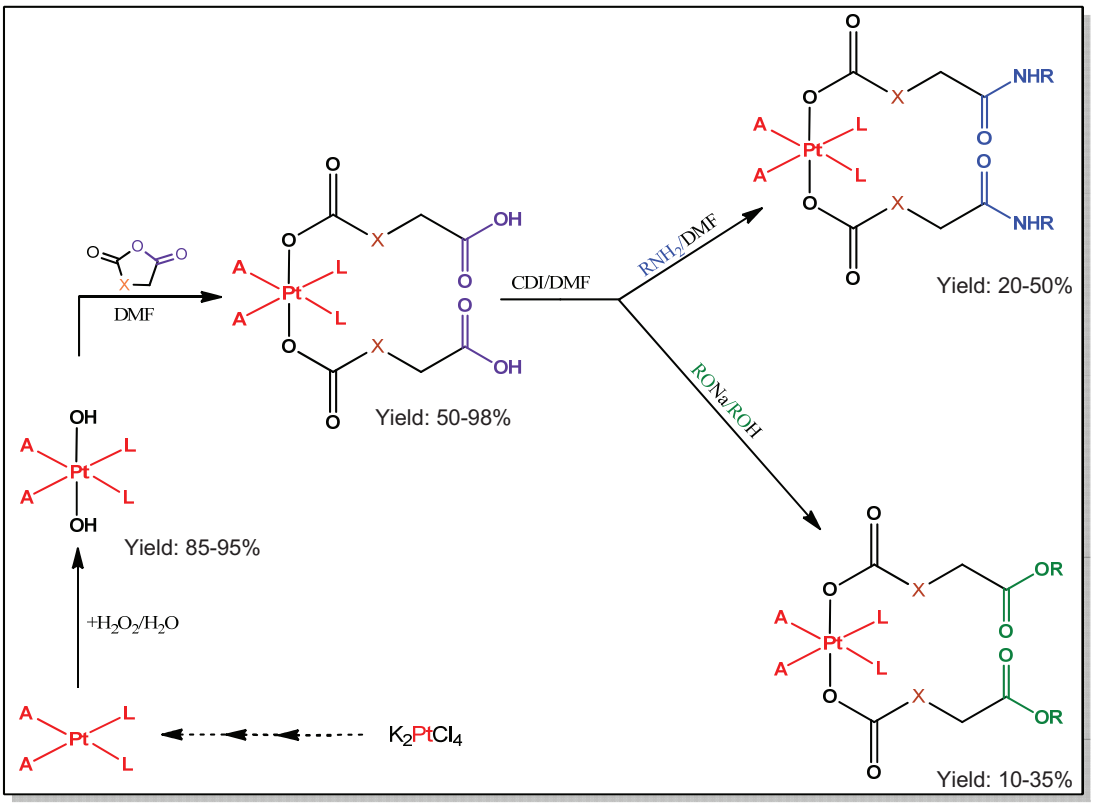

Figure 18. Scheme for the synthesis of novel bis-, tris- and tetrakis(carboxylato)platinum(IV) complexes, featuring different (ester or amide) functionality; X = CH_2, C_2H_4, $CH_2CH(CH_3)$ or $CH_2C(CH_3)_2$.

Main disadvantages of the method are the relatively low yields and the necessity of purification by column chromatography at the last stage, inconvenience in terms of future industrial manufacturing. These drawbacks can be overcome by preparing a monoester of the respective dicarboxylic acid, transforming it into an anhydride and letting the last one react with the chosen dihydroxidoplatinum(IV) complex (see **Fig. 19**).[i]

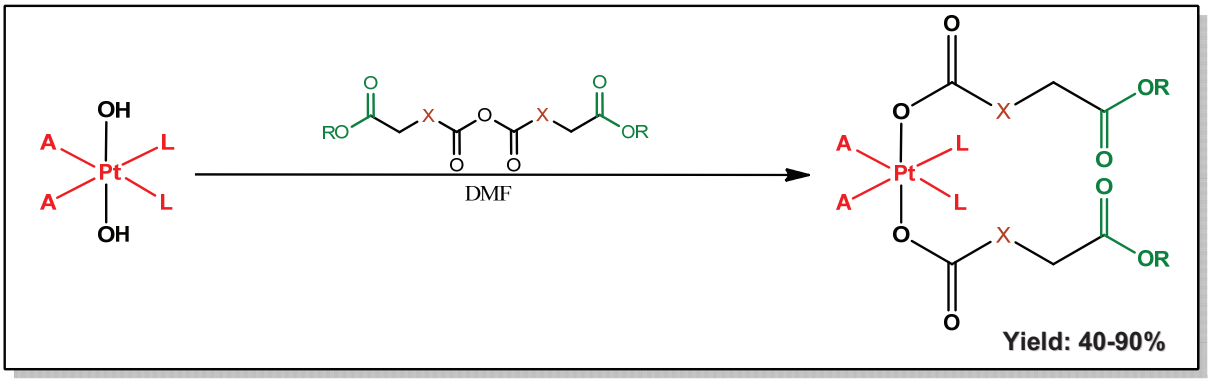

Figure 19. Alternative method for the synthesis of trans-carboxylato platinum(IV) complexes, featuring ester function.

Recently, the synthesis of *cis,cis,trans*-diamminedichloridobis(4-formylbenzoato) platinum(IV) complexes and their further derivatization by means of imine ligation were reported. The reaction between the aromatic aldehyde functionality of the complex and excess of hydrazide- or aminooxy-functionalized substrates resulted in yields over 90%.[156]

The listed approaches facilitate the formation of symmetric complexes with double functionality in axial position, when using *trans*- dihydroxidoplatinum(IV) compounds as precursors. The preparation of mixed *trans*-carboxylato platinum(IV) complexes using one-pot reaction between two different anhydrides and the dihydroxidoplatinum(IV) precursor was proposed by Song, Kim and Sohn. The final compounds were isolated

[i] Varbanov *et al.*, *unpublished data*

after chromatographic purification in relatively low yields (<30%).[157] In an alternative approach, suggested by the same group, monohydroxidotris(carboxylato)platinum(IV) complexes were first isolated and than treated with anhydrides of a second carboxylic acid in order to esterify the free axial OH ligand.[158] Lippard *et al.* have demonstrated, that a monohydroxidomonosuccinato complex is formed when oxoplatin reacts with stoichiometric amount of succinic anhydride in DMSO at RT.[74] Pichler *et al.* have shown, that monocarboxylato platinum(IV) complexes can be obtained by reaction of succinic anhydride with a *trans*-dihydroxidoplatinum(IV) precursor, bearing bulky equatorial amine ligands such as N,N-dimethyl-ethane-1,2-diamine[159].

An alternative method for unsymmetric acylation, with the aim of achieving a better tuning of pharmacological properties of platinum(IV) complexes, was recently published by *Fei Chin et al.*. In a first step, a monocarboxylated complex was generated by reaction of the Pt(IV) precursor with benzoic acid anhydride in DMSO under high dilution in a good yield. The free hydroxide was further derivatized with various anhydrides, producing complexes, bearing two different axial ligands.[160] Gibson and Hambley suggested the activation of carboxylic acid with coupling agents such as DCC (dicyclohexylcarbodiimide) or TBTU (tetramethyluronium tetrafluoroborate) prior to its reaction with the *trans*-dihydroxidoplatinum(IV) precursor. The obtained monocarboxylated complexes can be derivatized into mixed-carboxylato species following the same synthetic strategy.[146]

c) nucleophilic substitution

Despite the strong nucleophilic character of Pt(IV) coordinated hydroxides, they can be protonated at lower pH and participate in nucelophilic substitution reactions. This strategy is employed in the synthesis of Pt(IV) compounds, featuring chloride ions as

axial ligands by treating their dihydoxido analogues with concentrated HCl. The illustrated method is cheaper and more commonly used than the one described in **3.4.1** and **3.4.2**.[105] However, its applicability is restricted to diam(m)inedichloridodihydroxidoplatinum(IV) complexes, as carboxylato equatorial ligands would be also exchanged by chlorides under these conditions.

Song, Kim and Sohn have demonstrated, that compounds of the type [Pt(DACH)$(OH)_4$] can be carboxylated with pure acetic, propionic or butyric acid forming the respective tris(carboxylato)monohydroxido complexes with yields over 60%. Interestingly, regardless of the conditions (temperature and time of reaction), only three of the hydroxides have been carboxylated, leaving one underivatized OH at axial position. Contrary, when [Pt(DACH)$(OH)_4$] is suspended in 0.1 M HCl, all of the OH groups are exchanged for Cl (see **Fig. 20**).

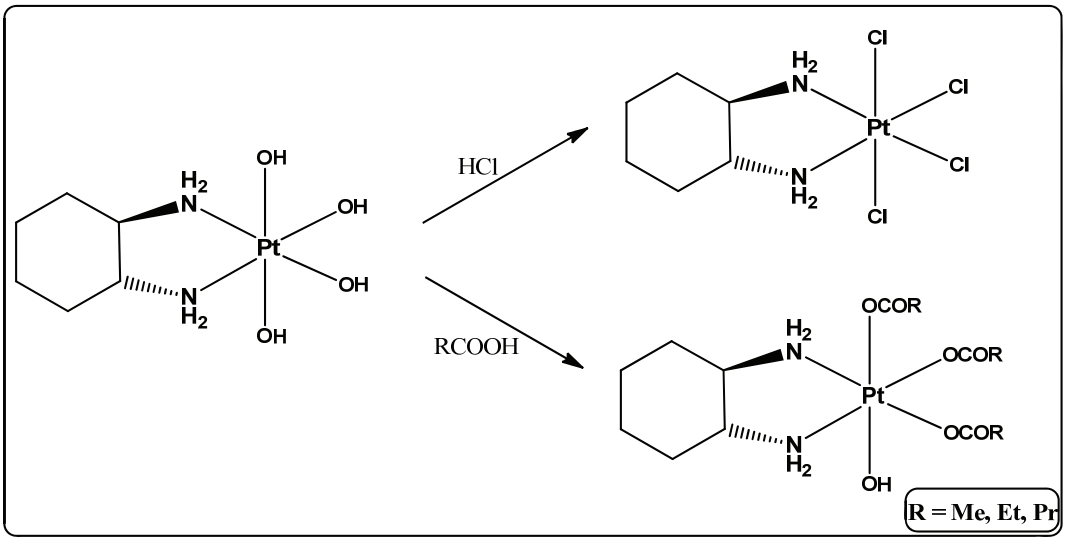

Figure 20. Nucleophilic substitution of (diamine)tetrahydroxidoplatinum(IV) complexes with carboxylic acids and with HCl.

The authors explained the observed phenomena by the different pKa values of the aquated ligands in [Pt(DACH)$(OH)_4$] and higher acidity of the last aqua ligand,

compared to that of the used carboxylic acids, but not to HCl. The presented method for preparation of diaminetris(carboxylato)monohydroxidoplatinum(IV) complexes is much more convenient and with higher yields formation, compared to the alternative electrophilic substitution.[161]

The preparation of tris(chelating) diaminebis(dicarboxylato)platinum(IV) complexes by dissolving [Pt(diamine)$(OH)_4$] type compounds in concentrated aqueous solution of dicarboxylic acids such as malonic, 3-methylmalonic, oxalic and 1,1'-cyclobutandicarboxylic, was recently developed in our group (see **Fig. 21**).[ii]

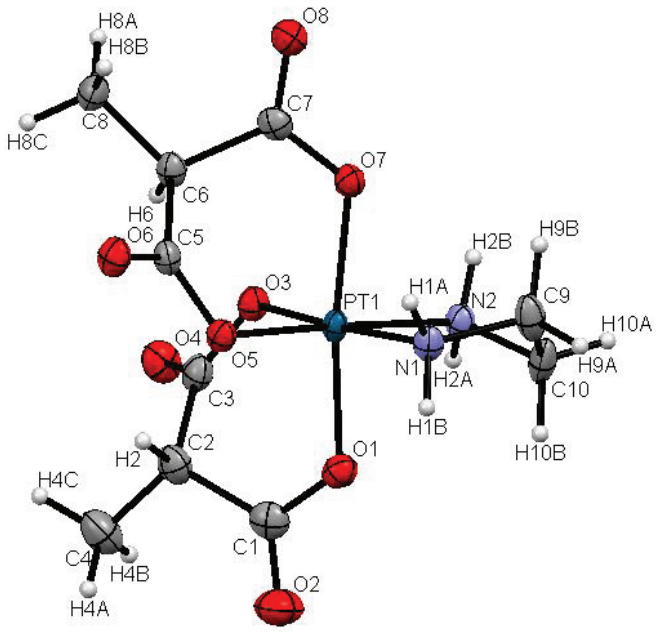

Figure 21. Crystal structure of (OC-6-22)-ethane-1,2-diaminebis(3-methylmalonato)platinum(IV); ORTEP diagram with ellipsoids at 60% probability.

d) derivatisation of the equatorial ligands

Galanski *et al.* have synthesized a diaminetetrachloridoplatinum(IV) complex featuring

[ii] Varbanov *et al*, Novel di- and trichelate am(m)inetetracarboxylatoplatinum(IV) complexes of the type [Pt(A)$(R(COO)_2)_2$], *unpublished data*.

N,N-bis(2-hydroxyethyl)ethane-1,2-diamine as carrier ligand and further derivatized it by means of carboxylation of the peripheral hydroxyl groups with acyl chlorides. The demonstrated approach provides the opportunity for selective conjugation of various bio-macromolecules with the carrier ligands of platinum-based prodrugs. [162]

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II. RESULTS

This PhD thesis is based on the following papers, which are presented in the original format:

Synthesis and characterization of novel bis(carboxylato)dichloridobis(ethylamine) platinum(IV) complexes with higher cytotoxicity than cisplatin.
H. Varbanov, S.M. Valiahdi, A.A. Legin, M.A. Jakupec, A. Roller, M. Galanski, B.K. Keppler, *Eur. J. Med. Chem.*, **2011,** 46, 5456-5464.

Novel tetracarboxylatoplatinum(IV) complexes as carboplatin prodrugs.
H.P. Varbanov, S.M. Valiahdi, C.R. Kowol, M.A. Jakupec, M. Galanski, B.K. Keppler, *Dalton Trans.*, **2012**, 41, 14404-14415.

Theoretical Investigations and Density Functional Theory Based Quantitative Structure–Activity Relationships Model for Novel Cytotoxic Platinum(IV) Complexes.
H.P. Varbanov, M.A. Jakupec, A. Roller, F. Jensen, M. Galanski, B.K. Keppler, *J. Med. Chem.*, **2013**, 56, 330-344**.**

1. Synthesis and characterization of novel bis(carboxylato)dichloridobis(ethylamine) platinum(IV) complexes with higher cytotoxicity than cisplatin.

H. Varbanov, S.M. Valiahdi, A.A. Legin, M.A. Jakupec, A. Roller, M. Galanski, B.K. Keppler, *Eur. J. Med. Chem.*, **2011,** 46, 5456-5464.

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Original article

Synthesis and characterization of novel bis(carboxylato)dichloridobis(ethylamine) platinum(IV) complexes with higher cytotoxicity than cisplatin

Hristo Varbanov, Seied M. Valiahdi, Anton A. Legin, Michael A. Jakupec, Alexander Roller, Markus Galanski*, Bernhard K. Keppler*

University of Vienna, Institute of Inorganic Chemistry, Währinger Strasse 42, A-1090 Vienna, Austria

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ABSTRACT

A series of six novel bis(carboxylato)dichloridobis(ethylamine)platinum(IV) complexes was synthesized and characterized in detail by elemental analysis, FT-IR, ESI-MS, HPLC, multinuclear (^{1}H, ^{13}C, ^{15}N, ^{195}Pt) NMR spectroscopy and in one case by X-ray diffraction. Cytotoxic properties of the complexes were evaluated in four human tumor cell lines originating from ovarian carcinoma (CH1 and SK-OV-3), colon carcinoma (SW480) and non-small cell lung cancer (A549) by means of the MTT colorimetrical assay. In addition, their octanol/water partition coefficients (log *P* values) were determined. Remarkably the most active (and also most lipophilic) compounds, having 4-propyloxy-4-oxobutanoato and 4-(2-propyloxy)-4-oxobutanoato axial ligands, showed IC_{50} values down to the low nanomolar range.

1. Introduction

Nowadays, 35 years after the serendipitous discovery of the cytotoxic potential of *cis*-diamminedichloridoplatinum(II) [1], more than 50% of anticancer therapy is platinum based. All platinum containing drugs used in the clinics are platinum(II) compounds (cisplatin, carboplatin, oxaliplatin, nedaplatin, lobaplatin and heptaplatin) [2]. However, it was shown, that Pt(IV) complexes also exhibit strong cytotoxic activity and can have some advantages in comparison to their Pt(II) analogues [3,4]. Consequences of the higher oxidation state are the introduction of two extra ligands and the change from planar to octahedral geometry. These characteristics, together with their higher kinetic inertness compared to their platinum(II) counterparts, opens up new possibilities in the design of novel platinum-based drugs (easier modulation of the pharmacokinetic properties, more opportunities for targeted therapy, oral administration, etc.) [5]. Nevertheless, no Pt(IV) complex has gained clinical approval up to now [2]. Four Pt(IV) compounds were in clinical trials (Fig. 1): tetraplatin was rejected after phase I because of a high general toxicity [6]; iproplatin was abandoned after phase III clinical trials, because it didn't show advantages compared with carboplatin [2]; satraplatin was rejected after the SPARC (Satraplatin and Prednisone Against Refractory Cancer) phase III clinical trials [7], because it didn't show a convincing benefit in terms of overall survival [8]; currently satraplatin is in phase I and II clinical trials in combination regimens [9]; its adamantylamine analogue LA-12 has passed phase I clinical trials.

Considering the mechanism of action of Pt(IV) complexes, it is accepted that they act as prodrugs via activation by reduction to their reactive Pt(II) species [10,11]. Pt(IV) based drugs would have better activity and lower side effects, when they are reduced primarily in the cell; contrary, an extracellular reduction would lead to deactivation and general toxicity. Consequently, the drug's potential strongly correlates with the rate of reduction which depends on the respective reduction potential [12]. Depending on the nature of the axial ligands, platinum(IV) complexes are reduced more easily in the following order: $CF_3COO > Cl > CH_3COO > OH$ [13] It was found that complexes with Cl^- as axial ligands were reduced very fast and showed a high general toxicity (tetraplatin [14]), on the other hand, complexes with axial hydroxido ligands were not reduced fast enough in the body to express their antitumor activity (iproplatin [15]). In the case of axial carboxylato ligands, an intermediate and optimal redox potential was observed [16], which led to promising results, obtained in preclinical and clinical evaluation of satraplatin and its adamantylamine analogue LA-12.

* Corresponding authors. Tel.: +43 1 4277 52601; fax: +43 1 4277 52680.
E-mail addresses: markus.galanski@univie.ac.at (M. Galanski), bernhard.keppler@univie.ac.at (B.K. Keppler).

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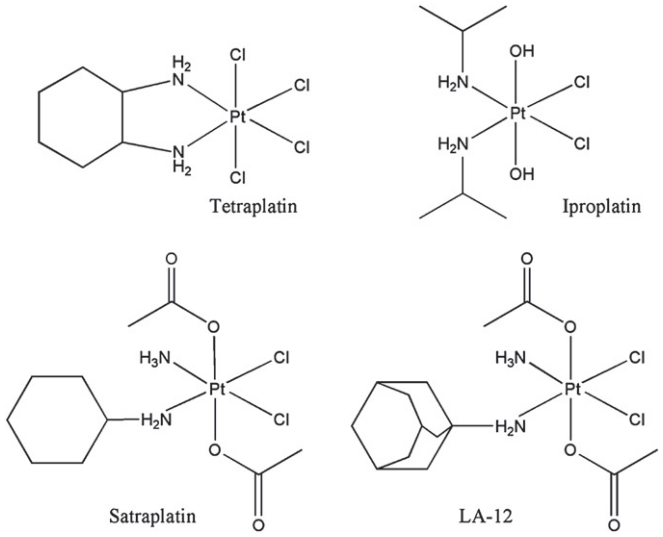

Fig. 1. Chemical structures of anticancer Pt(IV) complexes evaluated in clinical trials.

Recently, a convenient way for obtaining a series of dicarboxylatoplatinum(IV) complexes and modulation of their physicochemical properties such as solubility and lipophilicity was reported by our group [17–22]. In order to broaden the knowledge with respect to structure–activity relationships for that type of compounds and to find candidates with a promising physicochemical and pharmacological profile, we have synthesized a series of bis(carboxylato)dichloridobis(ethylamine)platinum(IV) complexes. The new compounds were fully characterized by elemental analysis, ATR IR, multinuclear NMR spectroscopy, HPLC and X-ray crystallography in one of the cases. Their cytotoxic properties were evaluated in four human tumor cell lines, originating from ovarian carcinoma (CH1 and SK-OV-3), colon carcinoma (SW480) and non-small cell lung cancer (A549), by means of the MTT colorimetrical assay. In addition, their octanol/water partition coefficients (log *P*) were determined.

2. Result and discussion

2.1. Synthesis

The new complexes were prepared according to the reaction scheme shown in Fig. 2, starting from K_2PtCl_4, which was converted to (SP-4-2)-dichloridobis(ethylamine)platinum(II) (complex **1**). Oxidation of **1** to the dihydroxido complex **2** was performed in aqueous solution, using 15% hydrogen peroxide as oxidizing agent.

Subsequent carboxylation of **2** with succinic anhydride was carried out in absolute DMF obtaining the dicarboxylato complex. The latter was used as starting material for the synthesis of complexes **4**–**8** via activation of its free carboxylic groups with CDI (1,1′-carbonyldiimidazole) in absolute DMF under argon atmosphere. To the imidazolide formed *in situ*, the respective alcoholate/alcohol mixtures or amine were added to obtain the respective esters (**4**–**6**) or amide (**8**). Purification of the crude products was performed with column chromatography and re-crystallization, when necessary.

When synthesizing complex **7** following the described procedure, a mixture of the desired diisopropylester (**7**) and asymmetric methylisopropylester (**7a**) in a ratio of 2:1 (according to ^{1}H NMR) was obtained. Unfortunately separation and isolation of compound **7** was not successful. Formation of the mixed methylisopropylester derivative **7a** was also confirmed by ESI-MS. Most likely **7a** was formed during separation via column chromatography. Apparently, reaction of **3** after CDI activation with isopropanol is not as fast as in the case of complexes **4**–**6**. As a result, the monoimidazolide platinum complex reacted with methanol, one of the constituents of the mobile phase. By increasing the reaction time from 24 to 72 h and avoiding the use of methanol in the mobile phase for purification, we were able to obtain exclusively pure **7** in satisfactory yield.

2.2. Spectroscopic characterization

Structures of the starting compounds (**1**, **2**) were proven by NMR and ATR IR spectroscopy and the new complexes (**3**–**8**) were fully characterized by elemental analysis, one- and two-dimensional

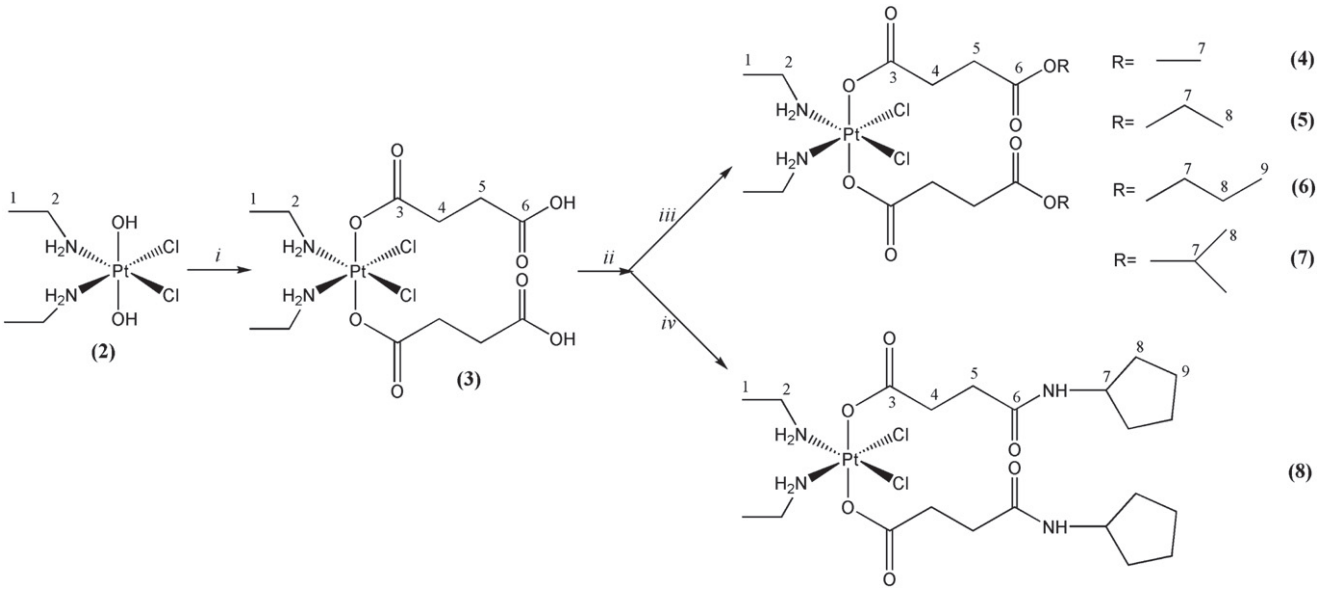

Fig. 2. Synthesis of novel bis(carboxylato)dichloridobis(ethylamine)platinum(IV) complexes with NMR numbering scheme; *i* = succinic anhydride/DMF, 70 deg., *ii* = CDI/DMF, 60 deg., *iii* = RONa/ROH, RT, *iv* = cyclopentylamine/DMF, RT.

multinuclear NMR (^{1}H, ^{13}C, ^{15}N, ^{195}Pt), ESI-MS and ATR IR spectroscopy and in the case of **4** also by X-ray diffraction.

Configuration of the novel platinum agents can best be established with the help of one- and two-dimensional multinuclear NMR spectroscopy. ^{1}H and ^{13}C chemical shifts of compounds **1**–**8** were found in the expected range, proving the supposed structure of the complexes. Correct assignment of the signals was based on ^{1}H^{1}H COSY, ^{1}H^{13}C HSQC and ^{1}H^{13}C HMBC spectra of the complexes. In ^{1}H NMR spectra, oxidation and subsequent derivatization can best be judged according to the shift of the NH_2 signal from 5.09 ppm in the Pt(II) complex **1** to 5.96 ppm in the dihydroxido complex **2**, and to 7.85–7.91 ppm in the dicarboxylato complexes **3**–**8**, respectively. Also indicative are the ^{15}N resonances, where the signal for NH_2 shifts from −40.9 ppm in complex **1** to around −21.3 ppm in compounds **3**–**8**. Additionally, successful derivatizations of complex **3** were observed in ^{13}C NMR spectra. The resonance of C-6 (uncoordinated COOH) was shifted upfield upon formation of esters **4**–**7** or amide **8** by ca. 2 ppm. ^{195}Pt NMR is very powerful technique for investigating the oxidation state and the coordination sphere of platinum complexes. ^{195}Pt signals for complexes **3**–**8** were detected in the region between 2849 and 2853 ppm, typical for compounds with *cis*,*cis*,*trans*-$Pt^{IV}Cl_2N_2O_2$ coordination [23] In comparison, the platinum(II) complex **1** resonates at −601 ppm, more than 3000 ppm upfield to the platinum(IV) analogues. As expected, derivatization of **3** had no influence on the ^{195}Pt signal, because the changes in the molecule are far away from the ^{195}Pt nucleus.

Oxidation of **1** and derivatization of **2** can also be followed in the IR spectra of the complexes. A new and intense signal at 3490 cm^{-1}, corresponding to ν_{PtO-H} can be observed in the spectrum of complex **2**, in comparison with that of **1**. After esterification with succinic anhydride this signal disappeared and in complexes **3**–**8** new strong bands in the region 1730–1630 cm^{-1} ($\nu_{C=O}$) were detected, proving the successful carboxylation. In esters **4**–**7**, bands with 10–25 higher reciprocal wavelengths were detected in comparison with the complex featuring free carboxylic groups (**3**). However, the signal around 1710 cm^{-1} is missing in compound **8**, because of amide formation and a strong band with a shoulder around 1639 cm^{-1}could be observed.

ESI-MS spectra have also confirmed the identity of the complexes. All new compounds (**3**–**8**) were measured in the positive as well as in the negative ion mode. In the positive ion mode, the peak assigned to $[M + Na^+]^+$ displayed the highest intensity, whereas in the negative ion mode the highest intensity was detected for $[M - H^+]^-$. However, a peak corresponding to $[M + Cl^-]^-$, could also be observed in the spectra of esters (complexes **4**–**7**). The detected *m*/*z* values as well as the isotopic distribution were in accordance with the expected chemical structures **3**–**8**.

2.3. Crystal structure of complex 4

The result from the X-ray diffraction analysis of **4** is shown in Fig. 3. Crystal data, data collection parameters and structure-refinement details are given in the Experimental section. Selected bond lengths and angles are listed in Table 1. The compound crystallized in the triclinic centrosymmetric space group *P*−1. The Pt(IV) atom has an octahedral coordination geometry with two ethylamine and two chlorido ligands in the equatorial plane and two 4-methoxysuccinates coordinated in axial positions. The Pt–N, Pt–Cl, and Pt–O bond lengths (Table 1) are well comparable with those, observed in structurally similar complexes [17,18]. Angles in the $PtCl_2N_2O_2$ octahedron were found between 86.26 and 92.69°, and between 172.70 and 178.18°, respectively. The torsion angles (Pt–O1–C1–O2, and Pt–O5–C6–O6) were found to be close to zero.

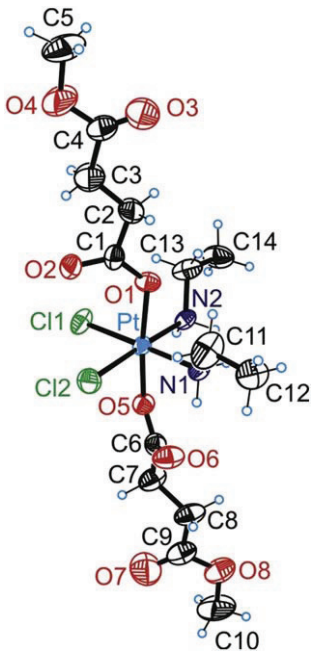

Fig. 3. ORTEP diagram of **4** displaying thermal ellipsoids at 50% probability.

2.4. Cytotoxicity in cancer cell lines

The new compounds were tested in comparison to cisplatin in four human tumor cell lines, originating from ovarian carcinoma (CH1, SK-OV-3), colon carcinoma (SW480) and non-small cell lung cancer (A549) with the help of the colorimetric microculture MTT assay. Except for CH1 cells, these cell lines are resistant to cisplatin, showing IC_{50} values about one order of magnitude higher than that in CH1 cells. The concentration-effect curves of the tested complexes are shown in Fig. 4 and the obtained IC_{50} values are summarized in Table 2.

Expectedly, the platinum(IV) precursor **3** featuring two COOH moieties showed the lowest cytotoxicity in all cell lines. In accordance with previously published data [18,19,22], lipophilicity as well as cellular accumulation are low for such types of complexes. Conversion of **3** to the corresponding ester derivatives **4**–**7** has a significant influence on antiproliferative potency. Whereas complex **4** is as cytotoxic as cisplatin in the cisplatin-sensitive CH1 cell line, complexes **5**–**7** are 3 to 17 times more cytotoxic than cisplatin in the same cell line. An analogous trend was also observed in cisplatin-resistant A549, SW480 and SK-OV-3 cells. Parallel to an increasing lipophilicity of the ester residue (Me, Et, Pr), the IC_{50} values are decreasing in all cell lines, yielding clear structure–activity relationships. The ^{i}Pr analogue **7** is similar in

Table 1
Selected bond lengths (Å) and bond angles (°) in complex **4**.

Bond lengths (Å)			
Pt–O1	2.039	Pt–N2	2.068
Pt–O5	2.039	Pt–Cl1	2.324
Pt–N1	2.063	Pt–Cl2	2.309
Bond angles (deg)			
N1–Pt1–O1	86.26	O1–Pt–O5	172.70
N1–Pt–N2	92.69	N1–Pt–Cl1	178.18
N2–Pt–Cl1	86.26	N2–Pt–Cl2	175.68
N1–Pt–Cl2	88.72	Pt–O1–C1–O2	−1.87
Cl1–Pt–Cl2	92.22	Pt–O5–C6–O6	−0.45

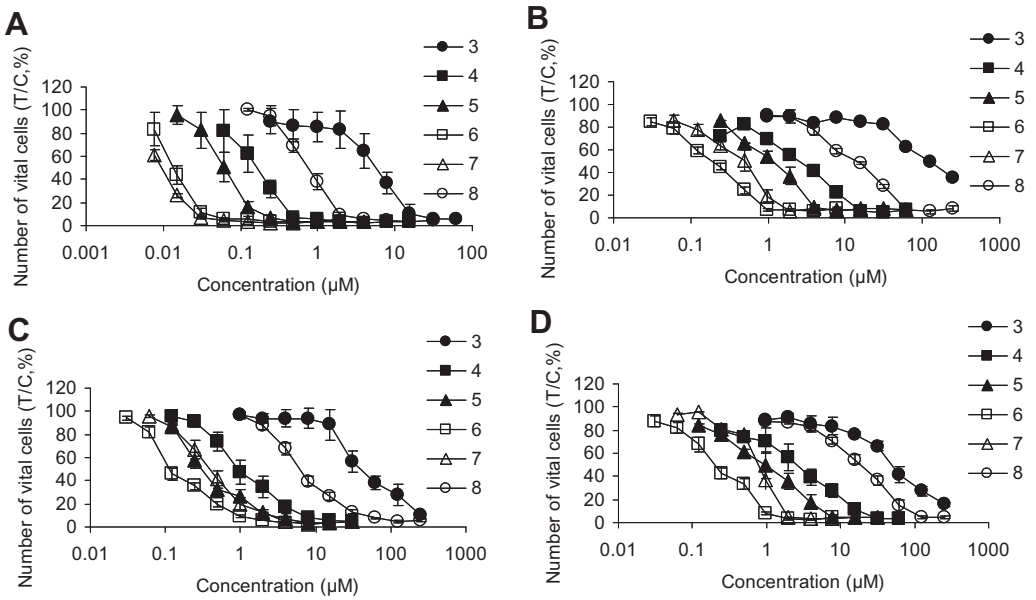

Fig. 4. Concentration-effect curves of complexes (**3**–**8**) in CH1 (A), SK-OV-3 (B), SW480 (C) and A549 (D) obtained by the MTT assay (96 h exposure).

cytotoxic potency to the Pr analogue **6** in CH1 cells, but somewhat less potent in the other cell lines. In analogy to previous observations [22], the cyclopentylamide derivative is equipped with a very low antiproliferative potency, despite its high lipophilicity (see below).

2.5. Lipophilicity vs. cytotoxicity

As an important factor for the pharmacokinetics and the cellular accumulation of the new complexes, the lipophilicity was determined by measuring octanol/water partition coefficients (log *P*) by two different methods; log *P* values, obtained by RP-HPLC and by the shake-flask method are shown in Table 3.

Complexes **4**–**8** have significantly higher log *P* values than that reported for cisplatin (−2.59) and platinum(II) complex **1** (−1.47) [24]. However, complex **3** is also more lipophilic than its Pt(II) congener (**1**), but its lipophilicity is pH-dependent, due to the presence of two underivatized carboxylic groups (lower lipophilicity under physiological conditions is expected). In Fig. 5, a semi-logarithmic graph plotting the cytotoxicity versus log *P* of the new complexes is shown. In case of compounds **3**–**7**, a linear dependency between lipophilicity and cytotoxicity could be observed – the more lipophilic a complex, the higher its cytotoxicity. The amide complex **8** does not match this trend – according to its lipophilicity, a higher cytotoxicity would be expected. The reasons for the latter finding are yet unclear.

2.6. Induction of apoptosis and necrosis

In order to compare the capacities of inducing apoptosis and necrosis of one representative, namely compound **7**, with those of cisplatin, growing SW480 cultures were treated with these compounds in various concentrations for 48 h, then double-stained with annexin V-FITC and propidium iodide and analyzed by fluorescence-activated cell sorting (FACS). This method allows to discriminate necrotic (stained by propidium iodide only), early apoptotic (stained by annexin V-FITC) and late apoptotic (stained by both) from viable (unstained) cells. From the dot plots (Fig. 6), it becomes obvious that the apoptosis-inducing potency of compound **7** is much higher than that of cisplatin. 50 μM of compound **7** reduce the amount of viable cells to 50% by induction of both apoptosis (29%) and necrosis (21%), while the same concentration of cisplatin has very little effect within the chosen exposure time in the intrinsically cisplatin-resistant SW480 (colon cancer) cells.

Table 2
Cytotoxicity of novel complexes (**3**–**8**) in comparison to cisplatin in four human cancer cell lines.

Compound	IC_{50} (μM)[a]			
	CH1	A549	SW480	SK-OV-3
3/R–COOH	5.6 ± 1.6	50 ± 8	40 ± 12	120 ± 17
4/R–COOMe	0.16 ± 0.05	2.5 ± 0.9	1.0 ± 0.3	2.4 ± 0.1
5/R–COOEt	0.061 ± 0.015	1.0 ± 0.4	0.30 ± 0.05	1.2 ± 0.3
6/R–COOPr	0.014 ± 0.002	0.20 ± 0.03	0.11 ± 0.01	0.19 ± 0.03
7/R–COO^{i}Pr	0.0094 ± 0.0012	0.78 ± 0.09	0.39 ± 0.07	0.49 ± 0.11
8/R–CONHR	0.75 ± 0.10	19 ± 3	6.1 ± 0.6	13 ± 1
Cisplatin	0.16 ± 0.03	1.3 ± 0.3	3.5 ± 0.3	1.9 ± 0.3

[a] 50% Inhibitory concentrations in CH1, A549, SW480 and SK-OV-3 cells in the MTT assay, 96 h exposure. Values are the means ± standard deviations obtained from three independent experiments.

Table 3
Log *P* values for complexes **3**–**8**, **R1** and **R2**, estimated by RP-HPLC and the shake-flask method.

Compound	Log *P* values, determined by RP-HPLC in different MeOH concentrations						Log *P*, determined by the shake-flask method
	0%	10%	20%	30%	40%	50%	
R1	−1.04	−1.03	−1.00	−0.95	−0.99	−0.88	−0.81
3	−0.70	−0.84	−0.79	−0.64	−1.14	−0.97	
4	0.30	0.25	0.17	0.08	0.14	−0.10	−0.12
5	0.90	0.87	0.83	0.73	0.78	0.85	0.64
6	1.38	1.41	1.44	1.49	1.47	1.48	1.44
7	1.30	1.33	1.36	1.41	1.37	1.39	
8	1.11	1.14	1.17	1.23	1.20	1.25	1.21
R2	1.39	1.41	1.44	1.48	1.44	1.46	1.69

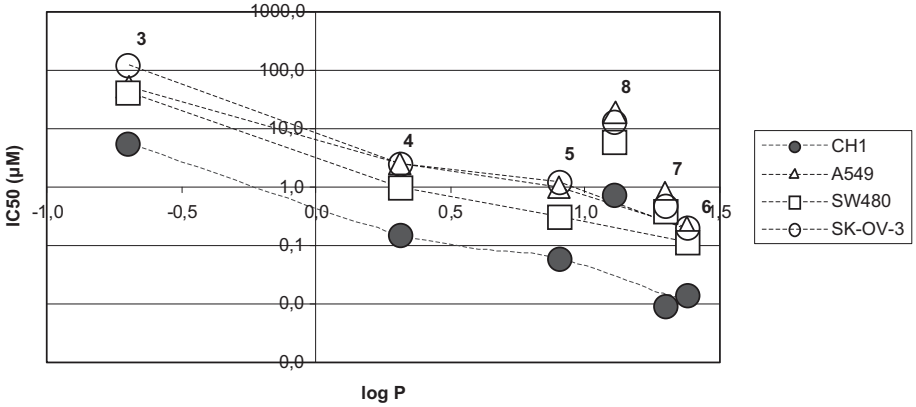

Fig. 5. Semi-logarithmic plot of lipophilicity (log *P* determined with RP-HPLC) of complexes **3**–**8** vs. cytotoxicity (IC_{50}) in CH1, A549, SW480 and SK-OV-3 cells.

A synopsis of all data (Fig. 7) further illustrates that compound **7** is a powerful apoptotic agent, which effectively causes cell death in a dose-dependent way.

3. Conclusions

Six novel bis(carboxylato)platinum(IV) complexes have been synthesized and fully characterized. The new compounds were investigated for their lipophilic properties and their in vitro cytotoxicity in four human tumor cell lines. Remarkably, IC_{50} values down to the nanomolar range, up to 32 times lower compared to cisplatin, were found. Whether the very high cytotoxicity is also accompanied by manageable systemic toxicity in vivo will be evaluated in future work.

4. Experimental protocols

4.1. Materials and methods

All reagents and solvents were obtained from commercial suppliers, and were used without further purification. Methanol and ethanol were dried, according to standard procedures. For column chromatography, silica gel 60 (Fluka) was used. (OC-6-33)-Dichloridobis(ethylamine)dihydroxidoplatinum(IV) (complex **2**, Fig. 2) was synthesized starting from K_2PtCl_4 and ethylamine, using Dhara's [25] method with some modifications. The resulting dichlorido complex **1** was oxidized with 15% H_2O_2.

^1H, ^{13}C, ^{15}N, ^{195}Pt and two-dimensional $^1H^1H$ COSY, $^1H^{13}C$ and $^1H^{15}N$ HSQC, and $^1H^{13}C$ HMBC NMR spectra were recorded with

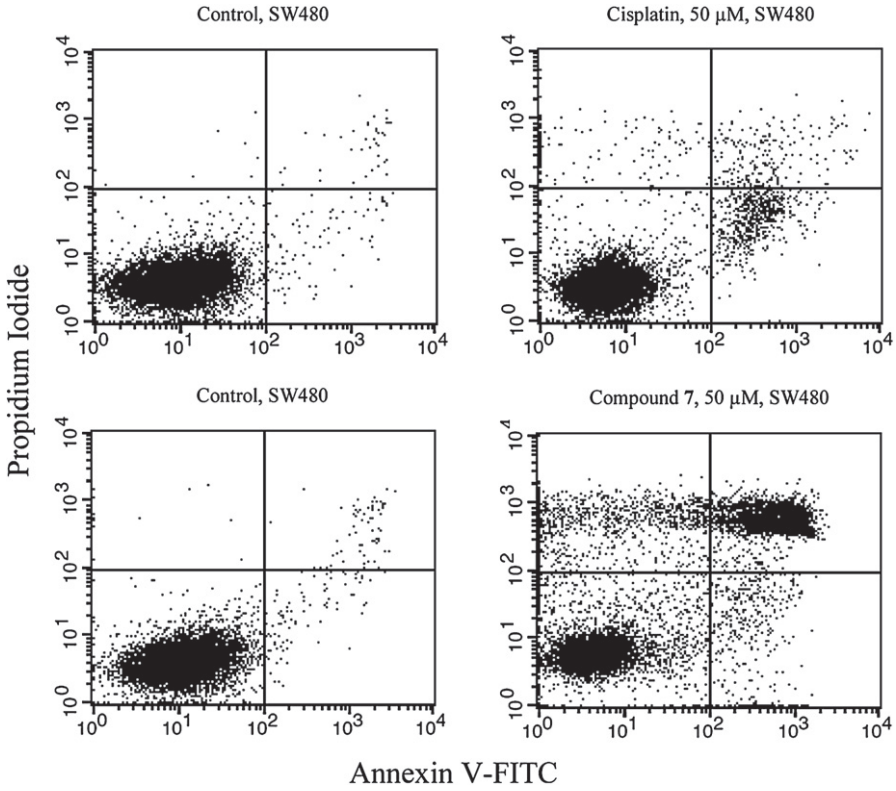

Fig. 6. FACS analysis of annexin V- and PI-stained SW480 colon cancer cells. Left column: untreated control; right column: after treatment with 50 μM cisplatin/compound **7** for 48 h. The upper left quadrant contains the necrotic (stained by PI only), the lower right early apoptotic (stained by annexin V-FITC only), the upper right late apoptotic (stained by both) and the lower left quadrant the viable (unstained) fraction of cell populations.

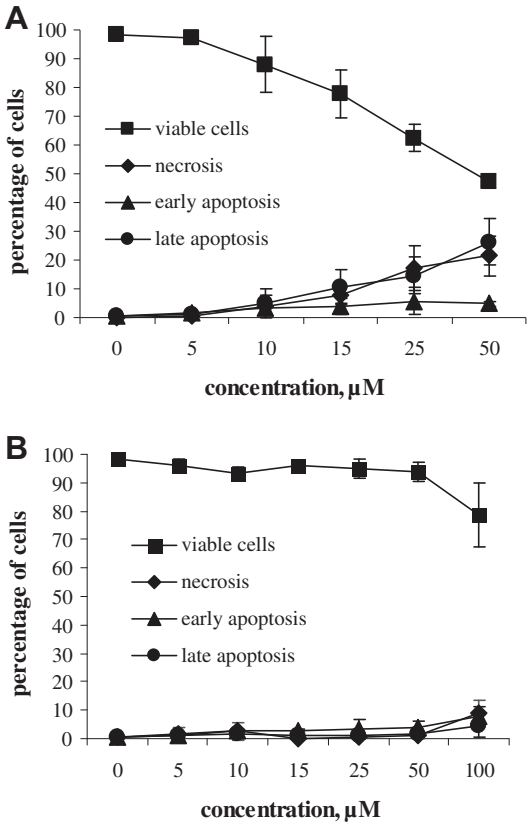

Fig. 7. Concentration-effect curves for compound **7** (A) and cisplatin (B) with regard to apoptosis and necrosis induction in SW480 cells after 48 h exposure, measured by FACS using annexin V-FITC/propidium iodide staining.

a Bruker Avance III 500 MHz NMR spectrometer at 500.32 (^{1}H), 125.81 (^{13}C), 107.55 (^{195}Pt), and 50.70 MHz (^{15}N) in DMF-d_7 at ambient temperature, using the solvent residual peak for ^{1}H and ^{13}C as internal reference. The splitting of proton resonances in the ^{1}H NMR spectra are defined as s = singlet, bs = broad singlet, d = doublet, t = triplet, and m = multiplet. ^{15}N chemical shifts were referenced relative to external NH_4Cl; whereas ^{195}Pt chemical shifts were referenced relative to external $K_2[PtCl_4]$ (see Fig. 2 for NMR numbering scheme).

IR spectra were obtained with a Perkin–Elmer 370 FT-IR 2000 instrument (4000–400 cm^{-1}) by using an ATR unit. Intensities of reported IR bands are defined as br = broad, s = strong, m = medium, and w = weak. Electrospray ionization mass spectrometry was carried out with a Bruker Esquire 3000 instrument using MeOH as solvent. Elemental analyses were performed with a Perkin–Elmer 2400 CHN-Elemental Analyzer by the Microanalytical Laboratory of the University of Vienna. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. Purity of novel compounds was additionally proved by analytical reversed-phase HPLC.

4.2. Synthesis

4.2.1. (OC-6-33)-Bis(3-carboxypropanoato)dichloridobis(ethylamine)-platinum(IV) (3)

Succinic anhydride (674.3 mg, 6.738 mmol) and 653 mg (1.675 mmol) of (*OC*-6-33)-dichloridobis(ethylamine)dihydroxidoplatinum(IV) were suspended in 9 mL of absolute DMF and the reaction mixture was stirred at 65 °C for 40 min and then for 2 more hours at room temperature. During this time, the solid material dissolved to form a yellow-brown solution. DMF was removed under reduced pressure. The residue was dissolved in acetone and filtered to give a clear, yellow solution. This solution was concentrated under reduced pressure, and subsequent addition of diethyl ether led to precipitation of a pale yellow solid. The precipitate was filtrated and dried in vacuo, while heating at 40 °C. Yield: 398 mg (40%). Anal. $C_{12}H_{24}Cl_2N_2O_8Pt$ (C, H, N). ESI-MS: *m*/*z* 612.6 $[M + Na^+]^+$, 588.7 $[M - H^+]^-$. ^{1}H NMR: δ = 12.52 (bs, 2H, COOH), 7.87 (bs, 4H, NH_2), 3.10 (m, 4H, H-2), 2.75 (m, 4H, H-4), 2.68 (m, 4H, H-5), 1.44 (t, $^{3}J_{H,H}$ = 7.2 Hz, 6H, H-1) ppm. ^{13}C NMR: δ = 181.3 (C-3), 174.1 (C-6), 39.9 (C-2), 31.2 (C-4), 29.9 (C-5), 14.3 (C-1) ppm. ^{15}N NMR7: δ = −21.2 ppm. ^{195}Pt NMR: δ = 2851 ppm. IR (ATR): 3241 m, 3197 br; 2977 br; 1710 s ($\nu_{C=O}$), 1642 m ($\nu_{C=O}$); 1357 m, 1344 m; 1238 s 1216 m, 1175 m; 1081 w, 1024 w (ν_{C-N}) cm^{-1}.

4.2.2. (OC-6-33)-Dichloridobis(ethylamine)bis((4-methoxy)-4-oxobutanoato)platinum(IV) (4)

CDI (241.1 mg, 1.4869 mmol) in absolute DMF (10 mL) was added to a solution of **3** (433.4 mg, 0.7342 mmol) in absolute DMF (6 mL), and the mixture was heated to 60 °C. After 10 min of being stirred, the solution was cooled to room temperature and CO_2 was removed by flushing with argon. Sodium methanolate (a piece of Na in 10 mL of absolute MeOH) in absolute MeOH was added and the solution was stirred for 24 h at room temperature. Methanol and DMF were removed under reduced pressure to form a yellow oil. The crude product was purified by column chromatography (EtOAc/MeOH, 7:1) to yield a yellow solid, which was dried in vacuo. Yield: 106 mg (23%). Anal. $C_{14}H_{28}Cl_2N_2O_8Pt$ (C, H, N). ESI-MS: *m*/*z* 640.8 $[M + Na^+]^+$, 616.5 $[M - H^+]^-$, 652.8 $[M + Cl^-]^-$. ^{1}H NMR: δ = 7.85 (bs, 4H, NH_2), 3.82 (s, 6H, H-7), 3.10 (m, 4H, H-2), 2.78 (m, 4H, H-4), 2.71 (m, 4H, H-5), 1.45 (t, $^{3}J_{H,H}$ = 7.2 Hz, 6H, H-1) ppm. ^{13}C NMR: δ = 181.0 (C-3), 173.1 (C-6), 51.2 (C-7), 39.9 (C-2), 31.1 ($^{3}J_{C,Pt}$ = 38.2 Hz, C-4), 29.8 (C-5), 14.3 ($^{3}J_{C,Pt}$ = 35.0 Hz, C-1) ppm. ^{15}N NMR: δ = −21.3 ppm. ^{195}Pt NMR: δ = 2853 ppm. IR (ATR): 3221 w, 3188 m; 2894 br; 1729 s ($\nu_{C=O}$), 1647 s ($\nu_{C=O}$); 1362 m 1333s; 1259 s, 1198s, 1176 s; 1088 m, 1026 w (ν_{C-N}); 683 w cm^{-1}. Crystals, suitable for X-ray data collection, were obtained after vapor diffusion of diethyl ether into a methanol solution of **4**.

4.2.3. (OC-6-33)-Dichloridobis((4-ethoxy)-4-oxobutanoato)-bis(ethylamine)platinum(IV) (5)

The synthesis was carried out as described for **4**. The crude product was purified by column chromatography (EtOAc/MeOH, 9:1), then recrystallized from ethyl acetate and diethyl ether to yield a pale yellow solid. The final product was dried in vacuo. Yield: 24 mg (16%). Anal. $C_{16}H_{32}Cl_2N_2O_8Pt$ (C, H, N). ESI-MS: *m*/*z* 669.1 $[M + Na^+]^+$, 644.3 $[M - H^+]^-$, 681.0 $[M + Cl^-]^-$. ^{1}H NMR: δ = 7.86 (bs, 4H, NH_2), 4.26 (m, $^{3}J_{H,H}$ = 7.1 Hz, 4H, H-7), 3.10 (m, 4H, H-2), 2.77 (t, 4H, H-4), 2.69 (t, 4H, H-5), 1.45 (t, $^{3}J_{H,H}$ = 7.2 Hz, 6H, H-1), 1.39 (t, $^{3}J_{H,H}$ = 7.1 Hz, 6H, H-8) ppm. ^{13}C NMR: δ = 181.0 (C-3), 172.7 (C-6), 60.2 (C-7), 39.9 (C-2), 31.1 ($^{3}J_{C,Pt}$ = 37.3 Hz, C-4), 30.0 (C-5), 14.4 ($^{3}J_{C,Pt}$ = 34.5 Hz, C-1), 14.0 (C-8) ppm. ^{15}N NMR: δ = −21.3 ppm. ^{195}Pt NMR: δ = 2850 ppm. IR (ATR): 3180 w, 3153 w; 2987 w; 1727 s ($\nu_{C=O}$); 1658 m, 1631 m ($\nu_{C=O}$); 1369 m; 1260 s, 1231 s; 1163 s; 1085 w; 1039 m, 1023 s (ν_{C-N}); 857 w, 681 m cm^{-1}.

4.2.4. (OC-6-33)-Dichloridobis(ethylamine)bis((4-propyloxy)-4-oxobutanoato)platinum(IV) (6)

The synthesis was carried out as described for **4**. The crude product was purified by column chromatography (EtOAc/MeOH, 11:1), then recrystallized from ethyl acetate and diethyl ether to yield a pale yellow solid. The final product was dried in vacuo. Yield: 33 mg (11%). Anal. $C_{18}H_{36}Cl_2N_2O_8Pt$ (C, H, N). ESI-MS: *m*/*z* 697.1 $[M + Na^+]^+$, 672.9 $[M - H^+]^-$, 708.9 $[M + Cl^-]^-$. ^{1}H NMR:

δ = 7.86 (bs, 4H, NH_2), 4.18 (t, $^3J_{H,H}$ = 6.7 Hz, 4H, H-7), 3.10 (m, 4H, H-2), 2.78 (m, 4H, H-4), 2.71 (m, 4H, H-5), 1.80 (m, $^3J_{H,H}$ = 7.3 Hz, 4H, H-8), 1.44 (t, $^3J_{H,H}$ = 7.2 Hz, 6H, H-1), 1.09 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-9) ppm. ^{13}C NMR: δ = 181.0 (C-3), 172.7 (C-6), 65.8 (C-7), 39.9 (C-2), 31.1 ($^3J_{C,Pt}$ = 31.1 Hz, C-4), 29.9 (C-5), 22.0 (C-8), 14.4 ($^3J_{C,Pt}$ = 34.7 Hz, C-1), 10.1 (C-9) ppm. ^{15}N NMR: δ = −21.4 ppm. ^{195}Pt NMR: δ = 2850 ppm. IR (ATR): 3222 m, 3192 m; 2970 w; 1731 s ($\nu_{C=O}$); 1671 m, 1654 s ($\nu_{C=O}$); 1370 m, 1326 m; 1164 s, 1088 w; 684 w cm^{-1}.

4.2.5. (OC-6-33)-Dichloridobis(ethylamine)bis((4-(2-propyloxy))-4-oxobutanoato)platinum(IV) (**7**)

CDI (184 mg, 1.135 mmol) in absolute DMF (6 mL) was added to a solution of **3** (325 mg, 0.551 mmol) in absolute DMF (5 mL), and the mixture was heated to 70 °C. After 10 min of being stirred, the solution was cooled to room temperature and CO_2 was removed by flushing with argon. 12 mL of sodium 2-propanolate (a piece of Na dissolved in 2-propanol, HPLC grade) was added to the solution and heated to 40 °C. The mixture was then stirred for 72 h at room temperature. 2-Propanol and DMF were removed under reduced pressure to form a yellow oil. The crude product was purified by column chromatography (EtOAc/2-propanol, 10:1) and then precipitated with Et_2O and cooled to 0 °C to give an almost white solid, which was dried in vacuo. Yield: 110 mg (30%). Anal. $C_{18}H_{36}Cl_2N_2O_8Pt$ (C, H, N). ESI-MS: *m/z* 696.8 $[M + Na^+]^+$, 673.4 $[M - H^+]^-$, 708.8 $[M + Cl^-]^-$. ^{1}H NMR: δ = 7.70 (bs, 4H, NH_2), 4.93 (m, $^3J_{H,H}$ = 6.3 Hz, 2H, H-7), 2.94 (m, 4H, H-2), 2.59 (m, 4H, H-4), 2.49 (m, 4H, H-5), 1.27 (t, $^3J_{H,H}$ = 7.2 Hz, 6H, H-1), 1.21 (d, $^3J_{H,H}$ = 6.3 Hz, 12H, H-8) ppm. ^{13}C NMR: δ = 180.9 (C-3), 172.0 (C-6), 67.4 (C-7), 39.8 (C-2), 30.9 ($^3J_{C,Pt}$ = 37.5 Hz, C-4), 30.1(C-5), 21.3 (C-8), 14.2 ($^3J_{C,Pt}$ = 32.9 Hz, C-1) ppm. ^{15}N NMR: δ = −21.3 ppm. ^{195}Pt NMR: δ = 2850 ppm. IR (ATR): 3282 m, 3206 m; 2981 w, 2937 w; 1722 s, 1702 s ($\nu_{C=O}$); 1668 s, 1637 s ($\nu_{C=O}$); 1375 m, 1362 m; 1304 s, 1265 s, 1239s; 1106 m, 1087 m cm^{-1}.

4.2.6. (OC-6-33)-Dichloridobis((4-cyclopentylamino)-4-oxobutanoato)bis(ethylamine)platinum(IV) (**8**)

CDI (106.5 mg, 0.6568 mmol) in absolute DMF (7 mL) was added to a solution of **3** (184.5 mg, 0.3126 mmol) in absolute DMF (5 mL), and the mixture was heated to 60 °C. After 10 min of being stirred, the solution was cooled to room temperature and CO_2 was removed by flushing with argon. Cyclopentylamine (75 μL, 0.7516 mmol) in 4 mL of absolute DMF was added to the solution and stirred for 30 h in the dark at room temperature. DMF was removed under reduced pressure to form a brown oil. The crude product was purified by column chromatography (EtOAc/MeOH, 4:1) and then precipitated with Et_2O (ultrasonic) and cooled to 0 °C to give an almost white solid, which was filtered off, washed with Et_2O and EtOAc and dried in vacuo. Yield: 64 mg (28%). Anal. $C_{22}H_{42}N_4O_6PtCl_2$ (C, H, N). ESI-MS: *m/z* 747.3 $[M + Na^+]^+$, 723.1 $[M - H^+]^-$. ^{1}H NMR: δ = 7.95 (d, $^3J_{H,H}$ = 6.9 Hz, 2H, NH-amide), 7.91 (bs, 4H, NH_2), 4.26 (m, $^3J_{H,H}$ = 6.8 Hz, 2H, H-7), 3.13 (m, 4H, H-2), 2.68 (m, 4H, C-4), 2.56 (m, 4H, H-5), 2.00 (m, 4H, H-8), 1.84 (m, 4H, H-9), 1.70 (m, 4H, H-9), 1.62 (m, 4H, H-8), 1.45 (t, $^3J_{H,H}$ = 7.1 Hz, 6H, H-1) ppm. ^{13}C NMR: δ = 181.8 (C-3), 171.3 (C-6), 51.0 (C-7), 40.0 (C-2), 32.7 (C-8), 32.1 (C-4), 31.8 (C-5), 23.8 (C-9), 14.4 (C-1) ppm. ^{15}N NMR: δ = 107.7 (CONH-amide), −20.3 (NH_2) ppm. ^{195}Pt NMR: δ = 2489 ppm. IR (ATR): 3352 m, 3210 br, 3073 br; 2871 w; 1639 s ($\nu_{C=O}$); 1535 s, 1356 w, 1258 m, 1246 w cm^{-1}.

4.3. Crystallographic structure determination

X-ray diffraction measurement was performed on a Bruker X8 APEXII CCD diffractometer. Single crystal of **4** was positioned at 40 mm from the detector, and 1839 frames were measured, each for 20 s over 1° scan width. The data were processed using SAINT software [26]. Crystal data, data collection parameters, and structure-refinement details are given in Table 4. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-H atoms were refined with anisotropic displacement parameters. H atoms were inserted in calculated positions and refined with a riding model. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the atoms to which hydrogens were bonded. Structure solution was achieved with *SHELXS-97* and refinement with *SHELXL-97* [27], and graphics were produced with *ORTEP-3* [28].

4.4. Determination of lipophilicity

Lipophilicity of new complexes was determined by the shake-flask method and by reversed-phased HPLC.

4.4.1. Shake-flask method

The log *P* determination of compounds (**4**, **5**, **6** and **8**) was carried out, according to the guidelines for the shake-flask method [29] with slight modifications [20]. Weighted amounts of platinum complexes were dissolved in HPLC-grade water, which was pre-saturated with *n*-octanol, and mixed by sonication for 5 min. Afterward, the solutions were centrifuged for 5 min and the concentration of Pt was determined by ICP-MS. Weighted amounts of that solutions were mixed with the same volume of *n*-octanol (pre-saturated with water) and shaken for 15. After phase separation, the Pt concentration in the aqueous phase was again determined by ICP-MS and the partition coefficients were calculated.

The platinum content in the aqueous phase was determined by ICP-MS (Agilent 7500ce, Waldbronn, Germany), equipped with a CETAC ASX-520 autosampler (Neuss, Germany), a Scott double pass spray chamber, and a MicroMist nebulizer. For the analysis, the samples were diluted 1:1000 with 2.5% HCl. Every sample contained 0.5 ppb In as internal standard (CPI International, Santa Rosa, CA, USA).

4.4.2. Reversed-phase HPLC method

HPLC analysis was performed on a Dionex Summit system controlled by the Dionex Chromeleon 6.60 software. The

Table 4
Crystallographic data for complex **4**.

	4
Empirical formula	$C_{14}H_{28}Cl_2N_4O_8Pt$
F_w	618.37
Space group	*P*−1
a [Å]	6.0519(3)
b [Å]	13.1190(6)
c [Å]	14.0840(5)
α [°]	79.521(2)
β [°]	83.694(2)
γ [°]	85.073(3)
V [Å^3]	1090.41(8)
Z	2
λ [Å]	0.71073
ρ_{calcd} [g cm^{-3}]	1.883
Crystal size [mm^3]	0.20 × 0.13 × 0.12
T [K]	100(2)
μ [mm^{-1}]	6.719
R_1[a]	0.0357
wR_2[b]	0.0858
GOF[c]	0.989

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
[b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.
[c] GOF = $\{\Sigma[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.

experimental conditions were as follows: Agilent ZORBAX Bonus-RP column (4.6 mm × 250 mm); 0.1% TFA water/MeOH based mobile phases; UV–vis detection set up at 210 nm; temperature of the column: 25 °C; flow rate: 1 mL min^{-1}; concentration of the investigated complexes: 2.5 mM, (1 mM KI as internal standard was added); 25 μL injection volume. The capacity factors $k' = (t_R - t_0)/t_0$ (t_R is the retention time of the species analyzed and k_0 is the retention time of the unretained substance, used as a standard (here KI)) of the investigated compounds were determined at different MeOH/water ratios (from 60:40 for the most lipophilic to 10:90 for the most hydrophilic compounds). Using the linear relationship between log *k*' and the percentage of MeOH in the mobile phase: $\log k' = \log k'_w - \%MeOH$. log *k*' values for all complexes were calculated for 0, 10, 20, 30, 40 and 50% of MeOH in the mobile phase. Complexes **3**–**8** and for comparison (OC-6-33)-dichlorido(ethane-1,2-diamine)-bis{(4-methoxy)-4-oxobutanoato}platinum(IV) (**R1**) and (OC-6-33)-bis{(5-butyloxy)-5-oxo-3-methylpentanoato}dichlorido(ethane-1,2-diamine)platinum(IV) (**R2**) described in Ref. [22] were investigated. Calibration curves for different MeOH concentrations were created on the basis of determined log *P* values of **4**-**6**, **8**, **R1**, and **R2**. The equations derived from the calibration curves for different percentage of MeOH together with their R^2 values, are shown in Table 5. From these equations log *P* values for all investigated complexes were calculated.

4.5. Cell lines and culture conditions

CH1 (ovarian carcinoma, human) cells were donated by Lloyd R. Kelland (CRC Center for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.). A549 (non-small cell lung cancer, human) and SW480 (colon carcinoma, human) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria), and SK-OV-3 (ovarian carcinoma, human) cells by Evelyn Dittrich (General Hospital, Medical University of Vienna, Austria). Cells were grown in 75 cm^2 culture flasks (Iwaki/Asahi Technoglass) as adherent monolayer cultures in Minimal Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, and 2 mM L-glutamine (all purchased from Sigma–Aldrich) without antibiotics. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

4.6. Cytotoxicity tests in cancer cell lines

Cytotoxicity in the cell lines mentioned above was determined by the colorimetric MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, purchased from Fluka). Cells were harvested from culture flasks by trypsinization and seeded in 100 μL aliquots in MEM supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, and 1% non-essential amino acids (100×) into 96-well microculture plates (Iwaki/Asahi Technoglass) in the following densities, to ensure exponential growth of untreated controls throughout the experiment: 1.5 × 10^3 (CH1), 3.5 × 10^3 (SK-OV-3), 4.0 × 10^3 (A549), and 2.5 × 10^3 (SW480) viable cells per well. Cells were allowed to settle and resume exponential growth in drug-free complete culture medium for 24 h, followed by the addition of dilutions of the test compounds in 100 μL/well of the same medium. After continuous exposure for 96 h, the medium was replaced by a 100 μL/well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 μL/well solution of MTT in phosphate-buffered saline (5 mg/mL) (all purchased from Sigma–Aldrich). After incubation for 4 h, medium/MTT mixtures were removed, and the formazan product formed by viable cells was dissolved in DMSO (150 μL/well). Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed as percentage of untreated controls, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration-effect curves by interpolation. Evaluation is based on means from three independent experiments, each comprising six replicates per concentration level.

Table 5
Results from the calibration curves $\log P = f(\log k')$ under different experimental conditions.

%MeOH	Equation	R^2
0	0.8842× −2.1484	0.92
10	0.9126× −1.6351	0.93
20	0.9586× −1.1251	0.95
30	0.9736× −0.4783	0.98
40	1.0174× +0.081	0.96
50	1.0063× +0.7582	0.98

Table 6
Viable, apoptotic and necrotic cell fractions (in %) of SW480 cells upon treatment with cisplatin or compound **7** for 48 h, analyzed by FACS using annexin V and PI staining.

Concentration, μM	Viable cells	Early apoptosis	Late apoptosis	Necrosis
Compound **7** (in %)				
0	98.3 ± 0.6	0.7 ± 0.3	0.8 ± 0.5	0.2 ± 0.1
5	97.2 ± 1.2	1.5 ± 0.8	1.0 ± 0.4	0.4 ± 0.3
10	87.9 ± 9.8	3.3 ± 1.9	5.1 ± 5.0	3.8 ± 3.7
15	77.7 ± 8.3	3.9 ± 2.9	10.6 ± 5.9	7.9 ± 2.6
25	62.4 ± 4.9	5.8 ± 4.9	14.7 ± 6.2	17.1 ± 7.8
50	47.5 ± 2.6	4.8 ± 0.8	26.3 ± 8.0	21.5 ± 7.0
Cisplatin (in %)				
0	98.1 ± 0.7	0.7 ± 0.6	0.5 ± 0.4	0.7 ± 0.5
5	96.1 ± 2.1	1.0 ± 0.2	1.1 ± 0.7	1.9 ± 2.2
10	93.5 ± 2.0	2.5 ± 0.8	1.5 ± 1.2	2.5 ± 3.1
15	95.9 ± 1.0	2.9 ± 0.3	1.0 ± 0.6	0.2 ± 0.1
25	94.9 ± 3.5	3.3 ± 3.4	1.3 ± 0.6	0.6 ± 0.5
50	93.8 ± 3.5	3.7 ± 2.6	1.6 ± 0.8	0.9 ± 0.7
100	78.7 ± 11.3	8.0 ± 3.5	4.6 ± 4.0	8.7 ± 4.7

4.7. Apoptosis/necrosis assay

Cell death was analyzed by fluorescence-activated cell sorting (FACS) using FITC-conjugated annexin V (BioVision, USA) and propidium iodide (PI; Fluka) staining (Table 6).

SW480 cells were seeded into 6-well plates (Iwaki/Asahi Technoglass, Gyouda, Japan) in amounts of 2 × 10^5 cells per well in complete medium (as described above) and allowed to settle for 24 h. The cells were exposed to cisplatin and compound **7** for 48 h at 37 °C. After the incubation, cells were gently trypsinized, washed with PBS, and suspended with FITC-conjugated annexin V (0.25 μg/mL) in binding buffer (10 mM HEPES/NaOH pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) at room temperature for 15 min. PI (1 μg/mL) was added shortly before the measurement. Stained cells were analyzed with a FACS Calibur instrument (Becton Dickinson, Franklin Lakes, NJ, USA) using Cell-QuestPro software. At least three independent experiments were conducted, and 10,000 cells were counted per analysis.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.09.006.

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2. Novel tetracarboxylatoplatinum(IV) complexes as carboplatin prodrugs.

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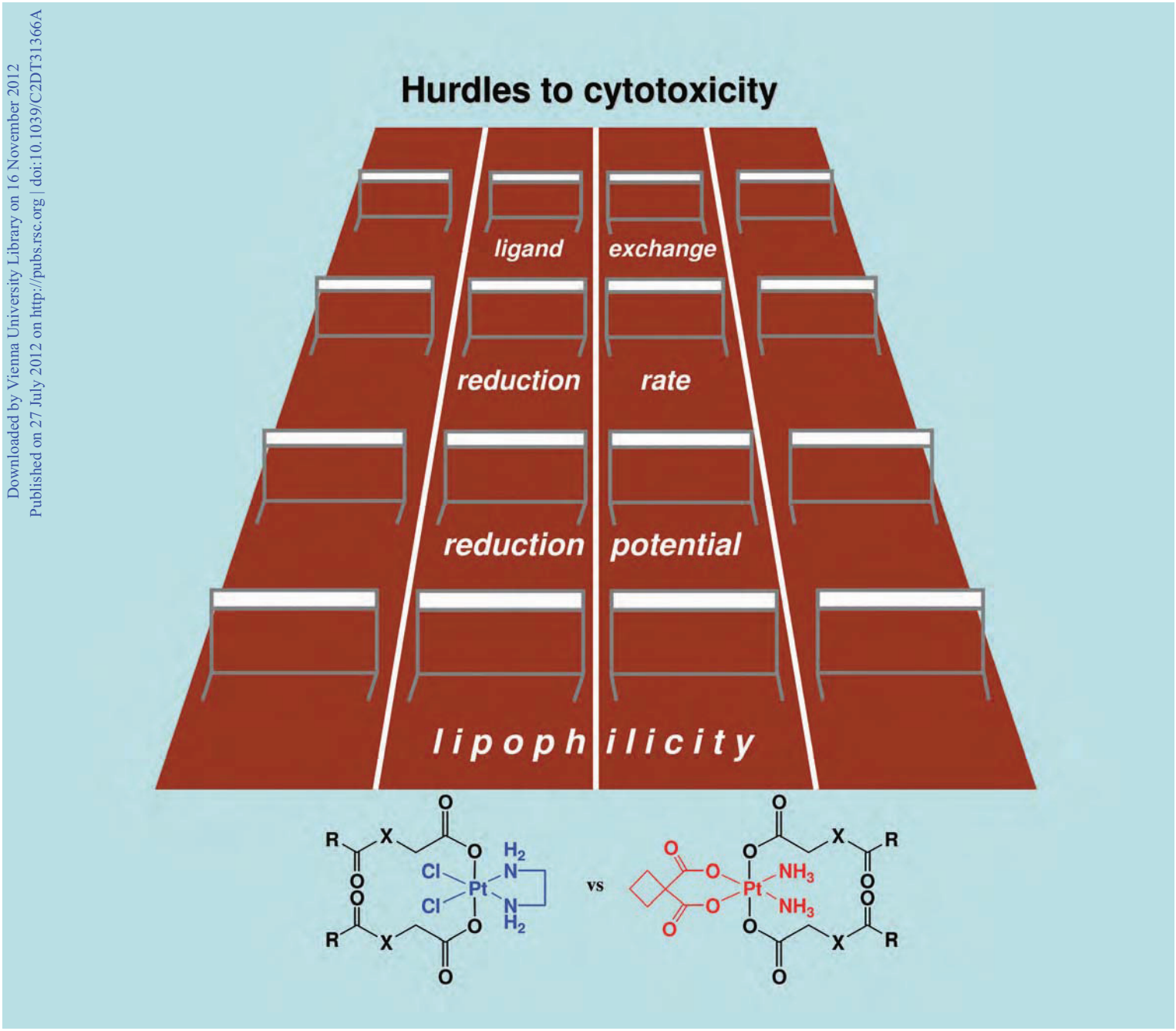

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PAPER

Novel tetracarboxylatoplatinum(IV) complexes as carboplatin prodrugs†

Hristo P. Varbanov, Seied M. Valiahdi, Christian R. Kowol, Michael A. Jakupec, Markus Galanski* and Bernhard K. Keppler*

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It is widely accepted that platinum(IV) complexes act as prodrugs and have to be activated by reduction to the respective platinum(II) analogs. Recently it could be shown that introduction of lipophilic carboxylato ligands in the axial position leads to diaminedichloridoplatinum(IV) compounds with exceptionally high cytotoxicity. With the aim of improving the antiproliferative properties of carboplatin, a series of twenty-one novel Pt(IV) complexes, featuring the equatorial ligand sphere of carboplatin as well as lipophilic axial carboxylato ligands, was synthesized. In depth characterization is based on elemental analysis, ESI-MS, ATR-IR, and multinuclear (^{1}H, ^{13}C, ^{15}N, and ^{195}Pt) NMR spectroscopy. Their cytotoxic activity in four cell lines (CH1, SK-OV-3, SW480, and A549), lipophilicity, electrochemistry and additionally the rate of reduction in the presence of ascorbic acid were investigated. In contrast to analogous diaminedicarboxylatodichloridoplatinum(IV) compounds, the cytotoxicity of novel diaminetetracarboxylato counterparts could not substantially be increased by simply enhancing their lipophilic character. It seems that not only the reduction potential, but also the rate of reduction has a tremendous influence on the cytotoxic properties. This has to be taken into account for the development of novel anticancer platinum(IV) agents.

Introduction

After Rosenberg's serendipitous discovery[1] opened up the way for the introduction of metal complexes in antineoplastic chemotherapy, several platinum(II) complexes (*i.e.* cisplatin, carboplatin and oxaliplatin) have become the mainstay of recent cancer treatment.[2] Nowadays, research in that field has focused on platinum(IV) and nonplatinum metal complexes. It has been expected that improvement of the pharmacological and toxicological profile in comparison to platinum(II) based drugs could be achieved.[3,4]

In this context it is worth mentioning that cytotoxic effects of platinum complexes were first found for a Pt(IV) compound, namely (OC-6-22)-diamminetetrachloridoplatinum(IV), a close analogue of cisplatin.[5] Platinum(IV) complexes are kinetically more inert than their Pt(II) counterparts. Reduction under hypoxic conditions accompanied by the loss of two ligands occurs more readily than ligand exchange reactions. Their physicochemical characteristics broaden the possibilities for the design of novel metal-based drugs in various ways, *e.g.* modulation of pharmacokinetic properties, additional opportunities for targeted therapy, oral administration, the prodrug concept, *etc*.[6] So far, three Pt(IV) complexes were studied in clinical trials (tetraplatin, iproplatin and satraplatin), but none of them gained clinical approval.[7] However, satraplatin currently entered new phase I and II clinical trials in combination regimens.[8] Generally, Pt(IV) complexes act as prodrugs *via* activation by reduction *in vivo* to the reactive Pt(II) species.[9–12] This mode of action explains the importance of the rate and mechanism of reduction as well as the final reduction products. An optimal pharmacological profile can be expected, provided that the prodrug is relatively stable in the blood stream and will preferably be reduced to the active platinum(II) species in the tumor cell or tissue. It has been shown that such redox behavior can be observed for Pt(IV) complexes with axial carboxylato ligands.[13] Consequently, platinum(IV) complexes were coupled to targeting moieties[14–20] or to pharmacologically relevant molecules ameliorating the cytotoxic properties of the platinum(II) fragment.[21–24]

A series of *cis*-diam(m)inebis(carboxylato)dichloridoplatinum(IV) complexes with high cytotoxic potency has been published recently.[25–29] It was found that increasing the lipophilicity of the axial ligands yields compounds with IC_{50} values in the nanomolar range.[27,28] Whether the latter concept can be transferred to kinetically more inert platinum(II) analogs such as carboplatin has not yet been investigated. Therefore, we intended to answer the following question within this project: Is it possible to significantly increase the antiproliferative properties of carboplatin just by synthesizing lipophilic prodrugs only differing in the axial ligands?

In line with that task, twenty-one novel Pt(IV) complexes, designed as prodrugs for carboplatin, were synthesized and completely characterized by elemental analysis, ESI-MS, ATR-IR,

University of Vienna, Institute of Inorganic Chemistry, Waehringer Strasse 42, A-1090 Vienna, Austria. E-mail: markus.galanski@univie.ac.at, bernhard.keppler@univie.ac.at; Fax: +43-1-4277-52680; Tel: +43-1-4277-52601

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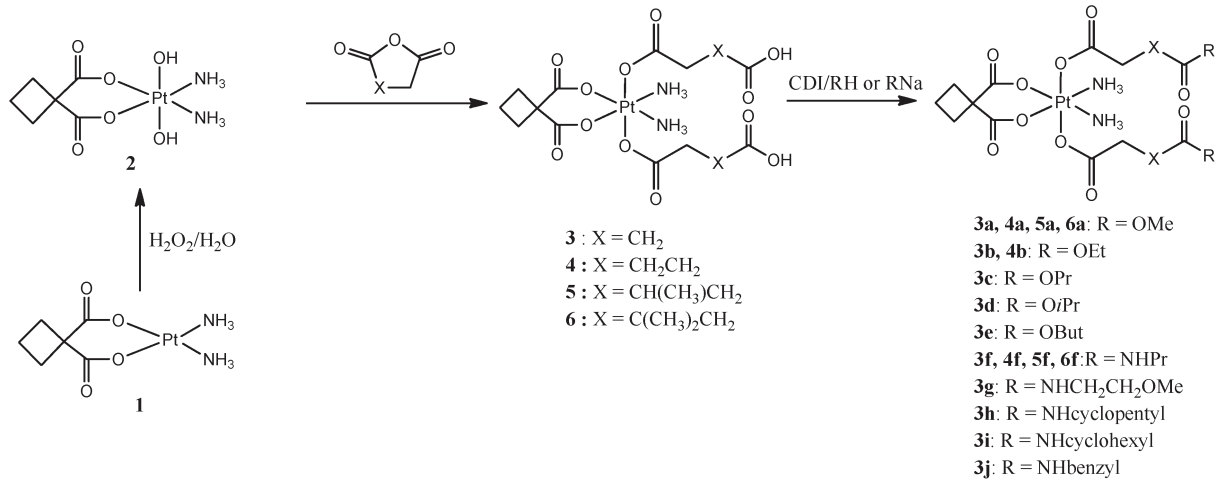

Fig. 1 Synthesis of novel tetracarboxylatoplatinum(IV) complexes.

and multinuclear NMR spectroscopy. Their cytotoxic activity in four human tumor cell lines, originating from ovarian carcinoma (CH1 and SK-OV-3), colon carcinoma (SW480) and non-small cell lung cancer (A549), was evaluated by means of the MTT colorimetric assay. In order to elucidate their biological behavior, lipophilicity as well as redox properties were determined.

Results and discussion

Synthesis

A general reaction scheme for the synthesis of novel complexes is shown in Fig. 1. The oxidation of carboplatin to the respective dihydroxido Pt(IV) analogue (**2**) using H_2O_2 in aqueous solutions at ambient temperature was performed successfully with yields over 90%. Complex **2** was subsequently converted to a series of tetracarboxylatoplatinum(IV) complexes using different cyclic anhydrides in DMF; complexes **3–6** were isolated with yields over 75%. The latter were used as the starting material for the synthesis of a plethora of amide and ester derivatives *via* activation of the free carboxylic groups with CDI in dry DMF, followed by reaction with the corresponding amine or alcoholate/alcohol mixtures. Pure products were isolated with the help of column chromatography and/or recrystallization with moderate yields (around 20% for the esters and 30% for the amides).

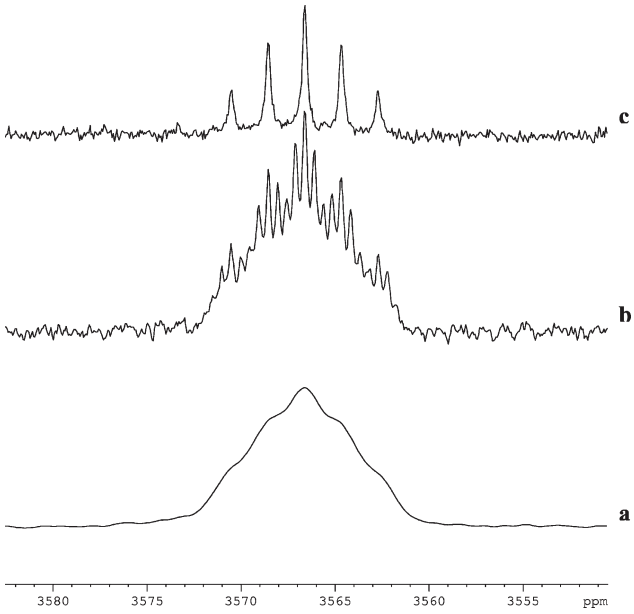

Fig. 2 ^{195}Pt NMR spectra of complex **6a** processed with line broadening factors of 100 Hz (a) and 10 Hz (b), respectively. ^{195}Pt NMR spectrum of **6a** measured with proton decoupling (c).

Spectroscopic characterization

The novel complexes were fully characterized by elemental analysis, one and two dimensional multinuclear NMR spectroscopy (^{1}H, ^{13}C, ^{15}N, ^{195}Pt), ESI-MS and ATR-IR. The oxidation of carboplatin and the following carboxylations are accompanied by significant shifts of the signals in the ^{195}Pt NMR spectra. A ^{195}Pt resonance for the platinum(II) complex (**1**) was detected at −88 ppm; upon oxidation to the corresponding dihydroxidoplatinum(IV) analogue, the chemical shift was found at 3268 ppm. Carboxylation of (**2**) resulted in a downfield shift of around 290 ppm with signals for complexes **3–6** between 3551 and 3567 ppm. Expectedly, further derivatization of **3–6** had no influence on the position of the Pt signals; chemical shifts of all novel target complexes ranged between 3551 and 3568 ppm.

Normally, ^{195}Pt NMR resonances are broad and unresolved; half height line widths of several hundred Hertz are common. Good signal to noise ratios are not easy to get in the case of ^{195}Pt NMR measurements. Consequently, large line broadening factors are used for processing of data in order to suppress unwanted noise (lb = 100 Hz, Fig. 2a). However, a fine structure of the signal is still visible which can be improved by decreasing the line broadening factor to 10 Hz (Fig. 2b). When measuring a ^{1}H decoupled ^{195}Pt spectrum, a nicely resolved quintet with a $^1J(^{14}N, ^{195}Pt)$ coupling constant of 205 Hz was obtained (Fig. 2c). The coupling of ^{14}N and ^{195}Pt with the protons of the coordinated ammine ligands is visible in the ^{1}H NMR spectrum

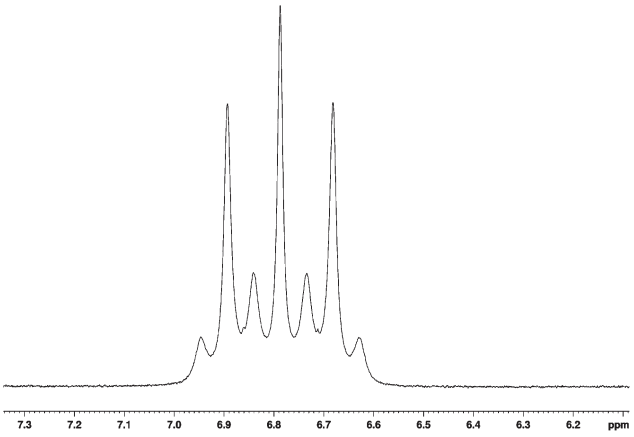

Fig. 3 NH_3 signal of complex **6a** in the ^1H NMR spectrum.

as exemplarily shown for complex **6a** (Fig. 3). The coupling constants $^1J(^{14}N, ^1H)$ = 53 Hz and $^2J(^{195}Pt, ^1H)$ = 53 Hz are in the expected range for platinum(IV) complexes.[30]

All signals in the ^1H and ^{13}C NMR spectra were found with expected chemical shifts and their correct assignment was performed on the basis of two dimensional $^1H^1H$ COSY, $^1H^{13}C$ HSQC and $^1H^{13}C$ HMBC spectra.

For complexes **5**, **5a** and **5f** a mixture of stereoisomers (RR : SS : RS = 1 : 1 : 2) was obtained due to the use of prochiral 3-methylglutaric anhydride for esterification of **2**. Nevertheless, this cannot be proven in the NMR spectra, as observed in previous work from our group.[27] Since the chiral information is situated on the axial ligands, which will be released during the reduction, complexes **5**, **5a** and **5f** were investigated without further chiral separation. Structures of the complexes were also proved by ESI-MS spectra, measured both in the positive as well as in the negative ion mode. The highest intensities were observed for peaks assigned to $[M + Na^+]^+$, $[M + H^+]^+$, $[M - H^+]^-$ and in some cases $[M + Cl^-]^-$. The *m*/*z* values as well as the isotopic distribution were in accordance with the theoretical values.

Crystal structure of complex 4

X-ray diffraction on a sample of **4** afforded a data set that was generally rather poor. However, the solution gave the gross structure of the complex, as shown in Fig. S1 in the ESI.†

Lipophilicity

Lipophilicity is one of the most important physicochemical parameters, characterizing the pharmacokinetic behavior and deducing the ability of drugs and drug candidates to pass through cell membranes. In particular this is true for platinum complexes (especially for Pt(IV)), which results in many efforts for establishing a reliable method for determination and prediction of log *P* (octanol–water partition coefficient) values of platinum-based cytostatics.[31–36] Verifying lipophilicity with RP-HPLC, based on chromatographic retention indexes, has many advantages in comparison with the classic shake-flask method, like independence from the compound's concentration, faster, robust and reproducible measurements, *etc*.[37] Measuring the isocratic and extrapolated retention factors log k_{30} and log k_w can provide relevant information about the lipophilic properties of the compounds even without converting them into log *P* values.[38]

Table 1 log k_{30} (log *k*, obtained with a mobile phase, containing 30% MeOH) and log k_w (values, extrapolated to 0% MeOH) for the new complexes

Compound	log k_w	log k_{30}
3	1.07	−0.15
4	1.35	0.14
5	2.06	0.64
6	2.27	1.13
3a	1.61	0.40
3b	2.16	0.95
3c	2.94	1.53
3d	2.71	1.42
3e	4.20	2.38
3f	2.23	0.80
3g	1.68	0.04
3h	3.13	1.54
3i	3.67	2.03
3j	3.32	1.75
4a	1.86	0.69
4b	2.54	1.22
4f	2.10	0.90
5a	2.77	1.20
5f	2.92	1.30
6a	2.97	1.57
6f	3.06	1.69

In previous studies, we have shown that for *cis*-diam(m)inodichloridobis(carboxylato)platinum(IV) complexes cytotoxicity, cellular accumulation as well as DNA platination increase with increasing log *P* within a series of analogues.[28,39] Herein, we have measured the lipophilicity of all new compounds with the help of RP-HPLC, using water/MeOH mobile phases. The obtained retention factors (log k_w and log k_{30}) are summarized in Table 1.

All novel complexes were more lipophilic than their Pt(II) precursor (carboplatin) and its oxidized form (**2**); the latter log *k* values could not be determined under the conditions used, because their retention times were lower than that of the standard, uracil.

Cytotoxicity in cancer cell lines

All novel compounds were tested in comparison to cisplatin and carboplatin in four human tumor cell lines, originating from ovarian carcinoma (CH1, SK-OV-3), colon carcinoma (SW480) and non-small cell lung cancer (A549), by means of the colorimetric MTT microculture assay. All of these cell lines, except for CH1 cells, are primarily cisplatin- and carboplatin-resistant (showing IC_{50} values about one order of magnitude higher than that in CH1 cells). IC_{50} values are summarized in Table 2, and complete concentration–effect curves in CH1 cells are depicted in the ESI (Fig. S2†).

All novel complexes showed cytotoxic activity in the cisplatin-sensitive CH1 cell line, with IC_{50} values ranging between 7.7 and 171 μM. However, cytotoxicity is lower in comparison to the clinically established platinum drugs cisplatin and carboplatin (IC_{50} 0.16 and 1.4 μM, respectively). Antiproliferative activity in cisplatin-resistant A549, SW480 and SK-OV-3 cancer

Table 2 Cytotoxicity of the novel complexes in comparison to cisplatin and carboplatin in four human cancer cell lines

	IC_{50} [a] (μM)			
Compound	CH1	A549	SW480	SK-OV-3
3	171 ± 1	>500	>500	>500
3a	32 ± 10	>500	>500	>500
3b	28 ± 4	>500	>500	>500
3c	24 ± 5	>500	>500	>500
3d	8.6 ± 1.7	>250	350 ± 39	—
3e	11 ± 6	>500	181 ± 44	—
3f	44 ± 8	>500	>500	>500
3g	62 ± 26	>500	>500	—
3h	15 ± 5	>500	>500	—
3i	28 ± 2	>500	>500	—
3j	31 ± 13	>500	>500	—
4	114 ± 23	>500	>500	>500
4a	33 ± 13	>500	>500	—
4b	7.7 ± 1.4	>500	>250	—
4f	89 ± 7	>500	>500	—
5	128 ± 48	>500	>500	>500
5a	23 ± 9	>500	>500	—
5f	49 ± 13	>500	>500	—
6	125 ± 35	>500	>500	>500
6a	22 ± 8	>500	>500	—
6f	33 ± 4	>500	>500	—
1 (carboplatin)	1.4 ± 0.4	91 ± 10	85 ± 28	67 ± 11
Cisplatin	0.16 ± 0.03	1.3 ± 0.4	3.5 ± 0.3	1.9 ± 0.3

[a] 50% Inhibitory concentrations in CH1, A549, SW480 and SK-OV-3 cells in the MTT assay, 96 h exposure. Values are the means ± standard deviations obtained from three independent experiments.

cells is generally negligible. In accordance with previous studies,[26,28] the complexes with free carboxylic groups (**3**, **4**, **5** and **6**) exert the lowest antiproliferative activity, probably due to their lower lipophilicity. The respective ester and amide derivatives are more cytotoxic, increasing with the lipophilicity of the alcoholate, resp. amide moiety. However, a substantial decrease of IC_{50} values with increasing lipophilicity of the side chains could not be observed. Contrary to our expectations, differences in cytotoxicity of, *e.g.*, methyl and propyl ester derivatives (**3a** and **3c**) are relatively small (IC_{50} values differing by a factor of 1.3). In the case of analogous complexes with two ethylamine and two chlorido ligands in the equatorial position, the propyl ester derivative is by more than one order of magnitude more cytotoxic than the methyl ester counterpart.[28]

A comparison of cytotoxicity of complex **3** and its ester and amide derivatives in the CH1 cell line is represented in Fig. 4. Overall, antiproliferative activity is dependent on lipophilicity. Esters (**3d** and **3e**) featuring an isopropyl or butyl ester moiety and cyclopentyl and cyclohexyl amide derivatives (**3h** and **3i**) show the lowest IC_{50} values. However, it is not possible to reach the cytotoxic potency of carboplatin by increasing the lipophilicity of platinum(IV) analogues.

Variation of the spacer (Fig. 5) between the two carbonyl atoms in the axial ligands of ester or amide derivatives revealed that derivatization of **2** with a lipophilic anhydride tends to be most favorable. However, the differences in cytotoxicity are not tremendous with increasing size of the spacer, which on the other hand decreases the solubility, and clear-cut structure–activity relationships with respect to the spacer cannot be formulated unequivocally.

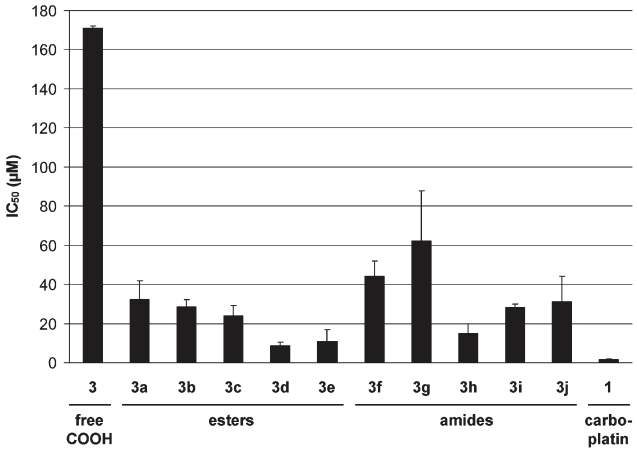

Fig. 4 Comparison of the IC_{50} values of complexes **3a–j** and carboplatin in CH1 cells.

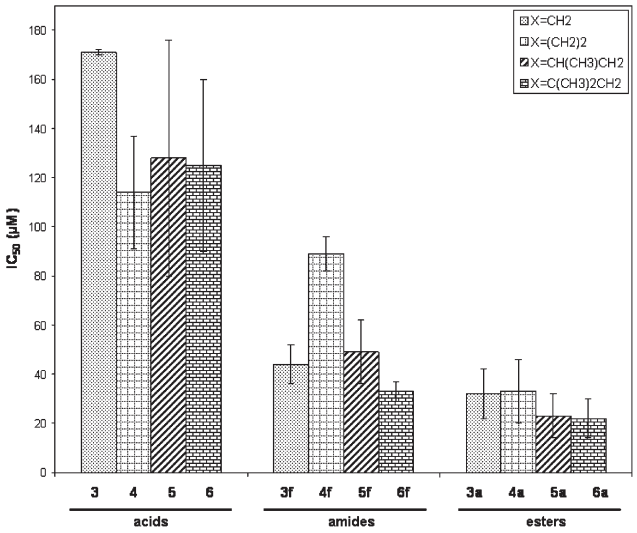

Fig. 5 Comparison of the IC_{50} values of complexes **3–6** and their propylamide and methyl ester derivatives in CH1 cells.

A semi-logarithmic plot of log k_w values (Table 1) *versus* IC_{50} values is shown in Fig. 6, demonstrating a rough correlation of IC_{50} values with lipophilicity, but with poor overall determination, indicating a considerable influence of additional factors on cytotoxicity. Recent studies on diam(m)inebis(carboxylato) dichloridoplatinum(IV) complexes have shown that with increasing lipophilicity, compounds with higher cytotoxicity compared to their Pt(II) counterparts can be obtained.[26–28] Nevertheless, there seem to be limits to this approach, as was demonstrated by this series of novel diammine tetracarboxylatoplatinum(IV) complexes, all of which are less cytotoxic than their precursor carboplatin. In order to find an explanation for this particular behavior, additional studies with respect to the redox behavior of these complexes were performed.

Redox behavior

Electrochemistry. As already mentioned above, the redox properties of Pt(IV) complexes have a significant influence on

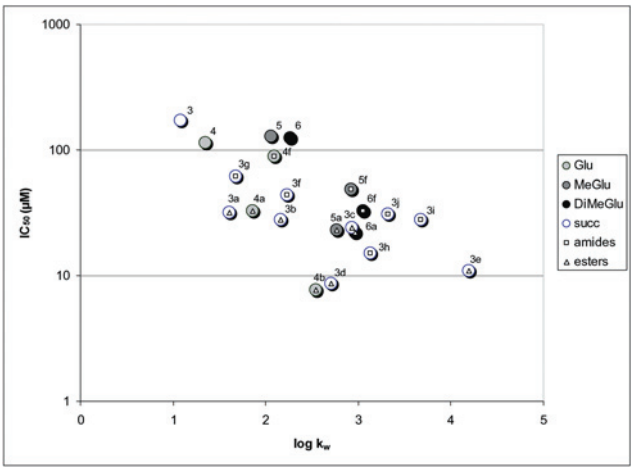

Fig. 6 Semi-logarithmic plot of lipophilicity (determined as log k_w with RP-HPLC) *vs.* cytotoxicity in CH1 cells (IC_{50}) for the new complexes (succ – complex **3** and its derivatives, Glu – complex **4** and its derivatives, MeGlu – complex **5** and its derivatives, and DiMeGlu – complex **6** and its derivatives).

Table 3 Summary of the electrochemical data

Compound	$E_p/Pt^{IV} \rightarrow Pt^{II}$
3	−0.69
3b	−0.70
3f	−0.68
4f	−0.74
5f	−0.70

Potentials in $V \pm 0.02$ *vs.* NHE measured at a scan rate of 100 mV s^{-1} in 0.15 M [*n*-Bu_4N][BF_4]/DMF.

their pharmacological profile. Fast reduction could result in deactivation of the respective platinum(II) species and high systemic toxicity, whereas very slow reduction could be responsible for the lack of anticancer activity. When platinum(IV) complexes exhibit axial carboxylato ligands, the reduction potential is situated in a medium range, which is believed to be a prerequisite for the platinum(IV) prodrug strategy.

In order to elucidate the influence of modifications in the axial carboxylato ligands on the redox potential and in last consequence on the cytotoxic properties, complexes **3**, **3b**, **3f**, **4f** and **5f** were investigated by cyclic voltammetry in DMF solution at a scan rate of 100 mV s^{-1} using 0.15 M [*n*-Bu_4N][BF_4] as the supporting electrolyte (Table 3, Fig. 7).

All complexes showed an irreversible reduction peak, common for Pt(IV) compounds (Fig. 7). The reduction potentials of the investigated complexes were found to be at *ca.* −0.7 V *vs.* NHE featuring no significant deviation in dependency on the structure of the axial ligand. These values are slightly more negative compared to those of the dichloridoethylendiamineplatinum(IV) analogues which are reduced at around −0.6 V.[39] Thus, the novel carboplatin prodrugs are harder to reduce and should therefore exhibit a slightly lower antiproliferative potency compared to the dichlorido compounds. However, as mentioned above the IC_{50} values of novel complexes are much higher than expected.

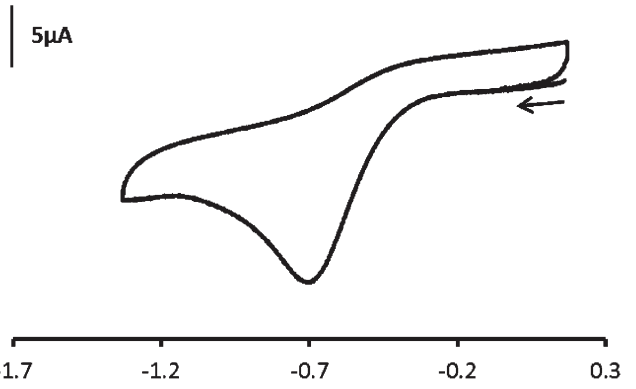

Fig. 7 Cyclic voltammogram of complex **3b** in DMF containing 0.15 M [*n*-Bu_4N][BF_4] at a scan rate of 100 mV s^{-1} using a glassy carbon working electrode.

Very recently, Gibson and Hambley *et al.* have shown that, in the case of platinum(IV) complexes featuring a dicarboxylato ligand in the equatorial position, rates of reduction by ascorbate do not necessarily go parallel to the electrochemical reduction potentials.[40]

They concluded that the reduction by ascorbate is very slow in the absence of coordinating hydroxido or chlorido ligands, which are capable of forming a bridge to the reducing agent and thereby facilitating the electron transfer. In line with this explanation, the tetracarboxylatoplatinum(IV) complexes in the present study (lacking any Cl^- or OH^- ligands) should be reduced very slowly.

Incubation with ascorbic acid

In order to judge the influence of the equatorial dicarboxylato leaving ligand on the rate of reduction, complex **3f** and its ethylenediaminedichlorido analogue (**M1**, the structure is shown in Fig. S3 in the ESI†) were incubated with a 25-fold excess of ascorbic acid. The fate of reduction was measured with the help of ^1H NMR spectroscopy in a D_2O buffered solution at physiological pH.

Complex **M1** was completely reduced after 24 h, with a half life of *ca.* 5 h. However, complex **3f** showed a very slow reduction rate; 50% of the complex were reduced after 3 weeks. Curves of the reduction (Fig. S4†) and comparative NMR spectra (Fig. S5 and S6) are given in the ESI.† The extremely slow reduction of **3f** might be the reason for its lower cytotoxicity *in vitro*.

Conclusions

A series of novel carboplatin prodrugs was synthesized and characterized in detail by various analytical techniques. Cytotoxicity was dependent on the lipophilicity of the axial ligands but could not be enhanced down to nanomolar concentrations as observed recently for analogs featuring chlorido ligands instead of 1,1-cyclobutanedicarboxylate. The reduction behavior in the presence of ascorbic acid was investigated and revealed a dramatic influence of the equatorial leaving group on the rate of reduction and in last consequence on the antiproliferative

potential. Nevertheless, further investigations are needed in order to get a detailed picture of the reduction process as well as of the mode of action of platinum(IV) complexes.

Experimental

Materials and methods

All reagents and solvents were obtained from commercial suppliers, and were used without further purification. Methanol and ethanol were dried, according to standard procedures. Water was purified through reverse osmosis, followed by double distillation. For column chromatography, silica gel 60 (Fluka) was used. High purity water used for the HPLC experiments was obtained from a Millipore Synergy 185 UV Ultrapure Water system (Molsheim, France).

^{1}H, ^{13}C, ^{15}N, ^{195}Pt and two-dimensional ^{1}H^{1}H COSY, ^{1}H^{13}C and ^{1}H^{15}N HSQC, and ^{1}H^{13}C HMBC NMR spectra were recorded with a Bruker Avance III 500 MHz NMR spectrometer at 500.32 (^{1}H), 125.81 (^{13}C), 107.55 (^{195}Pt), and 50.70 MHz (^{15}N) in DMF-d_7 or D_2O (in the case of carboplatin (**1**) and its dihydroxido Pt(IV) analogue (**2**)) at ambient temperature, using the solvent residual peak for ^{1}H and ^{13}C as the internal reference. The splitting of proton resonances in the ^{1}H NMR spectra is defined as s = singlet, bs = broad singlet, d = doublet, t = triplet, and m = multiplet. ^{15}N chemical shifts were referenced relative to external NH_4Cl, whereas ^{195}Pt chemical shifts were referenced relative to external $K_2[PtCl_4]$ (see Fig. 8 for the NMR numbering scheme).

IR spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer (4000–400 cm^{-1}) by using an ATR unit. Intensities of reported IR bands are defined as br = broad, s = strong, m = medium, and w = weak. Electrospray ionization mass spectrometry was carried out with a Bruker Esquire 3000 instrument using MeOH as the solvent. Elemental analyses were performed with a Perkin-Elmer 2400 CHN-Elemental Analyser by the Microanalytical Laboratory of the University of Vienna.

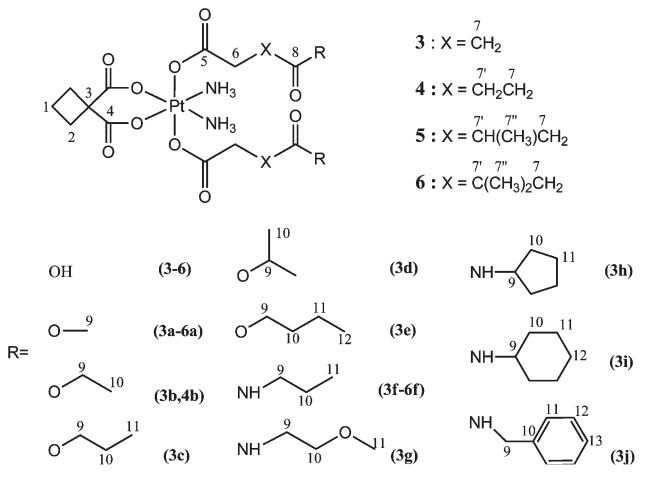

Fig. 8 NMR numbering scheme for novel tetracarboxylatoplatinum(IV) complexes.

Synthesis

The general reaction scheme is given in Fig. 1. Synthesis of precursors **1** and **2** is based on literature methods[41,42] with some modifications.

(SP-4-2)-Diammine(1,1-cyclobutanedicarboxylato)platinum(II) (1). Carboplatin was prepared, starting from K_2PtCl_4 *via cis*-$Pt(NH_3)_2I_2$, its activation with $AgNO_3$ (1.9 equiv.) and reaction of the formed diamminediaqua complex with the disodium salt (prepared *in situ*) of 1,1′-cyclobutanedicarboxylic acid (CBDA) (0.95 equiv.) in water. After reducing the solvent volume and cooling (to 4 °C), a white crystalline powder was collected, washed with small amounts of cold water and dried over P_2O_5 under vacuum. Yield: 56%. M_r = 371.25 g mol^{-1}. Elemental analysis, found: C 19.25, H 2.91, N 7.49. Calcd for $C_6H_{12}N_2O_4Pt$: C 19.41, H 3.26, N 7.55. ^{1}H NMR: δ = 2.79 (t, $^{3}J_{H,H}$ = 7.9 Hz, 4H, H-2), 1.81 (m, 2H, H-1) ppm. ^{195}Pt NMR: δ = −88 ppm. IR (ATR): 3258 s, 3190 br (ν_{N-H}); 2957 w; 1634 s, 1601 s ($\nu_{C=O}$); 1464 w; 1373 w, 1345 s, 1286 m, 1204 w cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)dihydroxidoplatinum(IV) (2). Carboplatin (**1**) (1.4862 g, 4.0038 mmol) was oxidized with 20 ml of 30% H_2O_2 in 20 ml of water at room temperature. The white product formed was filtered off, washed with cold water and dried over P_2O_5 under vacuum. The volume of the filtrate was reduced, cooled in the fridge for 24 h and the newly formed precipitate was also collected and dried. Yield: 1.5169 g, 93%. M_r = 405.26 g mol^{-1}. ^{1}H NMR: δ = 2.59 (t, $^{3}J_{H,H}$ = 8.1 Hz, 4H, H-2), 1.96 (m, 2H, H-1) ppm. ^{13}C NMR: δ = 180.7 (C-4), 55.8 (C-3), 32.1 (C-2), 15.7 (C-1) ppm. ^{195}Pt NMR: δ = 3268 ppm. IR (ATR): 3448 br (ν_{Pt-O-H}); 3217 br, 3093 br (ν_{N-H}); 2963 br, 2727 w; 1615 s, 1570 s ($\nu_{C=O}$); 1351 s cm^{-1}.

General procedure for synthesis of compounds 3–6. 4 equiv. of the corresponding anhydride and (OC-6-33)-diammine(cyclobutane-1,1-dicarboxylato)dihydroxidoplatinum(IV) (**2**) were suspended in dry DMF and the reaction mixture was stirred at 50 °C until the solid material dissolved to form a colorless to pale yellowish solution. DMF was then removed under reduced pressure (a white foam is formed). The residue was suspended in acetone, filtered off and dried under vacuum to obtain a white solid.

(OC-6-33)-Diamminebis(3-carboxypropanoato)(cyclobutane-1,1-dicarboxylato)platinum(IV) (3). Succinic anhydride (215 mg, 2.1500 mmol), 218 mg (0.5383 mmol) of **2** in DMF (6 mL), 2 h. Yield: 278 mg, 85%. M_r = 605.4 g mol^{-1}. Elemental analysis, found: C 27.95, H 3.51, N 4.62. Calcd for $C_{14}H_{22}N_2O_{12}Pt$: C 27.77, H 3.66, N 4.63. ESI-MS: *m*/*z* 627.1 $[M + Na^+]^+$, 605.1 $[M + H^+]^+$, 603.9 $[M - H^+]^-$. ^{1}H NMR: δ = 12.35 (bs, 2H, COOH), 6.73 (m, 6H, NH_3), 2.64 (t, $^{3}J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.53 (t, $^{3}J_{H,H}$ = 6.9 Hz, 4H, H-6), 2.47 (t, $^{3}J_{H,H}$ = 6.6 Hz, 4H, H-7), 1.90 (m, 2H, H-1) ppm. ^{13}C NMR: δ = 179.5 (C-5), 176.7 (C-4), 173.8 (C-8), 56.4 (C-3), 31.9 (C-2), 30.3 ($^{3}J_{C,Pt}$ = 37 Hz, C-6), 29.8 (C-7), 16.0 (C-1) ppm. ^{15}N NMR: δ = −53.8 ppm. ^{195}Pt NMR: δ = 3565 ppm. IR (ATR): 3237 br, 3161 br (ν_{N-H}); 2949 w; 1767 w, 1719 m 1632 s, 1594 m, 1546 w ($\nu_{C=O}$); 1407 w, 1349 s, 1328 s, 1286 w, 1178 m cm^{-1}.

(OC-6-33)-Diamminebis(4-carboxybutanoato)(cyclobutane-1,1-dicarboxylato)platinum(IV) (4). Glutaric anhydride (350 mg, 3.0675 mmol), 306 mg (0.7551 mmol) of **2** in DMF (8 mL), 3 h. The product was recrystallized from methanol, washed with acetone and dried under vacuum. Yield: 384 mg, 83%. M_r = 633.46 g mol^{-1}. Elemental analysis, found: C 30.24, H 4.00, N 4.29. Calcd for $C_{16}H_{26}N_2O_{12}Pt$: C 30.34, H 4.14, N 4.42. ESI-MS: *m/z* 654.9 $[M + Na^+]^+$, 633.9 $[M + H^+]^+$, 630.8 $[M - H^+]^-$. ^{1}H NMR: δ = 12.25 (bs, 2H, COOH), 6.78 (m, 6H, NH_3), 2.62 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.30 (t, $^3J_{H,H}$ = 7.6 Hz, 4H, H-7), 2.28 (t, $^3J_{H,H}$ = 7.6 Hz, 4H, H-6), 1.90 (m, 2H, H-1), 1.73 (m, 4H, H-7′) ppm. ^{13}C NMR: δ = 180.1 (C-5), 176.8 (C-4), 174.4 (C-8), 56.3 (C-3), 34.5 (C-6), 33.0 (C-7), 31.9 (C-2), 21.0 (C-7′), 16.0 (C-1) ppm. ^{15}N NMR: δ = −54.5 ppm. ^{195}Pt NMR: δ = 3551 ppm. IR (ATR): 3196 br, 3096 br (ν_{N-H}); 2953 m; 1698 m, 1637 s ($\nu_{C=O}$); 1411 w, 1332 s, 1234 m, 1087 w cm^{-1}.

(OC-6-33)-Diamminebis(4-carboxy-3-methylbutanoato)(cyclobutane-1,1-dicarboxylato)platinum(IV) (5). 3-Methylglutaric anhydride (528 mg, 4.1208 mmol), 411 mg (1.0141 mmol) of **2** in DMF (10 mL), 2 h. Yield: 520 mg, 78%. M_r = 661.52 g mol^{-1}. Elemental analysis, found: C 32.62, H 4.48, N 4.22. Calcd for $C_{18}H_{30}N_2O_{12}Pt$: C 32.68, H 4.57, N 4.23. ESI-MS: *m/z* 699.1 $[M + K^+]^+$, 683.3 $[M + Na^+]^+$, 662.2 $[M + H^+]^+$, 660.1 $[M - H^+]^-$. ^{1}H NMR: δ = 12.23 (bs, 2H, COOH), 6.80 (m, 6H, NH_3), 2.63 (t, $^3J_{H,H}$ = 7.9 Hz, 4H, H-2), 2.37 (m, 2H, H-7), 2.32 (m, 2H, H-6), 2.29 (m, 2H, H-7′), 2.12 (m, 2H, H-6), 2.08 (m, 2H, H-7), 1.90 (m, 2H, H-1), 0.93 (d, $^3J_{H,H}$ = 6.5 Hz, 6H, H-7″) ppm. ^{13}C NMR: δ = 179.7 (C-5), 176.8 (C-4), 173.8 (C-8), 56.3 (C-3), 42.2 (C-6), 40.6 (C-7), 31.9 (C-2), 27.8 (C-7′), 19.4 (C-7″), 16.0 (C-1) ppm. ^{15}N NMR: δ = −55.3 ppm. ^{195}Pt NMR: δ = 3559 ppm. IR (ATR): 3209 br, 3093 br (ν_{N-H}); 2963 w; 1704 m, 1637 s ($\nu_{C=O}$); 1458 w, 1342 s, 1221 m, 1169 w, 1080 w cm^{-1}.

(OC-6-33)-Diamminebis(4-carboxy-3,3-dimethylbutanoato)-(cyclobutane-1,1-dicarboxylato)platinum(IV) (6). 3,3-Dimethylglutaric anhydride (212 mg, 1.4194 mmol), 151 mg (0.3726 mmol) of **2** in DMF (6 mL), 4 h. Yield: 210 mg, 82%. M_r = 689.56 g mol^{-1}. Elemental analysis, found: C 34.88, H 4.88, N 4.08. Calcd for $C_{20}H_{34}N_2O_{12}Pt$: C 34.84, H 4.97, N 4.06. ESI-MS: *m/z* 712.2 $[M + Na^+]^+$, 688.7 $[M - H^+]^-$. ^{1}H NMR: δ = 12.14 (bs, 2H, COOH), 6.80 (m, 6H, NH_3), 2.63 (t, $^3J_{H,H}$ = 7.9 Hz, 4H, H-2), 2.34 (s, 4H, H-7), 2.32 (s, 4H, H-6), 1.90 (m, 2H, H-1), 1.06 (s, 12H, H-7″) ppm. ^{13}C NMR: δ = 179.4 (C-5), 176.8 (C-4), 173.2 (C-8), 56.3 (C-3), 46.9 (C-6), 45.1 (C-7), 32.3 (C-7′), 31.9 (C-2), 27.0 (C-7″), 16.0 (C-1) ppm. ^{15}N NMR: δ = −55.1 ppm. ^{195}Pt NMR: δ = 3567 ppm. IR (ATR): 3255 br, 3128 br (ν_{N-H}); 2964 m, 2879 w; 1712 m, 1697 m, 1638 s, 1611 s ($\nu_{C=O}$); 1365 s, 1345 s, 1229 s, 1159 m, 1108 w cm^{-1}.

General procedure for synthesis of ester derivatives 3a–e, 4a–b, 5a and 6a

2 equiv. of CDI (1,1′-carbonyldiimidazole) in dry DMF were added to a DMF solution of **3**, **4**, **5** or **6**, respectively, and the mixture was heated at 60°C for 10 min. After cooling to room temperature, CO_2 formed during the activation was removed by flushing the solution with argon for 10 min. Then sodium alcoholate in absolute alcohol (prepared *in situ* by dissolving a catalytic amount of Na in absolute alcohol) was added and the solution was stirred for 24 h (up to 72 h in some cases). The solvents were removed under reduced pressure and the residues purified by recrystallization or column chromatography.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((4-methoxy)-4-oxobutanoato)platinum(IV) (3a). CDI (136 mg, 0.9004 mmol) in DMF (5 mL), **3** (248.5 mg, 0.4105 mmol) in DMF (6 mL), MeONa/MeOH (10 mL). The crude product was purified by suspending in EtOAc, filtration of the yellow powder and recrystallization of the latter from water. The obtained white powder was filtered off, washed with a small amount of cold water and cold diethyl ether and dried in a vacuum desiccator over P_2O_5. Yield: 60 mg, 23%. M_r = 633.46 g mol^{-1}. Elemental analysis, found: C 30.18, H 3.90, N 4.43. Calcd for $C_{16}H_{26}N_2O_{12}Pt$: C 30.34, H 4.14, N 4.42. ESI-MS: *m/z* 655.0 $[M + Na^+]^+$, 633.9 $[M + H^+]^+$, 632.5 $[M - H^+]^-$, 669.0 $[M + Cl^-]^-$. ^{1}H NMR: δ = 6.74 (m, 6H, NH_3), 3.63 (s, 6H, H-9), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.54 (m, 4H, H-6), 2.48 (m, 4H, H-7), 1.90 (m, 2H, H-1) ppm. ^{13}C NMR: δ = 179.2 (C-5), 176.7 (C-4), 172.9 (C-8), 56.3 (C-3), 51.1 (C-9), 31.8 (C-2), 30.2 (C-6), 29.5 (C-7), 15.9 (C-1) ppm. ^{15}N NMR: δ = −54.0 ppm. ^{195}Pt NMR: δ = 3566 ppm. IR (ATR): 3256 br, 3204 br (ν_{N-H}), 3119 br; 2953 w; 1732 m, 1639 s ($\nu_{C=O}$); 1428 w 1315s, 1262 w, 1191 w, 1164 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((4-ethoxy)-4-oxobutanoato)platinum(IV) (3b). CDI (223 mg, 1.3753 mmol) in DMF (6 mL), **3** (416 mg, 0.6871 mmol) in DMF (8 mL), EtONa/EtOH (10 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 5 : 1), then isolated through suspending in EtOAc, filtration and washing with Et_2O. A white powder was obtained and dried under vacuum. Yield: 130 mg, 31%. M_r = 661.51 g mol^{-1}. Elemental analysis, found: C 32.49, H 4.47, N 4.31. Calcd for $C_{18}H_{30}N_2O_{12}Pt$: C 32.68, H 4.57, N 4.23. ESI-MS: *m/z* 699.0 $[M + K^+]^+$, 683.0 $[M + Na^+]^+$, 662.0 $[M + H^+]^+$, 659.7 $[M - H^+]^-$, 696.7 $[M + Cl^-]^-$. ^{1}H NMR: δ = 6.73 (m, 6H, NH_3), 4.08 (m, 4H, H-9), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.53 (m, 4H, H-6), 2.47 (m, 4H, H-7), 1.90 (m, 2H, H-1), 1.20 (t, $^3J_{H,H}$ = 7.1 Hz, 6H, H-10) ppm. ^{13}C NMR: δ = 179.3 (C-5), 176.7 (C-4), 172.4 (C-8), 56.3 (C-3), 60.0 (C-9), 31.8 (C-2), 30.2 (C-6), 29.8 (C-7), 15.9 (C-1), 13.8 (C-10) ppm. ^{15}N NMR: δ = −54.8 ppm. ^{195}Pt NMR: δ = 3564 ppm. IR (ATR): 3251 br, 3208 br (ν_{N-H}); 2980 w; 1727 m, 1640 s ($\nu_{C=O}$); 1424 w, 1317 s, 1093 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((4-propyloxy)-4-oxobutanoato)platinum(IV) (3c). CDI (0.2143 g, 1.3214 mmol) in DMF (9 mL), **3** (0.4000 g, 0.6607 mmol) in DMF (10 mL), *n*-PrONa/*n*-PrOH (15 mL). The crude product was purified by column chromatography (EtOAc/IPA, 3 : 1), then isolated from an Et_2O suspension, followed by MeOH/EtOAc recrystallization. The white powder obtained was dried under vacuum. Yield: 82 mg, 18%. M_r = 689.57 g mol^{-1}. Elemental analysis, found: C 34.70, H 4.74, N 4.04. Calcd for $C_{20}H_{34}N_2O_{12}Pt$: C 34.84, H 4.97, N 4.06. ESI-MS: *m/z* 734.0

$[M + 2Na^+ - H^+]^+$, 711.1 $[M + Na^+]^+$, 689.9 $[M + H^+]^+$, 687.9 $[M - H^+]^-$. ^{1}H NMR: δ = 6.74 (m, 6H, NH_3), 3.99 (t, $^3J_{H,H}$ = 6.7 Hz, 4H, H-9), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.54 (m, 4H, H-6), 2.48 (m, 4H, H-7), 1.90 (m, 2H, H-1), 1.61 (m, 4H, H-10), 0.91 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-11) ppm. ^{13}C NMR: δ = 179.3 (C-5), 176.7 (C-4), 172.5 (C-8), 65.6 (C-9), 56.3 (C-3), 31.8 (C-2), 30.2 (C-6), 29.8 (C-7), 21.8 (C-10), 15.9 (C-1), 9.9 (C-11) ppm. ^{15}N NMR: δ = −54.1 ppm. ^{195}Pt NMR: δ = 3565 ppm. IR (ATR): 3245 br, 3210 br (ν_{N-H}); 2969 w; 1730 m, 1641 s ($\nu_{C=O}$); 1318 s, 1259 w, 1090 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis((4-(2-propyloxy))-4-oxobutanoato)platinum(IV) (3d). CDI (230 mg, 1.4184 mmol) in DMF (10 mL), **3** (411 mg, 0.6789 mmol) in DMF (9 mL), i-PrONa/i-PrOH (10 mL). The crude product was purified by column chromatography (EtOAc/IPA, 2 : 1), then isolated from an EtOAc suspension, washed with cold Et_2O and dried under vacuum to obtain a white powder. Yield: 36 mg, 8%. M_r = 689.57 g mol^{-1}. Elemental analysis, found: C 33.58, H 4.86, N 4.08. Calcd for $C_{20}H_{34}N_2O_{12}Pt{\cdot}H_2O$: C 33.95, H 5.13, N 3.96. ESI-MS: *m*/*z* 711.0 $[M + Na^+]^+$, 687.8 $[M - H^+]^-$. ^{1}H NMR: δ = 6.74 (m, 6H, NH_3), 4.91 (m, 2H, H-9), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.52 (m, 4H, H-6), 2.44 (m, 4H, H-7), 1.90 (m, 2H, H-1), 1.20 (d, $^3J_{H,H}$ = 6.3 Hz, 12H, H-10) ppm. ^{13}C NMR: δ = 179.3 (C-5), 176.7 (C-4), 171.9 (C-8), 67.4 (C-9), 56.3 (C-3), 31.8 (C-2), 30.3 (C-6), 30.1 (C-7), 21.3 (C-10), 15.9 (C-1) ppm. ^{15}N NMR: δ = −54.1 ppm. ^{195}Pt NMR: δ = 3565 ppm. IR (ATR): 3230 br (ν_{N-H}); 2980 w; 1728 m, 1654 s, 1630 s ($\nu_{C=O}$); 1339 s, 1255 w, 1205 w, 1172 m, 1105 s cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((4-butyloxy)-4-oxobutanoato)platinum(IV) (3e). CDI (224 mg, 1.3814 mmol) in DMF (9 mL), **3** (400 mg, 0.6607 mmol) in DMF (10 mL), *n*-ButONa/*n*-ButOH (10 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 9 : 1), then recrystallized from MeOH, and dried under vacuum to obtain a white powder. Yield: 110 mg, 11%. M_r = 717.62 g mol^{-1}. Elemental analysis, found: C 36.60, H 5.14, N 3.93. Calcd for $C_{22}H_{38}N_2O_{12}Pt$: C 36.82, H 5.34, N 3.90. ESI-MS: *m*/*z* 739.2 $[M + Na^+]^+$, 716.1 $[M - H^+]^-$. ^{1}H NMR: δ = 6.73 (m, 6H, NH_3), 4.04 (t, $^3J_{H,H}$ = 6.7 Hz, 4H, H-9), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.54 (m, 4H, H-6), 2.48 (m, 4H, H-7), 1.90 (m, 2H, H-1), 1.58 (m, 4H, H-10), 1.36 (m, 4H, H-11), 0.91 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-12) ppm. ^{13}C NMR: δ = 179.3 (C-5), 176.7 (C-4), 172.5 (C-8), 63.9 (C-9), 56.3 (C-3), 31.8 (C-2), 30.6 (C-10), 30.2 (C-6), 29.8 (C-7), 18.9 (C-11), 15.9 (C-1), 13.3 (C-12) ppm. ^{15}N NMR: δ = −54.1 ppm. ^{195}Pt NMR: δ = 3565 ppm. IR (ATR): 3305 br, 3236 br (ν_{N-H}); 2959 w; 1728 m, 1616 s, 1571 s ($\nu_{C=O}$); 1424 m, 1399 s, 1169 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((5-methoxy)-5-oxopentanoato)platinum(IV) (4a). CDI (196 mg, 1.209 mmol) in DMF (8 mL), **4** (360 mg, 0.568 mmol) in DMF (8 mL), MeONa/MeOH (10 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 3 : 1) and isolated *via* suspending in Et_2O, filtration, washing with Et_2O, EtOAc and diisopropylester and drying under vacuum to obtain a white powder. Yield: 94 mg, 16%. M_r = 661.51 g mol^{-1}. Elemental analysis, found: C 32.33, H 4.32, N 4.26. Calcd for $C_{18}H_{30}N_2O_{12}Pt$: C 32.68, H 4.57, N 4.23. ESI-MS: *m*/*z* 683.1 $[M + Na^+]^+$, 661.0 $[M + H^+]^+$, 659.9 $[M - H^+]^-$. ^{1}H NMR: δ = 6.76 (m, 6H, NH_3), 3.63 (s, 6H, H-9), 2.62 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.34 (t, $^3J_{H,H}$ = 7.5 Hz, 4H, H-7), 2.27 (t, $^3J_{H,H}$ = 7.4 Hz, 4H, H-6), 1.90 (m, 2H, H-1), 1.74 (m, 4H, H-7′) ppm. ^{13}C NMR: δ = 180.0 (C-5), 176.8 (C-4), 173.4 (C-8), 56.3 (C-3), 50.9 (C-9), 34.4 (C-6), 32.7 (C-7), 31.9 (C-2), 20.9 (C-7′), 16.0 (C-1) ppm. ^{15}N NMR: δ = −54.4 ppm. ^{195}Pt NMR: δ = 3555 ppm. IR (ATR): 3240 br, 3210 br (ν_{N-H}); 2952 w; 1733 m, 1639 s ($\nu_{C=O}$); 1436 w, 1355 s, 1241 m, 1202 m, 1148 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((5-ethoxy)-5-oxopentanoato)platinum(IV) (4b). CDI (112.1 mg, 0.6913 mmol) in DMF (7 mL), **4** (219 mg, 0.3457 mmol) in DMF (5 mL), EtONa/EtOH (8 mL). The crude product was purified by column chromatography (EtOAc/IPA, 2 : 1) and isolated *via* suspending in EtOAc, followed by recrystallization from MeOH/EtOAc and final washing with cold Et_2O. The white powder obtained was dried under vacuum. Yield: 42 mg, 18%. M_r = 689.57 g mol^{-1}. Elemental analysis, found: C 33.98, H 4.65, N 3.92. Calcd for $C_{20}H_{34}N_2O_{12}Pt{\cdot}0.5H_2O$: C 34.38, H 5.05, N 4.01. ESI-MS: *m*/*z* 712.0 $[M + Na^+]^+$, 690.0 $[M + H^+]^+$, 686.8 $[M - H^+]^-$. ^{1}H NMR: δ = 6.77 (m, 6H, NH_3), 4.09 (m, 4H, H-9), 2.63 (t, $^3J_{H,H}$ = 7.9 Hz, 4H, H-2), 2.33 (t, $^3J_{H,H}$ = 7.5 Hz, 4H, H-7), 2.28 (t, $^3J_{H,H}$ = 7.4 Hz, 4H, H-6), 1.90 (m, 2H, H-1), 1.74 (m, 4H, H-7′), 1.21 (t, $^3J_{H,H}$ = 7.1 Hz, 6H, H-10) ppm. ^{13}C NMR: δ = 180.1 (C-5), 176.9 (C-4), 173.0 (C-8), 59.9 (C-9), 56.3 (C-3), 34.4 (C-6), 33.0 (C-7), 31.8 (C-2), 21.0 (C-7′), 16.0 (C-1), 13.9 (C-10) ppm. ^{15}N NMR: δ = −55.2 ppm. ^{195}Pt NMR: δ = 3554 ppm. IR (ATR): 3198 br (ν_{N-H}); 2980 w; 1733 w, 1718 m, 1622 s ($\nu_{C=O}$); 1416 w, 1358 s, 1327 s, 1207 m, 1106 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((5-methoxy)-5-oxo-(3-methyl)pentanoato)platinum(IV) (5a). CDI (240 mg, 1.480 mmol) in DMF (10 mL), **5** (481 mg, 0.727 mmol) in DMF (10 mL), MeONa/MeOH (10 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 5 : 1) and isolated *via* suspending in EtOAc, filtration, washing with Et_2O and EtOAc and drying under vacuum to obtain a white powder. Yield: 192 mg, 38%. M_r = 689.57 g mol^{-1}. Elemental analysis, found: C 34.74, H 4.76, N 4.04. Calcd for $C_{20}H_{34}N_2O_{12}Pt$: C 34.84, H 4.97, N 4.06. ESI-MS: *m*/*z* 726.9 $[M + K^+]^+$, 711.1 $[M + Na^+]^+$, 689.9 $[M + H^+]^+$, 687.8 $[M - H^+]^-$, 723.9 $[M + Cl^-]^-$. ^{1}H NMR: δ = 6.78 (m, 6H, NH_3), 3.63 (s, 6H, H-9), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.44 (m, 2H, H-7), 2.29 (m, 2H, H-6), 2.27 (m, 2H, H-7′), 2.15 (m, 2H, H-6), 2.13 (m, 2H, H-7), 1.90 (m, 2H, H-1), 0.91 (d, $^3J_{H,H}$ = 6.5 Hz, 6H, H-7″) ppm. ^{13}C NMR: δ = 179.5 ($^2J_{C,Pt}$ = 28 Hz, C-5), 176.8 (C-4), 172.8 (C-8), 56.3 (C-3), 50.9 (C-9), 42.0 ($^3J_{C,Pt}$ = 36 Hz, C-6), 40.2 (C-7), 31.9 (C-2), 27.8 (C-7′), 19.3 (C-7″), 16.0 (C-1) ppm. ^{15}N NMR: δ = −54.6 ppm. ^{195}Pt NMR: δ = 3560 ppm. IR (ATR): 3239 br, 3094 br (ν_{N-H}); 2876 w; 1732 m, 1632 s ($\nu_{C=O}$); 1437 w, 1331 s, 1211 m, 1155 m, 1079 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((5-methoxy)-5-oxo-(3,3-dimethyl)pentanoato)platinum(IV) (6a). CDI (180 mg, 1.110 mmol) in DMF (6 mL), **6** (375 mg, 0.544 mmol) in DMF (10 mL), MeONa/MeOH (8 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 6 : 1) and isolated *via* suspending in EtOAc, filtration,

washing with Et_2O and drying under vacuum to obtain a white powder. Yield: 125 mg (32%). M_r = 717.62 g mol^{-1}. Elemental analysis, found: C 36.51, H 5.35, N 3.87. Calcd for $C_{22}H_{38}N_2O_{12}Pt$: C 36.82, H 5.34, N 3.90. ESI-MS: *m/z* 761.8 $[M + 2Na^+ - H^+]^+$, 739.9 $[M + Na^+]^+$, 715.9 $[M - H^+]^-$. ^{1}H NMR: δ = 6.79 (m, 6H, NH_3), 3.61 (s, 6H, H-9), 2.63 (t, $^3J_{H,H}$ = 7.9 Hz, 4H, H-2), 2.39 (s, 4H, H-7), 2.29 (s, 4H, H-6), 1.91 (m, 2H, H-1), 1.03 (s, 12H, H-7″) ppm. ^{13}C NMR: δ = 179.2 (C-5), 176.8 (C-4), 172.2 (C-8), 56.3 (C-3), 50.6 (C-9), 46.8 ($^3J_{C,Pt}$ = 36 Hz, C-6), 44.6 (C-7), 32.5 (C-7′), 31.9 (C-2), 26.9 (C-7″), 16.0 (C-1) ppm. ^{15}N NMR: δ = −55.8 ppm. ^{195}Pt NMR: δ = 3567 ppm. IR (ATR): 3230 br, 3097 br (ν_{N-H}); 2956 m, 2876 w; 1735 m, 1618 s, 1570 w ($\nu_{C=O}$); 1436 w, 1332 s, 1227 m, 1149 m, 1112 m cm^{-1}.

General procedure for synthesis of amide derivatives 3f–j, 4f, 5f, 6f

2 equiv. of CDI (1,1′-carbonyldiimidazole) in dry DMF were added to a DMF solution of **3**, **4**, **5** or **6**, respectively, and the mixture was heated at 60 °C for 10 min. After cooling to room temperature, CO_2 formed during the activation was removed by flushing the solution with argon for 10 min. Then, 2.3 equiv. of the corresponding amine, dissolved in dry DMF, were added and the solution was stirred for 24 h (up to 48 h in some cases). Finally, DMF was removed under reduced pressure and the crude products were purified by column chromatography.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((4-propylamino)-4-oxobutanoato)platinum(IV) (3f). CDI (218 mg, 1.3444 mmol) in DMF (8 mL), **3** (407 mg, 0.6723 mmol) in DMF (8 mL), propylamine (112 µl, 1.3567 mmol) in DMF (2 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 2 : 1) and isolated from an EtOAc suspension, followed by recrystallization from MeOH and washing with a cold mixture of EtOAc and Et_2O. An almost white to pale yellow powder was obtained and dried under vacuum. Yield: 124 mg, 24%. M_r = 687.20 g mol^{-1}. Elemental analysis, found: C 34.43, H 5.21, N 8.15. Calcd for $C_{20}H_{36}N_4O_{10}Pt{\cdot}0.5H_2O$: C 34.48, H 5.35, N 8.04. ESI-MS: *m/z* 709.9 $[M + Na^+]^+$, 685.9 $[M - H^+]^-$. ^{1}H NMR: δ = 7.78 (bs, 2H, CONH), 6.73 (m, 6H, NH_3), 3.09 (m, 4H, H-9), 2.62 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.48 (t, $^3J_{H,H}$ = 7.1 Hz, 4H, H-6), 2.35 (t, $^3J_{H,H}$ = 7.6 Hz, 4H, H-7), 1.89 (m, 2H, H-1), 1.46 (m, 4H, H-10), 0.87 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-11) ppm. ^{13}C NMR: δ = 180.1 (C-5), 176.8 (C-4), 171.5 (C-8), 56.3 (C-3), 40.8 (C-9), 31.8 (C-2), 31.6 (C-7), 31.2 (C-6), 22.7 (C-10), 16.0 (C-1), 11.0 (C-11) ppm. ^{15}N NMR: δ = −54.3 (NH_3), 94.1 (NH-amide) ppm. ^{195}Pt NMR: δ = 3562 ppm. IR (ATR): 3236 br, 3087 br (ν_{N-H}); 2963 w; 1625 s, 1551 m ($\nu_{C=O}$); 1333 s, 1249 m, 1207 m, 1158 w cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis((4-(2-methoxy)ethylamino)-4-oxobutanoato)platinum(IV) (3g). CDI (159 mg, 0.9806 mmol) in DMF (5 mL), **3** (283 mg, 0.4675 mmol) in DMF (5 mL), 2-methoxyethylamine (101 µl, 1.1726 mmol) in DMF (4 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 1 : 1). A yellow sticky substance was isolated and purified again by column chromatography, using EtOAc/MeOH = 3 : 1 as mobile phase. The final product was obtained as a white powder through suspension in EtOAc, filtration, washing with Et_2O and drying under vacuum. Yield: 72 mg, 21%. M_r = 719.60 g mol^{-1}. Elemental analysis, found: C 32.86, H 4.90, N 7.47. Calcd for $C_{20}H_{36}N_4O_{12}Pt{\cdot}0.5H_2O$: C 32.97, H 5.12, N 7.69. ESI-MS: *m/z* 741.9 $[M + Na^+]^+$, 720.0 $[M + H^+]^+$, 718.1 $[M - H^+]^-$. ^{1}H NMR: δ = 7.86 (bs, 2H, CONH), 6.72 (m, 6H, NH_3), 3.39 (t, $^3J_{H,H}$ = 5.9 Hz, 4H, H-10), 3.31 (t, $^3J_{H,H}$ = 5.5 Hz, 4H, H-9), 3.28 (s, 6H, H-11), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.49 (t, $^3J_{H,H}$ = 7.1 Hz, 4H, H-6), 2.38 (t, $^3J_{H,H}$ = 7.5 Hz, 4H, H-7), 1.89 (m, 2H, H-1) ppm. ^{13}C NMR: δ = 180.1 (C-5), 176.9 (C-4), 171.8 (C-8), 71.1 (C-10), 57.9 (C-11), 56.4 (C-3), 38.9 (C-9), 31.9 (C-2), 31.6 (C-6), 31.2 (C-7), 16.0 (C-1) ppm. ^{15}N NMR: δ = −53.8 (NH_3), 88.2 (NH-amide) ppm. ^{195}Pt NMR: δ = 3561 ppm. IR (ATR): 3531 br, 3224 br (ν_{N-H}); 2945 w; 1629 s, 1551 m ($\nu_{C=O}$); 1338 s, 1257 m, 1210 w, 1121 w cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis((4-cyclopentylamino)-4-oxobutanoato)platinum(IV) (3h). CDI (210 mg, 1.295 mmol) in DMF (8 mL), **3** (392 mg, 0.648 mmol) in DMF (8 mL), cyclopentylamine (0.14 ml, 1.417 mmol) in DMF (3 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 3 : 1). The yellow powder, obtained *via* suspension in EtOAc, was recrystallized from MeOH, precipitated with EtOAc, filtered off, washed with Et_2O and dried under vacuum. A pale yellow to almost white powder was obtained. Yield: 184 mg, 38%. M_r = 739.67 g mol^{-1}. Elemental analysis, found: C 37.71, H 5.19, N 7.40. Calcd for $C_{24}H_{40}N_4O_{10}Pt{\cdot}H_2O$: C 38.04, H 5.59, N 7.39. ESI-MS: *m/z* 761.9 $[M + Na^+]^+$, 739.0 $[M + H^+]^+$, 738.1 $[M - H^+]^-$, 774.7 $[M + Cl^-]^-$. ^{1}H NMR: δ = 7.77 (d, $^3J_{H,H}$ = 6.7 Hz, 2H, CONH), 6.73 (m, 6H, NH_3), 4.07 (m, 2H, H-9), 2.62 (t, $^3J_{H,H}$ = 7.9 Hz, 4H, H-2), 2.46 (t, $^3J_{H,H}$ = 7.2 Hz, 4H, H-6), 2.32 (t, $^3J_{H,H}$ = 7.4 Hz, 4H, H-7), 1.89 (m, 2H, H-1), 1.82 (m, 4H, H-10), 1.65 (m, 4H, H-11), 1.52 (m, 4H, H-11), 1.43 (m, 4H, H-10) ppm. ^{13}C NMR: δ = 180.2 (C-5), 176.8 (C-4), 171.1 (C-8), 56.3 (C-3), 50.8 (C-9), 32.5 (C-10), 31.8 (C-2), 31.6 (C-7), 31.3 (C-6), 23.6 (C-11), 16.0 (C-1) ppm. ^{15}N NMR: δ = −54.3 (NH_3), 105.8 (NH-amide) ppm. ^{195}Pt NMR: δ = 3562 ppm. IR (ATR): 3260 br, 3086 br (ν_{N-H}); 2952 m, 2870 w; 1622 s, 1544 m ($\nu_{C=O}$); 1332 s, 1252 m, 1207 m, 1097 w cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((4-cyclohexylamino)-4-oxobutanoato)platinum(IV) (3i). CDI (190.7 mg, 1.176 mmol) in DMF (6 mL), **3** (356 mg, 0.588 mmol) in DMF (8 mL), cyclohexylamine (0.15 ml, 1.311 mmol) in DMF (2 mL). The crude product was purified by column chromatography (EtOAc/IPA, 2 : 1), then isolated *via* suspension in EtOAc, filtration, washing with EtOAc and Et_2O and drying under vacuum to obtain a white powder. Yield: 162 mg, 36%. M_r = 767.7 g mol^{-1}. Elemental analysis, found: C 40.29, H 5.50, N 7.22. Calcd for $C_{26}H_{44}N_4O_{10}Pt$: C 40.68, H 5.78, N 7.30. ESI-MS: *m/z* 789.1 $[M + Na^+]^+$, 768.0 $[M + H^+]^+$, 766.2 $[M - H^+]^-$. ^{1}H NMR: δ = 7.66 (d, $^3J_{H,H}$ = 7.6 Hz, 2H, CONH), 6.73 (m, 6H, NH_3), 3.59 (m, 2H, H-9), 2.62 (t, $^3J_{H,H}$ = 7.9 Hz, 4H, H-2), 2.47 (t, $^3J_{H,H}$ = 7.7 Hz, 4H, H-6), 2.33 (t, $^3J_{H,H}$ = 7.2 Hz, 4H, H-7), 1.89 (m, 2H, H-1), 1.79 (m, 4H, H-10), 1.69 (m, 4H, H-11), 1.56 (m, 2H, H-12), 1.27 (m, 4H, H-11), 1.18 (m, 4H, H-10), 1.16 (m, 2H, H-12) ppm. ^{13}C NMR:

δ = 180.1 (C-5), 176.8 (C-4), 170.6 (C-8), 56.3 (C-3), 47.9 (C-9), 32.7 (C-10), 31.8 (C-2), 31.7 (C-7), 31.3 (C-6), 25.6 (C-12), 24.9 (C-11), 16.0 (C-1) ppm. ^{15}N NMR: δ = −54.3 (NH_3), 108.1 (NH-amide) ppm. ^{195}Pt NMR: δ = 3562 ppm. IR (ATR): 3319 br, 3272 br (ν_{N-H}); 2929 m, 2854 w; 1658 s, 1639 s, 1538 m ($\nu_{C=O}$); 1442 w, 1330 s, 1256 w, 1209 w cm^{-1}.

(OC-6-33)-Diamminebis((4-benzylamino)-4-oxobutanoato)-(cyclobutane-1,1-dicarboxylato)platinum(IV) (3j). CDI (175 mg, 1.079 mmol) in DMF (7 mL), **3** (322 mg, 0.532 mmol) in DMF (8 mL), benzylamine (145 µl, 1.329 mmol) in DMF (5 mL). The crude product was purified by column chromatography (EtOAc/MeOH, 4 : 1) and then isolated *via* suspending in EtOAc. The obtained substance was recrystallized from MeOH, and precipitated with the help of acetone and ethyl acetate, filtered off, washed with diisopropyl ether and dried under vacuum to obtain a white powder. Yield: 140 mg, 34%. M_r = 783.7 g mol^{-1}. Elemental analysis, found: C 42.60, H 4.67, N 6.80. Calcd for $C_{28}H_{36}N_4O_{10}Pt$: C 42.91, H 4.63, N 7.15. ESI-MS: *m*/*z* 805.9 [M + Na^+]$^+$, 783.0 [M + H^+]$^+$, 781.9 [M − H^+]$^-$, 818.6 [M + Cl^-]$^-$. ^{1}H NMR: δ = 8.28 (t, $^3J_{H,H}$ = 5.5 Hz, 2H, CONH), 7.34 (m, 4H, H-12), 7.31 (m, 4H, H-11), 7.25 (m, 2H, H-13), 6.75 (m, 6H, NH_3), 4.38 (d, $^3J_{H,H}$ = 5.9 Hz, 4H, H-9), 2.64 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.54 (t, $^3J_{H,H}$ = 7.1 Hz, 4H, H-6), 2.44 (t, $^3J_{H,H}$ = 7.3 Hz, 4H, H-7), 1.89 (m, 2H, H-1) ppm. ^{13}C NMR: δ = 180.0 (C-5), 176.8 (C-4), 171.7 (C-8), 140.0 (C-10), 128.3 (C-12) 127.4 (C-11), 126.8 (C-13), 56.3 (C-3), 42.6 (C-9), 31.8 (C-2), 31.6 (C-7), 31.1 (C-6), 16.0 (C-1) ppm. ^{15}N NMR: δ = −54.5 (NH_3), 93.6 (NH-amide) ppm. ^{195}Pt NMR: δ = 3562 ppm. IR (ATR): 3258 br, 3076 br (ν_{N-H}); 2954 w; 1626 s, 1545 m ($\nu_{C=O}$); 1420 w, 1339 s, 1245 m, 1211 w cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-((5-propylamino)-5-oxopentanoato)platinum(IV) (4f). CDI (164 mg, 1.011 mmol) in DMF (8 mL), **4** (289 mg, 0.456 mmol) in DMF (5 mL), propylamine (85 µl, 1.021 mmol) in DMF (3 mL). The crude product was purified twice by column chromatography (EtOAc/MeOH, 1 : 1, followed by EtOAc/MeOH, 3 : 1), then isolated from an EtOAc suspension and washed with a cold mixture of EtOAc and Et_2O. The white powder obtained was dried under vacuum. Yield: 60 mg, 18%. M_r = 715.65 g mol^{-1}. Elemental analysis, found: C 36.58, H 5.34, N 7.58. Calcd for $C_{22}H_{40}N_4O_{10}Pt$: C 36.92, H 5.63, N 7.83. ESI-MS: *m*/*z* 737.1 [M + Na^+]$^+$, 716.1 [M + H^+]$^+$, 715.4 [M − H^+]$^-$. ^{1}H NMR: δ = 7.65 (bs, 2H, CONH), 6.78 (m, 6H, NH_3), 3.09 (m, 4H, H-9), 2.62 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.23 (t, $^3J_{H,H}$ = 7.5 Hz, 4H, H-6), 2.14 (t, $^3J_{H,H}$ = 7.4 Hz, 4H, H-7), 1.89 (m, 2H, H-1), 1.74 (m, 4H, H-7′), 1.46 (m, 4H, H-10), 0.87 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-11) ppm. ^{13}C NMR: δ = 180.3 (C-5), 176.9 (C-4), 172.0 (C-8), 56.4 (C-3), 40.7 (C-9), 35.1 (C-7), 35.0 (C-6), 31.8 (C-2), 22.8 (C-10), 22.0 (C-7′), 16.0 (C-1), 11.1 (C-11) ppm. ^{15}N NMR: δ = −54.4 (NH_3), 93.6 (NH-amide) ppm. ^{195}Pt NMR: δ = 3555 ppm. IR (ATR): 3250 br, 3088 br (ν_{N-H}); 2960 w; 1637 s, 1629 s, 1560 m ($\nu_{C=O}$); 1458 w, 1336 s, 1233 m cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-(3-methyl-(5-propylamino)-5-oxopentanoato)platinum(IV) (5f). CDI (222 mg, 1.3691 mmol) in DMF (8 mL), **5** (453 mg, 0.6848 mmol) in DMF (10 mL), propylamine (0.15 ml, 1.8170 mmol) in DMF (3 mL). The crude product was purified by column chromatography (EtOAc/MeOH/IPA, 3 : 1 : 1, followed by EtOAc/MeOH, 3 : 1 for the second column), then isolated from an EtOAc suspension and washed with a cold mixture of EtOAc and Et_2O. The white powder obtained was dried under vacuum. Yield: 252 mg, 49%. M_r = 743.70 g mol^{-1}. Elemental analysis, found: C 38.10, H 5.78, N 7.51. Calcd for $C_{24}H_{44}N_4O_{10}Pt{\cdot}0.5(H_2O)$: C 38.30, H 6.03, N 7.44. ESI-MS: *m*/*z* 782.0 [M + K^+]$^+$, 766.0 [M + Na^+]$^+$, 743.1 [M + H^+]$^+$, 742.2 [M − H^+]$^-$. ^{1}H NMR: δ = 7.68 (bs, 2H, CONH), 6.81 (m, 6H, NH_3), 3.10 (m, 4H, H-9), 2.63 (t, $^3J_{H,H}$ = 7.9 Hz, 4H, H-2), 2.29 (m, 2H, H-7′), 2.25 (m, 2H, H-6), 2.17 (m, 2H, H-7), 2.10 (m, 2H, H-6), 2.00 (m, 2H, H-7), 1.90 (m, 2H, H-1), 1.47 (m, 4H, H-10), 0.90 (d, $^3J_{H,H}$ = 4.7 Hz, 6H, H-7″), 0.88 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-11) ppm. ^{13}C NMR: δ = 180.0 (C-5), 177.0 (C-4), 171.5 (C-8), 56.4 (C-3), 42.9 (C-7), 42.7 (C-6), 40.7 (C-9), 32.0 (C-2), 28.6 (C-7′), 22.8 (C-10), 19.6 (C-7″), 16.0 (C-1), 11.2 (C-11) ppm. ^{15}N NMR: δ = −54.3 (NH_3), 96.7 (NH-amide) ppm. ^{195}Pt NMR: δ = 3560 ppm. IR (ATR): 3259 br, 3084 br (ν_{N-H}); 2962 m, 2875 w; 1624 s, 1551 m($\nu_{C=O}$); 1458 w, 1344 m, 1304 m, 1221 w, 1098 w cm^{-1}.

(OC-6-33)-Diammine(cyclobutane-1,1-dicarboxylato)bis-(3,3-dimethyl-(5-propylamino)-5-oxopentanoato)platinum(IV) (6f). CDI (152 mg, 0.9343 mmol) in DMF (10 mL), **6** (263 mg, 0.3810 mmol) in DMF (8 mL), propylamine (0.1 ml, 1.2112 mmol) in DMF (2 mL). The crude product was purified by column chromatography (EtOAc/MeOH/IPA, 4 : 1 : 1), then isolated from an EtOAc suspension and recrystallized from MeOH, suspended in EtOAc/Et_2O mixture, filtered off, washed with Et_2O and dried under vacuum to obtain a white powder. Yield: 98 mg, 33%. M_r = 771.76 g mol^{-1}. Elemental analysis, found: C 40.08, H 5.99, N 7.41. Calcd for $C_{26}H_{48}N_4O_{10}Pt$: C 40.46, H 6.27, N 7.26. ESI-MS: *m*/*z* 810.0 [M + K^+]$^+$, 794.0 [M + Na^+]$^+$, 771.2 [M + H^+]$^+$, 770.0 [M − H^+]$^-$, 806.9 [M + Cl^-]$^-$. ^{1}H NMR: δ = 7.60 (t, $^3J_{H,H}$ = 5.5 Hz, 2H, CONH), 6.83 (m, 6H, NH_3), 3.09 (m, 4H, H-9), 2.63 (t, $^3J_{H,H}$ = 8.0 Hz, 4H, H-2), 2.28 (s, 4H, H-6), 2.16 (s, 4H, H-7), 1.90 (m, 2H, H-1), 1.46 (m, 4H, H-10), 1.03 (s, 12H, C-7″), 0.88 (t, $^3J_{H,H}$ = 7.4 Hz, 6H, H-11) ppm. ^{13}C NMR: δ = 179.7 (C-5), 176.9 (C-4), 170.9 (C-8), 56.3 (C-3), 47.3 (C-6), 47.2 (C-7), 40.6 (C-9), 32.9 (C-7′), 31.9 (C-2), 27.5 (C-7″), 22.8 (C-10), 16.0 (C-1), 11.2 (C-11) ppm. ^{15}N NMR: δ = −55.5 (NH_3), 98.7 (NH-amide) ppm. ^{195}Pt NMR: δ = 3568 ppm. IR (ATR): 3266 br, 3075 br (ν_{N-H}); 2964 m, 2933 m; 1626 s, 1560 m ($\nu_{C=O}$); 1472 w, 1345 m, 1333 m, 1241 w cm^{-1}.

Determination of lipophilicity

Lipophilicity of the new compounds was determined as chromatographic retention factors (namely log k_{30} and log k_w) using reversed-phased HPLC.

The analysis was performed on a Dionex Summit system controlled by the Dionex Chromeleon 6.60 software. The samples were prepared by dissolving (with the help of ultrasonic) around 1.5 mg of each complex in a H_2O/MeOH mixture, followed by filtration through a 0.2 µm Nylon filter. The chromatographic conditions were as follows: an Agilent ZORBAX Bonus-RP column (4.6 mm × 250 mm); injection volume: 25 µl; flow rate: 1 ml min^{-1}; isocratic elution; temperature of the column: 25 °C;

UV-vis detection set up at 210 nm; uracil was used as an internal reference to determine the column dead-time (t_0); mobile phases containing different percentages of 0.1% TFA water solution and MeOH (the MeOH fraction ranged from 60% for the most lipophilic compounds to 10% for the most hydrophilic compounds); chromatograms for each complex were run with at least three different mobile phase compositions and at least two times.

The capacity factors $k = (t_R - t_0)/t_0$ (t_R is the retention time of the species analyzed and t_0 is the retention time of uracil) of the investigated compounds were calculated for all eluant compositions. The partition between the lipophilic stationary phase and water (0% MeOH) was determined by extrapolation, using the linear Soczewinski–Snyder relationship[43,44] between log k and the concentration of the organic modifier in the mobile phase:

$$\log k = \log k_w - S\varphi,$$

where log k is the capacity factor in the specific mobile phase composition, φ is the volume fraction of MeOH in the eluant, S is a constant for a given solute and a given HPLC system and log k_w corresponds to log k in pure water (buffer).

Electrochemical experiments

Cyclic voltammograms were measured in a three-electrode cell using a 2.0 mm diameter glassy carbon disc working electrode, a platinum auxiliary electrode, and a Ag/Ag^+ reference electrode containing 0.10 M $AgNO_3$, the potential of which was corrected using an internal standard of ferrocenium/ferrocene. Measurements were performed at room temperature using an EG & G PARC 273A potentiostat/galvanostat. Deaeration of solutions was accomplished by passing a stream of argon through the solution for 5 min prior to the measurement and then maintaining a blanket atmosphere of argon over the solution during the measurement. The potentials were measured at a scan rate of 100 mV s^{-1} in 0.15 M [n-Bu_4N][BF_4]/DMF, using [Fe(η^5-C_5H_5)$_2$] ($E_{\frac{1}{2}}$ = +0.72 V *vs.* NHE)[45] as the internal standard, and are quoted relative to the normal hydrogen electrode (NHE).

Incubation with ascorbic acid

Reduction of complex **3f** and its close analogue featuring ethane-1,2-diamine and two chlorido ligands in the equatorial position (**M1**) by ascorbic acid was monitored by ^{1}H NMR spectroscopy at ambient temperature. 1 mM solutions of the compounds were prepared in 50 mM phosphate buffer (in D_2O, pD = 7.4) and ^{1}H NMR spectra were measured from time to time over a period of 24 h in order to judge the stability of the complexes; changes in the NMR spectra could not be seen. Then, ascorbic acid (25 mM) was added and ^{1}H NMR spectra were recorded for 24 h in the case of **M1** and during three weeks for complex **3f**. The reduction was monitored by following the decrease of intensity of the CH_2 signal of the ethane-1,2-diamine ligand of **M1** resonating at 2.8 ppm. In the case of complex **3f**, the increase of intensity of CH_2 protons H2 at 2.8 ppm deriving from the cyclobutanedicarboxylato ligand of the reduced platinum(II) species was monitored. In both cases, integration of the signals was performed relative to the signals of the terminal methyl groups of the propylamine moieties.

Cell lines and culture conditions

CH1 (ovarian carcinoma, human) cells were a gift from Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.). A549 (non-small cell lung cancer, human) and SW480 (colon carcinoma, human) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria), and SK-OV-3 (ovarian carcinoma, human) cells by Evelyn Dittrich (General Hospital, Medical University of Vienna, Austria). Cells were grown in 75 cm^2 culture flasks (Iwaki/Asahi Technoglass) as adherent monolayer cultures in Minimal Essential Medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine and 1% non-essential amino acids (from 100× ready-to-use stock) (all purchased from Sigma-Aldrich) without antibiotics. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

Cytotoxicity tests in cancer cell lines

Cytotoxicity in the cell lines mentioned above was determined by the colorimetric MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, purchased from Fluka). Cells were harvested from culture flasks by trypsinization and seeded in 100 μL aliquots in MEM (see above) into 96-well microculture plates (Iwaki/Asahi Technoglass) in the following densities to ensure exponential growth of untreated controls throughout the experiment: 1.5×10^3 (CH1), 3.5×10^3 (SK-OV-3), 4.0×10^3 (A549), and 2.5×10^3 (SW480) viable cells per well. Cells were allowed to settle and resume exponential growth in a drug-free medium for 24 h, followed by the addition of dilutions of the test compounds in 100 μL per well of the same medium. Only for the less water soluble compound **3e**, a stock solution in DMSO/water was prepared (the DMSO content in the cytotoxicity assay did not exceed 0.5%). After continuous exposure for 96 h, the medium was replaced by a 100 μL per well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus a 20 μL per well solution of MTT in phosphate-buffered saline (5 mg mL^{-1}) (all purchased from Sigma-Aldrich). After incubation for 4 h, medium/MTT mixtures were removed, and the formazan product formed by viable cells was dissolved in DMSO (150 μL per well). Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed as a percentage of untreated controls, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration–effect curves by interpolation. Evaluation is based on means from three independent experiments, each comprising six replicates per concentration level.

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Electronic Supporting Information

Novel tetracarboxylatoplatinum(IV) complexes as carboplatin prodrugs

Hristo P. Varbanov, Seied M. Valiahdi, Christian R. Kowol, Michael A. Jakupec, Markus Galanski*, and Bernhard K. Keppler*

University of Vienna, Institute of Inorganic Chemistry, Waehringer Strasse 42, A-1090 Vienna, Austria

Contents

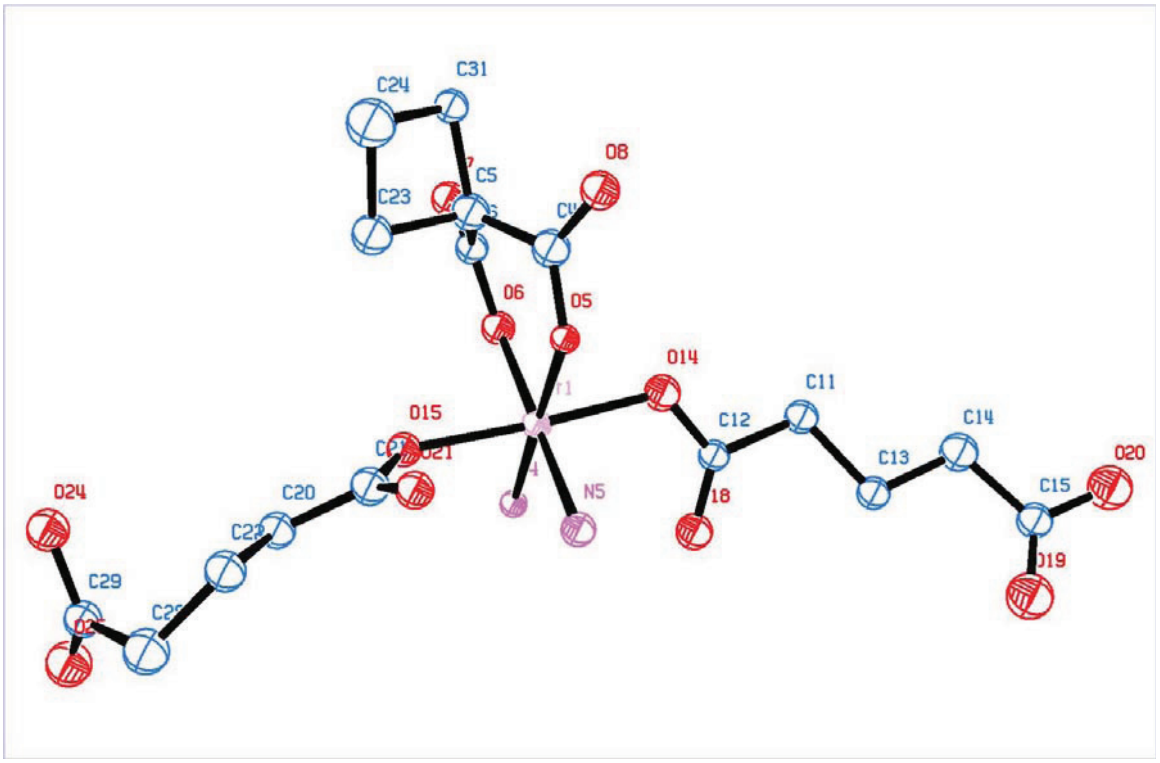

Fig. S1. ORTEP diagram of **4** displaying thermal ellipsoids at 55% probability level.

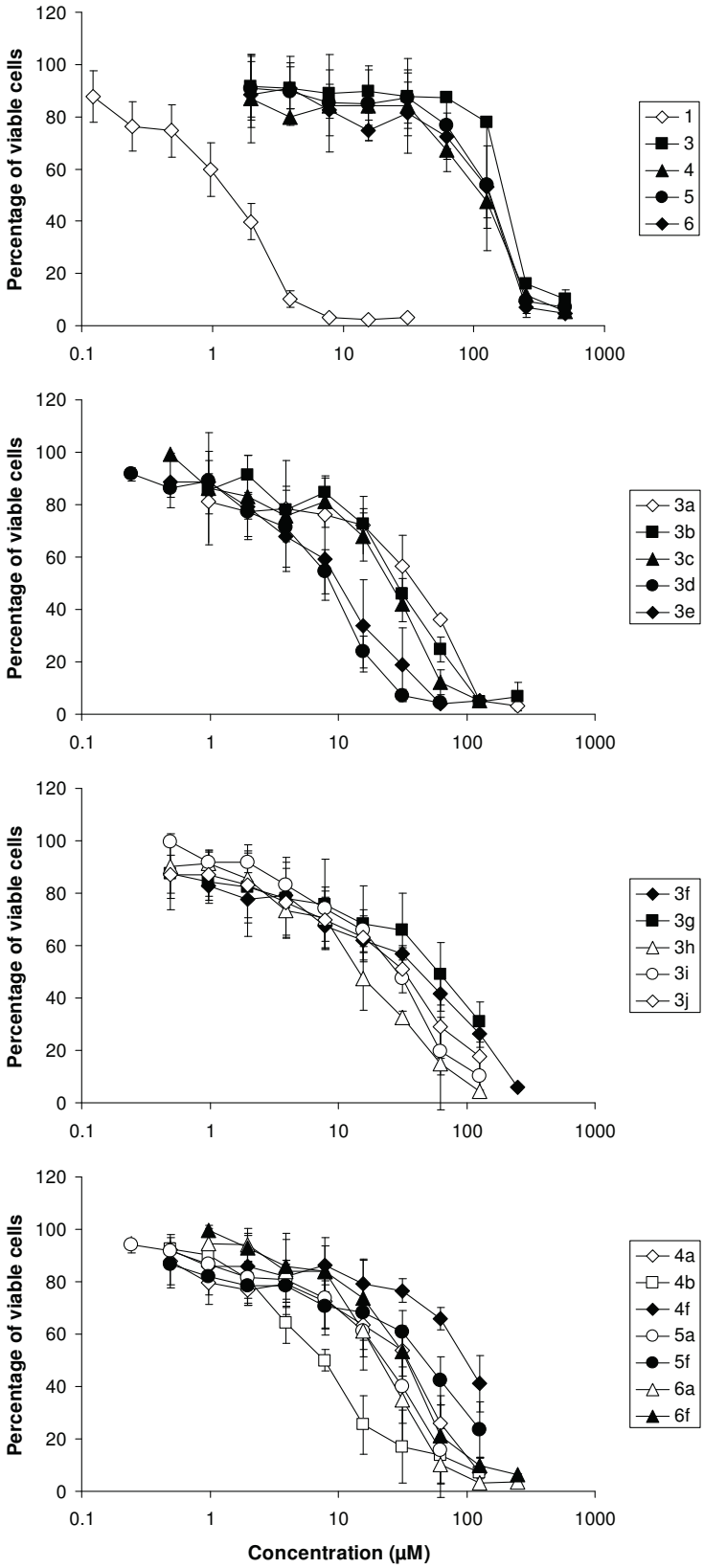

Fig. S2. Concentration–effect curves (means ± standard deviations) of investigated compounds in CH1 cells (MTT assay, exposure time 96 h).

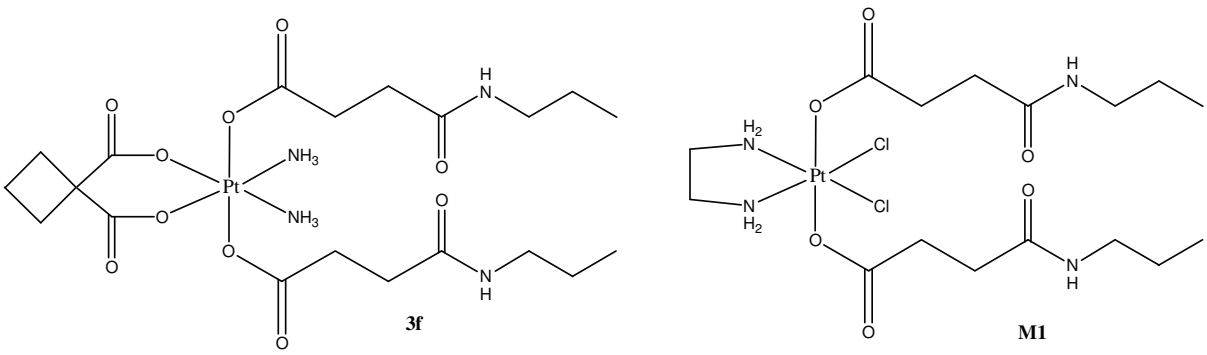

Fig. S3. Chemical structures of complexes **3f** and **M1**

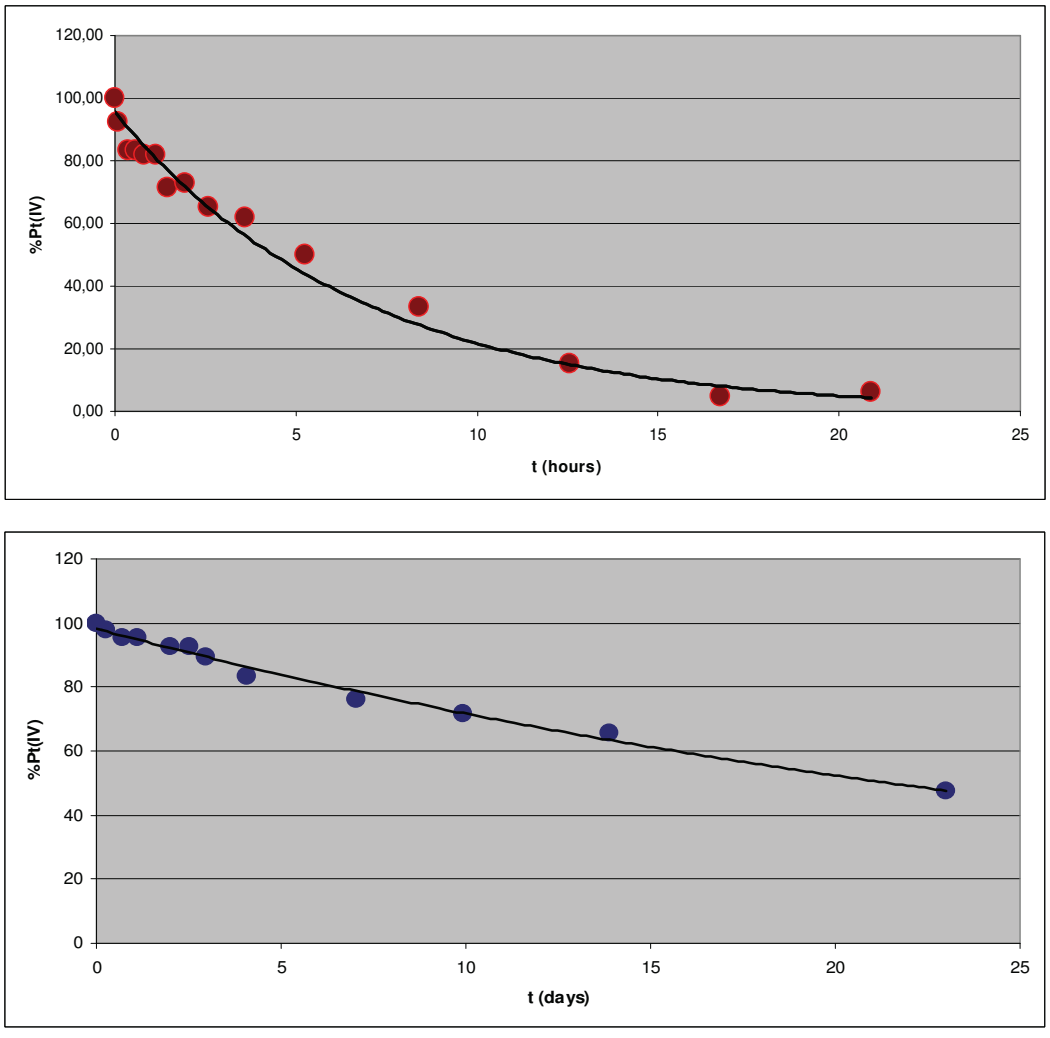

Fig. S4. Time dependent reduction of **M1** (top) and **3f** (bottom) in the presence of ascorbic acid; ambient temperature, pD =7.4, 1 mM complex, 50 mM phosphate buffer, 25 mM ascorbic acid.

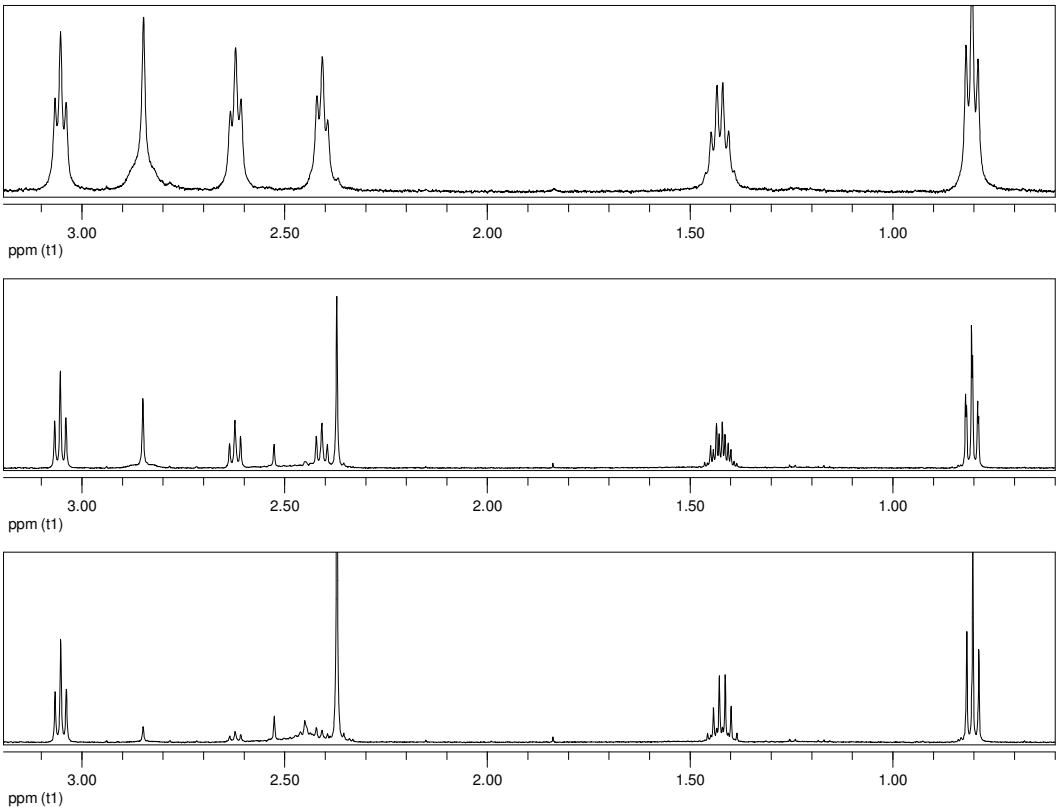

Fig. S5. ^{1}H NMR spectra of complex **M1** after addition of ascorbic acid (top – immediately, middle – after 5 hours, bottom – after 17 h); ambient temperature, pD =7.4, 1 mM complex, 50 mM phosphate buffer, 25 mM ascorbic acid.

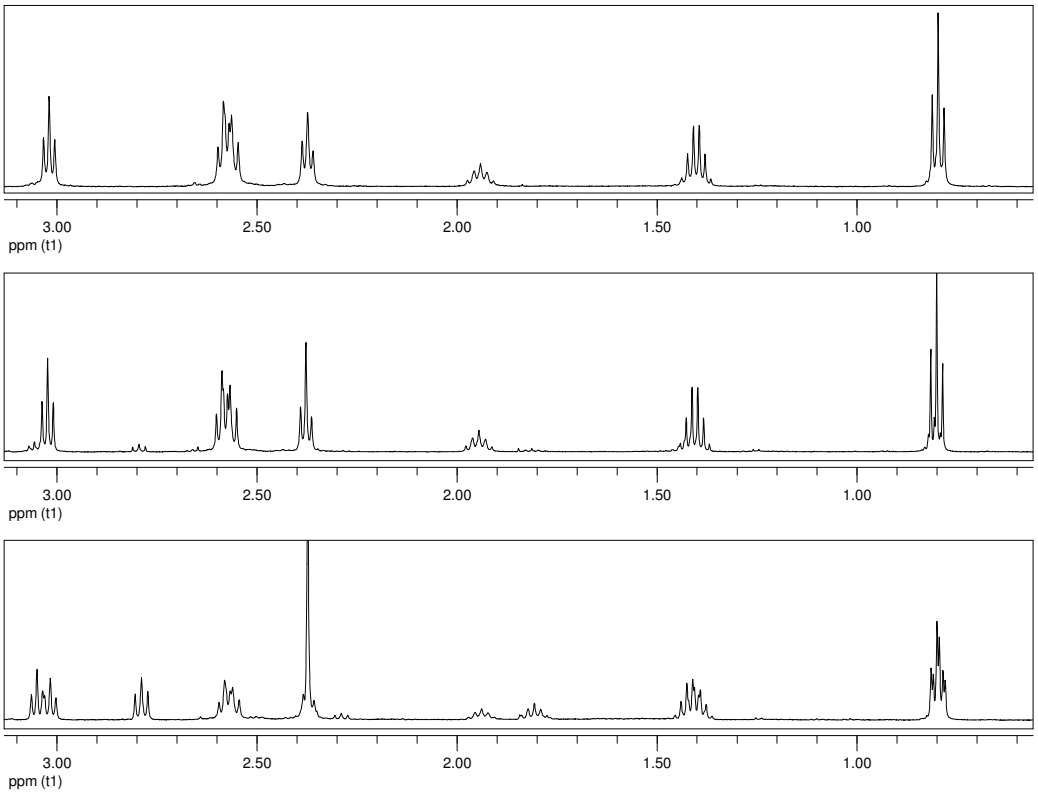

Fig. S6. ^{1}H NMR spectra of complex **3f** after addition of ascorbic acid (top – immediately, middle – after 3 days, bottom – after 23 days); ambient temperature, pD =7.4, 1 mM complex, 50 mM phosphate buffer, 25 mM ascorbic acid

Table S1. Comparison of redox potentials, halve life times of reduction by ascorbic acid and cytotoxicity for complexes **M1** and **3f.**

compound	Ep (V)	$t_{1/2}$	IC_{50} (CH1), µM	IC_{50} (SW480), µM
M1	**-0.60**	**5 h**	2.3±1.1[a]	31±15[a]
3f	**-0.68**	**21 d**	44 ± 8	>500

[a] data, taken from ref. [1]

[1] M.R. Reithofer, S.M. Valiahdi, M.A. Jakupec, V.B. Arion, A. Egger, M. Galanski, B.K. Keppler, *J. Med. Chem.*, 2007, **50**, 6692–6699.

3. Theoretical Investigations and Density Functional Theory Based Quantitative Structure–Activity Relationships Model for Novel Cytotoxic Platinum(IV) Complexes.

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Theoretical Investigations and Density Functional Theory Based Quantitative Structure−Activity Relationships Model for Novel Cytotoxic Platinum(IV) Complexes

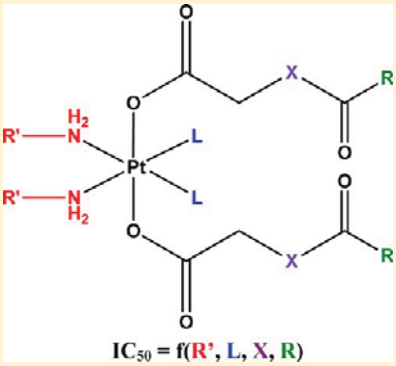
Hristo P. Varbanov,[†] Michael A. Jakupec,[†] Alexander Roller,[†] Frank Jensen,*[,‡] Markus Galanski,*[,†] and Bernhard K. Keppler[†]

[†]Institute of Inorganic Chemistry, University of Vienna, Währinger Strasse 42, A-1090 Vienna, Austria

[‡]Department of Chemistry, University of Aarhus, Langelandgade 140, 8000 Aarhus C, Denmark

Supporting Information

ABSTRACT: Octahedral platinum(IV) complexes are promising candidates in the fight against cancer. In order to rationalize the further development of this class of compounds, detailed studies on their mechanisms of action, toxicity, and resistance must be provided and structure−activity relationships must be drawn. Herein, we report on theoretical and QSAR investigations of a series of 53 novel bis-, tris-, and tetrakis-(carboxylato)platinum(IV) complexes, synthesized and tested for cytotoxicity in our laboratories. The hybrid DFT functional wb97x was used for optimization of the structure geometry and calculation of the descriptors. Reliable and robust QSAR models with good explanatory and predictive properties were obtained for both the cisplatin sensitive cell line CH1 and the intrinsically cisplatin resistant cell line SW480, with a set of four descriptors.



INTRODUCTION

Platinum complexes are among leading drugs in anticancer chemotherapy. Since the discovery of the cytotoxic effect of cisplatin and its Food and Drug Administration (FDA) approval in 1978, seven other Pt(II) compounds were introduced in clinics worldwide (carboplatin and oxaliplatin) or in selected countries (nedaplatin, lobaplatin, heptaplatin, miriplatin, and dicycloplatin).[1−3] Approximately 30 more Pt(II) and Pt(IV) complexes have been or are in clinical trials at different stages.[1] Despite the great medical success of platinum-based cytostatics, there are some major drawbacks that restrict their usage, mainly severe dose-limiting side effects, intrinsic or/and acquired resistance, and the uncomfortable and cost intensive way of administration (iv infusion). Thousands of metal compounds have been synthesized and investigated during the past decades with the aim of breaking these limitations. Nevertheless, in order to design a metal-based drug with improved pharmacological profile, details of the mechanism of action, toxicity, and resistance have to be studied[4] and structure−activity relationships have to be drawn. It is generally accepted that square planar platinum(II) complexes are acting like prodrugs, containing two carrier ligands and two leaving groups. The two leaving groups are exchanged in the cell, forming reactive aqua species capable of forming DNA adducts responsible for the cytotoxic effects of the compounds (Figure 1). Octahedral Pt(IV) complexes also possess antimalignant properties and can act as prodrugs for Pt(II) agents (reduction in vivo to the corresponding Pt(II) counterparts).

The first comprehensive SAR study of cytotoxic metal complexes was reported by Cleare and Hoeschele in 1973, where a wide variety of Pt(II) compounds was investigated for its antitumor activity in a sarcoma 180 mouse model.[5] Results from variation of carrier ligands, leaving groups, geometry, and charge and some physicochemical parameters like solubility and kinetics of hydrolysis affecting the antimalignant properties of cisplatin analogues were studied. The authors found that the cis geometry and neutral charge of the complexes, chloride or dicarboxylates as leaving groups, and primary amines as carrier ligands are crucial for the biological activity within the series studied. Today, different compound classes are known, violating the classical SAR set up by Cleare and Hoeschele, as for example complexes with trans geometry featuring high cytotoxicity.[6] Theoretical study attempts and a quantitative structure−activity relationship (QSAR) model for the anticancer activity of 26 Pt(II) complexes in vivo in mice models was reported in 1982.[7] Nevertheless, QSAR analysis results based on in vitro cytotoxicity of Pt(II) compounds in different cell lines were first published 23 years later.[8] Reliable models with good predictive strength, based on four molecular descriptors (chosen from 197), were obtained for a series of 16 Pt(II) complexes, including the clinically established drugs cisplatin, carboplatin, and oxaliplatin. The results confirmed the structure−activity relationships (SAR) reported by Cleare and Hoechele. Later, Sarmah and Deka reported QSAR and quantitative structure−properties relationship (QSPR) models for several platinum complexes, using density functional theory (DFT) and MM derived descriptors.[9] The authors showed that DFT and molecular

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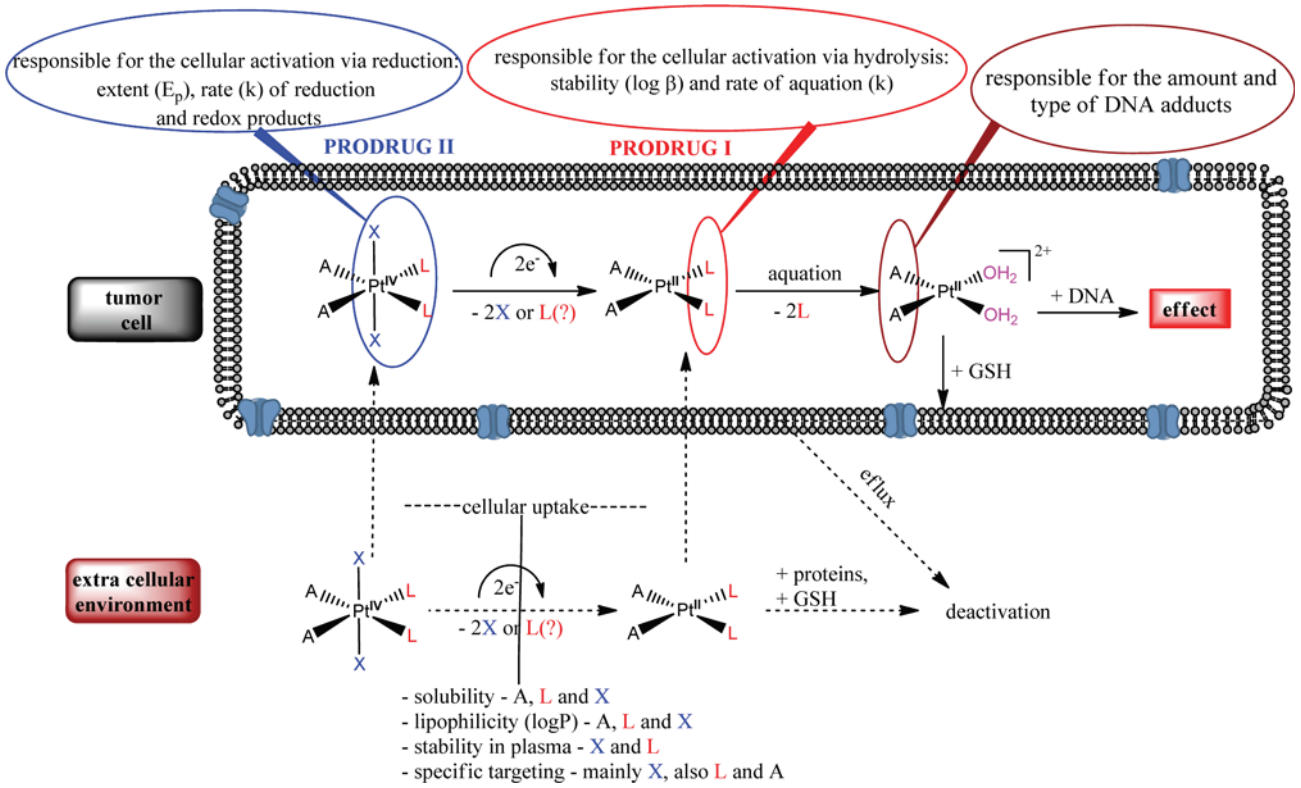

Figure 1. Scheme of the mechanism of action of platinum-based cytostatics.

mechanics (MM+) methods could be used successfully in the prediction of lipophilicity and cytotoxicity of platinum compounds. Furthermore, the usage of solvent models for calculation of the descriptors gave better results than those obtained in the gas phase.

As mentioned above, Pt(IV) complexes act as prodrugs of their Pt(II) counterparts and represent an important part of recent metal-based anticancer research. Their geometries and physicochemical features (octahedral coordination sphere with a maximum of six ligands, kinetic inertness in ligand-exchange reactions, reduction under hypoxic conditions, etc.) present advantages in fine-tuning of the pharmacological profile, providing the possibility for oral administration, targeted therapy, reduced side effects, etc.[10] As summarized in Figure 1, there are more parameters (in comparison with platinum(II) complexes), which should be taken into account when designing a Pt(IV) based drug. Some SARs based on a small set of Pt(IV) complexes have been established during the past decade.[11] It was shown that cytotoxicity of the compounds is dependent on their redox potential and lipophilicity and that these parameters have optimal values when the axial ligands are carboxylates.[12] However, it was found recently that redox potential does not always correlate with the rate of reduction and that the equatorial ligands can also play a crucial role.[13,14] Moreover, reduction of Pt(IV) complexes is not always accompanied by release of the axial ligands; in some rare cases a more complicated picture can be observed.[15,16]

cis-Diam(m)inebis(carboxylato)dichloridoplatinum(IV) and *cis*-diam(m)inetetrakis(carboxylato)platinum(IV) complexes with cytotoxicity ranging from low nanomolar to high micromolar IC_{50} values have recently been reported from our group (see Figure 2).[14,17−20] It was found that cytotoxicity in general increases with increasing lipophilicity of the axial ligands, but this effect is much more pronounced in the diam(m)inedichloridobis(carboxylato) complex series; moreover, compounds featuring amide moieties in the axial ligands are less effective than expected from their log *P* values.[20,21] The tetracarboxylato complexes have shown in principle a lower cytotoxic potency and a different redox kinetic behavior.[14] In order to find quantitative explanations of the phenomena and to rationalize the further development of antimalignant Pt(IV) complexes, we enlarged the series by including three diamminetris(carboxylato)platinum(IV) complexes, prodrugs of nedaplatin, and performed a QSAR study based on DFT calculated and constitutional molecular descriptors. Moreover, with the help of the calculations, we tried to better understand the redox behavior of the complexes in the series to explain the experimental data and to group them in subseries.

Up to now, there is only one report of a QSAR study for Pt(IV) complexes;[22] models based on the cytotoxicity of 23 compounds in two tumor cell lines were developed, using experimentally determined (log $P_{o/w}$ and E_p) and theoretical descriptors. Later, the authors suggested QSPR models able to predict the lipophilicity and the redox potential of Pt(IV) complexes, using (a slightly broadened) series from the QSAR study. The semiempirical method PM6 was used for optimization of the structures and calculation of the descriptors.[23] Total and polar surface area, orbital energies, atomic charges, and dipole moments were found to be significant descriptors.

To the best of our knowledge, there is still no QSAR study on Pt(IV) complexes based on DFT derived descriptors in the literature. Herein, we report theoretical and QSAR investigations on 53 novel Pt(IV) complexes (listed in Figure 2), synthesized and tested in our laboratories, using the hybrid DFT functional wb97x for optimization of the structure geometry and calculation of the descriptors. MLR, PCA, and simulated annealing were employed for the development of the statistical models.

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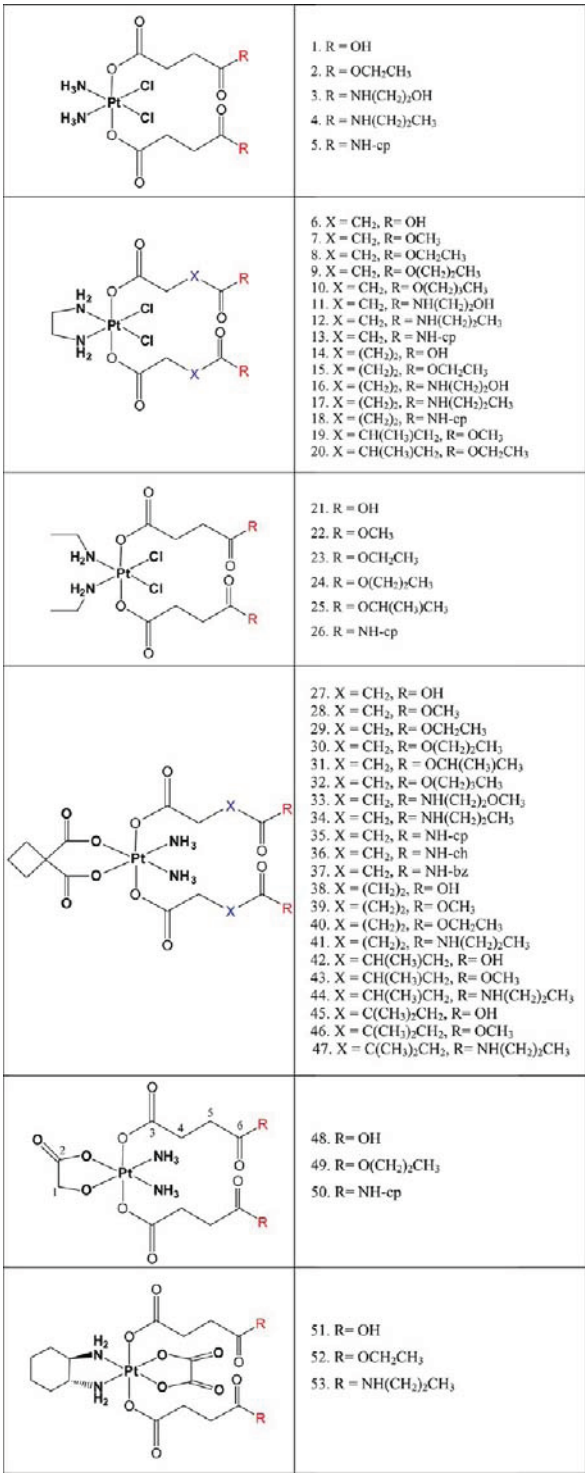

Figure 2. Schematic formulas of the investigated complexes.

RESULTS AND DISCUSSION

Synthesis and Characterization. The entire set of 53 Pt(IV) complexes, which are an object of this study, is presented in Figure 2. On the basis of the equatorial ligands, the compounds can be divided into six subseries, namely, derivatives of cisplatin (**1**−**5**), its ethylenediamine analogue (**6**−**20**), its bis(ethylamine) analogue (**21**−**26**), carboplatin (**27**−**47**), nedaplatin (**48**−**50**), and oxaliplatin (**51**−**53**). The axial ligands are represented by dicarboxylato chains, containing different spacers between the two carbonyl groups and diverse terminal moieties such as esters, amides, or free carboxylic acids. Synthesis and detailed characterization of compounds **1**−**20** and **51**−**53** are given in refs 17, 18, and 19, and those for **21**−**47** are given in refs 14 and 20. Nedaplatin derivatives (**48**−**50**) were synthesized using analogous procedures. Their detailed characterization is based on ^{1}H, ^{13}C, ^{15}N, and ^{195}Pt 1D and 2D NMR measurements. Interestingly, two different-shaped signals for the NH_3 groups can be observed in the ^{1}H spectra of compounds **48**−**50**: a broad signal at around 6.1 ppm and a multiplet around 6.6 ppm in which the ^{14}N−^{1}H and ^{195}Pt−^{1}H couplings can be seen. The existence of two signals in the ^{1}H and ^{15}N spectra can be explained by the unsymmetrical surroundings of the NH_3 groups, one is in trans position to carboxylate and the other one to alcoholate.

Cytotoxicity. All complexes (**1**−**53**) were tested for in vitro cytotoxicity in comparison with cisplatin, carboplatin, oxaliplatin, and nedaplatin in two human tumor cell lines (CH1 ovarian carcinoma and SW480 colon carcinoma), using the MTT colorimetric assay. The resulting IC_{50} values are listed in Table 1. The cell line CH1 is sensitive to the clinically applied platinum drugs, while the second one (SW480) is resistant to them with the exception of oxaliplatin. The set of compounds covers a large range of cytotoxicity with nanomolar IC_{50} values up to 174 μM in the cell line CH1 and from 0.1 μM to negligible activity (>500 μM) in the cell line SW480. In general, the diam(m)inebis(carboxylato)-dichlorido complexes (**1**−**26**, subset 1) show higher activity in comparison with the tri- and tetrakis(carboxylato)diam(m)-ine compounds (**27**−**53**, subset 2). With increasing the lipophilicity, complexes with higher cytotoxicity than the clinically applied platinum(II) drugs could be obtained in subset 1, while this observation is not valid for the compounds in subset 2. In general for all complexes in the set, cytotoxicity is dependent on lipophilicity, but this is much more pronounced for the diam(m)inedicarboxylatodichlorido complexes. When the axial ligands are compared, terminal ester groups are most favorable for antiproliferative activity, followed by amide derivatives; compounds featuring terminal carboxylic or hydroxy groups in the axial chain showed the lowest cytotoxic potency (see Figure 3). In subset 2, cytotoxicity of amide and ester derivatives is comparable. Lack of activity of all compounds from the subset (except for oxaliplatin analogues **51**−**53** and partially for nedaplatin analogues **49**−**50**) in the cisplatin-resistant cell line SW480 can be observed (Table 1).

Crystal Structure. The result of the X-ray diffraction analysis of **7** is shown in Figure S1 in Supporting Information. The compound crystallized in the triclinic centrosymmetric space group $P\bar{1}$. The Pt(IV) atom has an octahedral coordination geometry with one ethylenediamine and two chlorido ligands in the equatorial plane and two 4-methoxysuccinates coordinated in axial positions. The bond lengths and angles are well comparable with the crystal structure of analogous complex **6** previously published.[17] Interestingly, the orientations of the axial ligands in complex **7** are different from those observed in **6**. This is probably due to dissimilar crystal packing and H-bonding pattern. An analogous difference in the conformational behavior was observed in the structures of complexes **1** and **22**, whereas the 4-methoxysuccinate ligands in **22** have a straight orientation[20] while in **1** one succinate is twisted and the other is straight.[18]

Geometry Optimization. Comparison of geometry parameters, obtained after the optimization procedure in vacuum, in a water model and from the available X-ray data for compounds **1**, **6**, **7**, **22**, and **38** is shown in Table S1 (Supporting Information). A good agreement between experiment and calculation could be observed.

Table 1. Cytotoxicity of the Investigated Platinum(IV) Complexes in Comparison with the Clinically Applied Platinum(II) Drugs in the CH1 and SW480 Human Cancer Cell Lines

	IC_{50} (μM)[a]			IC_{50} (μM)[a]	
compd	CH1	SW480	compd	CH1	SW480
1	19 ± 1	136 ± 16	**30**	24 ± 5	>500
2	0.62 ± 0.32	3.8 ± 1.0	**31**	8.6 ± 1.7	350 ± 39
3	28 ± 2	183 ± 28	**32**	11 ± 6	181 ± 44
4	12 ± 4	48 ± 4	**33**	62 ± 26	>500
5	1.9 ± 0.2	24 ± 4	**34**	44 ± 8	>500
6	5.5 ± 2.2	95 ± 5	**35**	15 ± 5	>500
7	0.68 ± 0.20	16 ± 1	**36**	28 ± 2	>500
8	0.34 ± 0.11	4.1 ± 0.5	**37**	31 ± 13	>500
9	0.068 ± 0.024	0.63 ± 0.20	**38**	114 ± 23	>500
10	0.018 ± 0.007	0.22 ± 0.08	**39**	33 ± 13	>500
11	24 ± 3	142 ± 23	**40**	7.7 ± 1.4	>250
12	2.3 ± 1.1	31 ± 15	**41**	89 ± 7	>500
13	1.9 ± 0.2	19 ± 9	**42**	128 ± 48	>500
14	32 ± 19	160 ± 10	**43**	23 ± 9	>500
15	1.1 ± 0.2	3.5 ± 0.1	**44**	49 ± 13	>500
16	21 ± 8	90 ± 21	**45**	125 ± 35	>500
17	22 ± 12	43 ± 22	**46**	22 ± 8	>500
18	7.8 ± 1.0	21 ± 5	**47**	33 ± 4	>500
19	0.17 ± 0.05	2.9 ± 1.0	**48**	21 ± 6	>500
20	0.055 ± 0.006	0.96 ± 0.4	**49**	1.9 ± 0.3	100 ± 6
21	5.6 ± 1.6	40 ± 12	**50**	2.1 ± 0.3	161 ± 33
22	0.16 ± 0.05	1.0 ± 0.3	**51**	55 ± 28	44 ± 9
23	0.061 ± 0.015	0.30 ± 0.05	**52**	19 ± 5	14 ± 3
24	0.014 ± 0.002	0.11 ± 0.01	**53**	11 ± 2	12 ± 5
25	0.0094 ± 0.0012	0.39 ± 0.07	cisplatin	0.16 ± 0.03	3.50 ± 0.29
26	0.75 ± 0.10	6.1 ± 0.6	carboplatin	1.36 ± 0.40	85 ± 28
27	171 ± 1	>500	oxaliplatin[b]	0.33 ± 0.09	0.30 ± 0.08
28	32 ± 10	>500	nedaplatin	0.14 ± 0.05	6.3 ± 1.3
29	28 ± 4	>500			

[a]The reported 50% inhibitory concentrations are the means ± standard deviations obtained from three independent experiments. [b]Data taken from ref 24.

Analysis of the Calculated Physicochemical Parameters. The dipole moments (μ) vary from 3 to 15 D, implying that all the complexes are quite polar compounds (Table S2). Nevertheless, no trend in the alteration of this parameter within the investigated compounds could be found.

The energies of solvation (E_s and E_s') differ from 70 to 160 kJ mol^{-1} for all compounds. In general the complexes exerting carboxylic or amide groups in the axial ligands have higher solvation energies compared with their ester analogues. The distribution of the electron density, based on electrostatic potential (ESP) for compounds of the two subtypes, shows that the most electropositive regions in the molecule could be found around the nitrogen donor atoms and the most negative around the oxygen and chlorine atoms (Figure 4).

The natural population analysis (NPA) charge at Pt (q(Pt)) differentiates well the two major subtypes (in structure deviation and in cytotoxic activity): bis(carboxylato)dichlorido complexes (subset 1) versus tris- and tetrakis(carboxylato) complexes (subset 2). It also shows some minor discrimination in the two subtypes, dependent mainly on differences in the equatorial ligands. Expectedly, the deviations of the terminal fragments of the axial ligands do not affect the charge at the Pt atom. A plot of the NPA charge at Pt for the 53 investigated complexes (average values from the calculated conformers of compounds of **2** and **50** are used) is shown in Figure 5.

The described circumstances make q(Pt) a good descriptor for a further QSAR model. In Figure 6, a qualitative MO analysis of the frontier orbitals, together with their energies for complexes **22** and **38**, is shown. The low (negative) values of E_{HOMO} (<−9 eV) and the good correlating high ionization potentials (between 8 and 10 eV in vacuum and between 7 and 8.5 eV in water) are a logical consequence of the inability of Pt(IV) complexes to act as reductants. However, a clear tendency of the change of these parameters in the series cannot be observed.

The negative values of E_{LUMO} (Table S3, Figure 6) show that the compounds can act as oxidants and can be reduced relatively easily. The nedaplatin derivatives (complexes **48**−**50**) have higher (close to zero, even slightly positive for complexes **49** and **50b**) values of E_{LUMO}, which probably is connected with their eventual lower oxidative capability. In general, a clear relationship between the variations of E_{LUMO} with the structures in the series could not be found. Interestingly, the values of the electron affinity in gas phase (between 0.6 and 1.8 eV) and their corresponding vertical (between 2 and 3 eV) and adiabatic (around 4 eV) redox potentials in water do not correlate with each other as would be expected. In principle, the small differences observed between the values of the adiabatic redox potential in water imply that the investigated compounds can be reduced with relatively equal effort. The different tendencies for the vertical and adiabatic electron affinity in water also show that the acceptance of an electron in aqueous media is associated with significant

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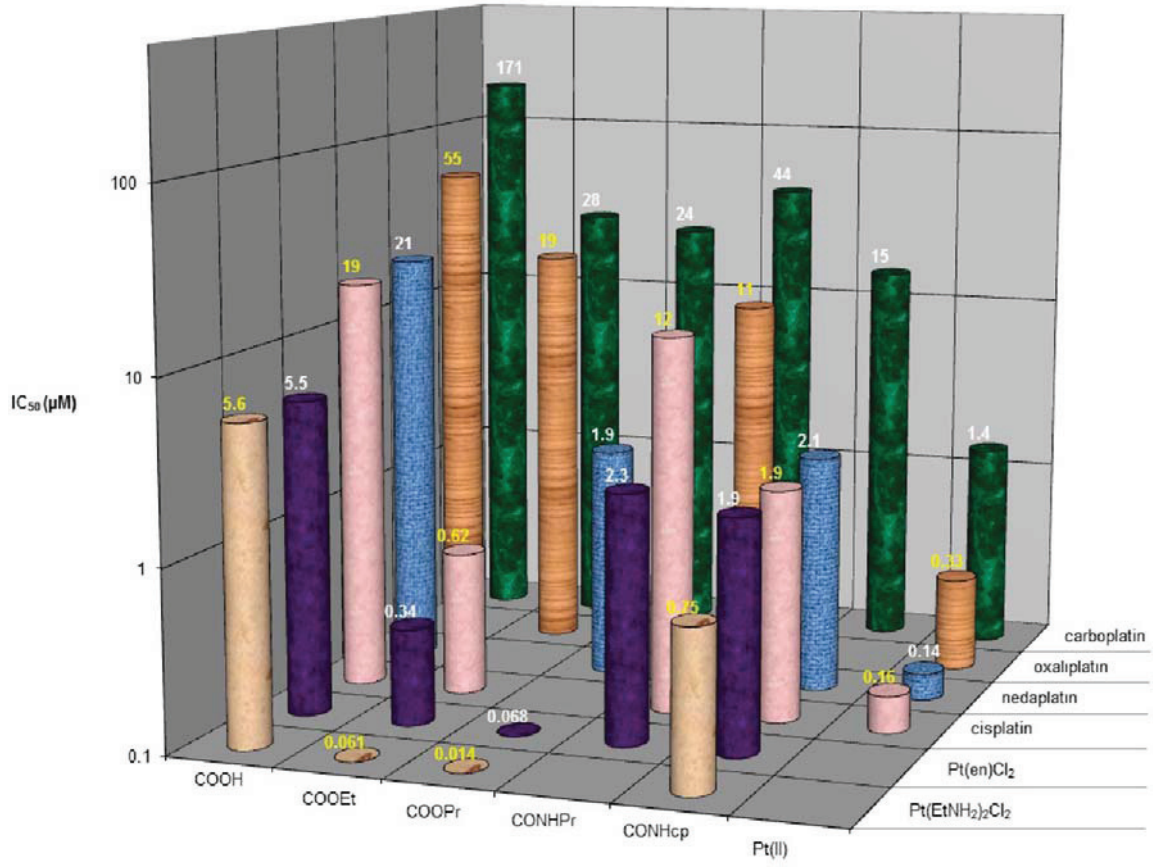

Figure 3. Comparative diagram of the cytotoxicity (IC_{50} values, logarithmic scale) of some Pt(IV) complexes from the series in the CH1 cell line, depending on their equatorial ligands (*y* axis) and the terminal moieties of the axial ligands (*x* axis), and the clinically approved Pt(II) drugs: cisplatin, carboplatin, oxaliplatin, and nedaplatin.

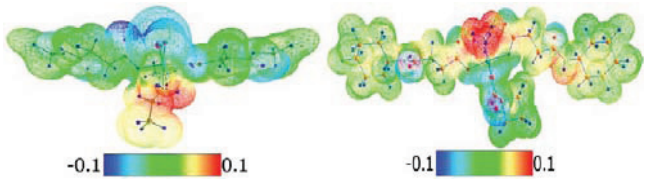

Figure 4. ESP color mapped electron density for complexes **22** (left) and **36** (right).

changes in the geometry and the energy of the system. It is interesting to follow how the geometry of the complexes has been changed with the acceptance of one electron. From the NPA charges it can be concluded that the extra electron mainly resides on the Pt atom (and the coordinated chloride, in the case of complexes of subset 1), a Pt(III) radical is formed, and expectedly the largest and important geometry alterations will be in the Pt coordination sphere. In Table 2, a comparison of the bond lengths changes between the neutral and the anion radical (both optimized in water) for complexes **24** and **28** is shown.

Table 2 shows that addition of an electron to a complex from subset 2 results in an elongation of $Pt-O_{ax}$ bonds while Pt−N and $Pt-O_{eq}$ in the equatorial sphere remain almost unchanged. These observations are in accordance with the expected reduction and loss of the axial ligands. Contrary to bis(carboxylato)-dichlorido complexes from subset 1 (with the exception of some of the cisplatin analogues, namely, complexes **1**, **2c**, and **3**), the axial Pt−O bonds have shifted insignificantly but the equatorial Pt−Cl and trans standing Pt−N have been elongated by more than 0.3 Å. In principle, the last findings correlate well with the shape of the LUMO orbitals which, for complexes from subset 2, are mainly situated around the axial ligands while for those from subset 1 they could be found in the square-planar sphere around platinum (Figure 6). Consequently, a different mechanism of reduction between the complexes of the two main subtypes is expected.

Reduction Model Studies. In order to gain a deeper insight into the mechanism of reduction of Pt(IV) prodrugs, further investigations based on two simple model systems, namely, (OC-6-33)-bis(acetato)diamminedichloridoplatinum(IV) (**M1**), representing complexes of subset 1, and (OC-6-33)-bis(acetato)diamminemalonatoplatinum(IV) (**M2**), representing complexes of subset 2 (Figure S2, Supporting Information), were performed. Applying again the most prominent hypothesis for the reduction pathway of Pt(IV) complexes featuring axial carboxylato ligands, which is an outer sphere reduction going via a Pt(III) intermediate,[15] the structures of **M1**, **M2**, and their analogous monoanionic Pt(III) radicals were optimized in a water model. The same was done for the respective intermediates of reduction, the pentacoordinate complex after cleavage (ionic or radical) of one ligand (acetate or chloride). The energy of the cleaved acetate and chloride in water was also calculated. The possible ligand dissociation reactions after one-electron reduction are presented schematically in Figure 7. The dissociation energies, calculated for possible ionic or radical cleavage of a ligand (chloride or acetate) from the neutral Pt(IV) complexes or from their anion radicals, are listed in Table 3.

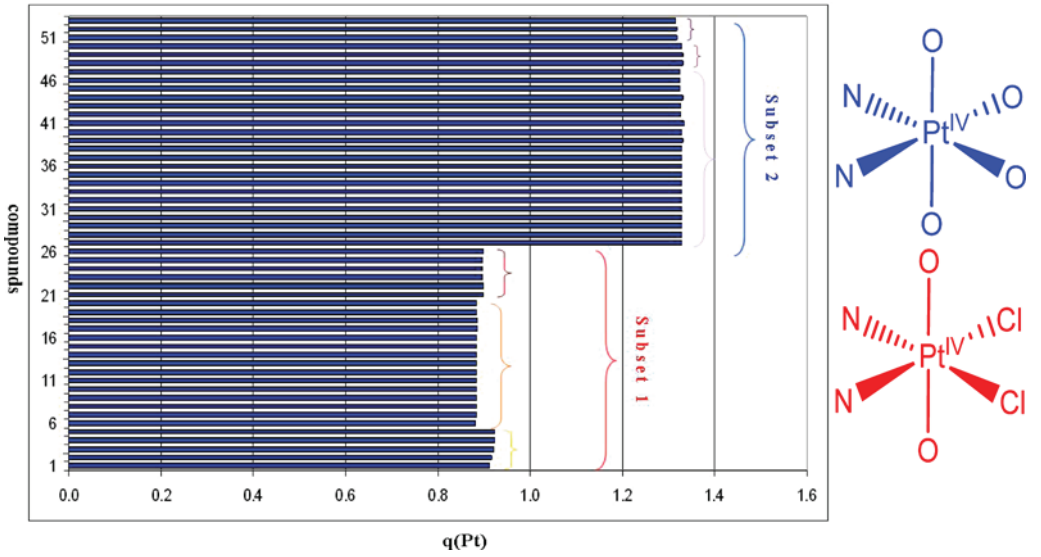

Figure 5. NPA charge at the Pt atom (in au), calculated for complexes **1**−**53**. A scheme of the coordination sphere of subsets 1 and 2 is presented on the right.

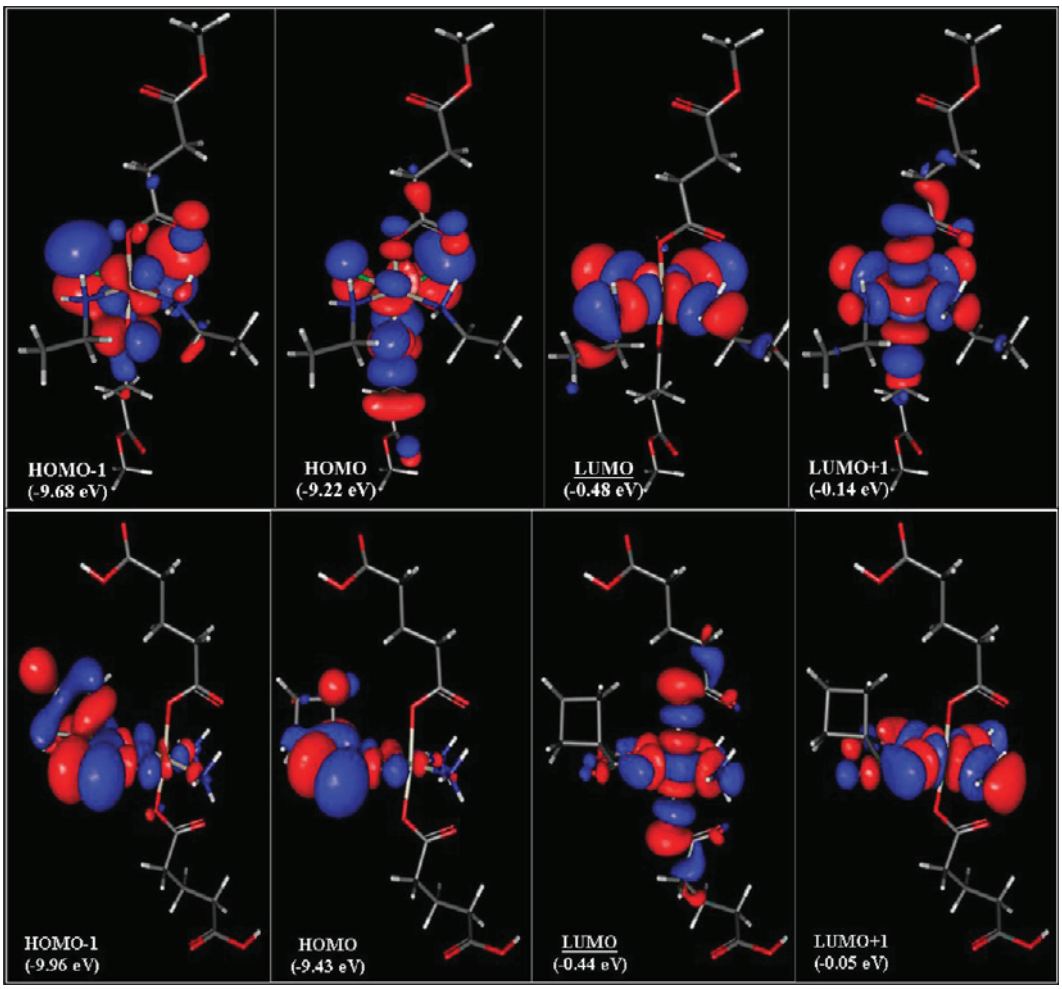

Figure 6. Frontier orbitals (with their energies) of complexes **22** (top) and **38** (bottom).

As presented in Table 3, reasonable dissociation energies are observed only when a ligand is cleaved from a Pt(IV) complex, which has already accepted an electron (and has been transformed into a Pt(III) radical). From the obtained values, it can be concluded that cleavage of equatorially bound chloride from the ionized complex **M1** requires less energy than dissociation of axial acetate. Furthermore, the dissociation of an axial carboxylato ligand appears to happen more easily in the case of

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Table 2. Bond Length Changes in Complexes 24 and 28 after the Acceptance of an Electron in Aqueous Medium

	complex	
Δ bond length (Å)	**24**	**28**
Pt−O1ax	0.04	0.35
Pt−O2ax	0.03	0.37
Pt−N1	0.34	0.01
Pt−N2	0.01	0.01
Pt−Cl1/Pt−O3eq	0.04	0.04
Pt−Cl2/Pt−O4eq	0.34	0.04

bis(carboxylato)dichlorido complexes (like **M1**) in comparison to tetracarboxylato species (like **M2**). The last findings correlate with the experimental data from electrochemical experiments, where it was found that bis(carboxylato)dichlorido(ethane-1,2-diammine)platinum(IV) complexes (**6**−**20**) have similar but slightly higher redox potentials (approximately −0.6 V vs NHE) than the corresponding carboplatin analogues (**27**−**47**) (approximately −0.7 V vs NHE).[14,21] Nevertheless, the electron affinity (the energy released after the attachment of an electron to a neutral complex) is much higher than the obtained dissociation energies: 380.2 kJ/mol for **M1** and 392.0 kJ/mol for **M2**. For this reason, reduction of both complexes with dissociation of acetate or chloride is possible, which is in agreement with Gibson's observation for more than one product of reduction of diacetatodiam(m)inedichloridoplatinum(IV) complexes[25] as well as with the nonaxial ligand loss reduction recently reported by Hambley/Gibson[13] and Cullinane.[26]

The thermodynamically comparable redox properties (in theory and experimentally) for both types of compounds were different with respect to their kinetic behavior. Compounds of subset 1 were reduced by ascorbic acid much more quickly than those from subset 2.[14] In order to learn more about the kinetics of reduction, modeling of the energy change as a function of elongation of the Pt−acetate bond (in both models) or Pt−Cl (in **M1**) was performed. Unfortunately, the attempts to find an energy barrier and the corresponding transition state are unsuccessful so far. It looks like that the rate-limiting factor of reduction is the transfer of an electron to the Pt(IV) atom, not breaking of the Pt−ligand bond as a result of the one-electron transfer. In this context the kinetics of reduction of platinum(IV) complexes are dependent not only on the compound itself but also on the bioreducing agent (ascorbate, glutathione, cysteine, methionine, etc.) and the surrounding pH.[27−29] How these factors influence the behavior of Pt(IV) complexes will be a matter of further investigation.

Table 3. Energies (in kJ/mol) Required for Dissociation of a Ligand from Complexes M1 and M2

reaction of reduction	M2-ac	M1-ac	M1-Cl
$Pt^{IV}L_6 \rightarrow (Pt^{IV}L_5)^+ + L^-$	305.6	303.7	246.7
$Pt^{IV}L_6 \rightarrow (Pt^{III}L_5)^\bullet + L^\bullet$	251.3	251.6	256.9
$(Pt^{IV}L_6)^{\bullet-} = (Pt^{III}L_6)^{\bullet-} \rightarrow (Pt^{III}L_5)^\bullet + L^-$	124.8	113.5	88.0

QSAR Analysis. Initial Screening of the Descriptors. From the initial screening of the correlation between properties and biological response, it was found that the most significant descriptors for the biological activity (as single parameters) in both cell lines are the number of H-bond acceptors (H_{acc}), charge at the Pt atom (q(Pt)), vertical and adiabatic electron affinities (E_{ea}s and E_{ea}s′), followed by the number of H-bond donors (H_{don}) (see Table 4).

Table 4. Significance of the Descriptors (as Single Parameters), Based on the Properties−Biological Response Correlation[a]

correlation with the response	CH1 cells	SW480 cells
strong (R > |0.5|)	H_{acc}, q(Pt), E_{ea}s′, H_{don}, E_{ea}s	H_{acc}, q(Pt), E_{ea}s
middle (R < |0.5|)	COOH, Es′, Es, E_{ea}	H_{don}, E_{ea}s′
weak (R < |0.3|)	E_{HOMO}, MW, E_i, H/Lgap, E_is	Es′, Es, COOH, MW, E_{HOMO}, E_{ea}, E_{LUMO}, E_is, V_m, E_i
very weak (R < |0.15|)	V_m, α, μ, SASA, E_{LUMO}	α, μ, SASA, H/Lgap

[a]for the abbreviations see the experimental section.

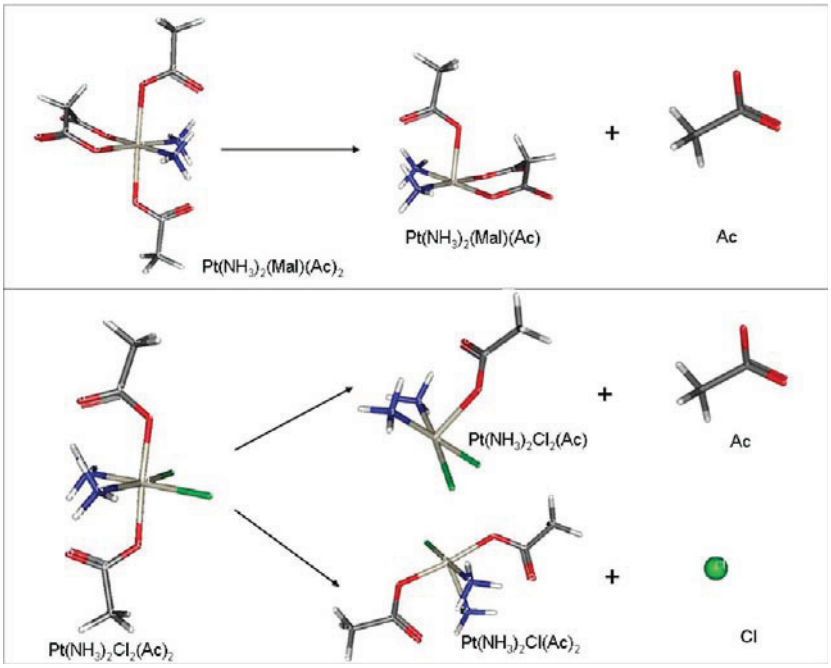

Figure 7. Scheme of possible reduction reactions to pentacoordinated Pt complex for **M1** (bottom) and **M2** (top).

A strong correlation between MW, V_m, and α can be found. The charge at Pt, the vertical redox potential (E_{ea}s), and the number of H-bond acceptors also have a strong correlation with each other. Expectedly, there is an excellent correlation between E_{HOMO} and the ionization energy (E_i) as well as among the vertical and adiabatic solvation energies. A good agreement between E_{LUMO} and E_{ea}, as well as among the HOMO/LUMO gap and first ionization energy in vacuum could be also observed during the descriptor analyses. From a group of descriptors having strong correlations with each other, only a single one is expected to contribute to a good QSAR model. With the aim of using the final QSAR model for screening purposes, it is advantageous to select the descriptor in each group as one that requires the least computational effort, e.g., the molecular weight is much easier to calculate than the molecular polarizability; vertical solvation energy can be calculated more quickly than adiabatic.

The most promising models based on a single descriptor or a combination of two, three, four, or five descriptors were chosen with the help of simulated annealing. It was demonstrated that the best merit for both cell line models could be derived by combination of four descriptors; utilizing more than five descriptors decreased the merit. How R^2 and Q^2 of the models change with increasing the number of the descriptors is shown in Figure S3 (Supporting Information).

QSAR Models for the Cell Line CH1. Statistical data for the best regression models for cytotoxicity in the cell line CH1 are summarized in Table 5. Additional statistical information for these and other models based on one, two, three, four, or five descriptors is presented in Table S4 (Supporting Information).

Table 5. Statistical Data for the Best Regression Models for Cytotoxicity in the Cell Line CH1 of the 53 Investigated Pt(IV) Complexes

no. of variables	descriptors	R^2	Q^2(LTOP)	rms
1	H_{acc}	0.51	0.48	0.78
2	H_{don}, H_{acc}	0.70	0.67	0.62
3	q(Pt), E_{ea}s′, H_{don}	0.79	0.75	0.53
4	α, q(Pt), E_{ea}s′, H_{don}	0.86	0.82	0.45
4	MW, q(Pt), E_{ea}s′, H_{don}	0.85	0.81	0.47
4	MW, E_{ea}s′, H_{don}, H_{acc}	0.85	0.82	0.46
4	α, E_{ea}s′, H_{don}, H_{acc}	0.85	0.82	0.46
5	MW, α, q(Pt), E_{ea}s', H_{don}	0.87	0.84	0.43
6	MW, α, q(Pt), E_{ea}s′, Es, H_{don}	0.88	0.84	0.43

Models derived by using only one descriptor (the good autocorrelating q(Pt) or H_{acc}), expectedly have low explanatory and predictive properties (R^2 and Q^2 under 50%). Essential improvement could be achieved by adding H_{don}; R^2 increased to nearly 70%, and the predictability was over 65%, respectively. Further enhancement could be obtained by adding a third descriptor to the best two-variable models (H_{don} and H_{acc} or q(Pt) and H_{don}). R^2 over 75% is achieved by including the adiabatic redox potential in water (E_{ea}s′). The developed models also showed high predictive strength (Q^2 > 75%) and robustness, which was proved in the external validation under severe conditions (Table S5, Supporting Information). Interestingly, by inclusion of the presence/absence of COOH in the combination of the H_{don} and H_{acc} model, only constitutional (easy to calculate) molecular descriptors with R^2 = 75% and Q^2 = 72% could be obtained (Table S4). The latter also showed good Pred.R^2 on the external validation and can be a good alternative for screening when quantum mechanical calculations are not possible or too expensive. Combining α or the autocorrelated MW with q(Pt) and H_{don} gave models with moderate explanatory (~72%) and predictive (~69%) properties, which totally failed in the external validation, partition **e** where all nedaplatin derivatives (**48**−**50**) are in the predictive set (Tables S5 and S6).

Reliable ($R^2 \geq 80\%$) and predictive ($Q^2 \geq 70\%$) models could be constructed only by adding a fourth descriptor. The best results are obtained via combining polarizability with the best two three-descriptor models, where R^2 of 86%, Q^2 of 82%, and AAR < 0.4 could be achieved. Using MW (easy to calculate and having strong correlation with α) instead does not decrease the quality of the models. The external validation proved the robustness and the predictive properties and showed that the most reliable model is built by using MW, E_{ea}s′, H_{don}, and H_{acc} as variables. Developing a model by adding a fifth descriptor can slightly increase the R^2 and Q^2 values only when autocorrelating descriptors (e.g., α and MW, E_{ea}s and E_{ea}s′) are included, which results in overfitting and fake higher predictability.

The complete regression equation for the final predictive model we have chosen is as follows:

$$pIC_{50}(CH1) = 0.006\,MW - 3.920E_{ea}s' - 0.417H_{don} - 0.363H_{acc} + 16.186$$

The plot of experimental and predicted pIC_{50} values of the model is shown in Figure 8.

In the PCA method all variables are combined into new descriptors that are ranked according their ability to describe the variation in the descriptor data. For the present case, 85% of the variance could be explained by five components, but a QSAR model using five components performs no better than the four-component MLR model (R^2 = 0.83). Since the PCA approach requires the calculation of all descriptors, the MLR approach is better suited for screening purposes. When PCA was applied on the four descriptors (MW, E_{ea}s′, H_{don}, and H_{acc}) used for developing our MLR model, three components, together explaining 88% of the variance, were obtained (their loading plots are shown in Figure S4, Supporting Information). The score plot, obtained by combination of the first two of them (PC1 and PC2, explaining 73% of the variability), grouped well the complexes in five clusters (Figure 9). The compounds from subsets 1 and 2 were split, depending on the terminal moieties of the axial ligands, separating the more active esters in one cluster from the less active amides and free carboxylic acids in another. Compounds **3**, **11**, and **16**, featuring terminal CH_2OH and equipped with the lowest cytotoxicity in subset 1, formed another cluster. Interestingly, nedaplatin derivatives (**48**−**50**) from subset 2 grouped together with the amides and free carboxylic acids from subset 1, and esters **17** and **18** (having three CH_2 groups spacer between the carbonyls in the axial chains) from subset 1 grouped together with amides and carboxylic acids from subset 2.

QSAR Models for the Cell Line SW480. Statistical data for the best regression models for cytotoxicity in the SW480 cell line are summarized in Table 6. Additional statistical information for these and other one- to five-variable regressions is presented in Table S6 (Supporting Information).

Using only one descriptor cannot give a model with good explanatory and predictive properties (R^2 and Q^2 under 65%). Including a second (q(Pt) and H_{don}) increases R^2 up to 73% and Q^2 to 70%. However, in order to reach values over 75%, models with a combination of three or four descriptors should be used. Increasing the number of variables to more than four gives models

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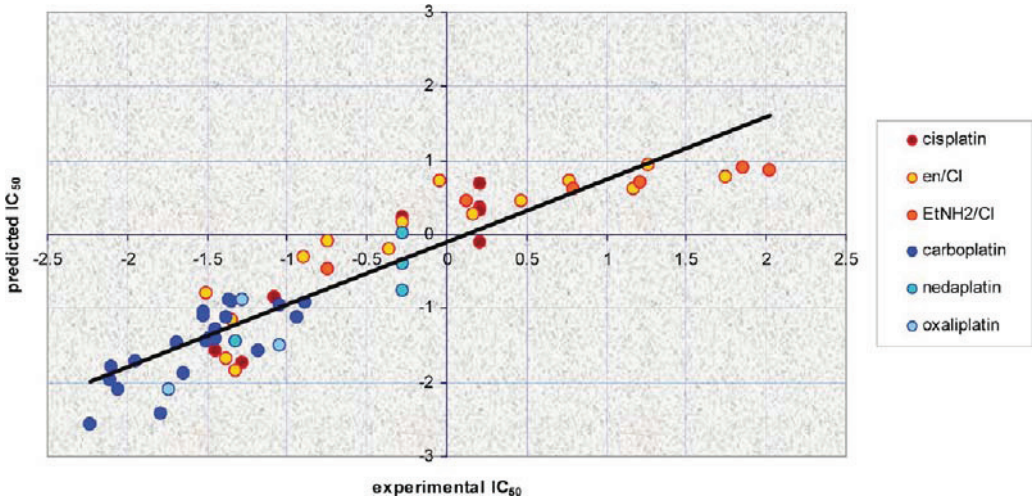

Figure 8. Predicted (with the selected four-variable model) vs experimental cytotoxicity in the cell line CH1. The coloring is based on the subtypes containing the same equatorial ligands.

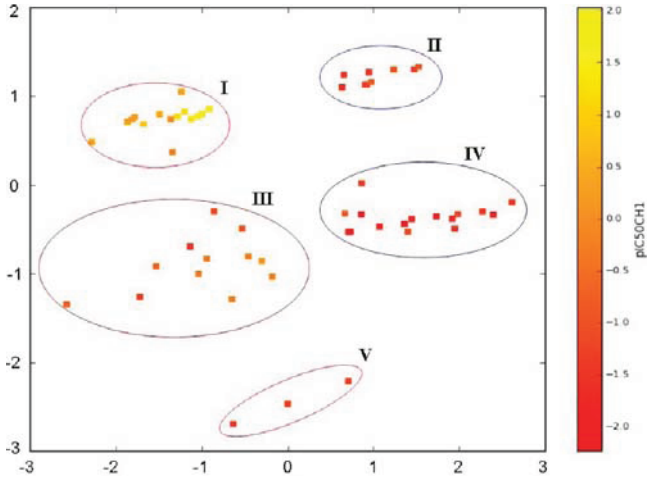

Figure 9. Scoring plot derived from PCA on the four descriptors (MW, $E_{ea}s'$, H_{don}, and H_{acc}) used in the proposed model for cytotoxicity in the CH1 cells: cluster I, esters from subset 1; cluster II, esters from subset 2; cluster III, amides and free carboxylic acids from subset I and nedaplatin derivatives (**48**−**50**); cluster IV, amides and free carboxylic acids from subset 2 and complexes **17** and **18** from subset 1; cluster V, compounds with terminal CH_2OH groups in the axial ligands.

with marginally higher R^2, but this is mainly due to overfitting, since they contain autocorrelated descriptors (E_{HOMO} or H/Lgap and E_i). The actual predictability of the best models, featuring three, four, or five descriptors, using external validation, is summarized in Table S7 (Supporting Information). The lowest predictive capability of the models could be observed on training sets **c** and **e** where most of the ethylenediamine derivatives or the oxaliplatin, nedaplatin, and part of the carboplatin analogues are moved to the predictive set.

The complete regression equations for the final predictive models we have chosen are the following:

$$pIC_{50}(SW480) = -0.024E_s - 0.353H_{don} - 0.534H_{acc} - 0.526\ COOH + 2.090 \quad (1)$$

$$pIC_{50}(SW480) = -0.637E_i + 4.162E_{ea}s - 4.522E_{ea}\,s' - 0.384H_{don} + 12.060 \quad (2)$$

Table 6. Statistical Data for the Best Regression Models for Cytotoxicity in the SW480 Cell Line of the 53 Investigated Pt(IV) Complexes

no. of variables	descriptors	R^2	Q^2(LTOP)	rms
1	H_{acc}	0.63	0.60	0.75
2	q(Pt), H_{don}	0.73	0.70	0.65
3	Es, H_{don}, H_{acc}	0.77	0.73	0.61
4	Es, H_{don}, H_{acc}, COOH	0.80	0.75	0.59
4	E_i, $E_{ea}s$, $E_{ea}s'$, H_{don}	0.80	0.76	0.58
4	E_i, E_{ea}, $E_{ea}s$, H_{don}	0.82	0.79	0.54
5	E_{HOMO}, E_i, E_{ea}, $E_{ea}s$, H_{don}	0.84	0.80	0.52
5	q(Pt), H/Lgap, E_i, $E_{ea}s$, H_{don}	0.82	0.78	0.56
6	E_{HOMO}, E_i, E_{ea}, Es, $E_{ea}s$, H_{don}	0.85	0.81	0.51
7	E_{HOMO}, E_i, E_{ea}, Es, Es′, $E_{ea}s$, H_{don}	0.86	0.80	0.52

$$pIC_{50}(SW480) = -1.094E_i - 2.634E_{ea} + 4.971E_{ea}s - 0.404H_{don} - 1.281 \quad (3)$$

The plot of the predicted vs experimental pIC_{50} values for the models is shown in Figure 10. Model 3 gives the best linear fit (R^2 = 0.82 vs R^2 =0.80 for models 1 and 2) and can predict better the activity of the oxaliplatin analogues (**51**−**53**), the only compounds from subset 2, showing some activity in the cell line SW480 (due to the DACH carrier ligand). On the other hand, model 1 showed higher predictive R^2 (near 60%) in the severe cross-validation partitioning **e** and in addition is built from easy to calculate molecular descriptors. It is therefore favorable for screening of new compounds.

As most of the compounds from subset 2 did not show cytotoxic activity in the SW480 cell line and IC_{50} could not be detected up to 500 μM, in the current study IC_{50} of 600, 1000, and 2000 μM were used as input for their cytotoxic activity. By an increase of these values from 600 to 2000, slightly better models with increased R^2 and Q^2 could be observed (Table S8); however, the AAR values increased too and the results from the external validation deteriorate slightly. The data, presented in Table 6, are based on the optimal input IC_{50} of 1000 μM. The tables in Supporting Information are based on the study using IC_{50} = 600 μM for complexes inactive in SW480 cells.

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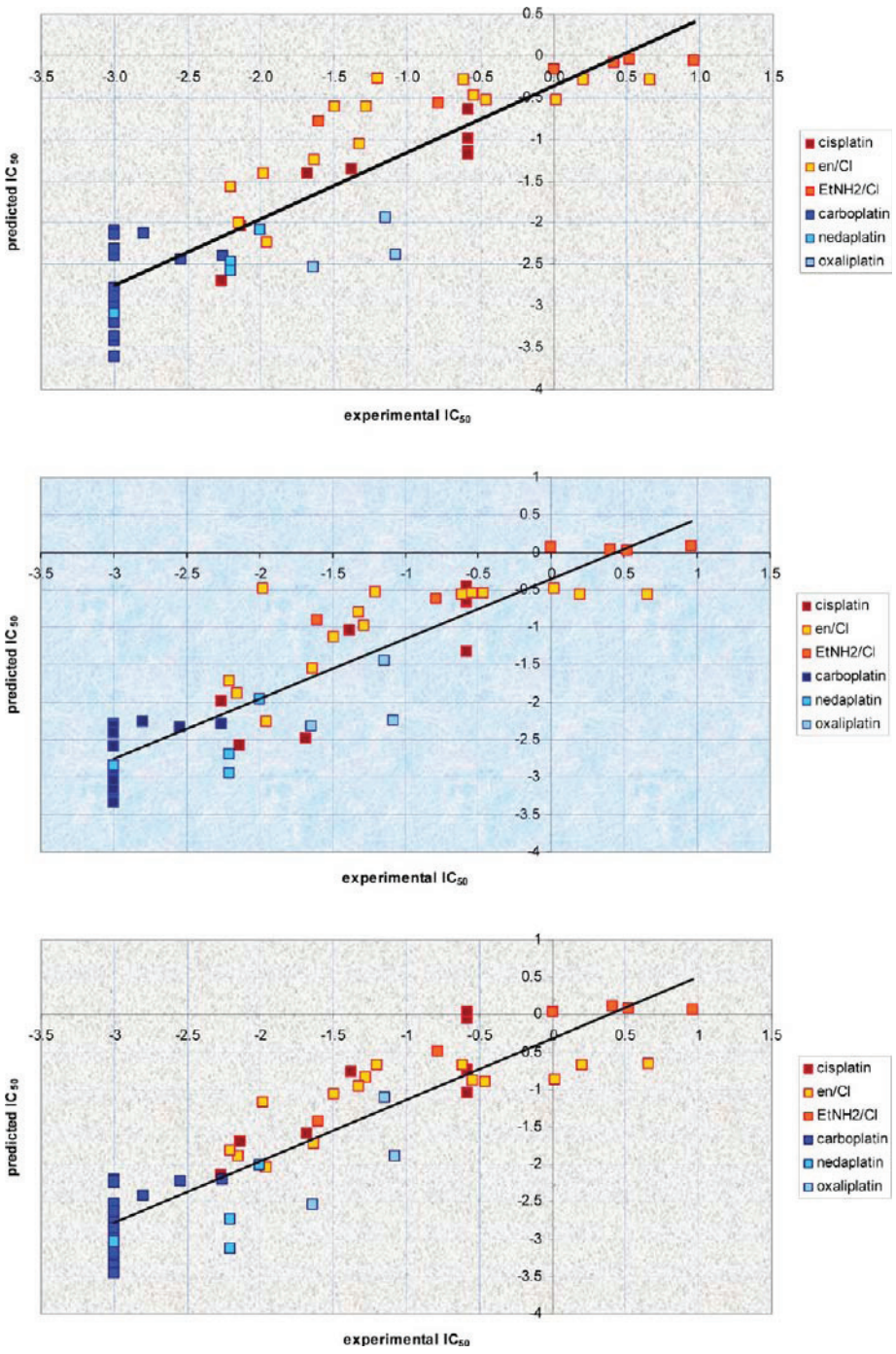

Figure 10. Predicted (with the selected four-variables model) vs experimental cytotoxicity in the SW480 cell line: top, model 1; middle, model 2; bottom, model 3. The coloring is based on the subtypes containing the same equatorial ligands.

Model 2 showed the smallest difference between the predicted pIC_{50} values for the inactive carboplatin analogues (-2.8 ± 0.4) for input value $pIC_{50} = -3.0$ ($IC_{50} = 1000\ \mu M$).

Applying PCA, using the descriptors from the chosen four-variable models, showed that 88−89% of the variance in the set can be explained by three components. By plotting of the scores of PC1 and PC3 (covering 60% of the variance) produced from model 3 descriptors combination, a nice clustering of the series could be observed (Figure 11). Similar to the clustering obtained with the CH1 cells model PCA, the compounds split into subsets 1 and 2 esters and subsets 1 and 2 amide and free carboxylic acids. Compounds **3**, **11**, and **16** are again in a separate cluster, and complexes **17** and **18** from subset 1 are in the subset 2 amides and acids cluster. In addition the $EtNH_2$ ester derivatives (**22**−**25**) built a subcluster.

Conformational Differences. Four different conformers for compound **2** and two different conformers for compound **50** were generated (Figure S5, Supporting Information), and their molecular properties were calculated. The descriptors with the smallest differences were q(Pt), α, E_{HOMO}, H/Lgap, E_i, E_is, E_{ea}s, and E_{ea}s′, with RSD < 2%. A moderate effect of the conformation was observed on V_m and SASA (RSD < 8%). The dipole moment (μ) and solvation energies (E_s and E_s') are more dependent on the conformation, where RSD rises to 17% in the

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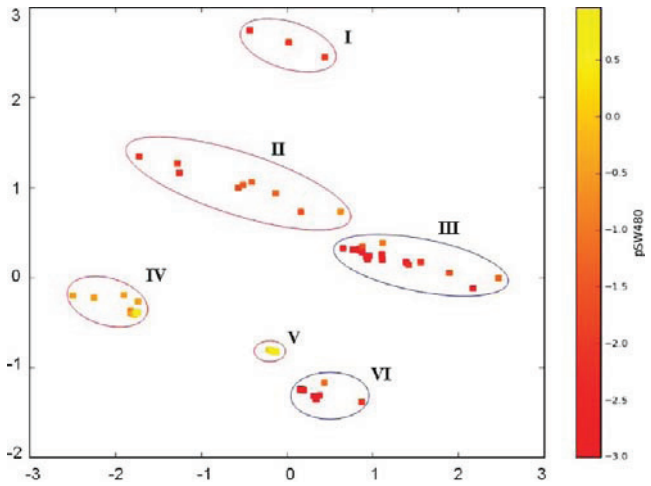

Figure 11. Score plot derived from PCA using four descriptors (E_i, E_{ea}, E_{ea}s, and H_{don}), applied for modeling the cytotoxicity in SW480 cells (model 3): cluster I, compounds with a terminal free CH_2OH group in the axial ligands; cluster II, amides and free carboxylic acids from subset 1; cluster III, amides and free carboxylic acids from subset 2 and **17** and **18** from subset 1; cluster IV, esters from subset 1 (without the $EtNH_2$ derivatives); cluster V, esters from subset 1/the $EtNH_2$ derivatives; cluster VI, esters from subset 2.

case of μ. The autocorrelating E_{LUMO} and E_{ea} showed great dependency on the conformation, which excludes them from the list of descriptors able to produce reliable models. The influence of the conformations of complex **2** on the predicted cytotoxicity from the best chosen four-descriptor models is summarized in Table 7.

In comparison to model 1, models 2 and 3 gave better results with respect to cytotoxicity in SW480 cells. However, the dependency on conformers was higher (high RSD values). This circumstance is expected, since the conformation has a significant impact on descriptor E_{ea}. The vertical and adiabatic redox potentials in water have small conformational dependence but also close values in the series, which pronounce the effect of the conformation to the predicted cytotoxicity.

Free–Wilson QSAR Model. In order to judge the contribution of different substituents to the biological activity of the compounds, Free–Wilson QSAR models for cytotoxicity of the complexes in the CH1 and SW480 cell lines were developed. The models are based on the concept that each substituent makes an additive and constant contribution to the biological activity regardless of substituent variation in the rest of the molecule.[30] Each compound was presented as a binary string with a length of 24 substituents (the different equatorial ligands, spacers between the two carbonyls, and terminal functional groups in the axial ligands). A term is equal to 1 when a substituent is present at a particular position and 0 when it is absent. The contribution of each substituent was calculated using MLR, and the following models were obtained:

$$\begin{aligned} pIC_{50}(CH1) = {} & -0.135A(NH_3) + 0.025A(en) \\ & + 0.212A(EtNH_2) - 0.049A(DACH) \\ & + 0.259L(Cl) - 0.280L(CBDA) \\ & + 0.082L(glyc) - 0.049L(ox) \\ & - 0.010X(succ) - 0.104X(Glu) \\ & + 0.107X(MeGlu) + 0.053X(DiMeGlu) \\ & - 0.277R(COOH) + 0.053R(COOMe) \\ & + 0.167R(COOEt) + 0.185R(COOPr) \\ & + 0.184R(COO\mathit{i}Pr) + 0.209R(COOBut) \\ & - 0.104R(CONHProp) \\ & - 0.014R(CONHcp) \\ & + 0.022R(CONHch) \\ & + 0.016R(CONHBz) \\ & - 0.232R(CONH(CH_2)_2OH) \\ & - 0.021R(CONH(CH_2)_2OMe) \end{aligned}$$

for which $R^2 = 0.90$, $Q^2 = 0.76$, and AAR = 0.28.

$$\begin{aligned} pIC_{50}(SW480) = {} & -0.140A(NH_3) - 0.034A(en) \\ & + 0.223A(EtNH_2) + 0.061A(DACH) \\ & + 0.341L(Cl) - 0.351L(CBDA) \\ & - 0.056L(glyc) + 0.061L(ox) \\ & - 0.053X(succ) - 0.038X(Glu) \\ & + 0.094X(MeGlu) + 0.048X(DiMeGlu) \\ & - 0.195R(COOH) - 0.007R(COOMe) \\ & + 0.146R(COOEt) + 0.177R(COOPr) \\ & + 0.085R(COO\mathit{i}Pr) \\ & + 0.206R(COOBut) \\ & - 0.066R(CONHProp) \\ & - 0.036R(CONHcp) \\ & + 0.012R(CONHch) \\ & + 0.012R(CONHBz) \\ & - 0.199R(CONH(CH_2)_2OH) \\ & + 0.012R(CONH(CH_2)_2OMe) \end{aligned}$$

Table 7. Average IC_{50} Values for Conformers of Complex 2, Derived from the Best Four-Variable QSAR Models, for CH1 and SW480 Cells in Comparison to Experimental Data[a]

cell line	CH1	RSD, %	SW480 model 1	RSD, %	SW480 model 2	RSD, %	SW480 model 3	RSD, %
exptl	0.62 ± 0.32	52	3.8 ± 1.0	26	3.8 ± 1.0	26	3.8 ± 1.0	26
linear fit	0.59 ± 0.46	80	10.9 ± 4.9	45	8.3 ± 8.6	104	4.6 ± 4.8	104
cross-validat predictions (LOOP)	0.60 ± 0.50	83	12.6 ± 6.4	52	8.6 ± 9.3	108	5.0 ± 5.5	110
ext validation[b]	0.91 ± 0.77	85	35.2 ± 19.5	55	42.3 ± 10.6	25	3.7 ± 3.6	97

[a]Results are presented as the mean ± sd. [b]Values from partitioning, where all the conformers are in the training set.

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for which R^2 = 0.91, Q^2 = 0.80, AAR = 0.26.

In the above equations, A is the carrier ligand, L the leaving groups, X the spacer between the two carbonyl groups in the axial ligands, and R the terminal functional group of the axial chains. Explanation of results and predictability of the models with the given set of substituents is good. The highest positive effect on the cytotoxicity in both cell lines have A = $EtNH_2$, L = Cl, R= COOEt, COOPr and COOBut. The lowest cytotoxic effect is due to A = NH_3, L = CBDA, R = COOH, and CONH-$(CH_2)_2$OH. The spacers between the carbonyl groups in the axial chains have a lower impact on the cytotoxicity in the series. In the model for SW480 cells, A = DACH and L = ox have a slightly positive effect on the cytotoxicity, contrary to the model for CH1 cells. In contrast, R = COO*i*Pr has a much higher positive effect on the pIC_{50} values in the cell line CH1 than in SW480 cells.

CONCLUSIONS

Reliable, robust, and predictive four-variable models for the in vitro cytotoxicity of bis-, tris-, and tetrakis(carboxylato)platinum-(IV) complexes in cisplatin sensitive CH1 cells and intrinsically cisplatin resistant SW480 cells were developed. The QSAR model of choice (R^2 = 85%, Q^2 = 82%) for CH1 cells was built using the combination of MW, H_{don}, H_{acc} and E_{ea}s′. For the SW480 cell line, models consisting of E_s, H_{don}, H_{acc}, and COOH (R^2 = 80%, Q^2 = 75%) and E_i, E_{ea}s, E_{ea}s′, and H_{don} (R^2 = 80%, Q^2 = 76%) were proposed. The autocorrelating descriptors q(Pt), H_{acc}, and E_{ea}s distinguished well the two main subtypes of compounds, namely, bis(carboxylato)dichlorido (subset 1) from tris- and tetrakis(carboxylato) (subset 2) complexes and showed some minor discrimination within the subsets, depending on different equatorial ligands. H_{acc} predicted a slightly higher activity for nedaplatin analogues compared to the other compounds in subset 2 (the case of CH1 cells), while q(Pt) and E_{ea}s discriminated oxaliplatin analogues as more active (the case of SW480 cells). The constitutional descriptor H_{don} discriminated the main functionalities on the axial ligands: amides and free carboxylic acids from esters. Therefore, the latter is crucial for building a good model. MW as a descriptor indicates the increase of lipophilicity (respectively, cytotoxicity) in the series with increasing the size of the axial chains or the size of equatorial amines. E_{ea}s′ and E_s, redox behavior and solubility correspond to important physicochemical parameters of Pt(IV) complexes and expectedly show significance for the prediction of the biological response. The results of the study represent a step toward a better understanding of the biological behavior of Pt(IV) carboxylato complexes and their further rational development.

EXPERIMENTAL SECTION

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Water was purified through reverse osmosis, followed by double distillation. For column chromatography, silica gel 60 (Fluka) was used. ^{1}H, ^{13}C, ^{15}N, ^{195}Pt, and two-dimensional ^{1}H−^{1}H COSY, ^{1}H−^{13}C and ^{1}H−^{15}N HSQC, and ^{1}H−^{13}C HMBC NMR spectra were recorded with a Bruker Avance III 500 MHz NMR spectrometer at 500.32 MHz (^{1}H), 125.81 MHz (^{13}C), 107.55 MHz (^{195}Pt), and 50.70 MHz (^{15}N) in DMF-d_7 or D_2O (in the case of nedaplatin and its dihydroxido Pt(IV) analogue) at ambient temperature. The splitting of proton resonances in the ^{1}H NMR spectra are defined as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, and m = multiplet. ^{15}N chemical shifts were referenced relative to external NH_4Cl, whereas ^{195}Pt chemical shifts were referenced relative to external $K_2[PtCl_4]$ (see Figure 2, compounds **48**−**50**, including NMR numbering scheme). IR spectra were recorded with a Bruker Vertex 70 FT-IR spectrometer (4000−400 cm^{-1}) by using an ATR unit. Intensities of reported IR bands are defined as follows: br = broad, s = strong, m = medium, and w = weak. Electrospray ionization mass spectrometry was carried out with a Bruker Esquire 3000 instrument using MeOH/H_2O as solvent. Elemental analyses were performed using a Perkin-Elmer 2400 CHN elemental analyzer at the Microanalytical Laboratory of the University of Vienna, Austria, and are within ±0.4% of the calculated values, confirming their ≥95% purity (see Table S9, Supporting Information).

Synthesis and characterization of complexes **1**−**47** and **51**−**53** are described in refs 14, 17, and 20. The synthetic procedure for compounds **48**−**50**, their precursor nedaplatin, and its dihydroxidoplatinum(IV) analogue is reported herein. Synthesis and characterization of compound **48** was reported recently.[22]

(SP-4-3)-Diammineglycolatoplatinum(II) (Nedaplatin). Nedaplatin was prepared starting from K_2PtCl_4 via *cis*-Pt$(NH_3)_2I_2$. An amount of 1.206 g (2.4972 mmol) of the latter was suspended in 36 mL of triply distilled water, and 870 mg (4.7564 mmol) of silver glycolate were added. The suspension was left stirring overnight in the dark, and then the obtained silver iodide was filtered through a sintered glass funnel with a filter paper disk (MN GF-3). The clear solution was stirred at room temperature in the dark for 4 h, and then Amberlite·HCl (conditioned with NaOH to its OH form) was added slowly in small portions to the solution of the complex while stirring until pH (9−10) was achieved. The mixture was left stirring overnight in the dark. Then Amberlite and traces of reduced Pt(0) were filtered off through a sintered glass funnel with a filter paper disk (MN GF-3). The volume of the filtrate was reduced and cooled in the fridge. The obtained precipitate was filtered off, washed with acetone, and dried in a vacuum desiccator over P_2O_5 to yield 498 mg of a white to pale yellow solid. Yield: 498 mg (69%). ^{1}H NMR: δ = 4.02 (s (with Pt satellites) H-1) ppm. ^{13}C NMR: δ = 194.8 (C-2), 68.1 (C-1) ppm. ^{195}Pt NMR: δ = −47 ppm. IR (ATR): ν = 3201 br, 2995 br (ν_{N-H}); 2889 br; 1613 s, 1578 s ($\nu_{C=O}$); 1443 w; 1337 s, 1319 m, 1060 w cm^{-1}. Anal. ($C_2H_8N_2O_3Pt$) C, H, N.

(OC-6-44)-Diammineglycolatodihydroxidoplatinum(IV). Nedaplatin (1.0995 g, 3.6267 mmol) was suspended in 22 mL of triply distilled water, and then an amount of 11 mL of 30% H_2O_2 was added. The mixture was stirred for 3 h at 30 °C (in the dark). The volume of the clear yellow solution obtained was reduced on a rotavapor, cooled in the fridge, and then precipitated with a sufficient amount of cold acetone. The precipitation was finalized with the help of ultrasonic waves and then the final product was filtered off, washed with acetone, and dried in vacuo to obtain a white to pale yellow solid. Yield: 1.3150 g (94%). ^{1}H NMR: δ = 4.30 (s + d, $^3J_{Pt,H}$ = 20.8 Hz, H-1) ppm. ^{195}Pt NMR: δ = 3222 ppm. IR (ATR): ν = 3462 br (ν_{PtO-H}); 3229 br, 3045 br (ν_{N-H}); 2791 w; 1653 s, 1588 m ($\nu_{C=O}$); 1346 s, 1308 s; 1060 w cm^{-1}.

(OC-6-42)-Diamminebis(3-carboxypropanoato)-glycolatoplatinum(IV) (48). Succinic anhydride (950 mg, 9.4934 mmol) and 800 mg (2.0607 mmol) of (OC-6-44)-diammineglycolatodihydroxidoplatinum(IV) were suspended in dry DMF (26 mL), and the reaction mixture was stirred at 60 °C for 8 h. During this time, the solid material dissolved to form a pale yellow solution. DMF was then removed under reduced pressure. The residue was suspended in acetone with the help of ultrasonic waves, filtered off, and washed with acetone. The pale yellow solid obtained was then dried in vacuo. Yield: 1.0865 g (98%). ^{1}H NMR: δ = 12.36 (bs, 2H, COOH), 6.47 (m, 3H, NH_3), 6.05 (bs, 3H, NH_3), 4.08 (bs, 2H, H-1), 2.56 (t, $^3J_{H,H}$ = 6.5 Hz, 4H, H-4 or H-5), 2.49 (t, $^3J_{H,H}$ = 6.5 Hz, 4H, H-4 or H-5) ppm. ^{13}C NMR: δ = 187.0 (C-2), 180.1 (C-3 or C-6), 174.0 (C-3 or C-6), 70.8 (C-1), 30.6 (C-4 or C-5), 29.8 (C-4 or C-5) ppm. ^{15}N NMR: δ = −58.6, −50.7 ppm. ^{195}Pt NMR: δ = 3460 ppm. IR (ATR): ν = 3201 br, 3109 br (ν_{N-H}); 2934 br; 1715 m, 1654 s, 1620 s, 1574 s ($\nu_{C=O}$); 1405 w; 1340 s, 1307 s, 1242 m, 1198 m, 1162 m, 1049 w cm^{-1}. Anal. ($C_{10}H_{18}N_2O_{11}Pt$) C, H, N.

(OC-6-42)-Diammineglycolatobis((4-propyloxy)-4-oxobutanoato)platinum(IV) (49). CDI (253 mg, 1.5566 mmol) in dry DMF (9 mL) was added to a solution of **48** (408 mg, 0.7593 mmol) in dry DMF (10 mL), and the mixture was heated to 60 °C. After 10 min of being stirred, the solution was cooled to room temperature and CO_2 was removed by flushing with argon. Sodium propanolate in *n*-propanol (9 mL) (a piece of Na (15 mg), dissolved in 10 mL of *n*-propanol) was added to the solution and stirred for 30 h at room temperature. Then

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propanol and DMF were removed under reduced pressure to form a yellow oil. The crude product was purified by column chromatography (EtOAc/MeOH, 4:1), then isolated from an EtOAc suspension, and dried in vacuo to yield a white to pale yellow powder. Yield: 95 mg (20%). ^{1}H NMR: δ = 6.57 (m, 3H, NH_3), 6.12 (bs, 3H, NH_3), 4.06 (bs, 2H, H-1), 4.01 (t, $^3J_{H,H}$ = 6.5 Hz, 4H, H-7), 2.57 (m, 4H, H-4), 2.53 (m, 4H, H-5), 1.62 (m, 4H, H-8), 0.91 (t, $^3J_{H,H}$ = 7.2 Hz, 6H, H-9) ppm. ^{13}C NMR: δ = 186.9 (C-2), 179.8 (C-3), 172.6 (C-6), 70.8 (C-1), 65.6 (C-7), 30.5 ($^3J_{Pt,C}$ = 41.0 Hz, C-4), 29.9 (C-5), 21.9 (C-8), 9.9 (C-9) ppm. ^{15}N NMR: δ = −58.3, −49.7 ppm. ^{195}Pt NMR: δ = 3464 ppm. IR (ATR): ν = 3301 br, 3212 br, 3048 br (ν_{N-H}); 2971 br; 1729 m, 1707 s, 1621 s, 1585 m ($\nu_{C=O}$); 1483 w; 1435 m, 1345 m, 1316 s, 1249 w, 1180 s, 1166 m, 1105 m, 984 w cm^{-1}. ESI MS (positive): m/z 643.9 $[M + Na^+]^+$. ESI MS (negative): m/z 620.8 $[M - H^+]^-$, 656.7 $[M + Cl^-]^-$. Anal. ($C_{16}H_{30}N_2O_{11}Pt{\cdot}0.5H_2O$) C, H, N.

(OC-6-42)-Diamminebis((4-cyclopentylamino)-4-oxobutanoato)glycolatoplatinum(IV) (50). CDI (176 mg, 1.0835 mmol) in dry DMF (7 mL) was added to a solution of **48** (284 mg, 0.5285 mmol) in dry DMF (9 mL), and the mixture was heated to 60 °C. After 10 min of being stirred, the solution was cooled to room temperature and CO_2 was removed by flushing with argon. Cyclopentylamine (115 μL, 1.1628 mmol) in 4 mL of dry DMF was added to the solution and stirred for 24 h at room temperature (the solution changed to a yellow suspension). DMF was removed under reduced pressure to form a pale brown solid. The crude product was purified by column chromatography, using EtOAc/MeOH = 2:1, and subsequently isolated from an EtOAc suspension, washed with EtOAc and Et_2O, and dried in vacuo to yield an almost white solid. Yield: 116 mg (33%). ^{1}H NMR: δ = 7.78 (d, $^3J_{H,H}$ = 6.6 Hz, 2H, CONH), 6.49 (m, 3H, NH_3), 6.06 (bs, 3H, NH_3), 4.09 (m, 2H, H-7), 4.06 (s, 2H, H-1), 2.50 (t, $^3J_{H,H}$ = 7.4 Hz, 4H, H-4 or H-5), 2.36 (t, $^3J_{H,H}$ = 7.3 Hz, 4H, H-4 or H-5), 1.83 (m, 4H, H-8), 1.66 (m, 4H, H-9), 1.52 (m, 4H, H-9), 1.44 (m, 4H, H-8) ppm. ^{13}C NMR: δ = 187.0 (C-2), 180.7 (C-3), 171.2 (C-6), 70.9 (C-1), 50.8 (C-7), 32.5 (C-8), 31.8 (C-4 or C-5), 31.5 (C-4 or C-5), 23.6 (C-9) ppm. ^{15}N NMR: δ = −58.7, −50.2, 106.7 ppm. ^{195}Pt NMR: δ = 3459 ppm. IR (ATR): ν = 3269 br, 3058 br (ν_{N-H}); 2958 br; 1697 m, 1632 m, 1571 s ($\nu_{C=O}$); 1483 m; 1441 s, 1339 w, 1319 m, 1255 m, 1195 m, 1106 s, 997 w cm^{-1}. ESI MS (positive): m/z 694.0 $[M + Na^+]^+$, 671.0 $[M + H^+]^+$. ESI MS (negative): m/z 669.9 $[M - H^+]^-$, 705.8 $[M + Cl^-]^-$. Anal. ($C_{20}H_{36}N_4O_9Pt{\cdot}0.5H_2O$) C, H, N.

Crystallographic Structure Determination. Yellow crystals of **7**, suitable for X-ray data collection, were obtained after slow evaporation of a MeOH/EtOAc solution. X-ray diffraction measurement was performed on a Bruker X8 APEXII CCD diffractometer. A single crystal was positioned at 40 mm from the detector, and 1638 frames were measured, each for 15 s over 1° scan width. The data were processed using SAINT software.[31] Crystal data, data collection parameters, and structure refinement details are given in Table S10 (Supporting Information). The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-H atoms were refined with anisotropic displacement parameters. H atoms were inserted in calculated positions and refined with a riding model. The isotropic thermal parameters were estimated to be 1.2 times the values of the equivalent isotropic thermal parameters of the atoms to which hydrogens were bonded. Structure solution was achieved with SHELXS-97 and refinement with SHELXL-97,[32] and graphics were produced with ORTEP-3.[33]

Cytotoxicity Assays. CH1 (ovarian carcinoma, human) cells were a gift from Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.). SW480 (colon carcinoma, human) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). Cells were grown in 75 cm^2 culture flasks (Iwaki/Asahi Technoglass) as adherent monolayer cultures in complete medium, i.e., minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine, and 1% v/v nonessential amino acids (from 100× ready-to-use stock) (all purchased from Sigma-Aldrich) without antibiotics. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. Cytotoxicity in the cell lines mentioned above was determined by the colorimetric MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide, purchased from Fluka). Cells were harvested from culture flasks by trypsinization and seeded in 100 μL aliquots in complete medium into 96-well microculture plates (Iwaki/Asahi Technoglass) in the following densities to ensure exponential growth of untreated controls throughout the experiment: 1.5 × 10^3 (CH1) and 2.5 × 10^3 (SW480) viable cells per well. Cells were allowed to settle and resume exponential growth in drug-free complete culture medium for 24 h, followed by the addition of dilutions of the test compounds in 100 μL/well of the same medium. After continuous exposure for 96 h, the medium was replaced by a 100 μL/well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM L-glutamine) plus 20 μL/well solution of MTT in phosphate-buffered saline (5 mg/mL) (all purchased from Sigma-Aldrich). After incubation for 4 h, medium/MTT mixtures were removed, and the formazan product formed by viable cells was dissolved in DMSO (150 μL/well). Optical densities at 550 nm were measured with a microplate reader (Tecan Spectra Classic), using a reference wavelength of 690 nm to correct for unspecific absorption. The quantity of viable cells was expressed as the percentage of untreated controls, and 50% inhibitory concentrations (IC_{50}) were calculated from concentration−effect curves by interpolation. Evaluation is based on the mean from three independent experiments, each comprising triplicates per concentration level.

Theoretical Calculations. All calculations were performed with the Gaussian 09 software package.[34] The starting structures for optimizations (complexes **1**, **6**, **7**, **22**, **38**) were taken from the available X-ray data;[14,17,18,20] the crystal structure of complex **7** is reported herein. The other compounds were modeled by modification of the latter. A complete conformational search is not feasible for systems with as many degrees of freedom as the present. Furthermore, there are very few force fields capable of handling Pt complexes. In order to see the influence of different conformations to the calculated parameters and to the QSAR models, four different conformers of compound **2** and two of compound **50** were modeled and their geometry was optimized (Figure S5, Supporting Information). In principle, the possible conformational uncertainties are in the chains of the axial ligands because the surroundings around the platinum atoms were taken from the crystallographic data. For compounds **19**, **20**, **42**, **43**, and **44**, which have two chiral centers in the axial carbon chains, the meso *RS* forms were taken for the calculations. These compounds were tested for cytotoxicity as a mixture of *RR*/*SS*/*RS* = 1:1:2 stereoisomers. In the particular case, stereochemistry will not affect essentially the activity because the chiral centers are in the middle of the carbon chains of the axial ligands, which are supposed to be lost after the activation of the complexes via reduction in vivo. In contrast, in the case of oxaliplatin, featuring DACH (1,2-diaminocyclohexane), the *R*,*R* configuration of the ligand should be respected (compounds **51**−**53**).

The DFT long-range corrected hybrid wb97x functional was used for all calculations[35] in connection with the Def2-SVP basis set[36] with effective core potential[37] for optimizing the geometries and calculation of the molecular descriptors. For the calculations of polarizability and dipole moment the basis set were augmented by a set of diffuse functions.

Geometry optimizations were performed in the gas phase and in a water solvent model by using the IEFPCM[38] method. Solvent accessible surface area (SASA) was extracted after single point energy calculation of the gas optimized structures in water environment with the ipcm[39] method, where the cavity is defined by a self-consistent isodensity contour in a water solvent model. Atomic charges were calculated using the NPA approach.[40]

The molar volume and the HOMO and LUMO energies were taken from the gas phase optimized geometries. The energies of solvation were calculated by extracting the energies in water environment (using the iefpcm method) with (adiabatic E_s') or without (vertical E_s) optimization from the total energies in gas phase. For estimation of the ionization potential and electron affinity, the energies of the corresponding anion and cation radicals were calculated in the gas phase and in solvent, with (only for the anion radicals) and without geometry optimization.

QSAR Analysis. QSAR Data Set. The $pIC_{50} = \log(1/IC_{50})$ values, used to develop the QSAR models, were taken from the MTT assays, described in refs 14 and 18−20 for complexes **1**−**47** and **51**−**53** and in the present paper for complexes **48**−**50**. The cytotoxicity data in CH1

and SW480 cells for all investigated compounds in comparison with the clinically approved platinum-based drugs cisplatin, carboplatin, oxaliplatin, and nedaplatin are summarized in Table 1.

By use of QM calculations, the following descriptors were extracted: molar volume (V_m), dipole moment (μ), polarizability (α), charge on the Pt atom (q(Pt)), energy of the HOMO and LUMO (and the corresponding HOMO−LUMO gap), SASA, vertical and adiabatic solvation energies (E_s and E_s'), vertical gas-phase ionization energies (E_i) and electron affinities (E_{ea}), and vertical and adiabatic oxidation (E_is) and reduction (E_{ea}s and E_{ea}s′) potentials in the water solvent model. In addition molecular weight (MW), number of H-bonds donors (H_{don}), numbers of H-bonds acceptors (H_{acc}), and presence/absence of carboxylic groups (COOH) in the axial ligands as constitutional molecular descriptors were used. The values of the used descriptors in the present study are summarized in Tables S3 and S4 (Supporting Information).

Chemometric Methods and Statistics. QSAR analysis was performed with the QSAR program[41] developed by the Ponder group and Schrödinger Strike 2.0 for Maestro application.[42] Standard multiple linear regression (MLR) and principal component analysis (PCA) methods were used to analyze the data, and simulated annealing was employed to identify the best combinations of descriptors. All descriptors were centered and autoscaled prior to analysis.

The robustness of the models and their predictivity were evaluated through R^2, Q^2 (R^2 of cross-validated predictions, using the leave-one-out procedure (LOOP) or leave-two-out procedure (LTOP)), AAR (average absolute error), and rms (root mean squared error). The actual predictive capability of every model was checked with external validation by splitting the data set into training and predictive sets. Five different ways of partitioning the data into training and predictive data sets were used in order to test the robustness of the QSAR model. In each case the training set encompassed 75% of the data while the remaining 25% was selected as (a) random, including 14 compounds representing every subtype, (b) including cisplatin and its bis(ethylamine) analogue derivatives (complexes **1**−**5**, counting all conformers for **2** and **21**−**26**), (c) including most of the ethylenediamine analogues (complexes **6**−**19**), (d) including most of the carboplatin analogues (**27**−**40**), and (e) including oxaliplatin, nedaplatin, and the other part of the carboplatin analogues (complexes **41**−**53**, counting both conformers of **50**). By use of the models derived from the training sets, the pIC_{50} values in the predictive sets were calculated and R^2 predictive, AAS, and RMS were measured.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures with ORTEP view of **7**, chemical structures of the model systems used in the reduction studies, dependency of the QSAR model properties from the descriptors number, loading plots derived from the PCA of the CH1 cells four variable model, and superposition of the optimized conformers of complexes **2** and **50**; tables with comparison of calculated and experimental geometrical parameters, calculated descriptors, and additional statistical data for chosen QSAR models and their external validation, dependency of the QSAR model properties from the input IC_{50} values used for the inactive compounds, elemental analysis data, structure refinement details for **7**; X-ray crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center with number CCDC 909001. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (deposit@ccdc.com.ac.uk).

■ AUTHOR INFORMATION

Corresponding Author

*For F.J.: phone, +45-87155908; e-mail, frj@chem.au.dk. For M.G.: phone, +43-1-4277-52600; fax, +43-1-4277-52680; e-mail, markus.galanski@univie.ac.at.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

FDA, Food and Drug Administration; ESP, electrostatic potential; SAR, structure−activity relationship; QSAR, quantitative structure−activity relationship; QSPR, quantitative structure−property relationship; DFT, density functional theory; MLR, multiple linear regression; MM, molecular mechanics; log $P_{o/w}$, logarithm of partition coefficient between *n*-octanol and water; E_p, redox potential; NPA, natural population analysis; PC, principal component; PCA, principal component analysis; RSD, relative standard deviation; CDI, 1,1′-carbonyldiimidazole

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Supporting Information

Theoretical investigations and density functional theory based quantitative structure activity relationships model for novel cytotoxic Pt(IV) complexes

Hristo Varbanov[†], *Michael A. Jakupec*[†], *Alexander Roller*[†], *Frank Jensen*[∫]*, *Markus Galanski*[†]*, *and Bernhard K. Keppler*[†]

[†] University of Vienna, Institute of Inorganic Chemistry, Währinger Strasse 42, A-1090 Vienna, Austria

[∫] University of Aarhus, Department of Chemistry, Langelandgade 140, 8000 Aarhus C, Denmark

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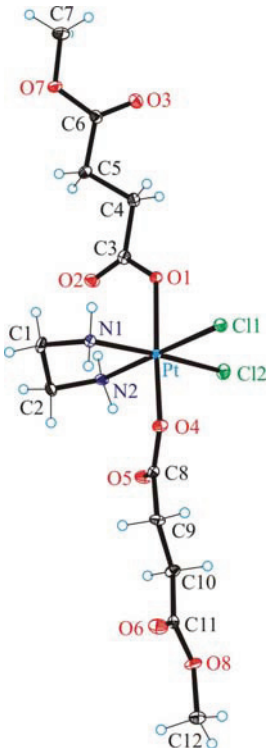

Figure S1. ORTEP view of **7** with atom labeling scheme. The thermal ellipsoids have been drawn at 50% probability level.

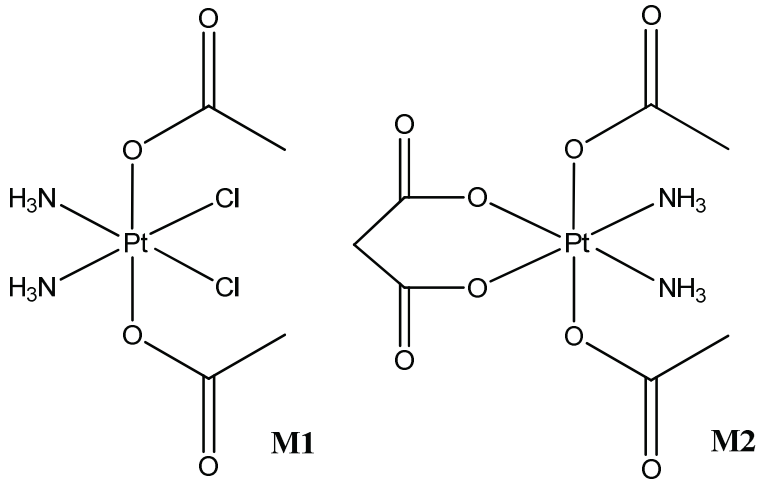

Figure S2. Chemical structure of model systems **M1** and **M2**

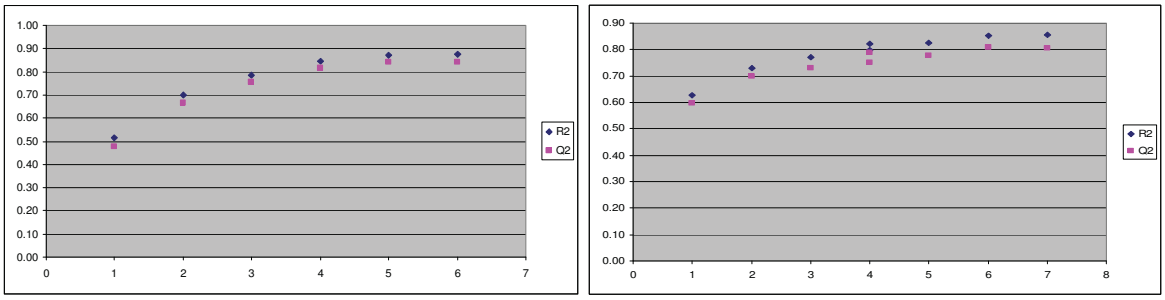

Figure S3. Dependency of the linear fit (R^2) and predictability (Q^2) of the QSAR models from the number of descriptors used for CH1 (left) and for SW480 (right) cells.

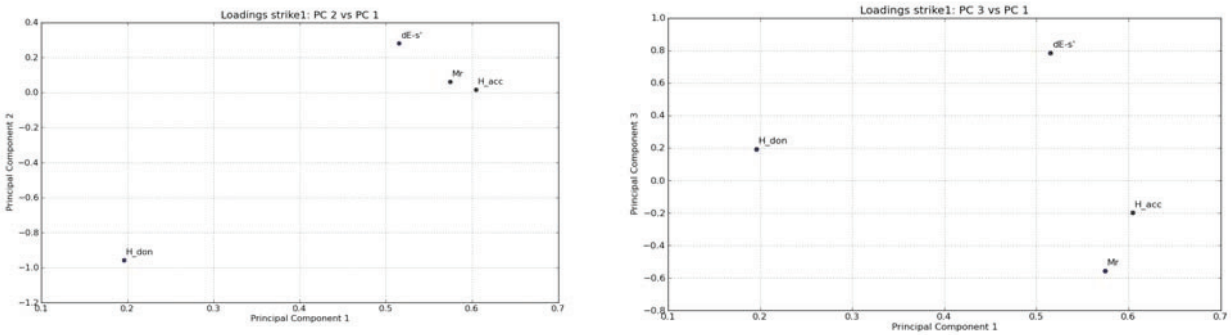

Figure S4. Loading plots derived from PCA on the four descriptors (MW, $E_{eas'}$, H_{don} and H_{acc}), used in the proposed model for cytotoxicity in CH1 cells.

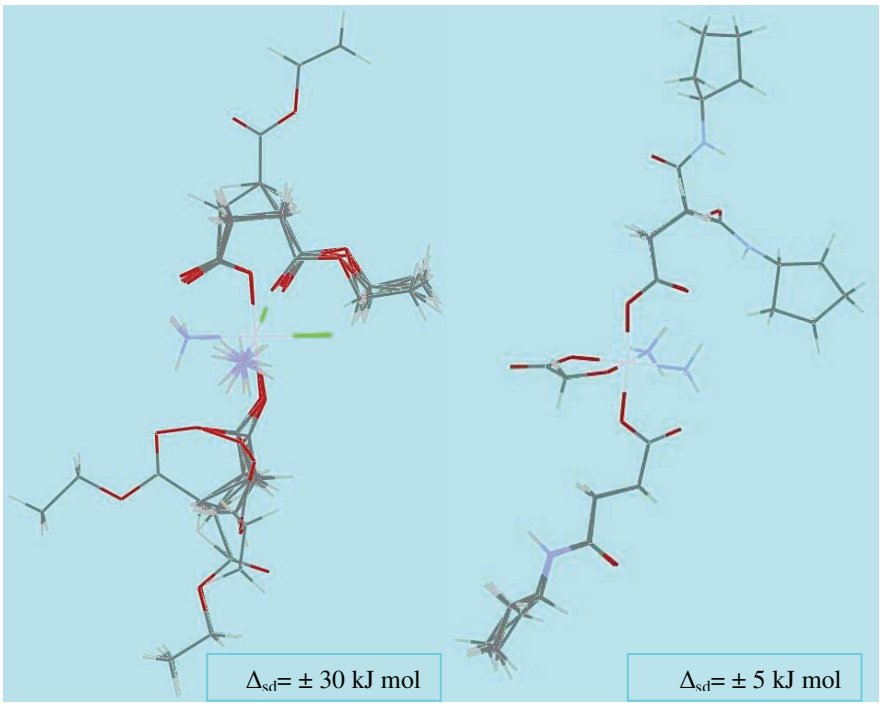

Figure S5. Superposition of the optimized four conformers of complex **2** (left) and two conformers of complex **50** (right).

Table S1. Comparison of crystal structure and wb97x optimized geometries in the gas phase and in a solvent model for **1, 6, 7, 22** and **38.**

bond lengths(Å), angles(°)		**1**	**6**	**7**	**22**	**38**
Pt-N	X-ray	2.050, 2.066	2.054, 2.054	2.050, 2.036	2.063, 2.068	2.050, 2.050
	DFT/gas	2.074, 2.085	2.085, 2.085	2.088, 2.076	2.107, 2.100	2.073, 2.073
	DFT/solv	2.062, 2.055	2.058, 2.058	2.060, 2.053	2.076, 2.080	2.050, 2.051
Pt-Cl/Pt-O_{eq}	X-ray	2.311, 2.319	2.318, 2.318	2.338, 2.307	2.309, 2.324	2.005, 2.031
	DFT/gas	2.310, 2.321	2.307, 2.307	2.318, 2.297	2.319, 2.306	1.965, 1.966
	DFT/solv	2.340, 2.334	2.338, 2.338	2.328, 2.348	2.335, 2.343	1.988, 1.990
Pt-O_{ax}	X-ray	1.993, 2.008	2.011, 2.011	2.000, 2.031	2.039, 2.039	1.997, 1.965
	DFT/gas	2.013, 2.018	2.016, 2.016	2.012, 2.018	1.998, 2.030	2.011, 2.017
	DFT/solv	2.013, 2.016	2.015, 2.015	2.017, 2.012	2.001, 2.025	2.011, 2.017
N-Pt-N	X-ray	90.2	83.7	83.3	92.7	93.1
	DFT/gas	91.0	83.1	83.5	95.9	93.2
	DFT/solv	90.1	83.4	83.4	93.9	91.3
Cl-Pt-Cl/ O_{eq}-Pt-O_{eq}	X-ray	94.4	91.2	89.6	92.2	95.1
	DFT/gas	95.1	94.7	94.1	94.4	97.7
	DFT/solv	93.7	93.0	92.4	93.8	95.4
O_{ax}-Pt-O_{ax}	X-ray	172.6	174.5	168.7	172.7	171.8

	DFT/gas	175.7	176.0	172.3	171.1	173.9
	DFT/solv	174.6	176.8	172.1	171.8	173.1
$Pt\text{-}O_{ax}\text{-}C$	X-ray	123.0, 125.3	125.5, 125.5	125.2, 126.7	123.7, 127.5	123.3, 127.7
	DFT/gas	122.6, 122.4	126.5, 126.5	126.8, 126.0	126.3, 126.7	122.8, 122.2
	DFT/solv	123.0, 123.3	126.2, 126.2	126.4, 125.9	128.6, 126.8	122.9, 123.3
Pt-N-C	X-ray	-	108.2, 108.2	109.2, 109.5	120.2, 118.7	-
	DFT/gas	-	108.2, 108.3	109.0, 107.5	120.6, 118.1	-
	DFT/solv	-	108.8, 108.8	108.3, 109.9	118.5, 120.5	-

Table S2. Calculated descriptors for the investigated compounds: molecular weight (MW), molar volume (Vm), polarizability (α), solvent accessible surface area (SASA), dipole moment (μ), charge at the Pt atom (q(Pt)), vertical and adiabatic energy of hydration (Es and Es'), number of H-bond donors and acceptors (H_{don} and H_{acc}), presence (1) or absence (0) of COOH group. The coloring is based on the subtypes, exerting the same equatorial ligands (Figure 2)

complex	MW (g/mol)	Vm (cm^3/mol)	α ($bohr^3$)	SASA ($bohr^2$)	μ (D)	q (Pt) (a.u.)	Es (kJ/mol)	Es' (kJ/mol)	H_{don}	H_{acc}	COOH
1	534.22	222.60	215.54	1121.38	5.217	0.913	86.035	89.785	4	8	1
2a	590.32	280.04	266.29	1105.02	4.895	0.919	78.408	82.114	2	8	0
2b	590.32	318.18	270.31	1034.83	4.996	0.917	92.614	96.406	2	8	0
2c	590.32	278.93	264.05	1143.75	6.813	0.917	71.440	75.377	2	8	0
2d	590.32	308.56	265.24	1232.36	6.339	0.918	70.790	74.999	2	8	0
3	620.35	295.30	284.66	1490.84	2.696	0.922	110.316	115.194	6	10	0
4	616.41	320.11	300.92	1240.14	3.139	0.923	90.276	95.023	4	8	0
5	668.49	351.10	340.09	1270.93	4.711	0.924	92.679	97.081	4	8	0
6	560.24	262.29	235.78	1249.41	10.038	0.883	111.868	119.697	4	8	1
7	588.30	279.03	263.82	1333.65	9.631	0.885	107.458	114.152	2	8	0
8	616.35	303.87	289.57	1465.76	9.456	0.885	107.375	114.120	2	8	0
9	644.40	350.94	314.84	1583.99	9.475	0.885	107.265	113.794	2	8	0
10	672.45	344.68	340.01	1866.04	9.426	0.885	107.383	113.902	2	8	0
11	646.38	313.16	308.55	1232.00	11.048	0.885	138.603	146.752	6	10	0
12	642.43	325.44	324.77	1368.51	9.604	0.885	122.957	130.222	4	8	0
13	694.51	357.09	364.41	1451.39	9.768	0.885	122.864	130.239	4	8	0
14	588.31	271.41	262.25	1272.38	5.813	0.884	105.143	110.849	4	8	1

15	644.42	320.71	312.35	1313.59	5.582	0.884	99.242	104.679	2	8	0
16	674.45	351.78	330.49	1229.71	4.147	0.885	129.568	135.961	6	10	0
17	670.50	340.28	339.25	1407.21	12.531	0.886	97.084	102.165	4	8	0
18	722.58	407.88	380.11	1339.52	9.977	0.886	104.990	110.228	4	8	0
19	644.40	321.27	311.74	1261.18	6.453	0.883	97.187	102.575	2	8	0
20	672.46	356.75	336.82	1203.31	6.300	0.884	97.318	102.509	2	8	0
21	590.32	277.70	269.56	1432.61	6.616	0.900	137.789	145.133	4	8	1
22	618.38	331.49	296.10	1714.83	9.048	0.899	111.916	118.178	2	8	0
23	646.43	296.25	321.51	1911.50	10.306	0.897	116.950	123.075	2	8	0
24	674.49	372.49	346.95	1425.27	10.418	0.897	116.643	123.350	2	8	0
25	674.49	371.93	345.56	1406.09	10.369	0.897	115.271	121.218	2	8	0
26	724.59	381.31	396.75	1879.12	5.916	0.899	124.879	131.664	4	8	0
27	605.42	298.95	264.44	1311.26	6.574	1.330	117.073	123.275	4	12	1
28	633.48	307.57	291.45	1320.90	9.854	1.330	109.316	115.441	2	12	0
29	661.53	338.83	316.66	1284.74	9.881	1.330	107.975	114.256	2	12	0
30	689.58	379.24	342.06	1310.12	9.949	1.330	107.649	114.011	2	12	0
31	689.58	371.64	340.04	1302.96	9.738	1.330	106.280	111.909	2	12	0
32	717.64	389.86	367.08	1219.40	9.938	1.330	107.711	114.122	2	12	0
33	719.69	396.75	360.53	1273.95	5.264	1.330	131.364	137.835	4	14	0
34	687.62	331.97	351.82	1665.79	6.335	1.330	121.395	127.642	4	12	0
35	739.69	402.95	391.02	1292.81	6.090	1.330	120.894	127.001	4	12	0
36	767.75	426.16	415.08	1864.06	6.278	1.330	119.904	126.080	4	12	0
37	785.67	450.17	427.26	2752.28	10.008	1.330	115.488	121.547	4	12	0
38	633.48	288.88	288.34	1311.57	10.410	1.330	128.700	137.113	4	12	1
39	661.53	340.69	312.80	1385.26	10.533	1.331	120.313	128.774	2	12	0
40	689.58	339.05	338.05	1399.50	10.027	1.330	118.948	127.480	2	12	0
41	715.67	424.16	370.92	1258.04	9.734	1.334	121.170	128.906	4	12	0

42	661.53	337.39	311.20	1426.34	9.210	1.326	126.238	132.477	4	12	1
43	689.58	359.24	335.02	1431.79	9.273	1.326	118.219	125.579	2	12	0
44	743.72	426.20	396.18	1425.48	12.598	1.331	112.826	120.966	4	12	0
45	689.58	361.41	334.48	1457.78	8.237	1.324	119.234	130.934	4	12	1
46	717.78	387.67	359.18	1613.85	8.780	1.324	111.339	120.691	2	12	0
47	771.78	495.12	420.93	1987.67	9.252	1.324	117.298	124.778	4	12	0
48	537.35	227.62	219.09	1063.21	6.278	1.331	108.407	113.363	4	11	1
49	621.51	343.68	296.51	1549.29	8.507	1.331	98.825	104.036	2	11	0
50a	671.62	341.22	345.91	1233.02	6.170	1.330	112.372	117.504	4	11	0
50b	671.62	362.46	340.48	1207.20	5.614	1.330	108.068	114.896	4	11	0
51	631.46	288.83	286.22	1354.69	12.809	1.318	153.353	164.242	4	12	1
52	687.57	355.00	337.82	1235.64	15.747	1.317	126.985	136.235	2	12	0
53	713.65	391.69	373.29	1457.55	11.945	1.317	137.753	146.294	4	12	0

Table S3. Calculated descriptors for the investigated compounds (all values are in eV): energies of HOMO (E_{HOMO}), LUMO (E_{LUMO}) and their gap (H/L gap)), vertical ionization energy (E_i) in vacuum and in water (E_is), vertical electron affinity in vacuum (E_{ea}) and in water (E_{ea}s), adiabatic electron affinity in water (E_{ea}s'). The coloring is based on the subtypes, exerting the same equatorial ligands (Figure 2).

complex	E_{HOMO}	E_{LUMO}	H/L gap	E_i	E_is	E_{ea}	E_{ea}s	E_{ea}s'
1	-10.005	-0.487	9.517	9.805	8.400	1.025	2.938	4.225
2a	-9.977	-0.447	9.530	9.740	8.279	0.989	2.944	3.976
2b	-10.086	-0.608	9.477	9.899	8.402	1.118	2.976	3.988
2c	-9.670	-0.109	9.561	9.397	8.348	0.716	2.858	4.102
2d	-9.680	-0.133	9.547	9.435	8.216	0.670	2.864	3.898
3	-9.702	-0.331	9.372	9.494	7.428	0.897	2.879	3.915
4	-9.592	-0.316	9.275	9.346	7.299	0.872	2.785	4.124
5	-9.477	-0.249	9.228	9.138	7.211	0.830	2.884	3.920
6	-9.796	-0.465	9.330	9.548	8.243	1.023	2.989	3.841
7	-9.827	-0.504	9.323	9.577	8.325	1.093	2.968	3.997
8	-9.813	-0.491	9.323	9.562	8.252	1.084	2.965	3.996
9	-9.812	-0.489	9.323	9.558	8.253	1.084	2.965	3.995
10	-9.810	-0.486	9.323	9.555	8.251	1.083	2.964	3.997
11	-9.513	-0.420	9.093	9.287	7.323	1.016	2.951	3.982
12	-9.485	-0.430	9.055	9.250	7.277	1.030	2.953	3.986
13	-9.408	-0.421	8.987	9.074	7.184	1.023	2.950	3.981
14	-9.930	-0.573	9.357	9.674	8.228	1.143	2.954	4.060
15	-9.887	-0.524	9.363	9.622	8.224	1.103	2.943	3.971
16	-9.379	-0.512	8.867	9.166	7.236	1.092	2.933	4.058

17	-8.932	-1.103	7.829	8.564	7.157	1.781	3.056	4.281
18	-8.596	-0.916	7.680	8.187	7.043	1.583	3.035	4.137
19	-9.880	-0.518	9.362	9.608	8.225	1.107	2.942	3.967
20	-9.874	-0.512	9.362	9.598	8.225	1.104	2.941	3.965
21	-9.425	-0.657	8.769	9.088	7.672	1.265	2.969	3.976
22	-9.219	-0.479	8.741	8.857	7.659	1.096	2.961	3.965
23	-9.210	-0.492	8.719	8.839	7.650	1.112	2.969	3.980
24	-9.210	-0.494	8.716	8.834	7.650	1.117	2.972	3.975
25	-9.200	-0.488	8.712	8.820	7.644	1.113	2.971	3.976
26	-9.104	-0.401	8.703	8.730	7.607	1.034	2.946	3.949
27	-9.510	-0.518	8.992	9.237	7.988	1.305	2.648	4.170
28	-9.431	-0.438	8.992	9.153	7.979	1.234	2.632	4.142
29	-9.421	-0.422	8.999	9.140	7.979	1.224	2.630	4.142
30	-9.419	-0.417	9.002	9.135	7.981	1.223	2.629	4.143
31	-9.410	-0.405	9.004	9.126	7.977	1.211	2.625	4.140
32	-9.415	-0.413	9.002	9.130	7.980	1.222	2.629	4.142
33	-9.453	-0.396	9.057	9.167	7.959	1.205	2.600	4.122
34	-9.410	-0.348	9.061	9.124	7.955	1.159	2.595	4.115
35	-9.400	-0.340	9.060	9.111	7.954	1.153	2.595	4.115
36	-9.390	-0.333	9.057	9.099	7.954	1.148	2.597	4.118
37	-9.409	-0.446	8.963	9.120	7.044	1.262	2.637	4.148
38	-9.430	-0.443	8.986	9.153	7.959	1.244	2.576	4.091
39	-9.413	-0.399	9.014	9.128	7.952	1.208	2.562	4.081
40	-9.412	-0.396	9.016	9.123	7.962	1.210	2.578	4.093
41	-9.354	-0.333	9.021	9.050	7.301	1.145	2.531	4.125
42	-9.455	-0.441	9.014	9.167	7.968	1.262	2.571	4.100

43	-9.446	-0.405	9.041	9.150	7.962	1.236	2.561	4.076
44	-8.995	-0.500	8.494	8.598	7.168	1.325	2.616	4.099
45	-9.400	-0.374	9.026	9.107	7.968	1.216	2.587	4.098
46	-9.358	-0.318	9.040	9.062	7.963	1.168	2.579	4.128
47	-9.283	-0.209	9.074	8.979	7.947	1.066	2.545	4.052
48	-9.212	-0.089	9.123	8.877	7.136	0.801	2.351	3.872
49	-9.166	0.010	9.176	8.793	7.133	0.722	2.334	3.838
50a	-9.133	0.088	9.400	8.747	7.116	0.649	2.303	3.806
50b	-9.312	-0.092	9.041	8.887	7.148	0.849	2.358	3.898
51	-9.327	-0.480	8.847	9.033	7.878	1.290	2.735	4.094
52	-9.130	-0.277	8.853	8.828	7.870	1.100	2.718	4.076
53	-9.109	-0.213	8.896	8.802	7.858	1.044	2.692	4.056

Table S4. QSAR models for the cytotoxicity in the cell line CH1 with simulated annealing chosen combination of descriptors. (Q^2, AAR´and RMS´ are the R^2, AAR and RMS values for cross validated predictions, using LOOP; the derived coefficients are for a model equation with auto scaled values of the descriptors).

Model	Number of variables	Used descriptors	R^2	Q^2	AAR	AAR'	RMS	RMS'	Coefficients
1	1	q(Pt)	0.4132	0.3920	0.6318	0.6431	0.8241	0.8389	-0.643
2	1	H_{acc}	0.5130	0.4953	0.5695	0.5795	0.7508	0.7643	-0.716
3	2	H_{don}, H_{acc}	0.6978	0.6769	0.4707	0.4878	0.5914	0.6115	-0.435, -0.651
4	2	q(Pt), H_{don}	0.6847	0.6643	0.4698	0.4843	0.6041	0.6233	-0.633, -0.522
5	3	H_{don}, H_{acc}, COOH	0.7450	0.7226	0.4160	0.4355	0.5433	0.5666	-0.363, -0.647, -0.229
6	3	q(Pt), $E_{ea}s'$, H_{don}	0.7867	0.7641	0.3794	0.4000	0.4968	0.5226	-0.516, -0.340, -0.513
7	3	q(Pt), H_{don}, COOH	0.7130	0.6866	0.4415	0.4620	0.5763	0.6023	-0.617, -0.466, -0.178
8	3	MW, q(Pt), H_{don}	0.7122	0.6800	0.4271	0.4505	0.5769	0.6087	0.187, -0.716, -0.541
9	3	α, q(Pt), H_{don}	0.7285	0.6987	0.4088	0.4306	0.5603	0.5905	0.224, -0.710, -0.546
10	3	q(Pt), dE_is, H_{don}	0.7219	0.6910	0.4305	0.4529	0.5674	0.5980	-0.652, -0.227, -0.639
11	3	$E_{ea}s'$, H_{don}, H_{acc}	0.7768	0.7542	0.3998	0.4210	0.5083	0.5334	-0.308, -0.444,

									-0.525
12	4	MW, E_{eas}', H_{don}, H_{acc}	0.8459	0.8228	0.3434	0.3695	0.4223	0.4529	0.309, -0.359, -0.456, -0.656
13	4	MW, q(Pt), E_{eas}', H_{don}	0.8458	0.8196	0.3324	0.3595	0.4225	0.4570	0.280, -0.621, -0.399, -0.539
14	4	q(Pt), E_i, E_{eas}', H_{don}	0.8137	0.7802	0.3611	0.3921	0.4643	0.5044	-0.584, -0.180, -0.336, -0.543
15	5	MW, α, q(Pt), E_{eas}', H_{don}	0.8713	0.8471	0.3178	0.3484	0.3859	0.4207	-1.056, 1.268, -0.487, -0.316, -0.541
16	5	α, E_{ea}, E_{eas}', H_{don}, H_{acc}	0.8505	0.8229	0.3393	0.3721	0.4160	0.4527	0.302, -0.051, -0.309, -0.462, -0.625

Table S5. External validation of the best models for cytotoxicity in CH1 cells, obtained with three, four or five descriptors:

Model N	Training set	Number of variables	Used descriptors	R^2	Q^2	**Pred R^2**	pred AAR	pred RMS
5	a	3	H_{don}, H_{acc}, COOH	0.751	0.7197	**0.7210**	0.402	0.5394
	b			0.7278	0.6879	**0.7828**	0.5039	0.7049
	c			0.7728	0.7383	**0.6263**	0.5655	0.6976
	d			0.7239	0.6924	**0.808**	0.3738	0.4831
	e			0.7532	0.7263	**0.7139**	0.4734	0.5492
6	a	3	q(Pt), $E_{ea}s'$, H_{don}	0.7931	0.7652	**0.7560**	0.3726	0.5044
	b			0.7699	0.7289	**0.8158**	0.4782	0.6492
	c			0.8085	0.7731	**0.7043**	0.4672	0.6206
	d			0.7778	0.7492	**0.8471**	0.3511	0.4311
	e			0.7868	0.7622	**0.7794**	0.3765	0.4822
8	a	3	MW, q(Pt), H_{don}	0.7329	0.6872	**0.6390**	0.4236	0.6136
	b			0.6382	0.5864	**0.7908**	0.4658	0.692
	c			0.7249	0.6782	**0.5843**	0.6199	0.7358
	d			0.6898	0.6414	**0.7452**	0.4671	0.5565
	e			0.8358	0.8132	**0.0635**	0.7271	0.9935
9	a	3	α, q(Pt), H_{don}	0.748	0.7056	**0.6593**	0.4084	0.596
	b			0.6512	0.6012	**0.8116**	0.443	0.6566
	c			0.7445	0.7029	**0.5970**	0.6029	0.7245
	d			0.7052	0.6587	**0.7662**	0.4502	0.5332
	e			0.8395	0.8176	**0.1942**	0.6843	0.9216
11	a	3	$E_{ea}s'$, H_{don}, H_{acc}	0.7804	0.7511	**0.7536**	0.3976	0.5068
	b			0.7606	0.7191	**0.8103**	0.4987	0.6589
	c			0.8018	0.7705	**0.7116**	0.4759	0.6129

	d			0.7652	0.7368	**0.8474**	0.3648	0.4307
	e			0.7649	0.7363	**0.8346**	0.3534	0.4176
	a			0.8614	0.8313	**0.7753**	0.4361	0.484
	b			0.8381	0.8027	**0.8546**	0.434	0.5767
12	c	4	MW, $E_{ea}s'$, H_{don}, H_{acc}	0.8736	0.8478	**0.754**	0.4838	0.566
	d			0.8303	0.7966	**0.9141**	0.2398	0.3232
	e			0.8459	0.8112	**0.842**	0.3824	0.4082
	a			0.8553	0.8199	**0.8023**	0.3662	0.454
	b			0.8422	0.8054	**0.8385**	0.4723	0.608
13	c	4	MW, q(Pt), $E_{ea}s'$, H_{don}	0.8644	0.8285	**0.7494**	0.4912	0.5713
	d			0.8297	0.7913	**0.9199**	0.2564	0.3119
	e			0.8729	0.8435	**0.6236**	0.5156	0.6299
	a			0.8275	0.773	**0.7422**	0.4167	0.5185
	b			0.7723	0.7215	**0.8454**	0.4307	0.5949
14	c	4	q(Pt), E_i, $E_{ea}s'$, H_{don}	0.8524	0.8155	**0.6043**	0.598	0.7179
	d			0.8176	0.7766	**0.8157**	0.3818	0.4734
	e			0.8163	0.774	**0.8161**	0.3614	0.4403
	a			0.8646	0.8154	**0.7701**	0.445	0.4896
	b			0.8486	0.8041	**0.8365**	0.4554	0.6117
15	c	5	α, E_{ea}, $E_{ea}s'$, H_{don}, H_{acc}	0.8785	0.8416	**0.7246**	0.5217	0.5989
	d			0.8352	0.7956	**0.9176**	0.2341	0.3165
	e			0.8494	0.8105	**0.8491**	0.3615	0.3989
	a			0.8769	0.8438	**0.8439**	0.34	0.4034
	b			0.8648	0.8296	**0.8802**	0.4132	0.5236
16	c	5	MW, α, q(Pt), $E_{ea}s'$, H_{don}	0.8894	0.857	**0.8073**	0.4262	0.501
	d			0.8574	0.8216	**0.9352**	0.2446	0.2806
	e			0.8787	0.8455	**0.7716**	0.4136	0.4906

Table S6. QSAR models for cytotoxicity in the cell line SW480 with simulated annealing chosen combination of descriptors (Q^2, AAR´and RMS´ are the R^2, AAR and RMS values for cross validated predictions, using LOOP; the derived coefficients are for a model equation with auto scaled values of the descriptors).

Model	Number of variables	Used descriptors	R^2	Q^2	AAR	AAR'	RMS	RMS'	Coefficients
1	1	q(Pt)	0.5453	0.5292	0.5847	0.5950	0.7388	0.7518	-0.738
2	2	H_{don}, H_{acc}	0.7009	0.6785	0.4446	0.4620	0.5992	0.6212	-0.321, -0.727
3	2	q(Pt), H_{don}	0.7196	0.7010	0.4173	0.4315	0.5802	0.5991	-0.731, -0.418
4	3	q(Pt), Es', H_{don}	0.7535	0.7247	0.4246	0.4495	0.5440	0.5749	-0.813, -0.220, -0.505
5	3	q(Pt), E_i, H_{don}	0.7587	0.7259	0.4083	0.4337	0.5382	0.5736	-0.811, -0.217, -0.454
6	3	q(Pt), E_{HOMO}, H_{don}	0.7552	0.7213	0.4121	0.4381	0.5422	0.5784	-0.812, 0.210, -0.461
7	3	α, q(Pt), H_{don}	0.7466	0.7208	0.4312	0.4539	0.5515	0.5789	0.176, -0.791, -0.436
8	3	Es', H_{don}, H_{acc}	0.7607	0.7282	0.4151	0.4419	0.5360	0.5712	-0.304, -0.423, -0.861
9	4	Es, H_{don}, H_{acc}, COOH	0.7893	0.7522	0.4084	0.4426	0.5030	0.5454	-0.334, -0.387, -0.867, -0.170
10	4	Es', H_{don},	0.7875	0.7507	0.4097	0.4438	0.5050	0.5470	-0.329, -0.377,

		H_{acc}, COOH							-0.869, -0.174
11	4	q(Pt), E_{LUMO}, E_i, H_{don}	0.7811	0.7486	0.4084	0.4395	0.5126	0.5494	-0.891, 0.167, -0.244, -0.457
12	4	E_i, E_{ea}, E_{ea}s, H_{don}	0.8094	0.7834	0.3891	0.4167	0.4783	0.5099	-0.307, -0.412, 0.845, -0.431
13	4	E_i, E_{ea}s, E_{ea}s', H_{don}	0.7965	0.7648	0.3900	0.4197	0.4943	0.5314	-0.185, 0.711, -0.365, -0.412
14	5	E_i, E_{ea}, Es, E_{ea}s, H_{don}	0.8230	0.7912	0.3784	0.4141	0.4610	0.5007	-0.281, -0.459, -0.147, 0.871, -0.486
15	5	q(Pt), H/Lgap, E_i, E_{ea}s, H_{don}	0.8145	0.7768	0.3798	0.4157	0.4719	0.5176	-0.329, 0.678, -0.818, 0.695, -0.409

Table S7. External validation of the best models for SW480 cells, obtained with three, four or five descriptors:

Model N	Training set	Number of variables	Used descriptors	R^2	Q^2	**Pred R^2**	pred AAR	pred RMS
4	a	3	q(Pt), Es´, H_{don}	0.7655	0.7280	**0.6834**	0.4844	0.6029
	b			0.6899	0.6304	**0.7981**	0.6115	0.7160
	c			0.7905	0.7440	**0.4651**	0.7380	0.8212
	d			0.7467	0.7087	**0.7074**	0.5710	0.7321
	e			0.8262	0.8028	**0.3439**	0.7227	0.8329
6	a	3	q(Pt), E_{HOMO}, H_{don}	0.7519	0.7042	**0.7481**	0.4249	0.5379
	b			0.6644	0.6111	**0.8211**	0.5277	0.6740
	c			0.8147	0.7812	**0.3419**	0.7643	0.9109
	d			0.7409	0.6936	**0.7483**	0.4843	0.6790
	e			0.8288	0.7975	**0.3705**	0.6516	0.8159
8	a	3	Es, H_{don}, H_{acc}	0.7915	0.7538	**0.6227**	0.5458	0.6582
	b			0.7294	0.6824	**0.7667**	0.6592	0.7697
	c			0.8040	0.7485	**0.5766**	0.6131	0.7307
	d			0.7385	0.6908	**0.7976**	0.4893	0.6088
	e			0.7836	0.7450	**0.6809**	0.4268	0.5809
9	a	4	Es, H_{don}, H_{acc}, COOH	0.8119	0.7644	**0.6638**	0.5392	0.6213
	b			0.7482	0.6909	**0.7892**	0.6224	0.7316
	c			0.8270	0.7681	**0.5750**	**0.6405**	0.7320
	d			0.7843	0.7340	**0.7593**	0.5677	0.6640
	e			0.8241	0.7848	**0.5864**	0.5129	0.6613
11	a	4	q(Pt),	0.7684	0.6919	**0.8088**	0.4027	0.4686

	b		E_{LUMO}, E_i, H_{don}	0.7034	0.6534	**0.8608**	0.5016	0.5945
	c			0.8178	0.7769	**0.4493**	0.7042	0.8333
	d			0.7571	0.7048	**0.8254**	0.4502	0.5656
	e			0.8439	0.8118	**0.5137**	0.5467	0.7171
12	a	4	E_i, E_{ea}, $E_{ea}s$, H_{don}	0.8045	0.7716	**0.8003**	0.4235	0.4789
	b			0.7451	0.6966	**0.8799**	0.4801	0.5522
	c			0.8531	0.8138	**0.5918**	0.6445	0.7174
	d			0.8033	0.7667	**0.7904**	0.4678	0.6196
	e			0.8409	0.8116	**0.4570**	0.6464	0.7578
13	a	4	E_i, $E_{ea}s$, $E_{ea}s'$, H_{don}	0.8009	0.7550	**0.7445**	0.4786	0.5416
	b			0.7312	0.6669	**0.8705**	0.4677	0.5734
	c			0.8577	0.8208	**0.5174**	0.6361	0.7801
	d			0.7834	0.7379	**0.7856**	0.4471	0.6266
	e			0.8195	0.7797	**0.5191**	0.6080	0.7131
14	a	5	E_i, E_{ea}, Es, $E_{ea}s$, H_{don}	0.8274	0.7486	**0.7697**	0.4388	0.5142
	b			0.7466	0.6722	**0.9020**	0.4415	0.4988
	c			0.8761	0.8364	**0.5470**	0.6869	0.7557
	d			0.8222	0.7824	**0.7861**	0.4741	0.6260
	e			0.8527	0.8251	**0.4405**	0.6629	0.7692
15	a	5	q(Pt), H/Lgap, E_i, $E_{ea}s$, H_{don}	0.8057	0.7410	**0.8381**	0.3838	0.4311
	b			0.7577	0.6872	**0.8924**	0.4661	0.5228
	c			0.8554	0.8006	**0.6248**	0.6118	0.6878
	d			0.8019	0.7461	**0.8268**	0.4338	0.5632
	e			0.8516	0.8181	**0.5809**	0.5569	0.6657

Table S8. Statistical parameters for the best four-variable models, using IC_{50} = 600, 1000 or 2000 μM as input for the inactive compounds from **subset 2**.

	model 1			model 2			model 3		
input IC_{50}	R^2	Q^2(LOOP)	AAR	R^2	Q^2(LOOP)	AAR	R^2	Q^2(LOOP)	AAR
600	0.79	0.75	0.41	0.80	0.77	0.39	0.81	0.78	0.39
1000	0.80	0.76	0.43	0.80	0.77	0.40	0.82	0.80	0.40
2000	0.80	0.77	0.47	0.81	0.78	0.43	0.83	0.80	0.42

Table S9. Elemental analysis data.

Compound	**Formula**	**MW**	**Calculated (%)**			**Found (%)**		
			C	**H**	**N**	**C**	**H**	**N**
nedaplatin	$C_2H_8N_2O_3Pt$	303.17	7.92	2.66	9.24	8.03	2.45	9.05
48	$C_{10}H_{18}N_2O_{11}Pt$	537.33	22.35	3.38	5.21	22.32	3.24	5.04
49	$C_{16}H_{30}N_2O_{11}Pt{\cdot}0.5H_2O$	630.50	30.48	4.96	4.44	30.41	4.59	4.39
50	$C_{20}H_{36}N_4O_9Pt{\cdot}0.5H_2O$	680.61	35.29	5.48	8.23	35.15	5.10	7.98

Table S10. Crystal data and details of data collection for **7**

Complex	**7**
empirical formula	$C_{12}H_{22}Cl_2N_2O_8Pt$
fw	588.31
space group	P-1
a, Å	8.2185(3)
b, Å	10.0375(4)
c, Å	12.4165(5)
V, Å^3	891.63(6)
Z	2
λ, Å	0.71073
ρ_{calcd}, g cm^{-3}	2.191
crystal size, mm	0.10 x 0.08 x 0.02
T, K	100
μ, mm^{-1}	8.211
R1[a]	0.0234
*w*R2[b]	0.0486
GOF[c]	0.998

[a] $R1 = \sum||F_o| - |Fc||/\sum|F_o|$, [b] $wR2 = \{\sum [w (F_o^2 - F_c^2)^2] / \sum[w(Fo^2)^2]\}^{1/2}$. [c] $GOF = \{\sum[w(Fo^2 - Fc^2)^2] /(n - p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.

III. CONCLUSIONS

In this PhD thesis, the synthesis, characterization, pharmacological, theoretical and QSAR investigations of 29 novel bis-, tris- and tetrakis(carboxylato)platinum(IV) complexes with potential antitumor activity are reported.

In the first part of the work, a series of six novel bis(carboxylato)dichloridobis(ethylamine)platinum(IV) complexes, featuring different axial ligands have been synthesized and fully characterized. Their lipophilicity (as log $P_{o/w}$ values) and cytotoxicity in four human tumor cell lines were evaluated and structure-activity relationships were drawn. The most active complexes demonstrated IC_{50} values in the low nanomolar range, significantly lower than that of cisplatin in both, cisplatin sensitive and resistant cell lines. Linear correlation between lipophilicity and cytotoxicity in the tested cell lines was observed in the series, while the most lipophilic compounds were the most cytotoxic. However, the complex, featuring an axial cyclopentylamide residue did not follow this trend, as a higher cytotoxicity could be expected from its log $P_{o/w}$ value.

In the second part, 21 new tetracarboxylatoplatinum(IV) complexes, designed as prodrugs of carboplatin were synthesized, characterized and their lipohilicity, cytotoxicitiy and redox potentials were determined. Contrary to corresponding diam(m)inebis(carboxylato)dichloridoplatinum(IV) compounds, cytotoxicity could not significantly be enhanced by variation of the axial ligands and their lipophilicity. Even the most lipophilic complexes possessed lower cytotoxic potency than carboplatin. In addition, the rate of reduction by ascorbic acid of a chosen diamminetetrakis(carboxylato)platinum(IV) compound and its diamine-bis(carboxylato)dichlorido analogue, featuring the same axial ligands were examined.

The considerable difference found between the redox kinetics is in accordance with large variance in cytotoxicity, but not with similar redox potentials.

In the last part of the work, theoretical and QSAR investigations on 53 platinum(IV) complexes, bearing the equatorial sphere of cisplatin, its bis(ethylamine) analogue, its ethylendiamine analogue, carboplatin, nedaplatin and oxaliplatin were performed. The compounds used for the study were synthesized, characterized and tested for cytotoxicity in our laboratories, more than halve of them within this PhD work. The hybrid DFT functional wb97x was utilized for geometry structure optimization and calculation of several molecular properties of the complexes. On basis of the computations, the compounds were divided in subsets and an attempt for explanation of their divergent physicochemical properties was made. Furthermore, reliable, robust and predictive models for their cytotoxic activity in CH1 and SW480 cells were developed. To the best of our knowledge, this was the first report of DFT descriptors-based QSAR models for the *in vitro* cytotoxicity of platinum(IV) complexes.

The influence of the type of axial and equatorial ligands on the cytotoxic potency of platinum(IV) complexes was studied by means of analytical and theoretical methods. For this purpose, appropriate procedures for the preparation of various platinum(IV) compounds, prodrugs of clinically applied cytostatics and their analogues were developed. The obtained results represent one further step towards a better understanding of the biological behavior of platinum(IV) carboxylato complexes and their subsequent rational development. However, there are still many open questions, concerning the fate of platinum(IV)-based drugs *in vivo* and the ways to control their extracellular and intracellular pharmacokinetics. The ongoing *in vivo* studies with chosen compounds from

the series, developed within this PhD work, together with further analytical and theoretical investigations are expected to contribute to a better fine-tuning of successful platinum(IV)-based chemotherapeutics.

Curriculum vitae

Personal information

Surname(s) / First name(s)	**Varbanov, Hristo Plamenov**
Address(es)	Starkfriedgasse 15 / 3205, 1180 Vienna, Austria
Telephone(s)	Mobile +4369917123262
E-mail(s)	hristo.varbanov@univie.ac.at; xhr_varbanov@abv.bg
Nationality	Bulgarian
Date and Place of birth	12th February 1984; Sofia, Bulgaria
Gender	Male
Marital status	Single

Education and training

Dates	15th February 2009 – to date
PhD Research	Novel antineoplastic platinum(IV) complexes: Synthesis, characterization, biological investigations and structure-activity relationships
Name and type of organisation providing education and training	Bioinorganic Chemistry group (M. Galanski, B.K. Keppler), Department of Inorganic Chemistry, University of Vienna, Vienna, Austria
Dates	05th September – 01st December 2011
Research work	DFT calculations of anticancer Pt(IV) complexes; building of QSAR and QSPR models.
Name and type of organisation providing education and training	Biomodelling group (F. Jensen), Department of Chemistry, Aarhus University, Aarhus, Denmark

Dates	September 2003 – December 2008
Title of qualification awarded	Magister of Pharmacy
Diploma thesis	Synthesis, chemometric and pharmacological investigation of novel platinum and palladium complexes with 5-methyl-5-(4-pyridil)hydantoin and its derivatives.
Name and type of organisation providing education and training	Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria

Dates	March 2005 – December 2008
Research work	Synthesis and characterization of new platinum and palladium complexes with hydantoin derivatives.
Name and type of organisation providing education and training	Department of Chemistry (A. Bakalova), Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria

Dates	05th June 2007 – 28th August 2007
Research work:	Chiral separation of aminoacids and hydantoin derivatives, using HPLC and micro HPLC techniques
Name and type of organisation providing education and training	Drug analysis group (G. Gübitz, M. Schmid), Department of Pharmaceutical Chemistry, Karl-Franzens-University Graz, Graz, Austria

Dates	February 2006 – February 2008
Specialization	Industrial Pharmacy
Name and type of organisation providing education and training	Faculty of Pharmacy, Medical University - Sofia, Sofia, Bulgaria

Dates	September 1998 - May 2003
Title of qualification awarded	Diploma of Completed Secondary Education
Name and type of organisation providing education and training	Chemistry class in the National High School of Mathematics and Science ‘acad. L. Chakalov’, Sofia, Bulgaria

Dates	September 1991 - June 1998
Title of qualification awarded	Basic Education Completion Certificate
Name and type of organisation providing education and training	131st primary school ‘K.A. Timiriazev’, Sofia, Bulgaria

Language skills

Mother tongue **Bulgarian**

Other language(s)

Self-assessment	Understanding		Speaking		Writing
	Listening	Reading	Spoken interaction	Spoken production	
English	C1	C1	C1	C1	C1
German	B2	B2	B2	B2	B2
Russian	B1	B1	B1	A2	A2

Professional skills and research interests

Synthesis and characterisation (using 1D and 2D NMR techniques, IR spectroscopy, MS, HPLC, TG, X- RAY, DFT methods) of metal complexes with biological activity
Theoretical, QSAR and QSPR studies of bioactive metal complexes
Analytics and bioanalytics

Publications in scientific journals

Theoretical Investigations and Density Functional Theory Based Quantitative Structure–Activity Relationships Model for Novel Cytotoxic Platinum(IV) Complexes.
H.P. Varbanov, M.A. Jakupec, A. Roller, F. Jensen, M. Galanski, B.K. Keppler; *J. Med. Chem.*, **2013**, 56, 330-344.

Novel tetracarboxylatoplatinum(IV) complexes as carboplatin prodrugs.
H.P. Varbanov, S.M. Valiahdi, C.R. Kowol, M.A. Jakupec, M. Galanski, B.K. Keppler; *Dalton Trans.*, **2012**, 41, 14404-14415. (with cover paper).

Synthesis and characterization of novel bis(carboxylato)dichloridobis(ethylamine) platinum(IV) complexes with higher cytotoxicity than cisplatin.
H. Varbanov, S.M. Valiahdi, A.A. Legin, M.A. Jakupec, A. Roller, M. Galanski, B.K. Keppler; *Eur. J. Med. Chem.*, **2011**, 46, 5456-5464.

Synthesis of palladium(II) complexes with 3-amino-5-methyl-5-(4-pyridyl)-hydantoin: cytotoxic and antimicrobial investigations and comparison with their platinum analogues.
H. Varbanov, R. Buyukliev, A. Bakalova, G. Momekov, R. Baykushev; *Trans. Met. Chem.*, **2010**, 35, 457–461

Novel Pt(II) and Pt(IV) complexes with 3-amino-5-methyl-5-(4-pyridyl)- 2,4-imidazolidenedione. Synthesis, physicochemical, chemometric and pharmacological investigation.
A. Bakalova, **H. Varbanov**, R. Buyukliev, S. Stanchev, G. Momekov, D. Ivanov; *Inorg. Chim. Acta*, **2010**, 363, 1568–1576.

3-Amino-5-methyl-5-(4-pyridyl)hydantoin.
H. Varbanov, R. Buyukliev, A. Bakalova, A. Roller; Acta Cryst., **2009**, E65, o953.

In Vitro Biochemical and Pharmacological Evaluation of a Novel Cytotoxic Dinuclear Pt(II) Complex with 3-amino-5-methyl-5-phenylhydantoin: Cytotoxicity, Induction of Apoptosis, DNA-binding and Processing of the DNA Adducts.
G. T. Momekov, I. Ugrinova, E. A. Pasheva, A. G. Bakalova, **H. P. Varbanov**, D. V. Ferdinandov, D. S. Ivanov, S. M. Konstantinov; *Annals of the New York Academy of Sciences*, **2009**, 1171, 649–658.

DFT study of the structure and spectral behavior of new Pt(II) complexes with 5-methyl-5(4-pyridyl)hydantoin.
A. Bakalova, **H. Varbanov**, S. Stanchev, D. Ivanov, F. Jensen; *Int. J. Quant. Chem.*, **2009**, 109, 826-836.

Palladium(II) complexes with 5-methyl-5-(4-pyridil)-2,4-imidazolidendione. Synthesis, thermogravimetric and cytotoxic investigation.
A. Bakalova, **H. Varbanov**, R. Buyukliev, G. Momekov, D. Ivanov*; J. Therm. Anal.and Cal.*, **2009**, 95, 241-246.

Synthesis, characterization and biological activity of Pt(II) and Pt(IV) complexes with 5-methyl-5-(4-pyridyl)-2,4-imidazolidenedione.
A. Bakalova, **H. Varbanov**, R. Buyukliev, G. Momekov, D. Ferdinandov, S. Konstantinov and D. Ivanov; *Eur. J. Med. Chem.*, **2008**, 43, 958-965.

Synthesis and crystal structure of new Pt(II) complex with 3-amino-5-methyl-5-phenyl hydantoin.
A. Bakalova, R. Petrova, B. Shivatchev, **H. Varbanov**; J. Coord. Chem., **2007**, 60, 15, 1701-1707.

Participation in conferences, workshops and summer schools:

XIth International Symposium on Platinum Coordination Compounds in Cancer Chemotherapy (ISPCC XI), October 2012 in Verona, Italy – oral presentation (in English): 'Pt(IV) bis-, tris- and tetracarboxylato complexes as potential anticancer drugs: synthesis, analytical, biological, DFT and QSAR studies'

11th European Biological Inorganic Chemistry Conference (EUROBIC 11), September 2012, Granada, Spain – poster presentation: 'Theoretical investigations and a DFT based QSAR model for novel cytotoxic Pt(IV) complexes'

7th Workshop on Inorganic Chemistry in Austria 2012 (WACÖ 2012), April 2012 in Innsbruck, Austria – oral presentation (in English): 'Novel tetracarboxylato platinum(IV) complexes as carboplatin prodrugs'

15th International conference on Bioinorganic Chemistry (ICBIC 15), August 2011 in Vancouver, Canada – poster presentation: 'Novel tetra- and tricarboxylato platinum(IV) complexes as carboplatin and nedaplatin prodrugs: synthesis, cytotoxicity and SAR'

10th European Bioinorganic Chemistry Conference (EUROBIC 10), June 2010 in Thessaloniki, Greece – poster presentation: 'Novel platinum(IV) complexes with high cytotoxicity'

6th Workshop on Inorganic Chemistry in Austria 2010 (WACÖ 2010), March 2010 in Linz, Austria – oral presentation (in English): 'Towards the development of novel anticancer platinum(IV) complexes'

Student Science Session of Pharmacy, November 2008 in Sofia, Bulgaria – oral presentation (in Bulgarian): 'Synthesis, chemometric and pharamacological investigation of new platinum and palladium complexes with 5-methyl-5(4-pyridyl)hydantoin and its derivatives'

38th International Conferences on Coordination Chemistry, July 2008 in Jerusalem, Israel - poster presentation: 'Theoretical and spectroscopic study of new platinum(II) complexes with 5-methyl-5(4-pyridyl)-2,4-imidazolidenedione' and co-author in poster: 'Comparative physicochemical and pharmacological investigation of new Platinum(II) and Palladium(II) complexes with 5-methyl-5(4-pyridyl)hydantoin'

8th International Symposium and Summer school on Bioanalysis, June 2008 in Nitra, Slovakia - poster presentation: 'Enantioseparation of new amino acid analogues and hydantoin derivatives using chiral HPLC techniques'

7th National Conference of chemistry for diploma and PhD students May 2008 in Sofia, Bulgaria - oral presentation (in Bulgarian): 'Theoretical, Spectroscopic and pharmacological investigation of new platinum complexes with 5-methyl-5(4-pyridyl)hydantoin'

Student Science Session of Pharmacy, November 2007 in Sofia, Bulgaria – oral presentation (in Bulgarian): 'Synthesis, characterisation and pharmacological study of new platinum complexes with 5-methyl-5(4-pyridyl)hydantoin'

13th International Conference on Biological Inorganic Chemistry, July 2007 in Vienna, Austria - poster presentation: 'New Pt(II) and Pt(IV) complexes with 5-methyl-5(4-pyridyl)-2,4-imidazolidenedione and different inorganic ligands. Synthesis, characterization and cytotoxic activity'

7th International Symposium and summer school on Bioanalysis, June 2007 in Pec, Hungary

37th International Conferences on Coordination Chemistry, August 2006 in CapeTown, South Africa – poster presentation: 'Synthesis and crystal structure of new Pt(II) complexes with some hydantoin derivatives'

Granted projects

Student research project 'Synthesis and physicochemical characterization of new platinum and palladium complexes with hydantoin derivatives and different inorganic ions. Pharmacological investigation for cytotoxic activity *in vitro* of the new compounds' from the Student Union of Medical University-Sofia, approved in 2007

Awards

Scholarship for excellence in chemistry from the foundation 'Evrika' on the name of acad. Rostislav Kaishev for the years 2005, 2006 and 2007

First prize in the competition 'Shimadzu' of the 'Union of Chemists in Bulgaria' for best diploma work for 2009 year