Palacký University Olomouc
Faculty of Science
Department of Biophysics

DOCTORAL THESIS

The study of interactions between antitumor effective heavy metal complexes with biomacromolecules using molecular biophysics methods

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Abstract

The conventional cisplatin [*cis*-diamminedichloridoplatinum(II)] is the most used and successful anticancer compound more than 50 years. The antitumor properties of cisplatin are attributed to its major adducts 1,2-GG-intrastrand cross-links formed in DNA, which is the major pharmacological target of platinum drugs, generally. Thus, acquired tumor resistance to cisplatin after wider application in clinical practice is mainly due to removal of major adducts from DNA by nucleotide excision repair (NER). In addition, undesirable side effects represent additional unfavorable aspect of the clinical use of cisplatin. Therefore, the current research of metallodrugs continues in two different directions. One direction is synthesis of polynuclear platinum complexes containing flexible bridged ligands. Such complexes exhibit a different DNA binding mode, which is responsible for different antitumor properties in comparison with cisplatin. This group of metallodrugs is represented in this thesis by trinuclear tridentate platinum complex {$[Pt_3Cl_3$ (hptab)$]^{3+}$ (hptab=N,N,N′,N′,N″,N″-hexakis(2-pyridylmethyl)-1, 3, 5tris(aminomethyl)benzene)} (**1**), which was studied because of its unique DNA binding and cross-linking mode. The second direction of current research is focused on synthesis of polynuclear platinum complexes with rigid linkers. These dinuclear azolato- bridged Pt^{II} complexes induced in DNA minimal conformational distortions, but, interestingly, they form the same adducts like cisplatin. This new class of metallodrugs is represented in this thesis by benzotriazolate-bridged dinuclear Pt^{II} complex [{*cis*-$Pt(NH_3)_2Cl$}$_2$ (μ-1, 2, 3-Btaz-H-N1,N3)]Cl (**2**). The early phases of the mechanism underlying biological properties of complex **2** were studied.

The primary objective of the study of trinuclear complex **1** was to fully investigate the binding of complex **1** to natural, high-molecular-mass DNA and plasmid DNAs with focusing on intramolecular and intermolecular cross-linking. Our results reveal that complex **1** forms mainly trifunctional intrastrand cross-links, i.e. when all three Pt^{II} centers of complex **1** are coordinated preferentially to G base residues. Hence, these trifunctional adducts induced in DNA extensive conformational distortions. Intriguingly, complex **1** can cross-link proteins to DNA and can

form interduplex interstrand cross-links under crowding conditions mimicking condition in cell nucleus. The latter fascinating phenomenon was identified for the class of trinuclear platinum complexes for the first time.

The study of benzotriazolate-bridged dinuclear Pt^{II} complex was focused on interactions with DNA. It was observed that adducts of dinuclear complex **2** distort DNA differently than adducts of cisplatin. Monofunctional DNA adducts of complex **2** are converted to more toxic bifunctional cross-links significantly more slowly than cisplatin. Furthermore, the binding of dinuclear complex **2** to DNA in tumor cells is inactivated by sulfur-containing compounds markedly more than that of cisplatin.

In addition, the attention was also paid to antitumor mononuclear analogues of cisplatin, namely {*cis*-[$PtCl_2$(*3Cl*Haza)$_2$]} (**3**) and {*cis*-[$PtCl_2$(*3I*Haza)$_2$]} (**4**) (*3Cl*Haza and *3I*Haza = 3- chloro-7-azaindole and 3-iodo-7-azaindole, respectively). This study revealed that reactions of these complexes (**3**, **4**) with sulfur containing compounds (which are responsible for the resistance of tumors to platinum drugs) are significantly retarded in contrast to those of cisplatin.

The results based on the investigation of interactions of platinum complexes examined within this thesis can expand the theoretical background needed for the design of new antitumor metallodrugs.

Abstrakt

Konvenční cisplatina (*cis*-diammindichloridoplatnatý komplex) je nejpoužívanějším a nejúspěšnějším metalofarmakem v léčbě rakoviny více než 50 let. Protinádorové vlastnosti cisplatiny jsou přičítány jejím hlavním aduktům 1,2-GG-vnitrořetězcovým můstkům vzniklým po vazbě na DNA, která je hlavním farmakologickým cílem platinovým léčiv. Opakované používání cisplatiny v klinické praxi vedlo k rezistenci nádorů vůči cisplatině, která spočívá zejména v odstranění tohoto poškození pomocí nukleotidových excizních oprav (NER). Nepříznivým aspektem použití cisplatiny v klinické praxi jsou také nežádoucí vedlejší účinky. Proto se dnešní výzkum metalofarmak vydává dvěma rozdílnými směry. První směr spočívá v syntéze vícejaderných platinových komplexů, jejichž reaktivní platinové jednotky jsou přemostěny flexibilními ligandy. Takové vícejaderné Pt^{II} komplexy mají rozdílný DNA vazebný modus, který vyvolává rozdílné protinádorové vlastnosti než cisplatina. Tuto skupinu metalofarmak v této disertaci reprezentuje trojjaderný platinový komplex {$[Pt_3Cl_3$ (hptab)$]^{3+}$ (hptab=N,N,N′,N′,N″,N″-hexakis(2-pyridylmethyl)-1, 3, 5tris(aminomethyl)benzen)} (**1**), který byl zkoumán pro svůj jedinečný vazebný a můstky tvořící modus. Druhý směr současného výzkumu se zabývá syntézou vícejaderných platinových komplexů, ve kterých jsou platinové jednotky přemostěny rigidními linkery. Tyto dvojjaderné Pt^{II} komplexy s azolátovým můstkem vyvolávají v DNA minimální konformační distorze, i když vytvářejí stejné adukty jako cisplatina. Nová skupina metalofarmak je v této disertační práci zastoupena dvojjaderným platinovým komplexem s benzotriazolátovým můstkem [{*cis*-$Pt(NH_3)_2Cl\}_2$ (μ-1, 2, 3-Btaz-H-N1,N3)]Cl (**2**), u něhož byla zkoumána počáteční fáze mechanismu zodpovědného za jeho hlavní biologické vlastnosti.

Primárním cílem studia trojjaderného komplexu **1** bylo prozkoumat jeho vazbu na DNA se zaměřením na formování vnitromolekulárních a mezimolekulárních můstků. Výsledky odhalily, že komplex **1** vytváří hlavně trifunkční vnitrořetězcové můstky, tzn., že všechny tři platinové jednotky jsou koordinovány ke guaninovým bázím v DNA. Tím dochází ke značným konformačním distorzím. Fascinujícím jevem je, že

trojjaderný komplex je schopný vytvářet koordinační můstky mezi DNA a proteiny a také meziduplexové mezivláknové můstky za podmínek napodobujících přirozený stav uvnitř buněčného jádra. Tento posledně jmenovaný jev byl u trojjaderných komplexů platiny pozorován poprvé.

Studium dvojjaderného Pt^{II} komplexu **2** bylo zaměřeno na jeho interakce s DNA. Bylo zjištěno, že adukty komplexu **2** vyvolávají v DNA odlišné distorze než adukty cisplatiny a také, že jeho monofunkční adukty se uzavírají na toxičtější bifunkční adukty pomaleji, než v případě cisplatiny. Vazba dvojjaderného komplexu **2** na DNA je inaktivována látkami obsahujícími síru (zodpovědnými za rezistenci nádorových buněk k platinovým cytostatikům) více než u cisplatiny.

Dále byly také studovány protinádorově účinná analoga cisplatiny, jmenovitě {*cis*-[$PtCl_2$(*3Cl*Haza)$_2$]} (**3**) a {*cis*-[$PtCl_2$(*3I*Haza)$_2$]} (**4**) (*3Cl*Haza a *3I*Haza = 3-chloro-7-azaindol a 3-iodo-7-azaindol) a bylo zjištěno, že tyto komplexy se vyznačují výrazně nižší reaktivitou s látkami obsahujícími síru v porovnání s cisplatinou.

Výsledky, kterých bylo dosaženo zkoumáním protinádorově účinných komplexů v rámci této disertační práce umožňují rozšířit teoretické zázemí důležité pro návrhy nových protinádorově účinných metalofarmak.

Acknowledgment

I would like to thank my supervisor prof. RNDr. Viktor Brabec, DrSc. for mentoring and comprehensive assistance during my Ph.D. study. Also, I would like to thank all my colleagues of the Department of Molecular Biophysics and Pharmacology, IBP, AS CR for creating a pleasant working environment and help to me during my research. Specially, I would like to thank my family for their support during my study.

Statutory declaration

I hereby declare that this doctoral thesis has been written solely by myself. All the sources quoted in this work are listed in the "Bibliography" section. All published or submitted results included in this work are approved by co-authors.

In Olomouc June 5, 2013

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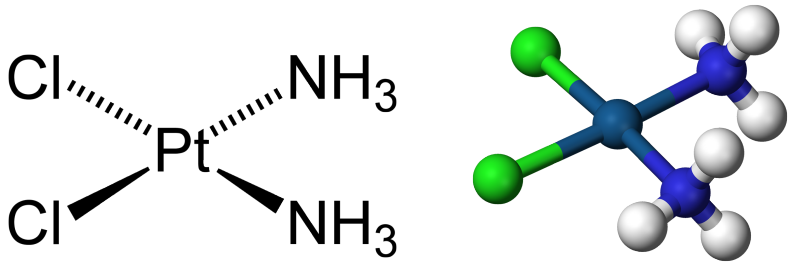
Contents

Chapter 1

Introduction

1.1 Discovery and development of anticancer drug cisplatin

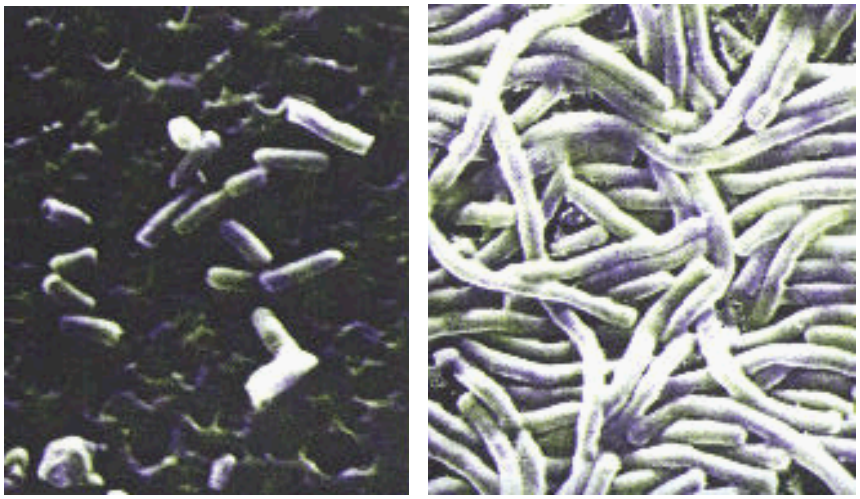
Cisplatin (cis-diamminedichloridoplatinum(II)) (Fig. 1-1), which is widely used in the clinic today, was synthesized for the first time in 1844 by Michele Peyrone [1]. More than a century later the tumor-inhibiting qualities of this substance were discovered by Barnett Rosenberg in 1969. Rosenberg examined the influence of an electric field on bacterial growth. He used platinum electrodes that were submerged in a solution containing ammonium chloride [2–5]. The bacteria grew up to 300 times their own length without any cell division (Fig. 1-2).



(a) **(b)**

Figure 1-1: Cisplatin, cis-diamminedichloridoplatinum(II) [6].

This selective influence on cell division, along with an unrestrained growth, was caused by cis-diamminetetrachloridoplatinum(IV), what was recognized by him later. It soon occurred to Rosenberg that compounds such as these might also be capable of inhibiting tumor growth. He proved that all these cis-configurated compound are active. In contrast to this, the corresponding trans-compounds were found to be inactive. This led him to synthesize some simple platinum complexes like cisplatin and to check their cytotoxic activity in experimental tumor models [7–12].



(a) **(b)**

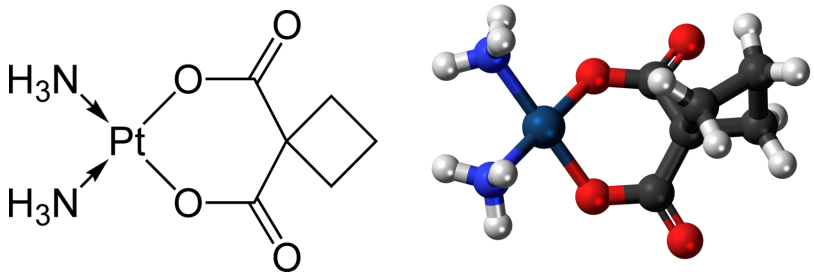
Figure 1-2: Scanning electron microphotograph of a) normal E. coli (Gram-negative rods); b) E. coli grown in medium containing a few parts per million of cisplatin. Cisplatin has inhibited cell division, but not growth, leading to long filaments [2].

Cisplatin entered clinical trials in 1971 and since then has become established as a highly effective drug for treating testicular tumors, ovarian carcinomas, bladder tumors, and head and neck cancer. Activity is also established in lung cancers, lymphomas, breast cancer, esophageal cancer and other tumor types [13]. Despite its success, cisplatin has several disadvantages. Its applicability is still limited to a relatively narrow range of tumors. Some tumors have a natural resistance to cisplatin, while others develop resistance after the initial treatment. Cisplatin also has limited solubility in aqueous solution and is administered intravenously. There are also significant problems in terms inducing severe side effects (especially kidney damage and

vomiting/nausea). Because of the drawbacks coupled with cisplatin toxicity there has been an impetus toward the development of improved platinum drugs. Broadening of chemoterapeutic arsenal depends on understanding existing agents with a view toward developing new modes of attack. It is therefore of great interest to understand details of molecular and biochemical mechanisms underlying the biological efficacy of the platinum compound [14].

1.2 Cisplatin analogues as a marketed platinum anticancer drugs

Over 3000 platinum compounds have been synthesized and tested for their biological activity [15]. Of these, however, fewer than 30 compounds have entered clinical trials [16]. Attempts to develop new anticancer platinum drugs have encountered difficulties in overcoming the drawbacks of cisplatin in actual clinical tests. At present, only four platinum drugs (anologues of cisplatin) are registered as marketed drugs (cisplatin, carboplatin, oxaliplatin and nedaplatin) and only one compound (oxaliplatin) has been approved by the FDA (Food and Drug Administration) for colorectal cancer since the release of cisplatin and carboplatin [17–19].

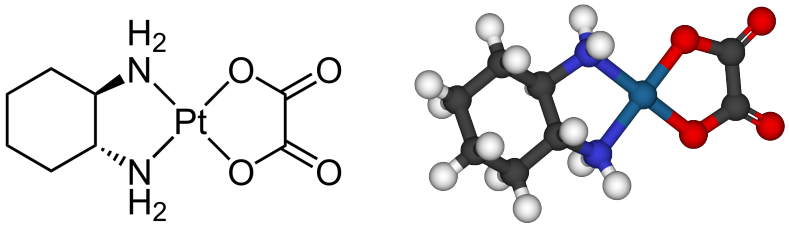


(a) **(b)**

Figure 1-3: Carboplatin, cis-diammine-1,1-cyclobutanedicarboxylato-platinum(II) [20].

Carboplatin (cis-diammine-1,1-cyclobutanedicarboxylato-platinum(II)) (Fig. 1-3). It was hypothesized that modification of cisplatin to contain less labile leaving groups could alter toxicity [21]. It was found that substitution of more stable ligands for the

chloride leaving groups did indeed diminish renal effects, while antitumor activity was retained [22]. Also, carboplatin did not require pretreatment with a rigorous hydration regimen. At effective doses, carboplatin produced substantially less nausea, vomiting, and neurotoxicity than cisplatin. Equivalence of carboplatin and cisplatin was demonstrated in the treatment of ovarian cancer [23], but in testicular, and head and neck cancer, cisplatin appears to be superior. Therefore, on the basis of superior therapeutic index, greater ease of administration, and more predictable individualized dosing, carboplatin has largely replaced cisplatin in the treatment of many, but not all platinum-sensitive tumors [21].



(a) **(b)**

Figure 1-4: Oxaliplatin, [1R,2R-diaminocyclohexane-(oxalato)platinum(II)] [24].

Oxaliplatin [1R,2R-diaminocyclohexane-(oxalato)platinum(II)] (Fig. 1-4) is the other analogue of cisplatin. Nevertheless, evidence for a lack of cross-resistance with cisplatin and carboplatin has been obtained *in vivo* [25]. It has been investigated that oxaliplatin in combination with 5-fluorouracil is potent against colorectal cancer. This important result lends substantial support to the hypothesis that structural modifications of the carrier ligand may greatly alter the spectrum of antitumor activity, and so overcome resistance. The major difference between the cisplatin, carboplatin and oxaliplatin is likely to be in the manner in which cellular proteins recognize and process platinum-DNA adducts [26].

1.3 Mechanism of anticancer action of cisplatin

The mechanism of action by which cisplatin manifests its selective toxicity to tumor cells is complex and includes cellular uptake and transport of the drug to the nu-

cleus, formation of DNA adducts in chromatin, and recognition by damage-response proteins [27]. Subsequent signal transduction pathways activated by this interaction between platinum-DNA damage and other nuclear proteins lead to cell-cycle arrest, attempts to repair the DNA lesions, and apoptosis or necrosis [28].

In aqueous solution, cisplatin undergoes stepwise aquation reactions in which the chloride ions are replaced by water [29] (Fig. 1-5). The loss of chloride ions results in formation of cationic mono- and diaqua complexes. Upon administration to the bloodstream as an intravenous injection, cisplatin maintains a relatively stable neutral state, because of the high concentration of chloride ion (∼100 mM), until the drug enters the cell. Inside the cell, however, the lower ambient chloride ion concentration (∼4–12 mM) facilitates cisplatin aquation to form the cationic aqua complexes [30]. Inside the cell, the activated platinum drug reacts with various cellular components, including DNA as the main biological target responsible for anticancer activity [31] and proteins, but also small molecules. Among small molecules are amino acids and oligopeptides with reactive side chains (Cys, Met, His, glutation) [32].

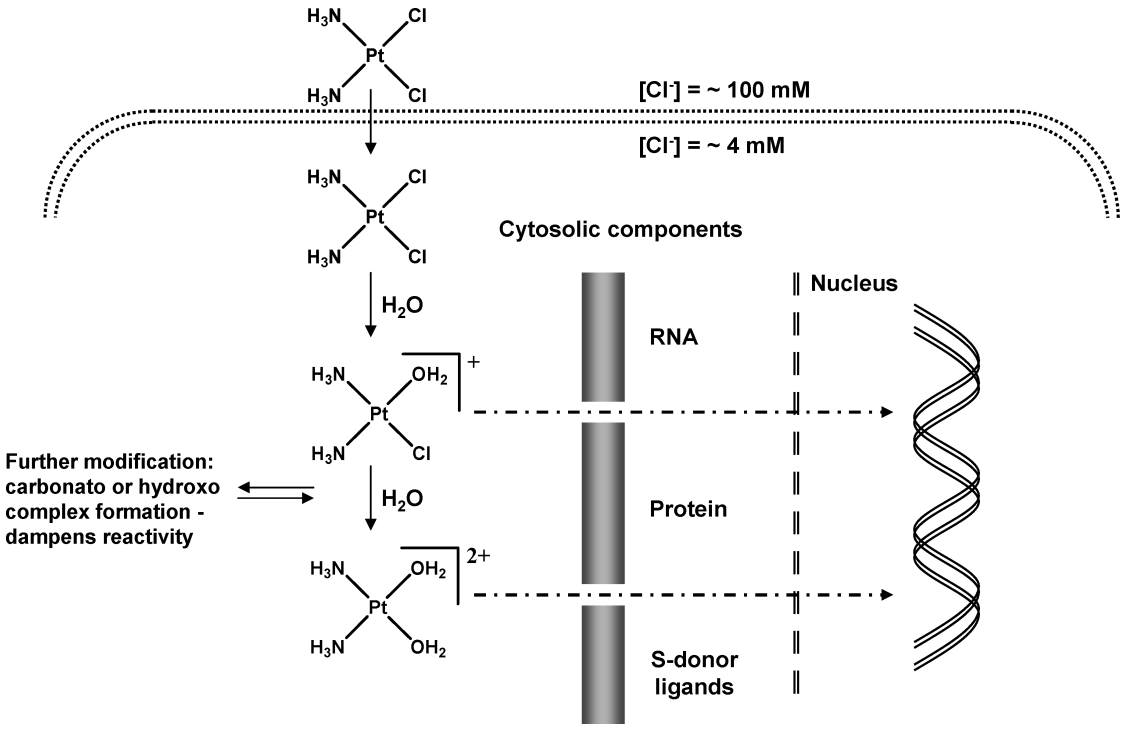


Figure 1-5: Aquation chemistry of cisplatin in the cell [31].

Cisplatin enters to cells primarily by passive diffusion (Fig. 1-6). But it is widely accepted that cisplatin can enter to cells even by active transport [31]. Cellular copper (Cu) transporting proteins are involved in both cisplatin uptake and efflux [33]. Whereas cisplatin uptake appears mediated by the copper transporter Ctr1, the copper transporting P_{1B} – type ATPases ATP7A and ATP7B (Menkes and Wilson diseases proteins) are suggested to be involved in cisplatin efflux. The link between cisplatin resistance and copper transporters was due to early observations of cisplatin resistance being associated with overexpression of the ATP7B and ATP7A proteins [34, 35].

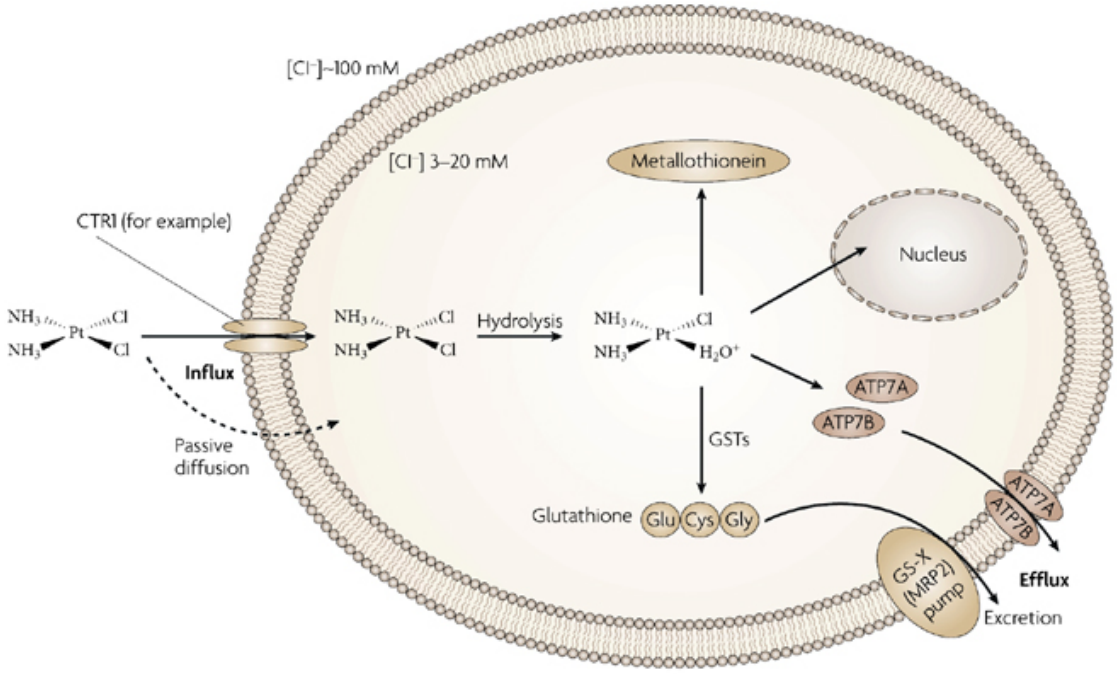


Figure 1-6: Cellular uptake of cisplatin and its mechanism of distibution *in vivo* [36].

1.4 Formation of Pt-DNA adducts and conformational changes in DNA

The anticancer drug cisplatin forms covalent adducts with DNA, which is its primary target among many potential cellular possibilities [21, 27]. DNA damage is recognized

with proteins which either bind to the cisplatin-DNA adducts or influence cellular pathways [21].

Cisplatin forms monofunctional adducts on DNA, preferentially at the N(7) guanine residues and at a much lesser extend at N(7) adenine residues, which are subsequently closed to bifunctional adducts creating mainly intrastrand cross-links.
The intrastrand cross-links are either between neighboring guanine residues or between guanine and adenine residues (65% 1,2-GG- or 25% 1,2-AG-intrastrand cross-links) [37, 38]. Between major aducts also belong 1,3-GXG-intrastrand cross-links (X=A, T, C). This type of cross-links is formed between two purine nucleotides separated by one or even more intervening nucleotides. A small percentage of interstrand cross-links, preferentially formed between guanine residues in the 5´-GC/5´-GC sequences, DNA-protein cross-links and monofunctional adducts are also present [39,40] (Fig. 1-7). Occurrence of interstrand cross-links is noticeably higher in negatively supercoiled DNA [41]. DNA-protein cross-links are also formed by the transformation of DNA monofunctional and intrastrand cross-links of cisplatin. In contrast, DNA interstrand cross-links of cisplatin are markedly more stable in the presence of DNA-binding proteins than intrastrand cross-links so that their transformation into DNA-protein cross-links is markedly more difficult [42].

The trans-diamminedichloridoplatinum(II) (transplatin; trans isomer of cisplatin) is clinically inactive compound. Transplatin can not form 1,2-intrastrand cross-links, the major DNA adducts of cisplatin, because of geometric constraints [21]. DNA adducts of transplatin are primary interstrand cross-links, which are formed between guanine and complementary cytosine residues, and monofunctional adducts [43, 44]. It is accepted that transplatin forms also intrastrand cross-links between nonadjacent guanine residues or between nonadjacent guanine and either adenine or cytosine residues [44, 45].

Carboplatin and oxaliplatin contain different leaving groups than the chloride ions of cisplatin and so exhibit different kinetics for DNA binding. They generate disparate adduct profiles from that of cisplatin [19].

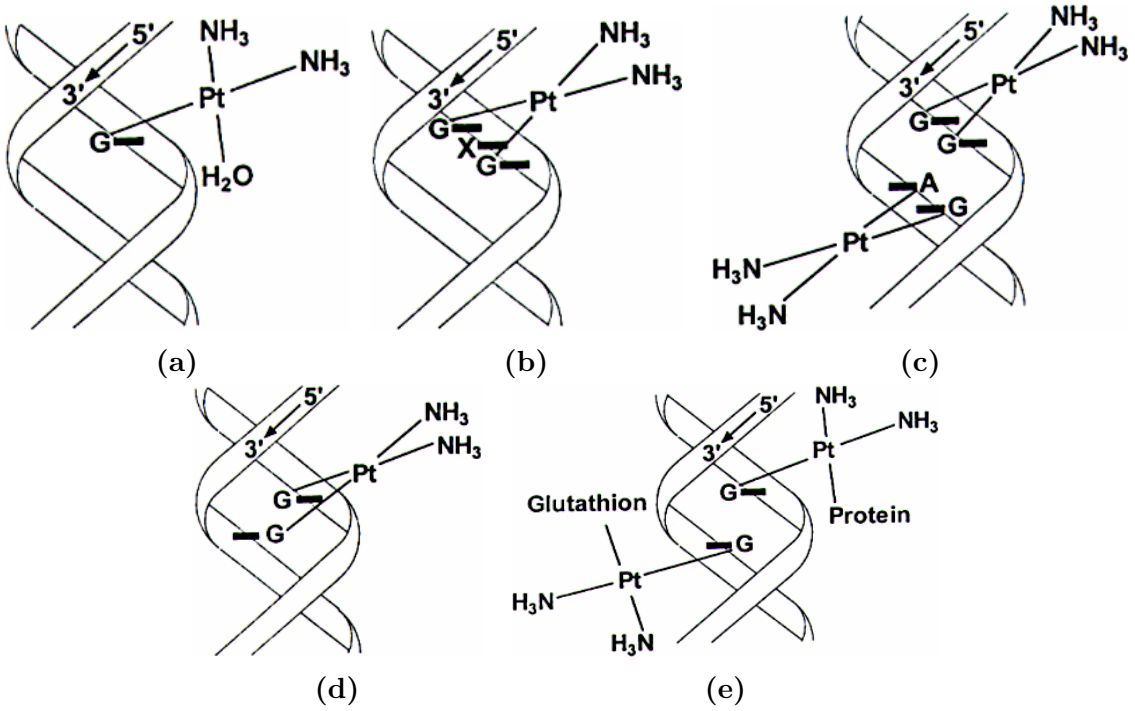
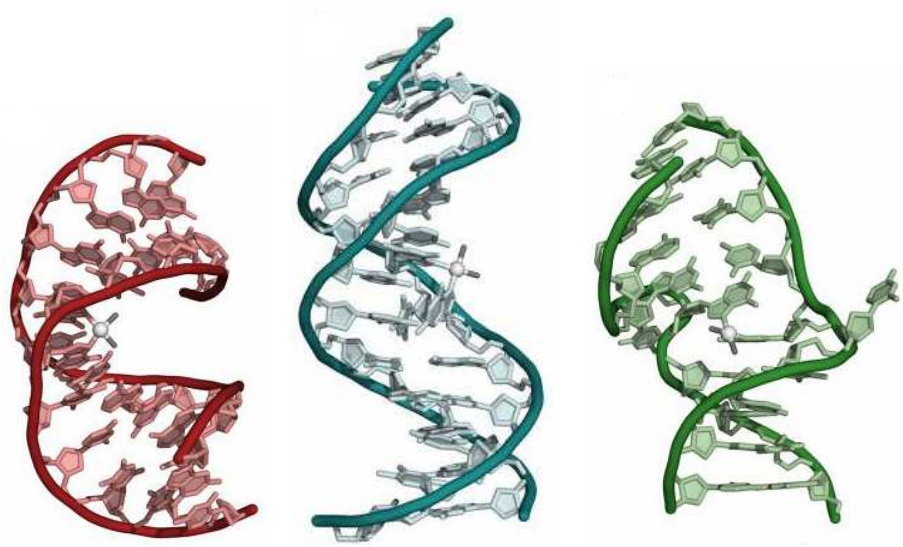


Figure 1-7: Types of cisplatin adducts. (a) Monofunctional adduct; bifunctional adducts: (b) 1,3-GXG-intrastrand cross-link; (c) 1,2-GG- and 1,2-AG-intrastrand cross-link; (d) interstrand cross-link; (e) DNA-protein cross-link [38].

Formation of cisplatin adducts significantly alters the structure of the target DNA. Cisplatin induces unwinding and bending as well as it destabilizes the normal B-DNA [46, 47]. Furthermore, approachability of the binding sites, conformation of the duplex, nucleotide sequence, electrostatic potential, flexibility, and the formation of transient reactive species can affect the DNA-binding mode of cisplatin [14].

The minor adducts formed in DNA by cisplatin (monofunctional adducts) at the guanine sites distort DNA and reduce its thermal stability [48, 49]. The most frequent 1,2-intrastrand adducts formed by cisplatin are considered crucial in the antitumor activity of cisplatin [14].

These major cisplatin adducts (1,2-intrastrand cross-links), unwind the DNA duplex in the site of platination by $\sim 13^\circ$ and bend it toward the major groove by approximately $35^\circ - 40^\circ$. The 1,3-intrastrand cross-links formed by cisplatin bend the helix axis toward a major groove by $\sim 30^\circ$ and locally unwind DNA by $\sim 19^\circ$ [51, 52]. On the other hand, the interstrand DNA cross-links formed by cisplatin bends the



(a) **(b)** **(c)**

Figure 1-8: Conformational changes in DNA induced by cisplatin. X-ray crystal and NMR structures of double stranded DNA containing adducts of cisplatin. (a) 1,2-GG intrastrand cross-link; (b) 1,3-GXG intrastrand cross-link; (c) interstrand cross-link [50].

helix by 47° toward the minor groove in which the platinum moiety is located and unwind the duplex by $76° - 80°$ [53] (Fig. 1-8). Although these platinum adducts display some degree of structural similarity, arising from coordination to the N(7) position of the guanine base, it is clear that each distorts duplex DNA in a distinctive manner. Moreover, the structures of DNA adducts formed by platinum drugs with nonleaving groups other than the ammine ligands of cisplatin display additional variations [19]. These nuances can convey distinctive recognition and processing by cellular proteins, which possibly translate also different roles in mediating the cytotoxicity and anticancer properties of the compounds [31].

1.4.1 Effects of platinum adducts on replication and transcription

The inhibition of DNA synthesis by cisplatin was reported early and is believed to contribute to the cytotoxicity of cisplatin [54]. Bifunctional adducts, intra- and interstrand cross-links, effectively inhibit DNA polymerases, whereas monofunctional

adducts seem not to block the polymerases as effectively [55]. Numerous studies demonstrated that the inhibition of DNA synthesis depends only on the concentration of cisplatin and not on the sensitivity of the cell line to the drug [56]. Only the level of cells arrested in the G2 phase correlates with cell line sensitivity to cisplatin. It is therefore likely that direct inhibition of DNA replication by cisplatin-DNA damage is not the key biological event that confers the unique properties of this anticancer agent [57]. Early *in vitro* studies reported the ability of cisplatin adducts to inhibit transcription elongation by various RNA polymerasis [58, 59]. Similar to the inhibition of DNA synthesis, RNA polymerases are strongly blocked by bifunctional adducts and not by monofunctional adducts [60, 61]. Accumulated data indicate a close relation between transcription inhibition by cisplatin adducts and its ability to kill the cells [31].

1.5 The response of cellular proteins to Pt-DNA adducts

Cisplatin forms a variety of DNA adducts, which are recognized by a number of cellular proteins. These proteins either bind to the cisplatin-DNA cross-links or influence cellular pathways. Included are proteins that participate in the DNA repair and p53-regulated pathways, transcription factors and structural proteins. The manner by which such proteins affect the processing of cisplatin-DNA adducts can determine whether a cell attempts to repair the damage or activates an irreversible cell death. Anticancer activity of cisplatin is mediated very likely by these proteins [21].

1.5.1 Nucleotide Excision Repair (NER)

Nucleotide excision repair is a primary process for repairing platinum-damaged DNA. Bacterial and mammalian cells deficient in NER are more sensitive to platinum compounds [27, 62]. On the other hand, a cisplatin-resistant tumor cells exhibit higher levels of repair activity [63, 64]. It was revealed that both cisplatin 1,2-GG and

1,2-AG-intrastrand cross-links are substrates for the mammalian excinuclease [65,66]. But the minor adducts of cisplatin (1,3-GXG intrastrand cross-links) are more efficiently repaired by NER than 1,2-intrastrand cross-links [65, 66]. Althought cisplatin interstrand cross-links also significantly distort DNA, they are not repaired by NER [67]. DNA damage induced by interstrand cross-links of cisplatin includes both DNA strings, therefore can not be repaired by NER, which exploits for final resynthesis the informations from the complementary string.

The first step of nucleotide excision repair mechanism by mammalian cells is to recognize damaged DNA with complex of proteins XPC-hHR23B. In particular, after damage recognition, TFIIH, XPA, and RPA are the next set of proteins to assemble on the DNA. Although the exact binding order of these proteins is controversial, they may be cooperatively recruited to the damage site [68, 69].

In the next step, XPB and XPD helicases, components of TFIIH, unwind the DNA in a process that requires ATP. XPC-HR23B is released when endonuclease XPG binds to this unfolded DNA. The structure-specific endonuclease XPF-ERCC1 is finally recruited to the NER complex, and dual incision occurs to remove platinated oligonucleotides. Excised oligonucleotides (24-32 nucleotides in length), containing a platinum lesion, and dual incision factors are then released from the DNA [70]. The final step of NER is filling the created gap with polymerases (ε, δ) and replications factors RPA, PCNA, RFC and DNA ligase I. Therefore, the result is double-stranded, undamaged DNA [71] (Fig. 1-9).

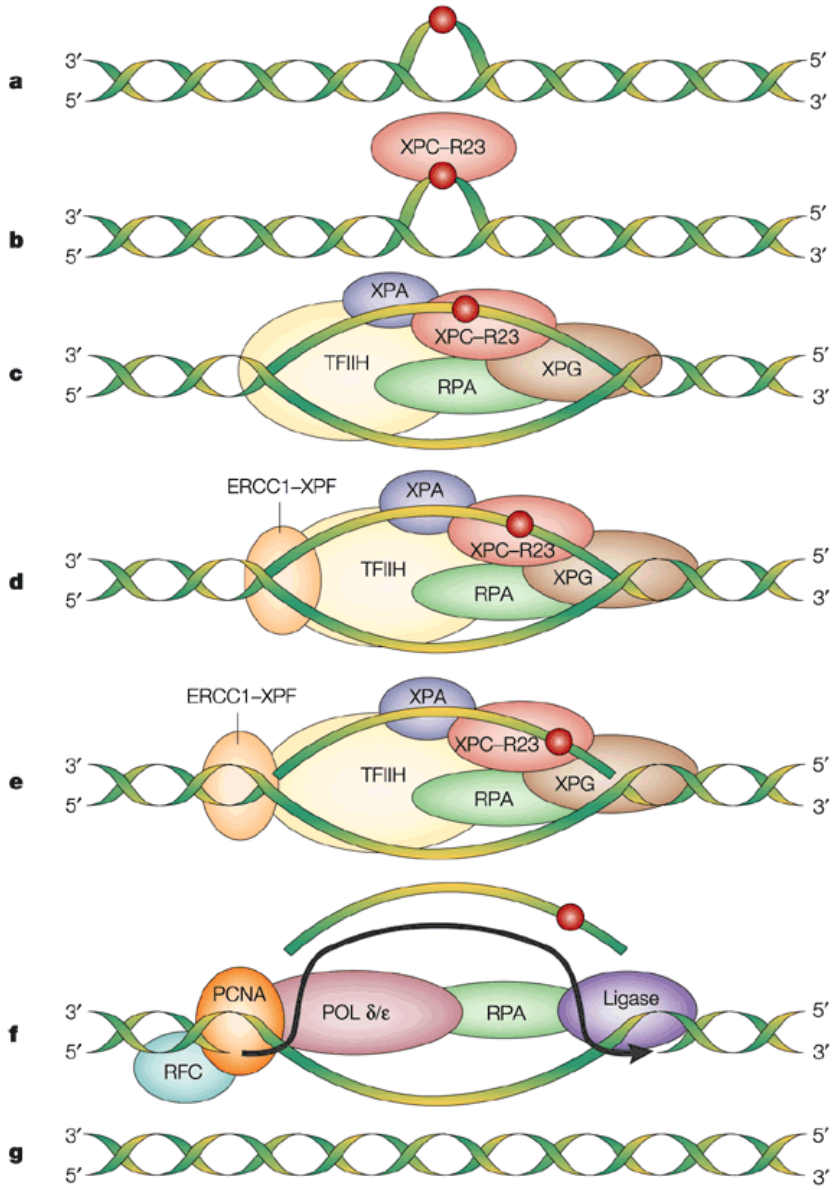


Figure 1-9: Mechanism of the nucleotide excision repair (NER) [71].

1.5.2 Mismatch-Repair (MMR) Proteins

Mismatch repair (MMR) is a repair system after replication of DNA that corrects mispaired and unpaired bases in the duplex [72]. MMR system repairs mismatches caused by adducts that are not removed by the NER system [73].

It has been demonstrated that cisplatin–DNA adducts are preferentially recognized and repaired by the MMR system, while oxaliplatin–DNA adducts are not [74–79]. DNA mismatch-repair proteins (hMSH2 and MutS) bind with greater affinity to cisplatin adducts than to oxaliplatin adducts [78]. In contrast, MutS shows low affinity for DNA adducts of the clinically inactive complexes such as transplatin and the monofunctional $[Pt(dien)Cl]^+$ (dien = diethylenetriamine) [78]. The highest affinity of cisplatin modified DNA can be explained on the basis of strong bend and distortion of the double helix induced by cisplatin. Other Pt compounds do not induce strong bending and unwinding of the double helix [78].

The DNA mismatch-repair system is critical in regulating cellular sensitivity to cisplatin, because the loss of mismatch repair due to lack of either hMSH2 or hMLH1 activity results in the resistance to cisplatin and carboplatin, drugs that form the same adducts in DNA, but the loss of mismatch repair does not cause resistance to oxaliplatin [77, 80–82].

In contrast to cisplatin and carboplatin, no difference in sensitivity between the DNA mismatch repair-proficient and -deficient cell lines for oxaliplatin, transplatin had been observed. These observations demonstrated a correlation between failure of the DNA mismatch-repair proteins to recognize the Pt adduct and low-level resistance [82]. The binding of mismatch-repair complex to Pt-DNA adducts appears to increase the cytotoxicity of the cisplatin adducts by activating downstream signaling pathways that lead to apoptosis [83–85]. Defective MMR was shown to result in a 2-4-fold increase in tolerance to cisplatin, which contributes significantly to the failure of cancer therapy [82, 86].

1.5.3 HMG - Domain Proteins

The mammalian proteins with high-mobility group (HMG) domain [87] are founding members of a class of gene regulatory molecules called “architectural transcription factors” [88] that specifically recognize and bind to cisplatin-modified DNA [89].

High-mobility-group protein B1 (HMGB1) is a very abundant non-histone nuclear protein, present in all mammalian tissues and cells and is highly conserved.

It appears to be involved in a large number of DNA transactions, including determination of nucleosome structure and stability, as well as transcription and replication [90]. HMGB1 has three structural domains: the N-terminal A-domain and the central B-domain are positively charged and bind to DNA, while the acidic terminal C-domain interacts with histones. The sequences of the two domains A and B of HMG1 are homologous to segments of about 70 amino acid residues called the HMG box [91].

The major DNA lesions formed with cisplatin are 1,2-GG- and AG-intrastrand cross-links [62]. These cisplatin-induced cross-links unwind the DNA helix by about 13° and bend it toward the major groove by between 26° and 35° [72, 92–94]. HMGB proteins, as well as other nuclear proteins that contain "B-box" motifs, selectively recognize and further bend DNA distorted by 1,2-intrastrand cisplatin adducts but do not bind to various minor cisplatin adducts such as 1,3-GXG intrastrand cross-links and monofunctional adducts [89,93]. HMGB1 can also apparently bind to interstrand cross-links induced in DNA by cisplatin [95]. The distorsions in DNA duplex induced by interstrand cross-links are quite different from those which are caused by 1,2-intrastrand cross-links. Which means that, there is no correlation between the bending or unwinding angles and HMG protein recognition [21].

Numerous studies have demonstrated that HMGB and other B-box containing proteins (e.g., an RNA polymerase 1 transcription factor; human mitochondrial transcription factor mtTFA; the testis-specific HMG domain protein tsHMG) inhibit NER of cisplatin adducts both *in vivo* and *in vitro* [65, 66, 96, 97]. The most likely mechanism for such inhibition is believed to be that DNA–HMGB–domain protein complexes protect cisplatin adducts from repair by a "shielding mechanism" that prevents access of NER factors to cisplatin lesions [98].

Support for a shielding mechanism comes from the demonstration that the HMGB proteins all specifically bind to, and inhibit repair of, 1,2-GG-intrastrand cross-links *in vivo* and *in vitro*, but do not bind to, or inhibit repair of 1,3-GXG-cross-links *in vitro* [65, 66].

1.5.4 Histone H1

The histone H1 protein is representative of non-sequence specific DNA-binding with structural function. This interaction is similar to that of other abundant chromosomal proteins (HMGB1, HMGB2) and reflect functions involving the organization and maintenance of chromatin structure. Histone H1 primary binds to altered DNA structures, including supercoiled DNA and four-way junctions [99]. Protein is associated with the linker DNA of chromatin. Proteins H1 interact with HMGB1 *in vitro*, and it has been proposed that HMGB1 functionally replaces H1 during the remodeling of chromatin that occurs during replication, transcription or repair [99, 100].

1.5.5 Glutathione (GSH), metalothionein (MT) and cellular resistance

Glutathione (GSH) - y-glutamylcysteinylglycine is a tripeptide thiol. It is the most abundant thiol in the cell [101]. As a potent nucleophile, GSH reacts with alkylating agents and with cisplatin. The reaction of GSH and cisplatin in a 2:1 molar ratio forms a GSH-platinum complex that is then eliminated from the cell by an ATP-dependent glutathione S-conjugate export pump [102]. GSH may protect cells by intercepting reactive platinum complexes before they can react with DNA. GSH also protects cells by supporting DNA repair, possibly by stabilizing repair enzymes such as DNA polymerase or by promoting the formation of deoxyribonucleotides [103].

Mammalian metallothionein (MT) is a small protein of 61-62 amino acids that contains 20 cysteine residues [104]. It has a presumed role in the detoxification of heavy metal ions. MT gene transcription is strongly induced by heavy metal ions, glucocorticoids, interferon, and stress. Cisplatin binds to MT, with 10 platinum atoms/molecule. The binding rate is much higher than for glutathione. Heavy metals may even induce the MT thiol content to a level higher than that of glutathione. When cisplatin binds to MT, it loses its amine ligands and displaces heavy metal ions [105].

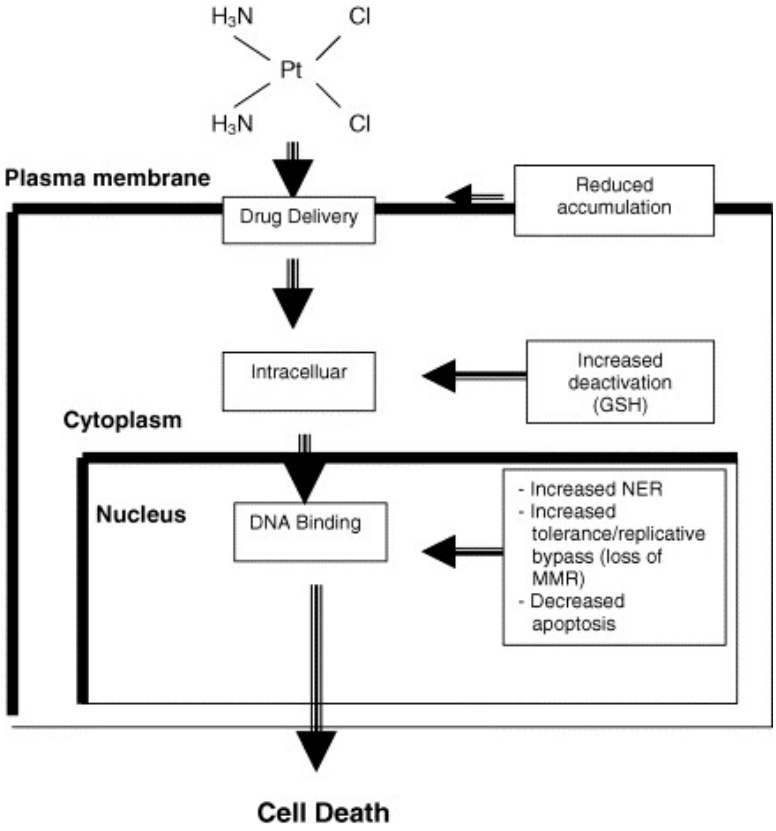


Figure 1-10: Schematic drawing of the major biochemical mechanisms of resistance to cisplatin. MMR, mismatch repair; NER, nucleotide excision repair; GSH, glutathione. Resistance mechanism may operate prior to or after binding of cisplatin to DNA [106].

In vitro studies have demonstrated that cellular resistance to cisplatin is multifactorial. Involved mechanisms include (Fig. 1-10): altered membrane transport; inactivation of the drug by cellular thiols such as GSH and metallothioneins; decreased Pt-DNA binding; and/or an increased Pt-DNA repair [107]. The role of the GSH system in cisplatin resistance has received much attention, because cisplatin as well as other platinum-containing compounds are electrophilic agents which are most reactive towards sulfurcontaining nucleophiles [108, 109].

Increased glutathione levels have been found in some cisplatin resistant cells. However, the capacity of the cell to synthesize GSH in response to stress may be more important than the steady state GSH level [110]. In some studies, cell lines selected with heavy metals showed increases in MT and became cross-resistant to cisplatin [105, 111, 112].

1.6 Programmed cell death

Programmed cell death (apoptosis) is generally defined as a consequence of the mechanism of action of cisplatin and a failure of the mechanism of resistance. Mechanism of resistance to cisplatin include reduced drug accumulation, reduced DNA platination and altered DNA repair [21].

Apoptotic cells have distinct morphology of cell death due to the biochemical and physical changes that occur in the cytoplasm, nucleus, and cell membrane [113]. Cisplatin induces two different modes of cell death, necrosis and apoptosis. Necrosis is characterized by a cytosolic swelling and early loss of plasma membrane integrity, while the characteristic features of cells undergoing apoptosis include cell shrinkage, chromatin condensation with activation of endogenous endonucleases, DNA fragmentation, membrane blebbing, and the formation of apoptotic bodies [113–116]. Platinum adducts can interfere with genomic activities by directly altering nucleosome dynamics and, thereby disrupt nuclear activities and trigger apoptosis [116]. In the 1980s, necrosis was considered the mode of cell-death induced by DNA-damaging agents because of the activity of poly (ADPribose) polymerase (PARP). By the 1990s, it was thought that most clinically effective anticancer agents that bind to DNA kill cancer cells by apoptosis [17].

Apoptosis is an irreversible event initiated by various extracellular stimuli, such as DNA damage (formation of DNA cross-links), heat shock, and growth factor deprivation, as well as by activation of apoptotic genes and the caspase cascade [117]. Initiation of apoptosis can occur either by the intrinsic pathway, which is thought to be mediated primarily by mitochondrial dysfunction or by the extrinsic pathway, which is mediated by cell surface-death receptors (e.g., FAS). Both of these pathways rely on the activation of caspases, which are commonly referred to as the executioners of apoptosis [113].

It was proved experimentally that cisplatin-induced programmed cell death in the cisplatin-sensitive and -resistant cells proceeds via caspase-3-independent extrinsic and dependent intrinsic pathways, respectively [118].

1.6.1 Role of tumor suppressor p53

The tumor suppressor protein p53 is one of the most commonly mutated proteins in human cancer. p53 is a protein of 393 amino acids containing two DNA binding domains. The first domain, located in the core of the protein, binds to a specific gene sequence, whereas the C-terminal DNA-binding domain is believed to recognize damaged DNA. Under normal conditions there is a low level of a latent form of p53, which is induced, activated, and stabilized under stress conditions including DNA damage. The p53 protein regulates DNA repair, cell-cycle arrest, and apoptosis by modification of other genes and their products, including those involved in transcription, DNA repair, and many signaling processes [119].

Two key features of p53 are required for its transcriptional activities: its ability to recognize and bind specific DNA sequences, and to recruit both general and specialized transcriptional co-regulators [120]. Activation of p53 protein can affect cell fate through the induction of either growth arrest at G1/S or G2/M cellcycle checkpoints or apoptotic cell death. p53 mediates G1–S arrest through p53-dependent transcriptional activation of the gene encoding the cyclin-dependent kinase inhibitor $p21^{WAF_1/Cip1}$ (p21) [121, 122]. However, p21 expression could be induced by chemotherapy irrespective of the p53 status of the tumor [123].

p53 expression enhances the sensitivity of p53-deficient cancer cells to cisplatin [124, 125] and accumulation of p53 also sensitizes cells to the drug [126]. In addition, p53 mutants have been detected in cisplatin-resistant ovarian carcinoma cells [127]. Several studies, however, reveal that p53 can have various effects on cisplatin cytotoxicity. p53-mediated sensitization to cisplatin is reversed by altering cell growth conditions [128] and p53 expression enhances cisplatin cytotoxicity in HeLa, but not in cisplatin-resistant HeLa cells [129].

1.6.2 NF-κB transcription factor

NF-κB is a family of transcription factors with five subunits (p52, p50, RelB, c-Rel, and RelA/p65) that form hetero- and homodimers which remain inactive in the cy-

toplasm when associated with IκB proteins [130]. Activation of IκB kinases (IκKα, IκKβ, and NF-κB essential modulator [NEMO]/IκKγ) results in the phosphorylation of the inhibitory IκB (IκBα, IκBβ, and IκBε) proteins bound to NF-κB. NF-κB is consequently released and binds to specific DNA sequences in target genes, designated as κB-elements, and regulates transcription of over 400 genes involved in immunoregulation, growth regulation, inflammation, carcinogenesis, and apoptosis [130, 131]. The NF-κB pathway can be activated by divergent stimuli including proinflammatory cytokines (e.g., TNF-α, interleukin-1 [IL-1]), T- and B-cell mitogens, bacteria, lipopolysaccharide (LPS), viruses, viral proteins, double-stranded RNA, and physical, chemical and cellular stresses [132].

NF-κB may up-regulate the expression of proteins that interfere with the death receptor apoptotic pathway. One of these target proteins is the FLICE-like inhibitory protein (FLIP) [133, 134]. FLIP shares a high degree of homology with caspase-8 but lacks protease activity and competes with caspase-8 for the binding to the Death-Inducing Signaling Complex (DISC). Thus, high levels of FLIP prevent caspase-8 recruitment to the DISC. Up-regulation of FLIP has been reported in many tumors. It is believed that FLIP expression may explain the resistance to death receptor apoptosis in some types of tumor [135].

NF-κB also induces the expression of the Inhibitors of Apoptosis (IAPs) [136–138] and some members of the anti-apoptotic BCL-2 family [139, 140]. It is generally accepted that NF-κB activation is responsible for apoptosis resistance. However, recent evidences support a pro-apoptotic role of certain dimers of NF-κB. It has been speculated that NF-κB may exert a dual function, either as an inhibitor or an activator of apoptotic cell death, depending on the levels of RelA and c-Rel [141]. Furthermore, NF-κB inhibits p53-induced apoptosis, by up-regulating anti-apoptotic genes, and downregulating p53 levels.

High levels of c-Rel have been found in solid tumor (non-small cell lung carcinoma and breast cancer) [142, 143]. NF-κB activation has been also identified in squamous cell carcinomas of the head and neck. Inhibition of NF-κB activity in these tumors inhibits cell survival and tumor growth [144]. Tumors with constitutive

NF-κB activation usually show increased resistance to chemotherapy. NF-κB may be responsible for blocking the efficacy of chemotherapy and radiation in some types of tumor cells [135].

Several studies have addressed the identification of putative NF-κB inhibitors as therapeutic agents for cancer. Since NF-κB activation is the result of a multi-step signaling pathway, these compounds may target different points of the signaling proces [135].

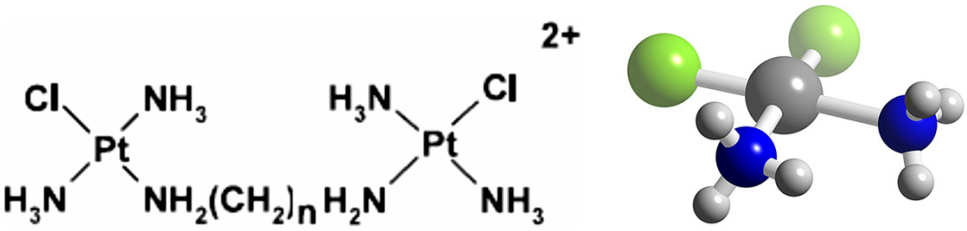
Chapter 2

Polynuclear platinum complexes

The tumor resistance to cisplatin is primarily attributed to recognition and removal of Pt^{II}-GG cross-links from DNA by proteins and enzymes through the nucleotide excision repair pathway [31,39,145]. One promising approach to overcome this problem is to design the new generation of Pt^{II}-based antitumour agents that form different DNA adducts and display a different spectrum of anti-cancer activity compared to cisplatin and its analogues [143,146,147]. It is generally believed that the antitumor effects of platinum compounds is covered in the DNA binding and the consequences for structure and function. Furthermore, hypothesis for design of new class of platinum complexes is created on the basis of change of antitumor activity by change of DNA binding [148]. Polynuclear platinum complexes exert their antitumor activity by the geometry, number of platinum centers and leaving groups in the coordination sphere of platinum atoms as well as the nature of the linkers bridging the platinum centers [149].

2.1 Polynuclear complexes with monofunctional Pt units

Platinum(II) complexes with two or three platinum-amine units linked by a variable-length diamine chain have been shown to be successful approaches lacking cross-



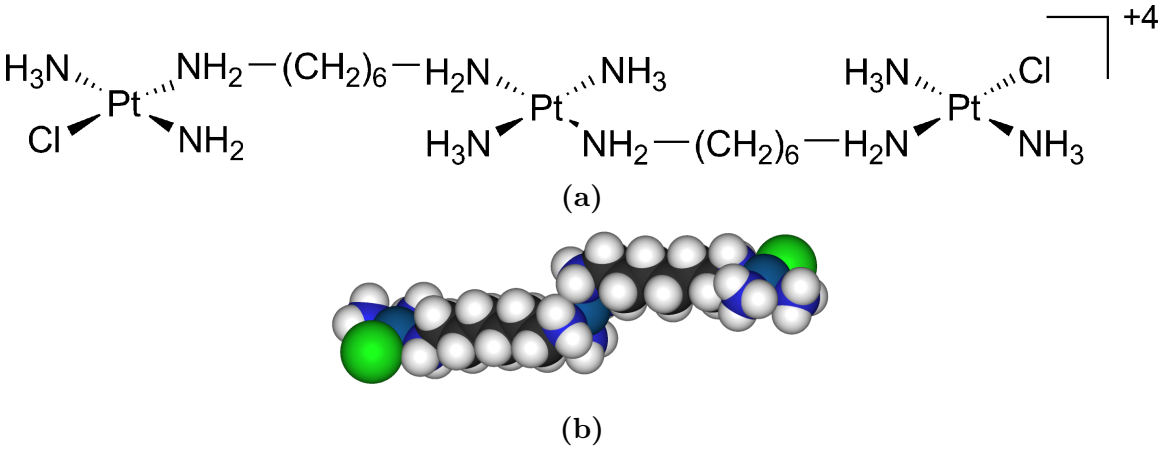
(a) (b)

Figure 2-1: *cis*-$PtCl_2(NH_3)_2$ [155].

resistance [150, 151]. These complexes have novel types of intra- and interstrand cross-links, suggesting that different DNA binding modes may exert different biological effects [152, 153]. Furthermore, these polynuclear platinum complexes undergo chemical degradation upon binding of reduced glutathione (GSH) under physiological conditions through the liberation of the terminal amine of the alkanediamine linker, which could result in deactivation as well as higher toxicity [154].

The first dinuclear compound was reported in 1988 by group of prof. Farrell and consisted of two cis-Pt (*cis*-$PtCl_2(NH_3)_2$) units linked by a flexible diamine chain (Fig. 2-1). It was intriguing, that dinuclear complex exerted activity in cisplatin-resistant cells and so overcomes resistance to cisplatin [21, 148].

Representative of trinuclear platinum complexes is BBR3464 described as two *trans*-$[PtCl(NH_3)_2]^+$ units linked by a non-covalent tetra-amine $[Pt(NH_3)_2\ \{H_2N(CH_2)_6NH_2\}_2]^{2+}$ unit (Fig. 2-2).



(a)

(b)

Figure 2-2: BBR3464 [31, 156].

This compound is the first platinum drug not based on the classical cisplatin structure which entered human clinical trials [21]. BBR3464 was in phase II stage of clinical trial before it was stopped due to significant toxicity namely neutropenias, diarrhoea and nausea [157]. Remarkable features of successful BBR3464 are the potency, the ten-fold lower maximum tolerated dose in comparison to cisplatin, and the broad spectrum of tumors sensitive to this complex [21]. It forms bifunctional DNA adducts through long-range inter- and intrastrand crosss-links and has (4+) charge which suggests, that with this advance, the paradigm of cisplatin-based antitumor agents is altered [21, 158].

2.1.1 DNA binding

Polynuclear complexes are characterized by rapid binding to DNA and formation of a significant percentage of long-range intra- and 1,2-, 1,4-, 1,6-interstrand cross-links and irreversible sequence-dependent conformational changes (B $\rightarrow$ Z; B $\rightarrow$ A) [159]. The structural features of complexes like both (*cis* and *trans*) geometries display useful antitumor activity. It has been demonstrated than *cis* isomer is more effective cross-linking agent that the *trans* isomer [148]. Linker properties such as length, flexibility and charge are also important factors that contribute to antitumor properties of the complexes [158]. The charge enhances cellular accumulation and in this manner, the cytotoxicity [160, 161].

Interstrand cross-links formed by BBR3464 occur in the 5$'\rightarrow$ 5$'$ direction (DNA helix is normally read from the 5$'$-side), but also in the opposite antiparallel 3$'\rightarrow$ 3$'$ direction [162] (Fig. 2-3). Nature of the cross-links govern the directionality [148]. The 1,2-interstrand CLs occurs in only the 3$'\rightarrow$ 3$'$ direction while the 1,4-interstrand CLs forms in both directions (5$'\rightarrow$ 5$'$ and 3$'\rightarrow$ 3$'$) in roughly equal proportions and 1,6-interstrand CLs occur in the 5$'\rightarrow$ 5$'$ direction [148]. While simple dinuclear compound (1,1/t,t) forms only cross-links in the 5$'\rightarrow$ 5$'$ direction [163]. Similar unwinding of DNA is induced by both isomers, but the 3$'\rightarrow$ 3$'$ bends slightly more than 5$'$ $\rightarrow$ 5$'$ isomers in the direction of the major groove, 21° and 15° respectively [148].

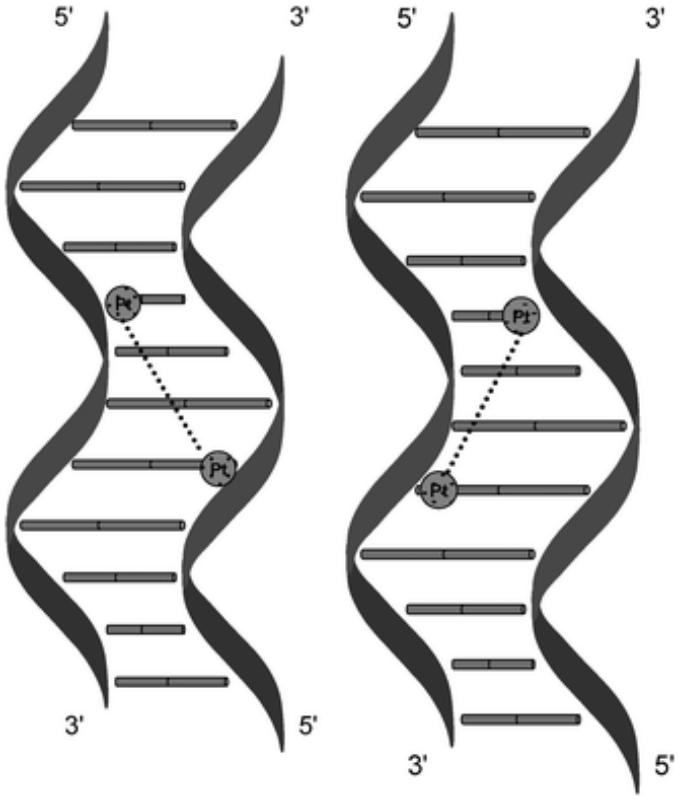


Figure 2-3: Schematic of directional isomers formed in 1,4-interstrand cross-links by polynuclear platinum complexes showing directionality [148].

The nucleotides covered by 1,4-interstrand CLs are mixture of syn and anti conformations and Watson-Crick hydrogen bonding. This observation suggests how delocalization of lesions beyond the binding site may occur. The $5' \rightarrow 5'$ 1,4-GG interstrand CLs show minimal distortion to the duplex while $3' \rightarrow 3'$ 1,4-GG interstrand CLs seem to be a highly distorted adduct [148, 164, 165]. The 1,4-cross-links induce the B $\rightarrow$ Z transition because of the appearance of the syn purine sugar conformation, an absolute requirement for the left-handed helix [166]. B-Z transition is a prerequisite for a good antitumor activity of the complexes [21]. This is in contrast to the major 1,2-intrastrand adduct of cisplatin which maintains the B-form in solution, although bends the helix significantly [72, 167].

The nature of template DNA may also uniquely affect processes, such as aquation kinetics, leading to specificity of the substrate. Dinuclear and trinuclear platinum

complexes show a kinetic preference for binding to single-stranded (ss) DNA over double-stranded (ds) DNA [148].

Polynuclear complexes in the presence of double-stranded DNA, in comparison with single-stranded DNA, have three fold slowing aquation [168]. The high affinity of polynuclear compounds for DNA, with a rapid electrostatic interaction because of charge effects coupled with a slow hydrolysis, could imply that DNA binding does not require activation by hydrolysis, a clear diference to the cisplatin case [21].

2.1.2 Protein recognition of DNA adducts

High mobility group HMGB1 proteins don´t recognize the 1,4-interstrand cross-links and this lack of recognition shows that this event is not critical to the cytotoxicity of BBR3464. The explanation can be that the prebending due to the 1,4-interstrand CL is small to be recognized by HMG-domain proteins or that the trinuclear complex restricts the additional DNA bending required for protein binding [148]. The central Pt(tetraamine) unit present in the minor groove may sterically block the protein recognition, which may have a biological consequence [148].

Trinuclear complex BBR3464 displays high activity in human tumor cell lines and *in vivo* murine xenografts characterized by both wild type and mutant p53 genes [169]. The p53 (tumor suppressor protein) is involved in DNA repair and induction of apoptosis by DNA-damaging agents [148]. It was demonstrated, that 1,4-interstrand CLs are resistant to nucleotide excision repair (NER), because no excision products were showed after incubation of 1,4-cross-links with repair proficient human or rodent cell-free extracts (CEF) [162].

2.2 Dinuclear azolato- and azinato-bridged Pt^{II} complexes

Recently, highly cytotoxic new class of dinuclear bifunctional azole and azine-bridged platinum(II) complexes were introduced by Komeda, Reedijk and their

coworkers [170–172]. These complexes own rigid bridging ligands, such as hydrazine and azoles [173]. Azolato-bridged platinum complexes contain the bridging by hydroxo anion, which has the function as leaving group to provide bifunctional coordination on DNA [153]. On the other hand, azinato-bridged platinum complexes contain the non-bridging chloride ions as a leaving groups [174]. Interestingly, azole-bridged dinuclear platinum complexes form 1,2-intrastrand GG cross-links like cisplatin, but with less local distortions upon binding DNA [170]. In fact, this kind of 1,2-intrastrand DNA adduct could be a trigger to induce cytotoxic effects and be favorable for escaping from the DNA recognition and repair systems in the cells [171]. It was demonstrated that dinuclear azolato-bridged platinum complexes show remarkably higher activity than cisplatin in several human tumor cell lines [171]. Dinuclear platinum(II) complexes with isomeric azines (pyrazine, pyrimidine and pyridazine) as bridging ligands show lower cytotoxicity than cisplatin for the human tumor cell lines and their cytotoxicity for the mouse cell lines is comparable to or higher than that of cisplatin [175]. This class of azine dinuclear compounds is extended with other azines such as quinazoline, phthalazine and 2,5-dimethylpyrazine as bridging ligands. These new dinuclear azine-bridged platinum complexes show considerable anticancer potential, but they have different steric and electronic properties [173].

Chapter 3

Aims of the Ph.D. thesis

The aims of this thesis are:

1. To study more fully the DNA binding mode of trinuclear Pt^{II} complex $\{[Pt_3Cl_3$ (hptab)$]^{3+}$ (hptab=N,N,N′,N′,N″,N″-hexakis(2-pyridylmethyl)-1,3,5tris(aminomethyl)benzene)$\}$ (**1**) in cell-free medium including also its unique intramolecular and intermolecular cross-linking capability.

2. To examine early phases of the mechanism underlying biological properties of benzotriazolate (Btaz)-bridged dinuclear Pt^{II} complex $[\{cis\text{-}Pt(NH_3)_2Cl\}_2$ (μ-1,2,3-Btaz-H-N1,N3)]Cl (**2**).

3. To investigate the interaction between cisplatin analogues $\{cis\text{-}[PtCl_2(3Cl\text{Haza})_2]\}$ (**3**) or $\{cis\text{-}[PtCl_2(3I\text{Haza})_2]\}$ (**4**) (*3Cl*Haza and *3I*Haza = 3-chloro-7-azaindole and 3-iodo-7-azaindole, respectively) and sulfur-containing compounds, which are associated with cellular resistance mechanisms.

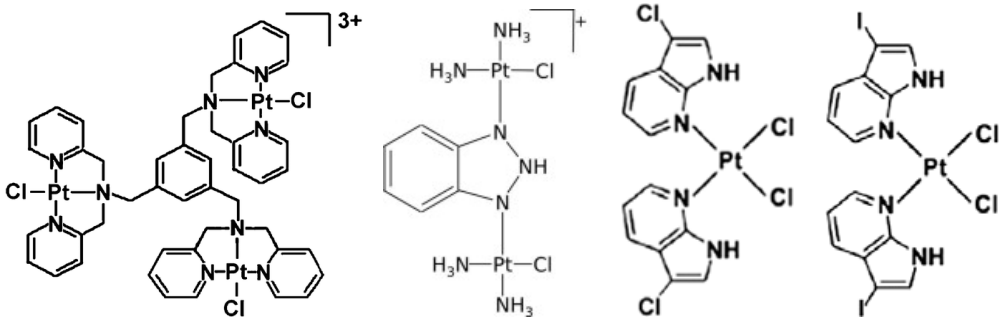
Chapter 4

Materials and methods

4.1 Chemicals

Trinuclear Pt^{II} complex $\{[Pt_3Cl_3$ (hptab)$]^{3+}$ (hptab=N,N,N′,N′,N″,N″-hexakis(2-pyridylmethyl)-1, 3, 5tris(aminomethyl)benzene)$\}$ (**1**) (Fig. 4-1a) was prepared as described previously [158,176]. Benzotriazolate-bridged dinuclear Pt^{II} complex [$\{cis$-$Pt(NH_3)_2Cl\}_2$ (μ-1, 2, 3-Btaz-H-N1,N3)]Cl (**2**) (Fig. 4-1b) was synthesized according to the previously published procedures [177]. The purity of complexes **1** and **2** was higher than 95% as established by combustion analysis carried out with a Hewlett-Packard 185 C, H, and N analyzer.

Cisplatin was obtained from Sigma (Prague, Czech Republic) (purity was $\sim$ 99.9% based on elemental and ICP trace analysis). Chloridodiethylenetriamineplatinum(II)



(a) **(b)** **(c)** **(d)**

Figure 4-1: Overview of platinum compounds used in this work

chloride ([PtCl(dien)]Cl) was a generous gift from Prof. G. Natile (University of Bari, Italy). The stock solutions of platinum compounds were prepared in 10 mM $NaClO_4$ and stored at $-20°C$ in the dark. The concentrations of platinum complexes in the stock solutions were determined by flameless atomic absorption spectrometry (FAAS). The concentrations of the platinum complexes indicated in the present work are related to the whole compound (not to the Pt content in the case of the trinuclear or dinuclear complexes).

The complexes {*cis*-[$PtCl_2$(*3Cl*Haza)$_2$]} (**3**) (Fig. 4-1c) or {*cis*-[$PtCl_2$(*3I*Haza)$_2$]} (**4**) (Fig. 4-1d) (*3Cl*Haza and *3I*Haza = 3-chloro-7-azaindole and 3-iodo-7-azaindole, respectively) were synthesized and characterized as described previously [178]. Stock solutions of complexes **3**, **4** were prepared at a concentration of 5×10^{-2}M in DMF, stored at 4°C in the dark, and diluted by water to the appropriate concentration just before use. The final concentration of DMF was less than 0.5%. The concentrations of platinum in the stock solutions and after dilution by water were determined by flameless atomic absorption spectrometry (FAAS).

Calf thymus (CT) DNA (42% G + C, mean molecular mass ca. 20,000 kDa) was prepared and characterized as described previously [179,180]. Plasmids pUC19 (2686 base pairs(bp)) and pSP73KB (2455 bp) were isolated according to standard procedures. The oligodeoxyribonucleotide duplex containing 50 base pairs bp 5′- CCAGATCTGATATCATCGATGAATTCGAGCTCGGTACCCGGGGATCCTCC / 5′-GGAGGATCCCCGGGTACCGAGCTCGAATTCATCGATGATATCAGATCTGG was from VBC-GENOMICS (Vienna, Austria). The purity of the oligonucleotides was verified by either high-performance liquid chromatography (HPLC) or gel electrophoresis. The duplex was formed by heating the mixture of the complementary single-stranded oligonucleotides at equal concentrations at 90°C for 5 min followed by incubation at 25°C for 4 h.

The Klenow fragment from DNA polymerase I (exonuclease minus, mutated to remove the 3′- 5′ proofreading domain) and restriction endonucleases were purchased from New England Biolabs (Beverly, MA). DNase I from bovine pancreas, nuclease P1 from Penicillium citrinum and alkaline phosphatase from calf intestine were

from Sigma-Aldrich (Prague, Czech Republic). Agarose was from FMC BioProducts (Rockland, ME), ethidium bromide (EtBr), urea, trichloroacetic acid (TCA), NaCN and dithiothreitol were from Merck KgaA (Darmstadt, Germany). Proteinase K and ATP were from Boehringer (Mannheim, Germany). Sodium dodecyl sulfate (SDS) was from Serva (Heidelberg, Germany). NF-κB protein (p50 homodimer) was kindly provided by Prof. M. Vasak (University of Zurich, Switzerland). Histone H1 was from Roche Diagnostics, GmbH (Mannheim, Germany). Glutathione (GSH), N,N´-dimethylformamide (DMF) were purchased from Sigma (Prague, Czech Republic) and rabbit metallothionenin isoform designated MT-2 (Zn7-MT-2) was a kind gift of Prof. M. Vasak (University of Zurich, Zurich, Switzerland). Radioactive products were from Amersham (Arlington Heights, IL, USA).

4.2 DNA platination in cell free media

Calf thymus (CT) or plasmid DNAs were incubated with the platinum complexes in 10 mM $NaClO_4$ at 37°C in the dark. After 24 h, the samples were exhaustively dialyzed against the medium required for subsequent biochemical or biophysical analysis. An Aliquot of these samples was used to determine the value of r_b (r_b is defined as the number of molecules of the platinum complex bound per nucleotide residue) by flameless atomic absorption spectrophotometry (FAAS) or by differential pulse polarography (DPP).

50-bp deoxyribooligonucleotide duplex [with a random nucleotide sequence (G + C content was 54%; see section 4.1)] was treated with trinuclear complex 1 for 24 h; the level of platination corresponded to $r_b = 0.023$. The platinated 50-bp duplex was repurified by ion-exchange fast protein liquid chromatography (FPLC). It was verified by platinum FAAS and by the measurements of the optical density that the modified 50-bp duplex contained three platinum atoms [181].

4.2.1 Polarograhic determination of DNA binding kinetics

Solutions of double-helical CT DNA at a concentration of 0.032 mg mL^{-1} were incubated with trinuclear complex **1** at an r_i of 0.05 or 0.1 and dinuclear complex **2** at an r_i of 0.1 in $NaClO_4$ (10 mM) at 37°C (r_i is defined as the molar ratio of free platinum complex to nucleotide phosphates at the onset of incubation with DNA). At various time intervals, an aliquot of the reaction mixture was withdrawn and assayed by DPP for platinum not bound to DNA. The amount of molecules of complexes **1** and **2** bound to DNA (r_b) was calculated by subtracting the amount of free (unbound) molecules of complexes **1** and **2** from the total amount of complexes **1** and **2** present in the reaction. No changes in the pH of the reaction mixture containing DNA and platinum compounds were measured within 48 h after mixing DNA with the platinum complex. The amount of the platinum compounds bound to DNA increased with time.

4.3 Characterization of DNA adducts by thiourea

Previous studies [182–185] have shown that under the appropriate conditions thiourea quantitatively reacts with DNA monoadducts of bifunctional Pt^{II} complexes without displacing platinum from the DNA. In other words, during the initial period of the reaction of DNA with bifunctional Pt^{II} complexes, when a significant part of the molecules is bound monofunctionally, the other coordination site can be blocked by thiourea [184]. This is because, although Pt–N bond has higher thermodynamic stability than Pt–S(thioether) bond, thioether sulfur is kinetically more favorable than guanine nitrogen when binding to Pt^{II} drugs.

Incorporation of [^{14}C] thiourea into DNA under controlled conditions (8.7×10^{-4}M, 10 min, 25°C [182]) was used to quantitate platinum-DNA mono- or bifunctional adducts of complexes **1** and **2**. The measurements were carried out in 10 mM $NaClO_4$ at 25°C. The molar ratio of free complex **1** and **2** to nucleotidephosphates at the onset of incubation with CT DNA, r_i, was 0.05 (DNA concentration was 0.24 mg·mL^{-1} (0.75 mM related to the phosphorus content), and the concentration of **1** and **2** was

3.75×10^{-5}M). Samples (120 μL) were withdrawn at various time intervals, and each sample was divided into two aliquots (60 μL). In one aliquot, conversion of the monofunctional adducts to bifunctional CLs, and in case of complex **1** eventual subsequent conversion of bifunctional adducts to trifunctional CLs, were blocked by addition of NaCl (final concentration was 0.15 M) and quick cooling to -20°C. The unbound complexes **1** and **2** were removed from these samples by centrifugation through a Sephadex G50 coarse column, and a molar ratio of covalently bound molecules of complexes **1** and **2** per nucleotide residue, r_b, were determined by FAAS. In the other aliquot, the conversion of the adducts was blocked by addition of [^{14}C] thiourea (final concentration was 8.7×10^{-4}M, and specific activity was 2.5 mCi·mmol^{-1}) and NaCl (final concentration was 0.15 M) so that the final volume of these samples was 1.0 mL. The samples were further incubated at 25°C for 10 min and subsequently layered on Millipore filters (diameter of pores was 0.1 μm); the unreacted thiourea and complexes formed between unbound **1** and **2** and thiourea were removed by washing the filters with 15 mL of 5% (v/v) trichloroacetic acid (TCA). The filters were dried under an infrared lamp and transferred to glass tubes, to which 5 mL of toluene scintillator was added. The radioactivity was measured on a liquid scintillation analyzer TriCarb 2800 TR (Perkin-Elmer) (2×2 min). The content of free coordination sites in DNA adducts of **1** and **2** not involved in the binding to DNA was determined as the amount (%) of radioactive thiourea bound to platinated DNA; the concentration of thiourea corresponding to 3-fold concentration of complex **1** (having three potential DNA binding sites) and 2-fold concentration of complex **2** (having two poteintial DNA binding sites) bound to DNA in each sample (in each time interval) determined by FAAS (r_b) was taken as 100%. It was also verified that, at 8.7×10^{-4}M thiourea, complete saturation of monofunctional or bifunctional adducts was obtained with no apparent reversal of platination [183, 184].

4.4 Characterization of DNA adducts by ethidium bromide (EtBr) fluorescence

EtBr as a fluorescent probe has been used to characterize perturbations induced in DNA by bifunctional adducts of several mononuclear platinum compounds [152, 186–188]. Double-helical DNA was first modified by bifunctional cisplatin, monofunctional [PtCl(dien)]Cl, trinuclear complex **1** or dinuclear complex **2** for 48 h. The levels of the modification corresponded to the values of r_b in the range between 0 – 0.04 or 0.02 – 0.08 for complex **1** or complex **2**, respectively.

These measurements were performed on a Varian Cary fluorescence spectrophotometer using a 0.5 cm quartz cell. Fluorescence measurements were performed at an excitation wavelength of 546 nm, and the emitted fluorescence was analyzed at 590 nm. The fluorescence intensity was measured at 25°C in NaCl (0.4 M) to avoid for EtBr, which corresponded to the saturation of all intercalation sites of EtBr in DNA [189].

4.5 HPLC analyses of enzymatically digested DNA

These analyses were performed using a Waters Breeze liquid chromatograph system equipped with 2489 UV/visible detector, 1525 binary HPLC pump, and a Discovery HSF5 HPLC column, 5 μm particle size (Sigma Aldrich-Supelco). The products were separated by reverse phase (RP) HPLC (isocratic elution with ammonium acetate (0.1 M, pH 4.5), in CH_3CN (3.9%) at 1 mL·min^{-1} flow rate). The following enzymatic digestion protocol was used in order to characterize the platinated 50-bp DNA fragment. The samples [1 nmol (30.7 μg) of the DNA] were incubated with 60 units of DNase I at 37°C. After 10 h, nuclease P1 (12 μg) was added, and the reaction was allowed to continue at 37°C for 24 h. Finally, alkaline phosphatase (5 units) was added and the incubation continued for an additional 4 h at 37°C. The samples

were digested with the enzymes in the buffers recommended by the manufacturers. The digested samples containing constituent nucleosides were then heated for 30 min at 50°C and centrifuged, the enzymes were separated by filters Nanosep NMWL:30K (Pall Filtron, Inc., Northborough, MA, USA) and the supernatant was analyzed by RP-HPLC. Each analysis was performed three times, and the data varied on average by ±1% from their mean.

4.6 Circular dichroism spectroscopy

Isothermal circular dichroism (CD) spectra of CT DNA were recorded using a Jasco J-720 spectropolarimeter equipped with a thermoelectrically controlled cell holder. The cell path length was 10 mm. CD spectra were collected using a Jasco J-720 spectropolarimeter. CD spectra of CT DNA at a concentration of 0.032 mg·mL^{-1} (1×10^{-4}M in nucleotides) modified by complex **2** at r_b in the range of 0.01 – 0.1 were recorded at 25°C in $NaClO_4$ (10 mM) with Tris-HCl (10 mM, pH 7.0) in the range of 200 – 400 nm.

4.7 Reactions with sulfur-containing compounds

Pt^{II} compounds have a strong thermodynamic preference for binding to sulfur donor ligands. Hence, before antitumor platinum drugs reach DNA in the nucleus of tumor cells, they may interact with various compounds, including sulfur-containing molecules. These interactions are generally thought to play a role in inactivation of platinum drugs, mechanisms underlying tumor resistance to platinum compounds and their side effects [190, 191]. Examples of endogenous thiols to which platinum complexes bind when they are administered intravenously or after they enter the cell are glutathione (GSH) and metallothioneins (MTs).

4.7.1 Reaction of benzotriazolate-bridged dinuclear Pt^{II} complex and cisplatin with GSH and MT-2

Reactions of GSH and mammalian MT-2 with complex **2** and cisplatin were investigated by monitoring UV absorption at 260 nm of solutions containing the platinum complex and GSH or MT-2 exactly as described in the previous work [192–194]; the absorbance at 260 nm reflects the presence of platinum–sulfur and disulfide bonds. The platinum compounds were mixed with GSH or MT-2 at 37°C in the medium of $NaClO_4$ (10 mM) plus phosphate buffer (0.1 mM, pH 7.0) in the dark in a nitrogen atmosphere. Reactions were initiated by mixing the platinum complex with the buffer followed by immediate addition of GSH or MT-2. The kinetic data were fitted by non-linear regression (GraphPad Prism) to one- and two-phase exponential association. The decision as to which fit was more appropriate for each dependence was made by comparing the fits of the two equations by using an F-test (GrahPad Prism).

4.7.2 Reaction of the *cis*-Pt(II)-dichlorido complexes containing 7-azaindole halogeno-derivates and cisplatin with GSH

Reactions of GSH and with complex **3**, **4** and cisplatin were investigated by monitoring UV absorption at 260 nm of solutions containing the platinum complex and GSH as described in the previous work [192, 195]; the absorbance at 260 nm reflects the presence of platinum–sulfur and disulfide bonds. The Pt^{II} complexes (30 μM) were mixed with GSH (15 mM) at 37°C in 4.6 mM NaCl plus 100 mM $NaClO_4$, pH 6.0 and 50% DMF. Reactions were initiated by mixing the Pt^{II} complex with the buffer followed by immediate addition of GSH. The kinetic data were fitted by nonlinear regression (GraphPad Prism) to one-phase or two-phase exponential association. The decision that fit to two-phase exponential association was more appropriate for each dependence was made by comparing the fits of two equations by using an F test (GrahPad Prism).

4.8 DNA melting

The melting curves of CT DNAs at the concentration of 32 μg·mL^{-1} were recorded by measuring the absorbance at 260 nm. The melting curves of unmodified or metalated DNA were recorded in the medium containing $NaClO_4$ (0.01 or 0.1 M) with Tris-HCl (1 mM, pH 7.4)/EDTA (0.1 mM). The melting temperature (t_m) was determined as the temperature corresponding to a maximum on the first-derivative profile of the melting curves. The t_m values could be thus determined with an accuracy of ± 0.3°C.

4.9 Unwinding of negatively supercoiled DNA

Unwinding of closed circular supercoiled pUC19 plasmid DNA was assayed by an agarose gel mobility shift assay [196]. The unwinding angle Φ, induced per one DNA adduct of the platinum complex, was calculated upon the determination of the r_b value at which the complete transformation of the supercoiled to relaxed form of the plasmid was attained. Samples of plasmid DNA were incubated with the platinum complex at 37°C in the dark for 24 h. The samples were subsequently subjected to electrophoresis on 1% native agarose gel running at 25°C in the dark with TAE buffer [Tris-acetate (40 mM, pH 8.0)/EDTA (1 mM)] and the voltage set at 18 V. The gels were then stained with EtBr, followed by photography with transilluminator.

4.10 Interstrand (intramolecular) cross-linking

Complexes **1** and **2** at varying concentrations was incubated for 24 h with 0.5 μg of a linear 2686-bp fragment of pUC19 plasmid linearized by *Eco*RI. The linear fragment was first 3′-end labeled by means of the Klenow fragment of DNA polymerase I in the presence of [α 32P]dATP. The platinated samples were analyzed for DNA interstrand CLs by previously published procedures [44, 197]. The number of interstrand CLs was analyzed by electrophoresis under denaturing conditions on alkaline agarose gel (1%). After the electrophoresis had been completed, the intensities of the bands corresponding to single strands of DNA and interstrand cross-linked duplex

were quantified. The frequency of interstrand CLs was calculated as % ICL/Pt = XL/5372r_b (the DNA fragment contained 5372 nucleotide residues), where % ICL/Pt is the number of interstrand CLs per adduct multiplied by 100, and XL is the number of interstrand CLs per molecule of the linearized DNA duplex and was calculated assuming a Poisson distribution of the interstrand CLs as XL = $-ln$A, where A is the fraction of molecules running as a band corresponding to the non-cross-linked DNA.

4.11 Interduplex cross-linking

At least two types of DNA interstrand cross-linking by multifunctional platinum complexes can exist, depending on whether the platinum complex coordinates to the bases in one DNA molecule (intraduplex interstrand CLs) or coordinates to the bases in separate DNA molecules (interduplex interstrand CLs). A large number of DNA cross-linking agents are known, but the reactive moieties of such compounds usually bind to the same DNA duplex because binding via one leaving group inevitably leaves the other in close proximity to other binding sites in the same duplex, leading to intraduplex interstrand cross-linking. However, if the reactive sites of the cross-linking agents are connected by a more rigid linker that forces those sites to point in appropriate directions and if the stereochemistry of the reactive sites of such cross-linking agents proves appropriate, such agents could bind to adjacent duplexes [198–200]. Such cross-linking agents might be effective interduplex cross-linkers in cases of two fragments of double-helical DNA molecules forced to lie together, i.e. for instance during recombination, at replication forks or sites of topoisomerase action [199, 201] or more generally in cellular environmental conditions [202, 203].

The double-stranded linearized pUC19 DNA (2686 bp) 3′-end-labeled with [α 32P] dATP was platinated by trinuclear complex **1** at r_b = 0.0001 – 0.005 or by cisplatin at r_b = 0.001 – 0.05 in the medium containing sodium acetate (0.2 M, pH 5.5) and ethanol (70%) in a reaction volume of 15 μL at 37°C for 48 h. Each reaction contained 500 ng of plasmid DNA. Then samples were stored on dry ice for 30 min and centrifugated at 14000g for 30 min at 4°C; supernatant was removed, and

pellet was dried and dissolved in TAE buffer. The amount of interduplex CLs was analyzed by electrophoresis in native agarose gel (0.5%). After the electrophoresis had been completed, the intensities of the bands corresponding to interduplex cross-linked and non-interduplex cross-linked duplexes were quantified. The frequency of interduplex CLs (% IICL/Pt) was calculated as % IICL/Pt = IXL/5372r_b (the DNA fragment contained 5372 nucleotide residues), where % IICL/Pt is the number of interduplex CLs per adduct multiplied by 100, IXL is the number of interduplex CLs per molecule of the linearized DNA duplex and was calculated assuming a Poisson distribution as IXL = $-ln$B, where B is the fraction of molecules running as a band corresponding to the non-interduplex cross-linked DNA.

4.12 DNA-protein cross-linking

Trinuclear complex **1** was investigated for its ability to form ternary DNA-protein complexes covalently linked by the platinum moiety. The proteins were chosen for these studies that bind to DNA with a relatively high affinity. The linker histone H1 was chosen as the representative of non-sequence specific DNA-binding proteins with structural function, whereas transcription factor NF-κB (p50 dimer) was chosen as the representative of a sequence specific DNA-binding protein with a regulation function [42].

A double-stranded linearized DNA was prepared by digesting the pUC19 plasmid with *Nde*I and *Eco*RI. The resulting two fragments, 2473 and 213 bp long, were separated on 1% agarose gel. The 213 bp fragment was cut from the agarose gel and fragment was elutioned to TAE buffer using electrophoresis. 213 bp fragment was extracted with phenol/chloroform (1:1), ethanol precipitated and dissolved in 10mM $NaClO_4$. DNA was 3´-end-labeled with [α 32P]dATP by using Klenow fragment [42]. The 213 bp fragment was modified with cisplatin and complex **1** for 24 h in 10mM $NaClO_4$ to an r_b value of 0.025. Radioactively labeled and platinated DNA (213 bp fragment) was incubated with the proteins (histone H1, NF-κB) overnight at room temperature. The ability to form CLs by platinum complexes between 213 bp

DNA fragment and proteins was assessed by 2% agarose (agarose and Metaphor agarose 1:1). Platinated and labeled DNA at a concentration of 10 nM was incubated with the proteins (histone H1 or NF-κB (p50 dimer)) at a concentration of 100 nM overnight at room temperature in the appropriate buffer: Tris-HCl (10 mM, pH 7.9) and NaCl (20 mM) (histone H1); HEPES (42 mM), KCl (42 mM), $MgCl_2$ (1 mM), EDTA (0.02 mM), DTT (210 mM), glycerol (2.5%), and Ficoll (2%) (NF-κB). The ability to form CLs by complex **1** or cisplatin between the 213 bp DNA fragment and proteins was assessed by 2% agarose (agarose and Metaphor agarose 1:1) after mixing the samples with the loading buffer (Tris-HCl (50 mM, pH 6.8), SDS (2%), bromophenol blue (0.1%), glycerol (10%)) and denaturing by heat at 90°C for 5 min. Gels were electrophoresed for 3 h at 40 V, dried and visualized with a bioimaging analyzer [42].

4.13 Other physical methods

Absorption spectra were measured with a Beckman DU-7400 spectrophotometer. FAAS measurements were carried out with a Varian AA240Z Zeeman atomic absorption spectrometer equipped with a GTA 120 graphite tube atomizer. For FAAS analysis, DNA was precipitated with ethanol and dissolved in HCl (0.1 M). DPP was performed with an EG&G Princeton Applied Research Corporation model 384B polarographic analyzer. HPLC purification of oligonucleotides was carried out on a Waters HPLC system consisting of a Waters 262 pump, Waters 2487 UV detector, and Waters 600S controller with MonoQ HR 5/50 GL column. The gels were visualized by using the BAS 2500 FUJIFILM bioimaging analyzer, and the radioactivities associated with bands were quantitated with the AIDA image analyzer software (Raytest, Germany).

Chapter 5

Results and discussion

This doctoral thesis is based on three papers published in peer-reviewed international journals. These full papers with comments on author's contribution are enclosed in the part Appendix. The overall results obtained, are briefly summarized in this chapter.

5.1 Unique DNA binding mode of antitumor trinuclear tridentate platinum(II) compound (paper I)

Polynuclear platinum complexes represent a class of the new generation of Pt^{II}-based antitumor agents that form different DNA adducts and display a different spectrum of anti-cancer activity compared to cisplatin and its analogues [158]. In an effort to design a new polynuclear platinum compound that is able to bind to DNA in a fundamentally new manner and consequently having novel preclinical properties, trinuclear trifunctional Pt^{II} complex $\{[Pt_3Cl_3$ (hptab)$]^{3+}$ (hptab=N,N,N′,N′,N″,N″-hexakis(2-pyridylmethyl)-1, 3, 5tris(aminomethyl)benzene)$\}$ was recently synthesized [158]. In this trinuclear and tridentate compound, the Pt^{II} centers are bridged by bulky semi-rigid aromatic linkers (Fig. 1, paper I). This new trifunctional agent exerted promising cytotoxic effects in human and mouse tumor cell lines [158, 176]. In addition, the ge-

ometry of leaving groups in the coordination sphere of platinum atoms in complex **1** does not undergo decomposition of the polynuclear structure upon reaction with sulfur nucleophiles, in contrast to another trinuclear, but bidentate Pt^{II} complex [{*trans*-$PtCl(NH_3)_2$}$_2$(μ-*trans*-Pt-$(NH_3)_2${$NH_2(CH_2)_6NH_2$}$_2$)]$^{4+}$ (BBR3464, Figure 1). Thus, also for this reason this trifunctional Pt^{II} complex can be considered a promising candidate for further preclinical and clinical testing. The entirely unique spatial configuration of leaving groups in trinuclear complex **1**, semirigid linkers bridging three Pt^{II} centers and three reactive groups predispose this trifunctional complex to a unique DNA binding mode.

Our primary objective in the present work was to understand more fully the DNA binding mode of complex **1** in a cell-free medium including also its unique intramolecular and intermolecular cross-linking capability. Comparisons of these results with those obtained earlier for conventional mononuclear cisplatin and other polynuclear Pt^{II} complex BBR3464 yielded new information that broadens the theoretical background needed for search and design of new, more effective antitumor metallodrugs.

5.1.1 DNA binding

The binding of complex **1** to natural double-helical CT DNA was performed in cell free media. It was demonstrated that the rate of binding of trinuclear complex **1** to natural double helical DNA is considerably higher than that of cisplatin and somewhat lower than that of other antitumor trinuclear Pt^{II} complex BBR3464. In this binding experiment, the time at which the binding reached 50% ($t_{50\%}$) was 60 min. The value of $t_{50\%}$ for the reaction of conventional mononuclear cisplatin and other antitumor trinuclear Pt^{II} complex BBR3464 with DNA under comparable conditions was $\sim$120 or 60 min, respectively [152, 204].

In this binding experiment, CT DNA was incubated with complex **1** at r_i of 0.05 and 0.1 and absolutely the same rate of the binding was observed. The binding experiments indicated that such platination reactions resulted in the coordination of all molecules of trinuclear complex **1**.

5.1.2 Characterization of DNA adducts

Characterization of adducts induced by trinuclear complex **1** in a 50-bp deoxyribooligonucleotide duplex [with a random nucleotide sequence (G + C content was 54%; see section 4.1] was carried out with RP-HPLC analysis of the products of enzymatic digest of platinated DNA fragment. Digestion of the unmodified 50-bp duplex produced mononucleoside peaks whose areas reflected the proper proportions and content of the single mononucleosides. On the other hand, digestion of the platinated sample resulted in a decrease of the integrated area of the deoxyriboguanosine peak by 17.8% and the deoxyriboadenosine peak by 8.1% (Fig. 4, paper I). The peaks for deoxyribocytidine and thymidine were not affected. This experiment showed the decrease of the deoxyriboguanosine and deoxyriboadenosine peaks corresponds to the loss of ∼4.8 guanine and ∼1.9 adenine residues, i.e. in total ∼6.7 base residues. These results came up to expectations if r_b value is 0.023 so 6.9 base residue in the 50-bp DNA fragment would be platinated in the case of the trifunctional binding of trinuclear complex **1**. This implies that, at a relatively low level of binding of complex **1** to DNA and after 24 h, this complex formed mainly trifunctional adducts. Furthermore, characterization of DNA adducts of complex **1** by HPLC analysis of enzymatically digested DNA revealed that guanine residues are the preferential DNA binding sites of complex **1** when polymeric DNA is modified with this complex in a random fashion and that complex **1** can also bind adenine residues, but to a lesser extent.

For characterization of DNA binding induced by platinum compounds can be used a fluorescent probe such as EtBr [152,205]. The binding of EtBr to DNA by intercalation is blocked in a stoichiometric manner by a wide spectrum of platinum compounds binding to DNA. The experiment demonstrated that the modification of DNA by bifunctional cisplatin, monofunctional [PtCl(dien)]Cl or trinuclear tridentate complex **1** resulted in a decrease of EtBr fluorescence (Fig. 2, paper I). Modification of DNA by monofunctional platinum complexes resulted in only a slight decrease of EtBr fluorescence intensity as compared with the control DNA-EtBr complex [152, 186–188].

In contrast, adducts of trinuclear complex **1** caused markedly more pronounced decrease of EtBr fluorescence intensity than that induced by the adducts of cisplatin. The explanation for this phenomenon of trinuclear complex **1** can be that complex **1** bound to CT DNA sterically block approach of EtBr to DNA and in this way hinder the EtBr to intercalate. The structures of DNA adducts of complex **1** may arise from one to three monofunctional substitutions on the polynucleotide. The conformational distortion in DNA induced by the adducts of trinuclear complex **1** is much more delocalized and extends over considerably more base pairs around the platination sites compared with the adducts of mononuclear complexes such as [PtCl(dien)]Cl and cisplatin. These obtained results are in agreement with capability of complex **1** to form DNA adducts which either span more base pairs around the binding sites or at least induce conformational distortions in DNA which extend over several base pairs.

In this context, complex **1** forms long-range cross-links preferentially at G residues similarly as other trinuclear Pt^{II} complex BBR3464. Nevertheless, the spatial configuration of leaving groups in both trinuclear Pt^{II} complexes is markedly different. Both complexes induce in DNA different conformational alterations [152, 206, 207].

Quantification of platinum-DNA mono- or bifunctional adducts of trinuclear complex **1** was performed by incorporation of [^{14}C] thiourea into DNA under controlled conditions [182] (see section 4.3). Incorporation of [^{14}C] thiourea into CT DNA was used to quantification of the adducts in which complex **1** have free coordination sites not involved in the binding to DNA. Thus, these adducts could be blocked by thiourea in mono- or bi- or even trifunctional lesions because it is expected that DNA adducts of complex **1** can be trifunctional CLs. The resulting data gained from the experiment demonstrated that in the early phase of the reaction of DNA with complex **1** (30 min) approximately 40% coordination sites in all molecules of complex **1** present in the reaction were already involved in DNA adducts. The amount of these coordination sites in complex **1** rapidly decreased with time of incubation. After 2 h, ∼90% coordination sites in all molecules of complex **1** were already involved in DNA adducts. Intriguingly, after 24 h, when all molecules of complex **1** were already bound to DNA

(*vide supra*), approximately 98% coordination sites in all molecules of complex **1** were already involved in DNA adducts (Fig. 3, paper I).

This result is consistent with the statement gained from binding experiment in cell free media. All three Pt^{II} centers of complex **1** were coordinated to DNA base residues after 24 h of incubation of DNA with complex **1**. Also, previous experimental findings [176] indicating that complex **1** formed trifunctional CLs in short synthetic oligodeoxyribonucleotide duplexes. These recorded figures are also supported with RP-HPLC analysis (Fig. 4, paper I) and experiment with EtBr (Fig. 2, paper I), which showed delocalized cross-links with span more than two base pair. The later long-range CLs may occur, because of distance between reactive sites: the Pt-Pt distances in complex **1** are in the range 0.73 – 0.84 nm [158] and the distance of neighboring base residues in B-DNA: approximately 0.34 nm. It is clear that the molecule of complex **1** can easily span 3-4 base pairs.

5.1.3 Conformation changes induced in DNA

Another proof that DNA binding mode of trifunctional complex **1** is markedly different from that of cisplatin was observed. This interesting finding was revealed by the different melting behaviour of DNA modified by complex **1** and cisplatin recorded in the media of low and high salt concentration (0.01 and 0.1 M $NaClO_4$, respectively). At low concentrations of $NaClO_4$ (0.01 M) an increase of t_m was observed even at relatively high levels of the modification of DNA by trinuclear complex **1** ($r_b = 0.045$). At high salt concentrations of 0.1 M the modification of DNA by complex **1** resulted in a decrease of t_m which became more pronounced with increasing r_b value (Fig. 5, paper I). On the other hand, at salt concentrations ranging from 0.01 to 0.2 M $NaClO_4$ the modification of DNA by cisplatin resulted in a decrease of t_m, which is in contrast with result obtained for complex **1**.

The different melting temperature of DNA modified by trinuclear complex **1** can be explained by three major factors affect the thermal stability of DNA modified by Pt^{II} complexes [208]. These major factors are (i) a destabilizing effect of conformational distortions due to the formation of CLs induced in DNA by platinum

coordination; (ii) stabilizing effects of DNA interstrand (intramolecular) cross-links which prevent dissociation of DNA strands; (iii) the positive charge on the Pt^{II} centers. Both the positive charge of platinum complexes and cations present in the medium stabilize DNA against thermal denaturation by decreasing electrostatic repulsion of negative charges of phosphate groups located at the complementary strands. The concentration of cations in the medium directly affect the stabilization effect of positive charge of platinum complexes. Thus, at high ionic strength (0.1 M $NaClO_4$) when the electrostatic stabilizing effects of complex **1** are markedly weakened, the decreases in t_m are caused by destabilizing effects of conformational distortions induced by lesions of complex **1** which dominate the stabilizing effects of interstrand CLs (in fact, complex **1** formed only 9% of interstrand CLs) (Fig. 7, paper I). On the other hand, at low ionic strength (0.1 M $NaClO_4$) was observed the increase in t_m because of dominated electrostatic stabilizing effect of complex **1**.

Capability of trinuclear complex **1** unwinds the negatively supercoiled pUC19 plasmid DNA was studied by migration of DNA in native agarose gel at various degrees of modification by this complex (Fig. 6, paper I). Complex **1** unwinds the negatively supercoiled DNA and reduces the number of supercoils resulting in decrease of electrophoretic mobility this DNA in compared with relaxed DNA. Unwinding of negative supercoiled DNA by complex **1** can be observed and quantified on the basis of decrease of migration this DNA through the native agarose gel. In contrast, the mobility of relaxed DNA was accelerated similarly to cisplatin whose bifunctional binding to DNA shortens and condenses the DNA helix [158]. Considerable decrease of the separation between supercoiled and relaxed DNA was observed with an increase of values of r_b. Both bands comigrated at the coalescence point which corresponded to the full relaxation of the supercoiled DNA. Beyond this point, the migration rate began to increase again as positive supercoils [158]. The unwinding angle is given by $\Phi = -18\sigma/r_{b(c)}$ where σ is the superhelical density and $r_{b(c)}$ is the value of r_b at which the supercoiled and relaxed forms comigrate [196]. Under the present experimental conditions, σ was calculated to be -0.0505 on the basis of the data of cisplatin for which the $r_{b(c)}$ was determined in this study and $\Phi = 13°$ was assumed. We determined the

DNA unwinding angle to be in the range $53^\circ \pm 3^\circ$ for complex **1**. This considerably large unwind of DNA is caused by formation of tridentate adducts by complex **1** in double helical DNA.

Polynuclear platinum complexes induced in DNA various types of interstrand and intrastrand cross-links. Many studies have been demonstrated that just mentioned types of cross-links caused the antitumor efficiency of platinum compounds. Therefore, the quantification of interstrand cross-linking efficiency of complex **1** in linearized and radioactively labeled pUC19 plasmid (2686 bp) was performed. Linearized DNA was modified at various r_b values by complex **1** and analyzed by agarose gel electrophoresis under denaturing conditions for the interstrand cross-links [44]. The interstrand cross-linked DNA migrated through the agarose gel more slowly as a higher molecular mass species than the 2686-base single-stranded (ss) DNA. The intensity of interstrand cross-linked fragments in the agarose gel rose with the increased values of r_b (Fig. 7, paper I). The fraction of non-cross-linked and cross-linked DNA was measured by radioactivity associated with the individual bands and frequency of interstrand CLs (% ICL per one molecule of the Pt complex) was calculated by technique described in section 4.10. We found that the intramolecular DNA interstrand cross-linking efficiency of complex **1** was only 9%, although slightly higher than that found for cisplatin (6% [44]), but markedly lower than that for trinuclear BBR3464 (20% [152]). Another intriguing feature of DNA binding mode of trinuclear complex **1** is that its interstrand CLs are diminished compared with BBR3464. Hence, major adducts formed in DNA globally modified for 24 h by complex **1** (in the absence of proteins and molecular crowding agents) appear to be unique tridentate intrastrand rather than intramolecular interstrand CLs.

Another unique feature of DNA binding mode of trinuclear tridentate complex is the fact that this complex can form another type of cross-links called interduplex CLs under molecular crowding conditions mimicking environmental conditions in cellular nucleus. Trinuclear complex **1** may coordinate to the bases in separate DNA molecules (interduplex interstrand CLs). This is possible because of stereochemistry reactive sides of complex **1** and rigid linker [198–200]. This interesting experiment was

performed that the radioactively labeled double stranded linearized pUC19 (2686-bp) was modified by complex **1** in medium containing 70% ethanol and high concentration of counter cations (0.2 M Na) because of minimizes the electrostatic repulsion between the sugar-phosphate backbones of two different duplex. Interduplex CLs were analyzed using of native agarose gel electrophoresis. The interduplex cross-linked duplex (two or more) migrated through the agarose gel more slowly as a higher molecular mass species than the non cross-linked double stranded linearized labeled pUC19 plasmid which migrates as a 2686-bp DNA molecule. The intensity of interduplex cross-linked fragments in the agarose gel rised with the increased values of r_b (Fig. 9, paper I). The fraction of non-interduplex cross-linked and interduplex cross-linked DNA was measured by radioactivity associated with the individual bands on the agarose gel and frequency of interduplex CLs (% IICL/Pt) was calculated as described in section 4.11. The frequency of these interhelical CLs tethered by platinum-DNA bonds was markedly higher (21%) than for mononuclear cisplatin ∼1%. The explanation of this result is that crowding conditions in the nucleus allow interduplex contacts that are sufficient for the interduplex cross-linking by trinuclear complex **1**, but not by cisplatin.

5.1.4 Recognition of DNA adducts by proteins

Trinuclear tridentate platinum complex **1** forms various types of cross-links such as intrastrand CLs, interstrand CLs or interestingly even interduplex CLs. Therefore, we decided to investigate complex **1** for its ability to form ternary DNA-protein complexes covalently linked by the platinum moiety. For this experiment, the proteins were chosen that bind to DNA with a relatively high affinity. The linker histone H1 was chosen as the representative of non-sequence specific DNA-binding proteins with structural function. Transcription factor NF-κB (p50 dimer) was chosen as the representative of a sequence specific DNA-binding protein with a regulation function. The first step of formation of ternary DNA-protein complex is that, relatively flexible DNA-binding proteins (such as NF-κB and the linker histone H1) come into close contact with DNA at the site of platination. After that, one molecule

of complex **1** can be bound simultaneously to base residue(s) in DNA and reactive group(s) in the protein, but only if a noncovalent preassociation takes place first. The experiment was performed that 213-bp fragment long of pUC19 plasmid and radioactively labeled was globally modified by complex **1** and cisplatin for 24 h to the r_b value of 0.025. Then the DNA fragment was mixed with histone H1 and NF-κB (the molar ratio protein/duplex was 8 or 34, respectively) and incubated overnight. DNA-Pt-protein cross-linking efficiency was assessed by agarose gel mobility shift assay (Fig. 10, paper I). Upon electrophoresis, DNA-protein CLs migrated through the agarose gel with significantly delayed mobility than free probe (fragments modified by complex **1** and cisplatin without proteins). The mechanism of formation of DPCLs mentioned above was supported by NaCN or proteinase K which these more slowly migrating fractions eliminated or converted them to these of unmodified probes (not shown), respectively. These results suggest that the species is a protein-DNA CL tethered by platinum-DNA and platinum-protein coordination bonds. The amount of radioactivity associated with the bands corresponding to DPCLs formed by complex **1** was higher compared to cisplatin. The complex **1** exhibited a significantly higher efficiency (ca. 2.6-fold) to form ternary DNA-protein CLs than cisplatin. The formation of DPCLs mediated by complex **1** raises the possibility of "suicide" lesions, which may irreversibly sequester various DNA-binding proteins, such as transcription factors or repair proteins [209, 210].

The another experiment was carried out to determine if differences exist in the recognition of the adducts of complex **1** by full-length HMGB1 protein, because the DNA conformational changes induced by complex **1** are very different from those induced by cisplatin. No recognition of DNA adducts of complex **1** by HMGB1 protein was observed (Fig. 8, paper I). This can be explained by the prebending, due to the adducts of complex **1**, is too small to be recognized by HMG domain proteins or that bulky adducts of complex **1** could restrict the additional DNA bending required for HMG domain binding [211, 212].

5.2 Mechanistic insights into toxic effects of a benzotriazolate-bridged dinuclear platinum(II) compound in tumor cells (paper II)

Polynuclear platinum complexes are a structurally unique class of anticancer drugs. Some of the polynuclear (mostly dinuclear) antitumor active Pt^{II} complexes described in the literature by group of prof. Farrell, contain flexible [213, 214] or semiflexible bridging ligands [149, 215], while others possess rigid linkers [174, 175, 216, 217]. It has been demonstrated that the complexes with flexible linkers form in DNA unique long-range cross-links (CLs), which aren't formed by mononuclear bifunctional Pt^{II} complexes [152, 218]. Recently, Reedijk, Komeda and their coworkers synthesized a new class of dinuclear bifunctional azolato- or azinato-bridged Pt^{II} compounds [153,171,174,175,219–221], described in section 2.2. Interestingly, the azolato-bridged dinuclear complexes form in DNA 1,2-intrastrand CLs which are identical of the major DNA adducts of cisplatin [221]. However, in contrast to cisplatin, these adducts formed by the dinuclear azolato-bridged Pt^{II} compounds induce only minimal conformational changes in double-helical DNA [219, 222]. Thus, we studied the new benzotriazolate (Btaz)-bridged dinuclear Pt^{II} complex [{*cis*-$Pt(NH_3)_2Cl$}$_2$ (μ-1, 2, 3-Btaz-H-N1,N3)]Cl(**2**) (Fig. 1, paper II) designed first by Stetsenko and coworkes [177]. This bifunctional Pt^{II} complex presents a combination of the two above-mentioned types of dinuclear Pt^{II} complexes such as it utilizes rigid aromatic ring of azole as a linker between two Pt atoms, featuring at the same time non-bridging chloride ions as leaving groups similar to azinato compounds.

Our primary objective of this present work was to examine toxicity in tumor cells and early phases of the mechanism underlying biological properties of dinuclear bifunctional platinum complex **2**. These results can provide a more rational basis for the design of new antitumor metallodrugs and chemotherapeutic strategies.

The comparative cellular experiments between dinuclear complex and cisplatin demonstrated:

1. The cytotoxic activity of complex **2** was markedly lower than that of cisplatin in human ovarian carcinoma cell lines and human breast cancer cells (Tab. 1, paper II). This result shows a difference in that mechanism of cytotoxicity of complex **2** and cisplatin.

2. The cellular platinum uptake was greater (∼4-7-fold) than that of cisplatin (Tab. 2, paper II). These results suggests that cellular uptake may not be responsible for reduced toxicity of complex **2** in tumor cell lines when compared with the effects of cisplatin.

3. DNA binding in cells exposed to complex **2** was lower than that of cisplatin (Tab. 3, paper II). This observation suggests that inside the cells must be some mechanism hindering of complex **2** to bind to DNA or/and reducing the amount of complex **2** bound to DNA.

4. Damage-induced by DNA repair synthesis in the plasmid modified by complex **2** was markedly lower that that of cisplatin (Fig. 3, paper II). Explanation of this feature is that DNA repair is implausible factor responsible for the reduced amount of adducts of complex **2** on DNA.

5.2.1 DNA-binding mode

The binding of dinuclear complex **2** to natural double-helical CT DNA was performed in cell free media. The amount of complex **2** bound to DNA increased with time and was quantitatively bound after 24 h. In this binding experiment, the time at which the binding reached 50% ($t_{50\%}$) was 11±1 min. (Fig. 2, paper II). The value of $t_{50\%}$ for the reaction of conventional mononuclear cisplatin with DNA under comparable conditions was ∼120 min. [204]. It was shown that the rate of binding of complex **2** to natural double helical DNA is considerably higher than that of cisplatin, so that the reduced biological activity of complex **2** is not due to the slower rate of initial binding of complex 2 to DNA.

Reaction of complex **2** and cisplatin with sulfur-containing compound such as glu-

tathione (GSH) and mammalian metallothionein (MT-2) was investigated. The reason for performing this experiment was that platinum(II) compounds have a strong thermodynamic preference for binding to sulfur donor ligands. The reaction between platinum complexes and sulfur-containing compounds play a role in inactivation of platinum drugs, mechanisms tumor resistance to platinum drugs and their side effects [190,191]. Dinuclear complex **2** and cisplatin at a concentration of 33 μM or 200 μM were mixed with 15mM GSH or 2.1 μM MT-2 at 37°C as described in section 4.7., respectively. The $t_{1/2}$ (half-times of the reactions, which mainly result in the formation of platinum-sulfur bonds) of the reaction of complex **2** or cisplatin with GSH were 47±4 min and 89±5 min, respectively (one-phase exponential association). And the half-times of the reaction of complex **2** or cisplatin with MT-2 were 106±7 and 190±9 min, respectively. From the results is noticed that complex **2** reacted with GSH and MT-2 significantly faster than cisplatin. Thus, efficiency of complex **2** to bind coordinatively DNA in tumor cells is inactivated by sulfur-containing compounds considerably more than that of cisplatin. Reasonable explanation of this observation is that deactivation of complex **2** by sulfur containing compounds contribute to its reduced DNA binding in cells and subsequently to its reduced cytotoxicity.

Circular dichroism (CD) spectra of CT DNA modified by dinuclear complex **2** to r_b values 0.02 – 0.1 was measured (Fig. 5, paper II) and compared with CD spectra earlier determined for cisplatin under the same experimental conditions [223]. The conservative CD spectrum of DNA is normally in the canonical B-conformation, neverthless upon binding platinum complexes to CT DNA, it considerably transforms at wavelengths below 300 nm. It was observed that the intensity of the positive band significantly decreased around 280 nm if DNA was modified by complex **2** already at relatively low values of r_b (Fig. 5, paper II). On the other hand, if DNA was modified by cisplatin, an increase of this band was noticed at relatively low values of r_b and at higher levels of the modification ($r_b > 0.6$), the intensity of this CD band began to decrease [223]. This result indicates that CD spectra of CT DNA was affected by complex **2** in different manner from cisplatin, but similarly to transplatin, which induces in DNA conformation nondenaturational alterations [223].

5.2.2 Characterization of DNA adducts

Quantification of platinum-DNA mono- or bifunctional adducts of dinuclear complex **2** and cisplatin was carried out by incorporation of [^{14}C] thiourea into DNA under controlled conditions [149, 182] (see section 4.3). The experiment demonstrated that in the early phase of the reaction of DNA with complex **2** (10 min) approximately ∼52% coordination sites in all molecules of complex **2** present in the reaction were already involved in DNA adducts. The amount of these coordination sites in complex **2** rapidly increased with time of incubation. After 2 h, ∼85% coordination sites in all molecules of complex **2** were already involved in DNA adducts. In other words, after two hours complex **2** formed in DNA ∼85% cross-links and only ∼15% of monofunctional adducts. In contrast, in the early phase of the reaction of DNA with cisplatin (10 min) approximately 92% coordination sites in all molecules of cisplatin were involved in DNA adducts. After 120 min, 97% coordination sites in all molecules of cisplatin were already involved in DNA adducts. These results (Fig. 6, paper II) show, that the rate of conversion of monofunctional DNA adducts of complex **2** to bifunctional CLs is considerably slower than that of cisplatin. The different biological activities of complex **2** and cisplatin can be explained with large differences in monofunctional adduct lifetimes. Definitely, monofunctional adducts of complex **2** react more rapidly with sulfur containing compounds than of cisplatin. This feature can contribute to the reduced activity of complex **2**.

5.2.3 Conformation changes induced in DNA

The melting behaviour of DNA modified by complex **2** and cisplatin was recorded in the media of high salt concentration (0.1 M $NaClO_4$). A considerably less decrease of t_m was observed for DNA modified by complex **2** than cisplatin. The different melting temperature of DNA modified by complex **2** can be explained by three major factors affect the thermal stability of DNA modified by Pt^{II}) complexes [208]. These factors are (i) a destabilizing effect of conformational distortions due to the formation of CLs induced in DNA by platinum coordination; (ii) stabilizing effects

of DNA interstrand (intramolecular) cross-links which prevent dissociation of DNA strands; (iii) the positive charge on the Pt^{II} centers.

Thus, at high ionic strength (0.1 M $NaClO_4$) when the electrostatic stabilizing effects of complex **2** are markedly weakened, the decreases in t_m are caused by destabilizing effects of conformational distortions induced by lesions of complex **2** which dominate the stabilizing effects of interstrand CLs (in fact, complex **2** formed only 6% of interstrand CLs like cisplatin) (Fig. 4, paper II). The observation of the melting behaviour of DNA suggests that major DNA adducts of complex **2** distort and destabilize double helix of DNA significantly less than major DNA adducts of cisplatin.

The comparative experiments between complex **2** and cisplatin, where were achieved of similar results:

1. Similar decrease of EtBr fluorescence in CT DNA induced by adducts of complex **2** or cisplatin. This result suggests that adducts of complex **2** induced in DNA conformation distorsion extends over approximately the same number of base pairs around the binding site like cisplatin.

2. DNA unwinding angle 13±2° induced by adducts of complex **2** was identical to that produced by the major adducts of cisplatin [196].

3. The interstrand cross-linking efficiency of complex **2** (6±0.5%) was identical to that of cisplatin (6% [44]).

4. *In vitro* RNA synthesis by T7 RNA polymerase on DNA revealed that adducts of complex **2** produced similar major stop sites like cisplatin, which are mainly at guanine residues and a few at adenine residues.

5.3 Insight into the toxic effects of the *cis*-Pt(II)-dichlorido complexes containing 7-azaindole halogeno-derivatives in tumor cells (paper III)

The new cisplatin analogues {*cis*-[$PtCl_2$(*3Cl*Haza)$_2$]}(**3**) or {*cis*-[$PtCl_2$(*3I*Haza)$_2$]}(**4**) (*3Cl*Haza and *3I*Haza = 3-chloro-7-azaindole and 3-iodo-7-azaindole, respectively) (Fig. 1, paper III) previously showed [178] promising *in vitro* cytotoxicity against the HOS (osteosarcoma), MCF7 (breast adenocarcinoma) and LNCaP (prostate adenocarcinoma) human cancer cell lines, i.e. against the tumor cell lines inherently resistant to cisplatin.

The primary objective of this paper was to study potential factors involved in the mechanism underlying the cytotoxic effects of complex **3**, **4** and compared with cisplatin.

The results indicate that efficient cellular uptake and different effects at the level of cell cycle regulation and reduced propensity of DNA adducts to repair are the main reasons for higher cytotoxicity of complexes **3** and **4**. Furthermore, the results show efficient DNA binding in cells exposed to these complexes and capability of circumventing resistance to cisplatin induced by alterations in cellular accumulation and DNA repair.

5.3.1 Reactions with reduced glutathione (GSH)

Platinum(II) drugs may react with various compounds including S-containing molecules (because of strong thermodynamic preference), before they reach the DNA in the nucleus of tumor cells, or even after they bind to DNA [190, 224]. Generally has been believed, that these reactions play a role in mechanisms underlying tumor resistance to Pt compounds, their inactivation, and side effects.

The reaction of complexes **3**, **4** and cisplatin with reduced form of glutathione was performed by using the UV absorption spectrophotometry such as described in section 4.7.2. The gained results demonstrated that complexes **3** and **4** react with GSH with a

rate similar to that of cisplatin and that the absorbance at 260 nm determined for the reaction of GSH with complexes **3** and **4** was approximately 1.2-fold lower than that determined for the reaction of GSH with cisplatin (Fig. 5, paper III). The absorbance at 260 nm reflects the presence of platinum-sulfur and disulfide bonds [192, 195].

The explanation of the result can be cover in the mechanism of the reaction between platinum complexes **3**, **4** or cisplatin and GSH. The initial products of the reaction between cisplatin or complexes **3**, **4** and GSH contain only one sulfur molecule, which were slowly transformed to bissulfur or bridged Pt species (polymers) with the 1:2 Pt:GSH stoichiometry on the basis of the trans effect of the N-donor ligands. The large differences in the trans effects of dissimilar types of N-donor ligands (NH_3, nHaza) are not observed. Thus, the more difficult binding of a second GSH to the Pt atom in *cis*-[PtCl(SG)(*n*Haza)$_2$] (in comparison with *cis*-[PtCl(SG)$(NH_3)_2$] initially arising from reaction of cisplatin with GSH) supported of thesis that bulkier nHaza sterically blocks the Pt atom in *cis*-[PtCl(SG)(*n*Haza)$_2$] more than a smaller NH_3 group in *cis*-[PtCl(SG)$(NH_3)_2$].

In sum, the steric properties of the N-donor ligands appear to be the dominating factor in determining the substitution kinetics of cisplatin and complexes **3** and **4** when bridged species (polymers) with the 1:2 Pt:GSH stoichiometry are formed.

Chapter 6

Conclusion

The results described and discussed in this thesis demonstrated that:

1. Trinuclear complex **1** forms in polymeric DNA mainly trifunctional intrastrand cross-links, which means that all three Pt^{II} centers of this complex are coordinated to DNA preferentially at guanine residues.

2. The adducts of trinuclear complex **1** induced in DNA conformational distortion, which are much more delocalized and extended over markedly more base pairs around the platination sites that in case of cisplatin.

3. Tridentate adducts of trinuclear complex **1** markedly unwind DNA double helix and thermally destabilize it.

4. Trinuclear tridentate complex **1** can form specific DNA lesions, which can efficiently cross-link proteins to DNA. It is an intriguing aspect of this unique DNA binding mode of complex **1**.

5. Another aspect of this trinuclear complex **1**, which is even more interesting and observed for the first time for antitumor trinuclear platinum complexes is that complex **1** can form interduplex interstrand cross-links. In a high yield trinuclear complex **1** can cross-link two DNA duplexes under the molecular crowding conditions mimicking environmental conditions in cellular nucleus.

6. Thus, the unique DNA binding mode of complex **1** with semirigid aromatic linkers can expand the theoretical background needed for the design of new compounds which exhibit a variety of biological effects and can be useful in nucleic acids research.

7. The dinuclear complex **2** forms DNA adducts, which distort DNA conformation differently than adducts of cisplatin.

8. Monofunctional DNA adducts of dinucler complex **2** are converted to more toxic bifunctional cross-links markedly more slowly than in case of cisplatin.

9. The binding of dinuclear complex **2** to DNA in tumor cells is inactivated by sulfur-containing compounds (GSH or MT-2) markedly more than that of cisplatin.

10. The results focused on the mechanism underlying biological effects of dinuclear complexes, such as dinuclear bifunctional azolato-bridged platinum compounds, should provide a more rational basis for the design of new antitumor metallodrugs and chemotherapeutic strategies.

11. Cisplatin analogues **3** and **4** show retarded reactivity toward sulfur-containing compounds (GSH) which may contribute to promising antitumor activity of these metallodrugs.

Bibliography

[1] Peyrone, M., *Annalen der Chemie und Pharmacie*, Band LI, 1ff, 1844.

[2] Renshaw, E., and Thomson, A. J., Tracer studies to locate the site of platinum ions within filamentous and inhibited cells of Escherichia coli. *Journal of Bacteriology*, 94, 6, pp. 1915-1918, 1967.

[3] Rosenberg B., VanCamp L., and Krigas Th., Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode. *Nature*, 205, pp. 698-699, 1965.

[4] Rosemberg, B., VanCamp, L., Grimley, E. B., and Thomson, A. J., The inhibition of growth or cell division in Escherichia coli by different ionic species of platinum(IV) complexes. *The Journal of Biolog. Chem.*, 242, 6, pp. 1347-1352, 1967.

[5] Rosemberg, B., Renshaw, E., VanCamp, L., Hartwick, J., and Drobnik, J., Platinum-induced filamentous growth in Escherichia coli. *Journal of Bacteriology*, 93, 2, pp. 716-721, 1967.

[6] Wikipedia. Cisplatin — Wikipedia, the free encyclopedia, 2013. [Online; accessed 07-May-2013].

[7] Kociba, R. J., Sleight, S.D., and Rosenberg, B., Inhibition of Dunning asc itic leukemia and Walker 256 carcinosarcoma with cis-diamminedichloroplatinum (NSC-119875). *Cancer Chemotherapy Reports*, 54, 5, pp. 325-328, 1970.

[8] Rosenberg, B., Possible mechanisms for the antitumor activity of platinum coordination complexes. *Cancer Chemotherapy Reports*, 59, 3, pp. 589-598, 1975.

[9] Rosenberg, B., Platinum complexes for the treatment of cancer. *Interdisciplinary Scince Reviews*, 3, 2, pp. 134-147, 1978.

[10] Rosenberg, B., *Biochemie*, 60, pp. 859-867, 1978.

[11] Rosenberg, B., VanCamp, L., Trosko, E.J., and Mansour, V.H., Platinum compounds: a new class of potent antitumour agents. *Nature*, 222, pp. 385-386, 1969.

[12] Rosenberg, B., and VanCamp, L., The successful regression of large solid sarcoma 180 tumors by platinum compounds. *Cancer Res.*, 30, 6, pp. 1799-1802, 1970.

[13] Rozencweig, M., von Hoff, D. D., and Slovik, M., Cisdiamminedichloroplatinum(II), a new anticancer drug. *Ann. Intern. Med.*, 86, pp. 803-812, 1987.

[14] Brabec, V., DNA modifications by antitumor platinum and ruthenium compounds: their recognition and repair. *Prog. Nucleic Acid Res. Mol. Biol.*, 71, pp. 1-68, 2002.

[15] Weiss, R. B., and Christian, M. C., New cisplatin analogues in development. A review. *Drugs*, 46, 3, pp. 360-377, 1993.

[16] Lebwohl, D., and Canetta, R., Clinical development of platinum complexes in cancer therapy: an historical perspective and an update. *Eur. J. Cancer*, 34, 10, pp. 1522-1534, 1998.

[17] Fuertes, M. A., Alonso, C., and Perez, J. M., Biochemical modulation of Cisplatin mechanisms of action: enhancement of antitumor activity and circumvention of drug resistance. *Chemical Reviews*, 103, 3, pp. 645-662, 2003.

[18] Kelland, L. R., Sharp, S. Y., O'Neill, C. F., Raynaud, F. I., Beale, P. J., and Judson, I. R., Mini-review: discovery and development of platinum complexes designed to circumvent cisplatin resistance. *Journal of Inorg. Biochem.*, 77, 1-2, pp. 111-115, 1999.

[19] Wong, E., and Giandomenico, C. M., Current status of platinum-based antitumor drugs. *Chemical Reviews*, 99, 9, pp. 2451-2466, 1999.

[20] Wikipedia. Carboplatin — Wikipedia, the free encyclopedia, 2013. [Online; accessed 07-May-2013].

[21] B. Lippert. *Cisplatin*. WILEY-VCH, 1999.

[22] Harrap K. R., Preclinical studies identifying carboplatin as a viable cisplatin alternative. *Cancer Treatment Reviews*, 12, Suppl. A, pp. 21-33, 1985.

[23] Aberts, D. S., Green, S., Hannigan, E. V., Otool, R., Stock-Novack, D., Anderson, P., Surwite, A., Malvlya, V. K., Nahas, W. A., and Jolles, C. J., Improved therapeutic index of carboplatin plus cyclophosphamide versus cisplatin plus cyclophosphamide: final report by the Southwest Oncology Group of a phase III randomized trial in stages III and IV ovarian cancer. *Journal of Clin. Oncology*, 10, 5, pp. 706-717, 1992.

[24] Wikipedia. Oxaliplatin — Wikipedia, the free encyclopedia, 2013. [Online; accessed 07-May-2013].

[25] Tashiro, T., Kowada, Y., Sakurai, Y., and Kidani, Y., Antitumor activity of a new platinum complex, oxalato (trans-l-1,2-diaminocyclohexane)platinum (II): new experimental data. *Biomedicine & Pharmacotherapy*, 43, 4, pp. 251-260, 1989.

[26] Mamenta, E. L., Poma, E. E., Kaufmann, W. K., Delmastro, D. A., Grady, H. L., and Chaney, S. G., Enhanced replicative bypass of platinum-DNA adducts in cisplatin-resistant human ovarian carcinoma cell lines. *Cancer Research*, 54, 13, pp. 3500-3505, 1994.

[27] Jamieson, E. R., and Lippard, S. J., Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chemical Reviews*, 99, 9, pp. 2467-2498, 1999.

[28] Siddik, Z. H., Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene*, 22, 47, pp. 7265-7279, 2003.

[29] Howe-Grant, M. E., and Lippard, S. J., In Metal Ions in Biological Systems (Sigel, H., ed.), 11, pp. 63-125, 1980, New York.

[30] Lim, M. C., and Martin, R. B., The nature of cis amine Pd(II) and antitumor cis amine Pt(II) complexes in aqueous solutions. *J. Inorg. Nucl. Chem.*, 38, 10, 1911-1914, 1976.

[31] Jung, Y., and Lippard, S. J., Direct cellular responses to platinum-induced DNA damage. *Chemical Reviews*, 107, 5, pp. 1387-1407, 2007.

[32] Dedon, P. C., and Borch, R. F., Characterization of the reactions of platinum antitumor agents with biologic and nonbiologic sulfur-containing nucleophiles. *Biochemical Pharmacology*, 36, 12, pp. 1955-1964, 1987.

[33] Dolgova, N. V., Olson, D., Lutsenko, S., and Dmitriev, O. Y., The soluble metal-binding domain of the copper transporter ATP7B binds and detoxifies cisplatin. *The Biochemical Journal*, 419, 1, pp. 51-56, 2009.

[34] Katano, K., Kondo, A., Safaei, R., Holzer, A., Samimi, G., Mishima, M., Kuo, Y. M., Rochdi, M., and Howell, S. B., Acquisition of resistance to cisplatin is accompanied by changes in the cellular pharmacology of copper. *Cancer Research*, 62, 22, pp. 6559-6565, 2002.

[35] Komatsu, M., Sumizawa, T., Mutoh, M., Chen, Z. S., Terada, K., Furukawa, T., Yang, X. L., Gao, H., Miura, N., Sugiyama, T., and Akiyama, S., Copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with cisplatin resistance. *Cancer Research*, 60, 5, pp. 1312-1316, 2000.

[36] Kelland, L., The resurgence of platinum-based cancer chemotherapy. *Nature Reviews Cancer*, 7, 8, pp. 573-584, 2007.

[37] Fichtinger-Schepman, A. M., Van der Veer, J. L., Den Hartog, J. H., Lohman, P. H., and Reedijk J., Adducts of the antitumor drug cis-diamminedichloroplatinum(II) with DNA: Formation, identification, and quantitation. *Biochemistry*, 24, 3, pp. 707-713, 1985.

[38] Eastman, A., The formation, isolation and characterization of DNA adducts produced by anticancer platinum complexes. *Pharmacol. Ther.*, 34, 2, pp. 155-166, 1987.

[39] Kartalou, M., and Essigmann, J. M., Recognition of cisplatin adducts by cellular proteins. *Mutation Research*, 478, 1-2, pp. 1-21, 2001.

[40] Brabec, V., Chemistry and structural biology of 1,2-interstrand adducts of cisplatin. *Platinum-Based Drugs in Cancer Therapy*, pp. 37-61, Humana Press Inc., Totowa, New Jersey, 2000.

[41] Vrana, O., Boudny, V., and Brabec, V., Superhelical torsion controls DNA interstrand crosslinking by antitumor c/s-diamminedichloroplatinum(II). *Nucleic Acids Research*, 24, 20, pp. 3918-3925, 1996.

[42] Chvalova, K., Brabec V., and Kasparkova, J., Mechanism of the formation of DNA-protein cross-links by antitumor cisplatin. *Nucleic Acids Res.*, 35, 6, pp. 1812-21, 2007.

[43] Eastman A., Jennerwein, M. M., and Nagel, D. L., Characterization of bifunctional adducts produced in DNA by trans-diamminedichloroplatinum(II). *Chemico-Biological Interactions*, 67, 1-2, pp. 71-80, 1988.

[44] Brabec, V., and Leng, M., DNA interstrand cross-links of trans-diamminedichloroplatinum(II) are preferentially formed between guanine and complementary cytosine residues. *Proc. Natl. Acad. Sei. U.S.A.*, 90, 11, pp. 5345-5349, 1993.

[45] Boudvillain, M., Dalbies, B., Aussourd, C., and Leng, M., Intrastrand cross-links are not formed in the reaction between transplatin and native DNA: Relation with the clinical inefficiency of transplatin. *Nucleic Acids Research*, 23, 13, pp. 2381-2388, 1995.

[46] Cohen, G. L., Bauer, W. R., Barton, J. K., and Lippard, S. J., Binding of cis- and trans-dichlorodiammineplatinum(II) to DNA: evidence for unwinding and shortening of the double helix. *Science*, 203, 4384, pp. 1014-1016, 1979.

[47] Poklar, N., Pilch, D. S., Lippard, S. J., Redding, E. A., Dunham, S. U., and Breslauer, K. J., Influence of cisplatin intrastrand crosslinking on the conformation, thermal stability, and energetics of a 20-mer DNA duplex. *Proc. Natl. Acad. Sci. U.S.A.*, 93, 15, pp. 7606-7611, 1996.

[48] van Garderen, C. J., Van den Elst, H., Van Boom, J. H., Reedijk, J., and Van Houte, L. P. A., A double-stranded DNA fragment shows a significant decrease in double-helix stability after binding of monofunctional platinum amine compounds. *J. Am. Chem. Soc.*, 111, 11, pp. 4123-4125, 1989.

[49] Brabec, V., Reedijk, J., and Leng, M., Sequence-dependent distortions induced in DNA by monofunctional platinum(II) binding. *Biochemistry*, 31, 49, pp. 12397-12402, 1992.

[50] Todd, R. C., and Lippard, S. J., Inhibition of transcription by platinum antitumor compounds. *Metallomics*, 1, 4, pp. 280-291, 2009.

[51] Bellon, S. F., and Lippard, S. J., Bending studies of DNA site-specifically modified by cisplatin, trans-diamminedichloroplatinum(II) and cis-[Pt(NH3)2(N3-cytosine)Cl]+. *Biophys. Chem.*, 35, 2-3, pp. 179-188, 1990.

[52] Teuben, J. M., Bauer, C., Wang, A. H. J., and Reedijk, J., Solution structure of a DNA duplex containing a cis-diammineplatinum(II) 1,3-d(GTG) intrastrand cross-link, a major adduct in cells treated with the anticancer drug carboplatin. *Biochemistry*, 38, 38, pp. 12305-12312, 1999.

[53] Coste, F., Malinge, J. M., Serre, L., Shepard, W., Roth, M., Leng, M., and Zelwer, C., Crystal structure of a double-stranded DNA containing a cisplatin interstrand cross-link at 1.63 A resolution: hydration at the platinated site. *Nucleic Acids Res.*, 27, 8, pp. 1837-46, 1999.

[54] Shimizu, M., and Rosenberg, B., A similar action to UV-irradiation and a preferential inhibition of DNA synthesis in E. coli by antitumor platinum compounds. *The Journal of Antibiotics*, 26, 4, pp. 243-245, 1976, Tokyo.

[55] Comess, K. M., Burstyn, J. N., Essigmann, J. M., and Lippard, S. J., Replication inhibition and translesion synthesis on templates containing site-specifically placed cis-diamminedichloroplatinum(II) DNA adducts. *Biochemistry*, 31, 16, pp. 3975-3990, 1992.

[56] Sorenson, C. M., and Eastman, A., Influence of cis-diamminedichloroplatinum(II) on DNA synthesis and cell cycle progression in excision repair proficient and deficient Chinese hamster ovary cells. *Cancer Research*, 48, 23, pp. 6703-6707, 1988.

[57] Grossmann, K. F., Ward, A. M., and Moses, R. E., Saccharomyces cerevisiae lacking Snm1, Rev3 or Rad51 have a normal S-phase but arrest permanently in G2 after cisplatin treatment. *Mutation Research*, 461, 1, pp. 1-13, 2000.

[58] Corda, Y., Job, C., Anin, M. F. Leng, M., and Job, D., Spectrum of DNA–platinum adduct recognition by prokaryotic and eukaryotic DNA-dependent RNA polymerases. *Biochemistry*, 32, 33, pp. 8582-8588, 1993.

[59] Lemaire, M. A., Schwartz, A., Rahmouni, A. R., and Leng, M., Interstrand cross-links are preferentially formed at the d(GC) sites in the reaction between cis-diamminedichloroplatinum (II) and DNA. *Proc. Natl. Acad. Sci. U.S.A.*, 88, 5, pp. 1982-1985, 1991.

[60] Mymryk, J. S., Zaniewski, E., and Archer, T. K., Cisplatin inhibits chromatin remodeling, transcription factor binding, and transcription from the mouse mammary tumor virus promoter in vivo. *Proc. Natl. Acad. Sci. U.S.A.*, 92, 6, pp. 2076-2080, 1995.

[61] Mello, J. A., Lippard, S. J., and Essigmann, J. M., DNA adducts of cis-diamminedichloroplatinum(II) and its trans isomer inhibit RNA polymerase II differentially in vivo. *Biochemistry*, 34, 45, pp. 14783-14791, 1995.

[62] Wozniak, K., and Blasiak, J., Recognition and repair of DNA-cisplatin adducts. *Acta Biochim. Pol.*, 49, 3, pp. 583-596, 2002.

[63] Weaver, D. A., Crawford, E. L., Warner, K. A., Elkhairi, F., Khuder, S. A., and Willey, J. C., ABCC5, ERCC2, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. *Mol. Cancer*, 4, 1, pp. 18, 2005.

[64] Ferry, K. V., Hamilton, T. C., and Johnson, S. W., Increased nucleotide excision repair in cisplatin-resistant ovarian cancer cells: role of ERCC1-XPF. *Biochem. Pharmacol.*, 60, 9, pp. 1305-1313, 2000.

[65] Huang, C. J., Zamble, J. T., Reardon, J. T., Lipard, S. J., and Sancar, A., HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. *Proc. Natl. Acad. Sci. U.S.A.*, 91, 22, pp. 10394-10398, 1994.

[66] Zamble, D. B., Mu, D., Reardon, T. J., Sancar, A., and Lippard, S. J., Repair of cisplatin–DNA adducts by the mammalian excision nuclease. *Biochemistry*, 35, 31, pp. 10004-10013, 1996.

[67] Bessho, T., Mu, D., and Sanncar, A., Initiation of DNA interstrand cross-link repair in humans: the nucleotide excision repair system makes dual incisions 5' to the cross-linked base and removes a 22- to 28-nucleotide-long damage-free strand. *Mol. Cell. Biol.*, 17, 12, pp. 6822-6830, 1997.

[68] Riedl, T., Hanaoka, F., and Egly, J. M., The comings and goings of nucleotide excision repair factors on damaged DNA. *The EMBO Journal*, 22, 19, pp. 5293-5303, 2003.

[69] You, J. S., Wang, M., and Lee, S. H., Biochemical analysis of the damage recognition process in nucleotide excision repair. *J. Biol. Chem.*, 278, 9, pp. 7476-7485, 2003.

[70] Leonetti, J. P., Mechti, N., Degols, G., Gagnor, C., and Lebleu, B., Intracellular distribution of microinjected antisense oligonucleotides. *Proc. Natl. Acad. Sci. U.S.A.*, 88, 7, pp. 2702-2706, 1991.

[71] Friedberg, E. C., How nucleotide excision repair protects against cancer. *Nature Reviews Cancer*, 1, 1, pp. 22-33, 2001.

[72] Takahara, P. M., Rosenzweig, A. C., Frederick, C. A., and Lippard, S. J., Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. *Nature*, 377, 6550, pp. 649-652, 1995.

[73] Silva, M. J., Costa, P., Dias, A., Valente, M., Louro, H., and Boavida, M. G., Comparative analysis of the mutagenic activity of oxaliplatin and cisplatin in the Hprt gene of CHO cells. *Environ. Mol. Mutagen.*, 46, 2, pp. 104-115, 2005.

[74] Chaney, S. G., Campbell, S. L., Bassett, E., and Wu, Y., Recognition and processing of cisplatin- and oxaliplatin-DNA adducts. *Crit. Rev. Oncol. Hematol.*, 53, 1, pp. 3-11, 2005.

[75] Fink, D., Zheng, H., Nebel, S., Norris, P., Abei, S., Lin, T. P., Nehme, A., Chirsten, R. D., Haas, M., and Macleod, C. L., In vitro and in vivo resistance to cisplatin in cells that have lost DNA mismatch repair. *Cancer Research*, 57, 10, pp. 1841-1845, 1997.

[76] Scheef, E. D., Briggs, J. M., and Howell, S. B., Molecular modeling of the intrastrand guanine-guanine DNA adducts produced by cisplatin and oxaliplatin. *Mol. Pharmacol.*, 56, 3, pp. 633-643, 1999.

[77] Vaisman, A., Varchenko, M., Umar, A., Kunkel, T. A., Risinger, J. I., Barret, J. C., Hamilton, T. C., and Chaney, S. G., The role of hMLH1, hMSH3, and hMSH6 defects in cisplatin and oxaliplatin resistance: correlation with replicative bypass of platinum-DNA adducts. *Cancer Res.*, 58, 16, pp. 3579-3585, 1998.

[78] Zdraveski, Z. Z., Mello, J. A., Fairnelli, C. K., Essigmann, J. M., and Marinus, M. G., MutS preferentially recognizes cisplatin- over oxaliplatin-modified DNA. *J. Biol. Chem.*, 277, 2, pp. 1255-1260, 2002.

[79] Yamada, M., O'Regan, E., Brown, R., and Karran, P., Selective recognition of a cisplatin-DNA adduct by human mismatch repair proteins. *Nucleic Acids Research*, 25, 3, pp. 491-496, 1997.

[80] Raymond, E. Faivre, S., Chaney, S., Woynarowski, J., and Cvitkovic, E., Cellular and molecular pharmacology of oxaliplatin. *Mol. Cancer Therapeut.*, 1, 3, pp. 227-235, 2002.

[81] Aebi, S., Kurdi-Haidar, B., Gordon, R., Cenni, B., Zheng, H., Fink, D., Christin, R. D., Boland, C. R., Koi, M., Fishel, R., and Howell, S. B., Loss of DNA mismatch repair in acquired resistance to cisplatin. *Cancer Research*, 56, 13, pp. 3087-3090, 1996.

[82] Fink, D., Nebel, S., Aebi, S., Zheng, H., Cenni, B., Nehme, A., Christin, R. D., and Howell, S. B., The role of DNA mismatch repair in platinum drug resistance. *Cancer Research*, 56, 21, pp. 4881-4886, 1996.

[83] Lin, D. P., Wang, Y., Scherer, S. J., Clark, A. B., Yang, K., Avdievich, E., Jin, B., Werling, U., Parris, T., Kurihara, N., Umar, A., Kucherlapati, R., Lipkin, M., Kunkel, T. A., and Edelmann, W., An Msh2 point mutation uncouples DNA mismatch repair and apoptosis. *Cancer Research*, 64, 2, pp. 517-522, 2004.

[84] Nehme, A., Baskaran, R., Abei, S., Fink, D., Nebel, S., Cenni, B., Wang, Y., Howell, S. B., and Christen, R. D., Differential induction of c-Jun NH2-terminal kinase and c-Abl kinase in DNA mismatch repair-proficient and -deficient cells exposed to cisplatin. *Cancer Research*, 57, 15, pp. 3253-3257, 1997.

[85] Nehme, A., Baskaran, R., Nebel, S., Fink, D., Howell, S. B., Wang, Y. J., and Christen, R. D., Induction of JNK and c-Abl signalling by cisplatin and oxaliplatin in mismatch repair-proficient and -deficient cells. *Br. J. Cancer*, 79, 7-8, pp. 1104-1110, 1999.

[86] Li, G. M., The role of mismatch repair in DNA damage-induced apoptosis. *Oncol. Res.*, 11, 9, pp. 393-400, 1999.

[87] Johns, E. W., The HMG Chromosomal Proteins, Academic Press, 1982, New York.

[88] Grosschedl, R., Giese, K., and Pagel, J., HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. *Trends Genet.*, 10, 3, pp. 94-100, 1994.

[89] Pil, P. M., and Lippard, S. J., Specific binding of chromosomal protein HMG 1 to DNA damaged by the anticancer drug cisplatin. *Science*, 256, 5054, pp. 234-237, 1992.

[90] Travers, A. A., Ner, S. S. and Churchill, M. E. A., DNA chaperones: a solution to a persistence problem?. *Cell*, 77, 2, pp. 167-169, 1994.

[91] King, C. Y., and Weiss, M. A., The SRY high-mobility-group box recognizes DNA by partial intercalation in the minor groove: a topological mechanism of sequence specificity. *Proc. Natl. Acad. Sci. U.S.A.*, 90, 24, pp. 11990-11994, 1993.

[92] Pil, P. M., Chow, C. S., and Lippard, S. J., High-mobility-group 1 protein mediates DNA bending as determined by ring closures. *Proc. Natl. Acad. Sci. U.S.A.*, 90, 20, pp. 9465-9469, 1993.

[93] Chow, C. S., Whitehead, J. P., and Lippard, S. J., HMG domain proteins induce sharp bends in cisplatin-modified DNA. *Biochemistry*, 33, 50, pp. 15124-15130, 1994.

[94] Chow, C. S., Barnes, C. M., and Lippard, S. J., A single domain in the high-mobility group 1 protein binds DNAs as small as 20 base pairs containing the major cisplatin adduct. *Biochemistry*, 34, 9, pp. 2956-2964, 1995.

[95] Kasparkova, J., Brabec, V., Recognition of DNA interstrand cross-links of cis-diamminedichloroplatinum(II) and its trans isomer by DNA-binding proteins. *Biochemistry*, 34, 38, pp. 12379-12387, 1995.

[96] Trimmer, E. E., Zamble, D. B., Lippard, S. J., and Essigmann, J. M., Human testis-determining factor SRY binds to the major DNA adducts of cisplatin and a putative target sequence with comparable affinities. *Biochemistry*, 37, 1, pp. 352-362, 1998.

[97] Trimmer, E. E., Essigmann, J. M., Cisplatin. *Essays Biochem.*, 34, pp. 191-211, 1999.

[98] Arioka, H., Nishio, K., Ishida, T., Fukumoto, H., Fukuoka, K., Nomoto, T., Kurokawa, H., Yokote, H., Abe, S., and Saijo, N., Enhancement of cisplatin sensitivity in high mobility group 2 cDNA-transfected human lung cancer cells. *Jpn. J. Cancer Res.*, 90, 1, pp. 108-115, 1999.

[99] Zlatanova, J., van Holde, K., The linker histones and chromatin structure: new twists. *Prog., Nucl. Acid Res., Mol. Biol.*, 52, pp. 217-259, 1996.

[100] Wolffe, A., Chromatin Structure and Function, Academic Press, 2nd ed., 1995, London.

[101] Meister, A., and Anderson, M. E., Glutathione. *Annu. Rev. Biochem.*, 52, pp.. 711-760, 1983.

[102] Ishikawa, T., and Ali-Osman, F., Glutathione-associated cis-diamminedichloroplatinum(II) metabolism and ATP-dependent efflux from leukemia cells. Molecular characterization of glutathione-platinum complex and its biological significance. *J. Biol. Chem.* 268, 27, pp. 20116-20125, 1993.

[103] Lai, G., Ozols, R., Young, R., and Hamilton, T. C., Effect of glutathione on DNA repair in cisplatin-resistant human ovarian cancer cell lines. *J. Natl. Cancer Inst.*, 81, 7, pp. 535-539, 1989.

[104] Hamer, D., Metallothionein1,2. *Annual Review of Biochemistry*, 55, pp. 913-955, 1986.

[105] Andrews, P., Murphy, M., and Howell, S., Metallothionein-mediated cisplatin resistance in human ovarian carcinoma cells. *Cancer Chemother. Phormacol.*, 19, 2, pp. 149-154, 1987.

[106] Pasetto, L. M., D'Andrea, M. R., Brandes, A. A., Rossi, E., Monfardini, S., The development of platinum compounds and their possible combination. *Critical Reviews in Oncology/Hematology*, 60, 1, pp. 59-75, 2006.

[107] Andrews, P. A., and Howell, S. B., Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. *Cancer Cells*, 2, 2, pp. 35-43, 1990.

[108] Mistry, P., Kelland, L. R., Abel, G., Sidhar, S., and Harrap, K. R., The relationships between glutathione, glutathione-S-transferase and cytotoxicity of platinum drugs and melphalan in eight human ovarian carcinoma cell lines. *Br. J. Cancer*, 64, 2, pp. 215-220, 1991.

[109] Hosking, L. K., Whelan, R. D. H., Shellard, S. A., Bedford, P., and Hill, B.T., An evaluation of the role of glutathione and its associated enzymes in the expression of differential sensitivities to antitumour agents shown by a range of human tumour cell lines. *Biochem. Pharmacol.*, 40, 8, pp. 1833-1842, 1990.

[110] Meister, A., Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacol. Ther.*, 51, 2, pp. 155-194, 1991.

[111] Andrews, P., Murphy, M., and Howell, S., Differential potentiation of alkylating and platinating agent cytotoxicity in human ovarian carcinoma cells by glutathione depletion. *Cancer Res.*, 46, 12 Pt 1, pp. 6250-6253, 1985.

[112] Kelley, S. L., Basu, A., Teicher, B. A., Hacker, M. P., Hamer, D. H., and Lazo, J. S., Overexpression of metallothionein confers resistance to anticancer drugs. *Science*, 241, 4874, pp. 1813-1815, 1988.

[113] Meyers, M., Hwang, A., Wagner, M. W., and Boothman, D. A., Role of DNA mismatch repair in apoptotic responses to therapeutic agents. *Environ. Mol. Mutagen.*, 44, 4, pp. 249-264, 2004.

[114] Wang, D., and Lippard, S. J., Cellular processing of platinum anticancer drugs. *Nature Rev. Drug Disc.*, 4, 4, pp. 307-320, 2005.

[115] Gonzalez, V. M., Fuertes, M. A., Alonso, and C. Perez, J. M., Is cisplatin-induced cell death always produced by apoptosis?. *Mol. Pharmacol.*, 59, 4, pp. 657-663, 2001.

[116] Perdomo-Arciniegas, A. M., Patarroyo, M. E., and Vernot, J.-P., Novel chimeric peptide inhibits Protein Kinase C and induces apoptosis in human immune cells. *Int. J. Pept. Res. Ther.*, 14, pp. 64-74, 2008.

[117] Kim, R., Tanabe, K., Uchida, Y., Emi, M., Inoue, H., and Toge, T., Current status of the molecular mechanisms of anticancer drug-induced apoptosis. The contribution of molecular-level analysis to cancer chemotherapy. *Cancer Chemother. Pharmacol.*, 50, 5, pp. 343-352, 2002.

[118] Henkels, K. M., and Turchi, J. J., Cisplatin-induced apoptosis proceeds by caspase-3-dependent and -independent pathways in cisplatin-resistant and -sensitive human ovarian cancer cell lines. *Cancer Research*, 59, 13, pp. 3077-3083, 1999.

[119] Manic, S., Gatti, L., Carenini, N., Fumagalli, G., Zunino, F., and Perego, P., Mechanisms controlling sensitivity to platinum complexes: role of p53 and DNA mismatch repair. *Curr. Cancer Drug Targets*, 3, 1, pp. 21-29, 2003.

[120] Laptenko, O., and Prives, C., Transcriptional regulation by p53: one protein, many possibilities. *Cell Death Differ.*, 13, 6, pp. 951-961, 2006.

[121] Segal-Bendirdjian, E., Mannone, L., and Jacquemin-Sablon, A., Alteration in p53 pathway and defect in apoptosis contribute independently to cisplatin-resistance. *Cell Death Differ.*, 5, 5, pp. 390-400, 1998.

[122] Slee, E. A., O'Connor, D. J., and Lu, X., To die or not to die: how does p53 decide? *Oncogene*, 23, 16, pp. 2809-2818, 2004.

[123] Nakamura, T., Ide, H., Hayashi, K., Euchi, R., and Takasaki, K., Expression of apoptosis-related proteins (p53, p21WAF1, bcl-2, and bax) and sensitivity to chemotherapy in squamous cell carcinoma of the esophagus. *Int. J. Clin. Oncol.*, 5, 2, pp. 89-96, 2000.

[124] Kanamori, Y., Kigawa, J., Minagawa, Y., Irie, T., Oishi, T., Shimada, M., Takahashi, M., Nakamura, T., Sato, K., and Terakawa, N., A newly developed adenovirus-mediated transfer of a wild-type p53 gene increases sensitivity to cis-diamminedichloroplatinum (II) in p53-deleted ovarian cancer cells. *Eur. J. Cancer*, 34, 11, pp. 1802-1806, 1998.

[125] Kigawa, J., Sato, S., Shimada, M., Kanamori, Y., Itamochi, H., and Terakawa, N., Effect of p53 gene transfer and cisplatin in a peritonitis carcinomatosa model with p53-deficient ovarian cancer cells. *Gynecol. Oncol.*, 84, 2, pp. 210-215, 2002.

[126] Naniwa, J., Kigawa, J., Akeshima, R., Kanamori, Y., Itamochi, H., Oishi, T., Iba, T., and Terakawa, N., Leptomycin B enhances CDDP-sensitivity via nuclear accumulation of p53 protein in HPV-positive cells. *Cancer Science*, 94, 12, pp. 1099-1103, 2003.

[127] Perego, P., Giarola, M., Righetti, S. C., Supino, R., Caserini, C., Delia, D., Pierotti, M. A., Miyashita, T., Reed, J. C., and Zunino, F., Association between cisplatin resistance and mutation of p53 gene and reduced bax expression in ovarian carcinoma cell systems. *Cancer Research*, 56, 3, pp. 556-562, 1996.

[128] Fan, J., and Bertino, J. R., Modulation of cisplatinum cytotoxicity by p53: effect of p53-mediated apoptosis and DNA repair. *Mol. Pharmacol.*, 56, 5, pp. 966-972, 1999.

[129] Minagawa, Y., Kigawa, J., Itamochi, H., Kanamori, Y., Shimada, M., Takahashi, M., and Terakawa, N., Cisplatin-resistant HeLa cells are resistant to apoptosis via p53-dependent and -independent pathways. *Jpn. J. Cancer Res.*, 90, 12, pp. 1373-1379, 1999.

[130] Baeuerle, P. A., and Baltimore, D. NF-κB: ten years after. *Cell*, 87, 1, pp. 13-20, 1996.

[131] Baud, V., and Karin, M., Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls, *Nat. Rev. Drug Discov.*, 8, 1, pp. 33-40, 2009.

[132] Aggarwal, B. B., Vijayalekshmi, R. V., and Sung, B., Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clin. Cancer Res.*, 15, 2, pp. 425-430, 2009.

[133] Kreuz, S., Siegmund, D., Scheurich, P., and Wajant, H. NFκB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. *Mol Cell Biol*, 21, 12, pp. 3964-3973, 2001.

[134] Micheau, O., Lens, S., Gaide, O., Alevizopoulos, K., and Tschopp, J. NF-κB signals induce the expression of c-FLIP. *Mol Cell Biol*, 21, 16, pp. 5299-5305, 2001.

[135] Dolcet, X., Llobet, D., Pallares, J., and Matias-Guiu, X., NF-κB in development and progression of human cancer. *Virchows Archive*, 446, 5, pp. 475-482, 2005.

[136] Deveraux, Q. L., Roy N., Stennicke, H. R., Van Arsdale, T., Zhou, Q., Srinivasula, S. M., Alnemri, E. S., Salvesen, G. S., and Reed, J. C., IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *The EMBO Journal*, 17, 8, pp. 2215-2223, 1998.

[137] Takahashi, R., Deveraux, Q., Tamm, I., Welsh, K., Assa-Munt, N., Salvesen, G. S., and Reed, J. C., A single BIR domain of XIAP sufficient for inhibiting caspases. *J Biol Chem*, 273, 14, pp. 7787-7790, 1998.

[138] Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V., and Baldwin, A. S. Jr, NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science*, 281, 5383, pp. 1680-1683, 1998.

[139] Chen, Q. M., and Tu, V.C., Apoptosis and heart failure: mechanisms and therapeutic implications. *Am J Cardiovasc Drugs*, 2, 1, pp. 43-57, 2002.

[140] Lee, J. U., Hosotani, R., Wada, M., Doi, R., Kosiba, T., Fujimoto, K., Miyamoto, Y., Tsuji, S., Nakajima, S., Nishimura, Y., and Imamura, M., Role of Bcl-2 family proteins (Bax, Bcl-2 and Bcl-X) on cellular susceptibility to radiation in pancreatic cancer cells. *Eur J Cancer*, 35, 9, pp. 1374-1380, 1999.

[141] Chen, F., Wang, M., O'Connor, J. P., He, M., Tripathi, T., and Harrison, L. E., Phosphorylation of PPARgamma via active ERK1/2 leads to its physical association with p65 and inhibition of NF-kappabeta. *J Cell Biochem*, 90, 4, pp. 732-744, 2003.

[142] Mukhopadhyay, T., Roth, J. A., and Maxwell, S. A., Altered expression of the p50 subunit of the NF-κB transcription factor complex in non-small cell lung carcinoma. *Oncogene*, 11, 5, pp. 999-1003, 1995.

[143] Sovak, M. A., Bellas, R. E., Kim, D. W., Zanieski, G. J., Rogers, A. E., Traish, A. M., and Sonenshein, G. E. Aberrant nuclear factor-κB/ Rel expression and the pathogenesis of breast cancer. *J Clin Invest*, 100, 12, pp. 2952-2960, 1997.

[144] Thornburg, N. J., Pathmanathan, R., and Raab-Traub, N. Activation of nuclear factor-κB p50 homodimer/Bcl-3 complexes in nasopharyngeal carcinoma. *Cancer Research*, 63, 23, pp. 8293-8301, 2003.

[145] Brabec, V., and Kasparkova, J., Molecular aspects of resistance to antitumor platinum drugs. *Drug Resist. Updates*, 5, 3-4, pp. 147-161, 2002.

[146] Reedijk, J., New clues for platinum antitumor chemistry: kinetically controlled metal binding to DNA. *Proc. Natl. Acad. Sci. U.S.A.*, 100, 7, pp. 3611-3616, 2003.

[147] Wheate, N. J., and Collins, J. G., Multi-nuclear platinum complexes as anticancer agents. *Coord. Chem. Rev.*, 241, 1-2, 133-145, 2003.

[148] Mangrum, J. B, and Farrell, N. P., Excursions in polynuclear platinum DNA binding. *Chemical Communications*, 46, 36, pp. 6640-6650, 2010.

[149] Olivova, R., Kasparkova, J., Vrana, O., Vojtiskova, M., Suchankova, T., Novakova, O., He, W., Guo, Z., and Brabec, V., Unique DNA Binding Mode of Antitumor Trinuclear Tridentate Platinum(II) Compound. *Molecular Pharmaceutics*, 8, 6, pp. 2368-2378, 2012.

[150] Qu, Y., Farrell, N., Interaction of bis(platinum) complexes with the mononucleotide 5'-guanosine monophosphate. Effect of diamine linker and the nature of the bis(platinum) complex on product formation. *J. Am. Chem. Soc.*, 113, 13, pp. 4851-4857, 1991.

[151] Kraker, A. J., Hoeschele, J. D., Elliott, W. L., Showalter, H. D. H., Sercel, D., N. P. Farrell, Anticancer activity in murine and human tumor cell lines of bis(platinum) complexes incorporating straight-chain aliphatic diamine linker groups. *J. Med. Chem.*, 35, 24, pp. 4526-4532, 1992.

[152] Brabec, V., Kasparkova, J., Vrana, O., Novakova, O., Cox, J. W., Qu, Y., Farrell, N., DNA modifications by a novel bifunctional trinuclear platinum phase I anticancer agent. *Biochemistry*, 38, 21, pp. 6781-6790, 1999.

[153] Komeda, S., Lutz, M., Spek, A. L., Yamanaka, Y., Sato, T., Chikuma, M., and Reedijk, J., A Novel Isomerization on Interaction of Antitumor-Active Azole-Bridged Dinuclear Platinum(II) Complexes with 9-Ethylguanine. Platinum(II) Atom Migration from N2 to N3 on 1,2,3-Triazole. *Journal of the American Chemical Society.* 124, 17, pp. 4738-4746, 2002.

[154] Oehlsen, M. E., Qu, Y. and Farrell, N., Reaction of polynuclear platinum anti-tumor compounds with reduced glutathione studied by multinuclear (1H, 1H-15N gradient heteronuclear single-quantum coherence, and 195Pt) NMR spectroscopy. *Inorg. Chem.*, 42, 18, pp.. 5498-5506, 2003.

[155] 3Dchem.com. Chemistry, structures and 3d molecules - *cis*-$\mathrm{ptcl_2(nh_3)_2}$. [Online; accessed 07-May-2013].

[156] Wikipedia. Triplatin tetranitrate — Wikipedia, the free encyclopedia, 2013. [Online; accessed 07-May-2013].

[157] Huq, F., Daghriri, H., Yu, J. Q., Tayyem, H., Beale, P., and Zhang, M., Synthesis, characterisation, activities, cell uptake and DNA binding of [[trans-PtCl(NH3)2] [μ-(H2N(CH2)6NH2)] [trans-PdCl(NH3)2](NO3)Cl. *European Journal of Medicinal Chemistry*, 39, 11, pp. 947-958, 2004.

[158] Zhao, Y., He, W., Shi, P., Zhu, J., Qiu, L., Linb, L., and Guo, Z. A positively charged trinuclear 3N-chelated monofunctional platinum complex with high DNA affinity and potent cytotoxicity. *Dalton Transactions*, 22, pp. 2617-2619, 2006.

[159] Farrell N., Polynuclear platinum drugs., *Metal Ions in Biological Systems*, 42, pp. 251-296, 2004.

[160] Montero, E. I., Benedetti, B. T., Mangrum, J. B., Oehlsen, M. J., Qu, Y., and Farrell, N. P., Pre-association of polynuclear platinum anticancer agents on a protein, human serum albumin. Implications for drug design. *J. Chem. Soc., Dalton Trans*, 43, pp. 4938-4942, 2007.

[161] Roberts, J. D., Peroutka, J., and Farrell, N., Cellular pharmacology of polynuclear platinum anti-cancer agents. *J. Inorg. Biochem*, 77, 1-2, pp. 51-57, 1999.

[162] Kasparkova, J., Zehnulova, J., Farrell, N., and Brabec, V., DNA interstrand cross-links of the novel antitumor trinuclear platinum complex BBR3464. Conformation, recognition by high mobility group domain proteins, and nucleotide excision repair. *J. Biol. Chem*, 277, 50, pp. 48076-48086, 2002.

[163] Kasparkova, J., Farrell, N., and Brabec, V., Sequence specificity, conformation, and recognition by HMG1 protein of major DNA interstrand cross-links of antitumor dinuclear platinum complexes. *J. Biol. Chem.*, 275, 21, pp. 15789-15798, 2000.

[164] Hegmans, A., Berners-Price, S. J., Davies, M. S., Thomas, D., Humphreys, A., and Farrell, N. P., Long range 1,4 and 1,6-interstrand cross-links formed by a trinuclear platinum complex. Minor groove preassociation affects kinetics and mechanism of cross-link formation as well as adduct structure. *J. Am. Chem. Soc.*, 126, 7, pp. 2166-2180, 2004.

[165] Cox, J. W., Berners-Price, S. J., Davies, M. S., Qu, Y., and Farrell, N., Kinetic analysis of the stepwise formation of a long-range DNA interstrand cross-link by a dinuclear platinum antitumor complex: evidence for aquated intermediates and formation of both kinetically and thermodynamically controlled conformers. *J. Am. Chem. Soc.*, 123, 7, pp. 1316-1326, 2001.

[166] Rich, A., The double helix: a tale of two puckers. *Nat. Struct. Biol*, 10, 4, pp. 247-249, 2003.

[167] Gelasco, A., and Lippard, S. J., NMR solution structure of a DNA dodecamer duplex containing a cis-diammineplatinum(II) d(GpG) intrastrand cross-link, the major adduct of the anticancer drug cisplatin. *Biochemistry*, 37, 26, pp. 9230-9239, 1998.

[168] Davies, M. S., Berners-Price, S. J., Cox, J. W., and Farrell, N., The nature of the DNA template (single- versus double-stranded) affects the rate of aquation of a dinuclear Pt anticancer drug. *Chem. Commun.*, 1, pp. 122-123, 2003.

[169] Pratesi, G., Perego, P., Polizzi, D., Righetti, S. C., Supino, R., Caserini, C., Manzotti, C., Giuliani, F. C., Pezzoni, G., Tognella, S., Spinelli, S., Farrell, N., and Zunino, F., A novel charged trinuclear platinum complex effective against cisplatin-resistant tumours: hypersensitivity of p53-mutant human tumour xenografts. *Br. J. Cancer*, 80, 12, pp. 1912-1919, 1999.

[170] Komeda, S., Ohishi, H., Yamane, H., Harikawa, M., Sakaguchi, K., and Chikuma, M., An NMR study and crystal structure of [cis-Pt(NH3)2(9EtG-κN7)2(μ-pz)][NO3]3 (9EtG = 9-ethylguanine) as a model compound for the 1,2-intrastrand GG crosslink *J. Chem. Soc., Dalton Trans.*, 17, pp. 2959-2962, 1999.

[171] Komeda, S., Lutz, M., Spek, A. L., Chikuma, M., and Reedijk, J., New antitumor-active azole-bridged dinuclear platinum(II) complexes: synthesis, characterization, crystal structures, and cytotoxic studies. *Inorg. Chem.*, 39, 19, pp. 4230-4236, 2000.

[172] Olivova, R., Stepankova, J., Muchova, T., Novohradsky, V., Novakova, O., Vrana, O., Kasparkova, J., and Brabec, V., Mechanistic insights into toxic effects of a benzotriazolate-bridged dinuclear platinum(II) compound in tumor cells. *Inorganica Chimica Acta*, 393, pp. 204-211, 2012.

[173] Kalayda, G. V., Komeda, S., Ikeda, K., Sato, T., Chikuma, M., Reedijk, J., Kasparkova, J., and Brabec, V., Synthesis, Structure, and Biological Activity

of New Azine-Bridged Dinuclear Platinum(II) Complexes as a New Class of Anticancer Compounds. *European Journal of Inorganic Chemistry*, 2003, 24, pp. 4347-4355, 2003.

[174] G. V. Kalayda, S. Komeda, K. Ikeda, T. Sato, M. Chikuma, J. Reedijk, Eur. J. Inorg. Chem. (2003) 4347.

[175] Komeda, S., Kalayda, G. V., Lutz, M., Spek, A. L., Yamanaka, Y., Sato, T., Chikuma, M. and Reedijk, J., New Isomeric Azine-Bridged Dinuclear Platinum(II) Complexes Circumvent Cross-Resistance to Cisplatin. *Journal of Medicinal Chemistry*, 46, 7, pp. 1210-1219, 2003.

[176] Zhu, J., Zhao, Y., Zhu, Y., Wu, Z., Lin, M., He, W., Wang, Y., Chen, G., Dong, L., Zhang, J., Lu, Y., and Guo, Z., DNA cross-linking patterns induced by an antitumor-active trinuclear platinum complex and comparison with its dinuclear analogue. *Chem. Eur. J.*, 15, 21, pp. 5245-5253, 2009.

[177] Yakovlev, K. I., Rozhkova, N. D., and Stetsenko, A. I., Mono- and binuclear platinum(II) complexes with benzotriazole. *J. Inorg. Chem.*, 41, 5, pp. 757-762, 1996.

[178] Starha, P., Travnicek, Z., Popa, A., Popa, I., Muchova, T., and Brabec, V., How to modify 7-azaindole to form cytotoxic Pt(II) complexes: highly in vitro anticancer effective cisplatin derivatives involving halogeno-substituted 7-azaindole. *J. Inorg. Biochem.*, 115, pp. 57-63, 2012.

[179] Brabec, V., and Palecek, E., The influence of salts and pH on polarographic currents produced by denatured DNA. *Biophysik*, 6, 4, pp. 290-300, 1970.

[180] Brabec, V., and Palecek, E., Interaction of nucleic acids with electrically charged surfaces. II. Conformational changes in doublehelical polynucleotides. *Biophys. Chem.*, 4, 1, pp. 76-92, 1976.

[181] Kasparkova, J., Marini, V., Najajreh, Y., Gibson, D., Brabec, V., Sato, T., Chikuma, M., and Reedijk, J., DNA Binding Mode of the Cis and Trans Geometries of New Antitumor Nonclassical Platinum Complexes Containing Piperidine, Piperazine, or 4-Picoline Ligand in Cell-Free Media. Relations to Their Activity in Cancer Cell Lines. *Biochemistry*, 42, 20, pp. 6321-6332, 2003.

[182] Boudny, V., Vrana, O., Gaucheron, F., Kleinwachter, V., Leng, M., and Brabec, V., Biophysical analysis of DNA modified by 1,2-diaminocyclohexane platinum(II) complexes. *Nucleic Acids Res.*, 20, 2, pp. 267-272, 1992.

[183] Eastman, A., Characterization of the adducts produced in DNA by cis-diamminedichloroplatinum(II) and cis-dichloro-(ethylenediamine)platinum(II). *Biochemistry*, 22, 16, pp. 3927-3933, 1983.

[184] Eastman, A., Reevaluation of interaction of cis-dichloro-(ethylenediamine)platinum(II) with DNA. *Biochemistry*, 25, 13, pp. 3912-3915, 1986.

[185] Page, J. D., Husain, I., Sancar, A., and Chaney, S. G., Effect of the diaminocyclohexane carrier ligand on platinum adduct formation, repair, and lethality. *Biochemistry*, 29, 4, pp. 1016-1024, 1990.

[186] Zakovska, A., Novakova, O., Balcarova, Z., Bierbach, U., Farrell, N., and Brabec, V., DNA interactions of antitumor trans-[PtCl2(NH3)-(quinoline)]. *Eur. J. Biochem.*, 254, 3, pp. 547-557, 1998.

[187] Brabec, V., Vrana, O., Novakova, O., Kleinwachter, V., Intini, F. P., Coluccia, M., and Natile, G., DNA adducts of antitumor trans-[PtCl2(E-imino ether)2]. *Nucleic Acids Res.*, 24, 2, pp. 336-341, 1996.

[188] Zerzankova, L., Suchankova, T., Vrana, O., Farrell, N. P., Brabec, V., and Kasparkova, J., Conformation and recognition of DNA modified by a new antitumor dinuclear PtII complex resistant to decomposition by sulfur nucleophiles. *Biochem. Pharmacol.*, 79, 2, pp. 112-121, 2010.

[189] Butour, J. L., Alvinerie, P., Souchard, J. P., Colson, P., Houssier, C., and Johnson, N. P., Effect of the amine nonleaving group on the structure and stability of DNA complexes with cis-[Pt(R-NH2)2(NO3)2]. *Eur. J. Biochem.*, 202, 3, pp. 975-980, 1991.

[190] Reedijk, J., Why does Cisplatin reach Guanine-n7 with competing s-donor ligands available in the cell? *Chem. Rev.*, 99, 9, pp. 2499-2510, 1999.

[191] Reedijk, J., and Teuben, J. M., In 30 years of Cisplatin, Chemistry and Biochemistry of a leading Anticancer Drug; Lippert, B., Ed.; Wiley-VCH, pp. 339-362, 1999.

[192] Hagrman, D., Goodisman, J., Dabrowiak, J. C., and Souid, A. K., Kinetic study on the reaction of cisplatin with metallothionein. *Drug Metab. Dispos.*, 31, 7, pp. 916-923, 2003.

[193] Kasparkova, J., Novakova, O., Vrana, O., Intini, F., Natile, G., and Brabec, V., Molecular aspects of antitumor effects of a new platinum(IV) drug. *Mol. Pharmacol.*, 70, 5, pp. 1708-1719, 2006.

[194] Suchankova, T., Vojtiskova, M., Reedijk, J., Brabec, V., and Kasparkova, J., DNA and glutathione interactions in cell-free media of asymmetric platinum(II) complexes cis- and trans-[PtCl2(isopropylamine)(1-methylimidazole)]: relations to their different antitumor effects. *J. Biol. Inorg. Chem.*, 14, 1, pp. 75-87, 2009.

[195] Dabrowiak, J. C., Goodisman, J., and Souid, A. K., Kinetic study of the reaction of cisplatin with thiols. *Drug Metab. Dispos.*, 30, 12, pp. 1378-1384, 2002.

[196] Keck, M. V., Lippard, S. J., Unwinding of supercoiled DNA by platinum ethidium and related complexes. *J. Am. Chem. Soc.*, 114, 9, pp. 3386-3390, 1992.

[197] Farrell, N., Qu, Y., Feng, L., and Van Houten, B., Comparison of chemical reactivity, cytotoxicity, interstrand cross-linking and DNA sequence specificity of bis(platinum) complexes containing monodentate or bidentate coordination spheres with their monomeric analogues. *Biochemistry*, 29, 41, pp. 9522-9531, 1990.

[198] Mullins, S. T., Annan, N. K., Cook, P. R., and Lowe, G., Bisintercalators of DNA with a rigid linker in an extended configuration. *Biochemistry*, 31, 3, 842-849, 1992.

[199] Carpenter, M. L., Lowe, G., and Cook, P. R., The structure of 4-way DNA junctions: Specific binding of bis-intercalators with rigid linkers. *Nucleic Acids Res.*, 24, 9, pp. 1594-1601, 1996.

[200] Matsumoto, L., Kurek, K., Larocque, K., Gustafson, G., Pires, R., Zhang, J., Tantravahi, U., and Suggs, J. W., Biological effects of a bifunctional DNA crosslinker - I. Generation of triradial and quadriradial chromosomes. *Mutat. Res.*, 426, 1, pp. 79-87, 1999.

[201] Huang, C.-H., Mirabelli, C. K., Mong, S., and Crooke, S. T., Intermolecular cross-linking of DNA through bifunctional intercalation of an antitumor antibiotic, luzopeptin A (BBM-928A). *Cancer Res.*, 43, 6, pp. 2718-2724, 1983.

[202] Ellis, R. J., and Minton, A. P., Cell biology: Join the crowd. *Nature*, 425, 6953, pp. 27-28, 2003.

[203] Zimmerman, S. B., and Minton, A. P., Macromolecular crowding - biochemical, biophysical, and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.*, 22, pp. 27-65, 1993.

[204] Bancroft, D. P., Lepre, C. A., and Lippard, S. J., 95195 $^{\mathrm{P}}$t NMR kinetic and mechanistic studies of cis-diamminedichloroplatinum and trans-diamminedichloroplatinum(II) binding to DNA. *J. Am. Chem. Soc.*, 112, 19, pp. 6860-6871, 1990.

[205] Prokop, R., Kasparkova, J., Novakova, O., Marini, V., Pizarro, A. M., Navarro-Ranninger, C., and Brabec, V., DNA interactions of new antitumor platinum complexes with trans geometry activated by a 2-metylbutylamine or sec-butylamine ligand. *Biochem. Pharmacol.*, 67, 6, pp. 1097-1109, 2004.

[206] Zehnulova, J., Kasparkova, J., Farrell, N., and Brabec, V., Conformation, recognition by high mobility group domain proteins, and nucleotide excision repair of DNA intrastrand cross-links of novel antitumor trinuclear platinum complex BBR3464. *J. Biol. Chem.*, 276, 25, pp. 22191-22199, 2001.

[207] Kasparkova, J., Zehnulova, J., Farrell, N., and Brabec, V., DNA interstrand cross-links of the novel antitumor trinuclear platinum complex BBR3464. Conformation, recognition by high mobility group domain proteins, and nucleotide excision repair. *J. Biol. Chem.*, 277, 50, pp. 48076-48086, 2002.

[208] Zaludova, R., Kleinwächter, V., and Brabec, V., The effect of ionic strength on melting of DNA modified by platinum(II) complexes. *Biophys. Chem.*, 60, 3, pp. 135-142, 1996.

[209] van Houten, B., Illenye, S., Qu, Y., and Farrell, N., Homodinuclear (Pt,Pt) and heterodinuclear (Ru,Pt) metal compounds as DNA-protein cross-linking agents: potential suicide DNA lesions. *Biochemistry*, 32, 44, pp. 11794-11801, 1993.

[210] He, C., and Verdine, G. L., Trapping distinct structural states of a protein/DNA interaction through disulfide crosslinking. *Chem. Biol.*, 9, 12, pp. 1297-1303, 2002.

[211] Ohndorf, U. M., Rould, M. A., He, Q., Pabo, C. O., and Lippard, S. J., Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. *Nature*, 399, 6737, pp. 708-712, 1999.

[212] Zamble, D. B., and Lippard, S. J., In 30 years of Cisplatin, Chemistry and Biochemistry of a leading Anticancer Drug; Lippert, B., Ed.; Wiley-VCH, pp. 73-110, 1999.

[213] Farrell, N., Qu, Y., Bierbach, U., Valsecchi, M., Menta, E., In 30 years of Cisplatin, Chemistry and Biochemistry of a leading Anticancer Drug; Lippert, B., Ed.; Wiley-VCH, pp. 479, 1999.

[214] Rauter, H., Di Domenico, R., Menta, E., Oliva, A., Qu, Y., and Farrell, N., Selective Platination of Biologically Relevant Polyamines. Linear Coordinating Spermidine and Spermine as Amplifying Linkers in Dinuclear Platinum Complexes *Inorg. Chem.*, 36, 18, pp. 3919-3927, 1997.

[215] Fan, D., Yang, X., Wang, X., Zhang, S., Mao, J., Ding, J., Lin, L., and Guo, Z., A dinuclear monofunctional platinum(II) complex with an aromatic linker shows low reactivity towards glutathione but high DNA binding ability and antitumor activity. *J. Biol. Inorg. Chem.*, 12, 5, pp. 655-665, 2007.

[216] Kozelka, J., Segal, E., and Bois, C., Cytotoxic activity of platinum (II) complexes with tri-n-butylphosphine. Crystal structure of the dinuclear hydrazine-bridged complex, cis,cis-[PtCl(PBu3n)2(mu-N2H4)PtCl(PBu3n) 2] (ClO4)2.2CHCl3. *J. Inorg. Biochem.*, 47, 2, pp. 67-80, 1992.

[217] Montet, Y., and Kozelka, J., Corigendum to "A dinuclear platinum(II) complex with hydrazine and chloro bridges: (NPrn4)[PtCl2(μ-Cl)(μ-N2H4)PtCl2]". *Inorganica Chimica Acta*, 298, 1, pp. 103-106, 2000.

[218] Kasparkova, J., Novakova, O., Vrana, O., Farrell, N., and Brabec, V., Effect of geometric isomerism in dinuclear platinum antitumor complexes on DNA interstrand cross-linking. *Biochemistry*, 38, 34, pp. 10997-11005, 1999.

[219] Teletchea, S., Komeda, S., Teuben, J.-M., Elizondo-Riojas, M. A., Reedijk, J., and Kozelka, J., A pyrazolato-bridged dinuclear platinum(II) complex induces only minor distortions upon DNA-binding. *Chem. Eur. J.*, 12, 14, pp. 3741-3753, 2006.

[220] Pantoja, E., Gallipoli, A., van Zutphen, S., Komeda, S., Reddy, D., Jaganyi, D., Lutz, M., Tooke, D. M., Spek, A. L., Navarro-Ranninger, C., and Reedijk, J., Three new asymmetric trans-amine(azole)dichloridoplatinum complexes that overcome cisplatin resistance and their reactions with 5'-GMP. *J. Inorg. Biochem.*, 100, 12, pp. 1955-1964, 2006.

[221] Komeda, S., Bombard, S., Perrier, S., Reedijk, J., and Kozelka, J. F., Kinetic study of azole-bridged dinuclear platinum(II) complexes reacting with a hairpin-stabilized double-stranded oligonucleotide. *J. Inorg. Biochem.*, 96, 2-3, pp. 357-366, 2003.

[222] Mlcouskova, J., Kasparkova, J., Suchankova, T., Komeda, S., and Brabec, V., DNA conformation and repair of polymeric natural DNA damaged by antitumor azolato-bridged dinuclear Pt(II) complex. *J. Inorg. Biochem.*, 114, pp. 15-23, 2012.

[223] Brabec, V., Kleinwächter, V., Butour, J. L., and Johnson, N. P., Biophysical studies of the modification of DNA by antitumour platinum coordination complexes. *Biophys. Chem.*, 35, 2-3, pp. 129-141, 1990.

[224] Wang, X., and Guo, Z., The role of sulfur in platinum anticancer chemotherapy. *Anti-Cancer Agents Med. Chem.*, 7, 1, pp. 19-34, 2007.

List of abbreviations

BBR3464	$[\{$*trans*-$PtCl(NH_3)_2\}_2(\mu$-*trans*-Pt-$(NH_3)_2\{NH_2(CH_2)_6NH_2\}_2)]^{4+}$
Bp	Base pair
C	Cytosine
CD	Circular dichroism
CL	Cross-link
CT	Calf-thymus
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DPCL	DNA protein cross-link
DPP	Differential pulse polarography
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
FAAS	Flameless atomic absorption spectrometry
G	Guanine
GSH	Glutathione
HMGB1	High-mobility group protein B1

HPLC	High-performance liquid chromatography
ICL	Interstrand cross-link
IICL	Interduplex interstrand cross-link
MT-2	Metallothionein-2
NF-κB	Nuclear factor κB
r_b	Number of molecules of the platinum compound coordinatively bound per nucleotide residue
r_i	Molar ratio of free platinum complex to nucleotide phosphates at the onset of incubation with DNA
SDS	Sodium dodecyl sulfate
UTC	Coordinated Universal Time

List of publications

1. **Olivova, R.**, Kasparkova, J., Vrana, O., Vojtiskova, M., Suchankova, T., Novakova, O., He, W., Guo, Z., and Brabec, V. Unique DNA Binding Mode of Antitumor Trinuclear Tridentate Platinum(II) Compound. *Molecular Pharmaceutics* 2011, 8(6), p. 2368-2378. ISSN 1543-8384. DOI: 10.1021/mp200298g. (IF=4.782, 3 times cited, as of May 30, 2013)

2. **Olivova, R.**, Stepankova, J., Muchova, T., Novohradsky, V., Novakova, O., Vrana, O., Kasparkova, J., and Brabec, V. Mechanistic insights into toxic effects of a benzotriazolate-bridged dinuclear platinum(II) compound in tumor cells. *Inorganica Chimica Acta* 2012, 393, p. 204-211. ISSN 00201693. DOI: 10.1016/j.ica.2012.06.002. (IF=1.846, 0 times cited, as of May 30, 2013)

3. Muchova, T., Pracharova, J., Starha, P., **Olivova, R.**, Vrana, O., Benesova, B., Kasparkova, J., Travnicek, Z., and Brabec, V. Insight into the toxic effects of the *cis*-Pt(II)-dichlorido complexes containing 7-azaindole halogeno-derivatives in tumor cells. *Journal of Biological Inorganic Chemistry* 2013. ISSN 1432-1327. DOI: 10.1007/s00775-013-1003-7.

PAPER I

Unique DNA Binding Mode of Antitumor Trinuclear Tridentate Platinum(II) Compound

R. Olivova, J. Kasparkova, O. Vrana, M. Vojtiskova, T. Suchankova, O. Novakova, W. He, Z. Guo, V. Brabec

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I declare that my role in preparation of this paper was as following:

Realization of all the experiments except electrophoretic mobility shift assays with HMGB1 protein.
Preparation of the manuscript.

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molecular pharmaceutics

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Unique DNA Binding Mode of Antitumor Trinuclear Tridentate Platinum(II) Compound

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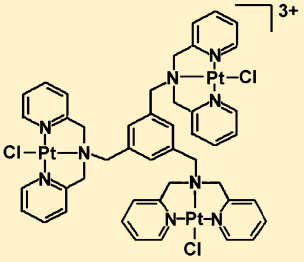
ABSTRACT: The new trinuclear tridentate Pt^{II} complex $[Pt_3Cl_3(hptab)]^{3+}$ (**1**; hptab = *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexakis(2-pyridylmethyl)-1,3,5-tris(aminomethyl)benzene) exhibits promising cytotoxic effects in human and mouse tumor cells including those resistant to conventional cisplatin (*Dalton Trans.* **2006**, 2617; *Chem. Eur. J.* **2009**, *15*, 5245). The present study is focused on the molecular pharmacology of **1**, in particular on its interactions with DNA (which is the major pharmacological target of platinum antitumor drugs), to elucidate more deeply the mechanism underlying its antitumor effects. Results obtained with the aid of methods of molecular biophysics and pharmacology reveal new details of DNA modifications by **1**. Complex **1** binds to DNA forming in the absence of proteins and molecular crowding agents mainly trifunctional intrastrand cross-links. In these DNA adducts all three Pt^{II} centers of **1** are coordinated to DNA base residues, which leads to extensive conformational alterations in DNA. An intriguing aspect of the DNA-binding mode of this trinuclear Pt^{II} complex **1** is that it can cross-link proteins to DNA. Even more interestingly, **1** can cross-link in the presence of molecular crowding agent, which mimics environmental conditions in cell nucleus, two DNA duplexes in a high yield—a feature observed for the first time for antitumor trinuclear platinum complexes. Thus, the concept for the design of agents capable of forming intramolecular tridentate DNA adducts, DNA−protein and interduplex DNA−DNA cross-links based on trinuclear tridentate Pt^{II} complexes with semirigid aromatic linkers may result in new compounds which exhibit a variety of biological effects and can be also useful in nucleic acids research.



KEYWORDS: *platinum drug, antitumor, DNA binding, interduplex cross-links, DNA−protein cross-links*

INTRODUCTION

More than forty years after antitumor activity of cisplatin was discovered in 1969,[1] the search continues for platinum compounds having novel preclinical properties, in particular activity in tumor cells exhibiting inherent or acquired resistance to cisplatin and its clinically used analogues and/or reduced side effects. The findings include structures that significantly violate the original structure−pharmacological activity relationship, including cationic polynuclear Pt^{II} complexes. This class of new antitumor platinum compounds was designed as a result of systematically testing the hypothesis that there is a correlation between clinical efficacy of platinum compounds and their ability to induce a certain sort of damage or conformational change in target DNA. In other words, platinum drugs that bind to DNA in a manner fundamentally different from that of conventional cisplatin and its mononuclear analogues can exhibit altered biological properties including the spectrum and intensity of antitumor activity.[2,3] The antitumor effects of polynuclear platinum complexes may be modulated by the geometry and number of platinum centers and leaving groups in the coordination sphere of platinum atoms as well as the nature of the linkers bridging the platinum centers.[4,5] In addition, location of leaving ligands in relation to the linkers in polynuclear Pt^{II} compounds determines their susceptibility to deactivating metabolic decomposition by the sulfur nucleophiles since substitution of the bond between Pt^{II} center and leaving group (for instance Cl) by a trans-influencing S donor results in bridge cleavage.[6,7]

In an effort to design a new polynuclear platinum compound that is able to bind to DNA in a fundamentally new manner and consequently having novel preclinical properties, trinuclear trifunctional Pt^{II} complex $[Pt_3Cl_3(hptab)]^{3+}$ (**1**; hptab = *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexakis(2-pyridylmethyl)-1,3,5-tris(aminomethyl)-benzene) was recently synthesized.[8] In this trinuclear and tridentate compound, the Pt^{II} centers are bridged by bulky semirigid aromatic linkers (Figure 1). This new trifunctional agent exerted promising cytotoxic effects in human and mouse tumor cell lines.[8,9] In addition, in contrast to another trinuclear but bidentate Pt^{II} complex $[\{trans\text{-}PtCl(NH_3)_2\}_2(\mu\text{-}trans\text{-}Pt(NH_3)_2\{NH_2(CH_2)_6NH_2\}_2)]^{4+}$ (BBR3464, Figure 1), which is in clinical trials,[4,5] the geometry of leaving groups in the

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PAPER II

Mechanistic insights into toxic effects of a benzotriazolate-bridged dinuclear platinum(II) compound in tumor cells

R. Olivova, J. Stepankova, T. Muchova, V. Novohradsky, O. Novakova, O. Vrana, J. Kasparkova, V. Brabec

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I declare that my role in preparation of this paper was as following:

Experimental realization of kinetics of binding, reactions with sulfur containing compounds, DNA melting, ethidium bromide fluorescence measurements, interstrand cross-link assay, unwinding of negatively supercoiled DNA, characterization of DNA adducts by [^{14}C] thiourea, Circular dichroism spectroscopy, preparation of the manuscript.

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Mechanistic insights into toxic effects of a benzotriazolate-bridged dinuclear platinum(II) compound in tumor cells

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ABSTRACT

We examined toxicity in tumor cells as well as some early phases of the mechanism underlying biological properties of benzotriazolate (Btaz)-bridged dinuclear Pt^{II} complex ([{*cis*-$Pt(NH_3)_2Cl$}$_2$(μ-Btaz-H)]Cl (**1**)). This dinuclear bifunctional Pt^{II} complex utilizes rigid aromatic ring of azole as a linker between two Pt atoms, featuring at the same time non-bridging chloride ions as leaving groups. The results of the present work demonstrate that the toxicity of **1** in several human tumor cell lines was lower than that of conventional cisplatin. The results obtained are consistent with the idea, and support the postulate that deactivation of **1** by sulfur-containing compounds is a significant factor contributing to reduced DNA binding of **1** in cells and subsequently to its reduced cytotoxicity. In addition, the observations that DNA adducts of **1** and cisplatin distort DNA conformation differently and that monofunctional DNA adducts of **1** are converted to more toxic bifunctional cross-links considerably more slowly in comparison with cisplatin may contribute to the reduced cytoxicity of **1**. The results of the present work afford further details on the mechanism underlying biological effects of bifunctional polynuclear Pt^{II} compounds and should provide a more rational basis for the design of new antitumor metallodrugs and chemotherapeutic strategies.

1. Introduction

Polynuclear platinum agents are a structurally unique class of anticancer drugs. Clinical profile and mechanism of action of polyplatin complexes are different from the established platinum mononuclear compounds, such as the cisplatin (*cis*-dimminedichloridoplatinum(II)) family. The biological activity of polynuclear platinum complexes may be modulated by the geometry and number of leaving groups in the coordination sphere of platinum atoms as well as by the nature of linkers connecting the platinum centers.

As regards molecular mechanisms of antitumor polynuclear Pt^{II} complexes, an attention has been so far paid to various types of polynuclear Pt^{II} complexes including several trans and cis Pt^{II} complexes. The Pt centers in these complexes were bridged by various linkers which differed in rigidity. Spatial configuration of leaving groups predisposed these polynuclear Pt^{II} complexes to react with their pharmacological target, presumably with DNA, in a unique way [1,2].

Some of the polynuclear (mostly dinuclear) antitumor active Pt^{II} complexes described in the literature contain flexible [3,4] or semi-flexible bridging ligands [5,6], while others possess rigid linkers [7–10]. The complexes with flexible linkers has been shown to form in DNA unique long-range cross-links (CLs) not formed by mononuclear bifunctional Pt^{II} complexes [11,12].

Recently, a new class of dinuclear bifunctional azolato- or azinato-bridged Pt^{II} compounds was synthesized by Reedijk, Komeda and their coworkers [9,10,13–17]. Both azolato- and azinato-bridged complexes contain a bridging hydroxide or non-bridging chloride ions as leaving groups, respectively and rigid linkers. Intriguingly, the structural properties predisposed the azolato-bridged dinuclear complexes to form in DNA the adducts identical to major DNA adducts of cisplatin, i.e. 1,2-intrastrand CLs [16]. However, in contrast to cisplatin, these adducts formed by the dinuclear azolato-bridged Pt^{II} compounds induce only minimal conformational changes in double-helical DNA [14,18]. This may overcome the problem of acquired cellular resistance since small DNA perturbations may be hardly recognized and removed from DNA by DNA repair systems. Consistent with this was the observation that the dinuclear azolato-bridged Pt^{II} compounds exhibited markedly higher toxic effects in some tumor cell lines than conventional mononuclear cisplatin [13,17,19]. Moreover, isomeric azines (pyrazine, pyrimidine, and pyridazine) as bridging ligands for the synthesis of dinuclear Pt^{II} complexes have been used as well

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PAPER III

Insight into the toxic effects of the *cis*-Pt(II)-dichlorido complexes 4 containing 7-azaindole halogeno-derivatives in tumor cells

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I declare that my role in preparation of this paper was as following:

Realization of the experiment with reduced form of glutathione (GSH).

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ORIGINAL PAPER

Insight into the toxic effects of *cis*-dichloridoplatinum(II) complexes containing 7-azaindole halogeno derivatives in tumor cells

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Abstract The cisplatin analogues *cis*-[$PtCl_2(3ClHaza)_2$] (**1**) and *cis*-[$PtCl_2(3IHaza)_2$] (**2**) (3ClHaza and 3IHaza are 3-chloro-7-azaindole and 3-iodo-7-azaindole, respectively) are quite toxic to ovarian tumor cells, with moderately better IC_{50} values than for cisplatin in the cisplatin-sensitive cell line A2780. We investigated potential factors which might be involved in the mechanism underlying the cytotoxic effects of **1** and **2** and compared these factors with those involved in the mechanism underlying the effects of conventional cisplatin. Our data indicate that the higher cytotoxicity of **1** and **2** originates mainly from their efficient cellular accumulation, different effects at the level of cell cycle regulation, and reduced propensity for DNA adduct repair. Studies of their reactivity toward cellular components reveal efficient binding to DNA, which is typically required for an active platinum drug. Further results suggest that **1** and **2** are capable of circumventing resistance to cisplatin induced by alterations in cellular accumulation and DNA repair. Hence, the latter two factors appear to be responsible for differences in the toxicity of **1** or **2**, and cisplatin in tumor cells. The results of this work reinforce the idea that direct analogues of conventional cisplatin-containing halogeno-substituted 7-azaindoles offer much promise for the design of novel therapeutic agents.

Keywords Platinum drugs · Cytotoxicity · Cellular uptake · Cell cycle · DNA damage · DNA repair

Abbreviations

3ClHaza	3-Chloro-7-azaindole
3IHaza	3-Iodo-7-azaindole
CT	Calf thymus
DMF	*N*,*N*′-Dimethylformamide
EtBr	Ethidium bromide
FAAS	Flameless atomic absorption spectrometry
GSH	Glutathione
IC_{50}	Compound concentration that produces 50 % cell growth inhibition
ICP-MS	Inductively coupled plasma mass spectroscopy
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
SD	Standard deviation

Introduction

The design of new potential platinum drugs to overcome tumor resistance to conventional cisplatin is an active area of bioinorganic chemistry and molecular pharmacology. This is because clinical use of platinum-based drugs is

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