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Picazoplatin, an Azide-Containing Platinum(II) Derivative for Target Analysis by Click Chemistry

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Supporting Information

ABSTRACT: Despite the broad use of platinum-based chemotherapeutics, identification of their full range of cellular targets remains a significant challenge. In order to identify, visualize, and isolate cellular targets of Pt(II) complexes, we have modified the chemotherapeutic drug picoplatin with an azide moiety for subsequent click reactivity. The new compound picazoplatin readily binds DNA and RNA oligonucleotides and undergoes facile post-labeling click reactions to alkyne-fluorophore conjugates. Pt-fluorophore click reactions in rRNA purified from drug-treated *Saccharomyces cerevisiae* demonstrate its potential for future *in vivo* efforts.

Platinum (II) compounds comprise up to 50–70% of anticancer treatment regimes in use today.[1] Unlike other classes of common therapeutics, such as antibiotics, however, a molecular-level understanding of the specific targets of Pt(II) drugs is lacking.[2] Pt(II) drugs preferentially target purine nucleobases, and drug binding to genomic DNA is an accepted cause of downstream apoptotic signaling.[3] Less than 10% of Pt (in the case of cisplatin) accumulates within genomic DNA,[4] and questions remain regarding additional targets that could impact cytotoxicity and resistance. A facile and sensitive method for drug target analysis would greatly aid such investigations.

One synthetic-based strategy for target identification involves modifying Pt(II) complexes to contain sterically small bioorthogonally reactive handles. Chemical transformations at this handle (such as reaction with a fluorophore) following drug treatment would allow for accurate Pt localization *in vivo* or subsequent purification of cellular targets.[2c] Post-treatment labeling circumvents problems associated with using compounds modified with bulky or charged fluorophores, which may not accurately represent cellular processing of the native drug.[2d] An ideal candidate for post-treatment labeling is the azide−alkyne dipolar cycloaddition, the prototypical click reaction.[5] Click chemistry has been broadly used for localization and functional studies of modified biomolecules, including proteins and nucleic acids.[6,7] Despite the importance of Pt(II) compounds in therapeutics and catalysis, applications of click reactions on Pt(II) complexes have been limited.[8] Click reactions have been used as a synthetic route to generate libraries of Pt-based therapeutics[8d−f] and for fluorescent postlabeling of a monofunctional Pt(II)-acridine derivative.[9] Our goal is to modify small bifunctional Pt(II) reagents, such as cisplatin and related compounds, (Figure 1) for post-treatment analysis.

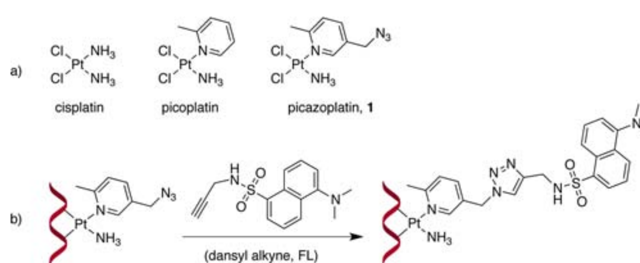

Figure 1. (a) The anticancer drugs cisplatin and picoplatin, with the novel picoplatin analogue **1**, picazoplatin. (b) Reaction scheme of biomolecule-bound picazoplatin clicking to a dansyl fluorophore.

We report the synthesis of the novel azide-functionalized Pt(II) complex **1** and present DNA oligonucleotide binding and subsequent click reactivity with alkyne-modified fluorophores (Figures 2 and 3). We extend these studies to show *in vitro* labeling of a structured RNA oligonucleotide (Figure 4) and *in vivo* treatment and post-labeling of yeast rRNA (Figure 5). This tandem Pt binding and post-modification technique enables visualization and quantification of the cellular targets of Pt(II) compounds and may be broadly applicable toward identification of other metal-ion based drug targets.

We designed complex **1** with the azide-functionalized 2-picoline ligand, similar to the well-known complex picoplatin (Figure 1a).[10] Since Pt(II) is capable of catalyzing hydroamination and other undesirable side reactions with alkynes,[11] the azide-functionalized complex remained the initial focus of this work. In addition, organoazide complexes of Pt, where the azide moieties are connected through organic linkers and not directly bound to the Pt center, are known compounds.[12] Furthermore, an azide-Pt(II) click reagent allows for the future use of nonlinear, ring-strained alkynes and thus copper-free click chemistry.[13] The steric hindrance provided by the 2-methyl group of picoplatin and its derivatives slows the kinetics of substitution reactions on Pt and is an important effector of therapeutic activity.[10,14] The presence of the 2-methyl substituent also prevents the formation of dipicoline adducts

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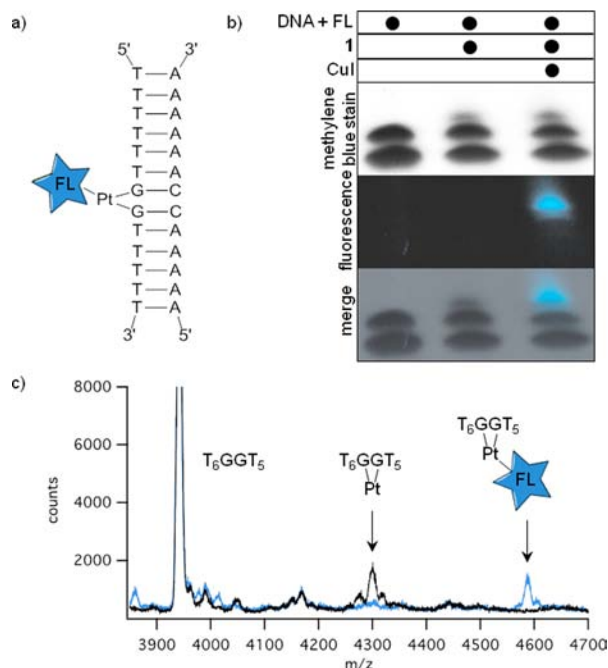

Figure 2. (a) DNA duplex with picazoplatin (**1**) bound and labeled with dansyl alkyne fluorophore. (b) dPAGE analysis showing the denatured complementary strands (left) and higher molecular weight Pt-bound species (center). Click reaction with the fluorophore results in fluorescence of the Pt-DNA band under UV light (right). (c) MALDI mass spectra of Pt-DNA (black) and Pt-DNA-click product (blue) showing the change in mass upon binding **1** and reacting with the fluorophore. Expected m/z of 4301.7 and 4590.8 compared to the observed 4300.03 and 4587.3, respectively. Conditions are outlined in the SI.

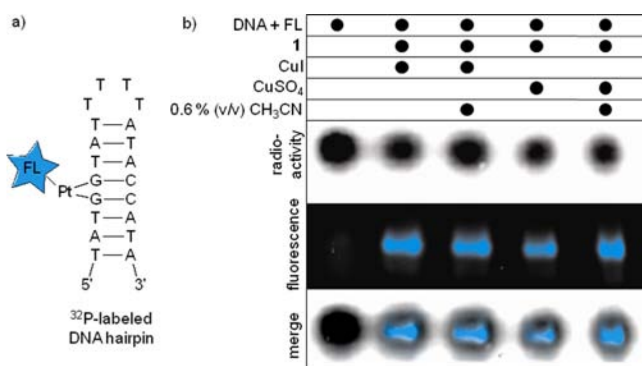

Figure 3. (a) DNA hairpin with **1** bound and labeled with the alkyne fluorophore. (b) dPAGE analysis showing fluorescent labeling of DNA hairpin by Cu-catalyzed click chemistry. Lane 1 contains nonplatinated DNA hairpin and shows no nonspecific DNA–fluorophore interaction. Lanes 2–5 contain platinated DNA under varying click condition sets (SI), all of which show successful DNA–fluorophore labeling.

and generally yields cleaner syntheses compared to other asymmetrically substituted cisplatin derivatives.[15] Complex **1** was synthesized and fully characterized by ^{1}H and ^{195}Pt NMR, IR, and HRMS (see Supporting Information, SI).

Cisplatin, picoplatin, and similar Pt compounds bind readily to 5′-GG-3′-containing short DNA oligonucleotides and hairpin structures.[3a,c,16] Binding of **1** to double-stranded DNA and click labeling were analyzed by dPAGE (Figure 2). Resolution between the denatured strands is clearly observed,

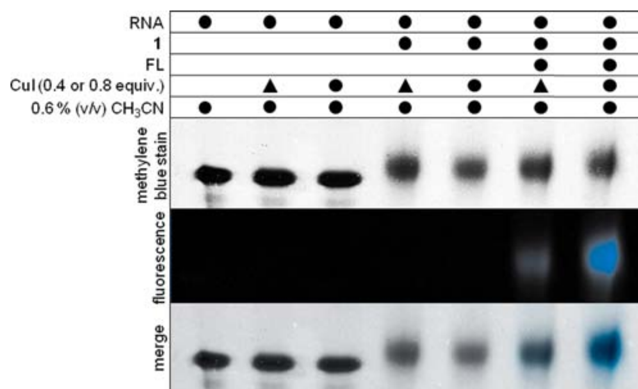

Figure 4. Fluorescent labeling of Pt-bound RNA SRL hairpin by click chemistry. Lanes 1–3: nonplatinated RNA is stable in click reaction conditions with 0.4 (triangle) or 0.8 equiv (circle) CuI. Lanes 4–7: SRL RNA treated with 4 equiv **1** shows increasing yields of fluorophore labeling following click reactions with 0.4 (lane 6) and 0.8 (lane 7) equiv CuI and the dansyl alkyne. Conditions are given in the SI.

and reaction with 2-fold excess **1** (4 h, 37 °C) results in a new band corresponding to Pt-bound T_6GGT_5 (Figure 1b, left and center lanes) in yields similar to those observed with the parent compound picoplatin (not shown). Reaction of the Pt-bound oligonucleotide with an alkyne fluorophore (dansyl propargylamine)[17] in the presence of CuI (4 h, 50 °C) results in fluorescent labeling of the Pt-bound species (Figure 2b, right lane). Observation of the expected click product with a single-stranded T_6GGT_5 construct was confirmed by MALDI mass spectrometry (Figure 2c).[18] The observed Pt-bound species (Figure 2c, black) shifts to a higher m/z as predicted from cycloaddition of the dansyl fluorophore (Figure 2c, blue). The click reaction efficiency appears to be high, as the Pt-bound peak disappears completely in the clicked (blue) spectrum.

Additional work examining nonspecific interactions between the dansyl alkyne fluorophore and the terminal azide of the Pt-bound DNA scaffold was performed with a pyrimidine-rich DNA hairpin (Figure 3a).[16] This sequence was selected to minimize potential Pt interstrand cross-link formation between hairpins (as was occasionally observed for single-stranded T_6GGT_5, data not shown). A Pt-bound species is not resolved under these gel conditions, but fluorescence detection of the subsequent click reaction clearly identifies a platinated species. No nonspecific fluorescent labeling is observed under Cu-free conditions (Figure 3, lane 1) following the reaction of the hairpin with 2-fold excess **1** (18 h, 37 °C). Proficient fluorescent labeling of the **1**-bound GG DNA hairpin is observed with either CuI-catalyzed (Figure 3, lanes 2–3) or $CuSO_4$-catalyzed click protocols (Figure 3, lanes 4–5) following incubation at 50 °C for 1 h.

The presence of Pt(II) lesions in cellular RNAs and their potential downstream consequences has become an increasingly important consideration given the many regulatory roles of RNA in essential cell processes.[19] Recent studies in our laboratory have demonstrated that in *Saccharomyces cerevisiae*, cisplatin-derived Pt species accumulate to a significantly greater extent in RNA vs DNA, with rRNA acting as a *de facto* cellular Pt sponge.[19a] In context of *Escherichia coli*[20] and *S. cerevisiae*[19a] rRNA, specific Pt binding sites have been identified within the small ribosomal subunit. Pt adducts are also observed in the sarcin-ricin loop (SRL), a universally conserved exogenous loop

essential for elongation factor binding and GTP-dependent translocation (unpublished data).[21] To establish potential for using Pt-click reagents on cellular RNA, we investigated the interaction and click reactivity of **1** on a model SRL RNA construct (Figure 4).

The sensitivity of unmodified (i.e., 2′-OH) RNA species toward degradation by Cu-mediated chemistry has previously limited studies of click reactions with RNA sequences like the SRL in the absence of protective ligands, such as TBTA or under O_2-free conditions.[22] We have independently observed significant degradation of our SRL RNA construct under various Cu-catalyzed click conditions (not shown). Recent work has demonstrated that trace amounts of acetonitrile stabilize free Cu(I) in aqueous solution while still facilitating efficient cycloaddition catalysis.[22] Here, we demonstrate substantial click product formation from picazoplatin-bound RNA in the presence of 0.6% v/v acetonitrile (Figures 4 and S7). Lanes 1−3 of Figure 4 show that the SRL RNA is stable in up to 0.8 mol equiv of Cu(I). In lanes 4−7, a supershift demonstrates Pt-adduct formation following RNA treatment with 4-fold excess **1** (18 h, 37 °C). Notably, lanes 6 and 7 demonstrate efficient Cu(I)-catalyzed click labeling to dansyl alkyne fluorophore (1 h, 50 °C). Thus, post-treatment labeling by click chemistry is also successful for the analysis of RNA-Pt adducts.

To assess the feasibility of identifying cellular picazoplatin-bound species, we extracted and labeled rRNA from **1**-treated *S. cerevisiae* with Alexa Fluor 488 DIBO alkyne (Figure 5). Lane 1 contains a 200 nucleotide RNA marker. Lanes 2−4 contain 5.8S rRNA isolated from cells treated for 6 h with 0 (lane 2), 250 or 500 μM picazoplatin (lanes 3,4) and reacted with an excess of alkyne fluorophore (18 h, 37 °C). Lane 2 indicates the absence of nonspecific labeling. Lanes 3 and 4 clearly demonstrate increasing picazoplatin accumulation on 5.8S rRNA *in vivo* and successful alkyne fluorophore labeling to an intact azide. These studies show the potential for analysis of Pt(II) drug targets and/or fluorescent imaging of Pt trafficking *in vivo* in fixed and permeabilized cells.

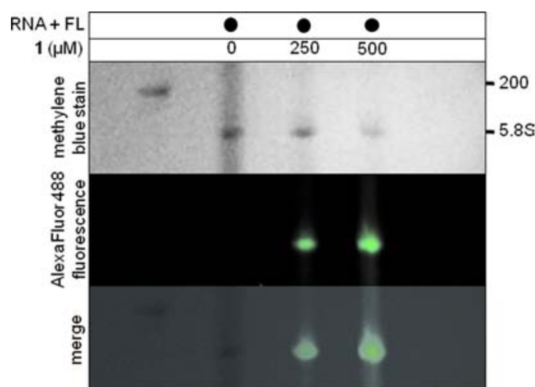

Figure 5. Fluorescent labeling by click chemistry of rRNA from **1**-treated *S. cerevisiae*. Lane 1 contains a 200 nucleotide RNA marker. Lanes 2−4 contain 5.8S rRNA (∼160 nucleotides) extracted from *S. cerevisiae* treated with 0, 250, or 500 μM picazoplatin and clicked to an excess of Alexa Fluor 488 DIBO alkyne (18 h, 37 °C). Proficient fluorescent labeling of the 5.8S rRNA is observed from *in vivo* **1**-treated samples. Conditions are given in the SI.

In summary, we report the successful synthesis of picazoplatin, an azide-containing picoplatin derivative for Pt-bound drug target analysis by click chemistry. Picazoplatin readily binds DNA oligonucleotides and undergoes high-yielding click reactions with alkyne fluorophores. Moreover, successful fluorophore labeling via acetonitrile-assisted click reactions was demonstrated on an RNA mimic of the ribosomal sarcin-ricin loop construct, demonstrating viability of this approach in the presence of unmodified (i.e., 2′-OH intact) RNA. Preliminary *in vivo* applications of this technique identified the 5.8S rRNA as a cellular Pt(II) drug target.

Future applications of Pt-click reactions could include the isolation, purification, and identification of *in vivo* targets of Pt(II) therapeutics using various alkyne-functionalized scaffolds. Reversal of Pt covalent linkages could be achieved by saturation with thiourea,[18a] releasing Pt-bound constituents for analysis via high-throughput sequencing, proteomics, or other analytical methods.[2c] The ability of Pt(II) compounds to cross-link RNA and RNA−protein complexes[23] suggests additional applications in RNA and ribonucleoproteins structure−function analyses. Such efforts toward using Pt-mediated click chemistry to isolate and quantify Pt-bound species are currently underway in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, syntheses, and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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