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Investigating the Anticancer Properties of Novel Functionalized Platinum(II)–Terpyridine Complexes

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Abstract: Novel platinum(II) complexes of 4′-substituted terpyridine ligands were synthesized and characterized. Each complex had a different biomolecule (amine, glucose, biotin and hyaluronic acid) as a targeting motif, potentially improving therapeutic outcomes. We demonstrated that complexes can self-assemble in water into about 150 nm nanoparticles. Moreover, the complexes were assayed in vitro toward a panel of human cancer cell lines (ovarian adenocarcinoma A2780, lung cancer A549, breast adenocarcinoma MDA-MB-231, neuroblastoma SHSY5Y) to explore the impact of the pendant moiety on the terpyridine toxicity. The platinum complex of terpyridine amine derivative, [Pt(TpyNH$_2$)Cl]Cl, showed the best antiproliferative effect, which was higher than cisplatin and [Pt(Tpy)Cl]Cl. Selective in vitro antiproliferative activity was achieved in A549 cancer cells with the Pt–HAtpy complex. These findings underline the potential of these novel platinum(II) complexes in cancer therapy and highlight the importance of tailored molecular design for achieving enhanced therapeutic effects.

Keywords: metallo-drugs; metal complexes; platinum; terpyridine

Citation: Panebianco, R.; Viale, M.; Giglio, V.; Vecchio, G. Investigating the Anticancer Properties of Novel Functionalized Platinum(II)–Terpyridine Complexes. *Inorganics* **2024**, *12*, 167. https://doi.org/10.3390/inorganics12060167

Academic Editors: Snežana Jovanović-Stević and Jovana V. Bogojeski

Received: 17 May 2024
Revised: 11 June 2024
Accepted: 13 June 2024
Published: 15 June 2024

1. Introduction

Platinum coordination compounds have a remarkable clinical history due to the discovery of cisplatin [1]. After the successful development of cisplatin, carboplatin, and oxaliplatin, numerous platinum compounds have been synthesized to uncover even more potent anticancer drugs [2,3].

Various amino ligands have been utilized to form platinum complexes [4]. In the last decade, many platinum compounds containing N-heterocyclic side chains have been investigated as anticancer agents, and some of them have also been tested in vivo [5].

In this context, a large and growing family of platinum(II) complexes of 2,2′:6′,6″-terpyridine (tpy) derivatives have been receiving increased attention [1,6–8].

Terpyridines are strongly chelating agents that have potential in medicinal chemistry. Terpyridines consist of three pyridine rings and act as tridentate chelators. Their fused aromatic structure makes them suitable ligands for various transition metals. The resultant complexes have been thoroughly investigated for their biological activity and physicochemical features [6,7,9].

Some diverse platinum(II) derivatives of tpy and its analogs have shown potent antiproliferative properties in vitro against several cancer cell lines, comparable to or even better than cisplatin [10,11]. The mechanism of action of platinum(II)–tpy complexes has been widely studied in vitro. It was observed that they are well suited to interacting with different biomolecules, including proteins and nucleic acids, such as RNA and DNA [7,12].

Nuclear DNA is believed to be the major biological target of many platinum-based agents, primarily due to platinum's ability to bind adjacent DNA base pairs. Tpy platinum

complexes have been investigated for their behavior as metallointercalation agents. The first structural evidence of their intercalation ability was reported by Lippard et al. [13]. They observed that the $[Pt(Tpy)Cl]^+$ complex could intercalate non-covalently between adjacent DNA base pairs, resulting in the unwinding of the DNA. Additionally, the labile chloride ligand can undergo a substitution reaction and bind DNA bases [14]. Indeed, $[Pt(Tpy)Cl]^+$ produced a mixture of intercalation and coordination with DNA due to the presence of the labile chloride ligand.

In recent years, extensive libraries of platinum(II)–terpyridine compounds have been prepared. Most developed systems include platinum complexes bearing π-conjugated heterocyclic ligands since large planar surfaces may improve the DNA intercalation via π–π stacking [15,16]. The design of particular tpy-based platinum(II) complexes can be accomplished through structural modification of the tpy, especially by introducing a substituent at the 4′ position, thereby tuning the properties of the complex or interactions with specific biomolecules.

Square-planar platinum(II)–terpyridyl complexes have a strong tendency toward the formation of intermolecular aggregates or oligomeric structures in the water solution via metal–metal interaction and the π–π stacking interaction of the terpyridine ligands [17–19]. The self-assembly of the metal complexes can be induced by polymers and solvents [20,21].

The family of terpyridine–platinum complexes offers different mechanisms of action involving diverse targets. Mainly, they have been developed as important topoisomerase I/II inhibitors [9,22], G-quadruplex DNA binders [23,24], telomerase inhibitors [25,26], DNA synthesis inhibitors [6,27,28], reactive oxygen species (ROS) activators [29], adenosine triphosphate (ATP) inhibitors [30], mitochondrion-targeted agents [31], photodynamic therapy agents [32], autophagy–lysosomal system inductors [33] and modulators of β-amyloid aggregation [34].

Most Pt-based drugs are non-specific regarding their target cell types. Thus, several attempts have been undertaken to make them more specific for targeting tumors of particular organs. Sugars and vitamins are attractive vectors for cellular targeting and delivery. By appending sugars, peptides, vitamins, and other biomolecules on the ligands of the desired complexes, the selective accumulation of drugs inside tumor cells may be achieved. Indeed, they are known to be transported through the blood and across the cellular membrane by binding of receptors.

Despite the interest in designing selective systems, few examples of platinum(II)–tpy complexes involve biomolecules as targeting agents, such as glycosyl or estrogen units [27,33,35–37].

For this purpose, here we report the synthesis of novel platinum(II) complexes with different 4′-substituted tpy ligands (Figure 1). They were functionalized with biomolecules such as glucose (Glc), biotin (Bio), hyaluronic acid (HA), and propylamine that may differently affect the cellular uptake. Glucoside conjugation has been explored by leveraging the Warburg effect of cancer cells characterized by glucose units crossing the membrane through GLUT transporters overexpressed in tumor cells [38]. Biotin is taken up via a high-affinity multivitamin transporter (SMVT) overexpressed in specific cancer cells, and it has been widely used as a targeting unit [39]. HA is a well-known targeting unit recognized by the CD44 receptor, overexpressed in cancer cells [40].

This work describes the synthesis and characterization of different platinum(II) complexes by varying the substituent on the tpy ligand (Figure 1). Their antiproliferative effect in vitro was investigated with regard to a representative panel of cancer cell lines.

To our knowledge, this is the first case in which a platinum(II)–terpyridine complex was covalently linked to a biocompatible polymer (HA) as a delivery strategy. Interestingly, promising and selective antiproliferative activity was achieved in A549 cancer cells with the Pt–HAtpy complex.

For comparative purposes, we also studied cisplatin and the [Pt(Tpy)Cl]Cl and [Pt(TpyCl)Cl]Cl complexes.

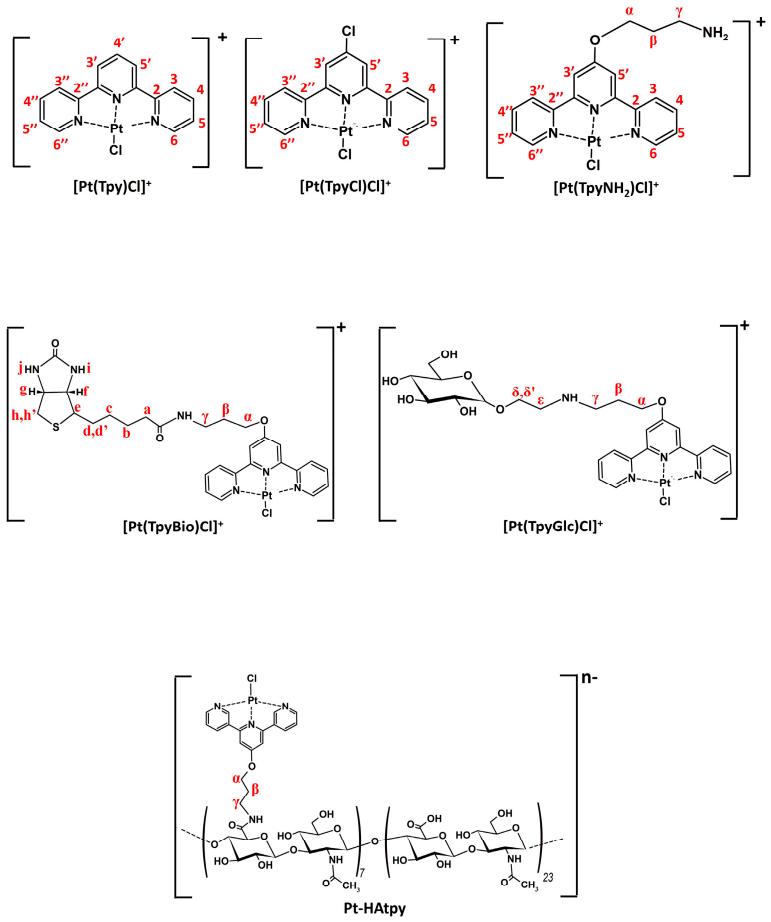

Figure 1. Schematic structure of platinum(II)–tpy complexes. In the complexes, chlorine serves as the counter ion. In the HA complex, COO^- groups can act as counterions.

2. Results and Discussion

2.1. Synthesis

The platinum complexes shown in Figure 1 were synthesized according to the general synthetic procedure reported in Figure 2. The platinum precursor cis[Pt(COD)Cl_2] was reacted directly with the respective terpyridine ligand (L) to yield the platinum complexes [Pt(L)Cl]Cl. The complexes are soluble in water.

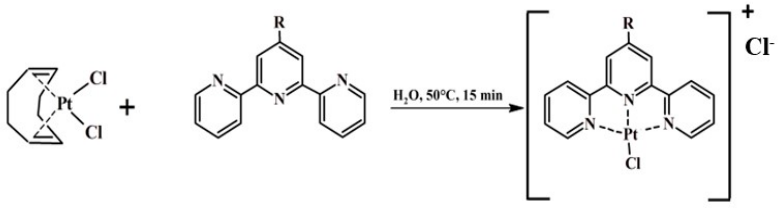

Figure 2. The schematic reaction of platinum(II) complexes of tpy derivatives.

The platinum(II)–terpyridine complexes were characterized by NMR, UV-Vis spectroscopy, and ESI-MS spectrometry, confirming their structure.

2.2. NMR Characterization

In the ^{1}H NMR spectra (Figures S1–S10), the pyridyl protons resonated in the aromatic region in the 7.30–9.50 ppm range, either as singlets or multiplets. Aromatic protons in the complexes are upfield shifted compared to the ligands due to metal complexation, which also determined the pyridine rings flipping from an antiperiplanar into a synperi-

planar conformation, as reported for other tpy derivatives. In the tpy derivatives, the proton signals of the alkyl chain appeared in the aliphatic region. [Pt($TpyNH_2$)Cl]Cl and [Pt(TpyGlc)Cl]Cl spectra were recorded in a NaCl solution to prevent chloride substitution with the amino group [41]. In the spectrum of [Pt($TpyNH_2$)Cl]Cl (Figure S3), in addition to aromatic signals, the signals of the propylenic chain can be found at 4.30, 3.30, and 2.29 ppm.

A glucose-based complex showed similar behavior (Figure S9). As reported for other metal complexes, the complexation produced a downfield shift of the signals of the moiety [42].

As for the NMR spectrum of the Pt–HAtpy complex (Figure S10), the aromatic signals are broad. In this case, we can hypothesize the coordination of carboxylic groups of HA with the formation of either intermolecular or intramolecular species with high molecular weight.

2.3. ESI-HRMS Characterization

Furthermore, ESI-mass spectra were recorded for the complexes (Figures S11–S17). Pt–HAtpy was not investigated due to the high molecular weight. Under electrospray ionization (ESI) conditions, some ions were observed from a 5 ppm solution of [Pt(L)Cl]Cl in water, including $[Pt(L)Cl]^+$, $[Pt(L)OH]^+$ and $[Pt(L)H_2O]^{2+}$. Characteristic isotopic peaks for platinum and chlorine-containing ions were clearly seen, and the isotopic patterns of these peaks confirmed the elemental composition of the observed ions. The most relevant peaks were assigned in the spectra. Both calculated and experimental isotopic patterns for selected peaks are reported in Table 1. As reported elsewhere, the exchange reaction of Cl^- in the platinum complex solutions was observed in the ESI-MS spectra [43]. In the spectra, $[Pt(L)Cl]^+$ and $[Pt(L)OH]^+$ species were consistently identified across all complexes. Additionally, in the case of [Pt($TpyNH_2$)Cl]Cl, a double-charged peak at m/z 258.5414 corresponding to the aqua species, $[Pt(TpyNH_2)H_2O]^{2+}$ was also detected (Figure S14). Moreover, the complex [Pt(TpyBio)Cl] exhibits a double-charged peak at m/z 363.5914, which was attributed to the species $[Pt(TpyBio)]^{2+}$ (Figure S19).

Table 1. ESI-MS peaks observed and calculated for the platinum complexes studied.

Complex (PtLCl)	Assignment	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Error (ppm)
$[Pt(Tpy)Cl]^+$	$[PtLCl]^+$ ($C_{15}H_{11}ClN_3Pt^+$)	463.0284	463.0251	7.1
	$[PtLOH]^+$ ($C_{15}H_{12}N_3OPt^+$)	445.0623	445.0591	7.2
$[Pt(TpyCl)Cl]^+$	$[PtLCl]^+$ ($C_{15}H_{10}Cl_2N_3Pt^+$)	496.9894	496.9835	11.9
	$[PtLOH]^+$ ($C_{15}H_{11}ClN_3OPt^+$)	479.0233	479.0189	9.2
$[Pt(TpyNH_2)Cl]^+$	$[PtLCl]^+$ ($C_{18}H_{18}ClN_4OPt^+$)	536.0811	536.0763	9.0
	$[PtLOH]^+$ ($C_{18}H_{19}N_4O_2Pt^+$)	518.1150	518.1104	8.9
	$[PtLH_2O{\cdot}H_2O]^{++}$ ($C_{18}H_{22}N_4O_3Pt^{++}$)	268.5664	268.5414	9.3
$[Pt(TpyGlc)Cl]^+$	$[PtLCl]^+$ ($C_{26}H_{32}ClN_4O_7Pt^+$)	742.1602	742.1534	9.2
	$[PtLOH]^+$ ($C_{26}H_{33}N_4O_8Pt^+$)	724.1941	724.1875	9.1
$[Pt(TpyBio)Cl]^+$	$[PtLCl]^+$ ($C_{28}H_{32}ClN_6O_6PtS^+$)	762.1587	762.1511	10.0
	$[PtLOH]^+$ ($C_{28}H_{33}N_6O_4PtS^+$)	744.1926	744.1859	9.0
	$[PtL]^{++}$ ($C_{28}H_{32}N_6O3PtS^{++}$)	363.5947	363.5914	9.1

2.4. UV-Vis Characterization

Platinum(II) complexes were also characterized by UV-Vis spectroscopy. UV-Vis spectra were recorded for all of the complexes in water or a NaCl solution (Figures S18–S23). All of the spectra showed the same characteristic absorption bands of [Pt(Tpy)Cl]Cl, suggesting the same coordination environment (Table 2). The intense bands in the UV region (250–280 nm) are due to the intra-ligand $\pi \rightarrow \pi^*$ transitions, characteristic of polypyridine ligands [44]. As reported for other metal complexes, the absorption between 300 and 350 nm is typical of the tpy change from the trans–trans conformation in solution to the planarized cis–cis conformation in the complex [45]. Finally, the moderately intense broad bands in the visible region (about 385 nm) of all the complexes can be assigned to $d\pi$ to π^* metal-to-ligand charge transfer (MLCT) transitions, as reported for similar tpy–platinum complexes [44,46,47]. The spectra confirmed the coordination of Cl^- [48].

Table 2. UV-Vis data (λ nm,ε, L $M^{-1}cm^{-1}$) of platinum(II)-tpy complexes in NaCl solution.

Complex	λ nm (ε, L $M^{-1}cm^{-1}$)
[Pt(Tpy)Cl]Cl	250 (18,400); 279 (14,800); 328 (7600); 344 (6200); 385 (sh,1800)
[Pt(TpyCl)Cl]Cl	249 (24,000); 281 (20,000); 306 (8500); 317 (8750); 329 (9500); 385 (2275)
[Pt(TpyNH2)Cl]Cl	243 (23,000); 282 (16,250); 312 (9000); 337 (sh,5250); 381 (sh,1625)
[Pt(TpyBio)Cl]Cl	247 (22,750); 282 (18,000); 329 (8500); 395 (2375)
[Pt(TpyGlc)Cl]Cl	245 (18,000); 282 (14,600); 313 (6800); 327 (6600); 390 (1700)
[Pt-HAtpy]	247 (27,142); 282 (21,142); 317 (9428); 390 (2314)

2.5. DLS Characterization

Several platinum(II) complexes have been shown to oligomerize in solution via weak bonding interactions. The [Pt(Tpy)Cl]Cl complex has been widely studied for its ability to self-assemble [44,49]. The supramolecular structure formed from platinum(II) terpyridine complexes is orchestrated by intermolecular interaction, mainly metal–metal and ligand–ligand π stacking interactions. To further investigate the synthesized complexes, their aggregation behavior was studied via dynamic light scattering (DLS) (Figure 3). The hydrodynamic volume values for platinum complexes depicted in Figure 1 are reported in Table 3.

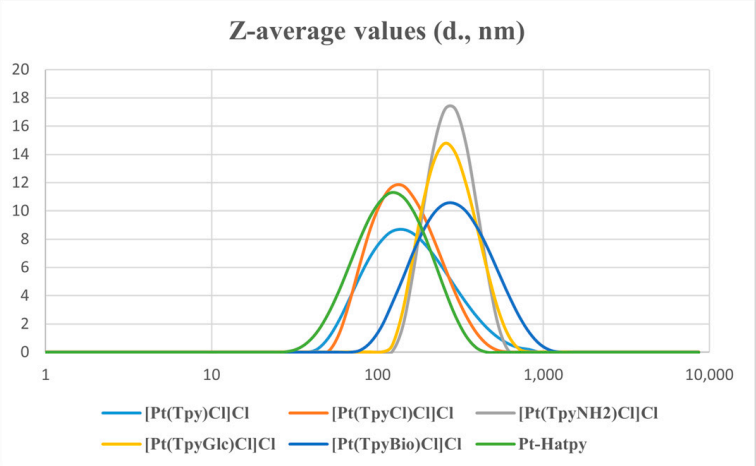

Figure 3. Distribution of hydrodynamic volumes of platinum(II)-tpy complexes.

Table 3. Hydrodynamic volume values (Z-average) of platinum(II)-tpy complexes.

Complex	Z-Average (d., nm)
[Pt(Tpy)Cl]Cl	153 ± 9
[Pt(TpyCl)Cl]Cl	137 ± 21
[Pt($TpyNH_2$)Cl]Cl	267 ± 6
[Pt(TpyBio)Cl]Cl	261 ± 32
[Pt(TpyGlc)Cl]Cl	193 ± 6
[Pt-HAtpy]	108 ± 2

The data suggested that all the complexes formed aggregates with hydrodynamic diameters of 150–300 nm. The side chain does not reduce the self-assembly. The complexes of $TpyNH_2$, TpyGlc, and TpyBio form bigger aggregates, probably because of higher solvation due to the hydrophilicity of the side chain. In the case of the hyaluronic acid derivative, the metal complex self-assembled into about 108 nm nanoparticles. This value suggests that the HA backbone modulates the aggregation and probably reduces the stacking between the tpy units.

2.6. Antiproliferative Activity of Pt-Containing Complexes

All the synthesized metal complexes were investigated in vitro and compared with cisplatin. The cell proliferation assays for platinum(II)-containing complexes were performed on A549 [obtained from Interlab Cell Line Collection (ICLC), Genova, Italy, HTL03001], A2780 (ovary, adenocarcinoma, HTL98008, ICLC), MDA-MB-231 (breast, adenocarcinoma, kindly provided by Dr. Antonio Daga, IRCCS Ospedale Policlinico San Martino, Genova, Italy), and SHSY5Y (neuroblastoma, HTL95013, ICLC) cell lines (Figure 4). IC_{50} values are listed in Table 4.

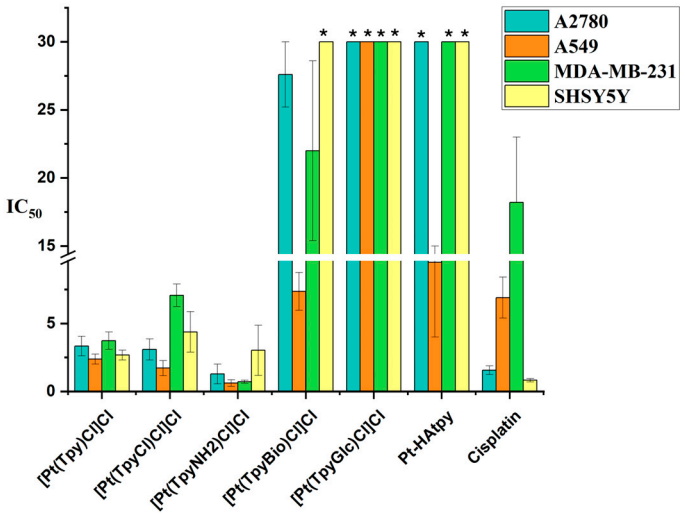

Figure 4. IC_{50} value (μM) distribution of platinum(II)–terpyridine complexes. * IC_{50} values > 30.

The most active compound was the complex [Pt($TpyNH_2$)Cl]Cl, exhibiting antiproliferative activity that was 2.1 to 16.4 times greater than that of cisplatin and all the other synthesized platinum(II) complexes.

It is noteworthy that the complexes [Pt(Tpy)Cl]Cl, [Pt(TpyCl)Cl]Cl, and [Pt($TpyNH_2$)Cl]Cl exhibited significantly lower IC_{50} values compared to classical cisplatin against A549 and MDA-MB-231 cells lines.

Also, it is important to note that the adenocarcinoma of the lung A549 cells, which are generally less sensitive to cisplatin compared to the other cell lines considered here, except for MDA-MB-231 cells [50,51], demonstrated a significantly higher sensitivity to nearly all tested platinum(II)-complexes. The increased sensitivity ranges from 1.1 for [Pt(Tpy)Cl]Cl to 4.8 for [Pt($TpyNH_2$)Cl]Cl.

Table 4. IC_{50} values (μM) of platinum(II)–terpyridine complexes. Cisplatin IC_{50}s are reported for comparison.

Complex	A2780	A549	MDA-MB-231	SHSY5Y
[Pt(Tpy)Cl]Cl	3.34 ± 0.72	2.38 ± 0.36	3.73 ± 0.64	2.68 ± 0.36
[Pt(TpyCl)Cl]Cl	3.09 ± 0.77	1.72 ± 0.55	7.07 ± 0.83	4.38 ± 1.49
[Pt($TpyNH_2$)Cl]Cl	1.29 ± 0.73	0.63 ± 0.24	3.03 ± 1.84	3.03 ± 1.84
[Pt(TpyBio)Cl]Cl	27.6 ± 2.4	7.36 ± 1.39	22.0 ± 6.6	>30
[Pt(TpyGlc)Cl]Cl	>30	>30	>30	>30
[Pt-HAtpy]	>30	9.5 ± 5.5	>30	>30
Cisplatin	1.56 ± 0.32	6.9 ± 1.5	18.2 ± 4.8	0.83 ± 12

The Pt–HAtpy complex did not exhibit antiproliferative activity in A2780, MDA-MB-231, and SHSY5Y cancer cells (IC_{50} > 30) but displayed selective toxicity in the A549 cell line, although this is characterized by an IC_{50} greater than that of cisplatin.

However, data suggested that the presence of hydrophilic substituents in the tpy moiety leads to a considerable decrease in activity, as in the case of Glc and HA derivatives. Similar behavior has been proven for iron and copper complexes of TpyGlc and HAtpy [42,52].

3. Materials and Methods

3.1. Chemicals

Commercially available reagents were used directly unless otherwise stated. Dichloro(1,5-cyclooctadiene)platinum(II) [Pt(COD)Cl_2] was obtained from Merk (Milan, Italy). 2,2′:6′,2″-Terpyridine (Tpy) and 4′-chloro-2,2′:6′,2″-terpyridine (TpyCl) were purchased from TCI (Tokyo, Japan). $TpyNH_2$, TpyBio, TpyGlc, and HAtpy were synthesized as reported elsewhere [42,52,53].

3.2. NMR Spectroscopy

^1H and ^{13}C NMR spectra were recorded at 25 °C with a Varian UNITY PLUS-500 spectrometer at 499.9 and 125.7 MHz, respectively, using standard pulse programs from the Varian library. Two-dimensional experiments (COSY, TOCSY, HSQC, and HMBC) were acquired using 1K data points, with 256 increments.

3.3. UV-Vis Spectroscopy

UV-Vis spectra were recorded with a Cary 3500 UV/Vis spectrophotometer equipped with a Peltier temperature control module. Complex solutions were prepared at a concentration of 0.2 mM in 0.9% NaCl or in water.

3.4. Dynamic Light Scattering

Dynamic light scattering (DLS) measurements were performed with Zetasizer Nano ZS (Malvern Instruments, UK) operating at 633 nm (He–Ne laser) at 25 °C. The mean hydrodynamic diameters (d) of platinum(II)–tpy complexes were calculated from intensity measurement after averaging ten measurements. The samples (4 mg/mL) were prepared in ultrapure filtered water (0.2 μm filter).

3.5. ESI-HRMS Spectrometry

The high-resolution mass spectra were acquired on Orbitrap Exploris™ Oe120 Thermo Fisher operated in positive electrospray ionization (ESI+) mode. The solutions of platinum complexes were prepared by dissolving an appropriate amount of the compound in water, immediately before the mass measurements. Sample water solutions were injected into the ion source at a concentration of 5 ppm at a flow rate of 30 μL/min, using nitrogen as the desolvation gas. The electrospray capillary voltage was optimized at 2.9 kV to maintain

satisfactory sensitivity and reduce in-source fragmentation. The resolution obtained ranged from 45,000 to 50,000 FMHM. Xcalibur 3.0 software was used to elaborate on mass spectra. Each species is indicated with the m/z value of the most intense peak of its isotopic cluster. For a more accurate structural assignment, the relative intensity of the peaks in each cluster was compared with that of the peaks in the corresponding simulated modeling.

3.6. Synthesis of Platinum Complexes

The metal complexes reported in Figure 1 were prepared according to the procedure reported elsewhere [54]. [Pt(COD)Cl_2] and tpy ligands were dissolved in water in a 1:1 molar ratio. HAtpy was mixed with [Pt(COD)Cl_2] in a 1:7 molar ratio since HAtpy holds seven terpyridine moieties [52]. Then, solutions were stirred at 50 °C.

After 15 min, the solids were dissolved and a red-orange clear solution was obtained. The solution was cooled at room temperature and then filtered with a cellulose filter (0.45 μm) to remove any unreacted COD-complex. Water was then evaporated and the collected red-orange solid was washed with diethyl ether and dried under vacuum.

[Pt(Tpy)Cl]Cl: ^{1}H NMR (500 MHz, D_2O) d(ppm): 8.10–8.01 (m, 3H, H-4′, H-6, H-6″), 7.85–7.80 (m, 6H, H-3′, H-5′, H-3, H-3″, H-4, H-4″), 7.35 (2H, t, H-5, H-5″). Yield: 68%.

[Pt(TpyCl)Cl]Cl: ^{1}H NMR (500 MHz, D2O) d(ppm): 8.21–8.15 (6H, m, H-3′, H-5′, H-4, H-4″, H-6, H-6″), 7.95 (2H, d, H-3, H-3″), 7.54 (2H, t, H-5, H-5″). Yield: 56%.

[Pt(TpyNH_2)Cl]Cl: ^{1}H NMR (500 MHz, D_2O) d(ppm): 8.05 (4H, m, H-6, H-6″ H-4, H-4″), 7.79 (2H, m, H-3, H-3″), 7.50 (4H, m, H-3′, H-5′, H-5, H-5″), 4.29 (2H, m, a of propylenic chain), 3.30 (2H, t, g of propylenic chain), 2.23 (2H, m, b of propylenic chain). Yield: 76%.

[Pt(TpyBio)Cl]Cl: ^{1}H NMR (500 MHz, D_2O) d(ppm): 8.24 (2H, d, H-6, H-6″), 8.13 (2H, t, H-4, H-4″), 7.91 (2H, d, H-3, H-3″), 7.57 (2H, s, H-3′, H-5′), 7.53 (2H, t, H-5, H-5″), 4.40 (1H, t, g), 4.22 (3H, m, a and f), 3.45–3.34 (2H, m, g), 3.14 (1H, m, e), 2.80–2.75 (1H, dd, h), 2.54 (1H, d, h′), 2.20 (2H, m, a), 2.07 (2H, m, b), 1.60–1.46 (4H, m, d, d′ and b), 1.27–1.20 (2H, m, c). Yield: 48%.

^{13}C{^{1}H} NMR (125 MHz, D_2O): 150. 94 (C-6, C-6″), 142.60 (C-4, C-4″), 129.30 (C-5, C-5″), 125.21 (C-3, C-3″), 110.84 (C-3′,C-5′), 68.32 (C-a), 61.93 (C-f), 60.25 (C-g), 55.47 (C-e), 39.78 (C-h), 35.70 (C-g), 35.41 (C-a), 28.04 (C-c), 27.5 (C-b), 25.26 (C-d, C-b).

[Pt(TpyGlc)Cl]Cl: ^{1}H NMR (500 MHz, D_2O) d(ppm): 8.05 (4H, m, H-6, H-6″ H-4, H-4″ of tpy), 7.80 (2H, d, H-3, H-3″ of tpy), 7.50 (4H, s, H-3′, H-5′ H-5, H-5″ of tpy), 4.50 (1H, d, J = 7.9 Hz, H-1 of Glc), 4.20 (2H, m, a of propylenic chain), 4.21 (1H, m, d of propylenic chain), 4.03 (1H, m, d′ of propylenic chain), 3.95 (1H, d, J = 12.1 Hz, H-6 of Glc), 3.60 (1H, ddJ =12.4, 5.7 Hz, H-6′ of Glc), 3.60–3.48 (2H, m, H-5 and H-3 of Glc), 3.43 (1H, t, J = 9.5 Hz, H-4), 3.39–3.23 (5H, m, g of the propylenic chain, e, H-4, H-2, of Glc), 2.29 (2H, m, b of the propylenic chain). Yield: 44%.

[Pt-HAtpy]: ^{1}H NMR (500 MHz, D_2O) d(ppm): 8.20–7.35 (10H, br. S, H6, H6″, H3, H3″, H3′, H5′, H4, H4″, H5 and H5″ of tpy), 4.46 (1H, d, H-1 of d-glucuronic acid unit), 4.39 (1H, d, H-1 of N-acetyl-d-glucosamine unit), 4.19 (2H, m, a of propylenic linker), 4.0–3.21 (m, H-2, H-3, H-4, H-5, H-6 of HA backbone and g propylenic linker), 2.06 (m, b of propylenic linker), 1.91 (s, CH_3 of HA). Yield: 44%.

3.7. Evaluation of Cell Proliferation Inhibition by the MTT Assay

Human cell lines A549 (lung, adenocarcinoma), A2780 (ovary, adenocarcinoma), MDA-MB-231 (breast, carcinoma), and SHSY5Y (neuroblastoma) were plated in appropriate concentrations in 180 μL of complete media RPMI for A549, A2780, and SHSY5Y (in this case supplemented with 2% glutamine) cells and DMEM for MDA-MB-231 cells, into flat-bottomed 96-well microtiter plates and centrifuged for 2 min at 1100 rpm. After 7–8 h, cells were treated with 20 μL of five concentrations of Pt complexes diluted in normal saline. Plates were then processed as described elsewhere [55].

IC_{50} values were calculated through the analysis of single concentration–response curves. Each experiment was repeated 3–6 times.

4. Conclusions

New platinum(II) complexes of terpyridine derivatives functionalized with biomolecules were synthesized and characterized. Their antiproliferative activity was tested against ovarian adenocarcinoma A2780, lung cancer A549, breast adenocarcinoma MDA-MB-231, and neuroblastoma SHSY5Y cell lines.

The platinum(II) complex with the terpyridine amino derivative, [Pt(TpyNH$_2$)Cl]Cl, showed the best IC$_{50}$ values in A2780, A549, and MDA-MB-231 cancer cells compared to all other complexes and even cisplatin, except for SHSY5Y cells, where cisplatin remains the most active drug.

However, it is particularly interesting that the MDA-MB-231 and A549 cell lines, derived from two of the most frequent neoplasia in women and men with regard to both the number of cases and deaths [56], responded very well to the treatment with the complexes [Pt(Tpy)Cl]Cl, [Pt(TpyCl)Cl]Cl, and in particular to [Pt(TpyNH$_2$)Cl]Cl.

Notably, the adenocarcinoma of the lung A549 cells, which are generally less sensitive to cisplatin, showed a significantly higher sensitivity to nearly all tested platinum(II) complexes, indicating that these complexes could be of interest for the treatment of this form of cancer.

Moreover, the introduction of the hyaluronic acid moiety in the Pt–HAtpy complex led to selective inhibition of cell proliferation in the A549 cancer line, although with a low antiproliferative activity compared to cisplatin and the other active platinum–tpy compounds.

In conclusion, the data suggested that the amino ligand enhances the antiproliferative activity of terpyridine-based platinum complexes. This could be related to the positive charge at the physiological pH of the amino group that may improve cellular uptake, as observed with copper and iron complexes containing the tpy amino derivative.

Further experiments are warranted to understand the mechanisms underlying these observations and clarify the role of the amino group.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/inorganics12060167/s1, Figures S1–S10: NMR spectra; Figures S11–S17: ESI-MS spectra; Figures S18–S23: UV-Vis spectra of platinum complexes.

Author Contributions: Data curation, R.P., M.V. and G.V.; Funding acquisition, G.V and M.V.; Investigation, R.P., M.V., V.G. and G.V.; Methodology, R.P., M.V., V.G. and G.V.; Supervision, G.V.; Writing—original draft, R.P.; Writing—review and editing, R.P., M.V., V.G. and G.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by EU funding within the NextGeneration EU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT), PRIN (Project no. 2022JXSA9C, SPlat-G) and the Italian Ministry of Health under grant "Ricerca Corrente", 2022-2772566.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

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