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Analysis of the DNA damage produced by a platinum–acridine antitumor agent and its effects in NCI-H460 lung cancer cells

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Abstract

High-performance liquid chromatography in conjunction with electrospray mass spectrometry (LC-ESMS) was used to structurally characterize the adducts formed by the platinum–acridine agent [PtCl(en)(*N*-(2-(acridin-9-ylamino)ethyl)-*N*-methylpropionimidamide)](NO₃)₂ (compound **1**) in cell-free DNA. Compound **1** forms monofunctional adducts exclusively with guanine, based on the fragments identified in enzymatic digests (dG*, dGMP*, dApG*, and dTpG*, where the asterisk denotes bound drug). The time course of accumulation and DNA adduct formation of compound **1** and the clinical drug cisplatin in NCI-H460 lung cancer cells at physiologically relevant drug concentrations (0.1 μM) was studied by inductively-coupled plasma mass spectrometry (ICP-MS). Compound **1** accumulates rapidly in cells and reaches intracellular levels of up to 60-fold higher than those determined for cisplatin. The hybrid agent shows unusually high DNA binding levels: while cisplatin adducts form at a maximum frequency of 5 adducts per 10⁶ nucleotides, compound **1** produces 25 adducts per 10⁶ nucleotides after only 3 h of continuous incubation with the lung cancer cells. The high overall levels of compound **1** in the cells and in cellular DNA over the entire 12-h treatment period translate into a rapid decrease in cell viability. Possible implications of these findings for the mechanism of action of compound **1** and the agent's potential to overcome tumor resistance to cisplatin are discussed.

1. Introduction

Platinum-based cytotoxic agents continue to be an important treatment option in the management of chemoresistant cancers despite the recent advent of novel targeted therapies.¹ Platinum–acridine hybrid anticancer agents derived from the prototypical complex PT-ACRAMTU ([PtCl(en)(ACRAMTU)](NO₃)₂ (where “en” stands for ethane-1,2-diamine and “ACRAMTU” stands for 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea) have shown cancer cell kill far superior to the drug cisplatin (*cis*-diamminedichloroplatinum(II), Figure 1).² Specifically, replacement of the thiourea donor group in PT-ACRAMTU with an amidine group has resulted in a set of analogues that have demonstrated cytotoxicity in the low-nanomolar concentration range.^{3, 4} The most active derivatives show a cytotoxic enhancement in non-small cell lung cancer (NSCLC) cells compared to the parent platinum–acridine and cisplatin of up to 100- and 500-fold,

respectively.^{4,5} Previous work has demonstrated that the DNA conformational changes caused by the unique monofunctional/intercalative adducts formed by compound **1** (Figure 1) are recognized and processed differently than the classical intrastrand cross-links formed by cisplatin.⁶ Using gel electrophoresis-based assays, we also demonstrated that compound **1** binds double-stranded DNA (dsDNA) much more rapidly ($t_{1/2} = 20$ min) than cisplatin ($t_{1/2} = 120$ min).⁷ On the basis of the cell-cycle perturbations (efficient suppression of DNA synthesis and S-phase arrest) and the overall cell kill potential observed in NSCLC cells, we reasoned that compound **1** might produce higher levels of potentially more cytotoxic DNA adducts that are less readily repaired than cisplatin-type damage.⁵

The goal of the current study was to determine the nature of the damage produced by compound **1** in native DNA and to correlate the frequency with which these adducts occur in whole cancer cells with the cytotoxic response triggered by this agent. To achieve this goal, we have used electrospray mass spectrometry (ES-MS) to structurally characterize the adducts formed in a cell-free environment, as well as inductively-coupled plasma mass spectrometry (ICP-MS) to study total platinum accumulation in cells and platinum adduct levels in cellular DNA. Cell proliferation assays using the NCI-H460 lung cancer cell line were also performed to monitor the time-dependent effects of intracellular platinum on cell viability.

2. Experimental

2.1 Materials

Compound **1** was synthesized according to the published procedure.³ Cisplatin was purchased from Sigma. Stock solutions of **1** and cisplatin were prepared in phosphate-buffered saline (PBS) or Tris buffer and stored at -20°C . Concentrations of **1** were determined spectrophotometrically using $\epsilon_{413} = 10571 \text{ M}^{-1} \text{ cm}^{-1}$ in PBS and $9500 \text{ M}^{-1} \text{ cm}^{-1}$ in 20 mM Tris buffer (pH 7.1). Calf thymus DNA (Sigma) was dissolved in 20 mM Tris buffer (pH 7.1). DNA concentrations (base pairs, bp) were determined from absorbances at 260 nm using Beer's law with $\epsilon_{260} = 12824 \text{ M}^{-1} \text{ cm}^{-1}$ (bp).⁸ HPLC-grade solvents were used in all chromatographic separations. Millipore water for the preparation of all buffers was obtained from a Milli-Q A10 synthesis water purification system. All other chemicals were molecular biology grade and purchased from Sigma or Fisher. DNase I (RNase-free) from bovine pancreas, and calf intestinal alkaline phosphatase (CIP) were supplied by New England BioLabs (Ipswich, MA, USA). Nuclease P_1 from penicillium citrinum was from Sigma and was dissolved in a buffer containing 40 mM sodium acetate, 10 mM ZnCl_2 , 100 mM NaCl and 50 % glycerol (pH 5.3). All enzymes were stored at -20°C . UV-visible spectra were recorded on a Hewlett-Packard 8453 spectrophotometer.

2.2 Incubations and enzymatic digestion

Calf thymus DNA ($c_{\text{DNA, bp}} = 7.0 \times 10^{-4} \text{ M}$) was incubated with compound **1** in Tris buffer at platinum-to-nucleotide ratios of 0.05, 0.005, and 0.0005 at 37°C for 18 h in dark (under these conditions, compound **1** has been demonstrated to be unreactive with this buffer⁷). After incubation, the platinum-modified DNA samples (200 μL) were digested using the following protocol (total incubation time 26 h at 37°C): i) + 20 μL DNase I stock buffer solution (100 mM Tris-HCl, 2.5 mM MgCl_2 , and 0.5 mM CaCl_2 ; pH = 7.6) + 40 units of DNase I (2 h); ii) +26 units DNase I (2 h); iii) + 16 units nuclease P_1 (2 h); iv) + 4 units nuclease P_1 (16 h); v) + 30 μL alkaline phosphatase buffer (1 M NaCl, 500 mM Tris-HCl, and 10 mM dithiothreitol; pH = 7.9) + 20 units alkaline phosphatase (2 h); and vi) + 12 units alkaline phosphatase (2 h). To inactivate the enzymes, the mixtures were heated at 70°C for 5 min and then allowed to cool to room temperature. Samples were centrifuged at 13000 rpm for 10 min and the supernatant was collected and dialyzed against Millipore water for

24 h at room temperature using a 100-Da-cutoff cellulose ester dialysis membrane (Fisher). To account for an increase in the sample volume during dialysis, the samples were lyophilized and redissolved in 250 μ L of 0.1% formic acid in water before LC-MS analysis.

2.3 High-performance liquid chromatography–electrospray mass spectrometry

LC–MS experiments were carried out on a Thermo Scientific LTQ XL Orbitrap system. Separations were achieved using a Thermo Scientific Hypersil Gold C18 column (100 mm $\times$ 1 mm, 1.9 μ m) attached to an Accela Open 1200 auto sampler. LC analysis consisted of 1- μ L injections using a gradient elution of 5–95 % B over 10 min at a flow rate of 100 μ L/min (solvent A: 0.1% formic acid; solvent B: 0.1% formic acid in methanol, Fisher Scientific Optima solvents). The electrospray and ion optics parameters were optimized on synthetic samples of one of the adducts, dG*. Representative values of positive-ion mode high-resolution mass spectra were: spray voltage, 4 kV; capillary temperature, 275 $^{\circ}$ C; capillary voltage, 33 V; tube lens voltage, 94 V; and a nebulizer spray of 50 arbitrary units. Full-scan Orbitrap data were collected at 60000 Hz resolution over a range of 200–2000 m/z and searched for $\pm$ 5 ppm of the expected isotopic masses. Target masses are reported to 2 decimal places.

2.4 Cell culture

The human non-small cell lung cancer cell line, NCI-H460, was obtained from the American Type Culture Collection (Rockville, MD, USA) and was cultured in RPMI-1640 media (HyClone) containing 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, 10 mM HEPES, and 110 mg/L sodium pyruvate supplemented with 10% fetal bovine serum (FBS), 10% penstrep (P&S), and 10% L-glutamine. Cells were incubated at a constant temperature at 37 $^{\circ}$ C in a humidified atmosphere containing 5% CO₂ and were subcultured every 2 to 3 days in order to maintain cells in logarithmic growth.

2.5 Drug treatment and sample preparation

To determine the Pt content in genomic DNA, DNA was extracted and purified using the Promega Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). 6×10^6 exponentially-growing NCI-H460 cells were seeded in a 60-mm cell culture dish with 2 mL of media and were allowed to attach. Cells were incubated in the presence of 0.1 μ M cisplatin or compound **1** at 37 $^{\circ}$ C for various time intervals. Incubations were performed in triplicate for each time point. To quench the incubations, cells were washed three times with 3 mL of cold PBS. The cells were then harvested by trypsinization and completely removed from the dishes by additional washings with cold PBS. Cell suspensions were centrifuged at 1500 rpm for 5 min at 4 $^{\circ}$ C. The DNA content was extracted according to the protocol provided by the manufacturer. The purity and concentrations of the isolated DNA were determined spectrophotometrically (triplicate readings at 260 and 280 nm). Whole-cell samples (3×10^6 cells) were incubated and collected using the same procedure. DNA solutions and whole-cell samples were lyophilized using a freeze dry system (FreeZone 4.5, Labconco, MO, USA) and were stored at -80° C until use.

2.6 Determination of platinum content by inductively-coupled plasma mass spectrometry

The concentration of Pt in the lyophilized samples was determined by quadrupole inductively-coupled plasma mass spectrometry (ICP-MS). Prior to analysis, study samples were subjected to a rigorous digestion procedure in the presence of high-purity, Ultrex-grade nitric (HNO₃) and hydrochloric (HCl) acids (J.T. Baker, Phillipsburg, NJ, USA). Nominal 0.200 mL aliquots of each acid were added to the study samples directly, along with 0.600 mL of approximately 18 M Ω quality deionized water (DI H₂O), obtained from a Pure Water Solutions (Hillsborough, NC, USA) system. Samples were then capped and placed in a

controlled temperature water bath (Model 1245PC, VWR International, Radnor, PA, USA) maintained at 90 °C. After 4 h, samples were removed from the bath and were allowed to cool to room temperature before adding 3 mL of DI H₂O. Samples were again capped and placed in a Beckman GS-6 centrifuge set to 5,000 rpm for 15 min.

After centrifugation, an aliquot (3 mL) of each digested sample was transferred to an ICP-MS autosampler tube and was fortified to contain a nominal 10 ng/mL concentration of the bismuth (Bi) internal standard. Prior to sample analysis, the Thermo (Waltham, MA, USA) XSeries2 ICP-MS was calibrated with Pt-containing acid matrix matched standards ranging from 0.001 to 0.100 ng Pt/mL. Single element Pt and Bi stock solutions traceable to the National Institute of Standards and Technology (NIST) were obtained from a commercial vendor (High Purity Standards, Charleston, SC, USA) and were used to prepare all solutions.

In addition to the study samples, several quality control samples were analyzed throughout the analysis to monitor method performance. Method blanks consisting of digestion acids were prepared with the study samples to monitor the analyte background contribution from the reagents and the procedure, and a method control sample (a fortified method blank) was prepared to assess analyte recovery in the absence of matrix. In addition, a mid-level calibration standard was analyzed immediately after the calibration, after a maximum of 20 samples and quality controls, and at the end of the analysis. In order for bracketed data to be considered acceptable, the determined concentration of the mid-level calibration standard was required to be within $\pm 10\%$ of its nominal value.

2.7 Cell viability assay

Cell viability was assessed using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) as described previously.⁵ Briefly, NCI-H460 cell suspensions were harvested and seeded into 96-well microplates at a density of 2000 cells/well. The cells were preincubated at 37 °C overnight and then treated with 0.1 μ M cisplatin or compound **1**. After incubation periods of 1, 3, 6, and 12 h, the drug-containing media was replaced with fresh media, and MTS (0.2% in PBS) solution was added to each well and incubated at 37 °C for 4 h. The absorbance of tetrazolium dye was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) reader. The fractions of viable cells was calculated as a percentage of untreated control and are reported as the mean $\pm$ standard deviation for 6 incubations at each time point.

3. Results and discussion

3.1 Adduct profile studied in cell-free, native DNA

To gain insight into the nature of the adducts formed by compound **1** and their nucleobase and base-pair step selectivity, calf thymus DNA was reacted with platinum drug. The DNA samples were then enzymatically digested and the mixtures analyzed by in-line high-performance liquid chromatography and electrospray mass spectrometry (LC-ESMS) using a modified protocol developed for the parent drug, PT-ACRAMTU.⁹ Briefly, incubations were performed under physiologically relevant conditions at various platinum-to-nucleotide ratios, and the platinum-treated DNA was digested with endonucleases and alkaline phosphatase to produce a mixture of unmodified and platinum-modified DNA fragments. A typical HPLC trace along with mass spectra recorded in positive-ion mode of each of the platinum-containing fractions is shown in Figure 2. Enzymatic degradation of the DNA afforded 4 platinated DNA fragments, **A1–A4**, the chemical composition of which could be unambiguously determined from the molecular parent and fragment ions (Table 1, Figure 3). In addition, the ions resulting from in-source collision-induced dissociation (CID) observed

for the undigested platinum-containing dinucleotides provided insight into the sequence selectivity of adduct formation.

The most abundant species observed in the digests proved to be the mononucleoside 2'-deoxyguanosine (dG) in which guanine is modified with a $[\text{Pt}(\text{en})(N\text{-(2-(acridin-9-ylamino)ethyl)-}N\text{-methylpropionimidamide})]^{3+}$ fragment (Figure 3, dG*, adduct **A1**). This observation confirms that compound **1**, in complete analogy to PT-ACRAMTU,⁹ forms monofunctional adducts with dsDNA in which the en ligand and amidine-linked acridine intercalator act as nonleaving groups. The corresponding platinum-modified 2'-deoxyguanosine 5'-monophosphate is also observed indicating incomplete removal of the 5' terminal phosphate group generated by enzymatic phosphodiester cleavage (Figure 3, 5'-dGMP*, adduct **A2**). In addition, two platinated deoxydinucleotides, d(ApG*) and d(TpG*), were identified (Figure 3, **A3** and **A4**). The characteristic fragmentation observed for these species, which leads to the formation of 5'-dGMP* ($[\text{M}+\text{H}]^{2+}$, m/z 453.64), indicates that platinum is bound to 3' guanine in the sequences 5'-AG and 5'-TG, and not 5' guanine at 5'-GA and 5'-GT steps (Figure 4). (The preferred mechanism of CID-induced fragmentation of the phosphodiester linkage involves depurination of the 5' nucleobase and subsequent cleavage of the 3'-O-P bond, which leads to cleavage products known as w-fragments.¹⁰) The presence of the fragments G* ($[\text{M}-\text{H}]^{2+}$, m/z 356.13) and dG* ($[\text{M}-\text{H}]^{2+}$, m/z 414.16) (Figure 4) provides additional evidence that platinum, indeed, is attached to guanine.

The DNA damage profile determined in this assay for compound **1** shares some features with that reported previously for PT-ACRAMTU. Unlike cisplatin, which forms bifunctional adducts in runs of adjacent purine bases, mainly in the sequences GG and 5'-AG, both hybrid agents form monofunctional adducts with guanine. Whereas PT-ACRAMTU has been shown to produce also a high percentage of adducts in which platinum is bound to adenine nitrogen (approximately 11% N7, 7% N3, and 2% N1),^{9, 11} no such binding mode has been detected in this assay for compound **1**. This observation is in agreement with a recent computational study at the density functional theory (DFT) level, which suggests that binding of compound **1** with adenine is kinetically disfavored.⁶ Another critical difference between PT-ACRAMTU and compound **1** appears to exist in the base-pair step selectivity of adduct formation. While compound **1** clearly favors platination of the 3' guanine base in the mixed-purine sequence 5'-AG, PT-ACRAMTU platinate the 3' adenine base at 5'-GA steps, as evidenced by the detection of the fragment dGpA* in enzymatic digests of calf thymus DNA treated with the latter compound.⁹ Likewise, no binding to adenine at 5'-TA steps, a dinucleotide site targeted by PT-ACRAMTU,⁹ is observed for compound **1**, which, instead, produces damage in the sequence 5'-TG. Both derivatives have a high affinity for guanine at 5'-pyrimidine/purine sites, the preferred binding site for DNA intercalators.¹² These findings are in complete agreement with a PCR-amplified Taq DNA polymerase stop assay, in which major stop sites caused by compound **1** on the sequencing gel were observed at 5'-CG and 5'-TG sites.³ Thus, replacing the thiourea donor in PT-ACRAMTU with amidine nitrogen to give compound **1** not only results in a greatly enhanced rate of platination of guanine in dsDNA, this modification also has consequences for the base and sequence specificity of platinum binding. Based on the computational analysis⁶ of the transition states and final adducts formed by compound **1** it can be speculated that the ability of the amidine-NH donor group to form a strong hydrogen bond with guanine-O6 may play a role in the binding selectivity of this derivative.

3.2 Platinum accumulation and levels of platinum–DNA damage in NCI-H460 lung cancer cells treated with compound **1** and cisplatin

Mechanistic studies performed in cell-free systems and in whole cells strongly suggest that the high cytotoxicity levels observed are mediated by the monofunctional DNA adducts formed by compound **1**. The frequency of formation of these adducts in genomic DNA in addition to the specific downstream events they trigger can be predicted to play a critical role in the mechanism by which compound **1** induces cell death. On the basis of the IC₅₀ values determined in this cell line for 72-h drug incubations, compound **1** proved to be ~150-fold more cytotoxic than cisplatin.⁵

The major goal of the experiments described here was to test if the cytotoxic responses produced by compound **1** and cisplatin in NCI-H460 lung cancer cells correlate with the platinum content in cellular DNA. The challenge with such an experiment in live cells is that the platinum levels should be determined at pharmacologically relevant concentrations of drug that affect cell viability without allowing loss of cell contents due to apoptosis. Another obstacle is that compound **1** kills NCI-H460 cells at nanomolar concentrations, which may lead to cellular and nuclear levels of platinum too low for detection of DNA adducts by LC–ESMS, the method used to characterize the damage in calf thymus DNA. To overcome these limitations and to assess the DNA damage quantitatively, an assay was designed in which NCI-H460 cells were treated with compound **1** and cisplatin at a dose (0.1 μ M) that has no major effect on cell morphology and attachment during the first 12 h of continuous incubation.⁵ Briefly, a well-defined number of NCI-H460 cells were incubated for 1, 3, 6, and 12 h with platinum drug, and the platinum content in whole cells and in the spectrophotometrically quantified DNA extracted from them was determined by ICP–MS, a technique that provides the lowest achievable detection limits.¹³ (For details of this assay, see Experimental.) (Note: ICP–MS measures element concentration but provides no information on species composition. Thus, the following discussion is based on the assumption that the total platinum content represents compound **1**, its (aquated) metabolites, and its adducts with cellular components.)

Compound **1** rapidly accumulates in NCI-H460 cancer cells with a maximum platinum level reached after an incubation period as short as 3 h (Figure 5a). At this time point, the content of the hybrid agent is approximately 60-fold higher than that determined for cisplatin at the same drug concentration and under the same incubation conditions. The intracellular concentration of compound **1** remains high across the entire incubation period with some decrease noted at the 6-h time point and partial recovery of platinum levels after 12 h of continuous incubation. By contrast, cisplatin accumulates in NCI-H460 cells at a much slower rate than compound **1**, which leads to significantly lower levels of platinum (Figure 5a). A similar situation is observed for the DNA lesions produced by compound **1**, which reach a maximum level after 6 h to then steadily decrease over time to approximately half that value at the 12 h time point. By contrast, cisplatin–DNA adducts have accumulated at a much slower rate at the 1-h time point and drop to control levels after 3 h and 6 h of incubation. At the 12-h time point, the cisplatin content increased to a level approximately 50% of that detected for compound **1** (Figure 5b). Under the specific conditions of this experiment, cisplatin produces 1–5 adducts per 10⁶ DNA nucleotides, a level of modification typically observed in cisplatin-treated cells and in DNA isolated from clinical samples.^{14, 15} Compound **1** produced irreversible damage with DNA at a significantly higher frequency of up to 25 adducts per 10⁶ nucleotides. Likewise, compound **1** showed considerably more efficient DNA binding in experiments in which NCI-H460 cells were incubated with compound **1** and cisplatin for 12 h, but at their respective 90% inhibitory concentrations (IC₉₀ values were 94 nM for compound **1** vs. 11 μ M for cisplatin, determined in 72-h incubations). In these experiments, cisplatin produced less than 3-fold

higher DNA adduct levels than compound **1** despite the more than 100-fold higher incubation concentration used (data not shown).

The accumulation of compound **1** and cisplatin in NCI-H460 cancer cells can be considered the net effect of drug uptake and efflux. Based on the data shown in Figure 5a, compound **1** enters cells at an unusually rapid initial rate. Rapid reaction with cellular DNA and other cellular constituents, as well as inefficient detoxification and efflux during the early stages of treatment may contribute to this effect. It appears that compound **1** and cisplatin enter NCI-H460 cells by distinctly different mechanisms. This can be expected based on the charge status of the two agents: while the clinical agent is a charge-neutral species, the platinum–acridine hybrid agent exists as a dicationic species at physiological pH. For cisplatin-type compounds, electroneutrality is one of the requirements for biological activity as stated by the Cleare–Hoeschele structure–activity rules,¹⁶ suggesting that passive diffusion across the cell membrane is a major pathway of uptake. Indeed, increasing lipophilicity in cisplatin derivatives has been demonstrated to facilitate drug uptake.¹⁷ Clearly, an alternative, active transport mechanism must exist for compound **1** and other classes of rule breakers, such as the cationic polynuclear platinum compounds, in which accumulation of positive charge favors cellular uptake.^{18, 19}

As a DNA-directed chemotherapy, compound **1** shows a clear advantage over cisplatin. At a given time point, the DNA binding levels in Figure 5b reflect the net effect of newly formed adducts and removal of platinum from the damaged DNA by the repair machinery. Several factors may contribute to the greater frequency of DNA lesions observed for compound **1**, especially after short incubation periods. Rapid uptake into the cells to produce high cytosolic levels of electrophile, as well as the DNA-targeted nature of compound **1** can be considered driving forces that enhance the target binding of this agent. As a cationic intercalator, compound **1** has a high intrinsic affinity for DNA and, unlike cisplatin, does not require an aquation step to produce a cationic form to promote electrostatic association with the negatively charged biopolymer.²⁰ Finally, the ability of compound **1** to undergo associative substitution reactions with nucleophilic DNA nitrogen much more rapidly than cisplatin may contribute to the higher DNA binding levels observed for the hybrid agent. The decrease in platinum levels with proceeding incubation time most likely indicates increased DNA damage recognition and slow removal of the damage caused by compound **1**.

3.3 Effect on cell viability

To test if the faster rates of cellular accumulation and DNA binding detected for compound **1** compared with cisplatin have an effect on the hybrid agent's cytotoxicity, NCI-H460 cells were treated with both agents for 1, 3, 6, and 12 h, and changes in cell viability were detected using a colorimetric cell proliferation assay. The results confirm that a higher level of DNA lesions indeed translates into a faster cell kill. At the final 12-h time point, compound **1** was able to reduce the number of viable cells to 37% of the control cells, whereas 74% of the cells remain viable after treatment with cisplatin (Figure 6). After an initial drop in the number of normally proliferating cells following exposure to cisplatin, no major change in viability is observed between the 3-h and 12-h time points. This is an important observation since compound **1** reduces the percentage of viable cells by ~40% during the same incubation period. It seems that cisplatin-treated cells, but not cells exposed to compound **1**, partially recover from the initial cytotoxic effects. This may be due to an early DNA damage response and efficient removal of the DNA cross-links formed by cisplatin. This would be in agreement with the platinum levels in cellular DNA at the 3-h and 6-h time points observed for cisplatin, which are indistinguishable from control levels (Figure 5b).

4. Conclusions

The platinum–acridine agent **1** forms a unique array of DNA adducts, similar to the prototypical analogue, PT-ACRAMTU, but at a faster rate and altered sequence specificity. The ability of compound **1** to rapidly produce high levels of permanent DNA damage, which may be favored due to efficient uptake into cells and the high reactivity with DNA bases, sets the mechanism of the hybrid agent apart from that of the clinical platinum drugs. In this regard it is noteworthy to mention that compound **1** shows a major advantage over cytotoxic non-intercalating monofunctional complexes, such as $[\text{PtCl}(\text{NH}_3)_2(\text{pyridine})]^+$ (“pyriplatin”).²¹ Pyriplatin forms DNA adduct at a slower rate than cisplatin and has proven to be less active in chemoresistant cancers compared with the clinically used platinum drugs cisplatin and oxaliplatin.²² Both the frequency and type of adducts formed by the platinum–acridines seem to be critical for inducing cell death in an aggressive and rapidly proliferating form of cancer, such as NSCLC. Compound **1** appears to be a potent inhibitor of DNA synthesis and transcription,^{5, 6} and its ability to trigger cancer cell kill most likely depends on the ability of the DNA lesions it produces to stall enzymes associated with these processes. Thus, in addition to the frequency of the monofunctional adducts, the ability of these lesions to evade repair and/or prevent replicative by-pass mechanisms may contribute to the enhanced biological activity of this type of agent. These aspects are currently being studied in cell-free systems.

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Fig. 1.
Structures of compounds studied.

Fig. 2.

(a) Reverse-phase HPLC elution profiles (total-ion count) of enzymatic digests of calf thymus DNA treated with compound **1** at $r_b = 0.05$. Unmodified mononucleosides elute at retention times < 4 min (dC elutes with the solvent front). The Pt-acridine-modified DNA fragments elute at 6.05 min (**A1**), 6.57 min (**A2**), 7.01 min (**A3**), and 7.28 min (**A4**). The asterisk indicates unreacted compound **1**. (b) Positive-ion mode electrospray mass spectra of adducts **A1**–**A4** showing peaks for the molecular parent and fragment ions (see Table 1). The adduct profiles recorded for $r_b < 0.05$ were qualitatively similar but showed lesser amounts of unreacted platinum complex and undigested dinucleotide adducts **A3** and **A4** (not shown).

Fig.3.
The structures of adducts **A1–A4** and fragment ions resulting from in-source collisionally activated dissociation.

Fig. 4. Positive-ion mode electrospray mass spectra (m/z from 350 to 460) for **A3** and **A4**. The insert shows the formation of w -fragments.

Fig.5.

(a) Cellular platinum content in NCI-H460 cells treated with 0.1 μM cisplatin or compound **1** for 0, 1, 3, 6, and 12 h. (b) Platinum content in cellular DNA extracted from NCI-H460 cells treated with 0.1 μM cisplatin or compound **1** for 0, 1, 3, 6, and 12 h. The data are presented as the mean $\pm$ standard deviation of 3 independent experiments.

Fig.6.

Viability of NCI-H460 cells after treatment with 0.1 μM cisplatin or compound **1** for 1, 3, 6 and 12 h, monitored by a colorimetric cell proliferation assay. The data shows the mean $\pm$ standard deviation of 6 incubations.

Table 1

Summary of Fragments for Platinum-DNA Adducts.

Adduct	Fragment	m/z	Assignment ^a
A1 , [Pt(LH)(en)dG] ³⁺	[A1-2H] ⁺	827.31	
	[Pt(LH)(en)(guanine)-2H] ⁺ <i>b</i>	711.26	a
	[Pt(en)(dG)(propan-1-imine)-H] ⁺	576.17	b
	[Pt(dG)-H] ⁺	460.12	c
	[A1-H] ²⁺	414.16	
	[Pt(LH)(en)(guanine)-H] ²⁺	356.13	a
	[N ⁴ -(acridin-9-yl)-N ² -methylethane-1,2-diamine+H] ⁺	252.15	d
A2 , [Pt(LH)(en)dGMP] ⁺	[Pt(LH)(en)(guanine)] ³⁺	237.76	a
	[A2] ⁺	907.27	
	[Pt(en)(dGMP)+H] ⁺	601.09	e
	[Pt(LH)(en)-2H] ⁺	560.21	f
	[A2+Na] ²⁺	465.13	
	[A2+H] ²⁺	453.64	
	[Pt(LH)(en)(dG)-H] ²⁺	414.16	g
	[Pt(LH)(en)(guanine)-H] ²⁺	356.13	a
	[Pt(LH)(en)-H] ²⁺	280.61	f
	[A3] ²⁺	570.69	
A3 , [Pt(LH)(en)ApG] ²⁺	[Pt(LH)(en)(dGMP)+H] ²⁺	453.64	h
	[Pt(LH)(en)(dG)-H] ²⁺	414.16	g
	[Pt(LH)(en)(guanine)-H] ²⁺	356.13	a
	[Pt(en)(dGMP)+2H] ²⁺	298.60	i
	[Pt(LH)(en)-H] ²⁺	280.61	f
	[A4] ²⁺	566.18	
A4 , [Pt(LH)(en)TpG] ²⁺	[Pt(LH)(en)(dGMP)+H] ²⁺	453.64	h
	[Pt(LH)(en)(dG)-H] ²⁺	414.16	g

Adduct	Fragment	<i>m/z</i>	Assignment ^a
	[Pt(LH)(en)(guanine)-H] ²⁺	356.13	a

^a See Figure 3.

^b en = ethane-1,2-diamine, LH = *N*-(2-(acridin-9-ylamino)ethyl)-*N*-ethylpropionimidamide, acridinium cation.