3’-Methyl-4-thio-1H-tetrahydropyranspiro-5’-hydantoin platinum complex as a novel deoxyribonuclease I inhibitor

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Abstract
Deoxyribonuclease I (DNase I) is one of the main nucleases involved in deoxyribonucleic acid (DNA) degradation during apoptosis. It catalyzes the hydrolytic cleavage of DNA, producing 5’-oligonucleotides. The inhibition of DNase I may serve as an important mechanism for protecting DNA against premature degradation during cell damage. Fourteen hydantoin-containing compounds, including two newly synthesized and seven previously synthesized metal complexes, along with five previously synthesized hydantoin ligands, were evaluated in vitro for their inhibitory properties against bovine pancreatic DNase I. As a result, the 3’-methyl-4-thio-1H-tetrahydropyranspiro-5’-hydantoin platinum complex (8) inhibited the enzyme with an IC50 value of 110.20 ± 24.20 µM, a potency 3-fold greater than that of the reference crystal violet (IC50 = 378.27 ± 47.75 µM). To understand the binding mode and mechanism of inhibition of compound 8 with DNase I, molecular docking calculations were performed. The analysis revealed that compound 8 interacts with the most important catalytic residues of DNase I. To the best of our knowledge, this is the first report of a platinum complex inhibiting DNase I.

Keywords
hydantoin, platinum complex, deoxyribonuclease I, molecular docking, synthesis

Introduction
Hydantoin, or imidazolidine-2,4-dione, is a non-aromatic, five-membered heterocycle highly esteemed in medicinal chemistry, captivating researchers for over a decade. Its appeal lies in features like five potential substituent sites, including two hydrogen bond acceptors and donors each, as well as its synthetic feasibility for core scaffolds via established cyclization reactions and its ability to accept various substituents with ease. These attributes have fostered the design and synthesis of numerous hydantoin derivatives with diverse biological activities, spanning antitumor, antimicrobial, anticonvulsant, antidiabetic, anti-inflammatory, anti-immune, antifibrinolytic,
antioxidant, antitussive, and cytoprotective effects. Hydantoins, along with their hybrids with other molecules, also serve as vital precursors in the chemical or enzymatic synthesis of significant non-natural α-amino acids and their conjugates, holding immense medical potential (Konnert et al. 2017; Cho et al. 2019; Elhady et al. 2019; Kalnik et al. 2021). Furthermore, several studies, including our own prior research (Cherneva et al. 2018a, 2018b, 2020, 2022; Bakalova et al. 2019), have explored the synthesis and biological activity evaluation of metal complexes of hydantoin derivatives.

One of the most well-studied deoxyribonucleosides is deoxyribonuclease I (DNase I), which is found in exocrine pancreatic tissue. It exhibits endonucleolytic activity, catalyzing the hydrolytic cleavage of deoxyribonucleic acid (DNA) to produce 5’-oligouronucleotides (Kreuder et al. 1984). Moreover, it is a key player in DNA degradation during apoptosis (Peitsch et al. 1993; Polzar et al. 1993; Rauch et al. 1997; Oliveri et al. 2001, 2004; Samejima and Earnshaw 2005). Inhibiting DNase I could play a crucial role in safeguarding DNA against premature degradation during cellular damage (Eulitz and Mannherz 2007).

Apoptosis, or programmed cell death, is an essential process in development during cellular damage (Eulitz and Mannherz 2007). It is a key player in DNA degradation during apoptosis (Peitsch et al. 1993; Polzar et al. 1993; Rauch et al. 1997; Oliveri et al. 2001, 2004; Samejima and Earnshaw 2005). Inhibiting DNase I could play a crucial role in safeguarding DNA against premature degradation during cellular damage (Eulitz and Mannherz 2007). DNase I inhibitors are highly sought-after targets for developing alternative strategies for preventing and treating various pathological conditions resulting from elevated DNase I levels and/or excessive apoptosis (Smelcerovic et al. 2020). Given the absence of a universally accepted “gold” standard DNase I inhibitor and the relatively limited number of known inhibitors, whether natural or synthetic (Kolarevic et al. 2014), there is an evident need for discovering new and potent DNase I inhibitors. Building upon our previous research efforts in this area (Kolarevic et al. 2018, 2019; Mavrova et al. 2018; Ilić et al. 2018, 2021; Bondžić et al. 2019; Kolarevic et al. 2019; Smelcerovic et al. 2020; Gagic et al. 2021a, 2021b; Gagic et al. 2022; Marković et al. 2023; Rusava et al. 2023a, 2023b), two new complexes were synthesized, and the DNase I inhibitory properties of 14 hydantoin derivatives were evaluated. These derivatives comprised five metal-free ligands, seven platinum, and two palladium complexes.

Materials and methods

Two new Pt(II) and Pt(IV) complexes bearing 3’-amino-4-thio-1H-tetrahydropyranospiro-5’-hydantoin as a ligand were studied using elemental analyses, IR, 1H, and 13C NMR spectra. Elemental analyses were conducted using a “EuroEA 3000 – Single” EuroVectorSpA apparatus (Milan, Italy). Corrected melting points were determined using a Bushi 535 apparatus (BushiLaborechnik AG, Flawil, Switzerland). IR spectra were recorded on a Thermo Scientific Nicolet iS10 spectrophotometer (Thermo Scientific, USA) in the range of 4000–400 cm⁻¹ using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). The 1H and 13C NMR spectra were recorded on a Bruker WM 500 (500 MHz) spectrometer. The splitting of proton resonances in the 1H NMR spectra was defined as s = singlet, bs = broad singlet, and m = multiplet.

Synthesis of platinum complexes

Aqueous ethanol solutions of the ligand (φ = 2:1) were added dropwise to aqueous solutions of K₂PtCl₄ and Na₂PtCl₆ (0.1 g). The reaction mixtures were stirred for approximately 10 hours, concentrated, and then cooled to 4 °C. The resulting yellow products were obtained, filtered, and dried in a vacuum desiccator. The purity was verified by elemental analysis. Thin-layer chromatography (TLC) was employed to identify the complexes, using CH₃COOC₂H₅/C₂H₅OH (φ = 2:1) as the eluent. The reaction between the ligand and K₂PtCl₄ and Na₂PtCl₆ yielded the desired Pt(II) and Pt(IV) complexes, as depicted in Scheme 1.


cis-[PtL₂Cl₂]. Yield: ca. 94%. M.p. 339 °C(dec). Anal. calc. for C₁₄H₁₂N₆O₃S₂Cl·Pt·C: 25.15; N: 12.58; H: 3.29; Found: C: 25.44; N: 12.70; H: 3.58. IR (cm⁻¹): ν(NH + NH) – 3500, 3249; ν(N=O) – 1776; ν(C=O) – 1723; δ(NH + NH) – 1604, 1414; ν(C-S) – 657. 1H-NMR (500 MHz, DMSO-d₆, δ, ppm): 8.71 (s, 1H, NH); 4.72 (bs, 2H, NH₂); 2.87–2.83 (m, 2H, CH₂-S(ax)); 2.70–2.65 (m, 2H, CH₂-C(eq)); 1.96–1.94 (m, 2H, CH₂-C(ax)); 1.83–1.78 (m, 2H, CH₂-C(eq)). 13C NMR (125 MHz, DMSO-d₆, δ, ppm): 174.61 (C=O-4); 155.99 (C=O-2); 58.46 (C-5); 34.65 (CH₂-S(eq)); 23.02 (CH₂-C).
cis-[PtL₂Cl₂]. Yield: ca. 44%. M.p. 278 °C(dec). Anal. calc. for C₁₄H₁₂N₆O₃S₂Cl·Pt·C: 16.22; N: 10.81; H: 3.09; Found: C: 16.54; N: 11.10; H: 3.37. IR (cm⁻¹): ν(NH + NH) – 3518, 3269; ν(N=O) – 1773; ν(C=O) – 1700; δ(NH + NH) – 1613, 1416 cm⁻¹; ν(C-S) – 658. 1H-NMR (500MHz, DMSO-d₆, δ, ppm): 8.65 (s, 1H, NH); 4.77 (bs, 2H, NH₂); 2.82–2.79 (m, 2H, CH₂-S(ax)); 2.77–2.75 (m, 2H, CH₂-S(eq)); 1.93–1.87 (m, 2H, CH₂-C(ax)); 1.74–1.70 (m, 2H, CH₂-C(eq)). 13C NMR (125 MHz, DMSO-d₆, δ, ppm): 174.39 (C=O-4); 155.96 (C=O-2); 58.48 (C-5); 34.64 (CH₂-S); 23.02 (CH₂-C).

Cytotoxic activity

A standard MTT colorimetric method was employed to evaluate the cell viability of the tested compounds. The method, originally described by Mosmann (1983) with...
some modifications (Konstantinov et al. 1999), is considered the simplest and fastest. In this method, the yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Cat. No. M5655-1G, Sigma’s Life Science, Steinhelm, Germany) is reduced to violet formazan by mitochondrial succinate dehydrogenase in viable cells. Eight separate wells were allocated for each concentration, and three separate microplates were used for each assay. The absorbance of MTT-formazan was determined using a microplate reader (Labxim LMR-1) at 580 nm.

**Evaluation of DNase I inhibition**

The inhibitory potential of hydantoin ligands and their metal complexes against bovine pancreatic DNase I was assessed *in vitro* using the method previously described by Kolarević et al. (Kolarević et al. 2018). This method relies on the spectrophotometric measurement of the formation of acid-soluble nucleotides at 260 nm, with crystal violet employed as a positive control.

**Molecular docking**

The optimized structure of compound 8 (for details, see Cherneva et al. 2020) underwent molecular docking calculations to the crystal structure of bovine pancreatic DNase I complexed with dinucleotide d(GCGATCGC)2 (PDB ID: 2DNJ) (Lahn and Suck 1991). Protein structure preparation tools followed by energy minimization using the AMBER12 force field implemented in Molecular Operating Environment (MOE) 2016.0801 were utilized to correct errors and optimize the target structure. Since the inhibitor's binding site on DNase I is currently unknown, the Site Finder tool was applied to identify potential binding pockets. Induced fit docking calculations were performed using the default placement method, Triangle Matcher, and scored via the GBVI/WSA dG function, which estimates the free energy of binding (∆G) of the ligand from a given pose.

**Results and discussion**

**Chemistry**

The platinum complexes 13 and 14 were synthesized by adding the ligand to an aqueous solution of K₂PtCl₄ and Na₂PtCl₄ in a molar ratio of 2:1 (Scheme 1). The compounds were characterized using spectroscopic techniques such as FT-IR, ¹H NMR, and ¹³C NMR.

**IR spectra**

In the IR spectra of the metal-free ligand, the stretching vibrations of ν(NH) and ν(NH₂) appeared in a broad absorption band in the range 3300–3150 cm⁻¹ due to the intermolecular hydrogen bonds. In the platinum complexes, the bands were slightly shifted to higher frequencies. In complexes 13 and 14, the deformation vibrations of the same groups were unaffected. In the ligand, they were observed at 1609 and 1411 cm⁻¹, while in the complexes, they were detected at 1604, 1613, and 1414 and 1416 cm⁻¹, respectively. Additionally, the stretching vibration of the C-S bond shifted from 623 cm⁻¹ in the ligand to 657 and 658 cm⁻¹ in the platinum complexes as a result of the binding of the ligand via the sulfur atom from the C-S group to the metal ion.

**NMR spectra**

In the ¹H NMR spectra of the newly synthesized complexes 13 and 14, the signal of the N-NH₂ protons was not shifted, indicating no complexation between the metal cation and the nitrogen of the NH₂ group. However, the proton signals of the CH₂-S groups in the platinum complexes, compared to those of the metal-free ligand, were shifted to the higher ppm, consistent with observations in other platinum complexes previously published (Cherneva et al. 2017). The differences for CH₂-C protons were smaller than those for CH₂-S protons.

**Biological behavior**

**Cytotoxic activity**

Four human tumor cell lines were utilized to assess the cytotoxic activity: human hepatocyte carcinoma Hep-G2, acute lymphoblastic leukemia REH, acute myeloid leukemia HL-60, and human urinary bladder carcinoma EJ. The IC₅₀ values of ligand 9, platinum complexes 13 and 14, and the reference cisplatin are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Cytotoxic activity of ligand 9, platinum complexes 13 and 14, and cisplatin.</th>
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<tbody>
<tr>
<td>Cell line</td>
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<tr>
<td>Compound</td>
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<td>Ligand 9</td>
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<td>Complex 13</td>
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<tr>
<td>Complex 14</td>
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<td>Cisplatin</td>
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The results indicate that the metal complexes exhibit higher cytotoxic activity than the ligand alone. This can be attributed to the presence of platinum metal in the complexes.

**Evaluation of DNase I inhibition**

A library of 12 previously synthesized hydantoin derivatives, comprising five metal-free ligands (1, 2, 5, 9, and 10), five platinum complexes (6–8, 11 and 12), and two palladium complexes (3 and 4), along with two newly synthesized platinum complexes (13 and 14), was subjected to an *in vitro* DNase I inhibition assay (Table 2). Platinum complex 8 inhibited the enzyme with an IC₅₀ value of 110.20 ± 24.20 μM, while the remaining studied ligands/complexes showed no inhibitory effect within the tested concentration range (IC₅₀ > 200 μM). Complex 8 demonstrated a 3-fold superior DNase I inhibitory property compared to crystal violet, which was utilized as a positive control (IC₅₀ = 378.27 ± 47.75 μM). Furthermore, it was previously demonstrated to be an effective inhibitor of xanthine oxidase (IC₅₀ = 19.33 μM) (Cherneva et al. 2020).
Molecular docking

The top-ranked potential binding site, identified via the Site Finder tool in the MOE 2016.0801 package, comprises the following residues: Asn7, Arg9, Glu39, Arg41, Tyr76, Glu78, Ser110, Arg111, His134, Ser135, Ala136, Pro137, Glu143, Asp168, Asn170, Tyr175, Thr203, Thr205, Ser206, Thr207, Tyr211, Asp251, and His252. This finding aligns with our previous studies on bovine pancreatic DNase I enzyme using another approach for binding site identification (Marković et al. 2023; Ruseva et al. 2023a, 2023b), as well as with other analogous studies on this enzyme (Ilić et al. 2018; Smelcerović et al. 2020; Gajić et al. 2021a, 2021b). The identified binding pocket is situated in the region where DNA binds DNase I (Fig. 1).

Figure 1. The top-ranked potential binding pocket resulting from the Site Finder tool in MOE is depicted using hydrophobic and hydrophilic alpha spheres. The oligonucleotide (represented in magenta) from the crystallographic structure with PDB ID: 2DNJ (Lahm and Suck 1991) is superimposed onto the structure.
The top-scored docking pose of compound 8 within the identified binding pocket is presented in Fig. 2. The formed complex is stabilized by four hydrogen bonds. Two hydrogen bonds are formed between one of the carbonyl oxygen atoms of the hydantoin moiety, which is oriented towards the substrate catalytic cavity of DNase I, the amino side chain group of Asn7, and the imidazole N3-H group of His134, respectively. One hydrogen bond is formed between the carbonyl oxygen atom in the second hydantoin system and the side chain OH-donor group of Ser110. The fourth hydrogen bond occurs between one of the sulfur atoms and the side-chain carboxyl group of Glu39.

The identified binding site on DNase I occupies the substrate’s major contact area, a shallow groove formed between the two central β-sheets and side loops (Fig. 3). The newly discovered inhibitor, compound 8, of DNase I forms contact with Ser110, which is known to bind the DNA molecule (Gajić et al. 2021a). Additionally, it interacts with two of the most important residues of the catalytic center, Glu39 and His134 (Oefner and Suck 1986; Lahm and Suck 1991; Jones et al. 1996; Guéroult et al. 2010). Thus, compound 8 likely exerts its inhibition mechanism via direct competitive binding to the substrate’s active site of DNase I.

**Conclusion**

In this study, we reported the synthesis of two new hydantoin platinum complexes as a continuation of our previous work. A total of fourteen hydantoin derivatives and their corresponding metal complexes were evaluated for their DNase I inhibitory properties. Among these, a platinum (IV) complex with 3'-methyl-4-thio-1H-tetrahydropranspiro-5'-hydantoin as a ligand (8) emerged as the most potent DNase I inhibitor, exhibiting activity three times higher than the “gold” standard crystal violet. Molecular docking revealed the binding mode of compound 8 on DNase I, indicating that the inhibitor interacts with key residues at the substrate’s catalytic center and likely exerts its effect through direct...
competitive inhibition of the catalytic site. To the best of our knowledge, this is the first reported platinum complex that possesses inhibitory activity on DNase I. The structure of compound 8 can serve as a basis for further hit-to-lead optimization in the design and development of novel, more potent DNase I inhibitors.

Acknowledgments

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