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Synthesis and Evaluation of Series of Diazine-Bridged Dinuclear Platinum(II) Complexes through in Vitro Toxicity and Molecular Modeling: Correlation between Structure and Activity of Pt(II) Complexes

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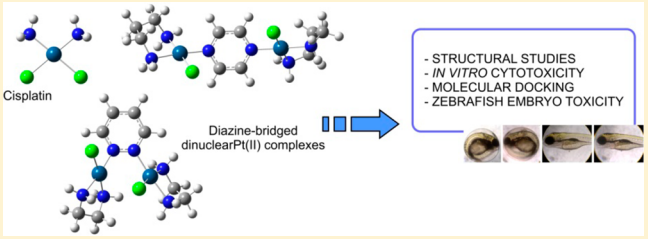
Supporting Information

ABSTRACT: Polynuclear Pt(II) complexes are a novel class of promising anticancer agents with potential clinical significance. A series of pyrazine (pz) bridged dinuclear Pt(II) complexes with general formulas $\{[Pt(L)Cl]_2(\mu\text{-pz})\}^{2+}$ (L, ethylenediamine, en; (±)-1,2-propylenediamine, 1,2-pn; isobutylenediamine, ibn; *trans*-(±)-1,2-diaminocyclohexane, dach; 1,3-propylenediamine, 1,3-pd; 2,2-dimethyl-1,3-propylenediamine, 2,2-diMe-1,3-pd) and one pyridazine (pydz) bridged $\{[Pt(en)Cl]_2(\mu\text{-pydz})\}^{2+}$ complex were prepared. The anticancer potential of these complexes were determined through in vitro cytotoxicity assay in human fibroblasts (MRC5) and two carcinoma cell lines (A375 and HCT116), interaction with double stranded DNA through in vitro assay, and molecular docking study. All complexes inhibited cell proliferation with inhibitory concentrations in the 0.5−120 μM range. While $\{[Pt(1,3\text{-pd})Cl]_2(\mu\text{-pz})\}^{2+}$ showed improved activity and $\{[Pt(en)Cl]_2(\mu\text{-pydz})\}^{2+}$ showed comparable activity to that of clinically relevant cisplatin, $\{[Pt(en)Cl]_2(\mu\text{-pydz})\}^{2+}$ was less toxic in an assay with zebrafish (*Danio rerio*) embryos, causing no adverse developmental effects. The in vitro cytotoxicity of all diazine-bridged dinuclear Pt(II) complexes is discussed in correlation to their structural characteristics.



INTRODUCTION

After Rosenberg's discovery of the antitumor activity of cisplatin (*cis*-$[PtCl_2(NH_3)_2]$, also known as *cis*-DDP and CDDP) it became one of the most often used metal-containing drugs in cancer chemotherapy.[1,2] Unfortunately, the side effects of cisplatin are severe and include dose-limiting toxicity such as nephrotoxicity, neurotoxicity, ototoxicity, and emetogenesis.[3] The so-called second-generation of platinum based drugs, such as carboplatin and oxaliplatin, were developed with the aims of obtaining better antitumor activity, increased solubility, and lower toxic side effects.[4,5] Interactions of platinum-based drugs with DNA, through covalent binding or intercalation of the aromatic ligands to cellular DNA, are responsible for their antitumor activity. This modified tertiary structure of DNA results in the death of the cancer cell through apoptosis.[6−8] Despite all the toxic side effects, platinum(II)-based cytotoxic agents are a part of more than 50% of clinically applied anticancer regimens and first-line chemotherapy for 12 different neoplasms.[9−11]

Polynuclear platinum complexes represent a novel class of promising antitumor agents with potential clinical significance.[4,11] These complexes contain two, three, or four platinum centers linked by a bridging diamine ligand. These linkers are commonly flexible linear aliphatic molecules with variable chain length,[12] which enables them to form long-range cross-links with DNA.[13,14] Some of the polynuclear complexes possess rigid linkers such as hydrazine and azoles.[15−17] These complexes are developed to minimize distortion of the DNA double helix in a cross-link.[18] Recently, another class of dinuclear platinum(II) complexes with six-membered heterocyclic diazines, namely, pyridazine (pydz), pyrimidine (pm), and pyrazine (pz) as bridging ligands, has been synthesized and they present a combination of the two above-mentioned types of linker ligands.[15,19] Two dinuclear platinum(II) complexes, namely, $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$[20] and $\{[Pt(en)Cl]_2(\mu\text{-pydz})\}Cl_2$,[21] have been synthesized in our laboratory and characterized by application of ^1H NMR spectroscopy and single-crystal X-ray diffraction. The obtained crystallographic results showed that in $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex, pyrazine acts as linear bridge between metal ions separated by ∼7 Å,[20] while in the pyridazine analogue, the two Pt(II) ions are only ∼3 Å

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apart.[21] As a continuation of our ongoing interest toward the coordination chemistry of platinum(II) with bridging nitrogen-containing heterocyclic ligands[20,21] in light of the facts that polynuclear Pt(II) complexes represent promising antitumor agents, in the present paper we report the synthesis, spectroscopic characterization, and biological evaluation of a whole series of $\{[Pt(L)Cl]_2(\mu\text{-pz})\}^{2+}$ type complexes (**3**–**9**), all differing in the chelated diamine ligand L (L is ethylenediamine, en (**3**); (±)-1,2-propylenediamine, 1,2-pn (**4**); isobutylenediamine, ibn (**5**); *trans*-(±)-1,2-diaminocyclohexane, dach (**6**); 1,3-propylenediamine, 1,3-pd (**7**); 2,2-dimethyl-1,3-propylenediamine, 2,2-diMe-1,3-pd (**8**); and pz is bridging pyrazine ligand), as well as $\{[Pt(en)Cl]_2(\mu\text{-pydz})\}^{2+}$ complex (**9**) (pydz is bridging pyridazine ligand) (Figure 1).

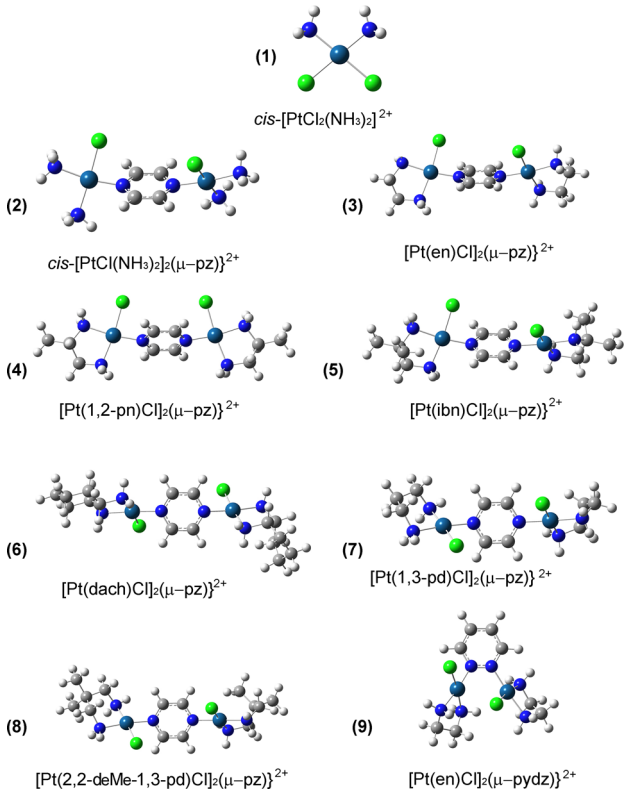


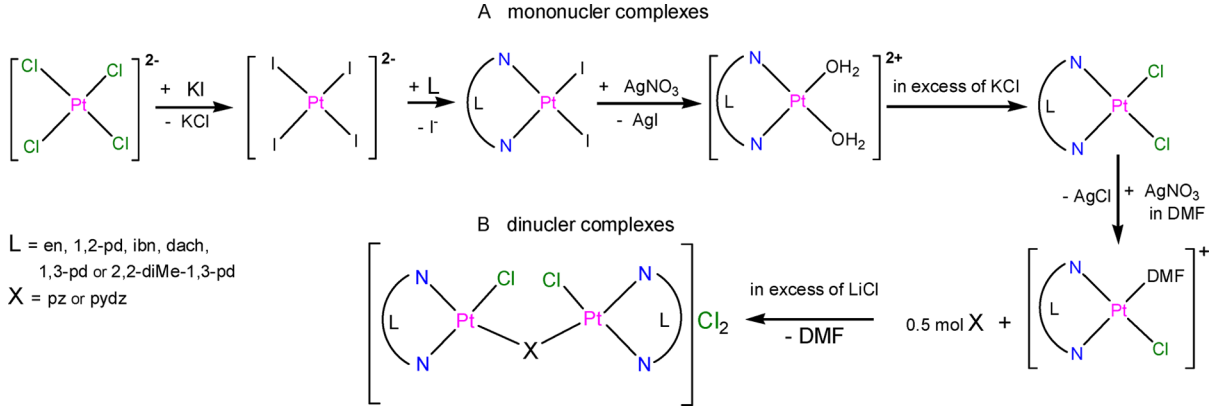
Figure 1. Structural representation of cisplatin (**1**) and diazine-bridged Pt(II) dinuclear complexes (**2**–**9**) analyzed in this study.

During this study for the first time in vitro cytotoxic activity of complexes **3**–**9** against human cell lines (fibroblast and cancer) was evaluated and their interaction with double stranded circular and chromosomal DNA was examined through in vitro activity assay and through molecular modeling with direct comparison with those for *cis*-$[PtCl_2(NH_3)_2]$ (**1**) and $\{cis\text{-}[PtCl(NH_3)_2]_2(\mu\text{-pz})\}Cl_2$ (**2**). In addition zebrafish embryo toxicity of potentially useful anticancer leads was evaluated.

RESULTS AND DISCUSSION

Synthesis and Structural Characteristics of Diazine-Bridged Dinuclear Pt(II) Complexes. Six pyrazine-bridged dinuclear $\{[Pt(L)Cl]_2(\mu\text{-pz})\}^{2+}$ complexes (**3**–**8**) (L is ethylenediamine, en; (±)-1,2-propylenediamine, 1,2-pn; isobutylenediamine, ibn; *trans*-(±)-1,2-diaminocyclohexane, dach; 1,3-propylenediamine, 1,3-pd; and 2,2-dimethyl-1,3-propylenediamine, 2,2-diMe-1,3-pd) and one pyridazine-bridged $\{[Pt(en)Cl]_2(\mu\text{-pydz})\}^{2+}$ complex (**9**) have been synthesized (Figure 1). The structures of all these complexes were confirmed by elemental microanalyses and NMR (^1H and ^{13}C) spectroscopy, and these data were correlated with those previously reported for the same complexes.[20−22] Moreover, the crystal structures of **3** and **9** were also confirmed by single-crystal X-ray diffraction analysis.[20,21] The schematic presentation of the reaction for the syntheses of these complexes is shown in Scheme 1. The mononuclear Pt(II) complexes of the type $[Pt(L)Cl_2]$ were prepared from equimolar amounts of K_2PtCl_4 and corresponding diamine ligand (L) according to a procedure published in the literature (Scheme 1A).[23−25] The mononuclear $[Pt(L)Cl_2]$ complex was reacted with an equivalent amount of $AgNO_3$ to replace one chloride by a DMF molecule. In order to obtain diazine-bridged dinuclear Pt(II) complex, the corresponding monoactivated $([Pt(L)Cl)DMF)]^+$ species was reacted with an equivalent amount of pyrazine or pyridazine ligand at room temperature (Scheme 1B). All complexes were crystallized as chloride salts from water solution in an excess of LiCl. Complexes **3**–**6** have the same pyrazine-bridged ligand but differ in the structure of the five-membered diamine ring (en, 1,2-pn, ibn, and dach) (Figure 1). Recently reactions between aqua derivatives of **3**–**6**, $\{[Pt(L)(H_2O)]_2(\mu\text{-pz})\}^{4+}$, and different methionine-containing peptides have been investigated by ^1H NMR spectroscopy.[20,23] This study showed that all investigated Pt(II) aqua complexes bind to the methionine side chain of the peptide and promote the cleavage

Scheme 1. Reactions for Preparation of Dinuclear Pt(II) Complexes (3–9)



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of the amide bond involving the carboxylic group of methionine. Moreover, it was demonstrated that the substituent in the ethylenediamine skeleton of these complexes, one methyl group in 1,2-pn (**4**), two methyl groups in ibn (**5**), or cyclohexane ring in dach (**6**), has influence on this hydrolytic reaction. Thus, the amount of hydrolyzed peptide was decreased by increasing the steric bulk of the corresponding dinuclear Pt(II) complexes (**3** > **4** > **5** > **6**). In comparison with the above-mentioned **3**–**6** all having five-membered ethylenediamine ring, complexes **7** and **8** contain six-membered 1,3-propanediamine ring (Figure 1). Finally, in complex **9** pyrazine-bridged ligand is replaced with less symmetrical pyridazine, for which we can assume that it also contributes to different reactivity of **9** in comparison with its analogue **3**. Additionally, the studies of the reactions between some dinuclear Pt(II) complexes and sulfur- or nitrogen-containing biomolecules showed that these complexes are quite stable in the broad pH range (2.0 < pH < 8.0) for more than 24 h.[20−22] According to the above-discussed results for different reactivity of dinuclear Pt(II) complexes with peptides in connection with their structural characteristics and those related to the facts that these types of complexes showed reasonable stability in the reactions with sulfur- and nitrogen-containing biomolecules in the broad pH range, in the second part of this work in vitro toxicity of **3**–**9** has been evaluated and discussed.

In Vitro Cytotoxicity and DNA Cleavage (Unwinding) Ability. The antiproliferative (cytotoxic) potential of diazine-bridged dinuclear Pt(II) complexes in comparison to cisplatin was tested on human lung fibroblasts (MRC5) and two carcinoma cell lines, melanoma (A375) and colon cancer (HCT116), by MTT assay after a 48 h treatment (Figure 2). Although all complexes showed the cytotoxic effect in all cell lines in concentrations of up to 50 μM, HCT116 appeared to be more sensitive in comparison to fibroblasts and melanoma, indicating some cell-line specificity (Figure 2). The cytotoxic profiles of each complex were constant for different cell lines but slightly different among complexes. Dose-dependent cytotoxicity exhibited by **1** was comparable to that of **2**, **3**, and **7**–**9**, while complexes **4**–**6** exhibited cytotoxicity in less dose-dependent manner. Indeed, the least cytotoxic was **5** with IC_{50} values being from 45- to 22-fold higher against fibroblasts and colon cancer in comparison to cisplatin, respectively (Table 1).

Diazine-bridged dinuclear Pt(II) complexes (**3**–**9**) generally appear to be less cytotoxic in comparison to cisplatin (**1**) and azine-bridged complex **2**, apart from the **7** and **9**. In fact, the only complex that showed 2-fold lower IC_{50} values in comparison to those of cisplatin for both melanoma and colon cancer was complex **7** (Table 1). Interestingly, structurally similar complex **8** showed desirable 3- to 5-fold lower activity against fibroblasts in comparison to carcinoma cell lines but slightly lower activity in comparison to complex **7**. This can be correlated with the fact that complex **7** is less sterically demanding with respect to complex **8** having two methyl groups attached on the middle carbon atom of 1,3-propanediamine ring. Also, higher activity of **3** in comparison with its analogues **4**–**6** contributed to the presence of different substituents in the ethylenediamine skeleton of the latter three complexes, i.e., one methyl group in **4**, two methyl groups in **5**, or cyclohexane ring in **6**. However, from the present investigation it can be concluded that complexes **3**–**6** with five-membered ethylenediamine ring showed lower IC_{50} values in comparison to those with six-membered 1,3-propanediamine

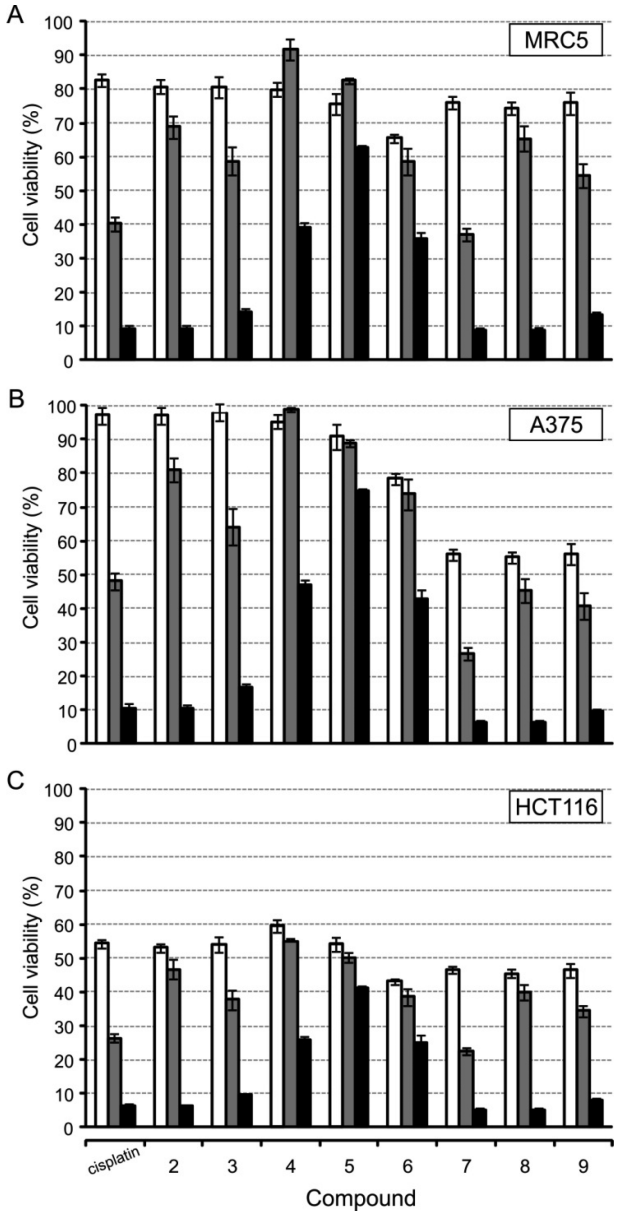

Figure 2. In vitro cytotoxicity (antiproliferative effect) profile of cisplatin and Pt(II) complexes **2**–**9** against (A) human lung fibroblast (MRC5), (B) melanoma (A375), and (C) colon cancer (HCT116) cell lines (□, 0.5 μM; gray box, 5 μM; ■, 50 μM concentration tested compound).

ring (**7** and **8**) (Table 1). All these complexes have the same pyrazine bridged ligand, but they differ in the ring size of the chelating diamine ligand. In all these complexes coordination around two Pt(II) ions is square planar but they differ in conformation of diamine rings. The diamine rings in complexes **3**–**6** adopt the usual twist conformation with an approximate 2-fold axis passing through the C1−C2 bond.[20] On the other hand, the six-membered chelate ring in complexes **7** and **8** is in a chair conformation. This conformation was established by X-ray crystallography for Pt(II) and Pd(II) complexes with different 1,3-propanediamine ligands.[26] From the above facts it can be assumed that different conformations between five-membered and six-membered rings in the above-mentioned complexes play crucial roles in their different activity. It is worth mentioning that complexes **7** and **8** contained 2 equiv of LiCl

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J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. In Vitro Antiproliferative Activity Given as IC_{50} ± SD in μM of the Cisplatin (1) and Complexes 2−9

compd	cell line		
	MRC5	A375	HCT116
cisplatin	4.5 ± 0.1	6 ± 0.1	1 ± 0.1
2	14.3 ± 0.5	20 ± 0.4	3.2 ± 0.1
3	20 ± 0.9	25 ± 0.4	3 ± 0.1
4	45 ± 0.8	50 ± 1	30 ± 0.8
5	100 ± 2	120 ± 3	45 ± 1
6	40 ± 0.8	45 ± 0.9	4 ± 0.1
7	4 ± 0.2	3 ± 0.1[a]	0.5 ± 0.1[a]
8	15 ± 0.4	5 ± 0.2	3 ± 0.3
9	6 ± 0.1	5 ± 0.1	3.3 ± 0.2

[a]Statistically significantly lower ($p < 0.05$) as compared to cisplatin.

as contaminant that itself in the applied concentrations did not have antiproliferative effect on the used cell lines (data not shown). Difference in the activity of pyrazine-bridged complex **3** with respect to the analogue pyridazine-bridged Pt(II) complex **9** can be explained from crystallographic results of these two complexes.[21] The X-ray results of **3** showed that the Pt···Pt distance in this complex is 6.7890(3) Å, comparable with the mean value of 6.815(8) Å obtained from 17 observations in the crystal structures containing a discrete pyrazine-bridged Pt(II) dimer, deposited in the CSD.[27] However, this distance for complex **9** having two ortho-arranged nitrogen donor atoms of pyridazine is much shorter (3.2535(4) Å) than in the closely related $\{[Pt(en)Cl]_2(\mu\text{-pz})\}^{2+}$ cation (**3**).[20] From the difference in the intramolecular distances of two Pt(II) ions of **9** and **3**, it can be assumed that the shorter distance in the first complex contributes to its better ability to form DNA adducts and higher cytotoxic activity.

Cytotoxicity of diazine-bridged Pt(II) complexes (**3**−**9**) was not previously examined, while in vitro cytotoxicity of the azine-bridged complex **2** was previously examined in several human tumor cell lines including melanoma (M19), colon cancer (WIDR), breast cancer (MCF7 and EVSA-T), renal cancer (A498), and non-small-cell lung cancer (H226).[15,19] Generally, **2** and its nitrate salt showed IC_{50} values higher than those of cisplatin, between 1.6- and 30-fold, with only comparable IC_{50} exhibited against ovarian cancer, indicating cell line and cancer-type specificity.[15] This is in agreement with data obtained in this study. On the other side, **2** showed improved activity with IC_{50} values 1.8- and 5.8-fold lower in comparison to cisplatin when tested against murine leukemia cell lines sensitive (L1210(0)) and resistant (L1210(cisPt)) to cisplatin, respectively, with the induction of apoptosis in this experimental system also reported.[19]

Since DNA is the primary target of Pt(II)-based antitumor complexes,[28] the ability of Pt(II) complexes to bind, unwind, and cleave DNA was investigated following the conversion of the supercoiled form of pUC18 plasmid DNA to the open circular and/or linear forms using agarose gel electrophoresis. Electrophoresis patterns of supercoiled pUC18 plasmid DNA in the presence of 50 μM **1**−**9** showed that all compounds had the ability to interact with DNA in vitro at physiological pH 7.4 (Figure 3). The supercoiled form of plasmid DNA was completely converted to circular and linear forms in the presence of **2**−**6** and **8**, while cisplatin (**1**) and Pt(II) dinuclear complexes **7** and **9** had only partially converted supercoiled DNA to other forms exhibiting quite similar pattern of plasmid DNA unwinding (Figure 3). Obtained results imply that

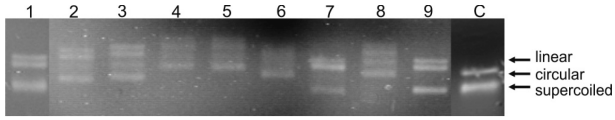

Figure 3. Agarose gel electrophoresis patterns of supercoiled pUC18 plasmid DNA (200 ng) incubated with cisplatin (**1**) and Pt(II) complexes **2**−**9** in the buffer at pH 7.4 (50 mM Tris-HCl, 50 mM NaCl) at 37 °C for 12 h.

complexes **2**−**6** and **8** could exhibit irreversible DNA degradation more readily than **1**, **7**, and **9**, which may lead to cell death by necrosis, therefore indicating potentially higher general toxicity of these compounds. However, all of these compounds exhibited lower antiproliferative activities in cytotoxicity assays in comparison to cisplatin (Table 1). Complex **7**, although with the highest activity against human cell lines, was mostly able to unwind supercoiled to circular form at concentration tested. Therefore, from the high interaction with circular double stranded DNA in vitro, cytotoxicity against human cell lines could not be predicted.

In Silico DNA Binding Ability of Diazine-Bridged Dinuclear Platinum(II) Complexes. Molecular docking is a powerful computational technique that can be used for the investigation of DNA−drug interactions, the identification of binding place (minor or major groove), and the prediction of complex binding affinities.[29,30] Molecular docking study was carried out to gain information about in silico DNA binding affinity for studied compounds **1**−**9**. The predicted top-ranking pose with the complex with lowest energy was applied for suggesting the best possible geometry of compounds inside the DNA double helix and the highest binding affinity of DNA. MolDock, Docking, Rerank, and Hbond scoring functions were used for the assessment of complexes DNA binding affinity. Top-ranked poses according to used scoring functions are presented in Table 2.

Table 2. Score Values (kcal/mol) for Cisplatin and Diazine-Bridged Pt(II) Dinuclear Complexes

compd	MolDock (kcal/mol)	Docking (kcal/mol)	Rerank (kcal/mol)	HBond (kcal/mol)
cisplatin[a]	−55.738	−54.669	−33.556	0
cisplatin[b]	−48.392	−52.322	−31.583	−2.176
2[a]	−97.320	−94.349	−56.667	0
2[b]	−96.699	−96.416	−48.246	−2.583
3[a]	−137.564	−135.115	−81.746	0
3[b]	−126.189	−125.065	−62.883	−0.051
4[a]	−141.673	−139.506	−76.378	−0.222
4[b]	−138.476	−135.245	−66.401	−1.071
5[a]	−136.797	−135.985	−81.047	−0.546
5[b]	−134.686	−133.195	−83.910	−2.329
6[a]	−154.521	−152.912	−90.716	−0.917
6[b]	−149.530	−146.665	−76.170	−0.991
7[a]	−137.554	−134.041	−83.667	0
7[b]	−121.797	−121.334	−66.648	−2.994
8[a]	−145.241	−141.051	−85.893	0
8[b]	−133.637	−131.236	−76.749	−4.976
9[a,b]	−105.609	−105.210	−58.2179	−2.57

[a]Best complex pose according to MolDock, Docking, and Rerank scoring functions. [b]Best complex pose according to Hbond scoring function.

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According to MolDock, Docking, and Rerank score values, the highest binding affinity has compound **6**. However, hydrogen bonds formed between complex and DNA are important for metal complex DNA binding and for further possible cytotoxic activity.[31,32] According to Hbond values the highest binding affinities have complexes **2**, **7**, **8**, and **9**. The more negative is the relative binding energy, greater is the binding propensity of the complex with DNA, which correlated well with the experimental in vitro DNA binding studies (Figure 3) and IC_{50} values for the cytotoxic activity of complex (Table 1). According to molecular modeling results complex **1** (cisplatin) formed eight hydrogen bonds with DNA (seven hydrogen bonds had binding energy values of −2.5 kcal/mol with bond lengths 2.62, 2.77, 2.82, 2.96, 3.03, 3.09, and 3.10 Å, respectively; one hydrogen bond had binding energy value of −0.84 kcal/mol with bond length value of 3.43 Å). Complex **2** formed nine hydrogen bonds (seven hydrogen bonds had binding energy values of −2.5 kcal/mol with bond lengths 2.62, 2.73, 2.75, 3.046, 3.058, 3.098, and 3.10 Å, respectively; one hydrogen bond had binding energy value of −2.44 kcal/mol with bond length value of 3.11 Å, and one hydrogen bond had binding energy value of −0.77 kcal/mol with bond length value of 3.44 Å). Complex **7** formed five hydrogen bonds with binding energy values of −2.5, −2.26, −2.21, −0.856, and −0.79 kcal/mol with bond length values of 3.098, 3.148, 2.60, 3.43, and 2.58 Å, respectively. Complex **8** formed four hydrogen bonds with binding energy values of −2.5, −2.5, −2.48, and −2.35 kcal/mol with bond length values of 2.66, 3.10, 2.92, and 3.13 Å, respectively. Complex **9** formed six hydrogen bonds with binding energy values of −2.5, −2.5, −1.79, −1.45, −1.18, and −1.13 kcal/mol with bond length values of 2.75, 2.99, 3.24, 2.9, 3.36, and 2.43 Å, respectively.

Best poses for complexes **7**−**9** according to Hbond values are presented in Figure 4. The minimum energy docked pose

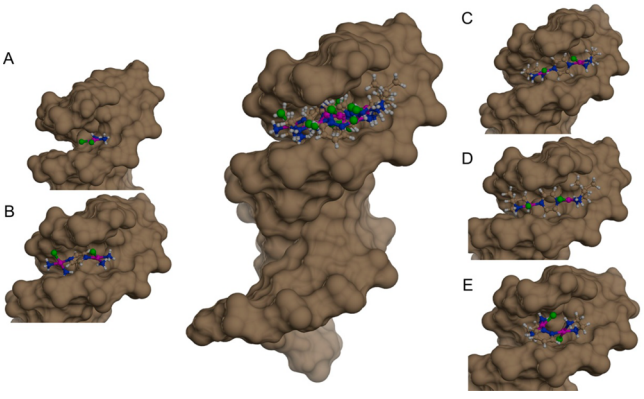

Figure 4. Computational docking model illustrating interactions between (A) cisplatin **1**, (B) complex **2**, (C) complex **7**, (D) complex **8**, and (E) complex **9** and DNA.

revealed that complexes **7** and **9** are very well fitted into the DNA minor groove. Docking poses suggest that complexes and DNA base pairs are arranged in such a way that they have effective $\pi-\pi$ stacking interactions. These interactions can lead to higher van der Waals interaction with the DNA functional groups which define the stability of groove, making the AT regions more preferable regions of dodecamer.[33] Complexes exhibited additional stabilization through the strong intermolecular hydrogen bonding interaction between the C-2 carbonyl oxygen of T and the N-3 nitrogen of A. The best binding pose in the AT stretches of the minor groove was indicative of an extensive H-bonding network. Structural analysis of the docking positions gave insight into the binding pattern of the complexes. Comparable semiempirical level of theory was successfully applied to metal complexes geometry optimization in similar docking studies.[34,35]

Mechanistic Insights of Cytotoxicity of Complexes 7 and 9 in Comparison to Cisplatin. It was established that Pt(II) complexes bind to DNA via coordination bonds distorting normal three-dimensional structure of double helix.[36] This subsequently induces cell death through apoptosis.[37] The ability to cause cellular chromosomal DNA damage was examined for Pt(II) complexes **7** and **9** and compared to that of cisplatin (Figure 5A). Because of specific

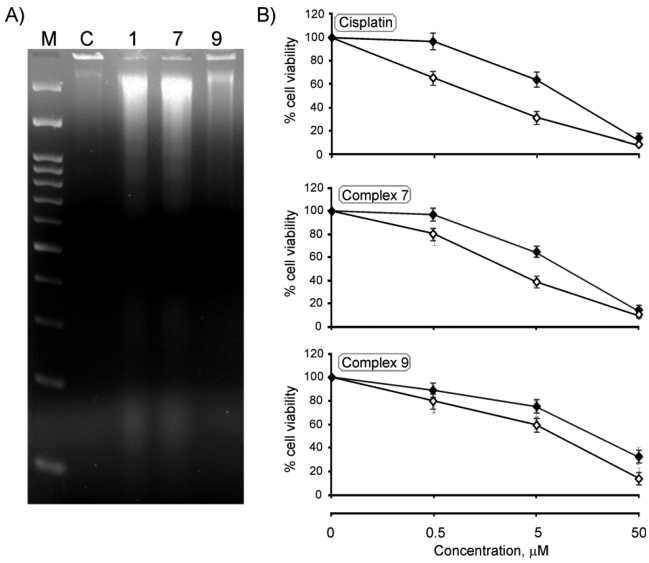

Figure 5. Cytotoxic effect of cisplatin and diazine-bridged Pt(II) complexes **7** and **9** on melanoma (A375) cells. (A) Cellular DNA degradation is induced by complexes **7** and **9** in comparison to cisplatin (**1**) and untreated cells (C). DNA molecular weight marker is in lane M (1 kb ladder, Nippon Genetics). (B) Cell viability was determined by MTT (white diamond) and CV (black diamond) assays upon treatment with a range of cisplatin and complex **7** and **9** concentrations for 48 h. Data are an average of three independent determinations.

structural characteristics, we assumed that **9** may exhibit a cytotoxic pathway in human cells different from that of cisplatin. Indeed, electrophoresis pattern of DNA extracted from melanoma cells (A375) treated with cisplatin and complex **7** was similar and revealed DNA damage, while DNA from the cells treated with complex **9** showed more resemblance to untreated cells with DNA degradation occurring to low extent (Figure 5A).

Cisplatin generally causes DNA damage subsequently followed by activation of mitochondrial cell death pathway which can also be observed as significant difference between IC_{50} values calculated from MTT and CV assays (Figure 5B). MTT viability screen indicates the number of metabolically active cells, while CV assay indicates the total number of live adherent cells; therefore, from the result, an agreement conclusion can be drawn if the cell mitochondria were the initial target of the tested compound. Complex **7** induced DNA fragmentation and affected mitochondrial respiration similar to cisplatin itself, while complex **9** most likely employs different

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mechanisms of action. From the shorter distance of two Pt(II) ions of **9** its better ability to form DNA adducts and high cytotoxic activity was proposed. It was previously shown that multinuclear Pt(II) complexes, such as a complex of two monofunctional [*trans*-$PtCl(NH_3)_2$] platinum units bridged by a platinum tetraamine unit [*trans*-$Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2]^{2+}$ (BBR3436),[38] maintain bifunctional binding mode on DNA and forms long-range cross-linking adducts, but when the cisplatin moieties were bridged by aromatic linkers, preferentially interstrand DNA cross-linking adducts were formed such as in the case of $\{[cis\text{-}PtCl(NH_3)_2]_2(4,40\text{-methylenedianiline})\}^{2+}$.[39] Cytotoxicity screening of numerous multinuclear Pt(II) complexes revealed the importance of the ligands, as well as that of tailoring groups.[1,40]

Toxicity of Cisplatin (1) and Diazine-Bridged Dinuclear Platinum(II) Complexes (7 and 9) on Zebrafish Embryos. The effects of cisplatin (**1**) and complexes **7** and **9** on development of *Danio rerio* embryos were examined at concentrations close to IC_{50} values determined in the cytotoxicity assessment (Table 1). None of tested substances, applied at concentrations from 0.5 to 50 μM, showed lethal or adverse developmental effects on treated embryos until 96 hpf (hpf = hours post fertilization) (Figure 6A). Low mortality rate

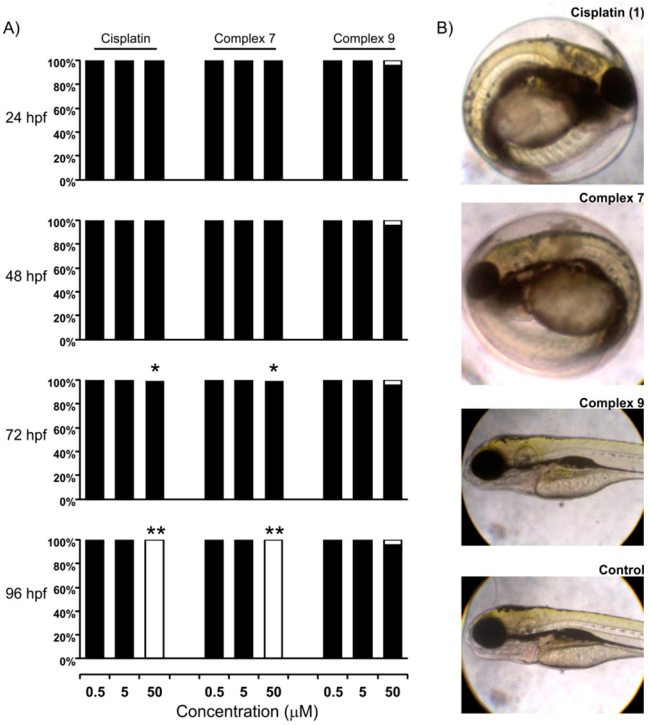

Figure 6. Effects of cisplatin (**1**) and two diazine-bridged dinuclear platinum(II) complexes (**7** and **9**) on development of zebrafish embryos: (A) at 0.5, 5, and 50 μM concentration from 24 to 96 h after fertilization (■, normal embryos; □, lethal embryos; ∗, at 72 hpf no hatching of embryos exposed to 50 μM; ∗∗, at 96 hpf prevented hatching of embryos was scored as lethal effect) and (B) images of zebrafish embryos at 96 hpf exposed to **1**, **7**, and **9** (50 μM).

(total 1 out of 24 embryos) observed at 50 μm of **9** corresponded to those observed in negative control (results not shown). At 96 hpf, all embryos treated with 50 μM of cisplatin and **7** have been prevented from hatching, while all embryos exposed to 50 μM of **9** hatched up to 72 hpf (Figure 6A). These data showed that while at tested concentrations **1**, **7**, and **9** were not teratogenic (neither skeletal nor cardiovascular abnormalities were observed), **9** was significantly less toxic than **1** and **7**, displaying no adverse effect on the embryos development (hatching) (Figure 6B). Hatching success is supposed to be a sensitive end point of the zebrafish embryo in toxicity assay, since no hatched embryos had a lethal outcome.[41] While no reference data are available in the literature on cisplatin and dinuclear Pt(II) complexes, it has been demonstrated that platinum and platinum nanoparticles had adverse impact on the hatching success on developing zebrafish embryos.[41,42]

The zebrafish allows high-quality in vivo validation of drug targets before clinical trials, serving as a very useful bioassay platform for toxicology studies.[43,44] Cisplatin was approved by Food and Drug Administration in 1978 in spite of some dose limiting side effects; hence, the data on cisplatin toxicology in wild type zebrafish that emerged as model later are not readily available in the literature. Likewise, there are no reports on embryotoxic effects on zebrafish of cisplatin derivatives such as oxaliplatin, carboplatin, tetraplatin or of any Pt(II) dinuclear complexes. In a few studies, concentration-dependent ototoxic, neurotoxic, and nephrotoxic effects of ciplatin and oxaliplatin were reported including work done on transgenic zebrafish (Brn3C: EGFP).[45] In these experiments, toxic effects were evaluated on 5-day-old embryos that were exposed to cisplatin, oxaliplatin, and related compounds for 24 h.[46,47] It was shown that 50 and 100 μM cisplatin significantly reduced hair-cell survival up to 81% and 55%, respectively, while at concentration of 400 μM cisplatin induced a lethal outcome for embryos.[46]

■ CONCLUSION

Over the past few years, modern medicinal chemistry has been providing us with new and alternative chemotherapeutic compounds with high cytotoxicity toward tumor cells, with reduced side effects in cancer patients. However, anticancer platinum complexes with their unique physicochemical properties still prevail in the field of metals in cancer chemotherapy with their use escalated and being sustained over decades.[11,48]

In summary, a series of diazine-bridged dinuclear platinum-(II) complexes was developed and evaluated in bioassays for potential further development toward novel anticancer agents. From these, although $\{[Pt(1,3\text{-pd})Cl]_2(\mu\text{-pz})\}^{2+}$ showed higher cytotoxic potential in comparison to cisplatin, {[Pt(en)Cl]2(μ-pydz)}$^{2+}$ stood out exhibiting similar in vitro cytotoxicity profile against different cell lines and desirable properties of being less embryotoxic in comparison to cisplatin. Therefore, this complex could serve as baseline for further derivatizations and development of new anticancer drug candidates.

■ EXPERIMENTAL SECTION

Synthesis and Characterization of Pt(II) Complexes. Materials. Distilled water was demineralized and purified to a resistance greater than 10 MΩ cm^{-1}. Ethylenediamine (en), (±)-1,2-propylenediamine (1,2-pn), isobutylenediamine (ibn), *trans*-(±)-1,2-diaminocyclohexane (dach), 1,3-propylenediamine (1,3-pd), 2,2-dimethyl-1,3-propylenediamine (2,2-diMe-1,3-pd), pyrazine or 1,4-diazine (pz), pyridazine or 1,2-diazine (pydz), and $K_2[PtCl_4]$ were obtained from Sigma-Aldrich. The *cis*-$[PtCl_2(NH_3)_2]$ (**1**) and {*cis*-$[PtCl(NH_3)_2]_2(\mu\text{-pz})\}Cl_2$ (**2**) were synthesized according to a procedure published in the literature.[15,49]

Measurements. The ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 spectrometer (^1H at 200 MHz, ^{13}C at 50 MHz) using 5 mm NMR tubes. The NMR samples were prepared in D_2O as

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J. Med. Chem. XXXX, XXX, XXX−XXX

solvent, and the total volume was 0.6 mL. Sodium trimethylsilylpropane-3-sulfonate (TSP) was used as an internal reference. Elemental microanalyses for carbon, hydrogen, and nitrogen parameters were performed using standard techniques by the Microanalytical Laboratory, Faculty of Chemistry, University of Belgrade.[50]

The mononuclear platinum(II) complexes of the type $[Pt(L)Cl_2]$ (L is en, 1,2-pn, ibn, dach, 1,3-pd, and 2,2-diMe-1,3-pd) were prepared according to a procedure published in the literature.[23−25] To $K_2[PtCl_4]$ (207.56 mg, 0.5 mmol) dissolved in 10 mL of water was added potassium iodide (332.02 mg, 2.0 mmol), and the mixture was heated at 50 °C for 5 min. Subsequently, an equimolar amount (1equiv, 1 mmol) of diamine ligand (L) was added to the obtained reaction mixture with heating (50 °C) and stirring continued for 30 min. All $[Pt(L)I_2]$-type complexes were crystallized from water at room temperature. The $[Pt(L)I_2]$ complexes were converted into the corresponding aqua derivatives by treatment with 1.98 equiv of $AgNO_3$ according to a previously published method.[51] The mixture was stirred overnight at room temperature in the dark. In each case, the formed solid AgCl was removed by filtration in the dark, and in the fresh solutions of the aqua complexes an excess of potassium chloride was added. The pale-yellow precipitate of $[Pt(L)Cl_2]$ complexes were removed by filtration, washed with methanol and then ether, and air-dried. The yield was between 80% and 90%. The experimental results of the elemental analysis for C, H, and N parameters for all Pt(II) complexes are in accordance with theoretical values calculated for $[Pt(L)Cl_2]$ type complexes. These complexes were used for further synthesis of the corresponding dinuclear platinum(II) complexes.

Synthesis of $\{[Pt(L)Cl]_2(\mu\text{-}X)\}Cl_2$ Complexes (3−9). The dinuclear platinum(II) complexes of the type $\{[Pt(L)Cl]_2(X)\}Cl_2$ (X is pyrazine, pz, or pyridazine, pydz) were synthesized by modification of the procedure published in the literature.[14,15,18,19] The mononuclear $[Pt(L)Cl_2]$ complex was converted into the corresponding monodimethylformamide (DMF) complex $[Pt(L)Cl(DMF)]NO_3$ by treatment with 0.98 equiv of $AgNO_3$. To a solution of $AgNO_3$ (49.26 mg, 0.29 mmol) in 5 mL of DMF was added a suspension of $[Pt(L)Cl_2]$ (0.30 mmol) in 10 mL of DMF. The mixture was stirred overnight at room temperature in the dark. The precipitated AgCl was removed by filtration, and the resulting pale yellow DMF solution of $[Pt(L)Cl(DMF)]NO_3$ was used as the starting material for the preparation of the required pyrazine or pyridazine-bridged platinum(II) complexes.

DMF solution of the X ligand (10.01 mg, 0.15 mmol) was added dropwise to the solution of $[Pt(L)Cl(DMF)]NO_3$. The mixture was stirred at room temperature in the dark for 24 h. The solvent was then rotary evaporated and the residue washed with ether. The crude product was dissolved in a minimal amount of 0.5 M LiCl aqueous solution. The obtained solution was left overnight in the dark. The pale-yellow precipitate of dinuclear Pt(II) complex was removed by filtration, washed with methanol and then ether, and air-dried.

Complexes containing six-membered diamine ring L crystallized with two molecules of LiCl and two water molecules ($\{[Pt(L)Cl]_2(\mu\text{-}X)\}Cl_2\cdot 2LiCl\cdot 2H_2O$) (**7** and **8**), which differs from complexes with five-membered chelate ring (L) crystallizing in $\{[Pt(L)Cl]_2(\mu\text{-}X)\}Cl_2$ (**4**, **6**, and **9**) molecular form. Depending of the type diamine (L) and bridging (X) ligand, the yield of $\{[Pt(L)Cl]_2(\mu\text{-}X)\}Cl_2$ complex was between 35% and 40%. The purity of the complexes was checked by elemental microanalysis and NMR (^1H and ^{13}C) spectroscopy. All these data confirmed >95% purity of the tested compounds and were in accordance with those previously reported for the same complexes.[20−22]

$\{[Pt(en)Cl]_2(\mu\text{-}pz)\}Cl_2$ (3). Yield 40.0% (43.93 mg, 0.06 mmol). Elemental analysis calculated (%) for $C_8H_{20}N_6Cl_4Pt_2$ (FW = 732.25): C 13.12, H 2.75, N 11.48. Found: C 13.16, H 2.98, N 11.19. ^1H NMR (200 MHz, D_2O): δ = 2.68−2.79 (m, 8H, en), 9.03 ppm (s, 4H, pz). ^{13}C NMR (50 MHz, D_2O): δ = 52.34, 153.46 ppm.

$\{[Pt(1,2\text{-}pn)Cl]_2(\mu\text{-}pz)\}Cl_2$ (4). Yield 33.3% (38.02 mg, 0.05 mmol). Elemental analysis calculated (%) for $C_{10}H_{24}N_6Cl_4Pt_2$ (FW = 760.31): C 15.80, H 3.18, N 11.05. Found: C 15.45, H 3.19, N 10.82. ^1H NMR (200 MHz, D_2O): δ = 1.34 (*d*, 3H, *J* = 6.6 Hz, 1,2-pn), 2.45−2.98 (m, 2H, 1,2-pn), 3.11−3.32 (m, H, 1,2-pn), 9.01 ppm (s, 4H, pz). ^{13}C NMR (50 MHz, D_2O): δ = 17.83, 54.52, 59.86, 153.44 ppm.

$\{[Pt(ibn)Cl]_2(\mu\text{-}pz)\}Cl_2$ (5). Yield 35% (41.39 mg, 0.05 mmol). Elemental analysis calculated (%) for $C_{12}H_{28}N_6Cl_4Pt_2$ (FW = 788.36): C 18.28, H 3.58, N 10.66. Found: C 17.84, H 3.63, N 10.47. ^1H NMR (200 MHz, D_2O): δ = 1.42−1.48 (m, 6H, ibn), 2.66 (s, 2H, ibn), 9.13 ppm (s, 4H, pz). ^{13}C NMR (50 MHz, D_2O): δ = 25.96, 60.09, 63.61, 153.36 ppm.

$\{[Pt(dach)Cl]_2(\mu\text{-}pz)\}Cl_2$ (6). Yield 32% (40.34 mg, 0.048 mmol). Elemental analysis calculated (%) for $C_{16}H_{32}N_6Cl_4Pt_2$ (FW = 840.43): C 22.87, H 3.84, N 10.00. Found: C 22.56, H 3.94, N 9.56. ^1H NMR (200 MHz, D_2O): δ = 1.27−1.62 (m, 4H, dach), 1.76−2.08 (m, 4H, dach,), 2.45−2.61 (m, 2H, dach,), 9.00 ppm (s, 4H, pz). ^{13}C NMR (50 MHz, D_2O): δ = 26.55, 34.56, 63.61, 65.15, 153.31 ppm.

$\{[Pt(1,3\text{-}pd)Cl]_2(\mu\text{-}pz)\}Cl_2\cdot 2LiCl\cdot 2H_2O$ (7). Yield 32% (44.94 mg, 0.051 mmol). Elemental analysis calculated (%) for $C_{10}H_{28}N_6Cl_6O_2Li_2Pt_2$ (FW = 881.12): C 13.63, H 3.20, N 9.54. Found: C 14.80, H 3.03, N 10.07. ^1H NMR (200 MHz, D_2O): δ = 1.84−1.95 (m, 2H, 1,3-pd), 2.71−2.88 (m, 4H, 1,3-pd), 9.03 ppm (s, 4H pz). ^{13}C NMR (50 MHz, D_2O): δ = 29.83, 44.56, 45.66, 153.57 ppm.

$\{[Pt(2,2\text{-}diMe\text{-}1,3\text{-}pd)Cl]_2(\mu\text{-}pz)\}Cl_2\cdot 2LiCl\cdot 2H_2O$ (8). Yield 30% (42.18 mg, 0.045 mmol). Elemental analysis calculated (%) for $C_{14}H_{36}N_6Cl_6O_2Li_2Pt_2$ (FW = 937.23): C 17.94, H 3.87, N 8.97. Found: C 18.04, H 3.72, N 9.17. ^1H NMR (200 MHz, D_2O): δ = 1.00 (s, 6H, 2,2-diMe-1,3-pd), 2.38 (2*s*, 2H, 2,2-diMe-1,3-pd,), 2.49 (2*s*, 4H, 2,2-diMe-1,3-pd,), 9.05 ppm (s, 4H pz). ^{13}C NMR (50 MHz, D_2O): δ = 25.57, 36.14, 53.97, 54.99, 153.58 ppm.

$\{[Pt(en)Cl]_2(\mu\text{-}pydz)\}Cl_2$ (9). Yield 40.0% (43.93 mg, 0.06 mmol). Elemental analysis calculated (%) for $C_8H_{20}N_6Cl_4Pt_2$ (FW = 732.25): C 13.12, H 2.75, N 11.48. Found: C 13.16, H 2.98, N 11.19. ^1H NMR (200 MHz, D_2O): δ = 2.78−2.82 (m, 8H, en), 8.14 (m, 2H, pz), 9.58 ppm (m, 2H, pz). ^{13}C NMR (50 MHz, D_2O): δ = 51.10, 137.33, 164.36 ppm.

Cell Viability Assays. Cell viability was tested by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and crystal violet (CV) colorimetric assays as previously described.[52,53] Assays were carried out after 48 h of cell incubation in the medium containing test compounds at concentrations ranging from 0.25 to 150 μM (including 0.5, 5, 50 μM). The results are presented as percentage of the control (untreated cells) that was arbitrarily set to 100%. The percentage viability values were plotted against the log of concentration and a sigmoidal dose−response curve was calculated by nonlinear regression analysis using GraphPad Prism software, version 5.0, for Windows (GraphPad Software, CA, USA). From these curves, IC_{50} values were obtained.

Gel Electrophoreses Study. The ability of **1**−**9** to cleave DNA was examined by following the conversion of the supercoiled form of pUC18 plasmid DNA (FI) to the open circular (FII) and/or linear forms (FIII), using agarose gel electrophoresis.[54] For the gel electrophoresis experiments, pUC18 plasmid DNA (200 ng) was treated with the cisplatin and Pt(II) complexes (50 μM) in buffer, and the contents were incubated for 12 h at 37 °C, then subjected to electrophoresis on a 1% (w/v) agarose gel containing 0.1 μg/mL ethidium bromide in TAE buffer (40 mM Tris acetate/1 mM EDTA, pH 7.4) buffer at 60 V for 2 h.

For the analysis of cellular DNA degradation, A375 cells (2×10^5 cells) were exposed to the concentrations equal to IC_{50} of cisplatin and Pt(II) complex **7** and **9** for 48 h. The DNA was subsequently extracted from the cells and analyzed by agarose electrophoresis (2% (w/v) agarose gel in a TAE buffer (40 mM Tris acetate/1 mM EDTA, pH 7.4) at 60 V for 2 h) as described previously.[19]

Molecular Docking. In this work, the geometry optimization of platinum(II) complexes has been carried out using semiempirical quantum chemistry method (PM6),[55,56] because of its excellent compromise between computational time and description of electronic correlation.[57] The calculations were performed with the Gaussian 09 molecular package.[58] The structure of B-DNA dodecamer (CGCGAATTCGCG)2 (PDB code 1BNA)[59] was used as a model to study the interaction between the metal complex and DNA.[60] The

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J. Med. Chem. XXXX, XXX, XXX−XXX

flexible compounds (platinum(II) complexes) were docked into rigid DNA structure using the Molegro Virtual Docker (MVD, version 2013.6.0.1).[61] MVD has been successfully applied in docking studies of metal complexes in DNA.[12] Hydrogen bonds and hydrophobic interactions between platinum(II) complexes and DNA were calculated. The binding site was computed with a grid resolution of 0.3 Å. The MolDock SE as a search algorithm was used with the number of runs set to 100. The parameters of docking procedure were the following: population size 50, maximum number of iterations 1500, energy threshold 100.00, and maximum number of steps 300. The number of generated poses was 5. The estimation of platinum(II) complexes and DNA interactions was described by the MVD-related scoring functions: MolDock, Docking, Rerank, and Hbond. A maximum population of 100 and maximum number of iterations of 10 000 were used for each run, and the 5 best poses were retained. Visualization of the docked pose has been done by using the CHIMERA (http://www.cgl.ucsf.edu/chimera/) molecular graphics program.

Toxicity against Zebrafish Embryos. For zebrafish embryotoxicity assessments general rules of the OECD Guidelines for the Testing of Chemicals were followed.[62] Adult wild type zebrafish (*Danio rerio*) obtained from a commercial supplier (Pet Centar, Belgrade, Serbia) and maintained under laboratory conditions for a several months were used in this study. Collected eggs at the 4- to 16-cell stage were exposed to three different concentrations of **1**, **7**, and **9** (0.5, 5, 50 μM) prepared by diluting the stock solution of each compound in the fish medium. Appropriate no-treatment controls were also included. Embryos were placed into 96-well plates containing 200 μL of solution, one embryo per well, and incubated at 27 °C. Twenty-four embryos have been used per group.

All embryos were staged and evaluated using standard procedures as previously described.[62−64] Apical endopoints (Table S1) were recorded at 24, 48, 72, and 96 h post fertilization (hpf) using an inverted microscope (CKX41; Olympus, Tokyo, Japan).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of complexes **2**−**9** and table of lethal and teratogenic effects in zebrafish. This material is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Muhammad, N.; Guo, Z. Metal-based anticancer chemotherapeutic agents. *Curr. Opin. Chem. Biol.* **2014**, *19*, 144−153.

(2) Rosenberg, B. In *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Wiley-VCH: New York, 1999; pp 3−30.

(3) Siddik, Z. Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene* **2003**, *22*, 7265−7279.

(4) Farrell, N. Metal complexes as drugs and chemotherapeutic agents. *Compr. Coord. Chem.* **2003**, *9*, 809−840.

(5) Kelland, L. The resurgence of platinum-based cancer chemotherapy. *Nat. Rev. Cancer* **2007**, *7*, 573−584.

(6) Wang, D.; Lippard, S. J. Cellular processing of platinum anticancer drugs. *Nat. Rev. Drug Discovery* **2005**, *4*, 307−320.

(7) Wang, X. Fresh platinum complexes with promising antitumor activity. *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 396−411.

(8) Jung, Y.; Lippard, S. J. Direct cellular responses to platinum-induced DNA damage. *Chem. Rev.* **2007**, *107*, 1387−1407.

(9) Wheate, N. J.; Collins, J. G. Multi-nuclear platinum drugs: a new paradigm in chemotherapy. *Curr. Med. Chem.: Anti-Cancer Agents* **2005**, *5*, 267−279.

(10) Wheate, N. J.; Walker, S.; Craig, G. E.; Oun, R. The status of platinum anticancer drugs in the clinic and in clinical trials. *Dalton Trans.* **2010**, *39*, 8113−8127.

(11) Zhang, J.; Wang, L.; Xing, Z.; Liu, D.; Sun, J.; Li, X.; Zhang, Y. Status of bi- and multi-nuclear platinum anticancer drug development. *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 272−282.

(12) Qu, Y.; Rauter, H.; Fontes, A. P. S.; Bandarage, R.; Kelland, L. R.; Farrell, N. Synthesis, characterization, and cytotoxicity of trifunctional dinuclear platinum complexes: comparison of effects of geometry and polyfunctionality on biological activity. *J. Med. Chem.* **2000**, *43*, 3189−3192.

(13) 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* **1999**, *38*, 6781−6790.

(14) Farrell, N.; Appleton, T. G.; Qu, Y.; Roberts, J. D.; Fontes, A. P. S.; Skov, K. A.; Wu, P.; Zou, Y. Effects of geometric isomerism and ligand substitution in bifunctional dinuclear platinum complexes on binding properties and conformational changes in DNA. *Biochemistry* **1995**, *34*, 15480−15486.

(15) Komeda, S.; Kalayda, G. V.; Lutz, M.; Spek, A. L.; Yamanaka, Y.; Sato, T.; Chikuma, M.; Reedijk, J. New isomeric azine-bridged dinuclear platinum(II) complexes circumvent cross-resistance to cisplatin. *J. Med. Chem.* **2003**, *46*, 1210−1219.

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

(17) van Zutphen, S.; Robillard, M. S.; van der Marel, G. A.; Overkleeft, H. S.; den Dulk, H.; Brouwer, J.; Reedijk, J. Extending solid-phase methods in inorganic synthesis: the first dinuclear platinum complex synthesised via the solid phase. *Chem. Commun.* **2003**, *7*, 634−635.

(18) Komeda, S.; Ohishi, H.; Yamane, H.; Harikawa, M.; Sakaguchi, K.-I.; Chikuma, M. An NMR study and crystal structure of $[\{cis\text{-}Pt(NH_3)_2(9EtG\text{-}\kappa N7)\}2(\mu\text{-pz})][NO_3]_3$ (9EtG = 9-ethylguanine) as a model compound for the 1,2-intrastrand GG crosslink. *Dalton Trans.* **1999**, 2959−2962.

(19) Kalayda, G. V.; Komeda, S.; Ikeda, K.; Sato, T.; Chikuma, M.; Reedijk, J. Synthesis, structure, and biological activity of new azine-bridged dinuclear platinum(II) complexes—a new class of anticancer compounds. *Eur. J. Inorg. Chem.* **2003**, 4347−4355.

(20) Asanin, D. P.; Zivkovic, M. D.; Rajkovic, S.; Warzajtis, B.; Rychlewska, U.; Djuran, M. I. Crystallographic evidence of anion···π interactions in the pyrazine bridged $\{[Pt(en)Cl]_2(\mu\text{-pz})\}Cl_2$ complex and a comparative study of the catalytic ability of mononuclear and binuclear platinum(II) complexes in the hydrolysis of N-acetylated L-methionylglycine. *Polyhedron* **2013**, *51*, 255−262.

(21) Rajkovic, S.; Rychlewska, U.; Warzajtis, B.; Asanin, D. P.; Zivkovic, M. D.; Djuran, M. I. Disparate behavior of pyrazine and pyridazine platinum(II) dimers in the hydrolysis of histidine- and

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J. Med. Chem. XXXX, XXX, XXX−XXX

methionine-containing peptides and unique crystal structure of {[Pt(en)Cl]$_2$(μ-pydz)}Cl$_2$ with a pair of NH···Cl$^-$···HN hydrogen bonds supporting the pyridazine bridge. *Polyhedron* **2014**, *67*, 279−285.
(22) Rajkovic, S.; Asanin, D. P.; Zivkovic, M. D.; Djuran, M. I. Synthesis of different pyrazine-bridged platinum(II) complexes and ^{1}H NMR study of their catalytic abilities in the hydrolysis of the N-acetylated L-methionylglycine. *Polyhedron* **2013**, *65*, 42−47.
(23) Hohmann, H.; Van Eldik, R. Rate and equilibrium data for substitution reactions of diaqua(ethylenediamine)palladium(II) with chloride in aqueous solution. *Inorg. Chim. Acta* **1990**, *174*, 87−92.
(24) Summa, N.; Soldatovic, T.; Dahlenburg, L.; Bugarcic, Z. D.; van Eldik, R. The impact of different chelating leaving groups on the substitution kinetics of mononuclear Pt(II)(1,2-*trans*-*R*,*R*-diaminocyclohexane)(X-Y) complexes. *J. Biol. Inorg. Chem.* **2007**, *12*, 461−475.
(25) Živković, M. D.; Ašanin, D. P.; Rajković, S.; Djuran, M. I. Hydrolysis of the amide bond in N-acetylated L-methionylglycine catalyzed by various platinum(II) complexes under physiologically relevant conditions. *Polyhedron* **2011**, *30*, 947−952.
(26) Appleton, T.; Hall, J. R. Complexes with six-membered chelate rings. IV. Preparation of some complexes of 1,3-diaminopropan-2-ol and 2-chloropropane-l,3-diamine with platinum and palladium. *Inorg. Chem.* **1972**, *11*, 112−117.
(27) Allen, F. H. The Cambridge Structural Database: a quarter of a million crystal structures and rising. *Acta Crystallogr.* **2002**, *B58*, 380−388.
(28) Zhao, Y.; He, W.; Shi, P.; Zhu, J.; Qiu, L.; Lin, L.; Guo, Z. A positively charged trinuclear 3N-chelated monofunctional platinum complex with high DNA affinity and potent cytotoxicity. *Dalton Trans.* **2006**, 2617−2619.
(29) Holt, P. A.; Chaires, J. B.; Trent, J. Molecular docking of intercalators and groove-binders to nucleic adds using autodock and surflex. *J. Chem. Inf. Model.* **2008**, *48*, 1602−1615.
(30) Warren, G. L.; Andrews, C. W.; Capelli, A.-M.; Clarke, B.; LaLonde, J.; Lambert, M. H.; Lindvall, M.; Nevins, N.; Semus, S. F.; Senger, S.; Tedesco, G.; Wall, I. D.; Woolven, J. M.; Peishoff, C. E.; Head, M. S. A critical assessment of docking programs and scoring functions. *J. Med. Chem.* **2006**, *49*, 5912−5931.
(31) Raman, N.; Sobha, S.; Mitu, L. Synthesis, structure elucidation, DNA interaction, biological evaluation, and molecular docking of an isatin-derived tyramine bidentate Schiff base and its metal complexes. *Monatsh. Chem.* **2012**, *143*, 1019−1030.
(32) Saquib, Q.; Al-Khedhairy, A. A.; Alarifi, S. A.; Dutta, S.; Dasgupta, S.; Musarrat, J. Methyl thiophanate as a DNA minor groove binder produces MT-Cu(II)-DNA ternary complex preferably with AT rich region for initiation of DNA damage. *Int. J. Biol. Macromol.* **2010**, *47*, 68−75.
(33) Filosa, R.; Peduto, A.; Di Micco, S.; de Caprariis, P.; Festa, M.; Petrella, A.; Capranico, G.; Bifulco, G. Molecular modelling studies, synthesis and biological activity of a series of novel bisnaphthalimides and their development as new DNA topoisomerase II inhibitors. *Bioorg. Med. Chem.* **2009**, *17*, 13−24.
(34) Alaghaz, A. M. A.; El-Sayed, B. A.; El-Henawy, A. A.; Ammar, R. A. A. Synthesis, spectroscopic characterization, potentiometric studies, cytotoxic studies and molecular docking studies of DNA binding of transition metal complexes with 1,1-diaminopropane-Schiff base. *J. Mol. Struct.* **2013**, *1035*, 83−93.
(35) Cincinelli, R.; Loana Musso, L.; Dallavalle, S.; Artali, R.; Tinelli, S.; Colangelo, D.; Zunino, F.; De Cesare, M.; Beretta, G. L.; Zaffaroni, N. Design, modeling, synthesis and biological activity evaluation of camptothecin-linked platinum anticancer agents. *Eur. J. Med. Chem.* **2013**, *63*, 387−400.
(36) Reedijk, J. New clues for platinum antitumor chemistry: kinetically controlled metal binding to DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3611−3616.
(37) Todd, R. C.; Lippard, S. J. Inhibition of transcription by platinum antitumor compounds. *Metallomics* **2009**, *1*, 280−91.
(38) Manzotti, C.; Pratesi, G.; Menta, E.; Di Domenico, R.; Cavalletti, E.; Fiebig, H. H.; Kelland, L. R.; Farrell, N.; Polizzi, D.; Supino, R.; Pezzoni, G.; Zunino, F. BBR 3464: a novel triplatinum complex, exhibiting a preclinical profile of antitumor efficacy different from cisplatin. *Clin. Cancer Res.* **2000**, *6*, 2626−2634.
(39) Zerzankova, L.; Kostrhunova, H.; Vojtiskova, M.; Novakova, O.; Suchankova, T.; Lin, M.; Guo, Z.; Kasparkova, J.; Brabec, V. Mechanistic insights into antitumor effects of new dinuclear cis Pt(II) complexes containing aromatic linkers. *Biochem. Pharmacol.* **2010**, *80*, 344−351.
(40) Brown, S.; Trotter, K.; Sutcliffe, O.; Plumb, J.; Waddell, B.; Briggs, N.; Wheate, N. Combining aspects of the platinum anticancer drugs picoplatin and BBR3464 to synthesize a new family of sterically hindered dinuclear complexes; their synthesis, binding kinetics and cytotoxicity. *Dalton Trans.* **2012**, *41*, 11330−11339.
(41) Osterauer, R.; Haus, N.; Sures, B.; Köhler, H. R. Uptake of platinum by zebrafish (*Danio rerio*) and ramshorn snail (*Marisa cornuarietis*) and resulting effects on early embryogenesis. *Chemosphere* **2009**, 975−982.
(42) Asharani, P. V.; Lianwu, Y.; Gong, Z.; Valiyaveettil, S. Comparison of the toxicity of silver, gold and platinum nanoparticles in developing zebrafish embryos. *Nanotoxicology* **2011**, 43−54.
(43) Mandrekar, N.; Thakur, N. L. Significance of the zebrafish model in the discovery of bioactive molecules from nature. *Biotechnol. Lett.* **2009**, *31*, 171−179.
(44) Spitsbergen, J. M.; Kent, M. L. The state of the art of the zebrafish model for toxicology and toxicologic pathology research: advantages and current limitations. *Toxicol. Pathol.* **2003**, *31*, 62−87.
(45) Choi, J.; Im, G. J.; Chang, J.; Chae, S. W.; Lee, S. H.; Kwon, S. Y.; Chung, A. Y.; Park, H. C.; Jung, H. H. Protective effects of apocynin on cisplatin-induced ototoxicity in an auditory cell line and in zebrafish. *J. Appl. Toxicol.* **2013**, *33*, 125−133.
(46) Hirose, Y.; Simon, J. A.; Ou, H. C. Hair cell toxicity in anti-cancer drugs: evaluating an anti-cancer drug library for independent and synergistic toxic effects on hair cells using the zebrafish lateral line. *J. Assoc. Res. Otolaryngol.* **2011**, *12*, 719−728.
(47) Ton, C.; Parng, C. The use of zebrafish for assessing ototoxic and otoprotective agents. *Hear. Res.* **2005**, *208*, 79−88.
(48) Sava, G.; Bergamo, A.; Dyson, P. Metal-based antitumour drugs in the post-genomic era: what comes next? *Dalton Trans.* **2011**, *40*, 9069−9075.
(49) Dhara, S. C. A rapid method for the synthesis of *cis*-[Pt(NH$_3$)$_2$Cl$_2$]. *Indian J. Chem.* **1970**, *8*, 193−194.
(50) University of Belgrade. Faculty of Chemistry. http://www.chem.bg.ac.rs/index-en.html (accessed Nov 9, 2014).
(51) Mahal, G.; Van Eldik, R. Kinetics and mechanism of the formation, aquation, and base hydrolysis reactions of a series of monodentate carbonato complexes of palladium(II). *Inorg. Chem.* **1985**, *24*, 4165−4170.
(52) Hansen, M. B.; Nielsen, S. E.; Berg, K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Methods* **1989**, *119*, 203−210.
(53) Mihajlovic, L. E.; Savic, A.; Poljarevic, J.; Vuckovic, I.; Mojic, M.; Bulatovic, M. Novel methylene modified cyclohexyl ethylenediamine-*N*,*N*′-diacetate ligands and their platinum(IV) complexes: influence on biological activity. *J. Inorg. Biochem.* **2012**, *109*, 40−48.
(54) Jamshidi, M.; Yousefi, R.; Nabavizadeh, S. M.; Rashidi, M.; Haghighi, M. G.; Niazi, A.; Moosavi-Movahedi, A.-A. Anticancer activity and DNA-binding properties of novel cationic Pt(II) complexes. *Int. J. Biol. Macromol.* **2014**, *66*, 86−96.
(55) Amin, E. A.; Truhlar, D. G. Zn coordination chemistry: development of benchmark suites for geometries, dipole moments, and bond dissociation energies and their use to test and validate density functionals and molecular orbital theory. *J. Chem. Theory Comput.* **2008**, *4*, 75−85.
(56) Stewart, J. J. P. Optimization of parameters for semiempirical methods V: modification of NDDO approximations and application to 70 elements. *J. Mol. Model.* **2007**, *13*, 1173−1213.

DOI: 10.1021/jm5017686
J. Med. Chem. XXXX, XXX, XXX−XXX

(57) Frison, G.; Ohanessian, G. A comparative study of semi-empirical, ab initio, and DFT methods in evaluating metal-ligand bond strength, proton affinity, and interactions between first and second shell ligands in Zn-biomimetic complexes. *J. Comput. Chem.* **2008**, *29*, 416−433.
(58) Frisch, M. J.; et al. *Gaussian 09*, revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.
(59) Drew, H. R.; Wing, R. M.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R. E. Structure of a B-DNA dodecamer: conformation and dynamics. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2179−2183.
(60) Suntharalingam, K.; Mendoza, O.; Duarte, A. A.; Mann, D. J.; Vilar, R. A platinum complex that binds non-covalently to DNA and induces cell death via a different mechanism than cisplatin. *Metallomics* **2013**, *5*, 514−523.
(61) Thomsen, R.; Christensen, M. H. MolDock: a new technique for high-accuracy molecular docking. *J. Med. Chem.* **2006**, *49*, 3315−3321.
(62) Fish Embryo Acute Toxicity (FET) Test. *OECD Guidelines for the Testing of Chemicals*; OECD: Paris, France, 2013; Test No. 236.
(63) Kimmel, C. B.; Ballard, W. W.; Kimmel, S. R.; Ullmann, B.; Schilling, T. F. Stages of embryonic development of the zebrafish. *Dev. Dyn.* **1995**, *203*, 253−310.
(64) Nagel, R. DarT: the embryo test with the zebrafish *Danio rerio*—a general model in ecotoxicology and toxicology. *ALTEX* **2002**, *19*, 38−48.

DOI: 10.1021/jm5017686
J. Med. Chem. XXXX, XXX, XXX−XXX