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## New trends in platinum and palladium complexes as antineoplastic agents

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## Abstract

The discovery of cisplatin (*cis*-Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>) as an antineoplastic agent has focused attention on the rational design of metal complexes that can be potentially used in cancer chemotherapy. Today, the pharmaceutical industry invests more than \$1 billion each year in the development of new metal-based drugs to improve biological activities, in terms of cellular selectivity, therapeutic efficiency and minimization of side effects. Chemotherapies based on transition metals play a key role in cancer treatment, and among them platinum and palladium are the most fruitful. This article reviews the main recent advances in the design and synthesis of platinum- and palladium-based drugs, their structural features and biological studies of them. The rationale for the choice of the ligand, related to leaving groups, the geometry of the complex and the oxidation state of the metal ion, is discussed. An overview of the main biological techniques and approaches for testing the interaction of these molecules with the biological environment, mainly DNA, to validate the effect is also provided.

**Keywords:** Platinum drugs / Palladium drugs / Inorganic Medicine / Cancer / Antineoplastic drugs

## 1. Introduction

Cancer is one of the main causes of morbidity and death worldwide, with approximately 14 million new cases and 8.2 million cancer-related deaths reported in 2012, thus affecting life expectancy and producing a negative impact on society [1].

The discovery of cisplatin (*cis*-Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>) as an antineoplastic agent has focused attention on the rational design of metal complexes that can be potentially used in cancer chemotherapy [2-6]. Today, the pharmaceutical industry invests more than \$1 billion each year in the development of new metal-based drugs with improved biological activities, in terms of cellular selectivity and therapeutic efficiency, but also to minimize side effects [7-9]. Serious side effects such as emesis, renal toxicity, bone marrow suppression, neurotoxicity, hearing loss and drug resistance are connected with the use of cisplatin in clinical application [10].

The economic interest in metal-based compounds for pharmaceutical uses is also attested by the appearance of more than 60 patents in the period from 2013 to 2015 concerning only platinum complexes for tumour treatments.

Despite the interest in new metal ions, platinum complexes are, at present, the only metal-based drugs currently used in clinical settings (Figure 1), and about 10 other platinum complexes are currently in clinical trials [11].

However, research on other metal complexes is constantly increasing, and many metal ions, mainly belonging to the class of noble metals, have been studied [12]. Among them, interest in palladium is increasing because it forms complexes similar to platinum but with different kinetics and stability of both the main ligand and the leaving groups and, in addition, at a lower cost.

Cisplatin is still considered the main architecture for the main development of platinum-based and other metal-based systems. This is because the platinum drugs used in the clinic up to now are similar to cisplatin, exerting the same mechanism of action in which the cross-linking of DNA by covalent bonds is predominant. At the same time, because these complexes are structurally derived from cisplatin, the drawbacks of cisplatin are inherited. This has resulted in many scientists being engaged in the design of novel metal complexes deriving not only from the cisplatin architecture but also from different architectures, such as monofunctional complexes [13,14] and positively charged multinuclear complexes [15]. Therefore, further molecular architectures and oxidation states are continuously being prepared with the aim to improve the efficacy and specificity in tumour treatments.

In Figure 2 the main approaches in the design of novel platinum-based drugs are reported, together with some examples of complexes that have been studied or are being studied.

In this review, we consider platinum and palladium complexes developed for antineoplastic aims in the period from 2013 to 2015. The complexes are grouped and discussed essentially following the guidelines in Figure 2. The approaches and results are discussed, focusing on the type of complexes

developed, their topology and the corresponding structural aspects. We also discuss the main biological approaches used to validate the efficacy of the complexes.

## 2. Cisplatin-like complexes

### 2.1. Platinum(II) complexes

Since the quite serendipitous discovery of cisplatin antitumour activity [16] (Figure 1), a plethora of strictly related platinum complexes have been invented, synthesized and tested. Carboplatin, oxaliplatin (both marketed worldwide), nedaplatin (approved in Japan), lobaplatin (approved in China), heptaplatin (approved in Korea) [3c,17] (Figure 1), and picoplatin are the result of the search for new and better performing (less toxic, broader activity spectrum, overcome drug resistance, etc.) anticancer platinum drugs originating from cisplatin by replacement of chloride and/or amine ligands. The rationale behind the design of cisplatin analogues rests both on the results of mechanistic investigations and on simple coordination chemistry considerations. These neutral complexes (which must be sufficiently water soluble), with general formula  $[\text{PtX}_2(\text{A})_n]$ , feature two leaving groups (X) in the *cis* position, with the other two positions of the square planar structure being typically occupied by the amine nitrogen atom provided by two monodentate ligands (A,  $n=2$ ) or one bidentate chelating diamine (A,  $n=1$ ). Once within the cell (chemical neutrality and high lipophilicity allow easy penetration of the complex into the cell), one or both X ligands are lost and replaced by water molecules. As a consequence, the donor atoms of the X anion, typically chloride, iodide and oxygen atoms, should bind the metal ion neither too weakly (to avoid toxic effects due to the high reactivity) nor too strongly (leading to inactive drugs). In this respect, the soft nature of the Pt(II) ion could help in the choice of suitable donor atoms. This is because the ligand A usually contains nitrogen as the donor atom, which guarantees thermodynamically stable bonds with platinum that are preserved even in the adduct formed with DNA. The latter is formed when the platinum ion of the aquated species covalently binds to the nitrogen atom at the 7-position of purines, losing the water molecules. The resulting adduct is quite stable kinetically, given that amines and heterocyclic nitrogen ligands have a low *trans* effect (another parameter that must be taken into account in the design of new cisplatin analogues [18]). Owing to the *cis* geometry of the metal complex, 1,2-intrastrand cross-links are formed between adjacent guanines as are 1,3-intrastrand cross-links (for geometric reasons, the *trans* isomer cannot form cross-links of this kind). The resulting intrastrand adduct, which distorts and bends DNA, causes different cellular responses, such as transcription inhibition, replication and cell-cycle arrest, DNA repair and apoptosis.

In addition, the solid-state structures of cisplatin [19,20] and most of the marketed [21-27], promising [28-31] and even not promising congeners have been determined by single-crystal X-ray diffraction, thus adding further details to the “classical” structure-activity relationships first recognized by Cleare and Hoeschele [32-35]. Knowledge of the molecular structure, other than providing obvious information (e.g. usual/unusual bond distances and angles about the Pt(II) ion, its square planar coordination geometry and the *cis* arrangement of the leaving groups), can suggest useful correlations with efficacy and with the observed physicochemical properties, as briefly outlined in the following. For example, single-crystal X-ray diffraction can reveal the enantiomer composition of a crystalline sample, and we know that chirality [36,37] can play an important role in determining the performance of a platinum-based anticancer drug, as evidenced by oxaliplatin [38]. On the other hand, it might be difficult for bulky N-donor ligands to penetrate the cell but, at the same time, once inside the tumour cell, they may play a positive role; in fact, they, due to their dimension, can hinder the recognition of DNA damage and its subsequent repair. In this respect, single-crystal X-ray diffraction can provide hints about the steric hindrance of the coordinated ligands and/or their relative repulsion by revealing, for example the relative orientation, intramolecular contacts, and deviations from the ideal geometry [39-42]. On the other hand, determination of the crystal structure and the subsequent study of the crystal packing can help to rationalize the tendency of the platinum drug to be involved in intermolecular interactions of the NH...O type, which are considered the first step in approaching DNA; hence, the presence in the platinum drugs of NH<sub>3</sub>/NH<sub>2</sub>R/NHR<sub>2</sub>, which are able to form hydrogen bonds with DNA peripheral phosphates, as non-leaving groups. In addition, the latter could contribute to stabilization of the drug-DNA adduct formed once the coordinating water molecules have been lost [43,44].

For all these reasons, it is interesting to analyse in depth the crystal packing of hydrated/solvated (solvent molecules should behave as hydrogen-bond acceptors) platinum drugs to form a picture of the hydrogen-bond donor ability of the platinum complexes [45,46]. Conversely, the lack of co-crystallized solvent molecules and/or complex self-association can be related to poor or even absent solubility [45,47]. This kind of analysis can provide indirect information on the hydrophilicity-lipophilicity balance, which is an essential requisite for a platinum-drug complex [47]. As a final remark, the occurrence in the crystal of stacking interactions between ad hoc functional groups bound to the metal ion suggests the likelihood of further stabilizing interactions in the drug-DNA adduct (see later).

In this context, modelling (ab initio, density functional theory, molecular mechanics/quantum mechanics and docking are the most popular techniques) is a helpful complementary approach

which can provide hints for the rational design of novel species [48,49] or can be used to obtain structural/energetic/electronic information about the metal complexes [50,51] and/or the postulated corresponding adducts [52,53]. In this section, the most recent and fruitful advances in the optimization of the structure of cisplatin-like compounds are described.

### **2.1.1. Leaving group**

It is well known that the nature of the leaving ligand plays a significant role in determining the toxicity and side effects of a platinum drug, because the capability of different ligands to leave the binding platinum atom can result in a remarkably altered biodistribution in vivo. In general, complexes with labile anionic leaving groups, such as the chloride anion in cisplatin, which hydrolyse relatively fast, are much more toxic. The resulting reactive platinum intermediates can efficiently bind biomolecules such as sulfur-containing amino acids and peptides or proteins in the blood, and their interactions with proteins in organs such as the kidney can cause severe adverse effects. In contrast, less labile leaving groups, such as iodide, give rise to inactive molecules. Complexes with weakly labile anionic leaving groups, such as the dicarboxylate moiety in carboplatin, which hydrolyse slowly, are divided into two types: (1) water-soluble platinum complexes, which are stable and have a longer half-time in the blood, are less toxic because they can be excreted efficiently via the kidneys but their antitumour abilities are clearly reduced; (2) water-insoluble (lipophilic) platinum complexes, which hydrolyse slowly, are cleared much more easily from the blood through tissue penetration; in this way, some typical toxic side effects derived from cisplatin-based therapy are absent because of the reduced requirement for renal excretion. Thousands of platinum complexes have been designed, synthesized and biologically evaluated, but none have entered worldwide clinical use except oxaliplatin (which exhibits potent anticancer activity as a result of the presence of the [(*R,R*)-1,2-diaminocyclohexane]platinum(II) fragment as well as because of its good aqueous solubility and stability derived from the dicarboxylate leaving ligand. Thus, it seems impossible or extremely difficult to obtain a platinum anticancer agent with the characteristics of both potent anticancer activity, such as cisplatin, and low toxicity together with good water solubility and stability, such as carboplatin [47].

### ***Dicarboxylates***

Bidentate dicarboxylates (e.g. 1,1-cyclobutanedicarboxylate and oxalate) are a type of common and effective alternative ligand to chloride anions. They can effectively improve the water solubility and/or lipophilicity of the resulting platinum complexes as compared with cisplatin. Moreover, the

chelating ring formed by the O,O-donors of the dicarboxylate and the platinum atom can increase the stability of the complexes and reduce the side effects of the corresponding platinum complexes. Furthermore, the nature of the leaving group determines different activities towards different cell lines. For example Xu et al. [54] demonstrated that by the introduction of an OH group at the 3-position of the 1,1-cyclobutane dicarboxylate in **C1a** to obtain **C1b** (Figure 4), the activity against the A549 and HCT cell lines increases but against the MCF-7 tumour cell line it decreases.

The same group [47] synthesized the ligands **C2** and **C3** (Figure 4), in which the 3-oxocyclobutane-1,1-dicarboxylate leaving group showed potent in vitro anticancer activity, comparable with that of cisplatin and oxaliplatin, and low short-term toxicity, similar to that of carboplatin; moreover, the complexes had acceptable solubility and stability in water. Chemical and biological results indicated that both the potent anticancer activity and the low apparent toxicity of the platinum complexes derived from the kinetic properties of the compounds; in fact, the introduction of a carbonyl group enhances both the water solubility and the lability of the leaving group.

Liu et al. [55] synthesized a series of oxaliplatin-like compounds in which the oxalate anion was replaced by 1,1-cyclobutane dicarboxylate derivatives in order to obtain complexes with the activity of oxaliplatin and to overcome the neurotoxicity of the oxalate anion. In particular, the derivative **C4** (Figure 4), bearing the acetyl group at the 3-position of the dicarboxylcyclobutane moiety, exhibited high antitumour activity and less toxicity than oxaliplatin, which was ascribed to its optimal water solubility.

Following the aim to increase both water solubility and cell membrane penetration, Sun et al. [56] synthesized a series of oxaliplatin derivatives with (*R,R*)-*N*-alkylcyclohexane-1,2-diamine as carrier ligands and 1-(methoxy- or methyl-substituted benzyl)azetidine-3,3-dicarboxylate anions as leaving groups. Generally, **C5a-C5f** (Figure 4), with an isopropyl substituent on the amine carrier ligand, exhibited higher activities in vitro than carboplatin against the MCF-7 human breast carcinoma and A549 human non-small-cell lung cell lines, although they were less potent than oxaliplatin. **C5e** exhibited cytotoxicity superior to that of carboplatin and comparable to that of oxaliplatin towards the two above tumour cell lines. The presence of the tertiary amine on the leaving group makes this complex particularly active in acidic media, such as the cytoplasm of cancer cells.

With regard to water solubility and the capability to cross the cell membrane, Liu et al. [57] designed three glucose-conjugated malonate-Pt(II) complexes (Figure 4, **C6**) to target tumour-specific active glucose transporters. The complexes exhibited 150 times higher aqueous solubility, 10 times improved cytotoxicity, and 30-fold increased therapeutic index in comparison with oxaliplatin.



As the leaving group was hydrolytically lost in the cytoplasm, this clearly represents an important issue in drug management. For this reason, as a valid alternative, the leaving group could be functionalized with bioactive moieties and thus the platinum complex could act as both a DNA cross-link inducer and an inducer of a different apoptosis mechanism.

For example Liu et al. [58,59] synthesized a series of new diamine platinum complexes containing a dichloroacetate moiety as a small-molecule cell apoptosis inducer; they did this by introducing 3-dichloroacetoxycyclobutane-1,1-dicarboxylate as the leaving group in platinum complexes **C7a-C7f** (Figure 5). Dichloroacetate can be efficiently released under physiological conditions via the hydrolysis of the ester bond, thus supporting the evaluation of these complexes as drug candidates.

Zhao et al. [60] synthesized a series of Pt(II) complexes of *N*-monoalkyl-(*R,R*)-1,2-diaminocyclohexanes with 3-(nitrooxy)cyclobutane-1,1-dicarboxylate as the leaving group (Figure 5, **C8**). These compounds showed high cytotoxicity towards human cancer cell lines, and their activity is enhanced by the release of NO in the presence of L-cysteine, based on the fact that thiols can induce the release of NO from organic nitrates in vitro. NO is well known for its biological functions in vasodilation, neurotransmission and the immune system and as a cell apoptosis inducer. Moreover, NO prodrugs may act synergistically with the platinum-based moieties on the tumour cells; such NO-donor compounds can improve the anticancer efficacy and can reduce the resistance of human cancer cells to platinum drugs.

Parker et al. [49] reported the design and synthesis of a novel Pt(II) complex of the histone deacetylase inhibitor belinostat conjugated to the malonate leaving group (**C9**, Figure 5). This complex is considerably cytotoxic to ovarian cancer cell lines, and favourable cytoselective properties as compared with cisplatin alone and belinostat alone.

### ***Other leaving groups***

To investigate the difference between the reactivity of halogens and carboxylate, Zhao et al. [61] synthesized three platinum complexes with both a chloride anion and a chelated carboxylate as leaving groups (**C10a-C10c**, Figure 6). All the compounds exhibited effective cytotoxicity towards tumour cell lines, nearly comparable to the cytotoxicity of cisplatin and oxaliplatin. Notably, the activity of **C10b** was about twofold better than that of oxaliplatin against the HCT 116 cell line. Chloride anion departs from the Pt(II) quickly, whereas the five- or six-membered ring formed by coordinated N,O-donors opened more slowly by the breaking of a Pt–O bond. This helped to further understand the mechanism of action of these complexes with biomolecules. The same research group [62] developed the dinuclear complexes **C11** (Figure 6), in which a dicarboxylate or a sulfate

ligand, in place of a chlorine, bridged two **C10c** units. The in vitro cytotoxicity of these compounds towards several cell lines was evaluated. All the compounds are cytotoxic towards the HepG2 cell line. Particularly, **C11d**, with the  $\text{SO}_4^{2-}$  bridge, exhibited better cytotoxicity than carboplatin or oxaliplatin towards all cell lines tested.

Valiahdí [63] described a series of bis(2-aminoalcoholato-*N,O*)platinum(II) complexes (Figure 6, **C12**) with low systemic toxicity; they achieved their full antitumour potency only in acidic conditions. These complexes are activated by protonation of the alcoholate oxygen, resulting in the cleavage of Pt–O bonds. The paradigm is that extracellular acidity is a frequent pathophysiological condition of solid tumours, and these compounds offer possibilities for improving the tumour selectivity for molecular therapy.

Cisplatin-like drugs with iodide as the leaving group are quite rare owing to their slow reaction rate. An example of a moderately active molecule has been reported by Savić et al. [50]; they synthesized a class of ligand in which the *cis*-PtI<sub>2</sub> moiety was coordinated by isobutyl, *n*-pentyl and isopentyl esters of (*S,S*)-1,3-propanediamine-*N,N'*-bis[2-(3-cyclohexyl)propanoic acid] (Figure 6, **C13**). The comparison of the structure-activity relationship indicated variations of cytotoxicity related to the drug/ligand lipophilicity, whereas the intracellular platinum content and DNA platination increased with increasing length and branching of the ester chain. The half maximal inhibitory concentrations (IC<sub>50</sub> values) ranged between 5 and 30  $\mu\text{M}$  and are of the same order as for cisplatin for all cell lines tested.

### 2.1.2. Amine carrier ligands

The structural characteristics of oxaliplatin, which has (*R,R*)-1,2-diaminocyclohexane as the carrier ligand instead of the two ammonia groups of cisplatin, suggested that 1,2-diaminocyclohexane may be a useful pharmacophore for the design of novel platinum-based complexes. Many platinum-based complexes containing 1,2-diaminocyclohexane have been previously, and some of them showed good results. However, to further explore such pharmacophores, many 1,2-diaminocyclohexane derivatives and other amine ligands have been prepared and used as platinum-based drugs. Some research groups reported a class of *N*-alkyl derivatives of (*R,R*)-1,2-diaminocyclohexane that can be used as carrier ligands for novel Pt(II)-based antitumour complexes. The introduction of the alkyl groups to 1,2-diaminocyclohexane may improve the properties of the resulting Pt(II) complexes as follows: (1) changing the substituted group may adjust the balance between the lipophilicity and hydrophilicity of the complex so that an optimal log *P* (*P* is the partition coefficient between an organic solvent and water) value of the complexes

can be expected; (2) the steric hindrance of the substituted group may change the mode of the interaction between the complex and DNA, which is the main target of the platinum-based drugs; this may subsequently affect antitumour properties.

Fang et al. [64] produced a series of novel Pt(II) complexes with *N*-monoalkyl (*R,R*)-1,2-diaminocyclohexane derivatives as amine ligands and 3-hydroxycyclobutane-1,1-dicarboxylate as the leaving group (Figure 7, **C14a-C14g**). In particular, **C14d**, with the *sec*-butyl group, showed much higher cytotoxic activity than carboplatin and oxaliplatin against the MCF-7 and A549 cell lines. Because of the use of *N*-monoalkyl (*R,R*)-1,2-diaminocyclohexane derivatives and 3-hydroxycyclobutane-1,1-dicarboxylate as ligands, the calculated log *P* values of the complexes are around 1; this suggests a good balance between lipophilicity and hydrophilicity. The same group [65] studied four Pt(II) complexes of (*R,R*)-*N*<sup>1</sup>,*N*<sup>2</sup>-dibutyl-1,2-diaminocyclohexane with two alkyl branches as steric hindrance (Figure 7, **C15a-C15d**). The in vitro cytotoxicity of these compounds indicated **C15d** is the most cytotoxic agent; it was more active than its parent molecule oxaliplatin against almost all the cell lines tested. In agarose gel electrophoresis studies, the kinetic reactivity of **C15d** with DNA is slow because of the steric hindrance effect, demonstrating that it may possess a molecular mechanism of action different from that of cisplatin. Nevertheless, the global effect is an apoptotic mechanism similar to that of cisplatin, which could induce apoptosis via a mitochondrial-dependent pathway.

Gay et al. [37] investigated the structure-activity relationships of several organic functionalities (C=C double bond, free OH group, MeO group, etc.) and the effect of the stereochemistry on the cytotoxic activity of selected Pt(II) complexes having differently functionalized 1,2-bis(aminomethyl)cyclohexane carrier ligands with a 1,4-diamino framework and iodide as labile ligands (**C16**, Figure 7). In comparison with cisplatin, these platinum complexes exhibited up to 16 times lower resistance factors in A2780cisR ovarian cancer cells. This cell line becomes drug resistant because of a combination of effects, among them reduced drug transport, enhanced DNA repair/tolerance and an elevated glutathione level with respect to the parental A2780 cells.

Another approach to improve the cellular accumulation of cisplatin-like compounds is to replace the aliphatic amine ligand with azaheterocycles or aromatic amines. Good results were obtained by the introduction of 7-azaindole halogen derivatives. Muchova et al. [66] synthesized ligands **C17** (Figure 7); they were toxic to ovarian tumour cells, with moderately better IC<sub>50</sub> values than cisplatin in the cisplatin-sensitive cell line A2780. The potential factors which might be involved in the mechanism underlying the cytotoxic effects of these compounds originate mainly from their efficient cellular accumulation. Other examples of N-heterocycle cisplatin-like complexes have

been reported by Štarha et al. [67] and Łakomska et al. [42] with use of 1,2,4-triazol-[1,5a]-pyrimidine and 7-azaindole, **C18** and **C17** (Figure 7), respectively. The applicability of these complexes and their fate in biological systems are characterized by their hydrolytic stability and by the thermodynamic aspects of their interactions with cysteine, reduced glutathione, and human serum albumin. Serebryanskaya et al. [68] studied a series of *cis*-tetraazole- and *trans*-tetraazole-containing Pt(II) and Pd(II) chloride complexes, discovering compound **C19** (Figure 8), with noticeable efficacy in vivo. Ferri et al. [69] synthesized a series of imidazole-based Pt(II) complexes (**C20**, Figure 8); they evaluated their cytotoxicity towards a cancer cell line partially resistant to cisplatin but sensitive to oxaliplatin. The design of these compounds, having a heterocycle and an amine N-donor, preserved the aliphatic amino function in order to display the optimum *trans* effect and introduced a chain of appropriate length at the imidazole moiety to improve the lipophilicity.

### **Delivery**

The main problems that limit the clinical use of cisplatin include acquired resistance and serious side effects such as neurotoxicity and nephrotoxicity. These shortcomings are closely related to the lack of tumour selectivity of cisplatin. So, in the past two decades, several strategies have been used to try to include targeting groups such as small ligands and drug delivery systems in platinum-based compounds. Huang et al. [39,40] introduced a series of cisplatin-like compounds with monoaminophosphonate ester as the carrier ligand and chloride as the leaving group (**C21**, Figure 8). Because alkaline phosphatase is overexpressed in the extracellular space of specific tumour cells such as ovarian and hepatic carcinoma cells, introduction of a phosphate group for targeted delivery appeared to be a reasonable strategy to increase solubility and to enhance transport through the cell membrane. Some phosphate groups also exhibit high affinity for calcium ions and have been used to design targeted drugs for treatment of bone cancer.

Another approach to attach the PtCl<sub>2</sub> scaffold to the target DNA was proposed by Wisnovsky et al. [70]. In their approach, **C22** (Figure 8), an analogue of cisplatin, was delivered to mitochondria of human cells by means of mitochondria-penetrating peptide linked to the Pt(NH<sub>3</sub>)<sub>2</sub> fragment by a β-diketonate acting as a leaving group. Mitochondria-penetrating peptides are short, cell-permeable peptide sequences, comprising alternating lipophilic and cationic residues that exhibit minimal toxicity towards human cells. **C22** induces apoptosis without damaging nuclear DNA, confirming that mitochondrial DNA damage is sufficient to mediate the activity of a platinum-based chemotherapeutic. This study was the first to demonstrate specific delivery of a platinum drug to mitochondria and to investigate the effects of directing Pt(II) ion outside the nucleus.

### *Active molecules as N-ligands*

Novakova et al. [71] synthesized the cisplatin-like complex **C23** (Figure 9), in which the two ammonia ligands are replaced by the cyclin-dependent kinase inhibitor boheminine. This compound exhibited a unique anticancer profile which may be associated with some features of the damaged DNA and/or its cellular processing; the mechanism of action was different from that of cisplatin because of (1) the slower rate of the initial binding to DNA, (2) the lower efficiency to form bifunctional adducts, (3) the reduced bending of the longitudinal DNA axis induced by the major 1,2-GG intrastrand cross-link, (4) the reduced affinity of high-mobility group proteins for the major adduct, (5) the enhanced efficiency of the DNA adducts to block DNA polymerization and to inhibit transcriptional activity of human RNA polymerase II and RNA transcription and (6) the slower rate of the reaction with glutathione. The same research group [46] studied the anticancer potential of Pt(II) complexes involving disubstituted and trisubstituted derivatives of the plant hormone kinetin as carrier ligands (**C24**, Figure 9). The complexes were able to circumvent cisplatin resistance in A2780cisR cells while additionally being significantly more cytotoxic to A2780 cells than cisplatin; this was mainly ascribed to hydrolytic stability and interactions with glutathione and guanosine monophosphate.

Jain et al. [72] studied the trinuclear Ru(III)/Pt(II) metal complex **C25** (Figure 9), in which the two ammonia molecules of cisplatin were replaced by two amine Ru(III) metalloligands, similar to what is done in the active anticancer agent NAMI-A, which is currently in clinical trials as an antimetastatic prodrug. The trinuclear complex is strikingly more effective than cisplatin and NAMI-A in the binding of RNA as well as in the inhibition of primer DNA synthesis.

Hui, Zhang and Chen [73] synthesized a series of dichloridoplatinum(II) complexes of podophyllotoxin. Among these, *cis*-[4 $\alpha$ -O-(2'',3''-diaminopropanoyl)podophyllotoxin]dichloride platinum(II) (**C26**, Figure 9) displayed the most potent cytotoxicity, with a sub-micromolar IC<sub>50</sub> value. This compound induces cell cycle arrest in the G<sub>2</sub>/M phase and inhibits the formation of microtubules in HeLa cells. Furthermore, it exhibits potent DNA cleavage capabilities.

Neves et al. [52] studied a series of chloride Pt(II) complexes of 2-hydroxy-3-(aminomethyl)-1,4-naphthoquinone Mannich bases (**C27**, Figure 9) exhibiting high cytotoxicity towards cancer cell lines. Natural and synthetic quinone derivatives have been widely investigated for cancer therapy because of their capability to form reactive oxygen species (ROS) in vivo. The PtCl<sub>2</sub> fragment substantially affects the chemical and biophysical properties of quinone ligands, leading to an

improvement of their DNA-binding properties and the generation compounds that cleave DNA and catalytically inhibit topoisomerase I.

Another approach to conjugate the effect of cisplatin with the inhibition of topoisomerase was proposed by Dallavalle's research group [48]; it involved two hybrid agents formed by 7-oxyiminomethylcamptothecin derivatives and diaminedichloridoplatinum(II) complex **C28** (Figure 9). These compounds contain camptothecin, an inhibitor of topoisomerase I, and the active principal of irinotecan. The compounds exhibited growth-inhibitory activity against a panel of human tumour cell lines, including sublines resistant to topotecan and platinum compounds. This supported the interpretation that the diaminedichloridoplatinum(II) complex conjugated with the camptothecin could result in a new synergic class of effective antitumour compounds.

Bérubé's group [74] designed and prepared new combi-molecules using 17 $\beta$ -acetyltestosterone and amino acid Pt(II) complexes linked at the 7a-position (**C29**, Figure 10) to target and to improve the antiproliferative activity of Pt(II)-based chemotherapy against prostate cancer cells.

### **Stereochemistry**

As in many cases the carrier amino ligand is an asymmetrical molecule, stereochemistry could influence the activity. Recently, Arnesano et al. [75] reviewed the effect of chirality in platinum drugs. It is well known that *trans*-(*R,R*)-1,2-diaminocyclohexane-containing platinum complexes exhibit better performance than the corresponding *trans*-(*S,S*)-1,2-diaminocyclohexane- and *cis*-(*R,S*)-1,2-diaminocyclohexane-containing platinum complexes [38]; this has been related to its flat shape that favours the first approach to DNA. Some articles have described this aspect; for example, Liu et al. [76] reported the 4*S*,5*S* isomer of **C30** (Figure 10) exhibits superior antitumour activity and less toxicity in comparison with the 4*R*,5*R* optical isomer as well as the parent heptaplatin (Figure 1).

#### **2.1.3. Cationic monofunctional complexes**

Cationic monofunctional Pt(II)-based anticancer agents with the general formula *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>(N-donor)Cl]<sup>+</sup> (**C31a** and **C31b**, Figure 11, are two examples) have received attention because of their unique mode of action, distinctive anticancer spectrum and promising antitumour activity both in vitro and in vivo. Studies of the mechanism revealed that monofunctional platinum compounds bind well to DNA and effectively inhibit transcription. Understanding the mechanism of action of novel monofunctional platinum compounds through rational drug design will aid in the further development of active agents. Wang et al. [77] synthesized and evaluated a monofunctional

platinum-based anticancer agent containing a bulky salicylanilide moiety, **C31c**. The compound bound to DNA as cisplatin, but did not block RNA polymerase II-mediated transcription as strongly as cisplatin, indicating that once the compound formed Pt-DNA lesions, the salicylanilide group was more easily recognized and removed. This study not only enriches the family of monofunctional platinum-based anticancer agents but the findings could also help in the design of more potent monofunctional platinum complexes.

Wang et al. [78] also developed cationic monofunctional Pt(II)-based anticancer agents (**C32**, Figure 11) in which the N-donor is a nicotinamide-like inhibitor of poly(ADP-ribose) polymerase 1; in clinical trials they were promising against cancer and other diseases. The complexes, in particular **C32c**, are able to enter cancer cells efficiently, to bind DNA well, and to block the cell cycle at G<sub>2</sub>/M phase, indicating that the conjugation of platinum with poly(ADP-ribose) polymerase 1 inhibitors could be a valid strategy to obtain more potent anticancer agents with improved biological activities with two distinct mechanisms of action.

#### 2.1.4. *Trans* platinum complexes

The *trans* isomer of cisplatin is inactive and, because of this lack of activity, all *trans* platinum complexes were assumed to be ineffective as antitumour agents. Now, it is recognized that the replacement of NH<sub>3</sub> with other amino ligands (L or L') in the *trans* position within the structure, *trans*-[PtCl<sub>2</sub>(L)(L')], can afford cytotoxicity in the micromolar range. For example, **C33** (Figure 11), named ampyplatin [41], forms DNA adducts similar to those of monofunctional Pt(II) complexes, such as **C31a**. Legin et al. [79] compared the activity of a series of *cis* and *trans* Pt(II) complexes in which ligands are guanidines; the cytotoxicity of *trans*-configured complexes is, in some cases, comparable to or higher than that of the *cis* congeners.

**C34** (Figure 11), recently synthesized and studied by Icel et al. [80,81], is a *trans*-dichlorido Pt(II) complex in which the two amine carrier ligands are 2-(2-hydroxyethyl)pyridine. This *trans* complex showed higher anticancer activity against different cancer cell lines than transplatin, cisplatin, carboplatin and oxaliplatin.

Fabijańska et al. [82] described the synthesis of *trans*-bis(3-aminoflavone)dichloridoplatinum(II) (**C35**, Figure 11) as a potential anticancer compound, and evaluated its biological behaviour. **C35** demonstrated a significant cytotoxic effect against human and murine cancer cell lines as well as weaker toxicity towards healthy cells (human peripheral blood lymphocytes) in comparison with cisplatin. The proapoptotic activity of this *trans* complex is markedly higher than that of cisplatin, suggesting that it may have an antitumour specificity different from that of cisplatin.

Herrera et al. [83] proposed a series of Pt(II) complexes to obtain more potent and selective metallodrugs (**C36**, **C37**, Figure 12); they bear aliphatic amines and ligands with specific DNA-targeting properties. The group developed six new *trans*-dichlorido platinum-based molecules containing dimethylamine or isopropylamine in the *trans* position with respect to a naphthalimide-based ligand. They contain a covalent DNA binder (*trans*-dichlorido platinum) connected through an aliphatic spacer to the intercalator naphthalimide moiety. The synergistic combination of the DNA binder and the DNA intercalator enhanced the antitumour activity of these *trans* platinum complexes. The distance of the naphthalimide to the metal centre was determinant for their cytotoxic activity. **C36a** and **C36c** showed the best cytotoxicity in both cisplatin-resistant and non-resistant cancer cell lines. Although **C36c** was the most active, **C36a** showed the best selectivity between cancer and normal cells. From cytotoxicity and DNA-interaction studies, these complexes seem to act through a combination of platination and naphthalimide-DNA interaction. Moreover, the subcellular uptake studies suggested that, in addition to DNA, these complexes might also interact with other cellular targets.

Del Solar et al. [84] reported the first study on the cytotoxic effect of sulfonamide platinum complexes; they synthesized a series of *trans*-*N*-sulfonamide Pt(II) complexes studied as antiproliferative agents in human solid tumours cells. The best compound was **C38** (Figure 12), formed by the *trans*-dichloride, dansyl sulfonamide and dimethyl sulfoxide (DMSO) at the platinum centre. Fluorescent cell assays showed that the final target is the cell nucleus.

Other examples of great interest as unconventional metallodrugs are the iodide analogues of cisplatin. Iodide complexes have not received great attention as pharmacological agents because of their low activity in aqueous solution and also because *cis*-[PtI<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>] was inactive as an anticancer agent [85]. There are only a few reports in the literature indicating a special reactivity of iodide platinum complexes towards important biological targets; for example, compounds **C16** (Figure 7) [37]. In contrast, *trans*-iodide complexes of Pt(II) afforded higher cytotoxicity values, exhibiting reactivity different from that of their *cis* counterparts. Moreover, both *trans*- and *cis*-type complexes bind to DNA models with retention of the amine spectator ligand. The lower reactivity of the *trans*-iodide compounds towards peptides might help their reactivity towards DNA and also their uptake into the cell. Examples of *trans*-iodide platinum complexes (**C39**, Figure 12) exhibiting biological activity have been reported by Parro et al. [86].

## 2.2. Palladium(II) complexes



The Pt(II) and Pd(II) coordination modes and chemical properties are quite similar, and as a consequence, their complexes are expected to show similar behaviour. This is the reason why Pd(II) has received much attention as an alternative to platinum in the search of new cisplatin analogues for antitumour treatments. However, Pd(II) complexes are somewhat less stable, from both a thermodynamic and a kinetic point of view, than the corresponding platinum analogues and are about  $10^5$  times more reactive [12,87]. As a consequence, palladium-based drugs can undergo a rapid hydrolysis before they reach the target; this results in both a low antitumour activity or even inactivity and toxicity. In addition, their relative lability and their tendency to predominantly form *trans* isomers might trigger *cis-trans* isomerization, thus leading, at least in the beginning, to inactive species. To overcome these drawbacks—that is, to reduce the reactivity of the Pd(II) ion—the carrier ligands should be bulky and feature strongly coordinating donor atoms such as nitrogen, sulfur, or phosphorus, preferably provided by a chelating ligand. In this case, solid-state structure characterization also helps to evidence strains [18,88], the *trans* effect [18], and intermolecular interactions holding together the crystal [89,90].

Further structural/electronic/energetic information can be obtained through modelling techniques, applied to studying the metal complexes as, for example, in [51], as well as their adducts with the biological target [89].

A valid strategy to obtain effective Pd(II) complexes is to stabilize the N–Pd interaction with the amine carrier ligand by use of secondary chelating bulky diamines to decrease the excessive reactivity. The use of sterically hindered amines lowers the reactivity and increases the lipophilicity of the complexes, making them suitable to enter cells. For example, Lj et al. [91] synthesized a series of Pd(II) dichlorido complexes in which the diamine carrier ligands were diethyl, dipropyl, dibutyl and dipentyl esters of (*S,S*)-ethylenediamine-*N,N'*-bis(2,2'-bis(4-hydroxybenzyl))acetic acid, a double tyrosine (**C40**, Figure 13). All compounds tested showed dose-dependent inhibition of cell viability for two different cell lines (MDA-MB-231 human breast cancer and A549 human lung cancer cell lines) and human chronic lymphocytic leukaemia cells. The most active complex was **C40d**, in which two pentyl groups are present as ester alkyl chains, confirming the crucial role of lipophilicity in cytotoxicity. Tanaka et al. [92] synthesized a glycoconjugate *cis*-PdCl<sub>2</sub> complex (**C41**, Figure 13) able to overcome cross-resistance to cisplatin both in vitro and in vivo.

In the same field, Matović et al. [89] obtained stable and biologically active Pd(II) complexes using an O,N,N,O tetradentate ligand in which, two N-donors are deprotonated secondary amides acting as a carrier group and two oxygen atoms, belonging to two carboxylate anions, act as a leaving group. The anionic complexes **C42a** and **C42b**, with malamido-*N,N'*-diacetic acid and oxamido-

*N,N'*-di-3-propionic acid (Figure 13), exhibited high activity on several tumour cells, thus revealing significant antitumour properties and less resistance of tumour cells in vitro than to cisplatin. From crystal structure and density functional theory calculations, the interaction with DNA is initially hydrogen bond driven, then nucleic acid bases bind the metal centre in an associative  $S_N2$  manner replacing the first and then the second carboxylate group and producing an intrastrand covalent interaction.

The use of soft ligands can be a valid strategy to improve stability and reduce the reactivity of palladium complexes. Motswainyana et al. [18] reported a series iminophosphine-chelating Pd(II) and Pt(II) complexes. The coordination geometry around the palladium and platinum atoms in the respective structures was distorted square planar at the metal centres. The complexes were evaluated in vitro for their cytotoxic activity against human breast cancer (MCF-7) and human colon cancer (HT-29) cells. They exhibited growth-inhibitory activities and selectivity that were superior to those of cisplatin. In particular, Pd(II) complexes **C43** (Figure 13) exhibited twice the activity of the Pt(II) complexes.

A monofunctional coumarin-based Pd(II) system having micromolar and sub-micromolar activity was reported by Ilić et al. [90] (**C44**, Figure 13). The Pd(II) ion is coordinated by a tridentate coumarin derivative 3-(1-(2-hydroxyethylamino)ethylidene)chroman-2,4-dione and by a chloride anion; the complex decreased viability of L929 mouse fibrosarcoma, U251 human glioma and B16 mouse melanoma cell lines in a dose-dependent manner, whereas the ligand exhibited no significant cytotoxicity. The cytotoxic effect of the complex was comparable to that of cisplatin; it is mediated by apoptosis associated with oxidative stress, mitochondrial depolarization and caspase activation. All these examples indicate that suitable Pd(II) complexes can be potential candidates for anticancer therapy.

### 3. Platinum(IV) complexes

Some problems linked to anticancer Pt(II)-based compounds, such as unselective binding to off-target biomolecules, side effects, and the need for intravenous administration, can be overcome by the use of Pt(IV) octahedral complexes which feature two additional binding sites. The relative kinetic inertness which characterizes the latter in comparison with the Pt(II) square planar congeners makes them suitable for oral administration, decreasing both the possibility of side reactions in the bloodstream (where it is estimated that about 90% of the administered cisplatin is deactivated) and undesirable side effects. They have the potential to be used as prodrugs based on an activation by reduction mechanism (hopefully intracellularly) providing the corresponding

square planar Pt(II) active complex. So far, four Pt(IV) complexes—namely, tetraplatin, iproplatin, satraplatin and LA-12 (Figure 3; for their solid-state structures, see [93-97]), have showed promising anticancer activity, but currently none of them have been approved for clinical use [98]. Studies of tetraplatin (tetrachlorido(*trans*-1,2-diaminocyclohexane)platinum(IV)) were abandoned because of severe neurotoxicity, whereas iproplatin (dichlorodihydroxidobis(isopropylamine)platinum(IV)) demonstrated lower activity than carboplatin and cisplatin. Satraplatin (bis(acetato)amminedichlorido(cyclohexylamine)platinum(IV)) was the first orally administered Pt(IV) complex with documented efficacy and acceptable safety in patients with hormone-refractory prostate cancer and small-cell lung cancer. Despite the positive outcome after completion of the Satraplatin and Prednisone Against Refractory Cancer (SPARC) phase III clinical trials, satraplatin was not approved by the FDA because it did not show a convincing benefit in terms of overall survival. However, clinical studies with satraplatin are still ongoing [99].

The reduction process, which occurs before the metal complex reaches DNA, is (generally) accompanied by the release of the axial ligands. As a consequence, the nature of the latter play a crucial role in determining the redox properties of the Pt(IV) species and hence the reactivity: for complexes featuring chloride ligands in equatorial positions, redox potentials and reduction rates correlate [17,100,101]. The order of the reaction rate with respect to the axial ligands is as follows: chloride  $\approx$  trifluoroacetate > carboxylate > hydroxide [101,102]. Consistently, cisplatin derivatives with trifluoroacetate ligands in axial positions undergo Pt(IV)→Pt(II) reduction more rapidly than the corresponding species with acetate donors, thus evidencing that the electron-withdrawing character of the axial ligands influences the reduction rate [103]. In addition, Pt(IV) complexes having trifluoroacetate and dichloroacetate as axial ligands can be unstable at neutral pH and undergo a rapid hydrolysis (thus affecting their physicochemical and pharmacological properties), whereas the corresponding complexes with monochloroacetato or acetato ligands are stable [102]. Incidentally, there are no significant differences in the metal coordination sphere of the corresponding complexes with dichloroacetate and monochloroacetato as determined by single-crystal X-ray diffraction [102].

Finally, the nature of axial ligands affects the balance between hydrophilicity [104] and lipophilicity [105,106] of the complex, thus influencing solubility, oral absorption [107] and cellular accumulation.

### 3.1. Axial leaving group

Zanellato et al. [106] discussed the biological properties of a series of cisplatin-based Pt(IV) prodrug candidates—namely, *trans,cis,cis*-[Pt(carboxylate)<sub>2</sub>Cl<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>], where carboxylate was acetate, butanoate, hexanoate, or octanoate (**C45**, Figure 14). The complexes exhibited a large range of lipophilicity and are stable from pH 1 to 9, making them suitable for oral administration. The transformation into their active Pt(II) metabolites was demonstrated in the presence of ascorbic acid, with pseudo-first order kinetics, the half-time of which smoothly decreased as the chain length of the carboxylic acid increased. As expected, the antiproliferative potency, tested on a large panel of human cancer cell lines, increased with the chain length; **C45c** and **C45d** were more active than cisplatin on all cell lines tested by about one or two orders of magnitude, respectively. Both complexes retained their activity also on a cisplatin-resistant cell line and exhibited a progressive increase of the selectivity compared with selectivity towards non-tumour cells. It is possible to overcome oxaliplatin chemoresistance in colorectal cancer cells by use of *trans,cis,cis*-bis(benzoato)dichloride[(*R,R*)-1,2-diaminocyclohexane]platinum(IV) (**C46**, Figure 14) bearing 1,2-diaminocyclohexane as the carrier the ligand; **C46** contains the same diamine present in oxaliplatin inserted in an octahedral Pt(IV) scaffold, with high lipophilicity conferred by the two benzoate axial ligands [105].

A great contribution to the study of the effect of the axial leaving group on Pt(IV) complexes has come from Austria thanks to an extended collaboration between chemical and medical academic and non-academic groups [99,108]. They reported a convenient synthetic procedure for the preparation of novel bis(dicarboxylato)platinum(IV) complexes and the modulation of their physicochemical properties of interest (e.g. solubility, lipophilicity and redox behaviour). The set of compounds (**C47**, Figure 14) tested covers a broad range of in vitro cytotoxicity (IC<sub>50</sub> values from 0.009 µM to more than 500 µM) in various cancer cell lines. Most of the compounds are active in vitro, and the results of experiments in vivo agree somewhat with the results of the cell culture experiments. In contrast, the data from cell culture experiments were not at all predictive for the in vivo activity after oral gavage. The mechanisms leading to this discrepancy between in vivo and cell culture experiments are so far speculative. One explanation might be rapid metabolism indicated by serum analysis, which showed that as early as 2 h after oral treatment the administered drugs had been completely metabolized and/or bound to proteins in serum. In addition, cytotoxicity tests conducted solely with human cancer cell lines do not consider the complex interactions between various tissue types and their impact on the pharmacokinetics and pharmacodynamics of the tested compounds. Further investigations of the role and activity of diverse drug metabolites are ongoing and will help to better understand the fate of anticancer drugs in vivo. Moreover, such

detailed knowledge can improve the prediction of in vivo anticancer activity, allowing the selection of the best drug candidates for further (pre)clinical development.

### 3.2. Conjugation with bioactive molecules

To improve the therapeutic effect of Pt(IV) prodrugs, a drug with a complementary mode of action can be coordinated (generally as a carboxylate derivative) to the Pt(IV) core. The final compound can operate on multiple targets with greater potency (provided the two activities are synergistic) and with fewer side effects than a single-target drug; a bifunctional or hybrid or dual-threat pharmaceutical agent as well as a combi-molecule can be conjugated. Gabano et al. [109] critically analysed the activity of Pt(IV) prodrugs conjugated with several fragments: with oestrogens, where binding to the oestrogen receptor increases the sensitivity to cisplatin of MCF-7 breast tumour cells; with dichloroacetate, which induces mitochondrial apoptosis; with paclitaxel (a cytotoxic analogue of taxol); with ethacrynic acid, which inhibits glutathione *S*-transferase, an enzyme involved in cisplatin resistance; and with valproic acid, a well-known antiepileptic drug that acts via inhibition of histone deacetylase. All these examples of combi-molecules have remarkable activity with respect to cisplatin, but the synergism with the active biomolecule was reported only in the case of paclitaxel and dichloroacetate conjugates, for which the activities of the combi-molecules were higher than those of an equimolar combination of cisplatin and bioactive compound. These contradictory results represent an example of a false positive because some bioactive ligands, such as valproic acid, act at millimolar concentrations, whereas cisplatin is administered in the micromolar range. Conversely, the oestrogens have a beneficial effect at the nanomolar level and can be toxic at the micromolar level. The increase of the effect of such combi-molecules with respect to cisplatin could be the result of a better cellular accumulation due only to the lipophilicity conferred by the bioactive ligand.

More recently, Ma et al. [110], reported a Pt(IV) prodrug derived from cisplatin with two dichloro chalcone derivatives acting as a p53 activator (**C48**, Figure 15). This single anticancer agent, called “chalcoptatin” by the authors, resulted in synergistically improved cytotoxicity in p53 wild-type but not p53-null human cancer cells. A mechanistic investigation was conducted. The prodrug effectively entered cancer cells and arrested the cell cycle at the S and G<sub>2</sub>/M phases, distinctively different from cisplatin. Chalcoptatin (**C48**) significantly induced p53 activation as well as the subsequent apoptosis pathways. This unique mode of action renders it remarkably cytotoxic and makes this compound among the first examples of a Pt(IV) prodrug that directly interacts with the

downstream pathway after the formation of Pt–DNA lesions. In this case, a synergistic mechanism between Pt(II) and chalcone was observed.

Cyclooxygenase (COX) is an enzyme involved in tumorigenesis and it is associated with tumour cell resistance to platinum-based antitumour drugs. Hey-Hawkins et al. [111] conjugated the NSAIDs indomethacin and ibuprofen with both Pt(II) and Pt(IV) analogues to explore the synergistic effects observed in combination treatments. The Pt(II) complexes revealed cytotoxic activities similar to the cytotoxic activity of cisplatin. In contrast, the Pt(IV) complexes **C49a**, **C49b**, **C50a** and **C50b** (Figure 15) exhibited remarkably higher cytotoxicity in all tumour cell lines tested and were able to completely overcome cisplatin-related resistance. Although the indomethacin conjugates were potent COX inhibitors, they appeared to execute their cytotoxic action via COX-independent mechanisms. The activities were similar in all cell lines independently of COX-2 expression and the COX potency of the conjugate or the respective NSAID. However, the potent Pt(IV) conjugates exhibited increased accumulation in the cells relative to that of cisplatin, probably resulting from their higher lipophilicity and kinetic inertness, thus leading to a clearly enhanced cytotoxicity. Investigations of this kind using conjugates are of fundamental importance to demonstrate that the observations from combination treatments are not directly transferable to dual-acting prodrugs and that the conjugates are important tools for the elucidation of the direct influence of COX inhibitors on platinum drugs in cells.

These few examples underline that the main difficulty in the interpretation of the activity of bifunctional Pt(IV) drugs is to determine whether synergism occurs or whether the increase of the activity is determined only by the improved pharmacokinetic properties of the complex.

### 3.3. Other platinum(IV) systems

Lorenzo et al. [104] synthesized and studied a series of Pt(IV) complexes having 1,2-bis(aminomethyl)carbocyclic or oxabicyclic carrier ligands (**C51**, Figure 16), bearing chloride and/or hydroxide ligands in the axial position and chloride or malonate ligands in the equatorial position as labile ligands. The introduction of bicyclic amines as carrier ligands allows one to improve the lipophilicity while limiting the increase of steric hindrance. The lipophilicity plays a crucial role in determining the cytotoxicity; in fact, the more active complex in vitro is **C51a**, formed by bicyclo[2.2.2]octane with amine ligands and four chloride ions as labile groups. Lipophilicity improves pharmacodynamic properties, but one of the major problems of platinum-based drugs is their poor pharmacokinetics owing to their very low water solubility. Clinically, platinum drugs are usually administered as a suspension in a physiological saline solution. To

improve the water solubility of the complexes, Lorenzo et al. worked at the level of the labile ligands, which are the first ligands exchanged during the absorption and distribution process of the drug. They introduced two hydroxide and a malonate bidentate ligands; in this way, **C51b** exhibited activity similar to that of cisplatin, with hydrophilic properties suitable for oral administration.

Another problem related to Pt(IV) compounds is the rapid and premature reduction *in vivo*; consequently, selection of a suitable ligand sphere to ensure appropriate activation kinetics is essential for clinical success. In line with these requirements, Varbanov et al. [100] explored the bischelate and trischelate diaminebis(dicarboxylato)platinum(IV) sphere of **C52** (Figure 16), which is believed to be optimal from hydrolysis kinetics considerations. Nine novel complexes were synthesized and studied; their cytotoxicity *in vitro* was mainly dependent on the amine carrier ligands, whereas the 1,2-diaminocyclohexane-containing compounds were the most potent species in all cell lines tested, with IC<sub>50</sub> values comparable to or superior to those of carboplatin. Surprisingly, the increase of the lipophilicity through the use of different carboxylate ligands had only a minor effect on the cytotoxicity within the series. The pronounced anticancer and especially antileukaemic activity combined with a low systemic toxicity observed in animal experiments indicates that **C52** might be a promising candidate for further preclinical development.

#### **4. Platinum(II) and palladium(II) as a structural moiety for non-covalent interaction with DNA**

DNA is an anionic polyelectrolyte that plays a fundamental role in the storage and expression of genetic information in a cell. The study of interaction of DNA with cationic metal complexes and the cytotoxic effects of such interactions has been a very active area during recent decades. These metal complexes bind to DNA through a series of binding modes, among them the main one is the covalent binding discussed in Section 2. However, metal systems can interact with DNA also by non-covalent interactions, the main ones being (1) electrostatic interactions of positively charged metal complexes with negatively charged phosphate groups of DNA, (2) groove binding, in which molecules bind in the DNA helix grooves involving hydrogen bonds and van der Waals interactions, and (3)  $\pi$ -stacking interactions, commonly observed between two planar aromatic groups. Among them, the most investigated is the latter, giving rise to the intercalating binding mode; it is the non-covalent  $\pi$ -stacking interaction resulting from the insertion of a planar aromatic ring, belonging to a ligand, between base pairs of the DNA double helix [112-116]. This way to bind DNA was first proposed by Lerman [117] to explain the strong affinity for DNA of some heterocyclic aromatic dyes, such as the acridines. In this context, the seminal work of Bond et al.

[118], which firstly showed, thanks to X-ray fibre diffraction measurements, structural evidence for interactions of this kind for the 2-hydroxyethanethiolato(2,2',2'-terpyridine)platinum(II) complex, must be mentioned. Both the presence of a planar ligand and the coordination geometry of the metal ion are important in determining the intercalating propensity of a given metal complex towards DNA. In addition, it can be postulated that the ability to intercalate depends on the extension of the aromatic region able to provide  $\pi$ -stacking interactions: the larger is the aromatic surface, the higher is the DNA binding affinity. Several studies seem to support this hypothesis [119,120], even if subtle hints [120] and different trends [121] make further investigations mandatory. As a consequence, a vast collection of different aromatic groups have been used to try to guarantee good intercalation properties for platinum- and palladium-based metal complexes, such as pyridine [113,116,122], bipyridine [122,123], terpyridine [124], phenanthroline [125,126], 8-hydroxyquinoline [127,128] and naphthalene [129], as well as several aromatic ligands bearing sulfur donor moieties, such as thiosemicarbazone, mainly for the palladium atom [130-132].

In this context, results from molecular and crystal structure determinations can offer valuable information about the overall shape of the metal complexes [130,123,132,133] (a planar arrangement would favour the intercalative interaction with DNA [113,114,127,131,133]) and the propensity of the aromatic moieties to be involved in  $\pi$ -stacking interactions with the neighbouring species in the crystal lattice [114,120,125,126,128].

The intercalation stabilizes, lengthens, stiffens, and unwinds the DNA double helix, and the degree of unwinding varies depending on the specific intercalator. The ethidium cation unwinds DNA by about  $26^\circ$ , whereas proflavine unwinds it by about  $17^\circ$ . These structural modifications of DNA can lead to functional modifications, or inhibition of transcription and replication or suppression of the DNA repair processes. All these effects make intercalators potent mutagens. Intercalative interactions between DNA duplexes and planar polycyclic aromatic organic intercalators, such as ethidium bromide (EB), acridine and its derivatives and benzo[*a*]pyrene have been thoroughly studied. Studies of bulky intercalators are quite rare.

Generally, the metal complexes that undergo intercalation with DNA can be divided in two main classes. For relatively inert coordinatively saturated square-planar Pt(II) and Pd(II) complexes with aromatic ligands, intercalation into DNA mainly involves the aromatic ligands. Metal complexes containing  $\sigma$ -bonded ligands having aromatic side arms for use as intercalators can be described as dual-function complexes: the aromatic side arms of the ligands can intercalate between DNA bases, whereas the metal coordinates directly to a DNA base.



Some recent examples of metal complexes in which the metal cation behaves as a structural moiety on binding with DNA, in the case of non-covalent interactions, are reported here for both Pt(II) and Pd(II) centres.

#### 4.1. Platinum(II) complexes

Despite the large number of Pt(II) complexes reported with 8-hydroxyquinoline and its derivatives, the mechanism of their anticancer activity remains unknown. Qin et al. [127] synthesized two Pt(II) complexes, (**C53** and **C54**, Figure 17) in which 8-hydroxyquinoline and 8-hydroxy-2-methylquinoline are present as ligands. Both complexes are very stable in solution and exhibited high cytotoxicity towards a number of human tumours but low cytotoxicity towards normal HL-7702 cells. Qin et al studied the cell cycle together with the apoptosis-inducing effect of the complexes; a number of crucial triggers in cell apoptotic pathways were also evaluated. Interactions of DNA with **C53** and **C54** were investigated, and the intercalation binding mode of these complexes, due to the planar aromatic structure of the corresponding quinolinol ligands, with DNA was demonstrated.

Pages et al. [120] and Garbutcheon-Singh et al. [125] synthesized numerous active complexes with the general formula  $[\text{Pt}(\text{I}_\text{L})\text{A}_\text{L}]^{2+}$ , where  $\text{I}_\text{L}$  is an aromatic intercalating ligand and  $\text{A}_\text{L}$  is an ancillary ligand. The intercalating ligands included 1,10-phenanthroline derivatives and dipyridoquinoxaline variants, whereas the ancillary ligands were the *R,R* or *S,S* isomers of 1,2-diaminocyclohexane or 1,2-diaminocyclopentane. They used different  $\text{I}_\text{L}$  and  $\text{A}_\text{L}$  combinations to modulate the chemical properties of the complexes with the aim of correlating the DNA binding affinity with the cytotoxicity of these complexes. As an example, **C55** (Figure 17) had much higher binding affinity than **C56** (Figure 17); this was explained by the larger aromatic surface area of dipyridoquinoxaline resulting in stronger  $\pi$ -stacking interactions between the ligand and the DNA base pairs. However, when the cytotoxicity was determined in the L1210 murine leukaemia cell line, all the dipyridoquinoxaline derivative-incorporating complexes were less active than those containing 1,10-phenanthroline. These results suggested that the contribution of the ancillary ligand should also be considered for the DNA binding of these complexes and thus for their cytotoxicity.

Special attention has been given recently to metal complexes with biphosphine ligands as potential therapeutic agents. Phosphine ligands in place of amines have been successfully used in gold-containing complexes used as therapeutic agents [134-137,138]. In this context Jamshidi et al. [112] synthesized three cationic platinum complexes containing bis(diphenylphosphino)ethane as a non-leaving carrier ligand. They investigated both the DNA binding properties and the anticancer

activity against Jurkat and MCF-7 cell lines, and all the complexes demonstrated a significantly higher level of anticancer activity compared with cisplatin. However, only **C57** (Figure 17) was capable of stimulating significantly the activity of caspase 3 and of inducing programmed cell death in Jurkat cancer cells. The study suggested that the interaction between DNA and the platinum complexes occurred according to a mixed-binding mode where intercalation, electrostatic binding and coordination were involved.

Zamora et al. [139] synthesized the steroidal 7-azaindole Pt(II) complexes **C58** and **C59** (Figure 17) with the aim of decreasing the main side effect associated with classic platinum-based anticancer compounds and of increasing the activity spectrum through more effective delivery of the drug to the desired target. They wanted to exploit the property of oestrogens to easily cross the cell membrane by passive diffusion and then to be bound by the oestrogen receptor in the cytoplasm and transferred to the nucleus. The biological potential of these stable compounds was evaluated towards the human breast cancer cells (T47D, cisplatin resistant) and epithelial ovarian carcinoma cells (A2780 and A2780cisR, acquired resistance to cisplatin). The results indicated great potential of these complexes to overcome cisplatin resistance and less toxicity towards normal human LLC-PK1 renal cells than cisplatin.

Platinum complexes bearing  $\pi$ -conjugated heterocyclic ligands such as terpyridine and its derivatives constitute a wide class of potential anticancer agents tested in recent years. Numerous examples of terpyridine-based platinum complexes have been reported in literature as efficient DNA intercalating units and active antitumour agents. In such systems, three nitrogen donor atoms are furnished by the terpyridine ligand, and the remaining fourth coordination position is quite variable. As an example of these systems, Zhang et al. [140] inserted a xanthine derivative such as caffeine in the fourth position of the square planar geometry of the Pt(II) ion, obtaining **C60** (Figure 17). The in vitro cytotoxicity of the complex was tested towards MCF-7, MDA-MB-231 and HT-29 tumour cells, and the values obtained, in the submicromolar range, exceeded the activity of cisplatin in the same conditions. Moreover, Zhang et al. highlighted the C2 atom coordination for the xanthine ligand might be responsible of the higher biological potencies of **C60** with respect to N5-coordinated analogues. Banerjee et al. [114] inserted a 4-amino-1,8-naphthalimide derivative as the fourth ligand in a Pt(II) terpyridine system. The resulting bifunctional complex (**C61**, Figure 17) exhibited high DNA binding affinity together with good cellular toxicity, comparable to that observed for cisplatin. This intercalating complex was readily taken up by breast and cervical cancer cell lines, inducing apoptosis.

Two Pt(II) terpyridine systems can be combined together to form bis-intercalating complexes whereby the remaining fourth coordination position was used to produce a bridge linking the two platinum centres. The cytotoxicity of such bis-intercalating systems is higher than their corresponding mononuclear units. Harper and Aldrich-Wright [141] synthesized a series of dinuclear Pt(II) terpyridine complexes connected by different linkers with the aim of exploring the effect of the chain length, flexibility, charge and stabilities on the cytotoxicity. In general, it was difficult to determine a structure-activity relationship: subtle structural differences reflected in evident variations in cytotoxicity as well as similar cytotoxicity were observed for complexes with large structural variations. In such a series, complexes that had an aliphatic thiol linker were more cytotoxic than cisplatin towards A2780cisR cells, with **C62** (Figure 18) being the most potent.

Organometallic compounds based on platinum have not received great attention as potential anticancer drugs probably because attention has been mainly focused on cisplatin analogues. In this context, Vezzu et al. [122] synthesized a series of structurally diverse cyclometallated platinum complexes and evaluated their cytotoxicity towards NCI-H522, HCC827 and NCI-H1299 human lung cancer cells and RV1 human prostate cancer cells. They demonstrated that in the square planar complexes based on tridentate ligands examined, the dissociation of a labile monodentate ligand, such the chloride in **C63** (Figure 18), was critical to obtain high cytotoxicity. **C63** was more cytotoxic than cisplatin in the cell lines tested; moreover, it interacted with DNA, triggering apoptosis by activation of poly(ADP-ribose) polymerase via the caspase 7 pathway. Recently, Mohammadi et al. [116] reported the cyclometallated platinum complex **C64** (Figure 18), based on deprotonated 2-phenylpyridine and a non-leaving lipophilic ligand, which exhibited higher cytotoxicity than cisplatin. The flexible chemical structure of **C64** was determining for the anticancer activity of the complex, which also showed an interesting property from a therapeutic point of view as the lack of a necrotic effect. The interaction between the complex and DNA was demonstrated to occur through a mixed binding mode, comprising partial intercalation and groove binding.

Gabano et al. [129] synthesized and evaluated two different platinum complexes with the formulas  $[\text{PtCl}_2(\text{NH}_3)(\text{L})]$  (**C65**) and  $[\text{PtCl}_2\text{L}_2]$ , where ligand L was the 2-aminonaphthalene group (Figure 18). The cytotoxicity of the complexes was tested against the A2780 ovarian carcinoma, A2780Cp8 cisplatin-resistant ovarian carcinoma and HCT 116 colon adenocarcinoma cell lines. **C65** showed cytotoxicity as good as that of oxaliplatin with regard to HCT 116 cells; this remarkable result is probably due to the presence of the aminonaphthalene ring, which mimics the effects of the diaminocyclohexane moiety in oxaliplatin, enabling **C65** to target tumours that respond poorly to

cisplatin. **C65** acted as a strong intercalator inducing a negative twist in the structure of supercoiled DNA. Moreover, **C65** was a strong binder of telomeric G-quadruplex structure, as it stacked on one external G-quartet and platinated the adenine residue close to it, with the result of cross-linking irreversibly the G-quadruplex structure.

Icsel et al. [113] reported the synthesis and biological studies of two complexes (**C66** and **C67**, Figure 18) based on substituted pyridine derivatives. The DNA binding studies revealed that the two complexes interacted with DNA; **C66** interacted with DNA by a single intercalative mechanism, whereas **C67** exhibited two types of interactions: intercalation and covalent binding. Both complexes have low cytotoxic activity against H1299 (human lung cancer) and 5RP7 (rat transformed fibroblast) cancer cell lines; however, **C66** had IC<sub>50</sub> values lower than those of transplatin and carboplatin.

Most of the Pt(II) terpyridine systems exhibiting anticancer activity reported to date bind to DNA via intercalation. However, some Pt(II) terpyridine complexes can also bind via other non-intercalative modes; Suntharalingam et al. [142] reported the synthesis and biological studies of **C68** (Figure 18), in which the terpyridine ligand has two sterically demanding groups, with the aim of favouring a non-intercalative interaction with DNA. All data were consistent with **C68** binding to the minor groove of DNA in a non-covalent manner. The almost planar structure of the complex fits in the minor groove of the DNA in a parallel manner with respect to the DNA backbone. **C68** has a promising toxicity profile, with high potency towards the cancerous U2OS (human osteosarcoma) and SH-SY5Y (neuroblastoma) cell lines (comparable to cisplatin) and reduced toxicity towards the normal GM05757 human fibroblast cell line. Moreover, **C68** selectively induced cell death in proliferating but not quiescent cells, and this is undoubtedly an attractive characteristic in terms of cancer therapy.

## 4.2 Palladium(II) complexes

Pd(II) derivatives have also been explored as an alternative to Pt(II)-based drugs. As already mentioned, Pd(II) displays similar chemistry to Pt(II) from many points of view; however, a significant difference between them is the high kinetic lability of Pd(II) complexes compared with Pt(II) complexes. Moreover, the better solubility of palladium complexes, compared with platinum complexes, seems to make palladium complexes more attractive.

Thiosemicarbazone and its derivatives have been widely used as ligands for palladium because of their pharmacological properties. Prabhakaran et al. [132] reported the synthesis and biological studies of four thiosemicarbazone palladium complexes. The DNA binding studies revealed that all

the complexes interacted with calf thymus DNA (CT-DNA) via an intercalation mode. The cytotoxicity of the complexes was tested against the A549 (lung cancer) and HepG2 (liver cancer) cell lines, and the results evidenced that the activity of **C69** and **C70** (Figure 19) was higher in A549 cells than that of cisplatin; moreover, **C69** had an IC<sub>50</sub> value similar to that of cisplatin in HepG2 cells. The intracellular concentrations of the complexes were determined; the accumulation of **C69** was higher than that of the other complexes, and this may contribute to the increased toxicity observed for this complex. Ramachandran et al. [130] described the synthesis, DNA and protein binding and in vitro toxicity of four Pd(II) complexes with 2-oxo-1,2-dihydroquinoline-3-carbaldehyde 4*N*-substituted thiosemicarbazones (**C71-C74**, Figure 19). All the complexes bind to the DNA helix via intercalation, and the binding constants revealed that the cationic nature of **C71** enhanced the binding affinity for DNA. Moreover, all the complexes possessed potent free radical scavenging activity compared with the standard antioxidants. From cytotoxicity studies, all the complexes exhibited antitumour activities against human cervical cancer cells (HeLa), human skin cancer cells (A431), human liver carcinoma cells (HepG2), and human laryngeal epithelial carcinoma cells (Hep-2), without affecting normal cells. Moreover, they exhibited better cytotoxicity towards the HepG2 cancer cell line, with **C71** showing an inhibitory activity about three times higher than that of cisplatin. Thiosemicarbazone derivatives were used by Hernández et al. [131] to synthesize a series of Pd(II) bischelate complexes that were evaluated for their cytotoxic activity against the H460 (human lung large cell carcinoma), M-14 (human amelanotic melanoma), DU145 (human prostate carcinoma), MCF-7 (human breast adenocarcinoma), HT-29 (human colon adenocarcinoma) and K562 (human chronic myelogenous leukaemia) cell lines. In general, all the complexes exhibited antiproliferative activity against all the human tumour cell lines investigated, with IC<sub>50</sub> values below 10 µM; moreover, **C75** and **C76** (Figure 19) exhibited the greater cytotoxic activity, with IC<sub>50</sub> values of 0.01-0.23 µM and 0.65-1.06 µM, respectively. The Pd(II) bischelate complexes act as intercalating agents between the pyrimidine and guanine bases of DNA of tumour cells, inducing conformational changes on the DNA double helix. In this context, the presence of the 3-hydroxy and 1-nitro substituent groups in the benzene and naphthalene aromatic rings of **C75** and **C76**, respectively, play an important role in the enhancement of the resulting antiproliferative activity.

Ulukaya and co-workers [115,143-145] reported the anticancer activity of a Pd(II) saccharinate complex with terpyridine (**C77**, Figure 19). This complex strongly binds DNA by a dual function: by metal intercalation, interacting with the DNA helix through the insertion of the planar terpyridine ring between the DNA base pair, and by covalent binding. The results obtained with the

5RP7 and NIH/3T3 cell lines clearly demonstrated that **C77** powerfully inhibited the growth of fibrosarcoma cells by inducing apoptosis. Moreover, cytotoxicity studies performed on the MCF-7 and MDA-MB-231 human breast cancer cell lines revealed that **C77** had a strong growth-inhibitory effect on MCF-7 cells rather than MDA-MB-231 cells. This Pd(II) complex induced apoptotic cell death through the caspase-dependent pathway in both cell lines. **C77** was able to significantly reduce the growth of tumour cells in an in vivo model. **C77** was also investigated in combination with niclosamide in breast cancer stem cells, highlighting the enhancement of the cytotoxic activity of both compounds [145]; in addition, it was tested in six different prostate cancer cell lines [146]. This complex seems important in terms of the cytotoxic effect of Pd(II) compounds on cancer stem cells that are thought to be responsible for both resistance to drugs and recurrence of the disease. Chu et al. [124] synthesized the terpyridine derivative-based complex **C78** (Figure 19), introducing the piperidylethoxy group as a substituent in 4'-phenylterpyridine; the aim was to increase the solubility of the molecule and its interaction with DNA. The cytotoxicity of **C78** was tested against eight different cell lines (HL-60, BGC-823, Bel-7402, KB, A549, HeLa, K562 and MCF-7), and the results indicated that the cytotoxicity was higher than that of cisplatin for all cell lines tested; in the Bel-7402 cancer cell line, **C78** exhibited a sevenfold higher cytotoxicity than cisplatin.

Gao et al. [126] studied the influence of the carbon chains bound to a Pd(II) metal centre on the DNA interactions and cytotoxic activities for a series of complexes with 1,10-phenanthroline as the auxiliary ligand and malonic acid derivatives as the main ligand (**C79-C83**, Figure 20). The DNA binding data indicated that the complexes interacted with the DNA helix by a strong stacking interaction between an aromatic group and the helix base pairs; the binding ability increased gradually as the length of the aliphatic chain became longer. All the complexes promoted the cleavage of pB322 DNA from supercoiled form I to the nicked form II, with **C83** exhibiting the most effective DNA cleavage activity in comparison with the other complexes at the same concentrations. The in vitro growth-inhibitory effect of the complexes was evaluated in HeLa and KB cells; the results indicated that the cytotoxicity increased gradually as the length of the aliphatic chain became longer, with **C83** showing cytotoxicity coefficients better than those of cisplatin for both cell lines.

Wang et al. [146] synthesized a series of complexes in which the Pd(II) ion was coordinated by an aromatic N-containing ligand and 4-toluenesulfonyl-L-amino acid dianion. The cell growth inhibitory studies revealed that both the amino acid and the diamine have significant effects on cytotoxicity, with effects related to tumour cell type. **C84** and **C85** (Figure 20) exhibited cytotoxicity higher than that of cisplatin in the MCF-7 cell line.

As observed for Pt(II) compounds, also among Pd(II) complexes special attention has been paid to metallacycle complexes with nitrogen donor ligands. In fact, metallated species may bind to DNA by means of intercalative interactions, mainly when such complexes contain planar and stable aromatic structures. Albert et al. [147] evaluated a series of cyclopalladated primary amine complexes by means of a structure-activity relationship analysis of the inhibition of cell proliferation activity against a panel of human adenocarcinoma cell lines (A549 lung cancer, MDA-MB231 and MCF7 breast cancer and HCT 116 colon cancer). **C86** and **C87** (Figure 20) exhibited the best antiproliferative activity and were more cytotoxic than cisplatin in all cell lines except A549. **C86** and **C87** were 14 and 19 times, respectively, more potent than cisplatin for the inhibition of the cisplatin-resistant HCT 116 human adenocarcinoma cell line. The complexes were not effective in removing the supercoils of plasmid DNA, pointing to a different mechanism of action or an alternative biomolecular target. The palladacycles **C86** and **C87** exercised their antiproliferative activity against A549 cells mainly through the induction of apoptosis.

An example of dinuclear palladacycle complexes was reported by Karami et al. [133] by the synthesis and biological studies of two complexes (**C88** and **C89**, Figure 20) in which the symmetric bidentate ligand 1,2-bis(diphenylphosphino)ethane bridges two identical cyclopalladate units. The DNA binding studies suggested that both complexes bind DNA by intercalation, with **C89** binding more strongly than **C88**. The in vitro toxicity of the two complexes was evaluated against human cervix carcinoma (HeLa), colon cancer (HT-29), leukaemia (K562) and human breast carcinoma (MCF-7) tumour cell lines. Both **C88** and **C89** exhibited high antiproliferative activity, with IC<sub>50</sub> values in the range 2-9 µM, values better than those of cisplatin in three cell lines (HeLa, HT-29 and K562). Karami et al. suggested that this high cytotoxicity could reflect the good solubility and lipophilicity of the complexes; in fact, the lipophilicity of the bridged complexes was related to the presence of two bulky PPh<sub>2</sub> groups from 1,2-bis(diphenylphosphino)ethane which facilitate the transport through the cell membranes. Moreover, both complexes interacted with DNA through intercalation, and the 1,2-bis(diphenylphosphino)ethane bridge confers more flexibility to the structure, giving the possibility to make more interactions with DNA. Hadizadeh et al. [148] provided a detailed study about the influence on cytotoxicity resulting from increasing the distance between the two Pd(II) ions in dinuclear complexes. In that study, Hadizadeh et al. synthesized three dinuclear Pd(II) complexes with dithiocarbamate ligands (**C90-C92**, Figure 20); they evaluated the effect of hydrocarbon chain length (propylene, butylene and octylene) in the structure of these complexes on the anticancer activity compared with that of cisplatin. All three complexes were highly cytotoxic to the AGS (human gastric carcinoma), HepG2 (hepatocellular carcinoma)

and KYSE-30 (oesophageal squamous cell carcinoma) cell lines, with  $IC_{50}$  values better than those of cisplatin in all the cell lines. The cytotoxicity values for the three complexes were rather similar, although **C90**, in which the propylene chain connected the two Pd(II) ions, had lower  $IC_{50}$  values in all the cell lines with respect to the other complexes. Cell cycle analysis revealed that **C90-C92** arrested the cell cycle at G<sub>2</sub>/M and S phases.

## 5. Photoactivated complexes

In the quest for even more selective anticancer therapies, several approaches to localize the cytotoxic effects of the drug to the tumour site have been investigated as already outlined. One of these approaches exploits the use of light and its interaction with matter, the beneficial effects of which have been known since antiquity. Transition metal complexes, and in particular those containing platinum and palladium, having a wide variety of electronic transition state available are suitable candidates for photoactivation, and the use of a suitable ligand can aid and amplify the effect. For example, charge transfer transitions such as metal-to-ligand charge transfer and ligand-to-metal charge transfer are usually intense, giving rise to the possibility of complete transfer of an electron from the metal to the ligand or vice versa in the excited state as well as irreversible photodecomposition (redox) reactions.

In cancer therapy, the main application used in the clinic is radiotherapy; it involves the use of high-energy radiation (X-ray) sometimes generated (internal radiotherapy) by specific radioactive metal isotopes such as cobalt-60, iridium-192, cesium-137 and palladium-103. Recently, the use of transition metal complexes has been investigated because their cytotoxic activity can be activated if they are irradiated by suitable light. Such activity is higher than that exerted in the dark and so is attributable to the activation of the species.

In all cases, the aim is to produce activated species that can be detrimental for cancer cells, acting with more efficiency or differently with respect to drugs currently in use. It was proved that ROS, free radicals and molecular oxygen in its reactive singlet state generated within cells and are the causes of DNA damage. However, photoactivated chemical reactions able to produce in situ the anticancer drug, starting from a non-toxic prodrug, are also being investigated.

The main approaches reported in recent years are X-ray radiotherapy, photodynamic therapy (PDT) and photoactivated chemotherapy (PACT); see Figure 26.

Non-invasive radiotherapy has been widely used clinically for decades, whereby high-energy X-rays or  $\gamma$ -rays are used to kill directly cancer cells, with all radiation converging exclusively on tumours; this reduces the effect of toxicity towards close tissues. Unfortunately, this type of



radiotherapy fails for hypoxic tumours, often resulting in insensitivity to the radiation [149]; at the same time, high dosages of radiation can damage normal cells, resulting in the development of secondary tumour. Heavy metal complexes and particularly platinum complexes have shown the possibility to enhance X-ray-induced cell death through Auger electron effects of the platinum atom under high-energy irradiation [150]. By X-ray irradiation, there is an Auger cascade effect due to inner-shell electrons in platinum; the emitted low-energy electrons give rise to ROS and free radicals around the platinum complex, thus damaging DNA by strand breaks.

PDT is another medical approved technique for the treatment of specific tumours and uses UV-vis energy. The technique has mainly been used to transform molecular oxygen from the fundamental triplet state  $^3\text{O}_2$  to the excited singlet state  $^1\text{O}_2$ ; this causes oxidative stress and the death of cancer cells [151-153]. PDT requires molecular oxygen in situ, low-energy light and a photosensitizer. The photosensitizer accumulates in the tumour tissue and is able to transfer efficiently the photon energy adsorbed to excite triplet oxygen into the singlet state and has an antitumour effect, avoiding the killing of normal cells. The photosensitizer must have strong absorbance at long wavelengths and a high  $^1\text{O}_2$  quantum yield. Porphyrin and porphyrinoid ligands and related metal complexes are often used often in this field because of their selectivity for malignant cells. PDT has the advantages of spatial and temporal control as well as the possibility of repeated doses [154-156], but because it needs dioxygen in situ, it is inactive in hypoxic conditions. Several platinum complexes that can be photoactivated by light have been investigated [157-159].

PACT is a more recent application against tumours; it also uses visible light and preferentially longer-wavelength red light, which is able to penetrate deep into tissue. In PACT, a suitable inert compound is transformed into the active anticancer agent through light irradiation; the process does not need the presence of molecular oxygen in situ and so can be applied in hypoxic conditions. The active compound can exert its action directly, behaving as a classic antitumour agent, but it can also generate in situ, during or because of the photoactivation, cytotoxic species such as ROS, free radicals and/or singlet state  $^1\text{O}_2$ .

Following these guidelines, Xie et al. [160] have recently reported four Pt(II) complexes obtained with 1,10-phenanthroline derivatives (**C93**, Figure 21). They have been tested as potential radiation sensitizers to increase the sensitivity of cancer cells to radiotherapy. In the absence of X-ray irradiation, the platinum complexes exhibited less antitumour activity in vitro against human cell lines less than cisplatin, with the compound with  $\text{X} = \text{COOH}$  being the most active. By X-ray irradiation, the inhibition of growth of A375 human melanoma cells increased in the presence of

such complexes; in contrast, they showed much lower cytotoxicity towards human normal cells. Such inhibition occurs by the induction of G<sub>2</sub>/M cell cycle arrest.

The complexes also dramatically inhibit the activity of thioredoxin reductase, an enzyme overexpressed in many cancer cells and identified as a cancer target for the design of new anticancer drugs [161]. In the presence of the complexes, intracellular ROS overproduction, attributed to the Auger electron effect of platinum under X-ray irradiation, was observed; this triggers DNA damage, thus increasing radiosensitivity and the inhibition of tumour reproduction.

Naik et al. [162] recently reported an example of PDT using a porphyrin motif. Porphyrins are widely used in PDT, but their platinum complexes are not, Naik et al. synthesized three tetraplatinum(II) complexes using a porphyrin scaffold (**C94-C96**, Figure 21) and investigated their light-induced anticancer properties in vitro. In each complex, the four Pt(II) atoms are located outside the porphyrin ring; each of them is stabilized by a pyridine, belonging to the porphyrin, and by two *trans* chloride ligands and one DMSO ligand (**C94**) or one chloride and two *trans* or *cis* ammonia ligands (**C95** and **C96**, respectively).

All the complexes exhibited excellent quantum yields of <sup>1</sup>O<sub>2</sub> production ( $\Phi$ ), which is the requirement for a compound to be used in PDT. A higher value ( $\Phi$  = 0.54) was obtained for **C96** than for **C94** ( $\Phi$  = 0.42) and **C95** ( $\Phi$  = 0.50), whereas for the precursor porphyrin,  $\Phi$  = 0.41.

The potential PDT activity was investigated in the dark and after irradiation with light of 420 nm; tetraplatinum(II) porphyrins **C94-C96** and the porphyrin precursor exhibited negligible toxicity in the dark towards both non-cancerous MRC-5 and HeLa cancer cells, whereas a photocytotoxicity 600-1200 higher than their toxicity in the dark towards HeLa cells was found only for the Pt(II) complexes. This ratio is defined as the phototoxic index (PI) and is the ratio between IC<sub>50</sub> in the dark and the IC<sub>50</sub> in light ( $PI = IC_{50(dark)}/IC_{50(light)}$ ). The best PI values were obtained with the ammonia Pt(II) complexes **C95** and **C96**, which had IC<sub>50</sub> values of 54 nM (PI = 655) and 37 nM (PI = 1210), respectively, on irradiation with light. Irradiation at lower energy (575 nm) also gave rise to photoactivity. The compounds also exerted phototoxicity (420 nm) towards cisplatin-sensitive A2780 and cisplatin-resistant CP70 human ovarian cancer cell lines (PI > 5260 and IC<sub>50</sub> = 19 nM for **C96**).

The activity has been correlated to the lipophilicity of the complexes, which favours their diffusion through the cell membrane, thus enhancing their cellular uptake. Confocal laser microscopy experiments confirmed a relevant uptake for the platinum porphyrins in HeLa cells, with selective accumulation in the nucleus. The lipophilicity allows **C96** to increase the nuclear platinum content more than 30-fold as compared with that observed with cisplatin. Viscometry and NMR

experiments performed with **C96** demonstrated that it was able to bind to DNA by coordination to the N7 atom of guanosine as well as by intercalation, although the N7 kinetic coordination is much slower than intercalation. The pronounced light-mediated cytotoxicity was attributed to a photoinduced DNA cleavage; **C96** did not cause any DNA cleavage on incubation in the dark, whereas it produced significant cleavage of the supercoiled configuration (intact DNA) and an increase in intensity of the nicked band (damaged DNA) on irradiation with light.

PACT is an approach towards better control of drug-action specificity through spatial and temporal activation [163-165]. PACT with coordination compounds is usually based on a photochemical reaction occurring at the metal centre [166,167]; instead Gamez et al. [168] reported the first example of a potential PACT compound obtained by the photochemical modification of a ligand(s) coordinated to a metal ion.

The new strategy exploited the photoswitchable property of 1,2-dithienylethene derivatives; these are photochromic molecules that can be converted into either their open or their closed forms on exposure to visible or UV light [169]. Gamez et al. [168] prepared two ligands each containing the photoswitchable 1,2-dithienylethene moiety able to form Pt(II) complexes (**C97** and **C98**, Figure 21); the difference between two ligands consists in the replacement of hydrogen (**C97**) with the more electronegative fluorine atoms (**C98**) in the cyclopentenyl ring. The open/closed forms of the Pt(II) complexes **C97** and **C98** displayed different DNA-interacting properties and cytotoxic behaviours. Complexes, as  $[(trans\text{-PtCl}_2(\text{DMSO}))_2\text{L}]$  complexes, were prepared by the reaction of 2 equiv of *cis*-[PtCl<sub>2</sub>(DMSO)<sub>2</sub>] with 1 equiv of the ligand in methanol at room temperature. The switching properties of **C97** and **C98** as well as those of their ligand precursors were verified by UV-vis spectroscopy in dichloromethane because the compounds are not soluble in water. In the absence of UV irradiation, characteristic bands attributed to the open form of the ligand were detected; irradiation with UV light at  $\lambda = 365$  nm converts the open form to the closed form, redshifting the  $\pi\text{-}\pi^*$  transitions of the species. This was observed both in the free form of the ligand and in the platinum species. On irradiation with visible light, the initial spectra are recovered for **C97** and **C98**, indicating the reversibility of the photocyclization process. However, the closed forms of both complexes was stable when it was kept in the dark for at least 24 h in dichloromethane solution, whereas it partially interconverts to the open form when kept in daylight. The affinity between DNA and the open and closed forms of **C97** and **C98** was investigated by competitive binding studies using EB as a reference. The main result is the significantly different behaviours of the open and closed forms of both complexes. The closed form showed an affinity towards CT-DNA 6.5 and 5.5 times higher than that of the open form for **C97** and **C98**,

respectively; in addition, **C97** displayed a slightly better DNA affinity than **C98**, attributed to the unfavourable electrostatic repulsion with the phosphate backbone of the double helix due to the fluorinated cyclopentenyl ring of **C98**.

The greater affinity of the closed complexes for duplex DNA was attributed to the different structural aspects between the open and closed forms. The open form is distinctly more voluminous than the closed form (about 12% longer and 25% wider) and, above all, the closed form is clearly more planar and more conjugated on ring closure, owing to a sterically favoured interaction with DNA.

The open/closed forms of the complexes have drastically distinct DNA-interacting behaviours, with the closed forms modifying the DNA shape more efficiently. The closed form of **C98** performs better than the close form of **C97**, which is the reverse behaviour compared with the competitive binding studies.

Even more interesting is the fact that each isomer (particularly for **C98**) exhibits a different cytotoxic behaviour towards various cancer cell lines. The open/closed forms of **C97** as well as the open form of **C98** exhibited very poor cytotoxicity properties, whereas the closed form of **C98** was active against most of the cancer cell lines tested. The best result was obtained with the DMS 53 small-cell lung cancer cell line, which is the most common and most virulent of neuroendocrine cancers ( $IC_{50} = 34.40$  mM, 48 h of incubation). However, compared with cisplatin, the closed form of **C98**, depending on the cell line, is about four to eight times less active.

Confocal microscopy indicated that all the compounds are capable of entering the cells; however, only the cytotoxic closed form of **C98** showed a diffuse cytoplasmic staining and it is the only species colocalized with the nucleus of A375 cells, thus suggesting that its high cytotoxicity may be due (in part at least) to its ability to reach the nucleus.

Sadler and co-workers [165, 170-175] have worked for several years on PACT, mainly regarding Pt(IV) complexes. They have focused on *trans*-diazido Pt(IV) complexes containing several substituents in the octahedral species. The diazido Pt(IV) complexes appeared to be very promising prodrugs [176] because they are inert and non-toxic in a biological environment in the dark and they exhibit high stability in the dark, allowing their potential release to the target, where they produce a potent cytotoxic effect on irradiation with visible light with new mechanisms of action [158, 177-179].

Recently, they reported a new series of photoactivable *trans,trans,trans*-diazidodihydroxodiamino Pt(IV) complexes able to form novel DNA adducts (**C99-C101**, Figure 21) [180]. They had demonstrated previously that replacement of one or two  $NH_3$  ligands with pyridine in the

*trans,trans,trans*-[Pt(N<sub>3</sub>)<sub>2</sub>(OH)<sub>2</sub>(NH<sub>3</sub>)<sub>2</sub>] led higher photocytotoxicity and visible-light activation [165,170]. The replacement of NH<sub>3</sub> with methylamine and/or thiazole at the Pt(IV) centre generated potent photocytotoxicity, in particular towards the cisplatin-resistant cell line A2780cisR [180]. The new compounds **C99**, **C100** and **C101** have been synthesized following an analogous method of Kauffman and Cowan [181,182]. The synthesis provides the oxidation by H<sub>2</sub>O<sub>2</sub> of the respective *trans*-diazido Pt(II) complexes **C102** and **C103** [165,183].

The compounds were stable in the dark for several months in biological conditions; because they do not react with 5'-guanosine monophosphate (5'-GMP) or L-ascorbic acid, they were stable in the dark with regard to hydrolysis and also glutathione reduction.

The complexes show three main absorption bands at 365, 420 and 450 nm, and their photoactivable cytotoxic activity was investigated by their irradiation at such wavelengths. Irradiation in aqueous solution resulted in the loss of the Pt(IV)–N<sub>3</sub> bond(s). Density functional theory and time-dependent density functional theory calculations attributed the photoreactivity to the presence of dissociative ligand-to-metal charge transfer/d-d excited states, which can be populated by irradiation with UV and visible light.

In the absence of light, **C99** and **C100** were not cytotoxic to several cell lines tested, whereas **C101** had IC<sub>50</sub> = 187 µM for A2780 cells. All of the compounds exhibited different and marked cytotoxic activity when irradiated; in particular, they exhibited potent activity towards A2780cisR cisplatin-resistant ovarian cancer cells on irradiation with UV or blue light and, when irradiated with blue light (420 nm), they were also highly cytotoxic to the A2780, OE19 and HaCaT cell lines.

They are more than an order of magnitude more potent towards HaCaT keratinocytes, A2780 ovarian cancer cells, and OE19 oesophageal carcinoma cells than cisplatin, also showing particular potency towards cisplatin-resistant human ovarian cancer cells (A2780cisR).

Their cytotoxicity, compared with that of similar compounds, highlighted that the replacement of the pyridine with a thiazole in **C101** increased the UV phototoxicity in some cell lines; the replacement of ammonia with methylamine in **C100** seemed to decrease the cytotoxicity of the thiazole without affecting the phototoxicity. All the results suggested that thioazole-containing complexes were more effective towards resistant cell lines, showing resistance factors close to 1 (i.e. lack of cross-resistance to cisplatin).

On irradiation, **C99** and **C100** exhibited significantly faster binding to 5'-GMP and DNA than their *trans* Pt(II) precursors or cisplatin. The products from photoreaction of **C99** or **C100** with 5'-GMP were investigated; the results indicated that one azido ligand and two bound hydroxyl groups were released during irradiation. A probably azidyl radical released together with a hydroxide radical

formed gave rise the reduction of Pt(IV) to Pt(II) and the formation of Pt(II)-5'-GMP complexes; the formation of a bisGMP adduct, afforded by the loss of the second azide, was detected in small amounts. The formation of Pt(II)-GMP species is in agreement with the density functional theory calculations on the lowest-lying triplet states of **C99** and **C100**. The highly distorted lowest-lying triplet geometry, showing an elongated Pt–N<sub>3</sub> distance and a reduced positive charge on the platinum centre, should favour azide release and the reduction of Pt(IV) to Pt(II).

**C99** and **C100** bind to DNA in a manner substantially different from that of cisplatin; this could account for their activity towards the A2780cisR cisplatin-resistant cell line.

Photoinduced binding with DNA oligonucleotide and with individual nucleotides revealed that photoactivated **C99** and **C100** formed both monofunctional and bifunctional DNA lesions, with preference for G and C, similarly to transplatin, but with significantly larger unwinding angles and a higher percentage of interstrand cross-links, with evidence for DNA strand cross-linking further supported by a comet assay.

The products of the photoinduced platination reactions of DNA caused by **C99** and **C100** showed similarities with the products of the dark reactions of the Pt(II) compounds *trans*-[PtCl<sub>2</sub>(MA)(Py)] and *trans*-[PtCl<sub>2</sub>(MA)(Tz)], where MA is methylamine, Py is pyridine and Tz is thiazole. Following photoactivation, **C100** reacted most rapidly with CT-DNA, followed by **C99**, whereas the dark reactions of both *trans* Pt(II) complexes with DNA were comparatively slow. **C99** and **C100** can therefore result in rapid potent photocytotoxicity and novel DNA lesions in cancer cells, with no activity in the absence of irradiation.

The promising results obtained with these systems have led to further investigation of **C99** to reveal in more detail the mechanism of the photoactivation/complex decomposition [180].

Irradiation of the Pt(IV) complex **C99** with 5'-GMP in aqueous solution at pH 7.4 resulted in different Pt(II) products depending on the irradiating wavelength. Irradiation at 450 nm gave the monoGMP Pt(II) complex [Pt(N<sub>3</sub>)(MA)(Py)(5'-GMP)]<sup>+</sup> (**C99a**<sup>+</sup>) as the major species and the bisGMP Pt(II) complex *trans*-[Pt(MA)(Py)(5'-GMP)<sub>2</sub>]<sup>2+</sup> (**C99b**<sup>2+</sup>). In addition to **C99a**<sup>+</sup> and **C99b**<sup>2+</sup>, two new compounds, **C99c** and **C99e**, were observed in high-performance liquid chromatography experiments on irradiation at 420 nm, whereas a further species (**C99d**) appeared on irradiation at 365 nm. These results are similar to those in the absence of dissolved molecular oxygen (i.e. under an argon atmosphere), thus excluding the possibility that dissolved molecular oxygen is involved in the Pt(IV) to Pt(II) reduction process. **C99c**, **C99d** and **C99e** contain NH<sub>3</sub> bound to Pt(II), suggesting that it is derived from the azide ligand.

By use of marked  $[^{15}\text{N}=\text{}^{14}\text{N}=\text{}^{14}\text{N}]^-$  azide ion, gaseous  $\text{N}_2$  directly released from  $\text{Pt-N}_3$  was detected. The photoreaction releases the  $\text{N}_3^\cdot$  radical but also probably the  $\text{OH}^\cdot$  radical (it was not detected because of its short lifetime).  $\text{O}_2$  is also formed; it is generated not by water molecules through energy transfer from a photosensitizer but directly from **C99**, and thus from coordinated hydroxide ligands. The presence of the singlet  $^1\text{O}_2$  form was detected; the quantity of generated  $^1\text{O}_2$  is higher the shorter the wavelength.

The production of ROS by the photodecomposition of the Pt(IV) complex **C99** leads to an unexpected oxidation of guanine during the photoreaction with 5'-GMP. The reaction pathways of the **C99**-5'-GMP photoreactions were investigated, and the findings suggested that the photodecomposition of **C99** involves the formation of platinum-nitrene intermediates and the formation of singlet oxygen, free azide, azidyl radicals and nitrogen gas. The oxidation of guanine is likely to arise from reactions of singlet oxygen and nitrene intermediates. The generation of singlet oxygen in the absence of oxygen gas and the oxidative damage to guanine may contribute to the potent photocytotoxic effects of this complex.

## 6. Other complexes

Many active complexes are difficult to rationalize in terms of molecular structure, both from the ligand and from the complex point of view. Currently, many of them, although active, are difficult to classify. Some of them have been tested in biological studies. In the last 3 years, several compounds have fallen in this category [184-191]; here we report those for which important results have been obtained in terms of antineoplastic as well as unconventional activity and a converging motif.

Polynuclear platinum and palladium complexes have been widely investigated [9] and are still being investigated in synthetic and related studies applied to different chemical structures (Figures 22-24). Polynuclear platinum complexes, structurally different from cisplatin, exhibit a different mode of DNA binding, circumventing cellular resistance. Mainly, the structural conformational changes induced by long-range interstrand and intrastrand cross-links are distinctly different from those induced by the mononuclear cisplatin and oxaliplatin [192]. Among them, the use of aliphatic polyamines is frequent because many of them, such as spermidine and spermine, are present in all prokaryotic and eukaryotic cells [193,194]. The prototype of this class (**C104**, Figure 22) has reached human phase II clinical trials [195]. In this period, other studies considered these motifs and obtained interesting results, such as the study of Menon et al. [196] on **C104** and **C105** (Figure 22), where the cyclohexyl fragment has been inserted as a carrier ligand. Both compounds exhibited

significant antiproliferative activity, and **C105** induces DNA interstrand cross-links, eliciting a unique dual mode of cell cycle arrest.

Silva et al. [197] investigated trinuclear Pt(II) and Pd(II) complexes obtained with dipropylentriamine (norspermidine) (**C106**, Figure 22) in a metal-to-ligand molar ratio of 3:1. The Pd(II) complex showed a stronger antiproliferative effect than the Pt(II) complex towards three breast cancer cell lines, whereas normal cells were less sensitive to both compounds. The Pd(II) species reduced the number of colonies formed in a soft agar assay performed with the breast cancer cell lines more so than the Pt(II) species. A genotoxicity screen indicated that they are not genotoxic at 25 mM concentration. Trinuclear Pt(II) complexes with linear polyamines were considered by Hamad et al. [198] for activity against human ovarian tumour models with *cis* geometry for terminal platinum centres; one of them (**C107**, Figure 22) was more active than cisplatin, and its activity has been ascribed to the formation of long-range interstrand and intrastrand adducts with DNA.

In research on polynuclear platinum and palladium complexes using linear polyamines Chtchigrovsky et al. [199] synthesized a series of *trans*-Pt(II) dinuclear complexes in which Pt(II) is also stabilized by one C–Pt(II) bond (**C108**, Figure 22); these compounds displayed cytotoxic activities in the micromolar range against many cancerous cell lines and did not cross-react with cisplatin in the A2780/DDP cell line. The slow formation of monoadducts to double-stranded DNAs as the major products was observed. These C–Pt and C–Pd compounds are organometallic systems recently investigated for their activity [200–204].

Several Berenil dinuclear Pt(II) complexes (Figure 23, **C109**) have been studied [205,206]. Two of them (where X is 3-ethylpyridine or 3-butylpyridine) exerted a strong antitumour effect on human breast cancer cells [205]. The results revealed apoptosis induction by those dinuclear Pt(II) complexes with the apoptotic potential being evidenced as primary lesions of DNA. Apoptosis induction, possibly enhanced by a contribution of targets other than DNA, seems to be an important factor in the mechanism of action of these compounds. The cytotoxic effects of both **C109** complexes cause mitochondrial dysfunction and activation of the caspase cascade. They might induce apoptosis by extrinsic and intrinsic pathways. The results in [206] demonstrated the remaining five Berenil dinuclear Pt(II) complexes enhanced oxidative stress formation in fibroblasts; this was ascribed to the increase of ROS generation, and to the decrease of antioxidant properties. Among all the complexes, those with two isopropylamine ligands are the most active; however, those with piperazine or 2-picoline ligands also increased the apoptotic profile compared with cisplatin, mainly relating to breast cancer cell metabolism.



Dinuclear Pt(II) tetraazolato-bridged complexes have been obtained and investigated in addition to previously synthesized similar species [207-209] (Figure 23, **C110**); the crystallographic characterization of one of them, cytotoxicity profiles, and in vivo antitumour efficacies were reported [210]. The cytotoxicity fingerprints of the complexes based on the JFCR39 cytotoxicity data were similar to one another but completely different from the fingerprints of clinical platinum-based anticancer drugs, thus suggesting that the new tetraazolate compounds inhibited cell growth by means of a different mechanism including cellular uptake, and they largely circumvent cisplatin resistance. The complex with X is  $\text{CH}_2\text{COOC}_2\text{H}_5$ , exhibited marked antitumour efficacy when tested in vivo on xenografts of PANC-1 pancreatic cancer in nude mice, highlighting the antitumour efficacy of these tetraazolate-bridged Pt(II) dinuclear complexes.

The synthesis and characterization of Pt(II) dinuclear complex **C111** (Figure 23) was reported in [211]; its cytotoxicity together with that of the Pd(II) analogue towards human breast cancer (MCF-7) and human colon cancer (HT-29) cell lines was evaluated. Although the free form of the ligand did not show appreciable activity, both complexes showed growth-inhibitory activities, even better than for cisplatin, with the Pt(II) complex having the highest cytotoxic activity ( $\text{IC}_{50} = 41 \mu\text{M}$ ).

The trinuclear Pt(II) complex **C112** (Figure 23) was synthesized [212]; in it the three Pt(II) centres are monofunctionalized with a chloride ligand. **C112** affects the conformation of DNA more efficiently and reacts with glutathione much more slowly than cisplatin. The DNA adducts formed are of 1,4-GG type as well as of lesser extent, and the 1,3-GG intrastrand cross-links are very different from those 1,2-GG intrastrand cross-links formed by cisplatin. Its cytotoxicity is higher than that of cisplatin towards the MCF-7 and A549 tumour cell lines. The cellular inhibition mode of the monofunctional trinuclear platinum complex was examined by flow cytometry in MCF-7 cells; **C112** arrests the cell cycle mainly in  $\text{G}_2$  or M phase, whereas cisplatin arrests the cell cycle in S phase. **C112** seems more accessible to DNA owing to the cationic nature and its stability in the presence of glutathione. **C112** may be useful in nucleic acid research as an effective GG cross-linking agent.

A series of diazine-bridged dinuclear Pt(II) complexes have been prepared and studied in bioassays as potential anticancer agents [213]. The interaction with double-stranded DNA was determined through in vitro cytotoxicity assay in human fibroblasts (MRC5) and two carcinoma cell lines (A375 and HCT 116). All the complexes inhibited cell proliferation with inhibitory concentrations  $\text{IC}_{50}$  in the 0.5–120  $\mu\text{M}$  range. The role of the bridging ligand and that of the tailored groups in the interaction with DNA were discussed. **C113** (Figure 23) exhibited higher cytotoxicity potential in comparison with cisplatin, whereas **C114** (Figure 23) exhibited activity comparable to that of

cisplatin and was less toxic in an assay with zebrafish embryos, causing no adverse developmental effects.

Polynuclear complexes have been tested as telomerase inhibitors; molecules able to stabilize certain G-quadruplex structures, such as human telomeric G-quadruplex DNA (leading to inhibition of the telomerase activity) [214] and to promote G-quadruplex DNA (leading to oncogene downregulation) [215] exerted inhibition of tumour cell proliferation. G-quadruplexes, which consist of four-stranded nucleic acids, are described as having a high-order secondary DNA structure and they have received attention regarding their potential use in anticancer therapies [216]. These guanine-rich sequences exist in the promoter regions of several oncogenes and the telomeres at the end of the chromosomes. Telomerase is active and upregulated in approximately 85% of tumour cells, thus the telomeric G-quadruplex has been considered a potentially effective antitumour target.

Zheng et al. [217] reported a tetranuclear Pt(II) complex obtained with two porphyrins (**C115** and **C116**, Figure 24) showing high affinity and excellent selectivity for the G-quadruplex structure rather than double-stranded DNA. Studies indicated their good anticancer activities through dual effects, inhibition of the telomerase activity and repression of oncogene expression. The work suggested that the modulation of the shape and configuration of multinuclear metal complexes working on the bridging ligands (the porphyrin in this case) is a strategy for constructing geometry-oriented G-quadruplex stabilizers and therefore anticancer drugs.

Similarly, they investigated two dinuclear Pt(II) complexes (**C117**, **C118**, Figure 24) and found that both complexes (mainly **C118**) were able to selectively stabilize the human telomeric G-quadruplex and to significantly inhibit the activity of telomerase, probably due to interactions with the sugar-phosphate backbone of the G-quadruplex. The  $IC_{50}$  is 0.113  $\mu$ M for **C118**, indicating that it is one of the strongest known telomerase inhibitors. V-shaped dinuclear Pt(II) complexes can act as selective G-quadruplex binders and telomerase inhibitors although mononuclear Pt(II) complexes also exerted similar behaviour but with higher  $IC_{50}$  values (10  $\mu$ M) [218].

Other dinuclear complexes have been studied [219] or previously discussed [62].

In recent years, sulfur-containing ligands such as dithiocarbamates and thiosemicarbazones and their transition metal complexes have received attention in the area of medicinal chemistry owing to their pharmacological properties, such as antiviral [220-222], antibacterial [223-226], antifungal [227-229], antiparasitic [230,231], and antitumor [131,232-238] activities. Among them, thiosemicarbazones are an important and versatile type of ligand because of the potential donor atoms that they possess; among which sulfur is of paramount importance in the metal-ligand

linkage. Pd(II) and Pt(II) complexes of thiosemicarbazone-derivatized molecules have been studied in depth by Matesanz and Souza [239]. Particularly, compounds in which the thiosemicarbazone side chain is attached at  $\alpha$  position to a nitrogen-containing heterocyclic ring—namely,  $\alpha$ -N-heterocyclic thiosemicarbazones—are strong metal chelating agents and, moreover, some of the ligands in their free form also showed antineoplastic activity. The biochemical mechanism of action involves, among other mechanisms, ribonucleotide reductase inhibition and non-covalent DNA binding [240-245]. In recent years many complexes of Pt(II) and Pd(II) with thiosemicarbazone ligands have been reported that exhibit considerable cytotoxic activity, particularly in cisplatin-resistant tumour cell lines. Pd(II) complexes are more active than Pt(II) complexes, probably due to greater affinity for sulfur ligands. Matesanz et al.[246] described four novel 2,6-diacetylpyridine bis(4*N*-tolylthiosemicarbazonato) Pd(II) and Pt(II) complexes (**C119a-C119d**, Figure 25). The ligands act as dianionic tetradentate donors coordinating to the metal centre in a square planar geometry through the *N*-pyridinic group, the *N*-iminic group and the sulfur atoms from one thiosemicarbazone arm; the fourth coordination position is occupied by the *N*-hydrazinic group of the other arm. The new compounds have been evaluated for antiproliferative activity in vitro against the NCI-H460, HepG2, MCF-7, A2780 and A2780cisR human cancer cell lines. The cytotoxicity data suggested that **C119a**, **C119b** and **C119c** may have important antitumour properties since they are capable of not only circumventing cisplatin resistance in A2780cisR cells but also of exhibiting high antiproliferative activity in MCF-7 breast cancer cells. A subsequent toxicity study in LLC-PK1 renal cells showed that none of these compounds are toxic in vitro in the concentration range tested. The same research group reported a similar system in which the two aromatic groups are *p*-chlorophenyl (**C119e** and **C119f**) [247]. Both the free form of the ligand and its Pd(II) and Pt(II) complexes have been evaluated for antiproliferative activity in vitro against the NCI-H460, T-47D, A2780 and A2780cisR human cancer cell lines and were active and capable of circumventing cisplatin resistance in A2780cisR cells. Both Pt(II) and Pd(II) complexes are particularly active on T-47D breast cancer cells. Parrilha et al. [248] synthesized metal complexes with 2-acetylpyridine-*N*<sup>4</sup>-ortho-chlorophenylthiosemicarbazone (**C120**, Figure 25); they were assayed for their cytotoxicity towards MCF-7 breast adenocarcinoma and HT-29 colon carcinoma cells. The thiosemicarbazone and its complexes are cytotoxic. The Pd(II) complex **C120b** was twice as toxic as the Pt(II) complex, with an IC<sub>50</sub> value ranging from 1.38 to 2.00  $\mu$ M, but they are also toxic to non-cancer cells.

Other examples of thiosemicarbazones studied by this research group are Pd(II) bischelate complexes of the type PdL<sub>2</sub> in which the ligands are 4-phenyl-1-(acetone)thiosemicarbazone

(**C121a**), 4-phenyl-1-(2-chlorobenzaldehyde)thiosemicarbazone (**C121b**), 4-phenyl-1-(3-hydroxybenzaldehyde)thiosemicarbazone (**C121c**), 4-phenyl-1-(2-naphthaldehyde)thiosemicarbazone (**C121d**) and 4-phenyl-1-(1-nitro-2-naphthaldehyde)thiosemicarbazone (**C121e**) (Figure 25) [131]. These complexes have a square planar geometry with two deprotonated ligands coordinated to Pd(II) through the azomethine nitrogen and thione sulfur atoms in a *cis* arrangement. The complexes exhibited higher antiproliferative activity than their ligands in the free form, with IC<sub>50</sub> values between 0.01 and 9.87 μM for different types of human tumour cell lines. In particular, **C121c** exhibited high antitumour activity against DU145 prostate carcinoma and K562 chronic myelogenous leukaemia cells, with low IC<sub>50</sub> values (0.01 and 0.02 μM, respectively).

Ramachandran et al. [130] synthesized and studied four new Pd(II) complexes of 2-oxo-1,2-dihydroquinoline-3-carbaldehyde thiosemicarbazones with triphenylphosphine as a co-ligand, discussed in Section 4 (Figure 19).

Shih et al. [249] proposed a Pd(II) complex with 3-arylsydnone-4-carbaldehyde-4'-phenylthiosemicarbazones (**C122**, Figure 25). The ligands bind the Pd(II) centre by an O,N,S tridentate coordination mode through sydnone carbonyl oxygen, azomethine nitrogen and thiolate sulfur atoms, with the fourth position occupied by a chloride anion. The carbonyl oxygen of the sydnone ring is a good electron donor for metal coordination. Shih et al. designed these ligands considering that several sydnone compounds, a class of amphionic aromatic rings, exhibited some pharmacological activity, including antimicrobial, anti-inflammatory, analgesic and antipyretic activity. Cytotoxicity was tested on several human tumour cell lines, and the results revealed that the palladium complexes **C122a-C122d** have greater antiproliferative activity than 5-fluorouracil, with IC<sub>50</sub> values in the range 0.45-2.25 μM. In particular, the complexes exhibited better activity than the corresponding ligands. Accordingly, the study of sydnonyl complexes with anticancer activity may support the development of these complexes in cancer therapy.

Mansour and Mohamed [51] used the ligand *N*-(2-thiazolyl)-1*H*-benzotriazole-1-carbothioamide, which can be considered a cyclic derivative of a thiosemicarbazone, to form the Pt(II) and Pd(II) complexes **C123a** and **C123b** (Figure 25); both complexes are cytotoxic to the MCF7 cell line, with the Pd(II) species more active than the Pt(II) species. The complexes tested have square planar geometry with the ligand chelating the metal ion in the deprotonated form and with the sulfur and one of the nitrogen atoms bearing a triazole. The other two coordination positions are occupied, in the case of Pd(II), by two ethanol molecules and, in the case of Pt(II), by an ethanol and a chloride ion; these ligands were lost when the complexes entered the cells.

## 7. Biological studies

The choice of the correct experimental procedure through which the biological activity of the drug is monitored and to possibly dissect its molecular mechanism of action is a crucial point. The exact mechanism of antitumour action of platinum-containing drugs (cisplatin as the reference compound) is not completely understood; however, DNA is often believed to be the main target of these drugs or at least the first target to be tested for novel metal complexes.

In the last decade some experimental approaches have become the gold standard for the characterization of the activity against DNA of platinum/palladium compounds. These approaches range from cell-free assays, through which it is possible to interpret the “secret” of the molecular action, to in vitro cellular assays, which are useful to monitor the biological activities. In addition, some of these compounds have been studied in vivo, by it which was possible to discover the real antitumour efficacy.

However, the question that most of the studies had to address before the successive in vitro cellular stage of the research was if the platinum or palladium complexes investigated are able to interfere in some way with the DNA structure. In other words, it was investigated if the molecule of interest was able to modify the DNA on the basis of the molecular similarity with cisplatin.

Cell-free assays are useful to directly investigate the interaction between DNA and complexes, simplifying studies. Nevertheless, cell-free assays are not devoid of limits in terms of interpretation of results and often require the use of multiple approaches. In this section we consider and discuss the cell-free biological tests used in recent years to “biologically” characterize platinum/palladium analogues as anticancer agents, mentioning the most used techniques, focusing on the major technical progress for each approach, and providing specific examples of the most representative studies. The methods are schematized in Figure 27:

### 7.1. UV-vis absorption spectrum analysis of CT-DNA

Electronic absorption spectroscopy in the UV-vis range is one of the most common techniques used to study the interaction of metal complexes with DNA. The procedure exploits the UV-vis absorption spectrum of DNA, which has a band (200-350 nm) in the UV region, with maximum absorption at 260 nm. Metal complexes may bind DNA in different ways depending on their structure, charge, and type of ligands. The kind of binding translates into different changes in the absorbance features. Addition of the complex to a DNA solution generally results in hyperchromism and a bathochromic shift of the band at around 260 nm, when compared with blank

DNA, which is generally accepted to be consistent with the interaction of binding of compounds to DNA. Several studies considered in this review monitored the changes of absorbance of a DNA solution (often CT-DNA) with increasing concentration of DNA for fixed amount of complex, and calculated the binding constant ( $K_b$ ). Icsel et al. [80] compared the binding strengths of different metal complexes, observing that Pt(II) complexes show somewhat higher DNA-binding affinity (fish sperm DNA in this case) than the corresponding Pd(II) complexes. In the case of compounds with supposed phototoxicity, spectroscopic approaches were useful to evaluate the changes in the absorption properties and photolysis of the metal complexes under investigation [180]. Absorption spectrum studies can also be performed at different wavelengths when the metal complex possesses intrinsic absorption features (e.g. at 665 nm for certain macrocycles with non-covalent abilities of DNA interaction) [250]. In addition, to evaluate the possible involvement of L-methionine as an accelerator of DNA-platinum complex formation, UV-vis spectrum analysis has also been used to monitor the possible binding of DNA with this amino acid [47].

## 7.2. Circular dichroism spectra

Cisplatin, considered the metal complex of reference, acts as an antitumour agent through the induction of DNA structural modifications that can be summarized by the formation of covalent adducts able to create intrastrand and interstrand DNA cross-links. Nevertheless, several studies postulated a mechanism of action involving also an intercalating ability of the metal complexes. The stabilization of specific secondary structures of DNA (e.g. G-quadruplex structures) by a non-covalent interaction can be experimentally followed by the circular dichroism (CD) approach. Several studies used the CD technique to evaluate DNA conformational changes resulting from the interaction between DNA and a metal complex. Usually, the approaches analyse the UV CD spectrum of CT-DNA (but also specific DNA sequences such as telomeric G-quadruplexes), which has a positive band at around 270-280 nm, due to base stacking, and a negative band at around 240-250 nm, due to the helicity of B-DNA (bands that are enhanced by non-covalent interactions). In particular, intercalation should stabilize the right-handed B conformation of CT-DNA, enhancing the intensities of the two bands. Thanks to this approach, researchers demonstrated the ability of some metal complexes to selectively bind and stabilize the G-quadruplex conformation of DNA, whereas other studies could not demonstrate the supposed intercalating abilities of the compounds of interest [187,217]. Notably, the CD spectrum can also change in a dose-dependent manner as demonstrated by Serebryaskaya et al. [68] and Manet et al. [250].

### 7.3. Impairment of DNA electrophoretic migration

The most used experimental approach is based on the theory that DNA molecules, whether covalently or not covalently modified, should change their abilities to migrate in the canonical agarose gel electrophoresis assay. This approach has proved extremely informative, simple to perform, and often suitable to monitor the alterations induced in the tertiary structure of DNA [37]. Usually circular plasmid DNA is used as the source of the polynucleotide (but also RNA [72]), which, after electrophoretic migration, should appear in three forms (bands): the band that migrates most rapidly represents the supercoiled closed circular form (SC form), the band that migrates most slowly represents the open circular nicked form (OC form) and the intermediate (less represented) band represents the linear form. Changes in the proportions of these bands, and/or in migration of the expected DNA forms, are interpreted as alterations of the DNA structure. However, the interpretation of the results is not always simple. Even though the speed of migration of a covalently closed circular DNA (under the same experimental conditions in terms of buffers, voltage, etc.) depends on the number of superhelicoidal turns, such migration should also be influenced by other factors, such as intercalation in the double helix or DNA fragmentation, both phenomena dependent on the drug activity. So there is not always a simple change in the proportion of the amount of each band but there is often a heterogeneous mix of different phenomena [129,251]. With use of this technique, several studies demonstrated the induction of structural alterations of DNA attributable to covalent cisplatin-like modifications for both palladium and platinum compounds. Several authors determined the effect of platinum or palladium complexes on DNA that can be summarized as follows: changes in both the amounts and the migration of the two major DNA species (SC and OC forms) probably due to covalent modification that induced different levels of supercoiling or the creation of a “nick” in one of the two DNA strands; decrease in the amounts of the SC and OC forms in favour of the linear form of the plasmid, due to the creation of a double strand break of DNA; global decrease in the amount of DNA and/or migration below the SC form due to massive degradation of DNA. An additional not expected phenomenon has been documented by Jamshidi et al. [112], in which the binding of the platinum complexes to the DNA caused the appearance of a DNA smear in the agarose gel (due to random degradation) but at a molecular weight higher than that of the single expected bands. They explained the phenomenon by the involvement of coordination binding between the platinum complexes and DNA, retarding the rate of migration of the modified DNA. The impairment of electrophoretic migration has been improved by the use of experimental variants that increase the potential of the assay. To monitor the interference with DNA migration, Wu et al. [212] investigated the possible

DNA binding modes of a monofunctional trinuclear platinum complex, examining different cross-links induced onto an 18-mer duplex derived from the breast or ovarian cancer susceptibility gene *BRCA1*. To gain further insight into changes in DNA conformation Hamad et al. [198], Utku et al. [251] and Prisecaru et al. [252] performed a drug-DNA incubation followed by targeted DNA digestion (enzymatic DNA restriction), analyzing in more detail the DNA structural alterations induced by the platinum complexes. To better understand the alteration of the tertiary structure of DNA, some researchers considered an additional experimental variant that is represented by the activity of DNA topoisomerase (type I). This enzyme, also called “nicking-closing enzyme” performs the conversion of supercoiled DNA to more relaxed DNA. This was used in some studies that monitored the inhibitory effect of platinum complexes of human topoisomerase I on supercoiled DNA or that simply caused a major unwinding of DNA, as deduced from the strong SC DNA band observed after treatment [48,52]. A few agarose gel electrophoresis assay studies were conducted to verify the abilities of palladium complexes to modify in some way the DNA structure; some authors found DNA modification attributable to DNA degradation or covalent adduct formation but, in general, at a concentration higher than the concentrations used for platinum complexes [80,126,147,253-256].

#### **7.4. Thermal denaturation of DNA**

The structural modification of DNA induced by chemicals can alter the stability of the double helix and, in turn, the melting temperature, at which the two strands can be separated. Therefore, thermal denaturation profiles provide a convenient way to detect binding and the influence of adduct formation on the stability of the DNA double helix. Some authors calculated the melting temperature and the changes of the melting curves as a function of the presence of metal complexes with DNA. The classic approach is based on the calculation of the spectrophotometric absorbance at 260 nm of a DNA solution (conventionally CT-DNA) at increasing temperature [80]. However, the thermal denaturation of DNA can also be done by fluorimetric approaches based on the use of an intercalating fluorescent dye such as SYBR® Green or EvaGreen®. The fluorescence resonance energy transfer approach, through which it is generally possible to detect spatial relationships between macromolecules (e.g. two DNA strands), has been used by several authors. Changes in fluorescence as a result of DNA denaturation are measured by use of a fluorescently labelled oligonucleotide mimicking the DNA sequence under investigation (e.g. human telomeric repeats, promoter-associated G-quadruplex DNA sequences such as c-kit and c-myc). The fluorescence



resonance energy transfer approach allowed a selective stabilization of human telomeric G-quadruplex DNA structure by the metal complex investigated to be determined [217,218,257].

### 7.5. Inhibition of DNA/RNA polymerase activities

Inhibition of the polymerase chain reaction (PCR), performed by DNA polymerase, is an enzyme-based approach used to indirectly assay the modifications of DNA structure induced by metal complexes. Xu et al. [257] determined the complete PCR inhibition by two platinum complexes using a semiquantitative PCR approach. This kind of assay has attracted the interest of the scientific community because it can be applied to investigate the activity of metal complexes against specific human genomic loci (e.g. telomeric DNA repeats). In particular, human telomeric DNA is characterized by a short G-rich tandem repeat sequence  $d[(TTAGGG)_n]$  that can form a structure named a G-quadruplex. Molecules that can bind and stabilize this structure inhibit the enzyme telomerase, which is more active in 85–90% of human cancer cells when compared with the normal cellular counterparts. Thus, the inhibition of telomerase activity could lead to a biological response in cancer cells and may be considered an appealing antineoplastic strategy for possible in vivo applications. To test the possible effect on the inhibition of telomerase activity, through G-quadruplex modification, some authors tested metal complexes using the assay named telomeric repeats amplification protocol [217]. In addition, some authors exploited the DNA primer extension by the enzyme reverse transcriptase in the presence of an RNA template. Changes in RNA structure, induced by the metal complexes under investigation, led to inhibition of the enzymatic activity [72].

### 7.6. Fluorescence-based assay

The fluorescent-based approaches have some advantages over other techniques that can be recapitulated as high sensitivity, wide linear concentration range and selectivity and are among the techniques most commonly used to study interactions between ligands and G-quadruplex structures. The fluorescent-based techniques can vary in terms of the kind of DNA intercalator used (e.g. EB, SYBR Green, etc.) and can be conducted in different ways:

- *Competitive EB displacement* is an experimental approach to monitor the binding affinity of the complexes towards DNA (CT-DNA), and is based on the use of the most common intercalating compound EB, measured at 600-610 nm at different metal complex concentrations. The addition of a new metal complex, supposedly able to replace EB, should result in a decreased fluorescence intensity. The quenching ability of a certain complex has

been evaluated, as an example, by the calculation of the Stern-Volmer constant, also called the quenching constant [113,120]. Competitive fluorescence binding to EB-saturated CT-DNA solutions was performed by Prisecaru et al. [252] using a high-throughput binding method, and they calculated the apparent DNA binding constant for the compound tested and the concentration required to displace 50% of the fluorophore. The same authors also calculated the relative kinetics of binding to DNA for each complex by measuring (at different intervals for 1 h) the fluorescence emission in the presence of saturated EB bound to CT-DNA.

- *Steady-state fluorescence spectra* have been recorded, at increasing concentration of DNA, to monitor the DNA targeting ability of the fluorescent Pt(II) terpyridine-based conjugate 1,8-naphthalimide [114]. By monitoring the fluorescent spectra, Zou et al. [258] demonstrated the differential emission of certain Pt(II) complexes when intercalated into double-stranded DNA or double-stranded RNA structures.
- *The fluorescence intercalator displacement assay*, a method developed by Monchaud et al. [259,260], is a more recent approach by which several studies demonstrated the ability of a compound to stabilize a specific DNA structure (e.g. G-quadruplex of human telomeric sequences or specific promoter regions such as c-kit or c-myc) [261].
- To determine the mode of interaction, the *DNA fluorescence quenching* method has been used under the experimental condition of limited EB and Hoechst 33258 bound to CT-DNA. The different abilities of the two ligands (Hoechst 33258 is a minor groove binding ligand showing high preference for AT sequences, whereas EB is a classic intercalator and binds DNA with weak preference to GC-rich sequences of polynucleotide) allowed the binding features of the trinuclear Pt(II) series under investigation to be specifically monitored [252].

The biological cell-free assays described in the text and related references are reported in Table 1.

## 8. Conclusions

Although metal-based drugs in cancer chemotherapy have a history of about 40 years, there is still growing interest in the design of new metal-based anticancer agents to overcome the problems of clinically used drugs but with maintenance of their efficacy. Starting from the progenitor drug cisplatin, first approved by the FDA in 1978 for the treatment of ovarian carcinoma, the second generation of square planar Pt(II) complexes was introduced to improve safety (carboplatin), to improve the spectrum of action (oxaliplatin), to overcome resistance (picoplatin) and to improve delivery (ProLindac, an oxaliplatin-like complex connected to a delivery polymer of

hydroxypropylmethacrylamide functionalized with a linker). All the Pt(II)-based drugs are administered intravenously or intraperitoneally because of sensitivity to low pH values. For oral activity, octahedral Pt(IV) complexes, such as satraplatin, have been developed. Today, about 10 new platinum complexes are in clinical trials, and hundred of new complexes are preclinically tested each year. The race to synthesize new platinum and palladium complexes engages many scientists, and the design of novel metal complexes not only deriving from the cisplatin architecture but also different from it, such as monofunctional complexes, positively charged multinuclear complexes, *trans* complexes and octahedral complexes, is an important area of drug discovery. To improve the efficacy, an extraordinary variety of ligands and leaving groups have been proposed. Among them, the derivatization of metal complexes with bioactive molecules is a disputable strategy because there is not always a synergistic effect between the metal centre and the bioactive compound. Despite this, the design and synthesis of combi-molecules is an intriguing task of bioinorganic chemistry.

A more recent application against tumour is the PACT strategy; it uses visible light, preferentially of longer wavelength (red light) able to penetrate deep into tissue, to activate a suitable inactive prodrug. In PACT a suitable inert compound, in this case a platinum or palladium complex, is transformed into an active anticancer agent through light irradiation. The active compound can exert its action directly, behaving as a classic antitumour agent, but it can also generate, during or because of the photoactivation, cytotoxic species such as ROS, free radicals and singlet-state  $^1\text{O}_2$ . In recent years, some UV-vis photoactivable metal complexes have been proposed as anticancer agents and promising biological studies have been published.

Finally, there is experimental evidence that some chemotherapeutic platinum complexes induce immunogenic cell death. One way in which chemotherapeutics can cause a tumour-specific immune response is by their triggering an immunogenic mode of tumour cell death, and thus these agents could act as "anticancer vaccines". It is probable that many highly promising immunogenic and/or immune-stimulating platinum and palladium candidates might have been neglected. The first attempt to explore the immune-modulating properties of platinum agents has been recently described by Wong et al. [262], opening the way to a new strategy for tumour therapy.

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## Abbreviations

2-Bu, 2-butyl; 2-Pn, 2-pentyl; Bu, butyl; CBDCA, 1,1-cyclobutanedicarboxylate; cBu cyclobutyl; cHex, cyclohexyl; cPn, cyclopentyl; DACH, 1R,2R-diaminocyclohexane; DMSO, dimethylsulfoxide; en, ethylenediamine; Et, ethyl; gluc, glycolate; Hept, heptyl; iBu, isobutyl; mal, malonate; Me, methyl; m-Tol, *meta* tolyl; o-Tol, *ortho* tolyl; p-Tol, *para* tolyl; NHcPn, cyclopentylamino; ox, oxalate; Pn, pentyl; py, pyridine; Ts, Tosyl; FID, Fluorescence intercalator displacement assay.

**Table 1.** Biological cell-free assays described in the text and related references.

Cell-free biological assays	References
UV-vis absorption spectrum analysis of calf thymus DNA	[47, 61, 64, 68, 71, 77, 78, 80, 83, 86, 112, 114, 116, 119, 120, 123, 126, 128, 132, 133, 139, 160, 180, 247, 250, 252, 253, 254]
Circular dichroism spectrum	[37, 40, 68, 112, 127, 187, 188, 212, 217, 218, 250, 253, 257]
Impairment of DNA electrophoretic migration	[37, 39, 40, 47, 48, 52, 53, 56, 61, 63, 64, 65, 71, 79, 80, 83, 88, 112, 122, 123, 126, 127, 129, 147, 180, 188, 198, 199, 200, 212, 251, 252, 253, 254, 255, 256]
Thermal denaturation of DNA	[80, 113, 217, 218, 257]
Inhibition of DNA/RNA polymerase activities	[72, 180, 217, 218, 257]
Fluorescence-based assays	[52, 77, 88, 113, 114, 116, 119, 120, 122, 123, 126, 127, 128, 132, 133, 252, 254, 258, 261]

## References

- [1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, *Int J Cancer* 136 (2015) E359–E386.
- [2] T.R. deBoer-Maggard, P.K. Mascharak, from *Ligand Design in Medicinal Inorganic Chemistry* Edited by T. Storr (2014) 355–374.

- [3] N.P.E. Barry, P.J. Sadler, *Pure Appl. Chem.* 86 (2014) 1897–1910.
- [4] C.-H. Leung, S. Lin, H.-J. Zhong, D.-L. Ma, *Chem. Sci.* 6 (2015) 871–884.
- [5] M. Ashfaq, T. Najam, S.S.A. Shah, M.M. Ahmad, S. Shaheen, R. Tabassum, G. Rivera, *Curr. Med. Chem.* 21 (2014) 3081–3094.
- [6] C.X. Zhang, S.J. Lippard, *Curr. Opin. Chem. Biol.* 7 (2003) 481–489.
- [7] A.R. Kapdi, I.J.S. Fairlamb, *Chem. Soc. Rev.*, 43 (2014) 4751–4777.
- [8] A. de Almeida, B.L. Oliveira, J.D.G. Correia, G. Soveral, A. Casini, *Coord. Chem. Rev.*, 257 (2013) 2689–2704.
- [9] J.J. Wilson, S.J. Lippard, *Chem. Rev.*, 2014, 114, 4470–4495.
- [10] T. Boulikas, *Cancer Ther.* 5 (2007) 351–376.
- [11] M. Nefees, G. Zijian, *Curr. Opin. Chem. Biol.* 19 (2014) 144–153.
- [12] S. Medici, M. Peana, V.M. Nurchi, J.I. Lachowicz, G. Crisponi, M.A. Zoroddu, *Coord. Chem. Rev.* 284 (2015) 329–350.
- [13] S.Wu, C. Zhu, C. Zhang, Z. Yu, W. He, Y. He, Y. Li, J. Wang, Z. Guo, *Inorg. Chem.* 50 (2011) 11847–11849.
- [14] K. Cheung-Ong, K.T. Song, Z. Ma, D. Shabtai, A.Y. Lee, D. Gallo, L.E. Heisler, G.W. Brown, U. Bierbach, G. Giaever, C. Nislow, *ACS Chem. Biol.* 7 (2012) 1892–1901.
- [15] K. Wang, E. Gao, *Anticancer Agents Med. Chem.* 14 (2014) 147–169.
- [16] B. Rosenberg, L. VanCamp, J.E. Trosko, V.H. Mansour, *Nature* 222 (1969) 385–386.
- [17] M. Galanski, M.A. Jakupiec, B.K. Keppler, *Curr. Med. Chem.* 12 (2005) 2075–2094.
- [18] W.M. Motswainyana, M.O. Onani, A.M. Madiehe, M. Saibub, N. Thovhogi, R.A. Lalancette, *J. Inorg. Biochem.* 129 (2013) 112–118.
- [19] G.H.W. Milburn, M.R. Truter, *J. Chem. Soc. A* (1966) 1609–1616.
- [20] V.P. Ting, M. Schmidtman, C.C. Wilson, M. T. Weller, *Angew. Chem., Int. Ed.* 49 (2010) 9408–9411.
- [21] S. Neidle, I.M. Ismail; P.J. Sadler, *J. Inorg. Biochem.* 13 (1980) 205–212.
- [22] B. Beagley, D.W.J. Cruickshank, C.A. McAuliffe, R.G. Pritchard, A.M. Zaki, R.L. Beddoes, R.J. Cernik, O.S. Mills, *J. Mol. Struct.* 130 (1985) 97–102.
- [23] M.A. Bruck, R. Bau, M. Noji, K. Inagaki, Y. Kidani, *Inorg. Chim. Acta* 92 (1984) 279–284.
- [24] A.S. Abu-Surrah, T.A.K. Al-Allaf, M. Klinga, M. Ahlgren, *Polyhedron* 22 (2003) 1529–1534.
- [25] T.C. Johnstone, *Polyhedron* 67 (2014) 429–435.
- [26] W. Qing-Kun, P. Shao-Ping, C. Yan-Wei, L. Yong-Nian, L. Chun-Fang, *Acta Crystallogr., Sect. E: Struct. Rep. Online* 65 (2009) m1687.

- [27] W. Qing-Kun, P. Shao-Ping, C. Yan-Wei, H. Shu-Qian, L. Yong-Nian, L. Chun-Fang, *Chin. Inorg. Chem.* 25 (2009) 1375–1378.
- [28] P. Bitha, R.G. Child, J.J. Hlavka, S.A. Lang Junior, Y.-I. Lin, R.C. Haltiwanger, C.G. Pierpont, *Inorg.Chim.Acta* 151 (1988) 89–93.
- [29] Y. Chen, Z. Guo, S. Parsons, P.J. Sadler, *Chem. Eur. J.* 4 (1998) 672–676.
- [30] A.R. Battle, R. Choi, D.E. Hibbs, T.W. Hambley, *Inorg. Chem.* 45 (2006) 6317–6322.
- [31] P. Shao-Ping, C. Yan-Wei, W. Qing-Kun, B. Yu-xing, L. Chun-fang, L. Xue-jie, S. Jia-lin, *Chin. New Drugs J.* 19 (2010) 1605–1608.
- [32] M.J. Cleare, J.D. Hoeschele, *Platinum Met. Rev.* 17 (1973) 2–13.
- [33] M.J. Cleare, *Coord. Chem. Rev.* 12 (1974) 349–405.
- [34] M.J. Cleare, *J. Clin. Hematol. Oncol.* 7 (1977) 1–25.
- [35] T.A. Connors, M.J. Cleare, K.R. Harrap, *Cancer Treat. Rep.* 63 (1979) 1499–1502.
- [36] F. Arnesano, G. Natile, *Coord. Chem. Rev.* 253 (2009) 2070–2081.
- [37] M. Gay, Á.M. Montaña, C. Batalla, J.M. Mesas, M.T. Alegre, *J. Inorg. Biochem.* 142 (2015) 15–27.
- [38] Y. Kidani, M. Noji, T. Tashiro, *Gan.* 71 (1980) 637–643.
- [39] K. Huang, Z. Chen, Y. Liu, Z. Li, J. Wei, M. Wang, G. Zhang, H. Liang, *Eur. J. Med. Chem.* 63 (2013) 76–84.
- [40] K. Huang, Z. Chen, Y. Liu, Z. Li, J. Wei, M. Wang, X. Xie, H. Liang, *Eur. J. Med. Chem.* 64 (2013) 554–561.
- [41] D. Xu, Y. Min, Q. Cheng, H. Shi, K. Wei, F. Arnesano, G. Natile, Y. Liu, *J. Inorg. Biochem.* 129 (2013) 15–22.
- [42] I. Łakomska, K. Hoffmann, A. Wojtczak, J. Sitkowski, E. Maj, J. Wietrzyk, *J. Inorg. Biochem.* 141 (2014) 188–197.
- [43] Reedijk, *Platinum Met. Rev.* 52 (2008) 2–11.
- [44] P.J.S. Miguel, M. Roitzsch, L. Yin, P.M. Lax, L. Holland, O. Krizanovic, M. Lutterbeck, M. Schürmann, E.C. Fusch, B. Lippert, *Dalton Trans.* (2009) 10774–10786.
- [45] H. Cui, R. Goddard, K. Pörschke, A. Hamacher, M.U. Kassack, *Inorg. Chem.* 53 (2014) 3371–3384.
- [46] R. Křikavová, L. Hanousková, Z. Dvořák, Z. Trávníček, *Polyhedron* 90 (2015) 7–17.
- [47] J. Zhao, S. Gou, F. Liu, *Chem. Eur. J.* 20 (2014) 15216–15225.
- [48] R. Cincinelli, L. Musso, S. Dallavalle, R. Artali, S. Tinelli, D. Colangelo, F. Zunino, M. De Cesare, G.L. Beretta, N. Zaffaroni, *Eur. J. Med. Chem.* 63 (2013) 387–400.

- [49] J.P. Parker, H. Nimir, D.M. Griffith, B. Duff, A.J. Chubb, M.P. Brennan, M.P. Morgan, D.A. Egan, C.J. Marmion, *J. Inorg. Biochem.* 124 (2013) 70–77.
- [50] A. Savić, L. Filipović, S. Arandelović, B. Dojčinović, S. Radulović, T.J. Sabo, S. Grgurić-Šipka, *Eur. J. Med. Chem.* 82 (2014) 372–384.
- [51] A.M. Mansour, M.F. Mohamed, *Inorg. Chim. Acta* 423 (2104) 373–383.
- [52] A.P. Neves, M.X.G. Pereira, E.J. Peterson, R. Kipping, M.D. Vargas, F.P. Silva Jr, J.W.M. Carneiro, N.P. Farrell, *J. Inorg. Biochem.* 119 (2013) 54–64.
- [53] J. Albert, R. Bosque, M. Crespo, J. Granell, C. López, R. Cortés, A. Gonzalez, J. Quirante, C. Calvis, R. Messeguer, L. Baldomà, J. Badia, M. Cascante, *Bioorg. Med. Chem.* 21 (2013) 4210–4217.
- [54] G. Xu, J. Zhao, S. Gou, J. Pang, *Bioorg. Med. Chem. Lett.* 25 (2015) 221–224.
- [55] W. Liu, Q. Ye, J. Jiang, L. Lou, Y. Xu, C. Xie, M. Xie, *Chem. Med. Chem.* 8 (2013) 1465–1467.
- [56] Y. Sun, Z. Cao, S. Gou, T. Hu, *Chem. Biodivers.* 11 (2014) 115–125.
- [57] P. Liu, Y. Lu, X. Gao, R. Liu, D. Zhang-Negrerie, Y. Shi, Y. Wang, S. Wang, Q. Gao, *Chem. Commun.* 49 (2013) 2421–2423.
- [58] W. Liu, J. Jiang, Y. Xub, S. Hou, L. Sun, Q. Ye, L. Lou, *J. Inorg. Biochem.* 146 (2015) 14–18.
- [59] W. Liu, J. Su, J. Jiang, X. Li, Q. Ye, H. Zhou, J. Chen, Y. Li, *Sci. Rep.* 3, article number 2464 (2013)
- [60] J. Zhao, S. Gou, G. Xu, L. Cheng, *Eur. J. Med. Chem.* 85 (2014) 408–417.
- [61] J. Zhao, S. Gou, F. Liu, Y. Sun, C. Gao, *Inorg. Chem.* 52 (2013) 8163–8170.
- [62] G. Xu, S. Gou, C. Gao, *Arch. Pharm. Chem. Life Sci.* 346 (2013) 308–313.
- [63] S.M. Valiahdi, A.E. Egger, W. Miklos, U. Jungwirth, K. Meelich, P. Nock, W. Berger, C.G. Hartinger, M. Galanski, M.A. Jakupec, B.K. Keppler, *J. Biol. Inorg. Chem.* 18 (2013) 249–260.
- [64] L. Fang, S. Gou, J. Zhao, Y. Sun, L. Cheng, *Eur. J. Med. Chem.* 69 (2013) 842–847.
- [65] H. Zhang, S. Gou, J. Zhao, F. Chen, G. Xu, X. Liu, *Eur. J. Med. Chem.* 96 (2015) 187–195.
- [66] T. Muchova, J. Pracharova, P. Starha, R. Olivova, O. Vrana, B. Benesova, J. Kasparkova, Z. Travnicek, V. Brabec, *J. Biol. Inorg. Chem.* 18 (2013) 579–589.
- [67] P. Štarha, J. Hošek, J. Vančo, Z. Dvořák, P. Suchy Jr, I. Popa, G. Pražanová, Z. Trávníček, *PLoS One* 9 (2014) e90341.
- [68] T.V. Serebryanskaya, T. Yung, A.A. Bogdanov, A. Shchebet, S.A. Johnsen, A.S. Lyakhov, L.S. Ivashkevich, Z.A. Ibrahimava, T.S. Garbuzenco, T.S. Kolesnikova, N.I. Melnova, P.N. Gaponik, O.A. Ivashkevich, *J. Inorg. Biochem.* 120 (2013) 44–53.

- [69] N. Ferri, G. Facchetti, S. Pellegrino, C. Ricci, G. Curigliano, E. Pini, I. Rimoldi, *Bioorg. Med. Chem.* 23 (2015) 2538–2547.
- [70] S.P. Wisnovsky, J.J. Wilson, R.J. Radford, M.P. Pereira, M.R. Chan, R.R. Laposa, S.J. Lippard, S.O. Kelley, *Chem Biol.* 20 (2013) 1323–1328.
- [71] O. Novakova, B. Liskova, J. Vystřcilova, T. Suchankova, O. Vrana, P. Starha, Z. Trávníček, V. Brabec, *Eur. J. Med. Chem.* 78 (2014) 54–64.
- [72] S.S. Jain, C.M. Anderson, F. DiRienzo, I.R. Taylor, K. Jain, S. Guha, N. Hoque, *Chem. Commun.* 49 (2013) 5031–5033.
- [73] X. Liu, L.L. Zhang, X.H. Xu, L. Hui, J.B. Zhang, S.W. Chen, *Bioorg. Med. Chem. Lett.* 23 (2013) 3780–3784.
- [74] S. Fortin, K. Brasseur, N. Morin, É. Asselin, G. Bérubé, *Eur. J. Med. Chem.* 68 (2013) 433–443.
- [75] F. Arnesano, A. Pannunzio, M. Coluccia, G. Natile, *Coord. Chem. Rev.* 284 (2015) 286–297.
- [76] W. Liu, J. Jiang, C. Xie, S. Hou, H. Quan, Q. Ye, L. Lou, *J. Inorg. Biochem.* 140 (2014) 126–130.
- [77] B. Wang, Z. Wang, F. Ai, W.K. Tang, G. Zhu, *J. Inorg. Biochem.* 142 (2015) 118–125.
- [78] B. Wang, H. Qian, S. Yiu, J. Sun, G. Zhu, *Eur. J. Med. Chem.* 71 (2014) 366–373.
- [79] A.A. Legin, M.A. Jakupec, N.A. Bokach, M.R. Tyan, V.Y. Kukushkin, B.K. Keppler, *J. Inorg. Biochem.* 133 (2014) 33–39.
- [80] C. Icsel, V.T. Yilmaz, F. Ari, E. Ulukaya, W.T.A. Harrison, *Eur. J. Med. Chem.* 60 (2013) 386–394.
- [81] A.Y. Oral, B. Cevatemre, M. Sarimahmut, C. Icsel, V.T. Yilmaz, E. Ulukaya, *Bioorg. Med. Chem.* 23 (2015) 4303–4310.
- [82] M. Fabijańska, K. Studzian, L. Szmigiero, A.J. Rybarczyk-Pirek, A. Pfitzner, B. Cebula-Obrzut, P. Smolewski, E. Zynera, J. Ochocki, *Dalton Trans.* 44 (2015) 938–947.
- [83] J.M. Herrera, F. Mendes, S. Gama, I. Santos, C.N. Ranninger, S. Cabrera, A.G. Quiroga, *Inorg. Chem.* 53 (2014) 12627–12634.
- [84] V. del Solar, A. Quiñones-Lombrana, S. Cabrera, J.M. Padrón, C. Ríos-Luci, A. Alvarez-Valdés, C. Navarro-Ranninger, J. Alemán, *J. Inorg. Biochem.* 127 (2013) 128–140.
- [85] M.J. Cleare, J.D. Hoeschele, *Bioinorg. Chem.* 2 (1973) 187–210.
- [86] T. Parro, M.A. Medrano, L. Cubo, S. Muñoz-Galván, A. Carnero, C. Navarro-Ranninger, A.G. Quiroga, *J. Inorg. Biochem.* 127 (2013) 182–187.
- [87] Ž.D. Bugarčić, J. Bogojeski, R. van Eldik, *Coord. Chem. Rev.* 292 (2015) 91–106.



- [88] N. Lease, V. Vasilevski, M. Carreira, A. de Almeida, M. Sanaú, P. Hirva, A. Casini, M. Contel, *J. Med. Chem.* 56 (2013) 5806–5818.
- [89] Z.D. Matović, E. Mrkalić, G. Bogdanović, V. Kojić, A. Meetsma, R. Jelić, *J. Inorg. Biochem.* 121 (2013) 134–144.
- [90] D.R. Ilić, V.V. Jevtić, G.P. Radić, K. Arsikin, B. Ristić, L. Harhaji-Trajković, N. Vuković, S. Sukdolak, O. Klisurić, V. Trajković, S.R. Trifunović, *Eur. J. Med. Chem.* 74 (2014) 502–508.
- [91] D.Lj. Stojković, V.V. Jevtić, G.P. Radić, D.S. Đačić, M.G. Curčić, S.D. Marković, V.M. Đinović, V.P. Petrović, S.R. Trifunović, *J. Mol. Struct.* 1062 (2014) 21–28.
- [92] M. Tanaka, H. Kataoka, S. Yano, H. Ohi, K. Kawamoto, T. Shibahara, T. Mizoshita, Y. Mori, S. Tanida, T. Kamiya, T. Joh, *BMC Cancer* 13 (2013) 237–245.
- [93] S. Neidle, C.F. Snook, B.A. Murrer, C.F.J. Barnard, *Acta Crystallogr. Sect.C: Cryst.Struct.Comm.* 51 (1995) 822–824.
- [94] Z. Lingmin, C. Liangwei, P. Shaoping, Y. Yikun, G. Wenggui, Y. Yao, *Powder Diff.* 18 (2003) 140–143.
- [95] A.R. Khokhar, X. Quanyun, S. Al-Baker, *J. Inorg. Biochem.* 52 (1993) 51–58.
- [96] C.F.J. Barnard, P.C. Hydes, W.P. Griffiths, O.S. Mills, *J. Chem. Res.* (1983) 302–303.
- [97] F. Zak, J. Turanek, A. Kroutil, P. Sova, A. Mistr, A. Poulova, P. Mikolin, Z. Zak, A. Kasna, D. Zaluska, J. Neca, L. Sindlerova, A. Kozubik, *J. Med. Chem.* 47 (2004) 761–763.
- [98] P. Sova, A. Mistr, A. Kroutil, M. Semerad, H. Chlubnova, V. Hruskova, J. Chladkova, J. Chladek, *Cancer Chemother. Pharmacol.* 67 (2011) 1247–1256.
- [99] S. Theiner, H.P. Varbanov, M. Galanski, A.E. Egger, W. Berger, P. Heffeter, B.K. Keppler, *J. Biol. Inorg. Chem.* 20 (2015) 89–99.
- [100] H.P. Varbanov, S. Göschl, P. Heffeter, S. Theiner, A. Roller, F. Jensen, M.A. Jakupec, W. Berger, M. Galanski, B.K. Keppler, *J. Med. Chem.* 57 (2014) 6751–6764.
- [101] C.K.J. Chen, J.Z. Zhang, J. Aitken, T.W. Hambley, *J. Med. Chem.* 56 (2013) 8757–8764.
- [102] E. Wexselblatt, R. Raveendran, S. Salameh, A. Friedman-Ezra, E. Yavin, D. Gibson, *Chem. Eur. J.* 21 (2015) 3108–3114.
- [103] S. Choi, C. Filotto, M. Bisanzo, S. Delaney, D. Lagasee, J.L. Whitworth, A. Jusko, C.R. Li, N.A. Wood, J. Willingham, A. Schwenker, K. Spaulding, *Inorg. Chem.* 37 (1998) 2500–2504.
- [104] J. Lorenzo, A. Delgado, A.M. Montaña, J.M. Mesas, M.T. Alegre, M.C. Rodríguez, F.X. Avilés, *Eur. J. Med. Chem.* 83 (2014) 374–388.
- [105] V. Gandin, C. Marzano, G. Pelosi, M. Ravera, E. Gabano, D. Osella, *Chem. Med. Chem.* 9 (2014) 1299–1305.

- [106] I. Zanellato, I. Bonarrigo, D. Colangelo, E. Gabano, M. Ravera, M. Alessio, D. Osella, J. Inorg. Biochem. 140 (2014) 219–227.
- [107] P. Wils, A. Warnery, V. Phung-Ba, S. Legrain, D. Sherman, J. Pharmacol. Exp. Ther. 299 (1994) 654–658.
- [108] B.R. Hoffmeister, M. Hejl, M.S. Adib-Razavi, M.A. Jakupec, M. Galanski, B.K. Keppler, Chem. Biodivers. 12 (2015) 559–574.
- [109] E. Gabano, M. Ravera, D. Osella, Dalton Trans. 43 (2014) 9813–9820.
- [110] L. Ma, R. Ma, Y. Wang, X. Zhu, J. Zhang, H.C. Chan, X. Chen, W. Zhang, S.K. Chiu, G. Zhu, Chem. Commun. 51 (2015) 6301–6304.
- [111] W. Neumann, B.C. Crews, M.B. Sarosi, C.M. Daniel, K. Ghebreselasie, M.S. Scholz, L.J. Marnett, E. Hey-Hawkins. Chem. Med. Chem. 10 (2015) 183–192.
- [112] M. Jamshidi, R. Yousefi, S.M. Nabavizadeh, M. Rashidi, M.G. Haghighi, A. Niazi, A. Moosavi-Movahedi, Int. J. Biol. Macromol. 66 (2014) 86–96.
- [113] C. Icel, V.T. Yilmaz, A. Golcu, E. Ulukaya, O. Buyukgungor, Bioorg. Med. Chem. Lett. 23 (2013) 2117–2122.
- [114] S. Banerjee, J.A. Kitchen, S.A. Bright, J.E. O'Brien, D.C. Williams, J.M. Kelly, T. Gunnlaugsson, Chem. Commun. 49 (2013) 8522–8224.
- [115] F. Ari, B. Cevatemre, E.I.I. Armutak, N. Aztopal, V.T. Yilmaz, E. Ulukaya, Bioorg. Med. Chem. 22 (2014) 4948–4954.
- [116] R. Mohammadi, R. Yousefi, M.D. Aseman, S.M. Nabavizadeh, M. Rashidi, Anticancer Agents Med. Chem. 15 (2015) 107–114.
- [117] L.S. Lerman, J. Mol. Biol. 3 (1961) 18–30.
- [118] P.J. Bond, R. Langridge, K.W. Jennette, S.J. Lippard, Proc. Natl. Acad. Sci. U. S. A. 72 (1975) 4825–4829.
- [119] S. Shahraki, H. Mansouri-Torshizi, Z.S. Nezami, A. Ghahghaei, F. Yaghoubi, A. Divsalar, A.A. Saboury, F.H. Shirazi, Iran. J. Pharm. Res. 13 (2014) 1279–1294.
- [120] B.J. Pages, F. Li, P. Wormell, D.L. Ang, J.K. Clegg, C.J. Kepert, L.K. Spare, S. Danchaiwijita, J.R. Aldrich-Wright, Dalton Trans. 43 (2014) 15566–15575.
- [121] S. Kemp, N.J. Wheate, D.P. Buck, M. Nikac, J.G. Collins, J.R. Aldrich-Wright, J. Inorg. Biochem. 101 (2007) 1049–1058.
- [122] D.A.K. Vezzu, Q. Lu, Y. Chen, S. Huo, J. Inorg. Biochem. 134 (2014) 49–56.
- [123] Y. Sun, D. Sun, W. Yu, M. Zhu, F. Ding, Y. Liu, E. Gao, S. Wang, G. Xiong, I. Dragutan, V. Dragutan, Dalton Trans. 42 (2013) 3957–3967.

- [124] W. Chu, Y. Wang, S. Liu, X. Yang, S. Wanga, S. Li, G. Zhou, X. Qin, C. Zhou, J. Zhang, *Bioorg. Med. Chem. Lett.* 23 (2103) 5187–5191.
- [125] K.B. Garbutcheon-Singh, P. Leverett, S. Myersa, J.R. Aldrich-Wright, *Dalton Trans.* 42 (2013) 918–926.
- [126] E. Gao, H. Fu, M. Zhu, C. Ma, S. Liang, J. Zhang, L. Li, L. Wang, Y. Li, W. Jiao, *Eur. J. Med. Chem.* 82 (2014) 172–180.
- [127] Q. Qin, Z. Chen, J. Qin, X. He, Y. Li, Y. Liu, K. Huang, H. Liang, *Eur. J. Med. Chem.* 92 (2015) 302–313.
- [128] P. Vranec, I. Potočňák, D. Sabolová, V. Farkasová, Z. Ipóthová, J. Pisarčíková, H. Paulíková, *J. Inorg. Biochem.* 131 (2014) 37–46.
- [129] E. Gabano, S. Gama, F. Mendes, M.B. Gariboldi, E. Monti, S. Bombard, S. Bianco, M. Ravera, *J. Biol. Inorg. Chem.* 18 (2013) 791–801.
- [130] E. Ramachandran, D.S. Raja, N.P. Rath, K. Natarajan, *Inorg. Chem.* 52 (2013) 1504–1514.
- [131] W. Hernández, J. Paz, F. Carrasco, A. Vaisberg, E. Spodine, J. Manzur, L. Hennig, J. Sieler, S. Blaurock, L. Beyer, *Bioinorg. Chem. Appl.* (2013) ID524701
- [132] R. Prabhakaran, P. Kalaivani, P. Poornima, F. Dallemer, R. Huang, V. Vijaya Padma, K. Natarajan, *Bioorg. Med. Chem.* 21 (2013) 6742–6752.
- [133] K. Karami, M. Hosseini-Kharat, H. Sadeghi-Aliabadi, J. Lipkowski, M. Mirian, *Eur. J. Med. Chem.* 73 (2014) 8–17.
- [134] V. Gandin, A.P. Fernandes, M.P. Rigobello, B. Dani, F. Sorrentino, F. Tisato, M. Bjornstedt, A. Bindoli, A. Sturaro, R. Rella, C. Marzano, *Biochem. Pharmacol.* 79 (2010) 90–101.
- [135] C. Marzano, V. Gandin, A. Folda, G. Scutari, A. Bindoli, M.P. Rigobello, *Free Radic. Biol. Med.* 42 (2007) 872–881.
- [136] G.D. Hoke, F.L. McCabe, L.F. Faucette, J.O. Bartus, C.M. Sung, B.D. Jensen, J.R. Heys, G.F. Rush, D.W. Alberts, R.K. Johnson, *Mol. Pharmacol.* 39 (1991) 90–97.
- [137] N. Pillarsetty, K.K. Katti, T.J. Hoffman, W.A. Volkert, K.V. Katti, H. Kamei, T. Koide, *J. Med. Chem.* 46 (2003) 1130–1132.
- [138] A. Bacchi, M. Carcelli, M. Costa, A. Fochi, C. Monici, P. Pelagatti, C. Pelizzi, G. Pelizzi, L.M.S. Roca, *J. Organomet. Chem.* 593 (2000) 180–191.
- [139] A. Zamora, V. Rodríguez, N. Cutillas, G.S. Yellol, A. Espinosa, K.G. Samper, M. Capdevila, Ò. Palacios, J. Ruiz, *J. Inorg. Biochem.* 128 (2013) 48–56.
- [140] J. Zhang, C. Che, I. Ott, *J. Organomet. Chem.* 782 (2015) 37–41.
- [141] B.W.J. Harper, J.R. Aldrich-Wright, *Dalton Trans.* 44 (2015) 87–96.

- [142] K. Suntharalingam, O. Mendoza, A.A. Duarte, D.J. Mann, R. Vilar, *Metallomics* 5 (2013) 514–523.
- [143] M.D. Coskun, F. Ari, A.Y. Oral, M. Sarimahmut, H.M. Kutlu, V.T. Yilmaz, E. Ulukaya, *Bioorg. Med. Chem.* 21 (2013) 4698–4705.
- [144] E. Ulukaya, F.M. Frame, B. Cevatemre, D. Pellacani, H. Walker, V.M. Mann, M.S. Simms, M.J. Stower, V.T. Yilmaz, N.J. Maitland, *PLoS One* 8 (2013) e64278.
- [145] D. Karakas, B. Cevatemre, N. Aztopal, F. Ari, V.T. Yilmaz, E. Ulukaya, *Bioorg. Med. Chem.* 23 (2015) 5580–5586.
- [146] L. Ma, K. Ge, R. Zhang, W. Fu, S. Li, S. Wang, G. Zhou, X. Qin, J. Zhang, *Eur. J. Med. Chem.* 87 (2014) 624–630.
- [147] J. Albert, R. Bosque, M. Crespo, G. García, J. Granell, C. López, M.V. Lovelle, R. Qadir, A. González, A. Jayaraman, E. Mila, R. Cortés, J. Quirante, C. Calvis, R. Messeguer, J. Badía, L. Baldomá, M. Cascante, *Eur. J. Med. Chem.* 84 (2014) 530–536.
- [148] S. Hadizadeh, N. Najafzadeh, M. Mazani, M. Amani, H. Mansouri-Torshizi, A. Niapour, *Biochem. Res. Int.* (2014) ID813457
- [149] G.H. Nguyen, M.M. Murph, J.Y. Chang, *Cancers* 3 (2011) 1232–1252.
- [150] K. Kobayashi, N. Usami, E. Porcel, S. Lacombe, C. Le Sech, *Mutat. Res.* 704 (2010) 123–131.
- [151] S. Pervaiz, M. Olivo, *Clin. Exp. Pharmacol. Physiol.* 33 (2006) 551–556.
- [152] J.P. Celli, B.Q. Spring, I. Rizvi, C.L. Evans, K.S. Samkoe, S. Verma, B.W. Pogue, T. Hasan, *Chem. Rev.* 110 (2010) 2795–2838.
- [153] P. Agostinis, K. Berg, K.A. Cengel, T.H. Foster, A.W. Girotti, S.O. Gollnick, S.M. Hahn, M.R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B.C. Wilson, J. Golab, *CA-Cancer J. Clin.* 2011, 61, 250–281.
- [154] E.D. Sternberg, D. Dolphin, C. Bruckner, *Tetrahedron* 54 (1998) 4151–4202.
- [155] D.E.J.G.J. Dolmans, D. Fukumura, R.K. Jain, *Nat. Rev. Cancer* 3 (2003) 380–387.
- [156] M. Ethirajan, Y. Chen, P. Joshi, R.K. Pandey, *Chem. Soc. Rev.* 40 (2011) 340–362.
- [157] N.J. Farrer, J.A. Woods, L. Salassa, Y. Zhao, K.S. Robinson, G. Clarkson, F.S. Mackay, P.J. Sadler, *Angew. Chem.* 122 (2010) 9089–9092.
- [158] N.J. Farrer, J.A. Woods, L. Salassa, Y. Zhao, K.S. Robinson, G. Clarkson, F.S. Mackay, P.J. Sadler, *Angew. Chem. Int. Ed.* 49 (2010) 8905–8908.
- [159] P.J. Bednarski, K. Korpis, A.F. Westendorf, S. Perfahl, R. Grnert, *Philos. Trans. R. Soc. A* 371 (2013) 20120118.

- [160] Q. Xie, G. Lan, Y. Zhou, J. Huang, Y. Liang, W. Zheng, X. Fu, C. Fan, T. Chen, *Cancer Lett.* 354 (2014) 58–67.
- [161] A. Bindoli, M.P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori, *Coord. Chem. Rev.* 253 (2009) 1692–1707.
- [162] A. Naik, R. Rubbiani, G. Gasser, B. Spingler, *Angew. Chem. Int. Ed.* 53 (2014) 6938–6941.
- [163] E.C. Glazer, *Isr. J. Chem.* 53 (2013) 391–400.
- [164] U. Schatzschneider, *Eur. J. Inorg. Chem.* (2010) 1451–1467.
- [165] N.J. Farrer, L. Salassa, P.J. Sadler, *Dalton Trans.* (2009) 10690–10701.
- [166] N.J. Farrer, P.J. Sadler, *Aust. J. Chem.* 61 (2008) 669–674.
- [167] A.M. Goodman, Y. Cao, C. Urban, O. Neumann, C. Ayala-Orozco, M.W. Knight, A. Joshi, P. Nordlander, N.J. Halas, *ACS Nano* 8 (2014) 3222–3231.
- [168] A. Presa, R.F. Brissos, A.B. Caballero, I. Borilovic, L. Korrodi-Gregório, R. Pérez-Tomás, O. Roubeau, P. Gamez, *Angew. Chem. Int. Ed.* 54 (2015) 4561–4565.
- [169] M. Irie, *Chem. Rev.* 100 (2000) 1685–1716.
- [170] P.J. Bednarski, F.S. Mackay, P.J. Sadler, *Anti-Cancer Agents Med. Chem.* 7 (2007) 75–93.
- [171] P. Müller, B. Schröder, J.A. Parkinson, N.A. Kratochwil, R.A. Coxall, A. Parkin, S. Parsons, P.J. Sadler, *Angew. Chem. Int. Ed.* 115 (2003) 349–353.
- [172] F.S. Mackay, J.A. Woods, H. Moseley, J. Ferguson, A. Dawson, S. Parsons, P.J. Sadler, *Chem. Eur. J.* 12 (2006) 3155–3161.
- [173] A.F. Westendorf, A. Bodtke, P.J. Bednarski, *Dalton Trans.* (2011) 5342–5351.
- [174] P.J. Bednarski, R. Grünert, M. Zielzki, A. Wellner, F. Mackay, P.J. Sadler, *Chem. Biol.* 13 (2006) 61–67.
- [175] A.F. Westendorf, L. Zerzankova, L. Salassa, P.J. Sadler, V. Brabec, P.J. Bednarski, *J. Inorg. Biochem.* 105 (2011) 652–662.
- [176] M.D. Hall, T.W. Hambley, *Coord. Chem. Rev.* 232 (2002) 49–67.
- [177] F.S. Mackay, J.A. Woods, P. Heringova, J. Kasparkova, A.M. Pizarro, S.A. Moggach, S. Parsons, V. Brabec, P.J. Sadler, *Proc. Natl. Acad. Sci. USA* 104 (2007) 20743–20748.
- [178] N.J. Farrer, J.A. Woods, V.P. Munk, F.S. Mackay, P.J. Sadler, *Chem. Res. Toxicol.* 23 (2010) 413–421.
- [179] A.F. Westendorf, J.A. Woods, K. Korpis, N.J. Farrer, L. Salassa, K. Robinson, V. Appleyard, K. Murray, R. Grnert, A.M. Thompson, P.J. Sadler, P.J. Bednarski, *Mol. Cancer Ther.* 11 (2012) 1894–1904.

- [180] Y. Zhao, J.A. Woods, N.J. Farrer, K.S. Robinson, J. Pracharova, J. Kasparkova, O. Novakova, H. Li, L. Salassa, A.M. Pizarro, G.J. Clarkson, L. Song, V. Brabec, P.J. Sadler, *Chem. Eur. J.* 19 (2013) 9578–9591.
- [181] G.B. Kauffman, D.O. Cowan, *Inorg. Synth.* 7 (1963) 239–245.
- [182] N. Farrell, L.R. Kelland, J.D. Roberts, M. Vanbeusichem, *Cancer Res.* 52 (1992) 5065–5072.
- [183] F.S. Mackay, S.A. Moggach, A. Collins, S. Parsons, P.J. Sadler, *Inorg. Chim. Acta* 362 (2009) 811–819.
- [184] F. Ari, N. Aztopal, C. Icsel, V.T. Yilmaz, E. Guney, O. Buyukgungor, E. Ulukaya, *Bioorg. Med. Chem.* 21 (2013) 6427–6434.
- [185] F. Ari, E. Ulukaya, M. Sarimahmut, V.T. Yilmaz, *Bioorg. Med. Chem.* 21 (2013) 3016–3021.
- [186] C. Mügge, R. Liu, H. Görls, C. Gabbiani, E. Michelucci, N. Rüdiger, J.H. Clement, L. Messori, W. Weigand, *Dalton Trans.* 43 (2014) 3072–3086.
- [187] M.T. Proetto, W. Liu, A. Molchanov, W.S. Sheldrick, A. Hagenbach, U. Abram, R. Gust, *Chem. Med. Chem.* 9 (2014) 1176–1187.
- [188] Z. Zhu, X. Wang, T. Li, S. Aime, P.J. Sadler, Z. Guo, *Angew. Chem. Int. Ed.* 53 (2014) 13225–13228.
- [189] Y. Wang, J. Hu, Y. Cai, S. Xu, B. Weng, K. Peng, X. Wei, T. Wei, H. Zhou, X. Li, G. Liang, *J. Med. Chem.* 56 (2013) 9601–9611.
- [190] N. Miklášová, E. Fischer-Fodor, R. Mikláš, L. Kucková, J. Kožíšek, T. Liptaj, O. Soritau, J. Valentová, F. Devínsky, *Inorg. Chem. Commun.* 46 (2014) 229–233.
- [191] N. Filipović, S. Grubišić, M. Jovanović, M. Dulović, I. Marković, O. Klisurić, A. Marinković, D. Mitić, K. Anđelković, T. Todorović, *Chem. Biol. Drug Des.* 84 (2014) 333–341.
- [192] J.B. Mangrum, N.P. Farrell, *Chem. Commun.* 46 (2010) 6640–6650.
- [193] A.E. Pegg, *Cancer Res.* 48 (1988) 759–774.
- [194] R.A. Casero Jr, P.M. Woster, *J. Med. Chem.* 52 (2009) 4551–4573.
- [195] N.P. Farrell, *Drugs Fut.* 37 (2012) 795–806.
- [196] V.R. Menon, E.J. Peterson, K. Valerie, N.P. Farrell, L.F. Povirk, *Biochem. Pharmacol.* 86 (2013) 1708–1720.
- [197] T.M. Silva, S. Andersson, S.K. Sukumaran, M.P. Marques, L. Persson, S. Oredsson, *PLoS One* 8 (2013) e55651.
- [198] S.A. Hamad, P. Beale, J.Q. Yu, F. Huq, *J. Biochem. Sci.* 21 (2014) 41.
- [199] M. Chtchigrovsky, L. Eloy, H. Jullien, L. Saker, E. Ségal-Bendirdjian, J. Poupon, S. Bombard, T. Cresteil, P. Retailleau, A. Marinetti, *J. Med. Chem.* 56 (2013) 2074–2086.

- [200] N. Cutillas, A. Martínez, G.S. Yellol, V. Rodríguez, A. Zamora, M. Pedreño, A. Donaire, C. Janiak, J. Ruiz, *Inorg. Chem.* 52 (2013) 13529–13535.
- [201] S.A. De Pascali, A. Muscella, C. Vetrugno, S. Marsigliante, F.P. Fanizzi, *Inorg. Chim. Acta* 412 (2014) 88–93.
- [202] A. Zamora, S.A. Pérez, V. Rodríguez, C. Janiak, G.S. Yellol, J. Ruiz, *J. Med. Chem.* 58 (2015) 1320–1336.
- [203] S. Aliwaini, A.J. Swarts, A. Blanckenberg, S. Mapolie, S. Prince, *Biochem. Pharmacol.* 86 (2013) 1650–1663.
- [204] N. Cutillas, G.S. Yellol, C. de Haro, C. Vicente, V. Rodríguez, J. Ruiz, *Coord. Chem. Rev.* 257 (2013) 2784–2797.
- [205] K. Bielawski, R. Czarnomysy, A. Muszyńska, A. Bielawska, B. Popławska, *Environ. Toxicol. Pharmacol.* 35 (2013) 254–264.
- [206] A. Gęgotek, A. Markowska, W. Łuczaj, A. Bielawska, K. Bielawski, E. Ambrożewicz, E. Skrzydlewska, *Adv Med Sci.* 58 (2013) 282–291.
- [207] S. Komeda, M. Lutz, A.L. Spek, M. Chikuma, J. Reedijk, *Inorg. Chem.* 39 (2000) 4230–4236.
- [208] S. Komeda, M. Lutz, A.L. Spek, Y. Yamanaka, T. Sato, M. Chikuma, J. Reedijk, *J. Am. Chem. Soc.* 124 (2002) 4738–4746.
- [209] S. Komeda, Y.L. Lin, M. Chikuma, *Chem. Med. Chem.* 6 (2011) 987–990.
- [210] S. Komeda, H. Takayama, T. Suzuki, A. Odani, T. Yamorif, M. Chikuma, *Metallomics* 5 (2013) 461–468.
- [211] W.M. Motswainyana, M.O. Onani, A.M. Madiehe, M. Saibu, *Bioorg. Med. Chem. Lett.* 24 (2014) 1692–1694.
- [212] S. Wu, X. Wang, Y. He, Z. Zhu, C. Zhu, Z. Guo, *J. Inorg. Biochem.* 139 (2014) 77–84.
- [213] L. Senerovic, M.D. Zivkovic, A. Veselinovic, A. Pavic, M.I. Djuran, S. Rajkovic, J. Nikodinovic-Runic, *J. Med. Chem.* 58 (2015) 1442–1451.
- [214] A.K. Todd, S.M. Haider, G.N. Parkinson, S. Neidle, *Nucleic Acids Res.* 35 (2007) 5799–5808.
- [215] N.W. Kim, M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, G. Coviello, W.E. Wright, S.L. Weinrich, J.W. Shay, *Science* 266 (1994) 2011–2015.
- [216] D.J Patel, A.T. Phan, V. Kuryavyi, *Nucleic Acids Res.* 35 (2007) 7429–7455.
- [217] X. Zheng, Q. Cao, Y. Ding, Y. Zhong, G. Mu, P.Z. Qin, L. Ji, Z. Mao, *Dalton Trans.* 44 (2015) 50–53.

- [218] C. Wei, Y. Wen, J. Wang, *Int. J. Biol. Macromol.* 55 (2013) 185–192.
- [219] D. Tsekova, P. Gorolomova, G. Gochev, V. Skumryev, G. Momekov, D. Momekova, G. Gencheva, *J. Inorg. Biochem.* 124 (2013) 54–62.
- [220] G. Pelosi, F. Bisceglie, F. Bignami, P. Ronzi, P. Schiavone, M.C. Re, C. Casoli, E. Pilotti, *Int. J. Pharm. Pharm. Sc.* 1 (2009) 62–70.
- [221] A. Karaküçü-Iyidoğan, D. Taşdemir, E.E. Oruç-Emre, J. Balzarini, *J. Med. Chem.* 46 (2011) 5616–5624.
- [222] P. Genova, T. Varadinova, A.I. Matesanz, D. Marinova, P. Souza, *Toxicol. Appl. Pharmacol.* 197 (2004) 107–112.
- [223] S. Thota, S.S. Karki, B.R. Bhukya, *J. Med. Chem.* 53 (2010) 8765–8769.
- [224] I. Kizilcikli, Y.D. Kurt, B. Akkurt, A.Y. Genel, S. Birteksöz, G. Ötük, B. Ülküseven, *Folia Microbiol.* 52 (2007) 15–25.
- [225] T. Rosu, A. Gulea, A. Nicolae, R. Georgescu, *Molecules* 12 (2007) 782–796.
- [226] M. Er, Y. Ünver, K. Sancak, E. Dügüd, *Arkivoc* 15 (2008) 99–120.
- [227] S. Chandra, M. Tyagi, *J. Serb. Chem. Soc.* 73 (2008) 727–734.
- [228] S.S. Konstantinović, B. C. Radovanović, S. P. Sovilj, and S. Stanojević, *J. Serb. Chem. Soc.* 73 (2008) 7–13.
- [229] R.V. Singh, N. Fahmi, M.K. Biyala, *J. Iran. Chem. Soc.* 2 (2005) 40–46.
- [230] L. Otero, M. Vieites, L. Boiani, A. Denicola, C. Rigol, L. Opazo, C. Olea-Azar, J.D. Maya, A. Morello, R.L. Krauth-Siegel, O.E. Piro, E. Castellano, M. González, D. Gambino, H. Cerecetto, *J. Med. Chem.* 49 (2006) 3222–3331.
- [231] P. Chellan, T. Stringer, A. Shokar, P.J. Dornbush, G. Vazquez-Anaya, K.M. Land, K. Chibale, G.S. Smith, *J. Inorg. Biochem.* 105 (2011) 1562–1568.
- [232] N.A. Lewis, F. Liu, L. Seymour, A. Magnusen, T.R. Erves, J.F. Arca, F.A. Beckford, R. Venkatraman, A. González-Sarriás, F.R. Fronczek, D.G. VanDerveer, N.P. Seeram, A. Liu, W.L. Jarrett, A.A. Holder, *Eur. J. Inorg. Chem.* (2012) 664–677.
- [233] E. Ramachandran, P. Kalaivani, R. Prabhakaran, N.P. Rath, S. Brinda, P. Poornima, V.V. Padma, K. Natarajan, *Metallomics* 4 (2012) 218–227.
- [234] U. Kulandaivelu, V.G. Padmini, K. Suneetha, B. Shireesha, J.V. Vidyasagar, T.R. Rao, K.N. Jayaveera, A. Basu, V. Jayaprakash, *Arch. Pharm.* 344 (2011) 84–90.
- [235] B. Atasever, B. Ülküseven, T. Bal-Demirci, S. Erdem-Kuruca, Z. Solakoğlu, *Invest. New Drugs* 28 (2010) 421–432.
- [236] M.X. Li, C.L. Chen, D. Zhang, J.Y. Niu, B.S. Ji, *Eur. J. Med. Chem.* 45 (2010) 3169–3177.



- [237] V. Vrdoljak, I. Đilović, M. Rubčić, S. K. Pavelić, M. Kralj, D. Matković–Čalogović, I. Piantanida, P. Novak, A. Rožman, M. Cindrić, *Eur. J. Med. Chem.* 45 (2010) 38–48.
- [238] K.S.O. Ferraz, L. Ferandes, D. Carrilho, M.C.X. Pinto, M.F. Leite, E.M. Souza–Fagundes, N.L. Speziali, I.C. Mendes, H. Beraldo, *Bioorg. Med. Chem.* 17 (2009) 7138–7144.
- [239] A.I. Matesanz, P. Souza, *Mini Rev. Med. Chem.* 9 (2009) 1389–1396;
- [240] T.S. Lobana, R. Sharma, G. Bawa, S. Khanna, *Coord. Chem. Rev.* 253 (2009) 977–1055.
- [241] J.M. Vila, T. Pereira, A. Amoedo, M. Graña, J. Martínez, M. López-Torres, A. Fernández, *J. Organomet. Chem.* 623 (2001) 176–184.
- [242] J.S. Casas, M.S. García-Tasende, J. Sordo, *Coord. Chem. Rev.* 209 (2000) 197–261.
- [243] A.C. Sartorelli, K.C. Agrawal, A.S. Tsiftoglou, E.C. Moore, *Adv. Enzyme Regul.* (1977) 117–139.
- [244] R.A. Finch, M.C. Liu, A.H. Cory, J.G. Cory, A.C. Sartorelli, *Adv. Enzyme Regul.* 39 (1999) 3–12.
- [245] R.A. Finch, M.C. Liu, S.P. Grill, S.P.W.C. Rose, R. Loomis, K.M. Vasquez, Y.C. Cheng, A.C. Sartorelli, *Biochem. Pharmacol.* 59 (2000) 983–991.
- [246] A.I. Matesanz, I. Leitaó, P. Souza, *J. Inorg. Biochem.* 125 (2013) 26–31.
- [247] A.I. Matesanz, C. Hernández, P. Souza, *J. Inorg. Biochem.* 138 (2014) 16–23.
- [248] G.L. Parrilha, K.S.O. Ferraz, J.A. Lessa, K. Navakoski de Oliveira, B.L. Rodrigues, J.P. Ramos, E.M. Souza-Fagundes, I. Ott, H. Beraldo, *Eur. J. Med. Chem.* 84 (2014) 537–544.
- [249] M. Shih, J. Chen, G. Lin, T. Lin, M. Sun, *J. Pharm. Pharmacol.* 66 (2013) 73–83.
- [250] I. Manet, F. Manoli, M.P. Donzello, E. Viola, A. Masi, G. Andreano, G. Ricciardi, A. Rosa, L. Cellai, C. Ercolani, S. Monti, *Inorg. Chem.* 52 (2013) 321–328.
- [251] S. Utku, A.B. Özçelik, F. Gümüő, S. Yılmaz, T. Arsoy, L. Açıık, A.Ç. Keskine, *J. Pharm. Pharmacol.* 66 (2014) 1593–1605.
- [252] A. Prisecaru, Z. Molphy, R.G. Kipping, E.J. Peterson, Y. Qu, A. Kellett, N.P. Farrell, *Nucleic Acids Res.* 42 (2014) 13474–13487.
- [253] M. Grazul, R. Sigel, C. Maake, E. Besic-Gyenge, I. Lorenz, P. Mayer, M. Czyz, E. Budzisz, *Polyhedron* 67 (2014) 136–144.
- [254] K.S. Prasad, L.S. Kumar, S. Chandan, R.M.N. Kumar, H.D. Revanasiddappa, *Spectrochim. Acta, Part A* 107 (2013) 108–116.
- [255] O. Kacar, Z. Adiguzel, V.T. Yilmaz, Y. Cetin, B. Cevatemre, N. Arda, A.T. Baykal, E. Ulukaya, C. Acilan, *Anti-Cancer Drugs* 25 (2014) 17–29.

- [256] Z. Adiguzel, A.T. Baykal, O. Kacar, V.T. Yilmaz, E. Ulukaya, C. Acilan, J. Proteome Res. 13 (2014) 5240–5249.
- [257] C. Xu, Y. Zheng, X. Zheng, Q. Hu, Y. Zhao, L. Ji, Z. Mao, Sci. Rep. 3, article number 2060 (2013).
- [258] T. Zou, J. Liu, C.T. Lum, C. Ma, R.C.-T. Chan, C.-N. Lok, W.-M. Kwok, C.-M. Che, Angew. Chem. Int. Ed. 53 (2014) 10119–10123
- [259] D. Monchaud, C. Allain, M. Teulade-Fichou, Bioorg. Med. Chem. Lett. 16 (2006) 4842–4845.
- [260] D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. De Cian, J. Mergny, M. Teulade-Fichou, Biochimie 90 (2008) 1207–1223
- [261] K.J. Castor, Z. Liu, J. Fakhoury, M.A. Hancock, A. Mittermaier, N. Moitessier, H.F. Sleiman, Chem. Eur. J. 19 (2013) 17836–17845.
- [262] D.Y.Q. Wong, W.W.F. Ong, W.H. Ang, Angew. Chem. Int. Ed. 54 (2015) 1–6.

## Captions to the figures

**Figure 1.** Platinum complexes currently used in clinical settings.

**Figure 2.** Main approaches in the design of platinum-based drugs.

**Figure 3.** Pt(IV) drugs that have been clinically tested.

**Figure 4.** Pt(II) complexes with dicarboxylate leaving groups.

**Figure 5.** Pt(II) complexes with dicarboxylate leaving groups.

**Figure 6.** Pt(II) complexes with other leaving groups.

**Figure 7.** Amine carrier ligands in Pt(II) complexes.

**Figure 8.** Amine carrier ligands in Pt(II) complexes and delivery systems.

**Figure 9.** Pt(II) complexes with active molecules as N-ligands.

**Figure 10.** Pt(II) complexes with active molecules as N-ligands.

**Figure 11.** Cationic and *trans* Pt(II) complexes.

**Figure 12.** *Trans* Pt(II) complexes.

**Figure 13.** Pd(II) complexes.

**Figure 14.** Pt(IV) complexes with axial leaving groups.

**Figure 15.** Pt(IV) complexes conjugated with bioactive molecules.

**Figure 16.** Other Pt(IV) complexes.

**Figure 17.** Pt(II) complexes for non-covalent interaction with DNA.

**Figure 18.** Pt(II) complexes for non-covalent interaction with DNA.

**Figure 19.** Pd(II) complexes for non-covalent interaction with DNA.

**Figure 20.** Pd(II) complexes for non-covalent interaction with DNA.

**Figure 21.** Photoactivated platinum complexes.

**Figure 22.** Polynuclear Pt(II) complexes.

**Figure 23.** Polynuclear complexes.

**Figure 24.** Polynuclear complexes.

**Figure 25.** Thiosemicarbazone-based complexes.

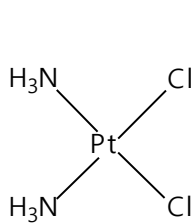
**Figure 26.** Main approaches in the development of photoactivated complexes

**Figure 27.** Main techniques in cell-free biological studies for antitumour investigation

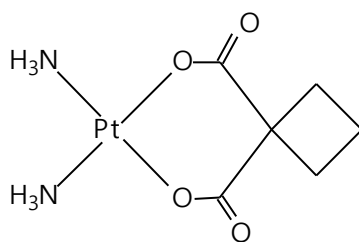
**Table 1.** Biological cell-free assays described in the text and related references.

<b>Cell-free biological assays</b>	<b>References</b>
UV-vis absorption spectrum analysis of calf thymus DNA	[47, 61, 64, 68, 71, 77, 78, 80, 83, 86, 112, 114, 116, 119, 120, 123, 126, 128, 132, 133, 139, 160, 180, 247, 250, 252, 253, 254]
Circular dichroism spectrum	[37, 40, 68, 112, 127, 187, 188, 212, 217, 218, 250, 253, 257]
Impairment of DNA electrophoretic migration	[37, 39, 40, 47, 48, 52, 53, 56, 61, 63, 64, 65, 71, 79, 80, 83, 88, 112, 122, 123, 126, 127, 129, 147, 180, 188, 198, 199, 200, 212, 251, 252, 253, 254, 255, 256]
Thermal denaturation of DNA	[80, 113, 217, 218, 257]
Inhibition of DNA/RNA polymerase activities	[72, 180, 217, 218, 257]
Fluorescence-based assays	[52, 77, 88, 113, 114, 116, 119, 120, 122, 123, 126, 127, 128, 132, 133, 252, 254, 258, 261]

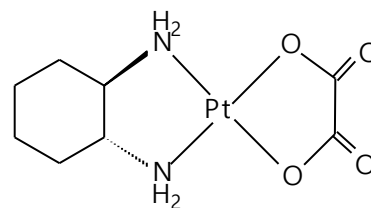
**Figure 1.**



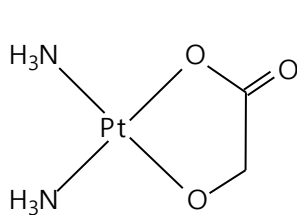
**Cisplatin**



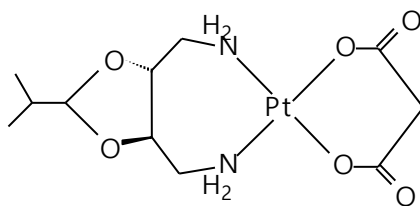
**Carboplatin**



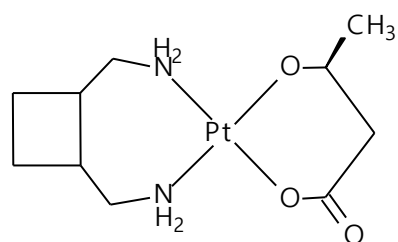
**Oxaliplatin**



**Nedaplatin**

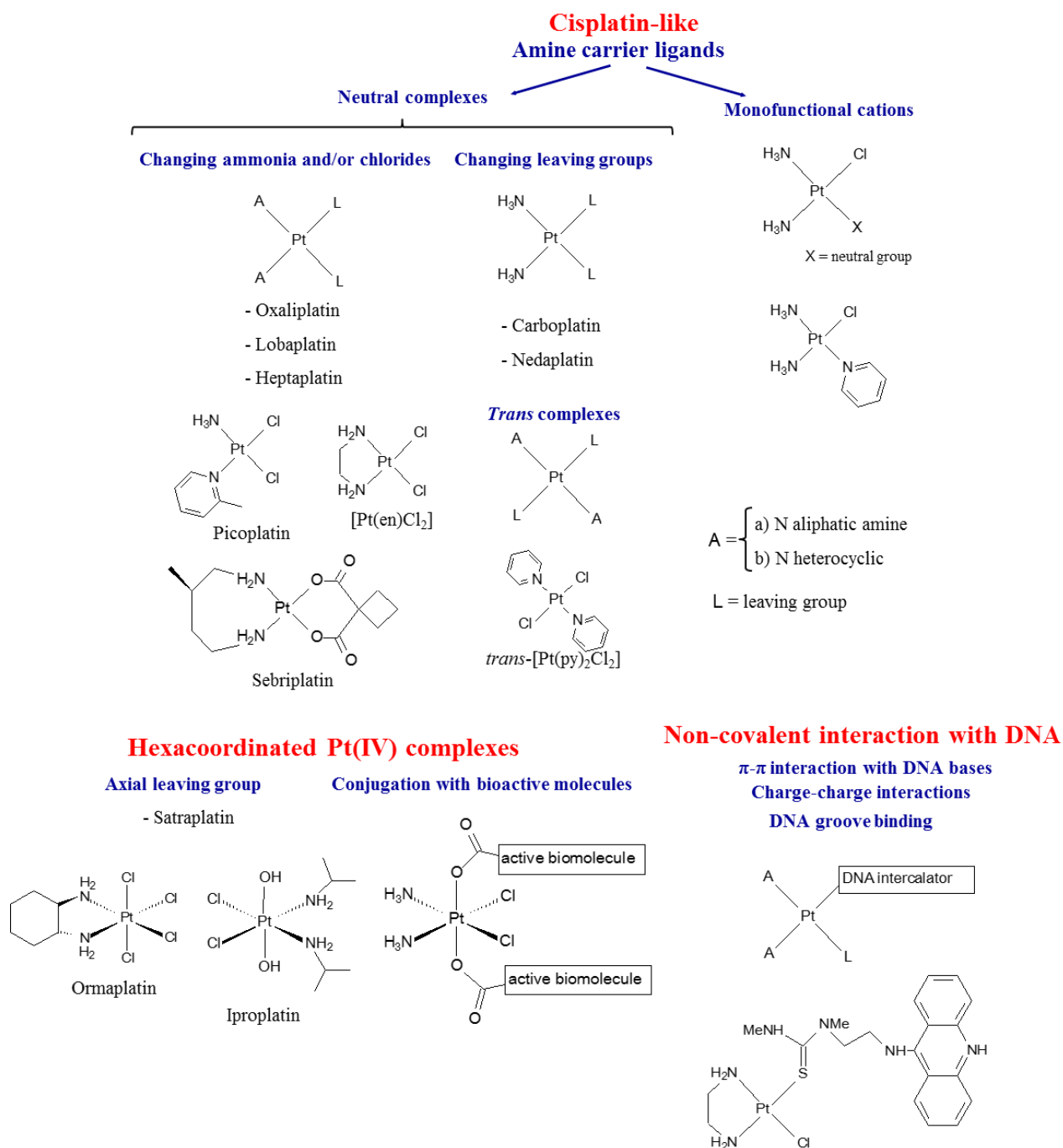


**Heptaplatin**

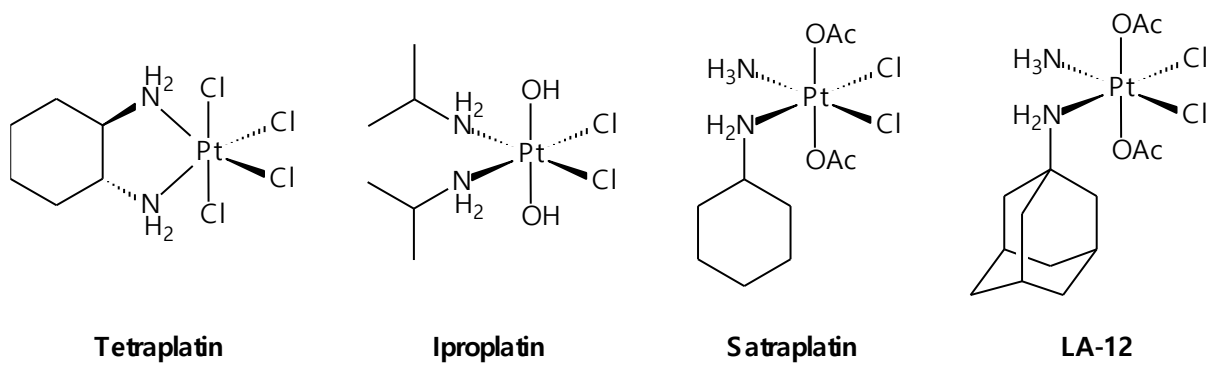


**Lobaplatin**

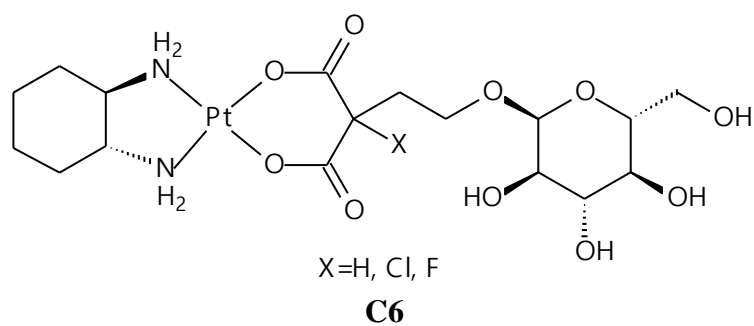
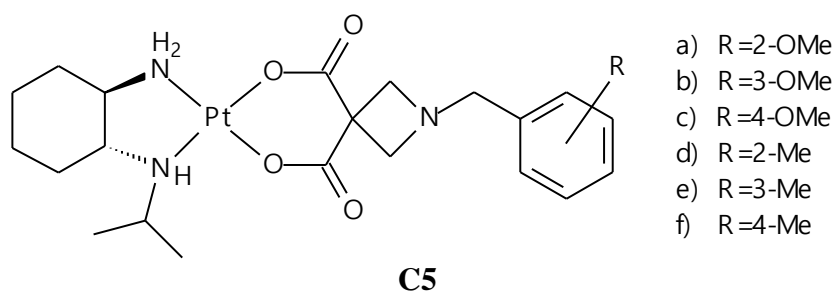
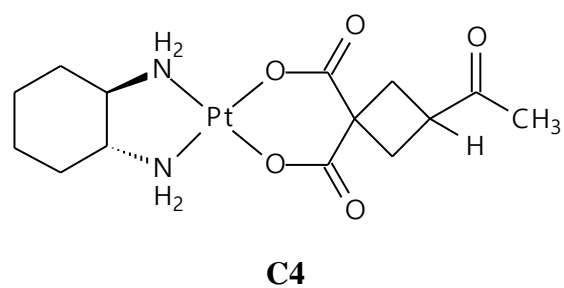
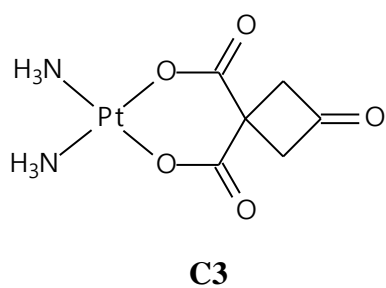
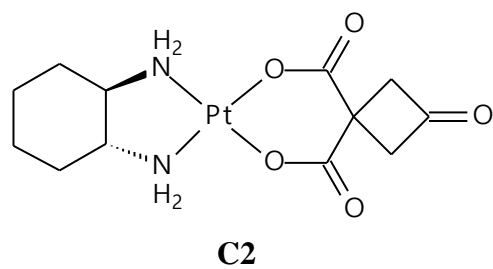
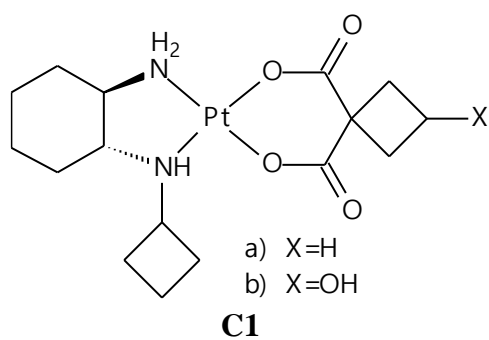
**Figure 2.**



**Figure 3.**

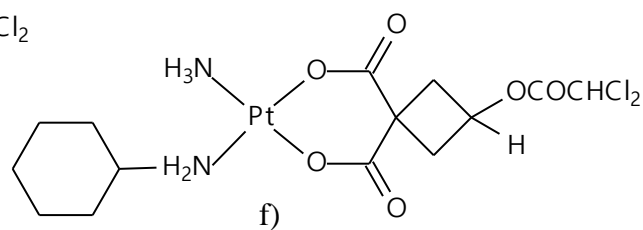
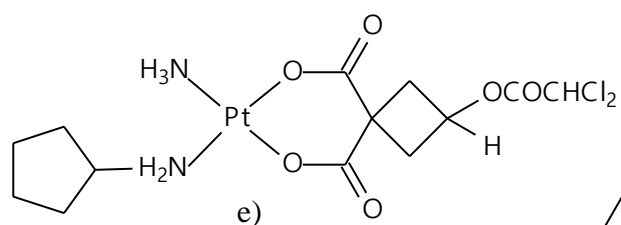
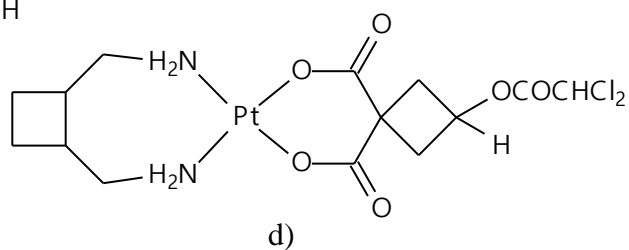
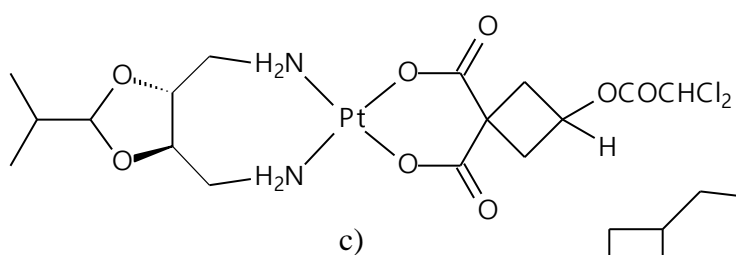
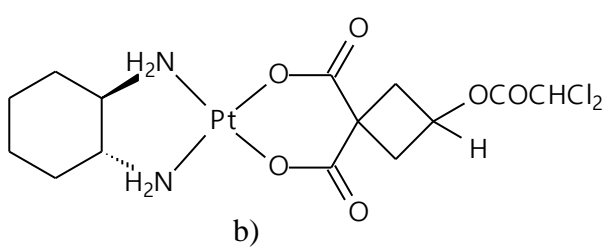
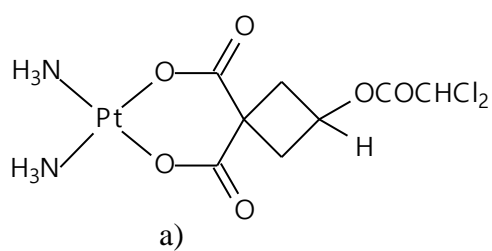


**Figure 4.**

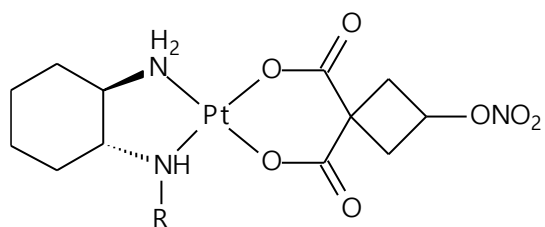




**Figure 5.**

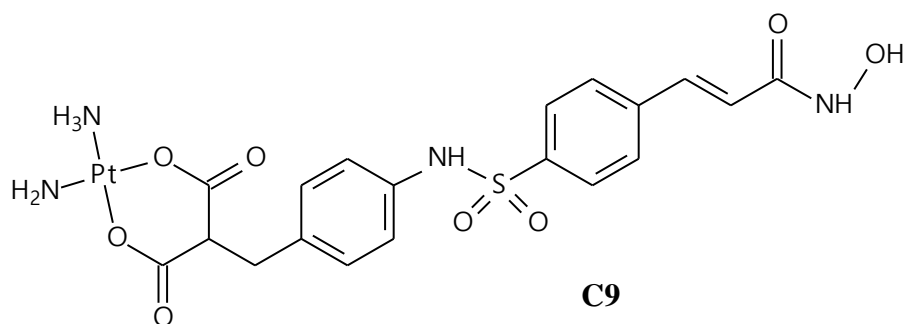


**C7**

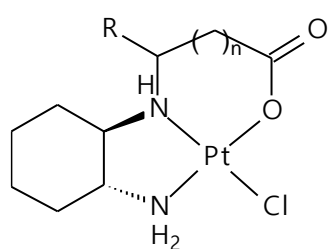


R = H, Pr, Bu, 2-Bu, tBu,  
2-Pn, cBu, cPn, cHex

**C8**

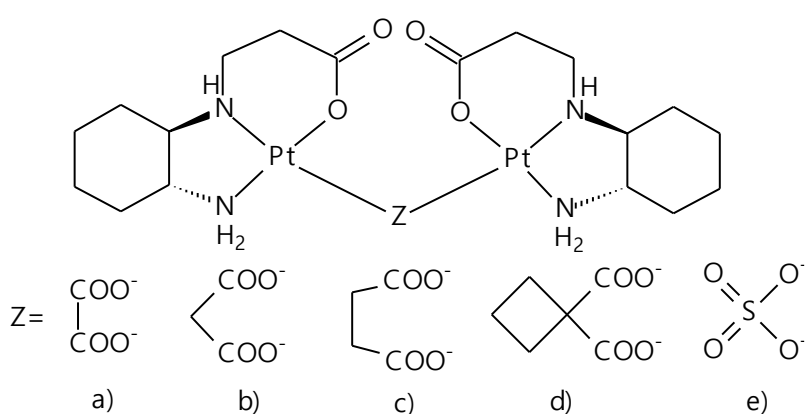


**Figure 6.**

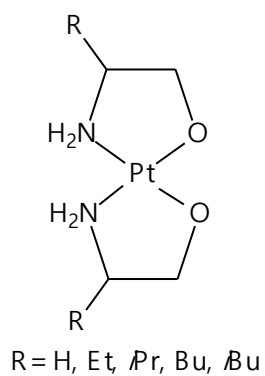


- a)  $n=0$ ,  $R=H$   
 b)  $n=0$ ,  $R=Me$   
 c)  $n=1$ ,  $R=H$

**C10**

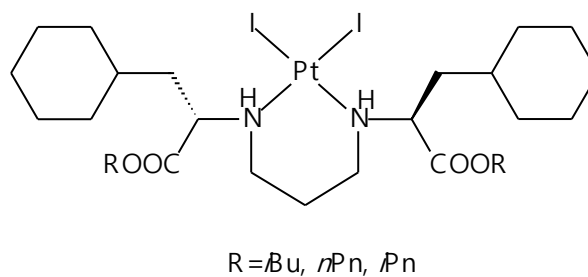


**C11**



$R=H, Et, iPr, Bu, tBu$

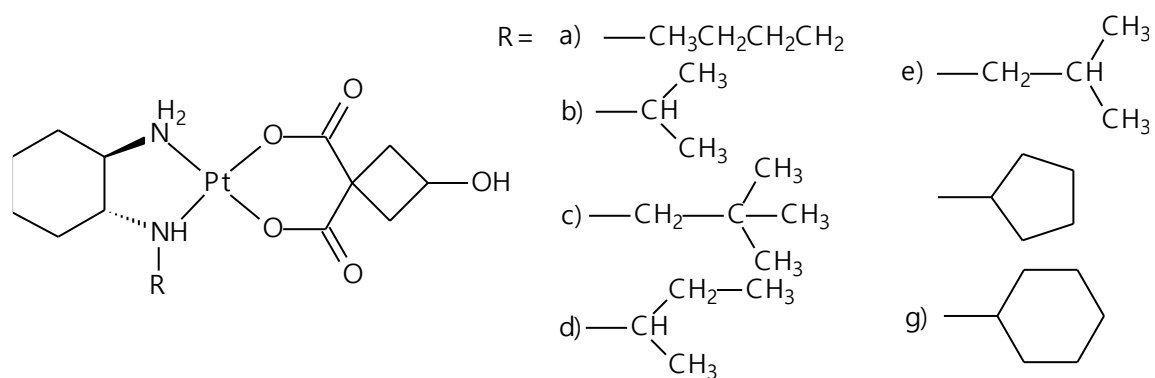
**C12**



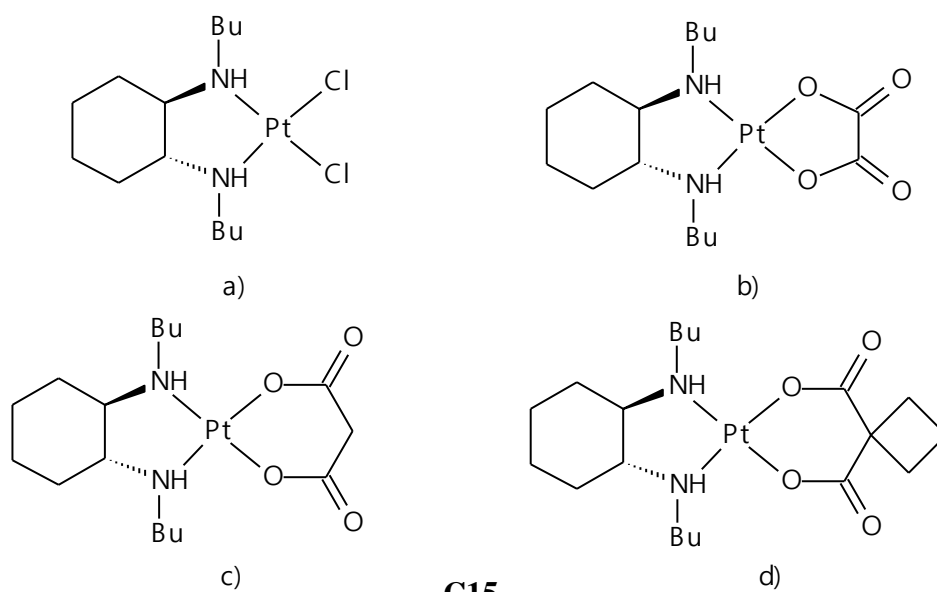
$R=Bu, nPr, iPr$

**C13**

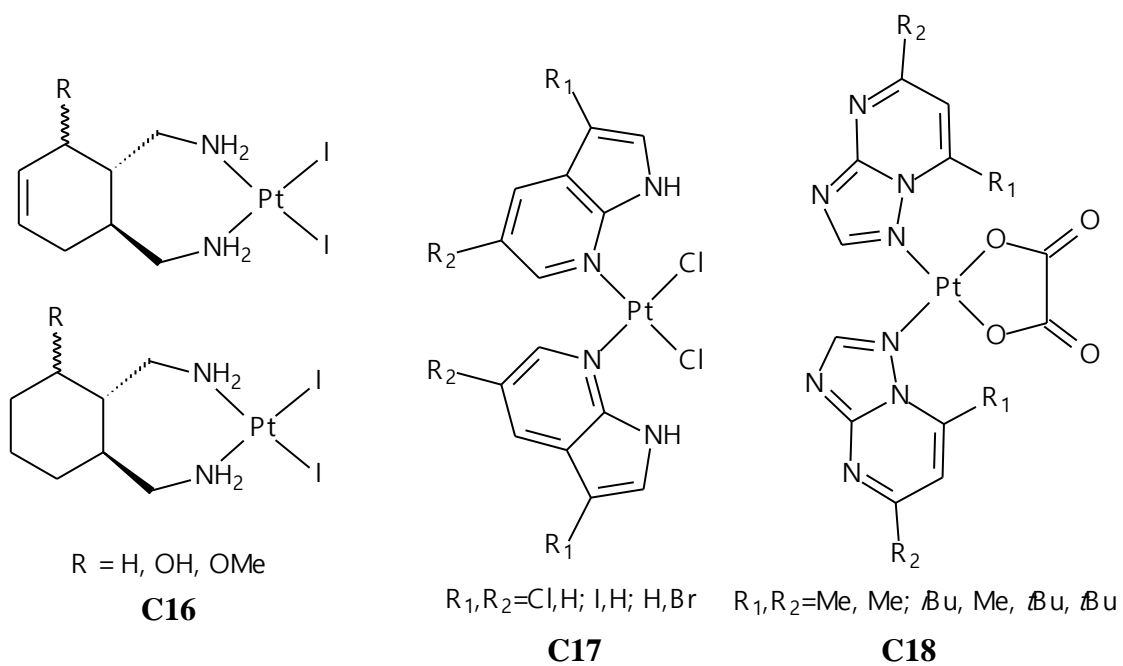
**Figure 7.**



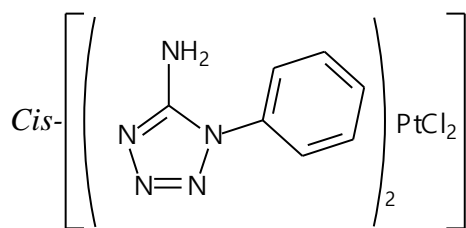
**C14**



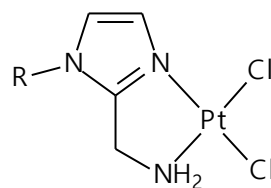
**C15**



**Figure 8.**

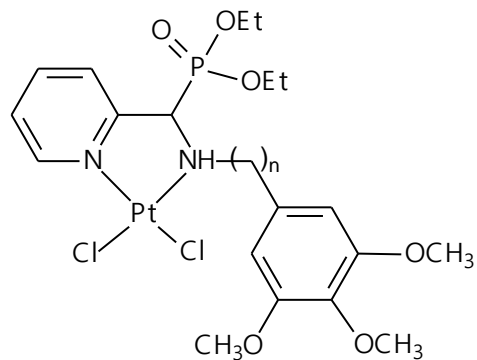
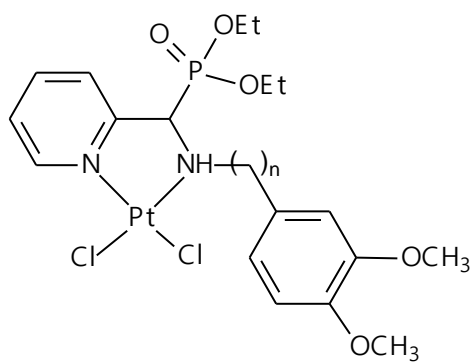
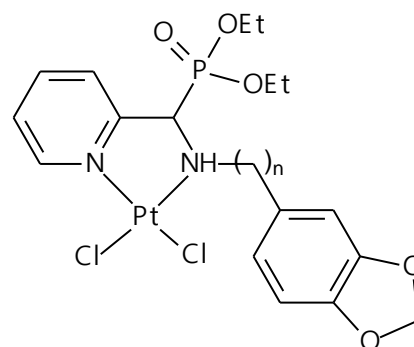
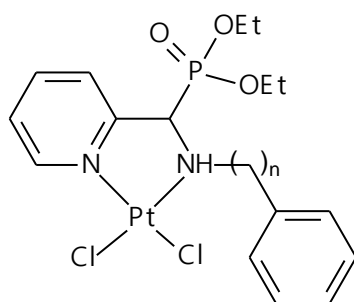


**C19**



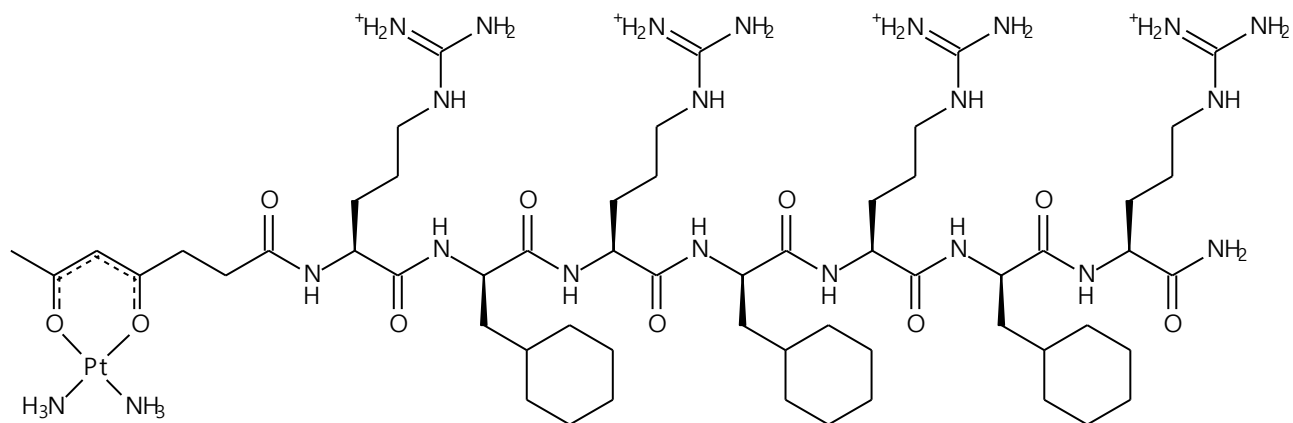
R = Saturated and unsaturated alkyl chains

**C20**



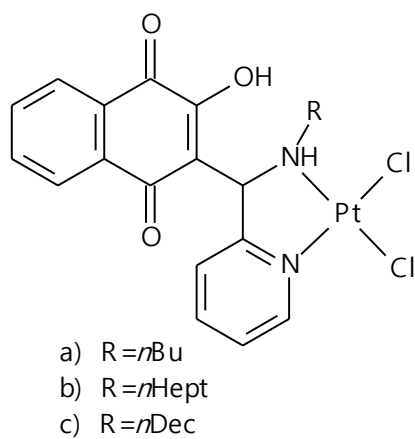
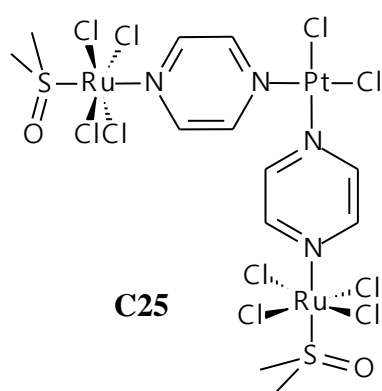
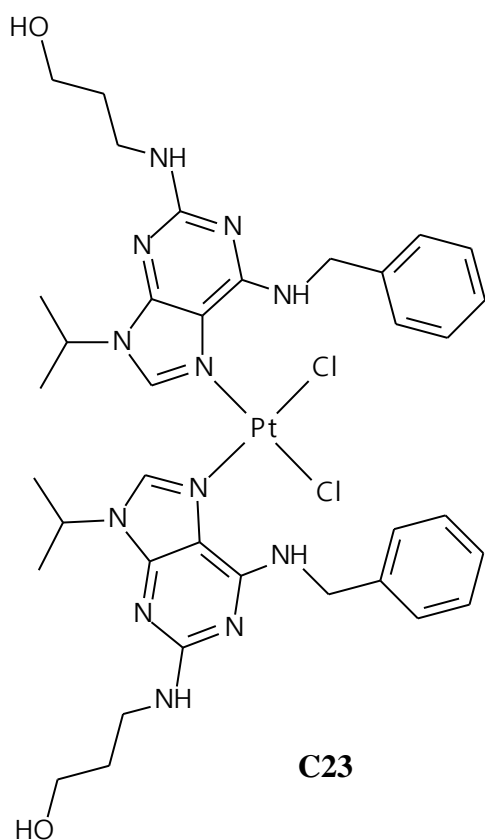
n=0,1,2

**C21**

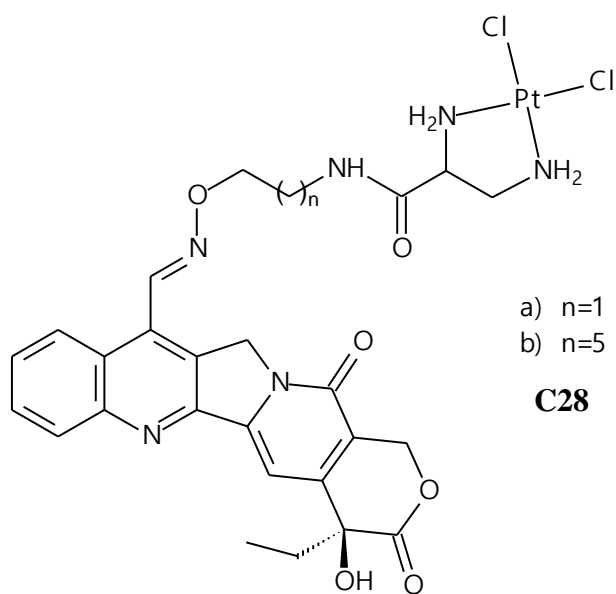
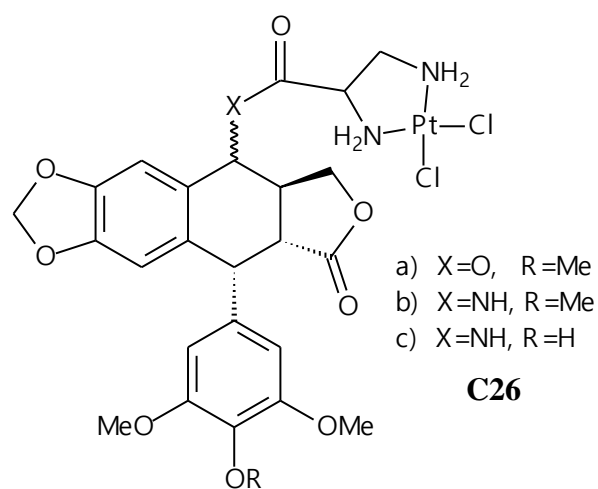
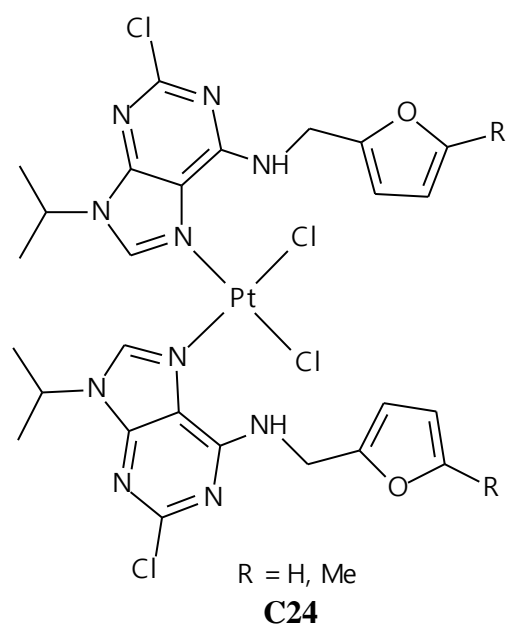


**C22**

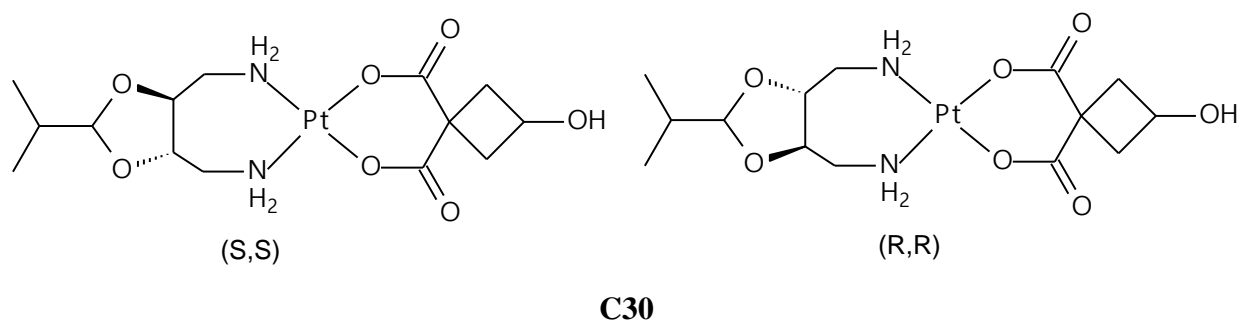
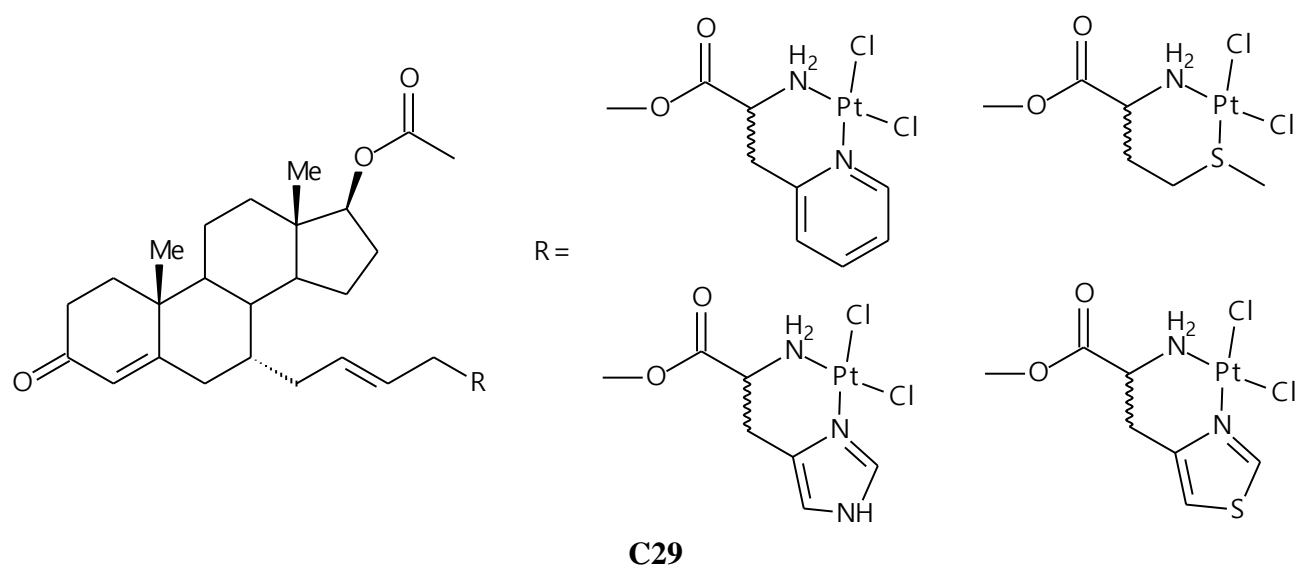
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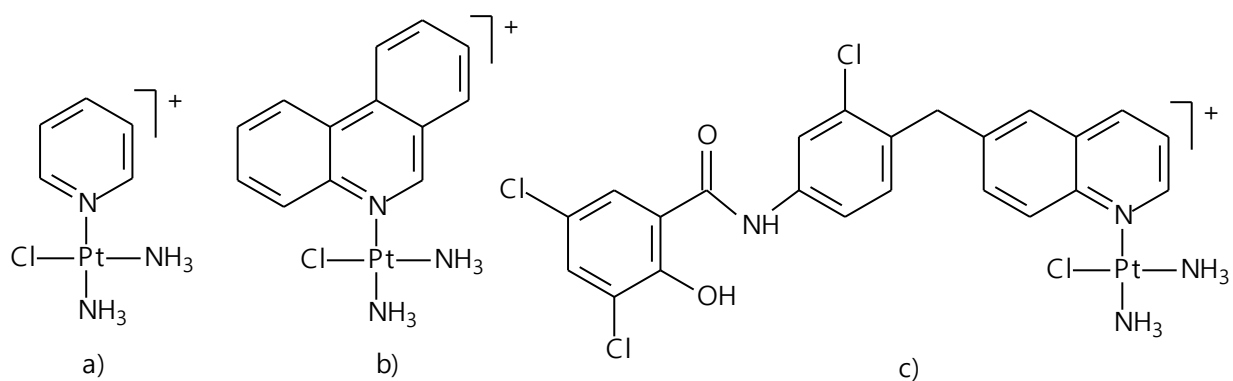
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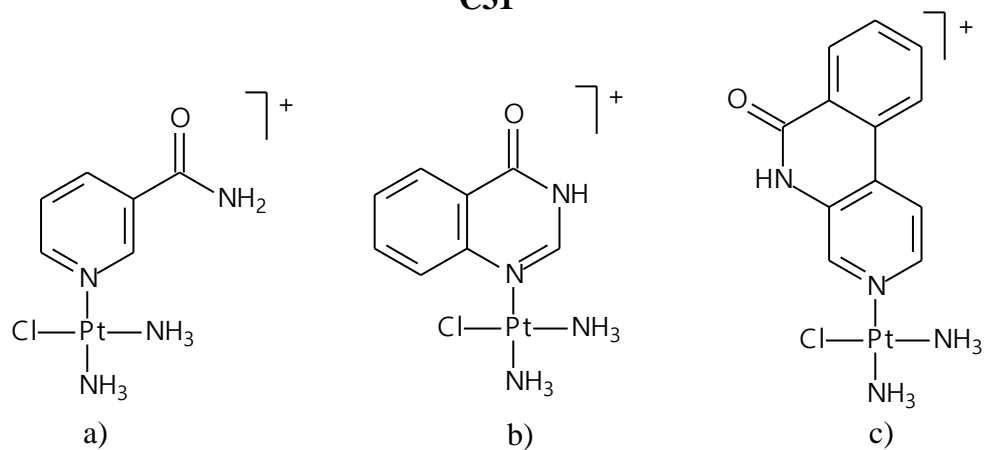
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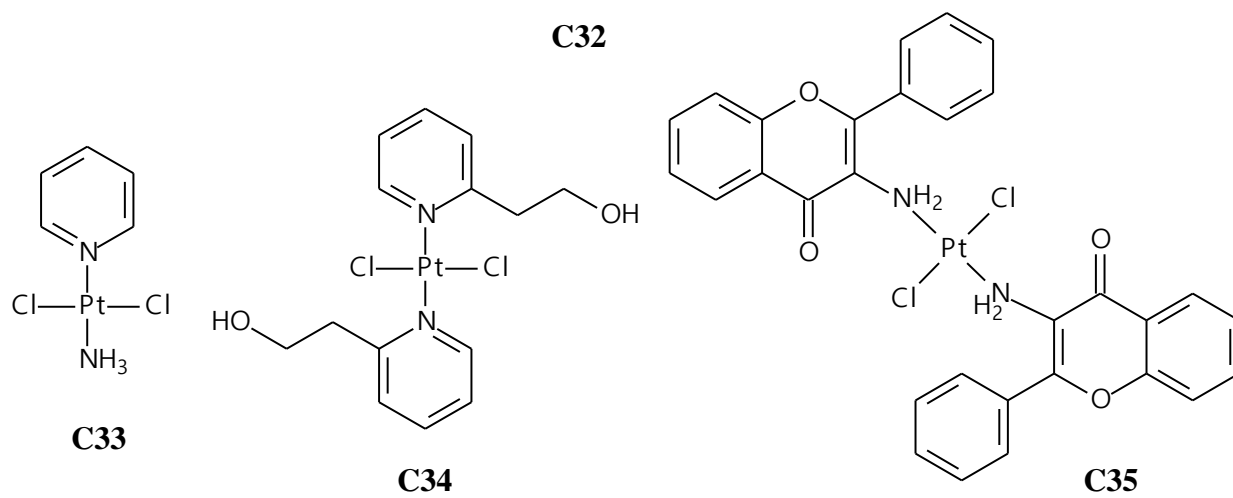
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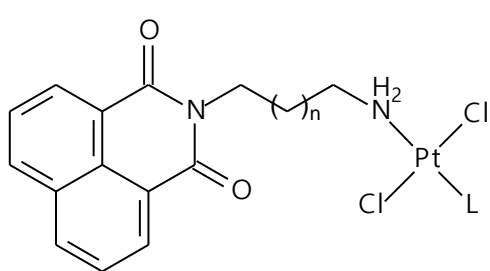
**C31**



**C32**



**Figure 12.**

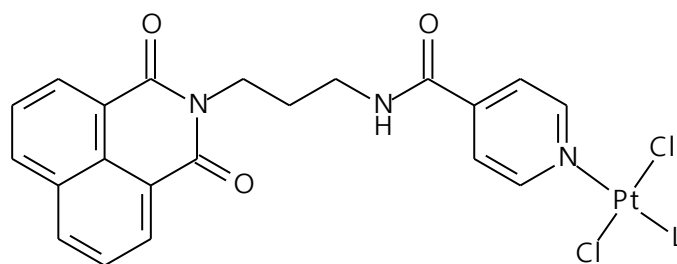


**C36a**  $n=1$ , L=dimethylamine

**C36b**  $n=4$ , L=dimethylamine

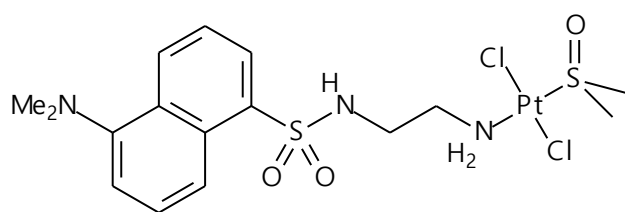
**C36c**  $n=1$ , L=isopropylamine

**C36d**  $n=4$ , L=isopropylamine

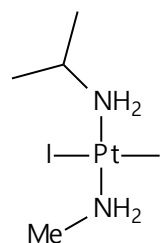


**C37a** L=dimethylamine

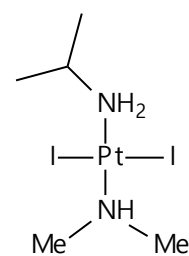
**C37b** L=isopropylamine



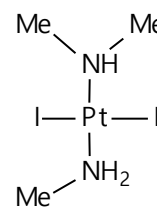
**C38**



a)



b)

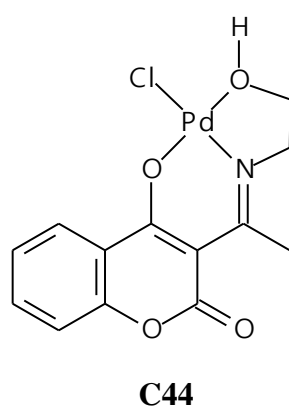
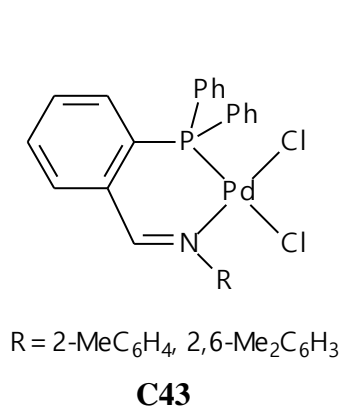
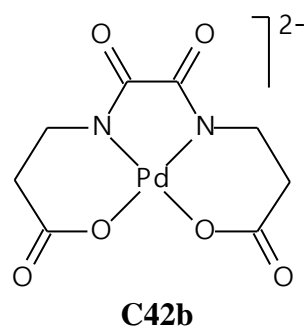
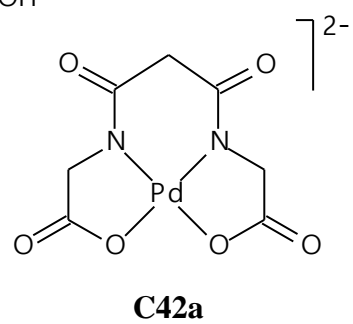
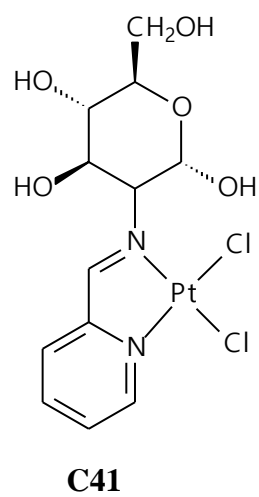
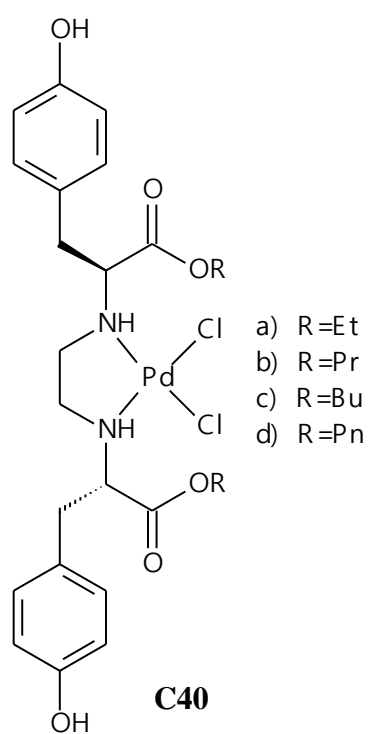


c)

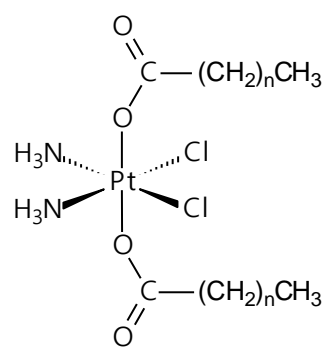
**C39**



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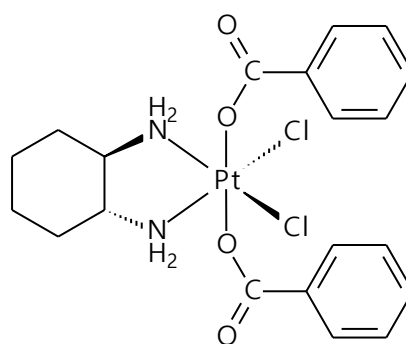


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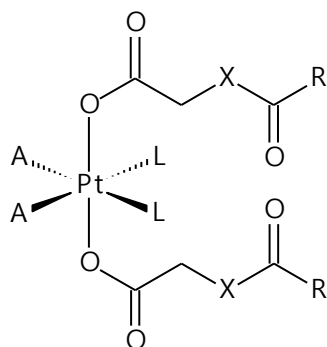


- a)  $n=0$       b)  $n=2$   
c)  $n=4$       d)  $n=6$

**C45**

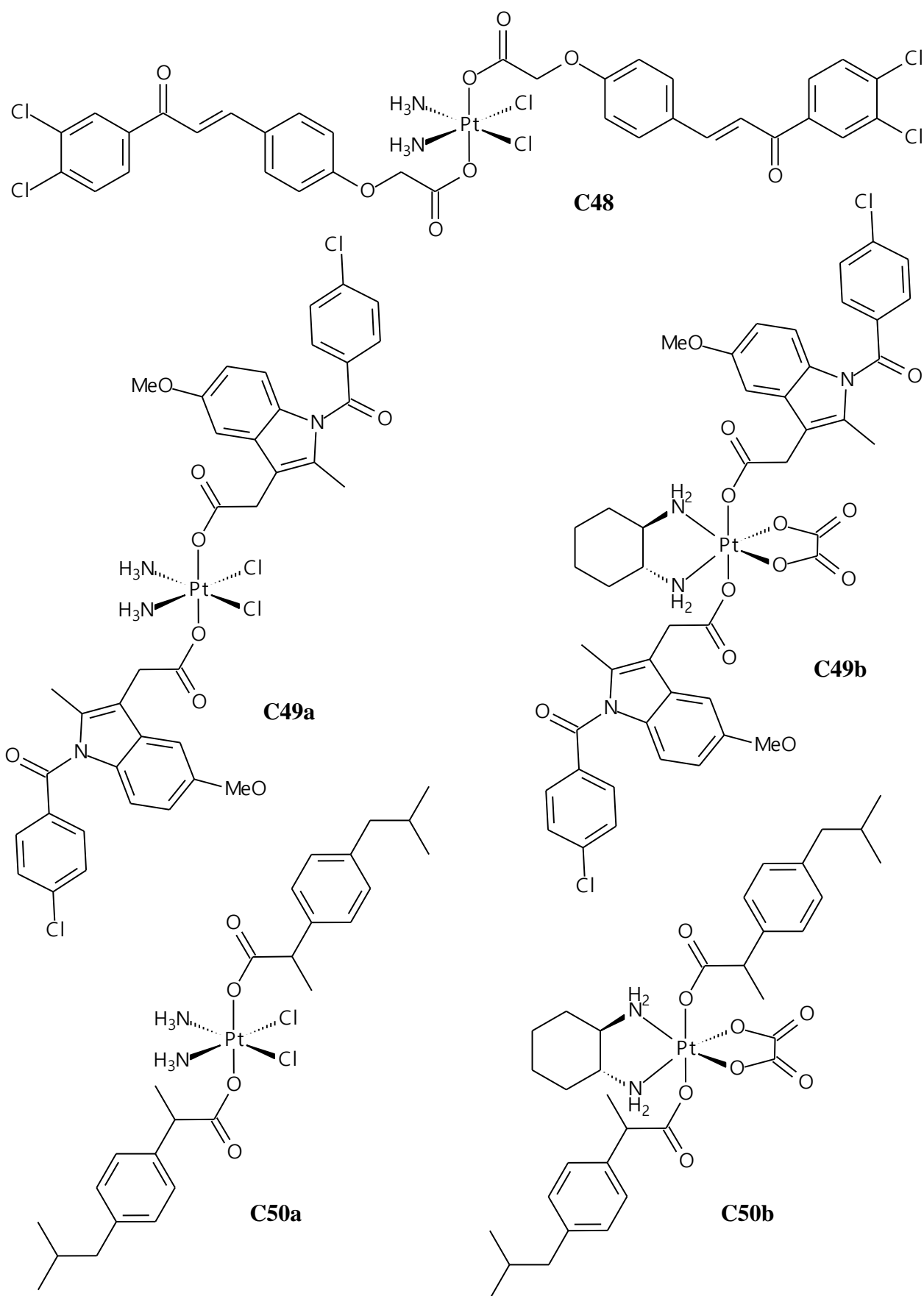


**C46**

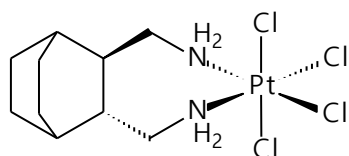
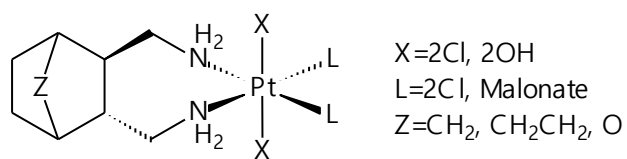


- C47** {  
2A=2NH<sub>3</sub>, en, 2EtNH<sub>2</sub>, DACH  
2L=2Cl, CBDCA, mal, ox, glyc  
X=CH<sub>2</sub>, (CH<sub>2</sub>)<sub>2</sub>, CH(CH<sub>3</sub>), C(CH<sub>3</sub>)<sub>2</sub>  
R=OH, OMe, OEt, OPr, OBu, OPn,  
OHex, OOct, NHPr, NHcPn

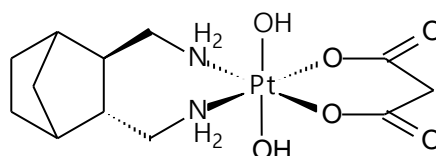
**Figure 15.**



**Figure 16.**

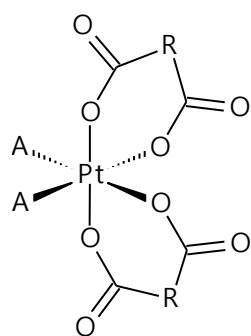


a)



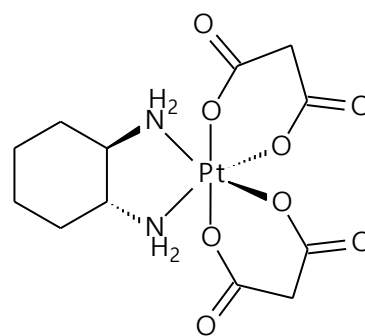
b)

**C51**

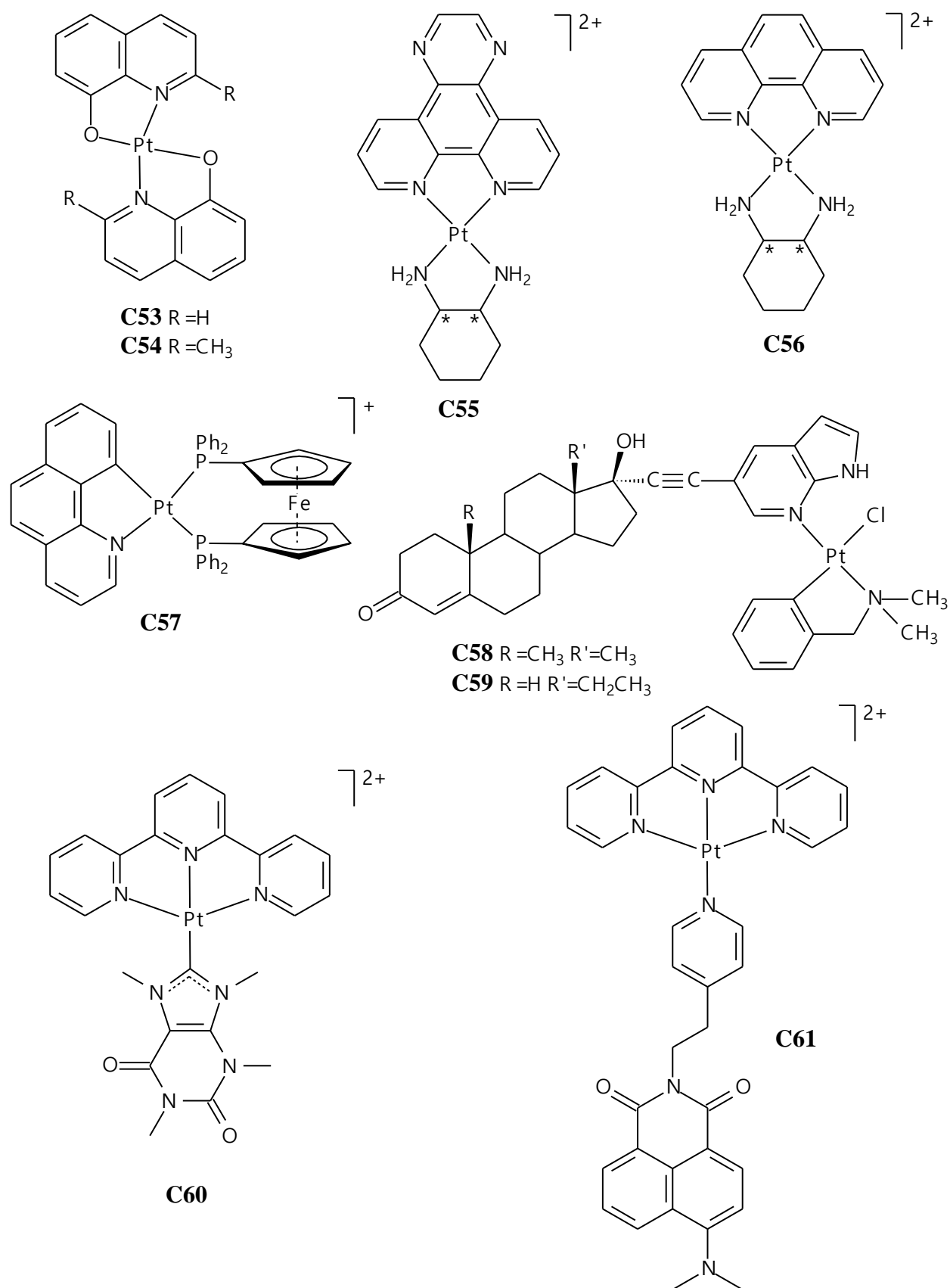


$A = 2NH_3, 2EtNH_2, NH_3 \text{ and } cHexNH_2, en, DACH$   
 $R = \text{nothing}, CH_2, CH(CH_3)$

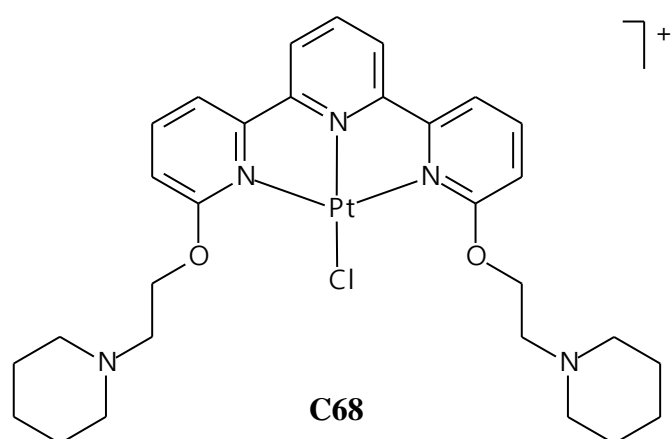
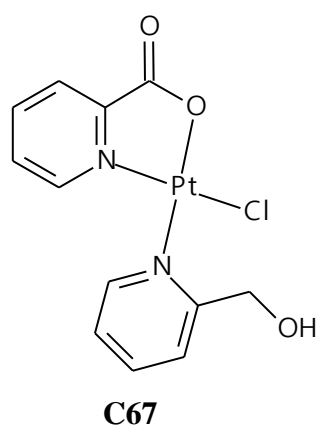
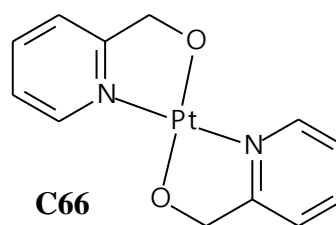
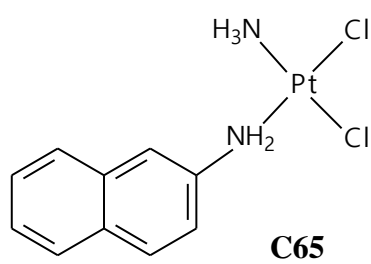
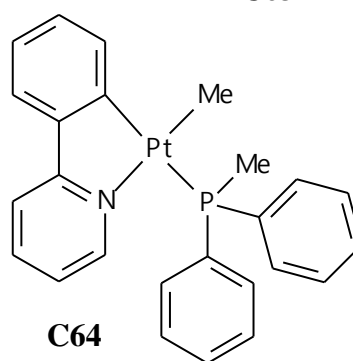
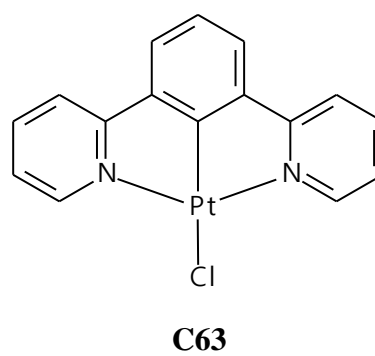
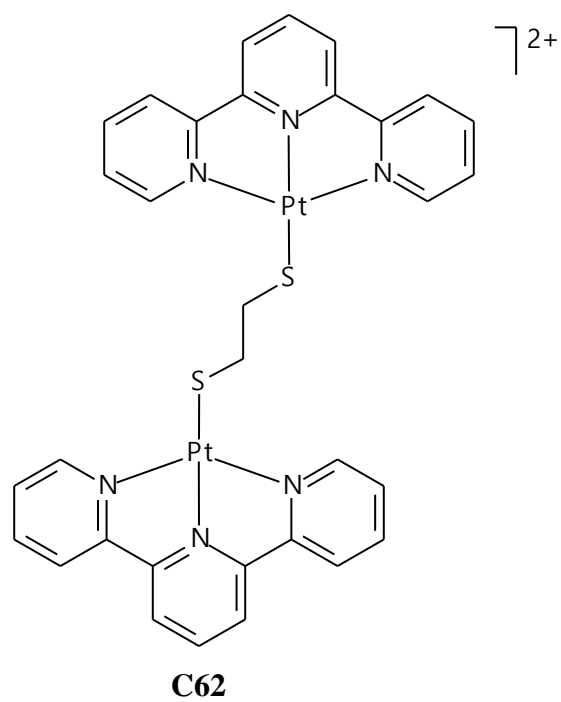
**C52**



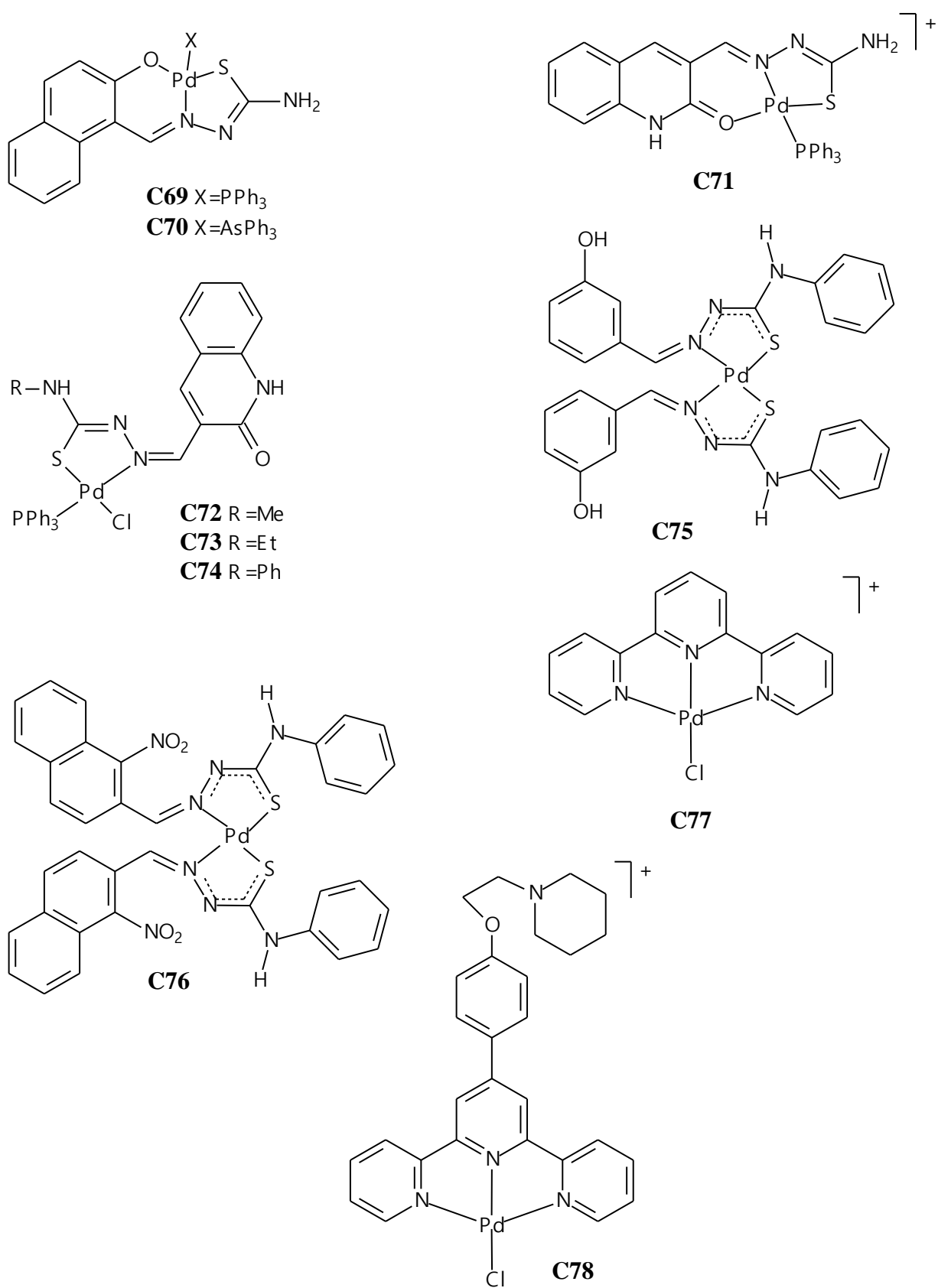
**Figure 17.**



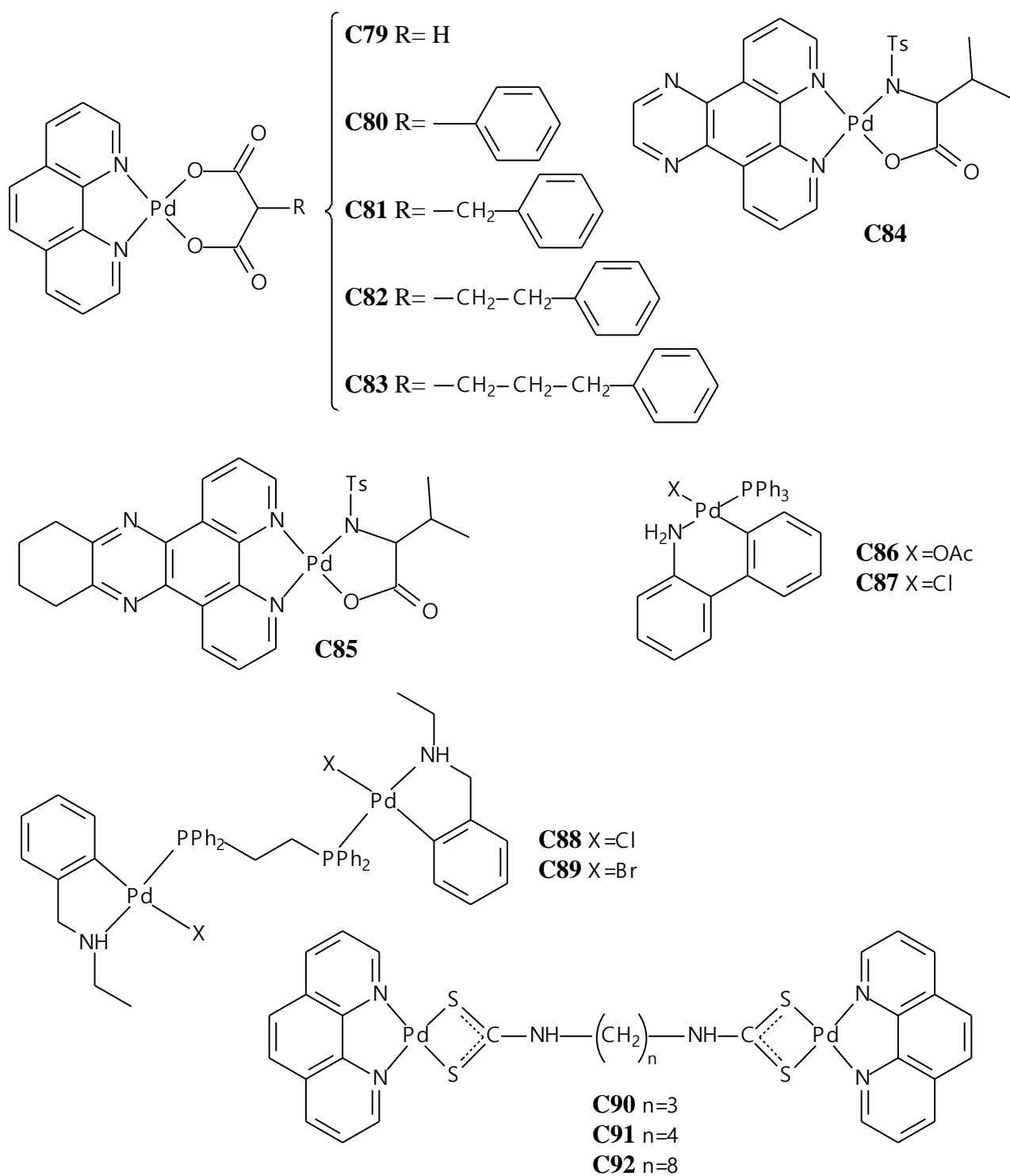
**Figure 18.**



**Figure 19.**

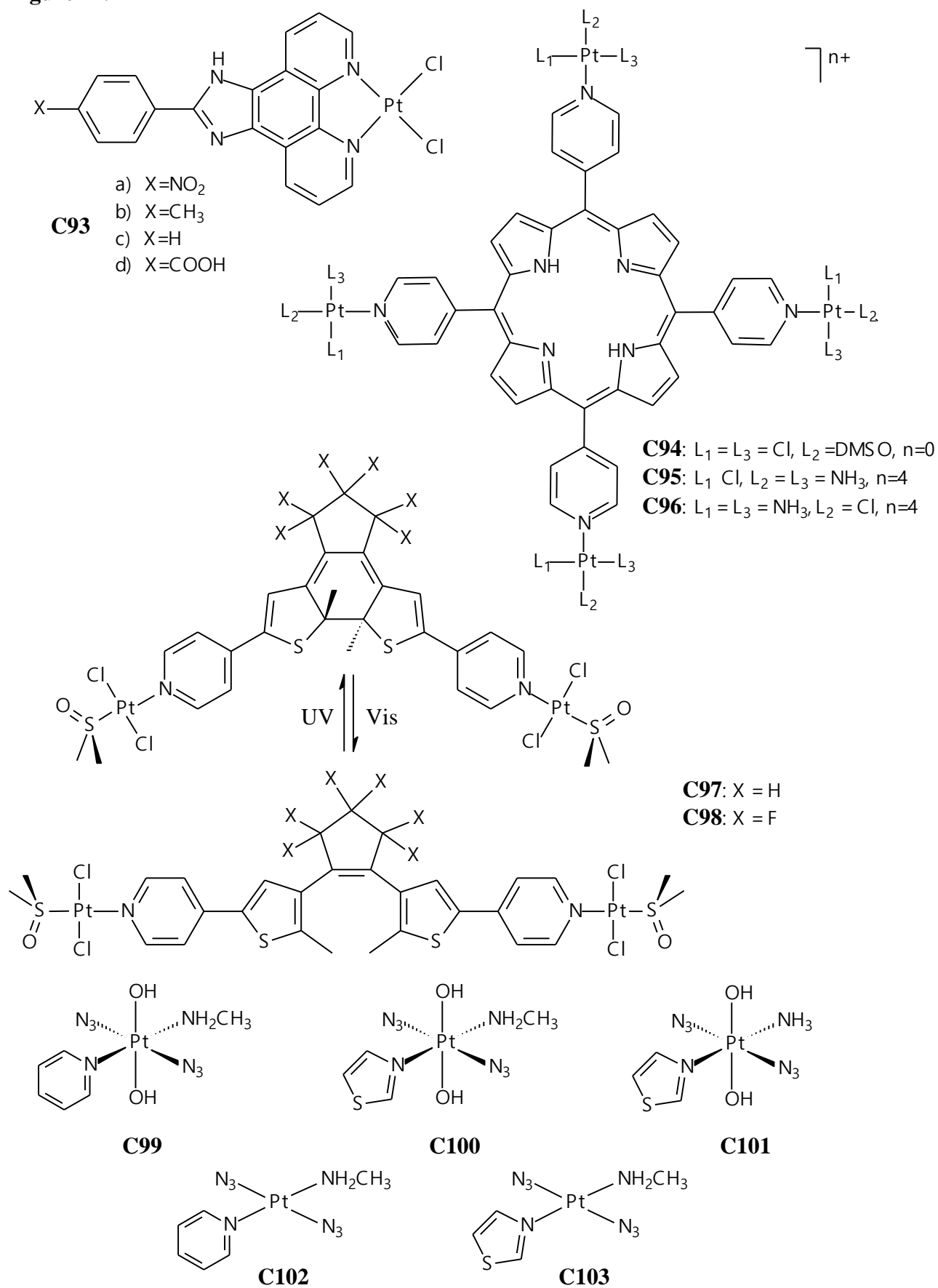


**Figure 20.**

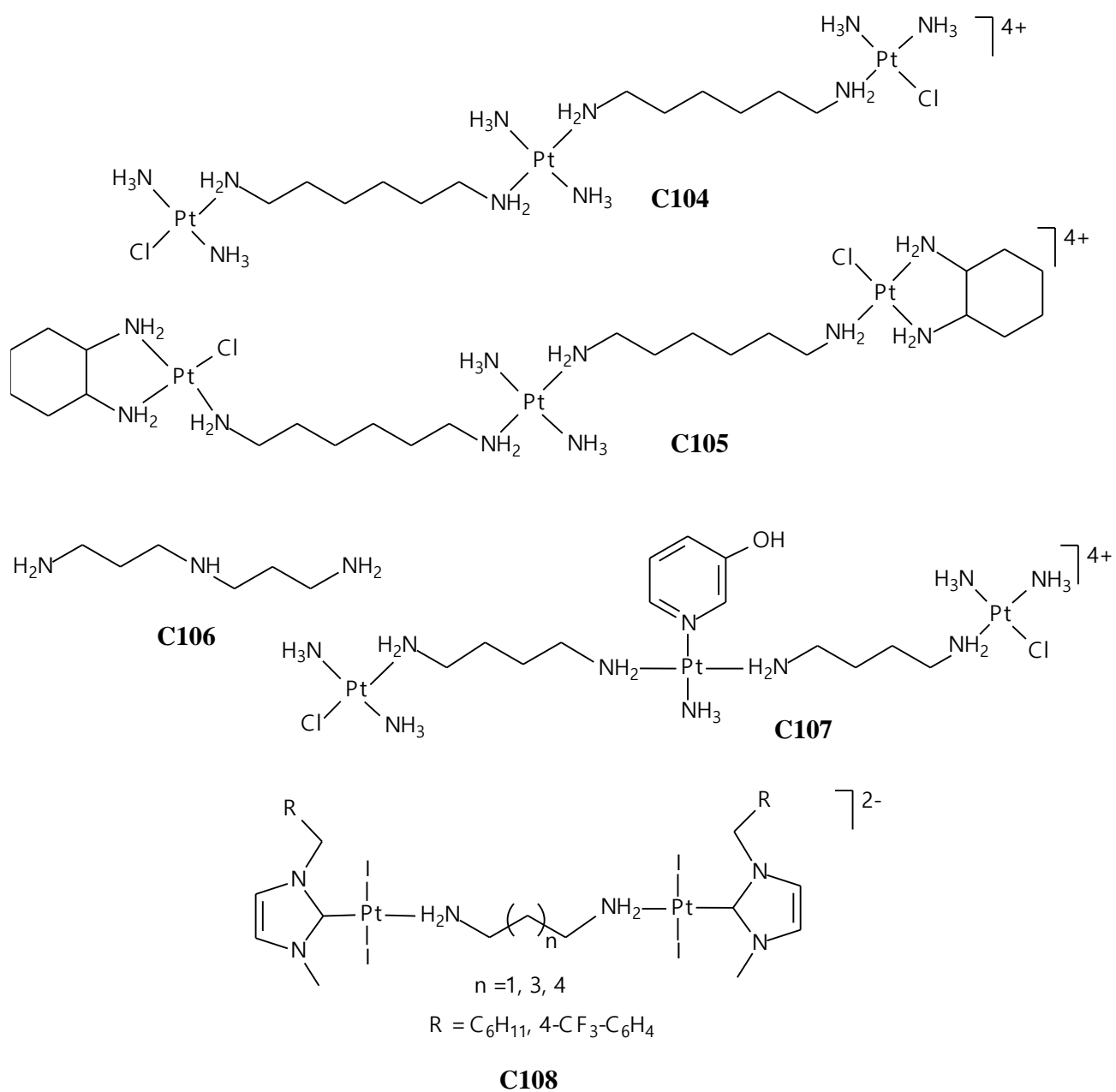




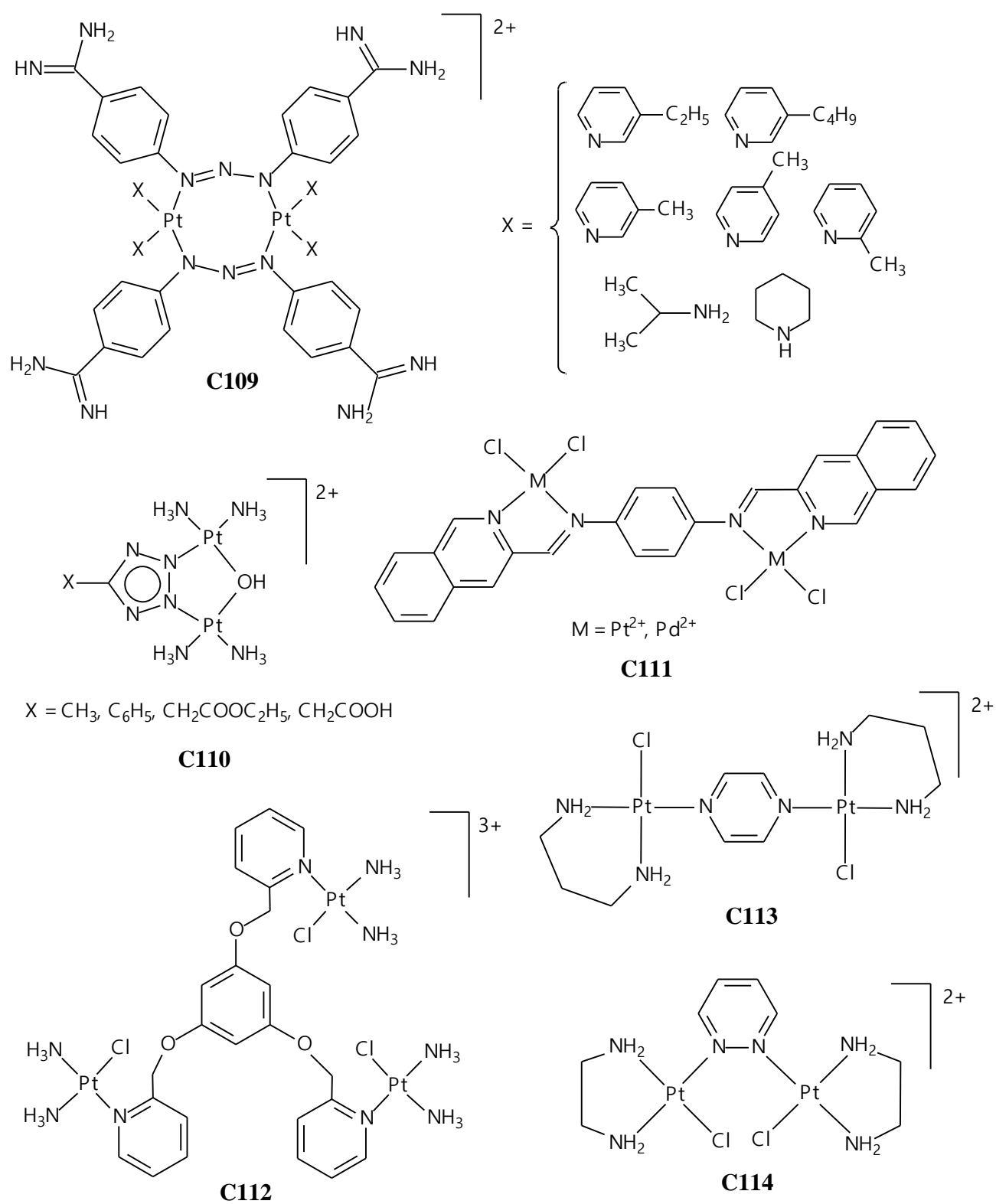
**Figure 21.**



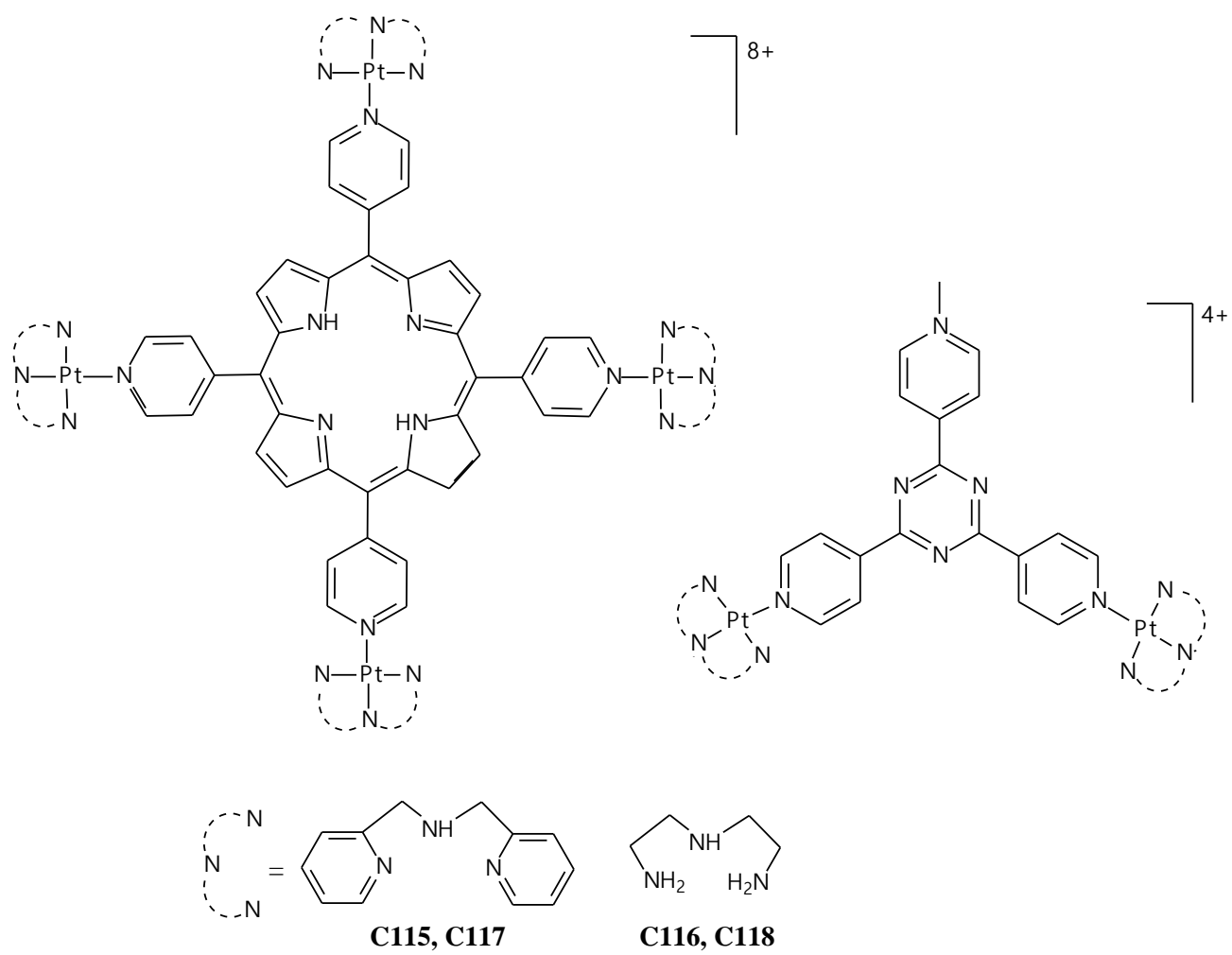
**Figure 22.**



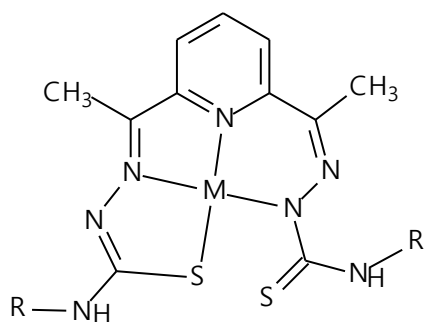
**Figure 23.**



**Figure 24.**



**Figure 25.**



**C119a** R=o-Tol, M=Pd<sup>2+</sup>

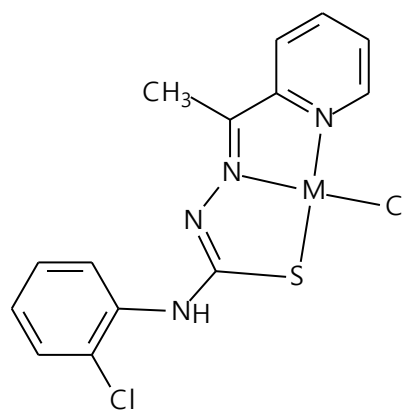
**C119b** R=p-Tol, M=Pd<sup>2+</sup>

**C119c** R=o-Tol, M=Pt<sup>2+</sup>

**C119d** R=p-Tol, M=Pt<sup>2+</sup>

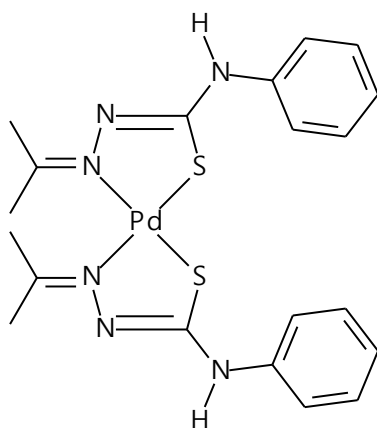
**C119e** R=p-ClC<sub>6</sub>H<sub>4</sub>, M=Pd<sup>2+</sup>

**C119f** R=p-ClC<sub>6</sub>H<sub>4</sub>, M=Pt<sup>2+</sup>

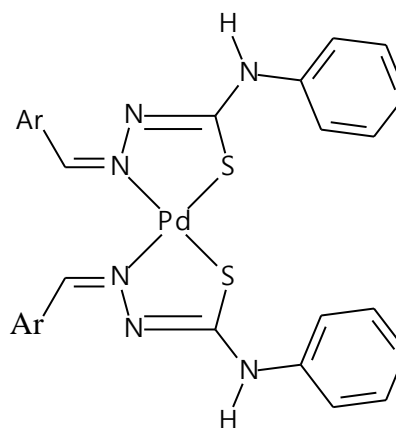


**C120a** M=Pt<sup>2+</sup>

**C120b** M=Pd<sup>2+</sup>



**C121a**

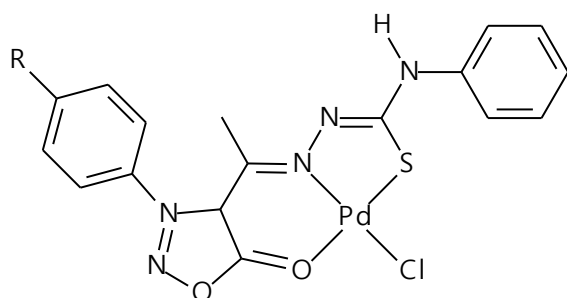


**C121b** Ar=2-ClC<sub>6</sub>H<sub>4</sub>

**C121c** Ar=3-HOC<sub>6</sub>H<sub>4</sub>

**C121d** Ar=1-naphthyl

**C121e** Ar=1-nitro-2-naphthyl

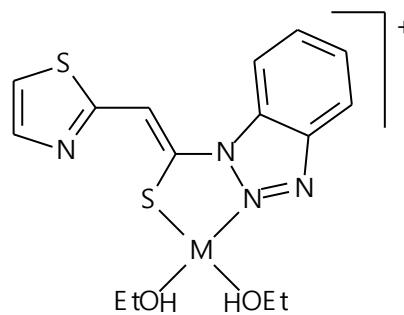


**C122a** R=H

**C122b** R=Me

**C122c** R=MeO

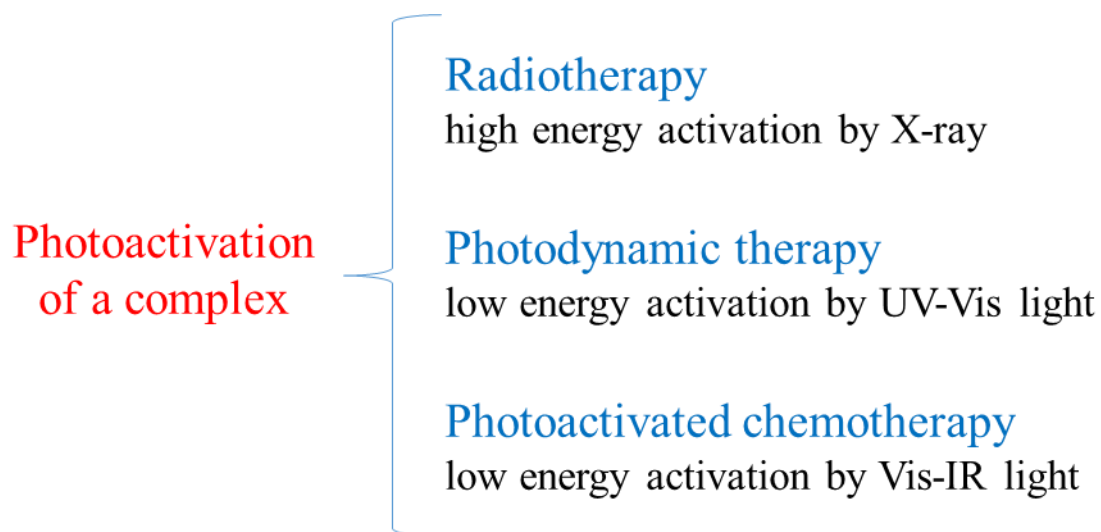
**C122d** R=EtO



**C123a** M=Pt<sup>2+</sup>

**C123b** M=Pd<sup>2+</sup>

**Figure 26.**



**Figure 27.**

