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Platinum(II) Complexes of 3-Hydroxypyridine-2-Carboxaldehyde, N(4)-Methyl and N(4)-Pyrrolidinyl Thiosemicarbazones: Synthesis, Characterization, and Primary Anticancer Screening against HeLa Cells, and Molecular Docking

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RESEARCH ARTICLE

Platinum(II) Complexes of 3-Hydroxypyridine-2-Carboxaldehyde, N(4)-Methyl and N(4)-Pyrrolidinyl Thiosemicarbazones: Synthesis, Characterization, and Primary Anticancer Screening against HeLa Cells, and Molecular Docking

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Abstract:

Background:

Thiosemicarbazones are an important class of synthetic organic compounds exhibiting promising biological activities, including antiviral, antibacterial, antitubercular, antiprotozoal, antimalarial, antifungal, enzyme inhibitory, and antitumor. Different α-(N)-heterocyclic thiosemicarbazones are potent inhibitors of ribonucleotide reductase enzyme that play a critical role in the DNA synthesis; moreover, some have been found 1000-fold more potent than the clinical drug hydroxyl urea.

Objective:

Different coordination complexes have been assessed for their efficacy to target MDR and surpass side effects associated with platinum drugs. In this work, we have prepared and investigated the anticancer potential of new platinum compounds of 3- hydroxy-2-formylpyridine thiosemicarbazones.

Methods:

Novel Pt(II) complexes were synthesized and characterized by elemental analyses, FT-IR, ^{1}H-NMR, UV-visible spectroscopy, and mass spectrometry. The *in vitro* anticancer activity of the synthesized compounds against HeLa cells by MTT assay was assessed. Protein-fixed and ligand-flexible docking studies were carried out using the Lamarckian genetic algorithm and Autodock 4.2 software.

Results:

The IC_{50} values of compounds **(3)** and **(4)** through MTT screening against HeLa cells were found to be 107.16 µM and 132.13 µM, respectively. The binding energy value for the complex [Pt(HyPyMe)Cl] was -6.49 kcal/mol. While for complex, [Pt(HyPyPyrd)Cl] was found to have a binding energy value of -6.83 kcal/mol.

Conclusion:

The spectroscopic and analytical data showed the mononuclear structures and square planar geometry of the Pt(II) complexes. The compounds exhibited moderate antineoplastic activity, and N(4)-methyl-substituted compound exhibited better anticancer activity. [Pt(HyPyMe)Cl] complex formed hydrogen bond interactions with guanine-6, guanine-7 and thiamine-8. While, [Pt(HyPyPyrd)Cl] interacted with guanine-7 and guanine-16 via hydrogen bond interaction.

Keywords: Antineoplastic activities, ESI-mass spectrometry, 3-hydroxypyridine, Platinum(II) complexes, Thiosemicarbazone, Molecular docking.

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1. INTRODUCTION

Cancer is the second leading cause of death, accounting for an estimated 9.6 million deaths globally in 2018, and major deaths (70%) from this disease occur in low- and middle-income countries. The present scenario projects the growing trend of cancer that has made it a significant public health concern [1]. Anticancer drugs in clinical use are obtained from different sources, *e.g.*, dyestuffs, natural products from plants, microbes, fungi, synthetic organic compounds, and metal coordination compounds [2]. The specificity of anticancer drugs that can differentiate between normal cells and cancer

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cells is an urgent requirement because it can reduce the side effects of the drugs [3]. Thiosemicarbazone derivatives have been found to inhibit topoisomerase II, one of the targets of anticancer drugs, like doxorubicin, mitoxantrone, or etoposide in breast [4] and prostate cancer [5]. Meanwhile, some thiosemicarbazone derivatives of (1)-3-menthone have been reported to show antiviral effects against the replication of HIV-l (lllB) and HIV-2 (ROD) in acutely infected MT-4 cells [6]. Thiosemicarbazones are an important class of synthetic organic compounds exhibiting promising biological activities, including antiviral, antibacterial, antitubercular, antiprotozoal, antimalarial, antifungal, enzyme inhibitory, and antitumour [7]. The pharmacological activity of thiosemicarbazones is greatly determined by its parent ring substitution on it as well as on the terminal nitrogen moiety [8, 9], and because of its potential biological importance, there has been growing interest in research on thiosemicarbazone [7, 10]. Thiosemicarbazones predominantly exist in the thione form in the solid state, whereas in solution, they exhibit thione-thiol tautomerism [11]. Structure-activity relationship studies revealed that the combination of soft donor atoms, nitrogen and sulfur, endows potent anticancer activity [12], especially α-(N)heterocyclic thiosemicarbazones show better antitumor activity [10]. Different α-(N)-heterocyclicthiosemicarbazones are potent inhibitors of ribonucleotide reductase enzymes that play a critical role in DNA synthesis; moreover, some have been found 1000-fold more potent than the clinical drug hydroxyl urea [13]. 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine) is in phase I and II clinical trials [14], which has demonstrated an overall response rate of 49% and a complete remission rate of 24% [15]. Thiosemicarbazones exhibit antitumor effects through a number of mechanisms, including reactive oxygen species (ROS), apoptosis induction, metastasis suppressor protein, ribonucleotide reductase (RR) inhibition, as well as cell cycle arrest [7]. 3-Hydroxy-2-formylpyridine thiosemicarbazone (3-HP) is one of the most effective anticancer agents with decreased toxicity [16]. Anticancer platinum drugs are in clinical use for the treatment of cervical, ovarian, testicular, head and neck, breast, bladder, stomach, prostate, and lung cancers, as well as for lymphoma, neuroblastoma, sarcoma, melanoma and multiple myeloma. The drawback associated with anticancer platinum complexes is the side effects and development of resistance [17].The NNS donor property of thiosemicarbazones for the metal ion is the key to its anticancer potency [18]. The coordination compounds have biological and chemical diversity distinct from organic drugs [2, 19]. Different coordination complexes have been assessed for their efficacy to target MDR and surpass the side effects associated with platinum drugs [20]. In this work, we have prepared and investigated the anticancer potential of new platinum compounds of 3-hydroxy-2-formylpyridine thiosemicarbazones.

2. METHODS

2.1. Materials and Measurements

4-methyl-3-thiosemicarbazide (Sigma-Aldrich), potassium tetrachloroplatinate (Sigma-Aldrich), and N-methylaniline, 3-pyrridol, hydrazine hydrate (98%), carbon disulfide, acetonitrile, and sodium chloroacetate were purchased from Hi-Media Laboratory Pvt. Ltd., Mumbai, India. The required solvents, such as methanol, ethanol, diethyl ether, formaldehyde, acetic acid, acetone, *etc*., were purchased from Merck, Hi-Media, Qualigens, Glaxo, Ranbaxy, S. D. fine chemical companies, India. All chemicals were used without further purification. Thin layer chromatography plates 60 GF 254 0.2 mm, E. Merck, Darmstadt, Germany, were used for TLC. Electronic spectra in DMF (concentration 5 x 10^{-5} mol/L) were recorded on Shimadzu-1700 spectrometer at the Department of Drug Administration, Government of Nepal, Bizulibazar, Kathmandu. Elemental analysis (C, H, and N) was obtained using a CHN-recorder MT-5 instrument, and NMR spectra were recorded in d_6-DMSO on Burker AV III, 500 MHz FT NMR spectrometer at IIT, Madras, and at Savitribai Phule Pune University, Pune, India on Burker AV III HD 500 MHz. IR spectra were recorded in the Shimadzu FTIR-200 spectrophotometer at the Department of Plant Resources, Government of Nepal, Thapathali, Kathmandu. LC/MS (Waters® Acquity UPLC™ and Q-T Premier™) UPLC was used for the separation of molecules at ultra-high pressure at Auburn University, USA. Q-T Premier Mass Spectrometer is a hybrid quadrupole, orthogonal acceleration time-of-flight mass spectrometer. UPLC/Q-T enables automated exact mass measurement of precursor and fragment ions. Data were recorded using electrospray ionization (ESI) mass spectrometry in positive mode. Determination of platinum and chlorine in the platinum complexes was done according to the method described in the published work [21].

2.2. Synthesis of Thiosemicarbazones

N(4)-Pyrrolidinylthiosemicarbazide, 3-hydroxy-2-formyl-pyridine, and thiosemicarbazones were synthesized and characterized following the procedure reported in our previous paper [22].

2.2.1. 3-hydroxy-2-pyridinecarboxaldehyde N(4)-Methylthiose-micarbazone: HHyPyMe (1)

^{13}C NMR; 178.60, 178.28, C(S); 153.90, 153.51,C(OH); 143.76, C(6); 141.55 C(2); 139.90, 139.37, 138.34, C(4); 128.35, 126.59 C(7); 125.7, 124.75, 124.61, C(5); 31.51, C(N_4).

2.2.2. 3-hydroxy-2-pyridinecarboxaldehyde N(4)-Pyrrolidinyl-hiosemicarbazone:

HHyPyPyrd (2). ^{13}C NMR; 176.21, C(S); 154.33,C(OH); 147.22, C(6); 141.15, C(2);137.71, C(4); 125.29,C(7); 124.48, C(5); C(N_4). 176.21-178.60, 125.29-128.35

Elemental analysis, ^{1}H NMR, IR and UV-Vis spectra of the ligands; HHyPyMe and HHyPyPyrd are previously published [22].

2.3. Synthesis of Platinum Complexes

Platinum complexes were synthesized by stirring an equimolar solution of thiosemicarbazone and potassium tetrachloroplatinate as mentioned before [21].

2.3.1. Chloro (3-hydroxy-2-pyridinecarboxaldehyde N(4)-methylthiosemicarbazonato) Platinum(II): [Pt(HyPyMe)Cl] (3).

Red-brown solid. Yield: 91%, m.p. 198-202 °C. Anal. found C, 21.75; H, 1.98; N, 12.67; Pt, 44.28; Cl, 7.97.; Calc. for $C_8H_9N_4SOPtCl$: C, 21.85; H, 2.06; N, 12.74; Pt, 44.36; Cl, 8..06;.IR ($cm^{-1,}$ vs: very strong, s: strong, m: medium, w: weak, sh: shoulder and br: broad): ν(OH) 3240m, ν(C=N), ring breath 1573s, 1527s,1458m, 1404m, ν(C-O) 1242s, ν(N-N) 1095s, ν(CS) 802s ν (PtN_{azo}) 509m, ν(PtS) 331s, ν(PtCl) 293s. ^{1}H-NMR (ppm) (s: singlet, d: doublet, t: triplet): 8.17 (1H, s, C7H), 8.26 (1H, d, C6H), 7.57 (1H, s,C4H), 7.60 (1H, d,C5H), 8.26 (1H, s, N4H), 2.96, (1H, d, N4CH). ^{13}C-NMR (ppm): 181.96, C(S); 155.02, C(OH); 138.84, C(2); 128.31,C(4); 144.53, C(6); 128.03,C(5); 147.23, C(7); 33.47, $C(N_4)$. Electronic spectra (nm, b: broad): 286(0.939)s, 424(0.883)s, 532(0.239)b. Mass spectrum (*m/z*): 440.99 $[M+H]^+$ (cal. 440.98).

2.3.2. Chloro (3-hydroxy-2-pyridinecarboxaldehyde N(4)-pyrrolidinylthiosemicarbazonato) platinum (II): [Pt(HyPy Pyrd)Cl] (4).

Brick red. Yield: 95%, m.p. 265-268 °C. Anal. found C, 27.35; H, 2.59; N, 11.62; Pt, 40.58; Cl, 7.24.; Calc. for $C_{11}H_{13}N_4SOPtCl$: C, 27.53; H, 2.73; N, 11.68; Pt, 40.65; Cl, 7.39;.IR ($cm^{-1,}$ vs: very strong, s: strong, m: medium, w: weak, sh: shoulder and br: broad): ν(OH) 3201s, ν(C=N), ring breath 1582s, 1550m,1496s, 1450s, ν(C-O) 1288br, ν(N-N) 1095s, ν(CS) 771m ν (PtN_{azo}) 462m, ν(PtS) 354m, ν(PtCl) 308s. ^{1}H-NMR (ppm) (s: singlet, d: doublet, t: triplet): 8.08 (1H, s, C7H), 8.21 (1H, d, C6H), 7.50 (1H, s,C4H), 7.50 (1H, s,C5H), 1.91, 3.59 (8H, s, N4CH). ^{13}C-NMR (ppm): 181.35,180.07, C(S); 154.70,C(OH); C(2); 139.02,C(4); 142.70, C(6); 127.94, 127.69 C(5); 147.73, C(7); 25.40,56.58, 51.79, 51.06, 49.67, $C(N_4)$. Electronic spectra (nm, b: broad): 304 (0.375)s, 428(0.138)s, 536(0.102)b. Mass spectrum (*m/z*): 481.02 $[M+H]^+$ (cal. 481.01).

3. RESULTS AND DISCUSSION

3.1. Infra-red Spectroscopy

Thiosemicarbazones that had exhibited a strong band in the region 3207-3305 cm^1 for ν(OH) stretch [23] were found intact in the Pt(II) complex and shifted to higher energy due to comparatively weaker hydrogen bonding by the –OH group in the complex compared to that in the free thiosemicarbazones. The band next to ν(OH) in the range of 3101-3207 cm^{-1} assigned to ν(NH) [24] in the thiosemicarbazones was found missing in the complexes due to deprotonation of the ligand upon complexation [25]. An intense band at 1603-1563 cm^{-1}, characteristic of thiosemicarbazones corresponding to ν(C=N) stretching vibration [26] and its negative shift (1582-1573 cm^{-1}) in the complexes, indicated the involvement of azomethine nitrogen in coordination with Pt(II). On the other hand, the ν(N-N) band observed at 1051-1045 cm^{-1} in the thiosemicarbazones was observed shifted to higher wavenumbers 1095 cm^{-1} in the platinum complexes that occurred due to coordination through the azomethine nitrogen and deprotonation of thiosemicarbazones, resulting in electronic delocalization [26, 27].

The ν(C=S) stretch observed at 831-845 cm^{-1} in the free thiosemicarbazones shifted to lower wavenumbers (13-66 cm^{-1}), indicating the coordination through the thiol sulfur of the deprotonated ligand in the complexes [24]. The coordination through N and S was further approved by the appearance of new bands at the 509-462 cm^{-1} and 354-331 cm^{-1}, corresponding to ν(Pt-N_{azo}) and (Pt-S), respectively, in the complexes [26, 28]. Coordination of pyridine nitrogen was indicated by the negative shift of the ring band in the complexes compared to that in the free thiosemicarbazones [28]. The fourth coordination site in the tetracoordinated Pt(II) complex was occupied a chloride ion as the new band corresponding to Pt-Cl appeared in the complexes at 308-293 cm^{-1} [26, 28]. The shift in the band position of different groups suggested the formation of Pt(II) complexes, with the deprotonated ligand coordinating as NNS chelating system.

3.2. NMR Spectroscopy

The azomethine (=N-NH) proton signal in the free thiosemicarbazones at 11.76-11.60 ppm was absent in the ^{1}H-NMR spectra of the platinum(II) complex, indicating its deprotonation. The HC=N proton signals shifted to the upfield in the complexes and appeared at 8.17-8.08 ppm. This shift was from the coordination of azomethine nitrogen with the Pt(II) center. The pyridine protons signals afforded irregular changes in the chemical shifts in the platinum(II) complexes with a downfield shift of C(4)H, C(5)H, and C(6)H [26, 28]. Upfield shifts of the N(4)H and N(4)CH protons were observed in the case of Pt(II) complexes compared to that of free thiosemicarbazones. This observation supports the involvement of C=S chromophore in the coordination *via* thioenolization [29]. The peaks observed at 11.49-9.77 ppm due to –OH protons in the spectra of the free ligands were absent/broadened in the spectra of the Pt(II) complexes, which was attributed to strong hydrogen-bonding interactions with the highly polar solvent DMSO. The proton signals of the coordinated thiosemicarbazones shifted mostly upfield in the complexes through coordination by sulfur, azomethine nitrogen, and pyridine nitrogen atoms. This may be due to the delocalization of the negative charge throughout the deprotonated thiosemicarbazone that caused increased shielding of all protons [30]. Another factor for the upfield shift of the proton signal includes the flow of charge from the electron-rich platinum (d^8) into the thiosemicarbazone skeleton, including the aromatic rings (л back bonding) (Fig. **1**) [31, 32].

The ^{13}C NMR spectra of the synthesized thiosemicarbazones exhibited signals corresponding to the expected chemical structure. The aromatic carbons of the thiosemicarbazones were observed at 124.48-154.33 ppm, and the signals of C-OH and C(6) were found to shift downfield in the complexes. The signals of the imine (C=N) and (C=S) carbon atoms in the free thiosemicarbazones appeared at 125.29-128.35 ppm and 176.21-178.60 ppm, respectively [33]. These signals were most influenced by coordination, leading to a downfield shift in the platinum complexes, and appeared at 147.23-147.73 ppm and 180.07-181.96 ppm, respectively, indicating the coordination of the metal center to the azomethine nitrogen and sulfur in the thiol form to the metal ion (Fig. **1**) [34, 35].

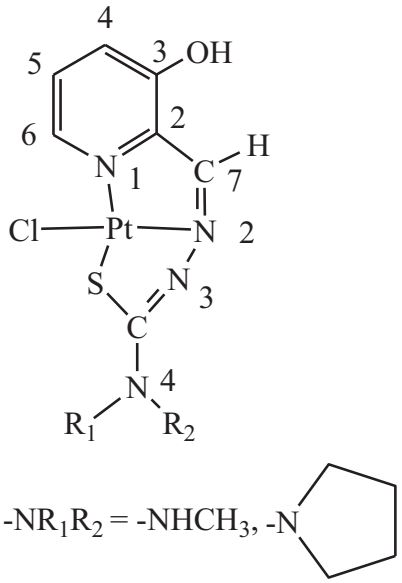

Fig. (1). Structure of platinum complexes.

3.3. UV-Visible Spectroscopy

The UV-Vis spectrum of $K_2[PtCl_4]$ [36] exhibited a band at 216 nm, corresponding to Cl→Pt(II) charge transfer and three d-d transitions at 330 nm, 400 nm, and 425 nm, respectively. The broad bands at 336-342 nm ascribed to n→π* transition of the pyridine ring, azomethine C=N and thioamide N=CS in the free thiosemicarbazones, noticeably shifted to shorter wavelengths in the Pt(II) complexes at 286-304 nm, indicating the coordination of ligand to the metal ion [37]. This demonstrates the coordination of azomethine nitrogen and CS sulfur to Pt(II) [38, 39]. The band at a higher wavelength was assigned to a combination of ligand-to-metal (L →M) and metal-to-ligand (M→L) charge transfer transitions [40, 41]. The square planar Pt(II) complexes with d^8 system exhibit three spin-allowed d-d transitions from ground state $^1A_{1g}$ to excited states $^1A_{2g}$, $^1B_{1g}$ and ^1E_g in order of increasing energy. The electronic spectra of the complexes displayed three bands at 400 nm, 424 - 428 nm, and 532 - 536 nm, respectively, indicating the square planar geometry around the Pt(II) ion (Fig. **1**) [42].

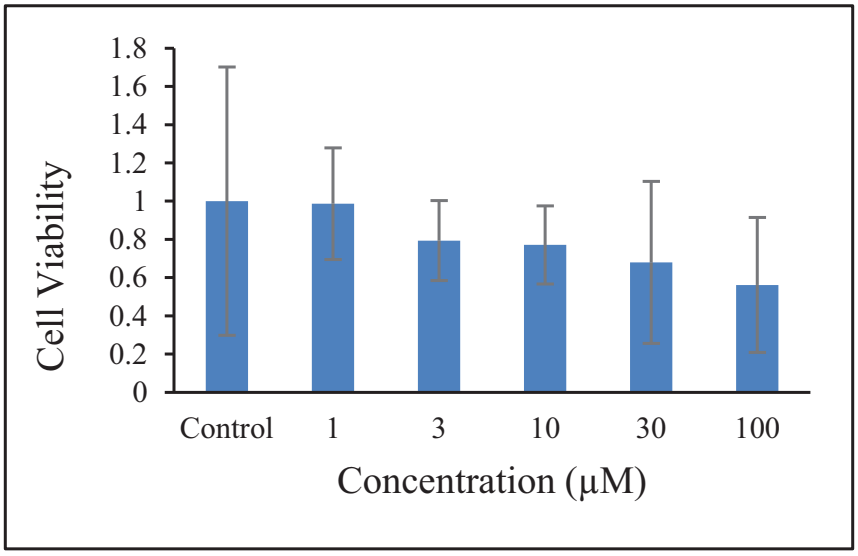

Fig. (2). Bar graph showing the effect of various concentrations of [Pt(HyPyMe)Cl] (3) on cell viability; IC_{50} = 107.16 μM.

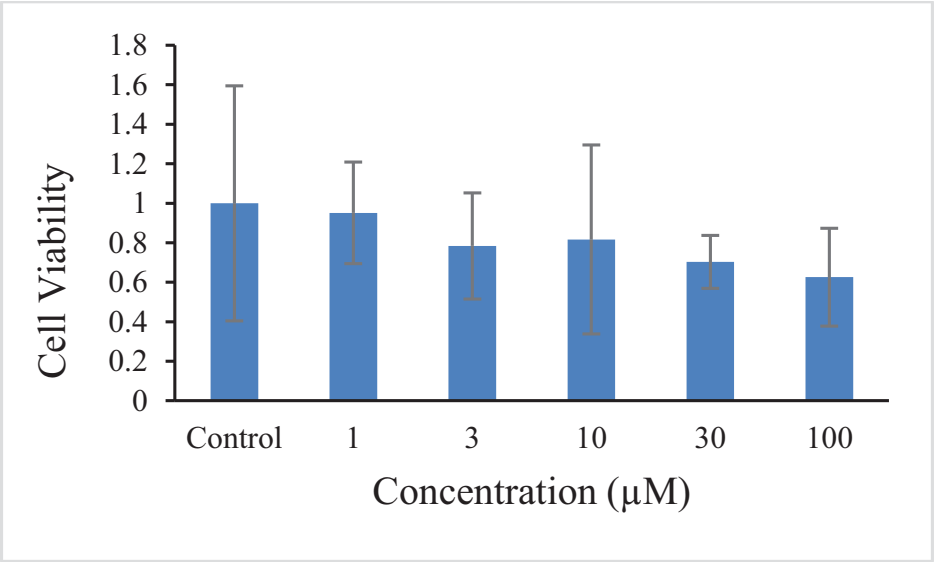

Fig. (3). Bar graph showing the effect of various concentrations of [Pt(HyPyPyrd)Cl] (4) on cell viability; IC_{50} = 132.13 µM.

3.4. Mass Spectrometry

The ESI spectrum of Pt(II) complexes [Pt(HyPyMe)Cl] **(3)** and [Pt(HyPyPyrd)Cl] **(4)** displayed molecular ion peaks at m/z 440.99 $[M+H]^+$ and 481.02 $[M+H]^+$, respectively. Intensity of the molecular ion peak of Pt(HyPyMe)Cl] **(3)** and [Pt(HyPyPyrd)Cl] **(4)** was 100% and 28%, respectively (Figs. **2** and **3**). Platinum has several isotopes, e.g., ^{194}Pt, ^{195}Pt, ^{196}Pt, ^{198}Pt, and chlorine has two isotopes, ^{35}Cl and ^{37}Cl, in different natural abundances [38]. The peaks with different isotopes with variable intensity were seen in the spectra of Pt(II) complexes and appeared as cluster of ions [43]. Other peaks of various fragments of the complexes were also observed, and their intensities corresponded to their stability [26].

4. BIOLOGY

4.1. Cell Culture

HeLa (cervical cancer cell) was obtained from NCCS Pune, India. It was maintained in DMEM media with 10% FBS and 1% penicillin-streptomycin, and kept in a CO_2 incubator with 5% CO_2 at 37 °C.

4.2. Cytotoxicity Assay

Effects of the test compounds on cell viability, expressed as the percentage of cell inhibition, were determined by crystal violet assay. Approximately 3.5 × 10^3 cells per well were seeded in 96-well plates. Cells were allowed to attach to the surface for 24 hr, followed by compound treatment. After incubation for 72 hr, 90 µL of crystal violet solution (0.5%) was added to each well. The plates were incubated for 30 min at room temperature. Subsequently, the crystal violet solution was removed, and the dye was dissolved with 150 µL of methanol. Absorbance was recorded at 570 nm with the use of a microplate reader (Bio-Tek Synergy HT Multi-Detection Microplate Reader). The percentage cell inhibition was calculated by the formula [(control-exp)/control) × 100]. Three separate experiments were performed to determine the half inhibitory concentrations (IC_{50}).

concentrations of [Pt(HyPyMe)Cl] **(3)** on various concentrations of [Pt(HyPyPyrd)Cl]

cell viability; IC_{50} = 107.16 µM. **(4)** on cell viability; IC_{50} = 132.13 µM.

The anticancer mechanisms of the platinum complexes are complicated. One established mechanism of platinum complexes as an anticancer agent is the release of leaving groups from the complex and the formation of active aquated species that inhibit DNA synthesis through the formation of inter-and intrastrand crosslinks with DNA. A factor responsible for the imperfect activity of the platinum complex is the decreased cellular platinum accumulation due to the involvement of a series of transporters. One of the drawbacks of the anticancer platinum complex is the development of resistance in tumor cells developed by detoxification components, thereby preventing the formation of Pt-DNA adducts. The concept of the selective target in chemotherapy is the future objective in designing the anticancer drug [44].

5. MOLECULAR DOCKING

5.1. Methodology

Docking studies were carried out using AutoDock 4.2. Gasteiger charges were added to the ligand, and a maximum of six active torsions were given to the lead compound using AutoDock tool. Our previously reported method was used for docking simulations [45]. The evaluation of docking results and analysis of their surface with graphical representations were carried out using the Discovery Studio visualizer.

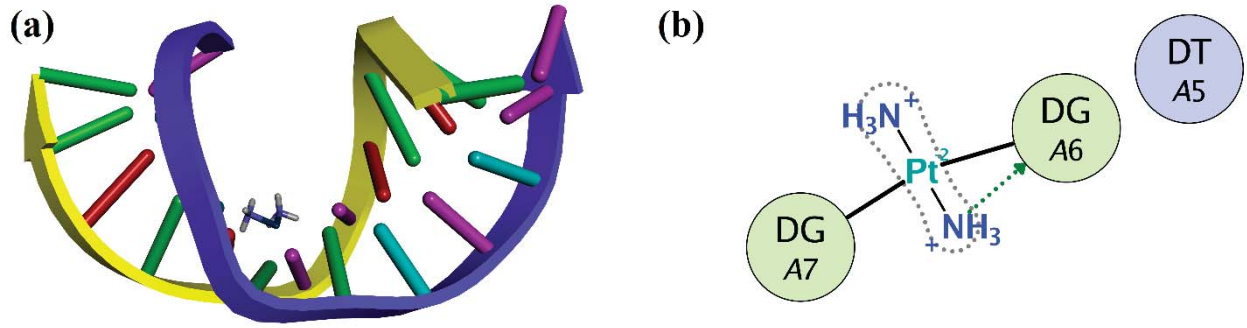

Fig. (4). Binding orientation (left) and 2D interaction plot of cisplatin obtained by docking simulations.

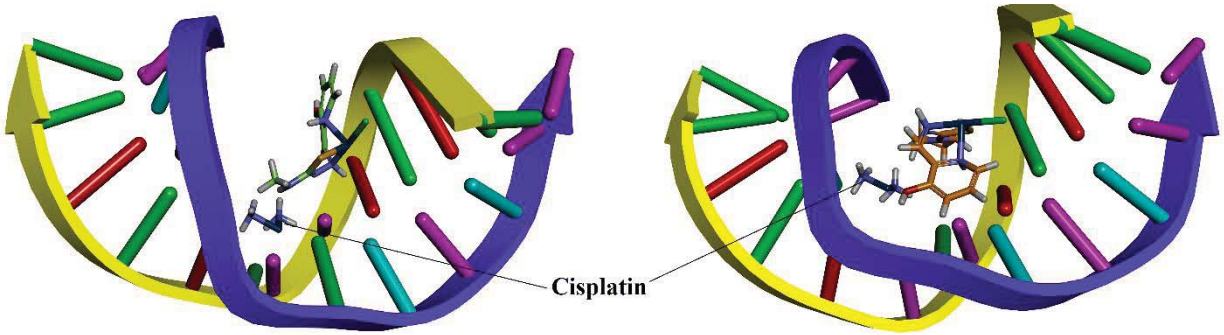

Fig. (5). Schematic of (left) complex [Pt(HyPyMe)Cl]–DNA and (right) complex [Pt(HyPyPyrd)Cl] –DNA obtained by docking simulations.

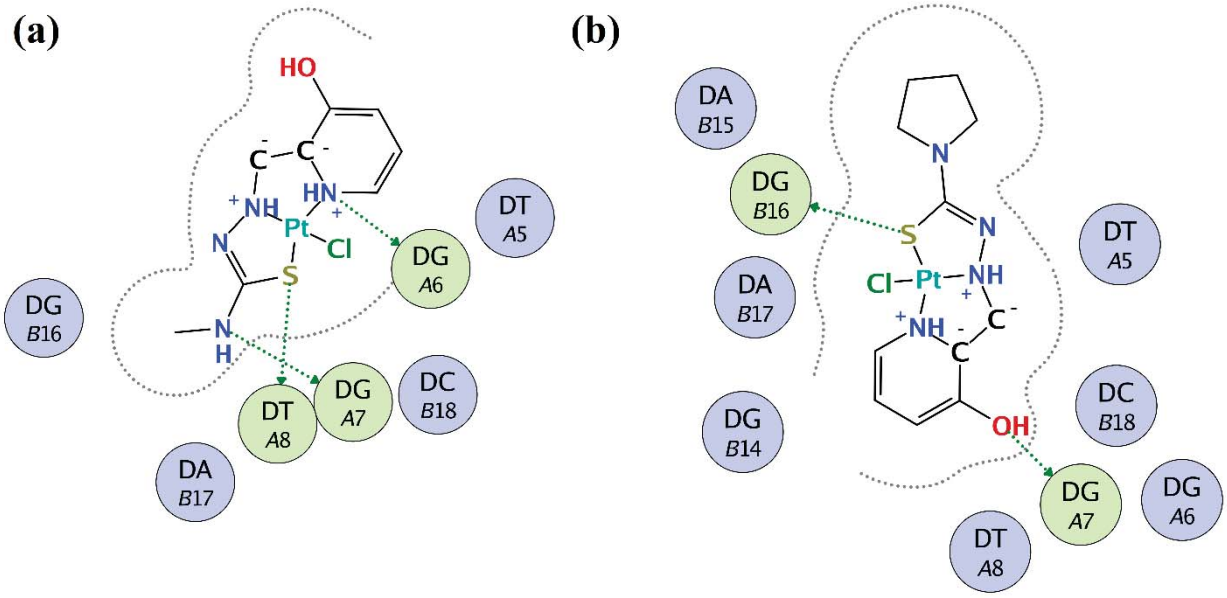

Fig. (6). 2D interaction plots of (left) complex [Pt(HyPyMe)Cl]–DNA and (right) complex [Pt(HyPyPyrd)Cl] –DNA obtained by docking simulations.

6. DISCUSSION

Protein-fixed and ligand-flexible docking studies were carried out using the Lamarckian genetic algorithm and Autodock 4.2 software. Three-dimensional (3D)

deoxyribonucleic acid (DNA) duplex structure was downloaded from the protein data bank (PDB). The accession code for the downloaded DNA duplex was 3LPV. Anticancer drug cisplatin formed covalent bonds with guanine-6 and guanine-7. 3D binding orientation and interaction plot are shown in Fig. (**4**).

Platinum complexes, [Pt(HyPyMe)Cl] and [Pt(HyPyPyrd)Cl], were docked within the cisplatin region. Low binding energy values were considered the comparison criteria between the two Pt-complexes. The studied complexes fit well into the major groove of cisplatin. The 3D binding orientations of the two complexes are shown in Fig. (**4**). The binding energy value for the complex [Pt(HyPyMe)Cl] was -6.49 kcal/mol. While for the complex, [Pt(HyPyPyrd)Cl] was found to have a binding energy value of -6.83 kcal/mol (Fig. **5**). This showed the Pt-complexes to be tightly fitted in the cisplatin binding site. 2D interaction plots of both complexes are shown in Fig. (**6**). [Pt(HyPyMe)Cl] complex formed hydrogen bond interactions with guanine-6, guanine-7 and thiamine-8 (Fig. **6a**). While, [Pt(HyPyPyrd)Cl] interacted with guanine-7 and guanine-16 via hydrogen bond interaction (Fig. **6b**).

CONCLUSION

Two Pt(II) complexes, Pt(HyPyMe)Cl]**(3)** and [Pt(HyPyPyrd)Cl]**(4)**, containing thiosemicarbazone anion, were synthesized and characterized. Their spectroscopic properties suggested that they corresponded to NNS binding sets, and the fourth coordination site was occupied by a chloride ion in a distorted square planar geometry. Mass spectra of the compounds, 440.99 $[M+H]^+$**(3)** and 481.02 $[M+H]^+$**(4)**, further established the formation of the proposed compounds. The IC_{50} values of compounds **(3)** and **(4)** through MTT screening against HeLa cells were found to be 107.16 μM and 132.13 μM, respectively.

LIST OF ABBREVIATIONS

(RR) = Ribonucleotide Reductase

(ESI) = Electrospray Ionizationprotein data bank (PDB)

(PDB) = Protein Data Bank

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data sets used and/or analysed during this study are available from the corresponding author upon request.

FUNDING

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CONFLICT OF INTEREST

Dr. Paras Nath Yadav is the Editorial Advisory Board Member of the journal Current Indian Science.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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