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State of art in the chemistry of nucleoside-based Pt(II) complexes

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ABSTRACT

After the fortuitous discovery of the anticancer properties of cisplatin, many Pt(II) complexes have been synthesized, to obtain less toxic leads which could overcome the resistance phenomena. Given the importance of nucleosides and nucleotides as antimetabolites, studying their coordinating properties towards Pt(II) ions is challenging for bioorganic and medicinal chemistry. This review aims to describe the results achieved so far in the aforementioned field, paying particular attention to the synthetic aspects, the chemical-physical characterization, and the biological activities of the nucleoside-based Pt(II) complexes.

1. Introduction

Metals play essential roles in the biochemistry of living cells and are necessary for the correct functioning of several enzyme machineries [1]. Metal ions are also involved in regulating the immune system [2,3], triggering the defence against pathogens and cancer cells [4]. Therefore, metal-mediated activation strategies have been studied to unravel the mechanisms of innate immune stimulation and the activation of T cells [4]. In addition, the coordinating properties of metals like platinum [5,6], gold [7], and ruthenium [8,9] have been extensively explored for the synthesis of complexes endowed with interesting anticancer properties [10,11]. In 2020 about twenty million new cases of cancer in the world were detected, and about 10 million deaths were due to the disease [12]. It is worth pointing out that this picture does not consider the influence of the Covid-19 pandemic, which could change trends in the coming years. Indeed, it cannot be excluded that in the future there will be a short-term decrease in new diagnoses due to the interruption or slowdown of screening programs and an increase in mortality and diagnoses of advanced-stage cancers in some contexts. Despite the recent advances in monoclonal antibody-based therapies [13,14] and personalized medicine [15], chemotherapy remains the most effective weapon in the presence of inoperable or highly aggressive tumors [16]. Chemotherapy was born in 1965 after a serendipitous experiment performed by Rosenberg, who discovered that cisplatin (**1**, Fig. 1) could induce filamentous growth in bacteria and inhibit cell division. [17] Cisplatin is a small planar molecule that contains a Pt(II) ion bonded to two ammonia groups placed on the same side and to two chloride ligands.

This molecule amazed the scientific world in the 1970s because of its strong effectiveness against certain types of solid tumors. [18] It is accepted that the main target of cisplatin is nuclear DNA. After entering the cells either by passive diffusion or by a copper membrane transporter (CTR1)-mediated active uptake, cisplatin loses one chloride ligand and forms a mono-aqua complex, the actual electrophile that can react with the nucleic acid. After that, the mono-aqua complex binds to the guanine N7 atom and loses the water molecule. The remaining chloride is displaced by another water molecule, allowing cisplatin to bind adjacent guanine on the same strand. Thus, DNA undergoes a substantial modification of its secondary structure, which may lead to cell death (Fig. 2) [19].

Despite its benefits, cisplatin cannot be considered an ideal drug as it elicits toxicity. Many cancer cells either show innate resistance to the drug or acquire resistance during therapy [18]. Starting from cisplatin, several Pt(II)-based compounds have been synthesized by introducing in the place of the ammonia ligands lipids [20–22], peptides [23,24], sugars [25,26], and natural products [27] to obtain drugs with better toxicity profiles and able to overcome the intrinsic and acquired resistance. Some of them have shown interesting antiproliferative features.

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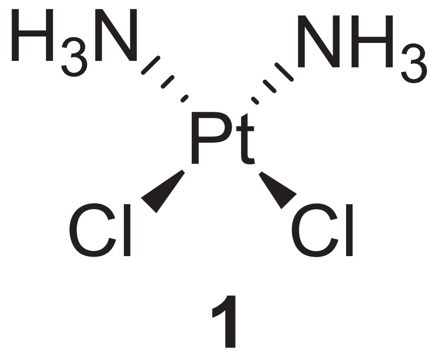


Fig. 1. The structure of cisplatin (**1**).

In the vast panorama of complexes synthesised, we can distinguish between neutral or charged Pt(II) complexes. If the latter are prepared as cisplatin derivatives, they can be represented with the general formula $[Pt(3L)Cl]^+$ (L: ammonia or amine). By the presence of only one labile chloride ligand around the metal, they can be referred to as mono-functional complexes [28].

As several anticancer drugs currently approved are nucleoside and nucleotide-based (Table 1) [29–31], studying the ligand properties of modified nucleosides and nucleotides towards Pt(II) metal centres is an appealing field for the preparation of novel antineoplastic agents.

In addition, the presence of protein transporters on cellular membranes involved in the molecular recognition and internalization of nucleosides and nucleotides makes them even more intriguing for constructing new metal-based antineoplastic agents [43]. In light of the importance of such complexes, in this paper, an overview of the synthesis, the physico-chemical characterization, and biological features of the most interesting Pt(II) complexes embodying purine and pyrimidine nucleosides as ligands will be presented.

2. Pt(II) complexes with purine nucleosides as ligands

2.1. Reaction of adenosine, inosine and guanosine with K_2PtCl_4

Before describing the research aimed at the study of Pt(II) complexes having as ligands modified nucleosides, a mention should be made about the reactivity of simple purine nucleosides towards the usual platinating agent K_2PtCl_4. In a relatively recent paper, Hadjiliadis *et al.* elucidated the structures of the complexes obtained by the reaction of adenosine, inosine, and guanosine with K_2PtCl_4 [44]. When two equivalents of adenosine (**2**, Scheme 1) were reacted with one equivalent of K_2PtCl_4, the 1:2-complex **3** was obtained.

The platination of the purine N^7 position was demonstrated by analyzing the ^1H NMR spectrum of complex **3**. After metallation, the H8 proton became more acidic and easily exchanged with deuterium and resonated downfield with respect to the same signal in the free nucleosides. On the other hand, the *cis* geometry of the complex was deduced through the Kurnakov test [45]. Differently, the same reaction performed on inosine (**4**, Scheme 2) and guanosine (**5**) gave a mixture of compounds. For both nucleosides, after the formation of the N^7-Pt bond, the N^1-H proton became more acidic and the formation of polymeric species with N^1,O^6 chelates in the presence of N^7,O^6 and N^1,N^7 bridges could not be excluded. When the same reaction was performed in a 2 M NaCl aqueous solution, the 2:1 complexes (**6** for inosine and **7** for guanosine) were isolated and characterized as for the complex **3**. For both complexes **6** and **7**, the high $[Cl^-]$ prevented the hydrolysis of chloride ligands and deprotonation of N^1-H protons.

2.2. Studies of the platination of acyclovir (8), ribavirin (14), valganciclovir (15) and didanosine (16)

Considering that antiviral drugs can inhibit the growth of cancer cell lines [46–48], some research groups have exploited the coordinating properties of some nucleoside-based antivirals towards Pt(II) centers, to improve their clinical efficacy and accelerate the availability and design of new drugs. In this frame, Natile *et al.* studied the coordinating properties of acyclovir (**8**, Scheme 3), a guanosine analogue endowed with powerful anti-herpetic activity [49]. In the first instance, cisplatin (**1**) was reacted with $AgNO_3$ in *N*,*N*-dimethylformamide (DMF), thus obtaining the ion **9**. The latter, after treatment with acyclovir (**8**), gave the mono-functional complex **10** in a 70 % yield. The success of N^7 purine position platination was demonstrated through inspection of the ^1H NMR spectrum of complex **10**, which revealed a downfield shift of the H8 proton with respect to the same signal belonging to free acyclovir. The geometric orientation of ligands around the Pt(II) in complex **10** was not determined.

The anti-leukemic activity of complex **10** was assessed on the murine P388 cell system using cisplatin as a positive control (Table 2). Notwithstanding complex **10** was markedly less potent than cisplatin, it was as effective as cisplatin when equitoxic dosages were administered *in vivo* to P388 leukaemia-bearing mice. The complex **10** was also active against a cisplatin-resistant subline of the P388 leukaemia (P388/cisplatin), thus suggesting a different mechanism of action with respect to cisplatin.

To shed light on the mechanism of action of complex **10**, in parallel experiments, circular pBR322 DNA was reacted with complex **10** and

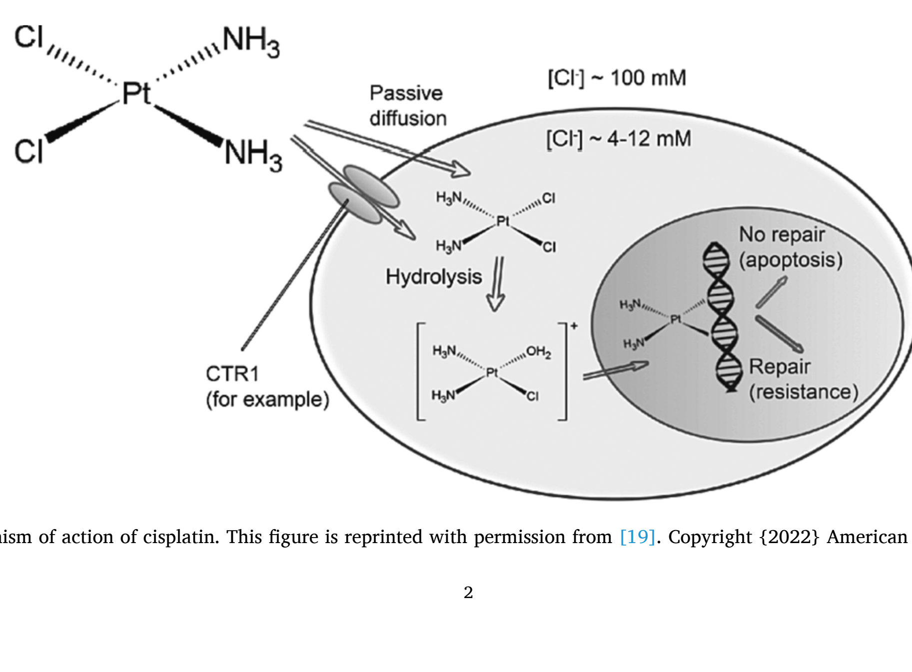


Fig. 2. Mechanism of action of cisplatin. This figure is reprinted with permission from [19]. Copyright {2022} American Chemical Society.

Table 1
Approved nucleoside-based antineoplastic drugs.

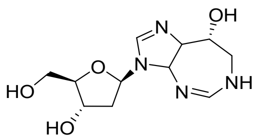
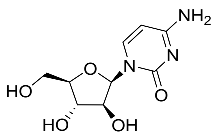
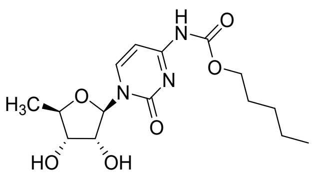
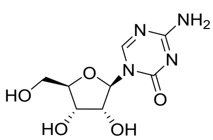
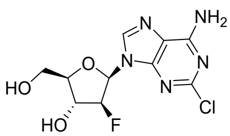
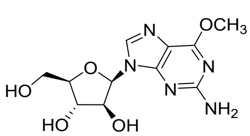
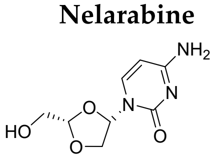
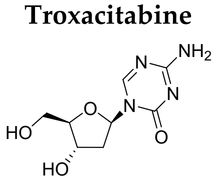
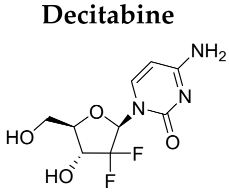
Name	Type	Targeted tumour (approval date)
 **Pentostatin**	Adenosine analogue	Hairy cell leukaemia (1991) [32]
 **Cytarabine**	Cytidine analogue	Remission induction in acute non-lymphocytic leukaemia of adults and pediatric patients (1998) [33]
 **Capecitabine**	Cytidine analogue	Colon and rectum cancers (1998) [34]
 **Azacytidine**	Cytidine analogue	Myelodysplastic syndrome (2004) [35]
 **Clofarabine**	Adenosine analogue	Acute lymphoblastic leukaemia (2004) [36]
 **Nelarabine**	Guanosine analogue	Acute lymphoblastic leukaemia (2005) [37]
 **Troxacitabine**	Cytidine analogue	Advanced and/or metastatic renal cell carcinoma; leukaemias and myelodysplastic syndromes, lymphoproliferative neoplasms and multiple myeloma; pancreatic cancer and lung cancer (2005) [38]
 **Decitabine**	Cytidine analogue	Myelodysplastic syndrome (2006) [39]
 **Gemcitabine**	Cytidine analogue	Various carcinomas: non-small cell lung cancer, pancreatic cancer, bladder cancer, breast cancer, ovarian cancer (1995) [40]
	Adenosine analogue	Treatment of adult patients with B-cell chronic lymphocytic leukaemia (CLL) (2008) [41]

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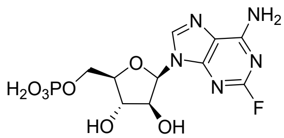
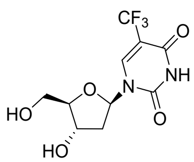
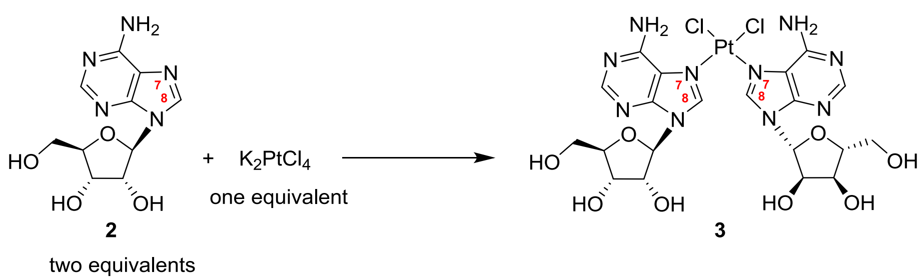
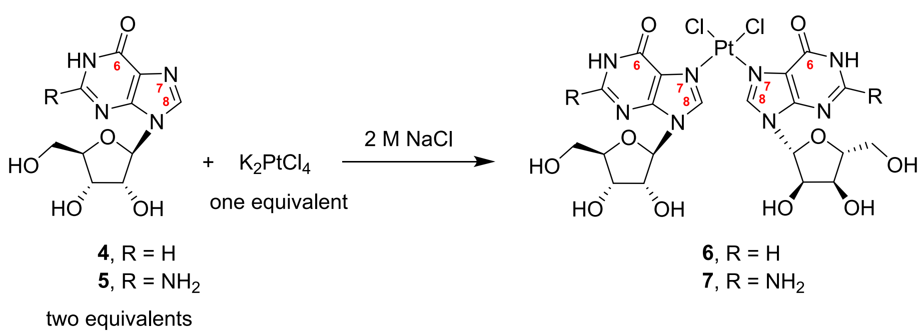
Table 1 (*continued*)

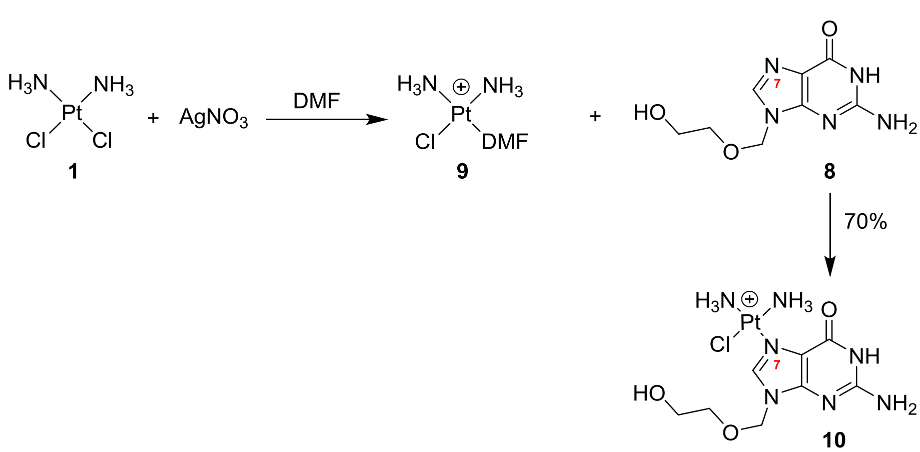
Name	Type	Targeted tumour (approval date)
 **Fludarabine**		
 **Trifluridine**	Thymidine analogue	Metastatic colorectal cancer (2015) [42]



Scheme 1. The structure of complex **3** obtained by the reaction of two equivalents of adenosine (**2**) with one equivalent of K_2PtCl_4.



Scheme 2. The structure of complexes **6** and **7** obtained by reaction of two equivalents of inosine (**4**) and guanosine (**5**) with one equivalent of K_2PtCl_4 in 2 M NaCl.



Scheme 3. Synthesis of the mono-functional Pt(II) complex **10** having acyclovir (**8**) as ligand.

Table 2
In vitro and *in vivo* antileukemic activity (P388 system) of cisplatin and complex **10**.

Entry	ID_{50} (μM)[a]	Dose (mg/kg)	P388 (%T/C)[b]	P388/cisplatin (%T/C)[b]
Cisplatin	2	0.6	209	97
10	108	50	211	140

[a] ID_{50} = complex concentration inhibiting 50 % cell growth *in vitro*.
[b] The *in vivo* effects on survival time of tumour-bearing mice are expressed as %T/C, *i.e.* mean survival time (x100) of treated animals versus controls.

cisplatin. The DNA was amplified *in vitro* by a polymerase, and the synthesis products were analyzed by polyacrylamide gel electrophoresis. In correspondence with the platinated sites, the polymerase stopped, thus determining premature chain terminations. Although for both the complexes, the sites of DNA synthesis termination on the DNA template corresponded to runs of two or more guanines (complex **10** showed little affinity for multiple guanines), complex **10** determined additional stop bands corresponding to cytosine residues in 3′GCT and 3′CGGC sites. Based on these results, it could be deduced that cisplatin and complex **10** reacted differently with DNA, thus suggesting that a different mechanism of action could occur.

Lakomska *et al.* synthesized the Pt(II) complex **11** (Scheme 4) having as ligand the antiviral drug ribavirin (**14**), a guanosine analogue that in combination with interferon-alpha is efficacious against HCV [50]. For that purpose, the nucleoside analogue **14** was reacted with *cis*-[Pt(II) $Cl_2(DMSO)_2$] (**17**) and the complex **11** was recovered in a 73 % yield. When **17** is used as platinating agent, there are generally-two main advantages in the synthesized complexes: 1) the water solubility is improved, 2) carriers can be tethered to the complexes exploiting the final DMSO displacement. The structure of complex **11** was ascertained by single crystal X-ray diffraction and NMR analyses. In the ^{1}H NMR spectrum of complex **11**, N^5-H and N^7-H protons shifted with respect to the same signals belonging to ribavirin (**14**). In detail, N^5-H proton downfield shifted (+0.55 ppm), whereas the N^7-H proton upfield shifted (−1.42 ppm). The latter evidence could be related to the deprotonation of the carboxamide group and coordination with the metal. Taken together, the NMR data confirmed the metallation through N^4 and N^7 positions. The ^{195}Pt NMR resonance of complex **11** was detected at −3103 ppm, that is a typical value for the square-planar Pt(II) complexes in which two adjacent corners are occupied by two nitrogen atoms and the other two positions by DMSO and Cl.

In another paper, Shahabadi *et al.* studied the interaction of complex **11** with calf thymus DNA (CT-DNA) by using several spectroscopic techniques [51]. When analyzed by CD spectroscopy, CT-DNA shows the typical spectrum of a right-handed B form DNA that consists of two bands: a positive one at 275 nm due to the base stacking and a negative one at 245 nm due to the right-handed helicity. Modifications of the CD signals are helpful features to detect changes in the DNA secondary structures upon interaction with metal complexes [52]. By exposing the CT-DNA to increasing amounts of complex **11** ([complex]/[DNA] = 0.0, 0.6, 0.7 and 0.8, Fig. 3), the authors observed that both the positive and negative signals in the CD spectra of CT-DNA increased. They attributed

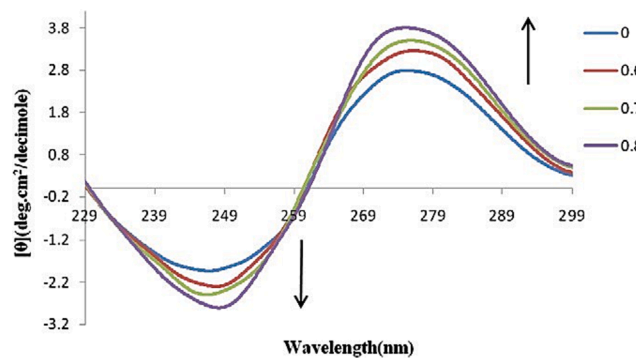
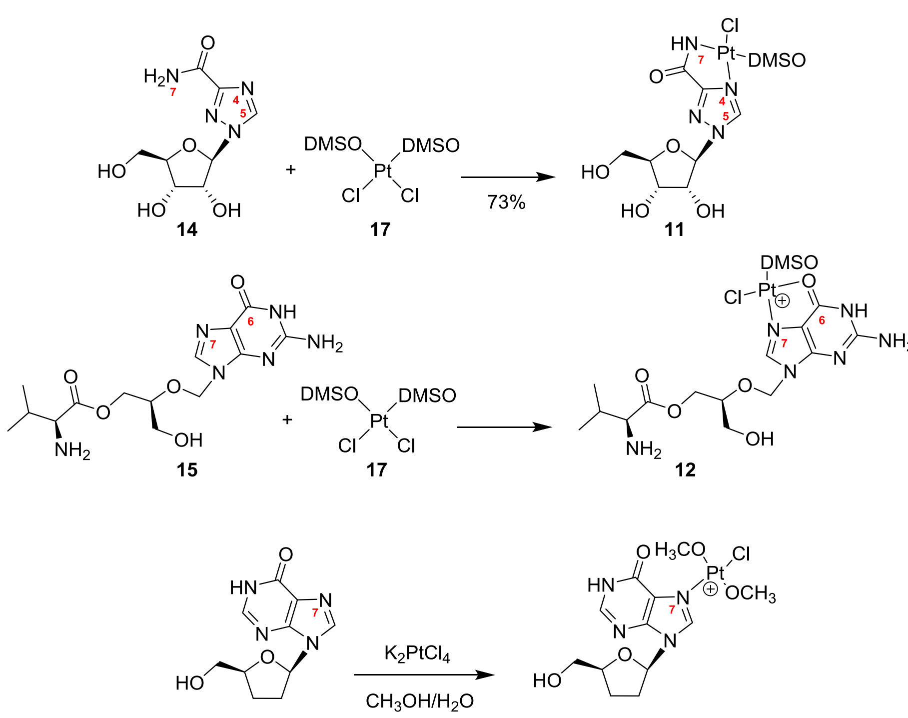


Fig. 3. Circular dichroism spectra of CT-DNA (5.0 × 10^{-5} M) in Tris–HCl (50 mM) in the presence of increasing amounts of the Pt(II) complex **11** ([complex]/[DNA] = 0.0, 0.6, 0.7 and 0.8). This figure is republished with permission of Elsevier, from [51]; permission conveyed through Copyright Clearance Center, Inc. License Number: 5430910193376.



Scheme 4. Synthesis of the Pt(II) complexes **11**, **12**, and **13** having ribavirin (**14**), valganciclovir (**15**), and didanosine (**16**) as ligands, respectively.

these results to a predominant intercalative process involving π-π* stacking and stabilization of the right–handed B form of CT-DNA. Conversely, groove bindings usually produce a decrease of the intensity of both the positive and negative bands, accompanied by a bathochromic shift [52].

The predominant intercalative binding mode of complex **11** towards the CT-DNA was also supported by viscosity and fluorescence studies.

Shahabadi *et al.* synthesized also the Pt(II) complexes **12** and **13** starting from the antiviral drugs valganciclovir (**15**) [53] and didanosine (**16**) [54], respectively. By exposing the CT-DNA to increasing amounts of both complexes, no conclusive evidence about the binding mode with the CT-DNA could be deduced by CD spectroscopy. On the other hand, competitive fluorescence studies using Hoechst 33258, a known minor groove DNA binder [55], allowed to conclude that both complexes could behave as minor groove binders since the alterations in the fluorescence intensities of CT-DNA-Hoechst systems showed that some Hoechst molecules were released into solution.

2.3. Studies of platination reactions of modified inosines

Inosine has been extensively used as starting material for the preparation of new linear [56–62] and cyclic [63–69] nucleosides and nucleotides endowed with biological activities. The introduction of alkyl substituents at the N^1 purine position was revealed to be one of the key steps for obtaining the molecular frameworks. In a pioneering work Piccialli *et al.* described a successful example of synthesis of N^1-alkylinosine derivatives (i.e., N^1-propylinosine) *via* formation of a N^1-4-nitrophenylinosine intermediate [70]. Thereafter, our group discovered that the exposition of the ribose-protected N^1-2,4-dinitrophenyl inosine **18** to alkyl/ω-hydroxyalkyl/ω-aminoalkyl amines (**19a–c**) afforded high yields of the N^1-alkyl/ω-hydroxyalkyl/ω-aminoalkyl inosines (**20a–c**) (Scheme 5) [57–59]. The mechanism of the reaction has been studied in detail by our group: briefly, the strong electron-withdrawing 2,4-dinitrophenyl group (DNP) renders the *C*2 purine atom very reactive towards amines. After the opening and following reclosure of the pyrimidine ring by the nitrogen, which has attacked the *C*2 purine atom, the N^1 substituted inosines are obtained[59].

The pendant OH groups in the nucleosides **20b** were exploited for the synthesis of analogues of cyclic adenosine diphosphate ribose (cADPR), a secondary messenger involved in the Ca^{2+} homeostasis [64–72]. On the other hand, the terminal NH_2 groups in the nucleosides **20c** were exploited for the first solid-phase synthesis of inosine-based dinuclear platinum(II) complexes [73]. The peculiarity of the newly synthesized complexes was the presence of two far mono-functional Pt(II) centers on a semi-rigid molecular scaffold.

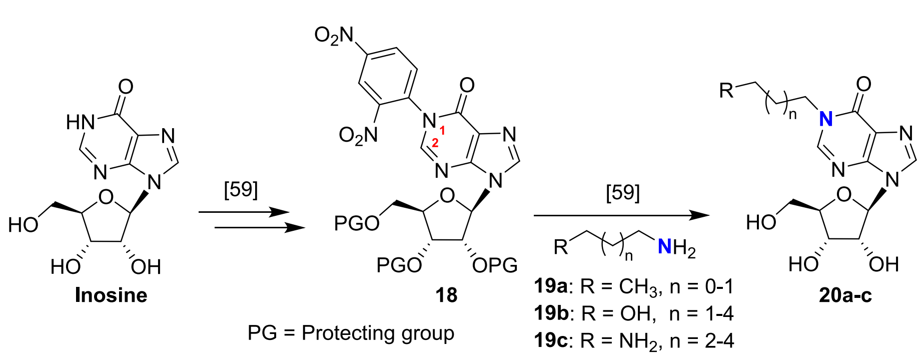
As a proof of concept, three different Pt(II) moieties were attached to the pendant NH_2 group of a flexible hexylamine chain attached to the hypoxanthine N^1 position and to the purine N^7 atom. The solid-phase synthesis for the preparation of the complexes **21a–c** is described in Scheme 6. Inosine was attached to a polystyrene monomethoxytrityl chloride resin (MMT-Cl) through the 5′-OH sugar position, thus obtaining the support **22**. After protection of 2′- and 3′-OH functionalities, the introduction of the DNP group to inosine N^1 atom (support **23**) and treatment with 1,6-diamminohexane, the support **24** was obtained. By exposing the resin-bound nucleoside **24** to the pre-activated platinating complexes **26a–c**, having *trans* or *cis*-diamino ligands, as well as an ethylenediamine ligand around the metal center, the bis-platinated supports **27a–c** were produced, from which the inosine-tethered platinum(II) complexes **21a–c** were released through a final acidic treatment. The structures of complexes **21a–c** (yields 67–70 %) were ascertained by 2D-nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS) data. In Fig. 4 the ^{1}H NMR spectra of free ligand inosine N^1-6-aminohexane and complex **21b** are reported. The presence of the N^7-Pt bond in the complex **21b** was evidenced by the downfield shift of H8 proton resonance (+0.51 ppm) with respect to the same signal in the free nucleoside. After metallation, the H8 proton became more acidic and partially exchanged with deuterium. As a consequence of H/D exchange, a reduction of H8 proton signal integration was observed. Platination of the pendant NH_2 group was demonstrated by the high field shift of the methylene proton resonances (ω-CH_2) flanking the Pt-NH_2 bond (−0.24 ppm). The geometric orientation of ligands around the Pt(II) in complex **21b** was not determined.

The cellular response to complexes **21a–c** vs cisplatin in a short-term exposure assay was evaluated in four human tumor cell lines, namely ovarian A2780, cervical HeLa, breast MCF-7 and lung A549 cells by determining their ATP levels in terms of relative light units (RLUs). Interestingly, the 50 % inhibitory concentration (IC_{50}) measured for the complex **21b** was fivefold lower than that obtained from cisplatin in the MCF-7 cell line (Table 3). The response to the complex **21b** in the short-term exposure assay was particularly interesting, especially in the light of the high aggressivity of the breast cancer cell line used for the biological experiments.

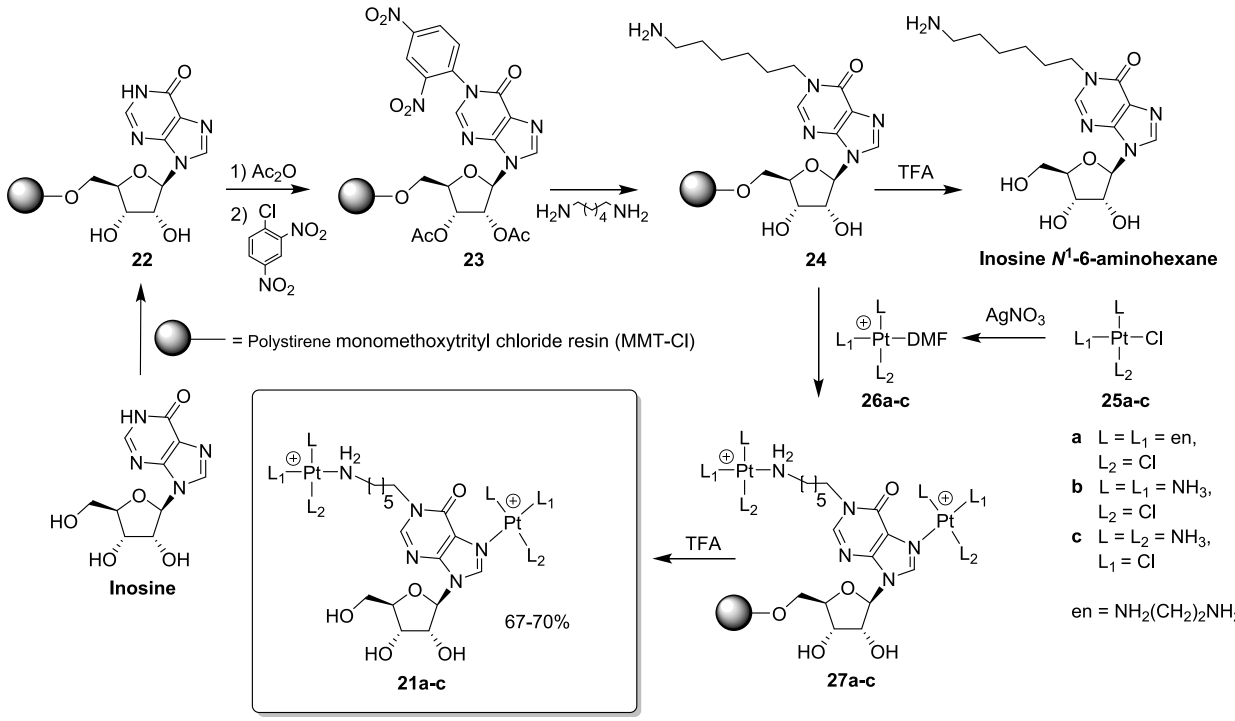
2.4. Studies of platination reactions of adenosine and its analogues

Although the reactivity of adenine base towards Pt(II) centers has been explored in detail [74], very few articles investigated the reactivity of the nucleoside adenosine. Longato *et al.* discovered that neutral phosphine ligands in cisplatin analogues influenced the reactivity of Pt (II)-adenine adducts [75]. As a continuation of their work, the authors reported on the synthesis, characterization and cytotoxicity of four new Pt(II)-adenosine complexes having the general formulas *cis*-$[L_2Pt\{adenosine(\text{-}H),N^1N^6\}]_2(NO_3)_2$ (**28** L = $P(CH_3)_3$; **29** L = PPh_3, Scheme 7) and *cis*-$[(PPh_3)_2PtNH = CR_1\{adenosine(\text{-}2H)\}]NO_3$ (**30a** $R_1 = CH_3$; **30b** R_1 = Ph) [76].

When adenosine was reacted with *cis*-$[L_2Pt(\mu\text{-}OH)]_2(NO_3)_2$ in water, two different products were isolated, depending on the nature of the ligand L. If L = $P(CH_3)_3$ (**31a**), the bridged dinuclear species **28** was obtained in a 85 % yield, in which two adenosine molecules chelated two Pt(II) centers through the N^1 and N^6 purine atoms. The N^1,N^6-coordination for both adenosine ligands in complex **28** was ascertained by X-ray analysis. The monitoring of the reaction through a ^{31}P NMR



Scheme 5. Inosine N^1 functionalization reaction.



Scheme 6. The synthesis of the inosine-containing Pt(II) complexes **21a–c**.

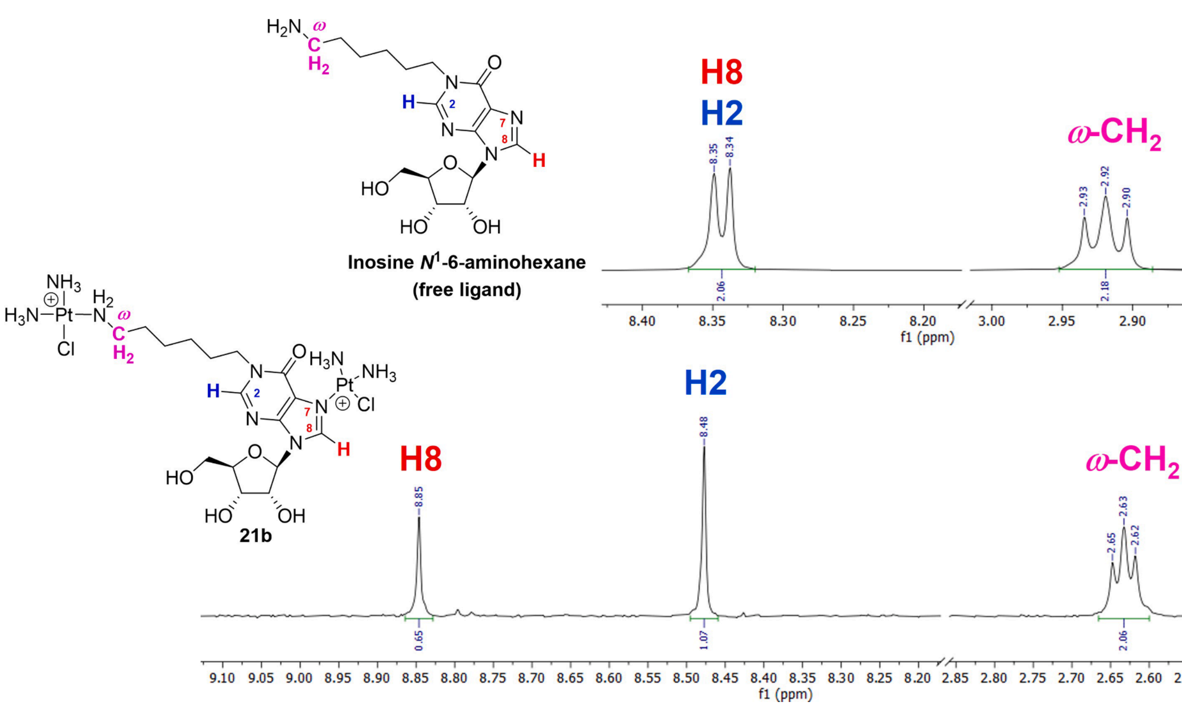
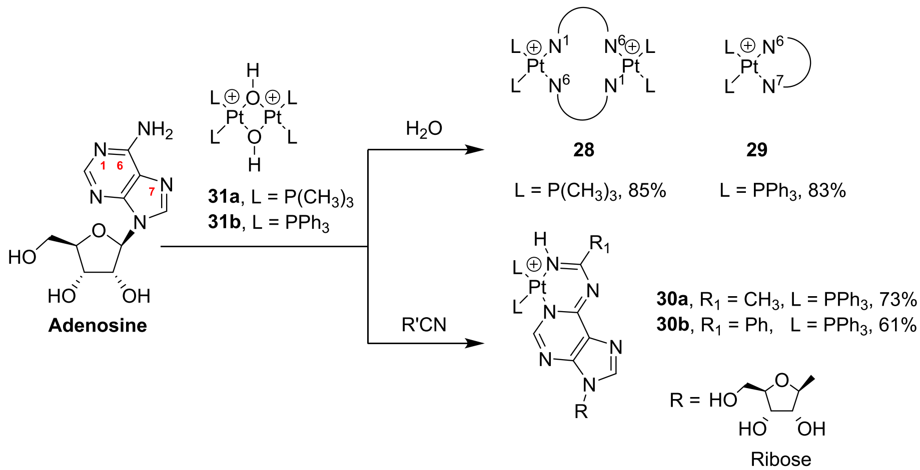


Fig. 4. Expansions of ^1H NMR (400 MHz, D_2O) spectra of complex **21b** (A) and free ligand inosine N^1-6-aminohexane (B) that show the effects of N^7 and ω-NH_2 platinations on H8 purine atom chemical shift and methylene proton (ω-CH_2) resonances flanking the Pt-NH_2 bond, respectively.

Table 3
Cytotoxic effects of complex **21b** and cisplatin on MCF-7 cell line in a short-term exposure assay.

Entry	(IC_{50}, μM)
Cisplatin	2.36 ± 1.4
21b	0.47 ± 0.14

experiment allowed to detect the formation of a mixture of complexes that slowly afforded the more stable complex **28**. Differently, in the presence of the less basic L = PPh_3 (**31b**), the multimerization process was prevented and the mononuclear species **29** was isolated in a 83 % yield. The N^6,N^7-coordination of the adenosine ligand was determined by inspection of 1D/2D NMR spectra. Interestingly, when the reaction of adenosine was performed with **31b** in acetonitrile or benzonitrile as solvents, complex **29** was initially formed;however, after a few days, it



Scheme 7. The synthesis of Pt(II) complexes in which two ligand positions were occupied by N^1,N^6 (**28**), N^6,N^7 (**29**), and N^1,NH(amidine) (**30a–b**) adenosine positions.

converted into the amidine complexes **30a** (73 %) and **30b** (61 %), respectively.

The biological effect of complexes **28**–**30** was evaluated over a wide panel of cancer cell lines and satisfactory results were obtained from the amidine complex **30b** against the human cervical carcinoma cell line resistant to cisplatin (A431/cisplatin). The authors calculated a resistance factor four times lower than cisplatin on the A431/cisplatin cell line, and attributed the biological activity to a different mechanism of action with respect to cisplatin (Table 4).

To determine the effect of the adenine base on the anticancer properties of Pt(II) complexes, our group also focused on the synthesis of one dinuclear platinum complex carrying a N^6-(6-aminohexyl)adenosine as a ligand of mono-functional cisplatin units (**32**, Scheme 8) [77]. For its synthesis, we started from the commercially available 6-chloropurine riboside **33**, which was reacted with an excess of 1,6-diaminohexane to obtain the nucleoside **34** (86 %). After the platination of both N^7 purine position and the terminal amino group of the hexylamine side chain with the activated platinating agent **26b**, complex **32** was achieved (60 %). The structure of complex **32** was supported by 1D/2D NMR and high-resolution mass spectrometry (HR MS) data. In the ^{1}H NMR spectrum of complex **32** the downfield shift of the H8 proton compared with the resonance of the same atom in the nucleoside **34** (Δppm = 0.6) was a proof of the presence of the N^7-Pt bond. Further evidence of N^7 purine position metallation was also the increased acidity of the H8 proton, whose intensity in the ^{1}H NMR spectrum lowered because of exchange with D_2O. As in the ^{1}H NMR spectrum of complex **32** the chemical shift of the H2 proton did not significantly change with respect to the value found in compound **34**, equilibria involving migrations of the platinum moieties from N^7 to N^1 of the nucleobase could be excluded. In the ^{13}C NMR spectrum of complex **32**, a 5 ppm downfield shift of the ω-methylene carbon indicated that the NH_2 platination succeeded. The geometric orientation of ligands around the Pt(II) in complex **32** was not elucidated.

Regarding the biological activity, complex **32** was able to inhibit the MCF7 cell line proliferation slightly better than cisplatin at the lowest concentration tested.

Table 4
Cytotoxic effects of complex **30b** and cisplatin on A431 and A431/cisplatin cancer cell lines.

Entry	A431 (IC_{50}, μM)	A431/cisplatin (IC_{50}, μM)	R.F.[a]
30b	23.12 ± 1.35	18.48 ± 1.21	0.8
cisplatin	1.89 ± 1.67	5.81 ± 1.33	3.1

[a] Resistant factor (R.F.) is defined as IC_{50} resistant/parent line.

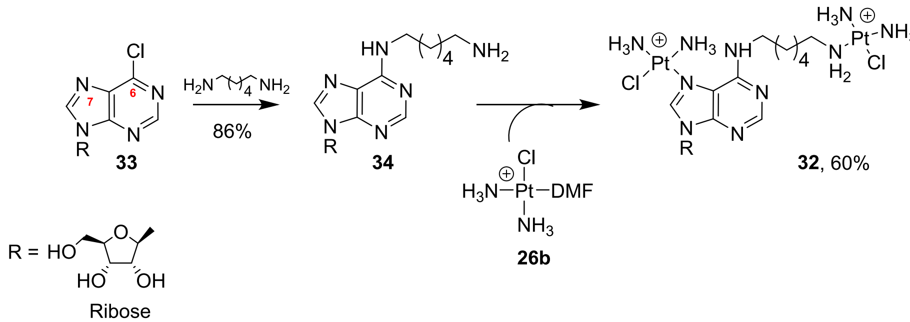
N^6-benzyl adenosines and some substituted benzyl derivatives are the transporter forms of plant hormones [78]. These compounds have shown antitumoral properties against several human tumour cell lines. In this frame, Travnicek *et al.* have studied the coordinating properties of nine N^6-benzyl (**33a–i**, Table 5) and four 2-chloro-N^6-benzyl adenosine derivatives (**34a–d**) towards the platinating agent K_2PtCl_4 [79].

The authors exploited the displacement of the C6-Cl halogen carbon atom of nebularine **35** and 2-chloronebularine **36** by benzyl amines **37a–i** and **38a–d** (Scheme 9; Table 2 for the R_2-R_5 substituents of the phenyl ring) and recovered the ligands **33a–i** and **34a–d**, respectively. Unexpectedly, the platination reactions of ligands **33a–i** and **34a–d** with K_2PtCl_4 afforded the *trans*-isomers **39a–i** and **40a–d** (65–75 % yields) and not the corresponding *cis*-isomers. The authors attributed this unexpected behaviour to the very low thermodynamic stability of the *cis*-isomers, which converted in short time into the *trans*-isomers.

The sites of platination were assessed for all the complexes through the analysis of ^{1}H–^{15}N gs-HMBC spectra, in which only the purine N^7 chemical shifts were significantly influenced by the coordination of the nucleosides with Pt(II) atoms. The presence of ^{195}Pt signals in the range −2071 to −2091 ppm in the ^{195}Pt NMR spectra, whose position and intensity did not change in time, was a typical spectroscopic feature of *trans*-platinum(II) dichlorido complexes [79].

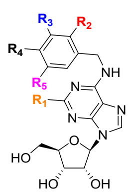
Unfortunately, the complexes **39a–i** and **40a–d** did not show any cytotoxic effects up to the highest concentration tested (50.0 μM concentration, $IC_{50} > 50.0$ μM) against breast adenocarcinoma (MCF7) and osteosarcoma (HOS) cancer cell lines. At first glance, these results may not be surprising, as the complexes **39a–i** and **40a–d** are analogues of transplatin, a biologically inactive stereoisomer of cisplatin. However, several reported *trans*-Pt(II) complexes with *N*-donor heterocycle ligands displayed interesting antitumor activities and were active against cisplatin-resistant cancer cell lines, contravening the general structure–activity rule that only the presence of two leaving groups arranged in *cis* geometry around the metal could afford Pt(II) complexes endowed with antineoplastic activities [80,81]. As the presence of sulphur ligands in some transplatin derived complexes was revealed to be important for their bio-activation and cytotoxicity, the authors reacted the complexes **39i** and **40c** with L-methionine and acquired mass spectra both immediately after the mixing and after 12 h of incubation. Only after 12 h the ions [PtCl(**33i**)(L-Met)]$^+$ and [PtCl$_2$(**34c**)(L-Met)-H]$^-$ were detected in a very low abundance. The slow kinetics of ligand exchange between complexes **39i** and **40c** and L-methionine could explain, in principle, the negligible cytotoxicity found in the two screened cancer cell lines.

7-Deazaadenosine, also known as tubercidin, (Scheme 10), is a natural adenosine analogue endowed with antibiotic and antitumor properties. Tubercidin shows a potent *in vitro* cytotoxicity against the murine



Scheme 8. The synthesis of the adenosine-containing Pt(II) complex **32**.

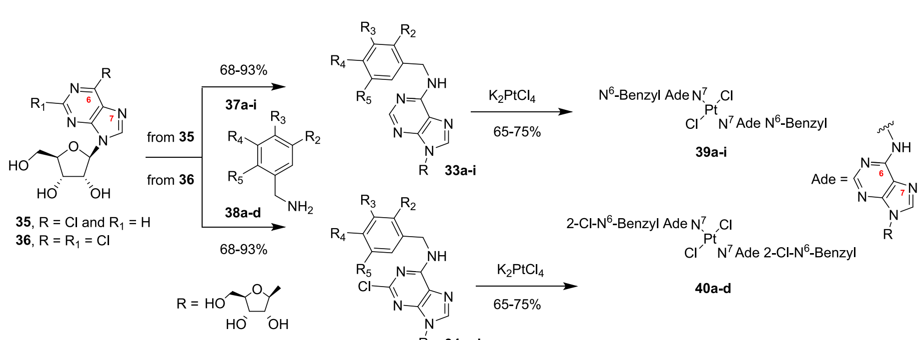
Table 5
N^6-Benzyl (**33a–i**) and 2-chloro-N^6-benzyl adenosine derivatives (**34a–d**) used in the reactions with K_2PtCl_4.

Ligand	R_1	R_2	R_3	R_4	R_5
					
33a	H	OCH_3	H	H	H
33b	H	H	H	OCH_3	H
33c	H	Cl	H	H	H
33d	H	H	H	Cl	H
33e	H	OH	H	H	H
33f	H	H	OH	H	H
33g	H	OH	OCH_3	H	H
33h	H	H	H	F	H
33i	H	H	H	CH_3	H
34a	Cl	H	OH	H	H
34b	Cl	H	H	OH	H
34c	Cl	OH	OCH_3	H	H
34d	Cl	OH	H	H	CH_3

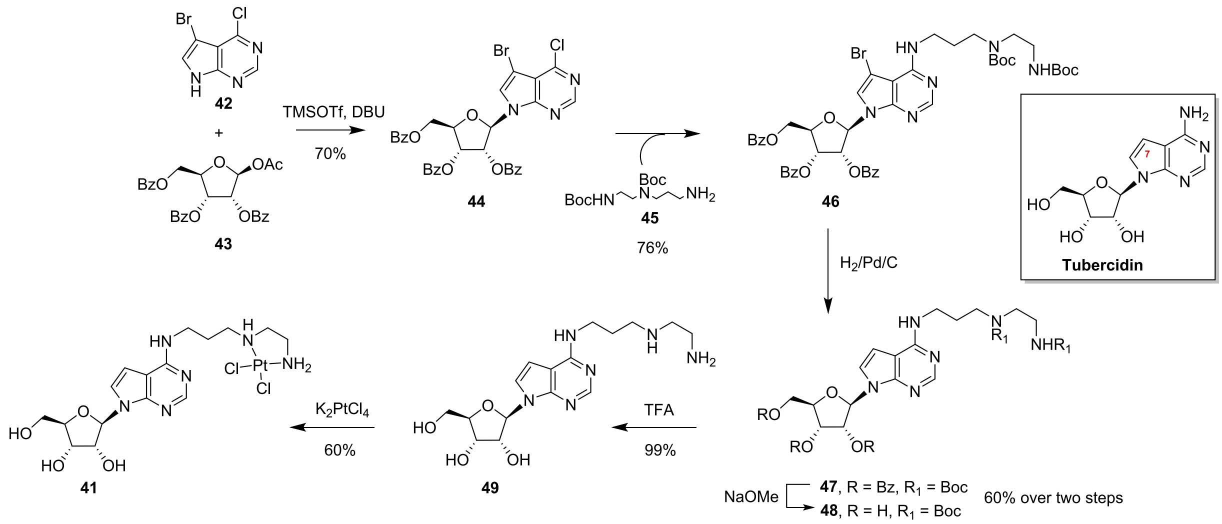
P388 and the human lung adenocarcinoma A549 cell lines. In the cell, tubercidin is phosphorylated by kinases to the corresponding triphosphate which elicits damage to nucleic acid functions after its incorporation into DNA or RNA [82]. A lot of research has been devoted to the preparation of tubercidin analogues to reduce its substantial toxicity [82,83]. Interestingly, the C6 alkyl-, aryl- and heteroaryl-substituted analogues showed interesting biological properties [83]. Our group reasoned that the nucleophilicity of two vicinal amino groups on the same alkyl chain installed on the C6 purine position could be exploited for the obtainment of new neutral Pt(II) complexes [84,85]. The lack of the purine N^7 atom would assure the sole metalation of the two adjacent amino groups. As a model compound, our group prepared the tubercidin-Pt(II) complex **41**, in which a propyl chain connected the cisplatin-like moiety to the nucleoside scaffold [86]. For the synthesis of complex **41** we started from the coupling reaction between 7-bromo-6-chloro-7-deazapurine **42** and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose **43**, under the silyl-Hilbert-Johnson conditions using trimethylsilyl triflate (TMSOTf) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [87], then the nucleoside **44** was obtained (70 %). The C6-Cl halogen displacement was carried out by the primary amino group of compound **45**, readily obtainable starting from the Michael addition of *tert*-butyl(2-aminoethyl)carbamate to acrylonitrile (76 %). After reduction of the C-Br bond (**46** → **47**), removal of the benzoate (**47** → **48**, 60 % over two steps) and Boc protecting groups (99 %), the nucleoside **49** with the two free vicinal amino groups was yielded.

The compound **49** was then reacted with K_2PtCl_4, and the Pt(II) complex **41** was recovered by filtration as a pale-yellow solid in a 60 % yield. Its structure was determined by NMR spectroscopy. The platination of the diamino ethane moiety in the complex **41** was confirmed by the downfield shift of the ^{13}C resonances of CH_2NH_2 (Δppm $\approx$ 5) and CH_2NH (Δppm $\approx$ 3 each) compared with the same signals belonging to the diamine **49**. With complex **41** in our hand, we evaluated its propensity to react with the model DNA duplex **d50/51**, monitoring the behaviour by CD spectroscopy. The duplex **d50/51**, obtained by hybridizing equimolar amounts of oligodeoxynucleotides (ODNs) **50** and **51** (Table 6), is characterized by the presence of three GG boxes, which represent the points of contact with the cisplatin-like moiety. The complex **41**, tubercidin and cisplatin were incubated in two and ten equiv. excess [88] with respect to the duplex **d50/51** and the CD spectra of the resulting mixtures were acquired after 24, 48 and 72 h of incubation (Fig. 5). The CD spectrum of the **d50/51** displayed a negative band at 239 nm related to the characteristic helicity of the right-handed B form and a positive band at 273 nm diagnostic of the base-stacking [52].

Only after the incubation of **d50/51** with ten equivalents of the complex **41** and cisplatin considerable conformational changes were observed. Both complex **41** and cisplatin reduced the intensity of the 273 nm band with hypsochromic and bathochromic shifts of the



Scheme 9. The synthesis of *trans*-[$PtCl_2$(N^6-benzyl Ade)$_2$] (**39a–i**) and *trans*-[$PtCl_2$(2-Cl-N^6-benzyl Ade)$_2$] (**40a–d**).



Scheme 10. The synthesis of the tubercidin-containing Pt(II) complex **41**.

Table 6
The oligodeoxynucleotides (ODNs) **50** and **51** used to form the model duplex DNA **d50/51**.

ODN	Sequence
50	d(5′-**GG**AGACCAGA**GG**-3′)
51	d(5′-CCTCT**GG**TCTCC-3′)
d50/51	d(5′-**GG**AGACCAGA**GG**-3′)d (3′-CCTCT**GG**TCTCC-5′)

negative and positive signals, respectively. Taken together, these data are consistent with a disruption of the B-DNA double helix and indicate non-intercalative interactions of the complexes with the double-stranded DNA [52].

Interestingly, the most pronounced changes were elicited by complex **41** after 24 and 48 h of incubation. The CD profiles obtained after 72 h of incubation of complex **41** and cisplatin with **d50/51** matched, leaving us to deduce that both complexes could have a similar mechanism of action. For the presence of several polar functions in the complex **41**, its higher reactivity than cisplatin towards the double helix could derive from an initial pre-association with the double helix **d50/51**, which favoured the interaction of the cisplatin-like moiety with the GG boxes at earlier time points. Considering that the interaction of tubercidin with **d50/51** did not produce any appreciable conformational change in the duplex structure, it may be concluded that DNA is not its biological target.

The cytotoxicity profiles of complex **41** were evaluated on three different human cancer cell lines, namely cervical adenocarcinoma cells (HeLa), low metastatic (A375) and metastatic melanoma cells (WM266), as well as on healthy human dermal fibroblasts (HDF), using cisplatin as a positive control. Complex **41** presented a good effect on HeLa cell viability, but was less active than cisplatin on WM266 and only slightly effective on A375 and on healthy HDF (Table 7). Notwithstanding complex **41** showed lower cytotoxicity than cisplatin in all the cell lines tested, its greater selectivity toward tumor cells and non-cytotoxicity on the normal HDF cell line could be exploited in multi-drug therapy approaches. A poor cellular uptake or deactivation process intra/extra-cellular environments could explain the weak *in vitro* biological activity of complex **41**, despite it was able to induce faster conformational changes than cisplatin to the DNA double helix **d50/51**.

3. Pt(II) complexes with modified thymidines as ligands

5-Fluorouracil (5-FU) and 3′-azido-3′-deoxythymidine (AZT) are two thymine and thymidine analogues extensively used in clinical practice as antitumor and antiviral agents, respectively. As 5-FU is used in combination with cisplatin for the treatment of several cancers [89,90], research has been devoted to the construction of thymidine-containing Pt(II) complexes, to evaluate the properties of compounds containing the pyrimidine nucleoside and a Pt(II) moiety in the same molecular scaffold.

Osella *et al.* prepared complex **52** (Scheme 11) starting from the unprotected thymidine [91]. They exploited the highest acidity of the N^3-H proton to obtain regioselectively the nucleoside **54** (80 %) by reaction of deprotonated thymidine with 1,3-diiodopropane **53**. The iodinate analogue **54** was then reacted with ethylenediamine **55**, affording the diamine **56** (50 %). Finally, the reaction of the nucleoside **56** with K_2PtCl_4 gave the Pt(II) complex **52** (60 %). As complex **52** was poorly soluble in common organic solvents as well as in water, the spectroscopic characterization was performed only through ESI MS experiments. In the mass spectrum recorded in DMSO/water (1:1) the base peak (m/z = 651) corresponded to the $[M - Cl + DMSO]^+$ ion, which formed after Cl^- displacement by DMSO. For its very low solubility in biological media, complex **52** could not be screened over cancer cell lines.

Picciali *et al.* [92] reported on the synthesis of complex **57** (Scheme 12) in which a Pt(II) moiety with two bulky PPh_3 groups was directly bonded to the N^3 thymidine atom, to slow down the reaction rate with DNA and affect the mechanism of action. The 3′,5′-di-*O*-acetylthymidine **58** was reacted with $Pt(PPh_3)_4$ in the presence of KCl, affording after oxidative addition the *cis* complex **59** as a mixture of two rapidly interconverting diastereomers (80 %). These latter were detected through the analysis of ^1H and ^{31}P NMR spectra, and their presence was ascribed to the restricted rotation around the N^3-Pt bond. The *cis* geometry was confirmed by analyzing the ^{31}P NMR spectrum, in which two overlapped sets of doublets centered at 8.6 and 15.3 ppm showed two different values of $^1J_{Pt-P}$. Complex **59** was stable in the presence of *S*-donor ligands (DMSO and CS_2) and D_2O. To obtain a more soluble complex in biological media, the acetyl groups in complex **59** were removed by an aqueous ammonia treatment, and compound **57** was recovered (90 %). No *cis*–*trans* isomerization was observed during the final deprotection reaction step.

In a later paper, Romanelli *et al.* reported on its preliminary biological activity, performing an arrested polymerase chain reaction (PCR) test and an antiproliferative assay on human chronic myelogenous K652 cells, using cisplatin as a positive control (Table 8) [93]. For the inhibition of PCR, the found IC_{50} was 3.1 ± 0.9 μM, whereas for the inhibition of the cell growth the found IC_{50} was 2.95 ± 1.11 μM.

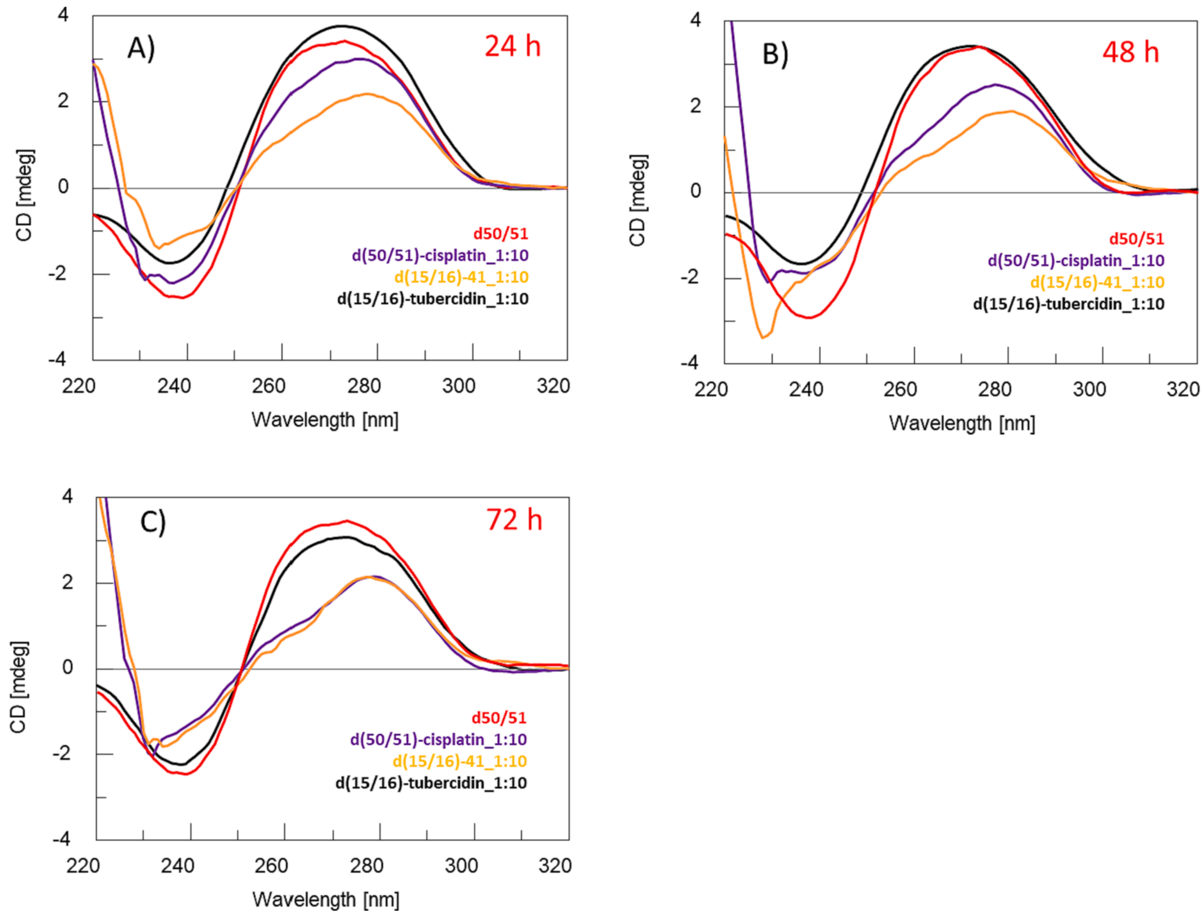


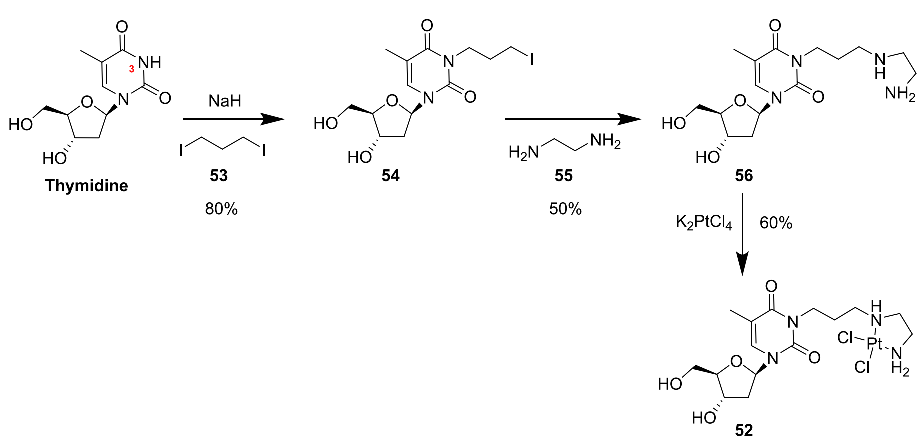
Fig. 5. Overlapped CD spectra of **d50/51** (red line) recorded before and after 24 (panel **A**), 48 (panel **B**) and 72 h (panel **C**) of incubation with 10 equiv. of **41** (orange line), cisplatin (violet line) and tubercidin (black line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 7
IC_{50} values obtained after 72 h incubation with complex **41** and cisplatin on HeLa, A375, WM266 and HDF cell lines.

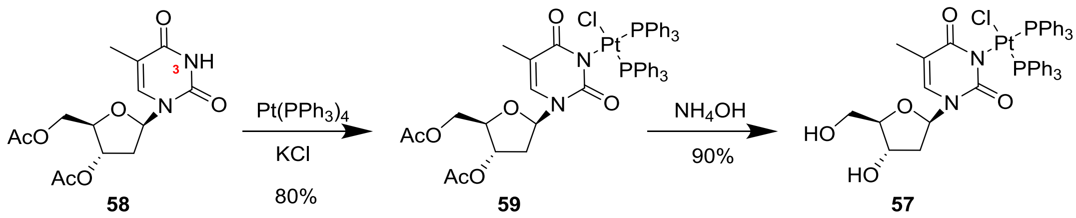
Entry	IC_{50} [μM]			
	HeLa	A375	WM266	HDF
41	55.1 ± 14.6	>100	91.0 ± 11.9	>100
Cisplatin	1.8 ± 0.75	1.3 ± 0.28	2.4 ± 0.40	6.3 ± 1.8

To exclude that the biological effects could be due to a PPh_3 release, this latter was also screened in both assays. As for PPh_3 $IC_{50} > 50$ and 5.07 ± 1.00 μM were found respectively for the inhibition of PCR and cell growth, it was hypothesized that: 1) complex **57** and not PPh_3 could influence the differential inhibition of DNA replication, 2) the stability of complex **57** in the presence of DMSO and CS_2 could also be held against cellular Pt-detoxifying agents, such as thiourea and glutathione.

The evidence that Pt(II)-DMSO-pyridine complexes could lose the heteroaromatic ligand in solution when reacted with biomolecules [94] prompted Church *et al.* to synthesize complex **60** and explore its



Scheme 11. The synthesis of the thymidine-containing Pt(II) complex **52**.



Scheme 12. The synthesis of the thymidine-containing Pt(II) complex **57**.

Table 8
Effects of cisplatin, complex **57** and triphenylphosphine on PCR and cell growth of K562 cells.

Entry	Inhibition of PCR (IC_{50}, μM)	Inhibition of cell growth (IC_{50}, μM)
Cisplatin	8.5 ± 1.5	2.10 ± 0.91
57	3.1 ± 0.9	2.95 ± 1.11
PPh_3	>50	5.07 ± 1.00

reactivity against the DNA pUC18 circular plasmid (Scheme 13) [95]. For the preparation of complex **60** they started from the ribose protected thymidine **61**, on which the pendant pyridinyl moiety was bonded to the N^3 thymidine atom through the Mitsunobu reaction (**61** → **62**, 65 %) using tributyl phosphine (PBu_3) and 1,1′-(azodicarbonyl)dipiperidine (ADDP) as reagents [96]. After deprotection of the sugar moiety (**62** → **63**, 62 %) and treatment with $PtCl_2(DMSO)_2$ complex **60** was recovered as a not crystallizable white solid (60 %).

The platination site was clearly demonstrated by the downfield shifts of the protons and carbons located in the proximity of the metal. Unfortunately, NMR spectroscopy did not allow to determine the *cis/trans* geometric disposition of the ligands around the Pt(II) center. Despite ^{195}Pt NMR spectroscopy could be helpful to discriminate between *cis/trans* isomers in $PtCl_2$(DMSO)Py complexes (Py = substituted pyridines), the authors found for complex **60** a ^{195}Pt resonance at −3124 ppm, that did not help them assigning the correct structural isomer.

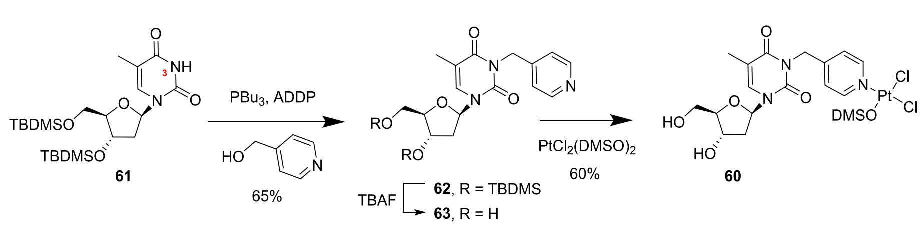
In a successive experiment, the authors probed the reactivity of complex **60** against the pUC18 circular plasmid DNA (lane 1: DNA; lane 3: DNA/**60**, 1:1; Fig. 6) by gel electrophoresis, using cisplatin as a positive control (lane 5, DNA/cisplatin, 1:1). As the complex **60** was poorly soluble in the phosphate buffered solution, a little amount of DMF was used to increase its solubility. To verify the effect of DMF and nucleoside scaffold on DNA conformation and stability, in parallel experiments, the plasmid was incubated both with the organic solvent (lane 2) and with compound **63** (lane 4). After the run, the gel was stained with ethidium bromide and exposed to UV light. As is evident from Fig. 6, DMF and nucleoside **63** did not affect the DNA mobility.

In the presence of Pt-DNA adducts, the supercoiled DNA could be cleaved into fragments or denatured. If the DNA fragments are large enough, the exposition to the intercalating agent should allow the detection of slower migrating bands on the gel. Incubating the plasmid with complex **60**, the DNA signal was completely lost. The latter observation was attributed to strong cleavage or denaturation processes suffered by the supercoiled DNA. The high reactivity showed by complex **60** towards DNA strongly supported the *cis* geometry of the ligands around the metal, as *trans* Pt(II) complexes are less reactive and induce other damages to the double helix [28,97,98].

As the intact thymine base may be involved in fundamental recognition processes [99], the synthesis of some thymine-containing Pt(II) complexes, in which the platinum-based moiety was tethered to the sugar residue or an amino acid, will be presented. Shieh *et al.* prepared a Pt(II) complex in which the thymidine 3′-OH was esterified with the COOH function of Sulindac, an anti-inflammatory drug currently investigated for the treatment of colon and breast cancers (**64**, Scheme 14) [100]. As currently approved Pt(II)-based drugs (cisplatin, carboplatin, and oxaliplatin) are effective against several carcinomas, the authors expected a synergic effect using Sulindac as one of the four Pt(II) ligands. Furthermore, the free thymidine 5′-OH could be exploited to form gene carriers releasing the drug *in situ*.

After Sulindac was coupled to 5′-*O*-TBDMS-2′-deoxythymidine **65** using 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC) as a condensing agent, compound **66** was obtained. The removal of the TBDMS group under acidic conditions afforded the nucleoside **67**, which was then reacted with the pyridine *N*-oxide **68** in the presence of K_2PtCl_4, and the Pt(II) complex **64** was obtained as a yellow solid in a 53 % yield. The *cis* configuration of the two sulfur atoms in complex **64** was elucidated by X-ray diffraction analysis, and its stability was checked under physiological conditions (>99 % after ten days). To evaluate a preliminary biological effect of complex **64** an MTT test was performed over oral cancer OECM1 cells using cisplatin as a positive control. Interestingly, complex **64** significantly reduced the cell viability compared to cisplatin after 48 h of incubation and was inactive towards normal cells at a concentration lower than 10 μM. Considering these newsworthy results, complex **64** could represent a valuable tool for targeted chemotherapy.

The 2-deoxyribose residues of nucleosides possess the two 3′-OH and 5′-OH groups in an *erythro* relative configuration. The alcoholic functionalities may be converted into amino groups that can be used for chelating bio-metals. Considering the importance of stereochemistry in the biological activity of metal-based sugars, Prokop *et al.* reported on the synthesis of the two thymidine-based cisplatin analogues **69** and **76** (Scheme 15), in which the relative configurations at the C3′ and C5′ carbons were *threo* and *erithro* respectively [101]. The common precursor for the preparation of both complexes was identified in thymidine. After mesylation reaction of the OH groups (86 %) followed by mesylate displacement by NaN_3 treatment, the C3′ carbon configuration could be inverted (**70** → **71**, 80 %). The reduction of both azide functionalities afforded the diamine **72** (95 %), and the final treatment with K_2PtCl_4 gave the desired *threo* complex **69** in a 94 % yield. When



Scheme 13. The synthesis of the thymidine-containing Pt(II) complex **60**.

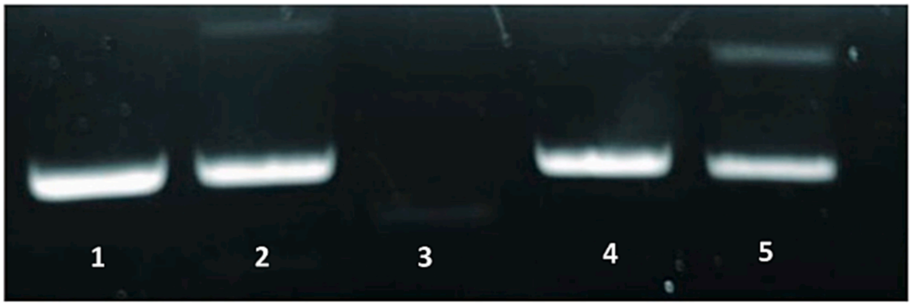
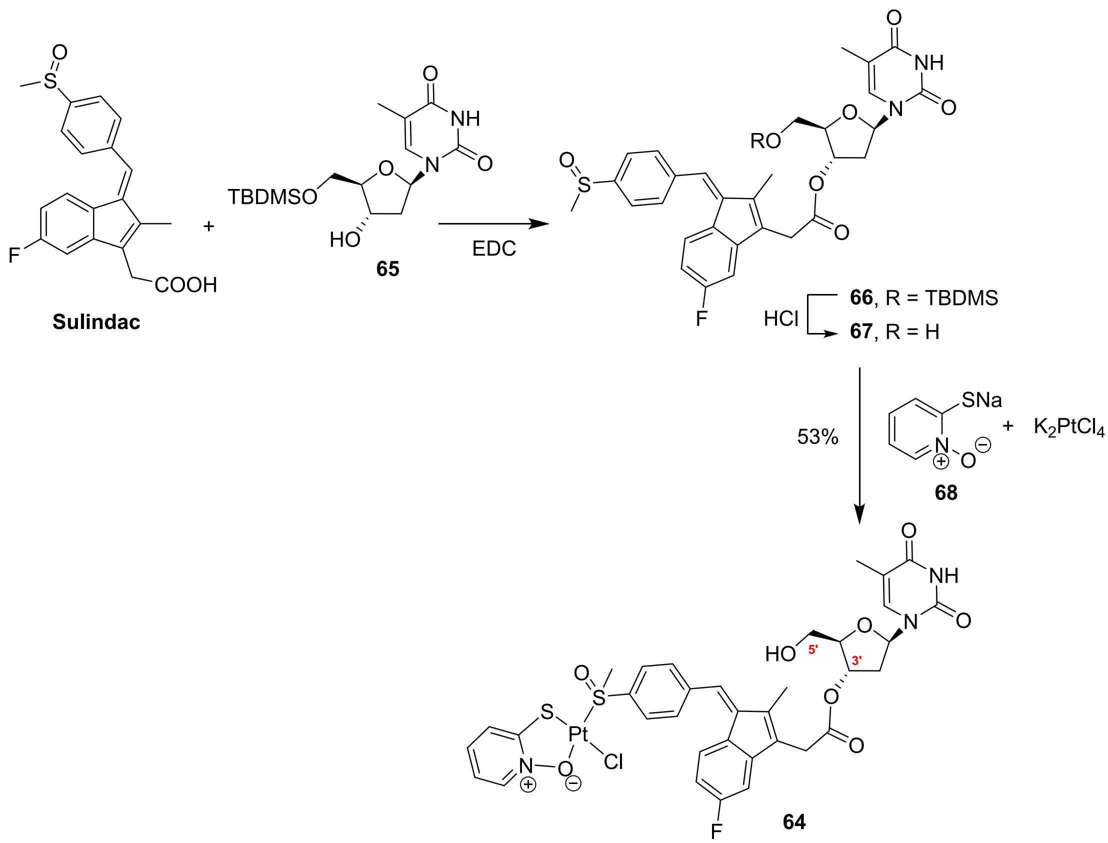


Fig. 6. Effect of complex **60** on the migration of pUC18 circular plasmid DNA (1:1 ratio). Lane 1: 15 μM DNA; lane 2: 15 μM DNA + 20 % DMF; lane 3: 15 μM DNA + 20 % DMF + **60**; lane 4: 15 μM DNA + 20 % DMF + **63**; lane 5: 15 μM DNA + 20 % DMF + cisplatin (DNA/cisplatin, 1:1). This figure is republished with permission of Elsevier, from [95]; permission conveyed through Copyright Clearance Center, Inc. License Number: 5430910535900.



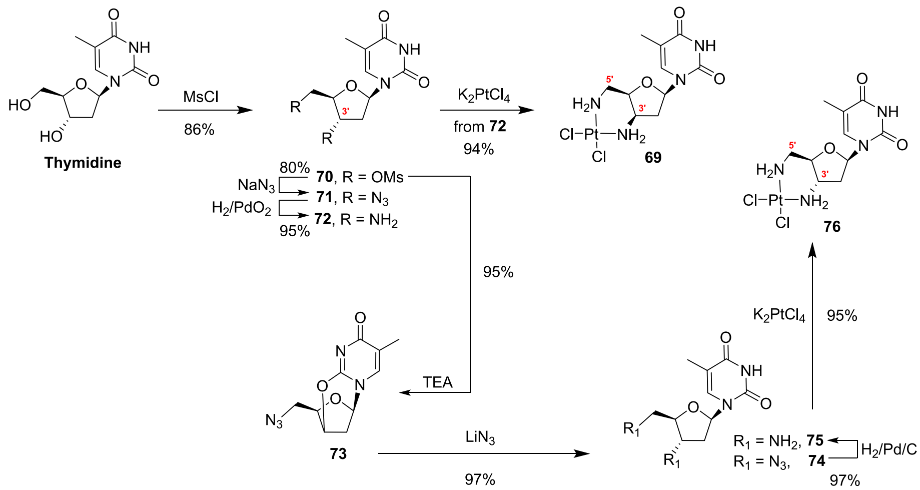
Scheme 14. The synthesis of the thymidine-containing Pt(II) complex **64**.

compound **70** was treated with triethylamine (TEA), the anhydro nucleoside **73** (95 %) could be produced. After the α-face attack of N_3^- ion to the C3′ carbon, the *erythro* bis-azide **74** was yielded (97 %). The reduction of both azide functionalities afforded the diamine **75** (97 %) and the final treatment with K_2PtCl_4 gave the desired *erythro* complex **76** in a 95 % yield. The structures of complexes **69** and **76** were supported by single crystal X-ray analyses and NMR experiments. As the platination reactions involved the sugar moieties, only little downfield shifts of C3′ and C5′ atoms were detected. Furthermore, since no changes in chemical shifts of protons and carbons belonging to thymine residues were observed with respect to the diamines **72** and **75**, it could be excluded that the bases were involved in the reaction.

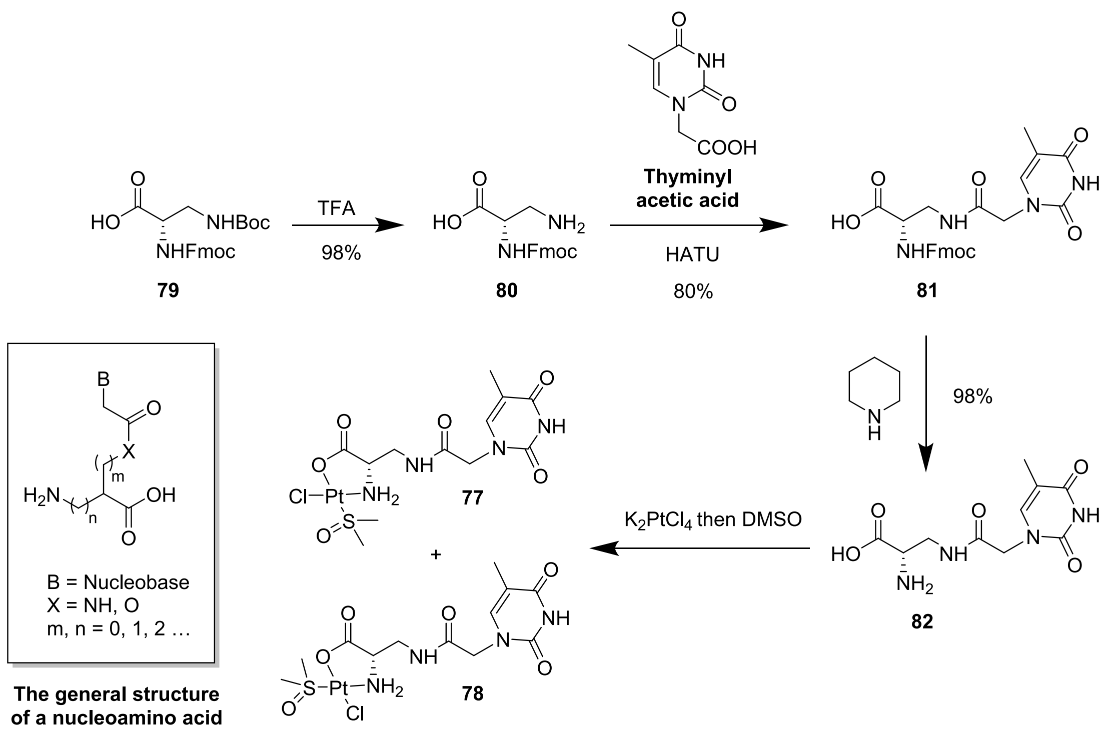
Both the complexes were screened over a lymphoma cell line (BJAB) that was poorly responsive to cisplatin treatment. Complex **69** displayed significant antiproliferative effects (80 % growth inhibition after 96 h at 40 μM) and induced apoptosis (50 % after 96 h at 60 μM). The apoptosis was mediated by a caspase activation that was associated with loss of mitochondria membrane potential and was dependent on Bcl-2. In addition, complex **69** was also active on leukaemia cell lines resistant to vincristine and daunorubicin treatments (NALM-6/VCR and NALM-6/DAUNO).

Nucleoamino acids (Scheme 16) are molecules in which purine or pyrimidine bases are joined to amino acid scaffolds and have been used as building blocks to synthesise nucleopeptides [102]. Nucleopeptides were revealed as a valuable tool in antisense/antigene strategies or to produce new ordered self-assembling superstructures [103–105]. The simplest amino acid which can be used to produce a nucleoamino acid is L-2,3-diaminopropanoic acid (DAPA). As the α-amino and α-carboxylic functions of DAPA can be exploited to chelate Pt(II) ions, Musumeci *et al.* have recently prepared the first examples of Pt(II)-nucleoamino complexes (**77** and **78**) in which a thyminyl residue was tethered to a Pt(II)-DAPA moiety [106].

For the preparation of complexes **77** and **78** the authors started from Fmoc-L-DAP(Boc)-OH **79**. After Boc removal (**79** → **80**, 98 %), 1-[bis



Scheme 15. The synthesis of the thymidine-containing Pt(II) complexes **69** and **76**.



Scheme 16. The synthesis of the thymidine-containing Pt(II) complexes **77** and **78**.

(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5–b]pyridinium-3-oxid hexafluorophosphate (HATU)-mediated coupling with thyminyl acetic acid (**80** → **81**, 80 %), and Fmoc cleavage the free ligand thyminylamino acid **82** was obtained (98 %). Unfortunately, the treatment of compound **82** with K_2PtCl_4 afforded unstable and difficult-to-handle complexes. Given the stabilizing and solubilizing properties of DMSO after the displacement of labile ligands around Pt(II), the crude obtained after the reaction with K_2PtCl_4 was immediately treated with DMSO, and the two complexes **77** and **78** were isolated after selective precipitations. The presence in the ^{1}H NMR spectra of signals at 5.68 and 6.29 ppm for **77** and 5.63 and 6.25 ppm for **78** was diagnostic of protons belonging to nitrogen bound to Pt(II) center. In addition, CH-α protons and carbons as well as carboxylate carbons of both complexes were markedly downfield shifted with respect to the corresponding signals in the nucleoamino acid **82**. The presence of DMSO as a Pt(II) ligand was confirmed by the appearance in ^{1}H and ^{13}C NMR spectra of CH_3-S-Pt(II) signals, downfield shifted with respect to those belonging to a CH_3 in a free DMSO molecule. Lastly, the geometry of ligands around platinum was determined through NOESY experiments. In particular, a correlation between the protons of the amino group coordinating the Pt(II) and the DMSO methyl groups was only detected in complex **77**.

The reactivity of complexes **77** and **78** was probed against four model ODNs forming different secondary structures (Table 9), and the behaviours were monitored by CD spectroscopy.

Interestingly, both the synthesized complexes were able to induce important conformational changes only after reaction with ODN **51** (Fig. 7, panels a and b) and with the monomolecular G-quadruplex obtained by ODN **85** (Panels c and d).

Regarding the effect with ODN **51**, a decrease over time of the band at 275 nm accompanied by a red shift of the band maximum was observed. More marked changes in the CD band were observed when complex **78** was added to the ODN **51**. Furthermore, after adding

Table 9
The ODNs used for studying the reactivity with the complexes **77** and **78**.

ODN	Sequence	Secondary structure formed after annealing
51	d(5′-CCTCT**GG**TCTCC-3′)	Random coil with one GG box
83	d(5′-CGCGAATTCGCGTTTCGCGAATTCGCG-3′)	Self-complementary hairpin B-DNA duplex without GG boxes
84	d(5′-CATGT**GG**TTCTGTTTCAGAACCACATG-3′)	Self-complementary hairpin B-DNA duplex with one GG box
85	d(5′-TTA**GGG**TTA**GGG**TTA**GGG**TTA**GGG**TT-3′)	Monomolecular G-quadruplex

complex **77** and **78** to the G-quadruplex, both the main band at 290 nm and the shoulder at 267 nm significantly decreased over time. In parallel experiments, the reactivity of cisplatin with ODN **51**, DNA duplexes derived from ODNs **83** and **84**, and G-quadruplex derived from ODN **85** was also monitored by CD spectroscopy. The collected data are consistent with a disruption of the B-DNA double helix and are indicative of non-intercalative interactions of the complexes with the double-stranded DNA [51,86]. Whether cisplatin was able to interact significantly with the ODN **51** and DNA duplex containing the GG box, it was not able to disrupt both the DNA duplex without the GG box and the G-quadruplex structure obtained by ODN **85**. Taken together, these data supported the selectivity of complexes **77** and **78** towards the G-quadruplex structure with respect to cisplatin. It should point out that the monomolecular G-quadruplex used in this study was derived from the 26-mer human telomere sequence (tel26) [107,108]. As the presence of these non-canonical DNA structures was found in some regulating regions of the human genome [109,110], the search for new molecules capable of stabilizing or destabilizing G-quadruplex structures is an exciting field that could allow understanding the role of the G-quadruplexes in the progression of tumours [111–115].

The cytotoxicity profiles of complexes **77**, **78**, and free ligand **82** were evaluated on three different human tumor cell lines, namely cervical adenocarcinoma cells (HeLa), metastatic melanoma cells (WM266), and healthy human dermal fibroblasts (HDF) . The complexes showed good antiproliferative activity on HeLa cells with a cell viability reduction of 23 % or 29 % at 25 μM and 55 % or 54 % at 50 μM for **77** or **78** respectively, and were not cytotoxic against the HDF cell line (Fig. 8). However, the cytotoxicity of complexes **77** and **78** were lower than cisplatin on all the treated tumor cells. Considering the poor reactivity of the complexes towards the model DNA duplexes, it is plausible that the moderate cytotoxic effect observed on the HeLa cell line may be derived from a mechanism of action different from that of cisplatin.

4. Conclusions

The efforts to obtain chemotherapeutics that were less toxic and more active on cancer cell lines resistant to cisplatin led to the preparation of a wide range of Pt(II)-complexes, exploiting the ligand properties of several molecular scaffolds towards the metal center. Nucleosides and their analogues can act as ligands of Pt(II) ions. Despite their great biological importance, only a few Pt(II) complexes having nucleosides in the coordination sphere of the metal have been synthesized so far. Additionally, among all the synthesized nucleoside-based Pt (II) complexes, no significant examples with cytidine analogues as ligands are reported in the literature, notwithstanding the cytidine scaffold has been used to produce many anticancer and antiviral drugs on the market. The results summarized in this review have shown that some nucleoside-based Pt(II) complexes induce damage to DNA in a shorter time than cisplatin and display cytotoxicity selectively against cancer cells. The weaker *in vitro* biological activity elicited by other complexes

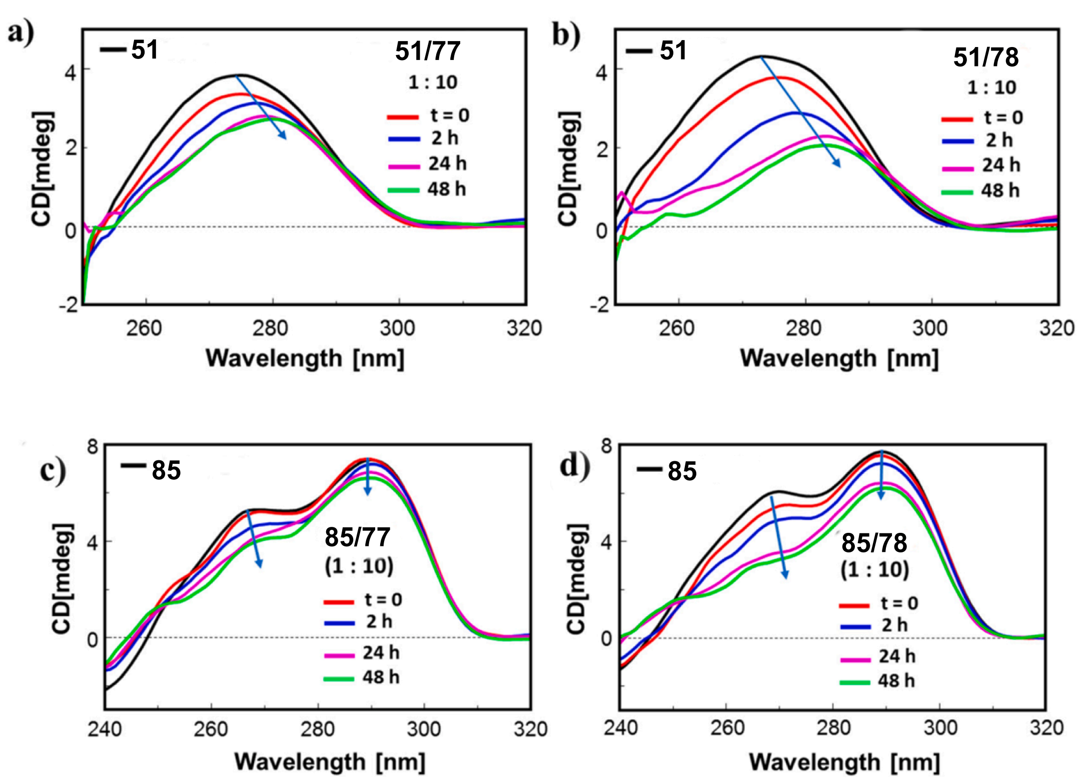


Fig. 7. Panels a) and b): Overlapped CD spectra of ODN **51** in the absence (black lines) and presence of **77** and **78**, respectively, at different time points (0, 2, 24, 48 h) after the addition of the target molecules. Panels c) and d): Overlapped CD spectra of G-quadruplex obtained from ODN **85** in the absence (black lines) and presence of **77** and **78**, respectively, at different time points (0, 2, 24, 48 h) after the addition of the target molecules. For all the experiments the ratio ODN/complex was 1:10.

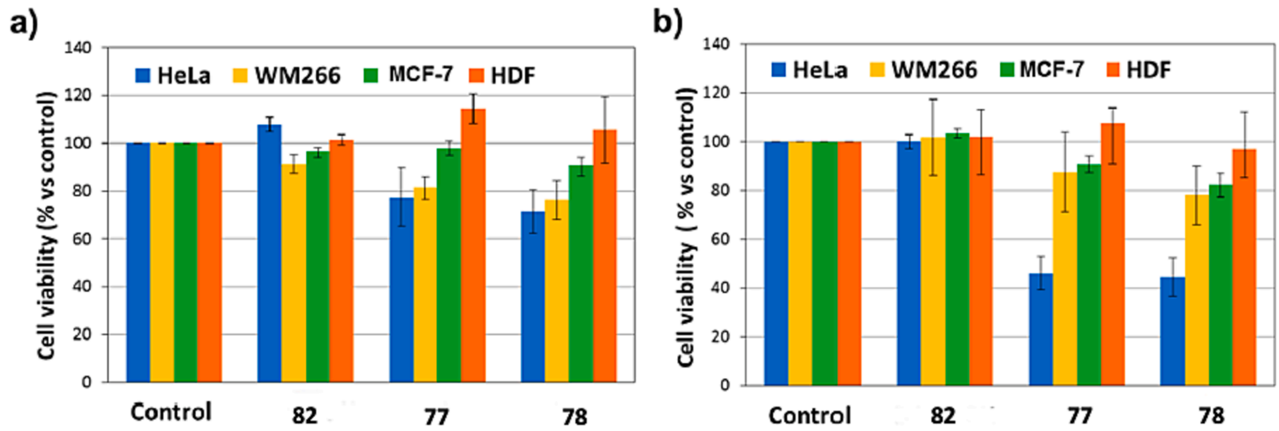


Fig. 8. Cell viability assay on human cervical adenocarcinoma (HeLa), metastatic melanoma (WM266), breast adenocarcinoma (MCF-7), and human fibroblasts (HDF). Cells were incubated with **77**, **78**, and **82** at 25 (**a**) or 50 (**b**) μM concentration at 37 °C for 48 h . Control represents vehicle-treated cells.

against cancer cell lines with respect to cisplatin could be ascribed either to deactivation processes in intra/extracellular environments before they reach their target or to poor cellular uptake. All these things considered, studies should be directed towards the inorganic/organic drug delivery of nucleoside-based Pt(II) complexes to obtain biocompatible systems that are resistant to biological fluids and that display specificity towards the target [18,116–118].

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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