

Supplementary Information

A ruthenium-platinum metal complex that binds to Sarcin Ricin Loop RNA and lowers mRNA expression

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Metal Complex	Molecular Weight	Color	pH	Solubility
Cisplatin	300.01 g/mol	Pale Yellow	4.05	2.53 mg/mL
IT127	784.0 g/mol	Dark Yellow	3.42	>63 mg/mL
NAMI-A	458.18 g/mol	Dark Orange	4.07	>29 mg/mL

Table S1: Chemical and physical properties of Cisplatin, IT127, and NAMI-A. The pH was tested over the course of four hours by dissolving the metal complexes in deionized water. All three solutions (Cisplatin, NAMI-A, IT127) became slightly more acidic as time progressed. The pH of Cisplatin started at 4.05 (0 hour) and decreased to 3.97 (4 hours). The pH of IT127 started at 3.42 and decreased to 3.21. The pH of NAMI-A started at 4.07 and decreased to 3.84. The pH data is not of significant relevance because in our work, all experiments were done in buffered solutions. According to the Merck Index, Cisplatin has a solubility of 2.53 g/mL which translates into an 8 mM solution. In our experiments, we made readily soluble IT127 at a concentration of 63 mg/mL (80 mM) and NAMI-A at a concentration of 29 mg/mL (63 mM). IT127 has the highest solubility of the three complexes due to the presence of the specific functional groups. The synthesis, X-ray structure, and characterization of IT127 were published in 2012 (Anderson et al, Inorganic Chemistry). This article has been referenced in the current manuscript. Additional chemical information for IT127 can be found in this citation.

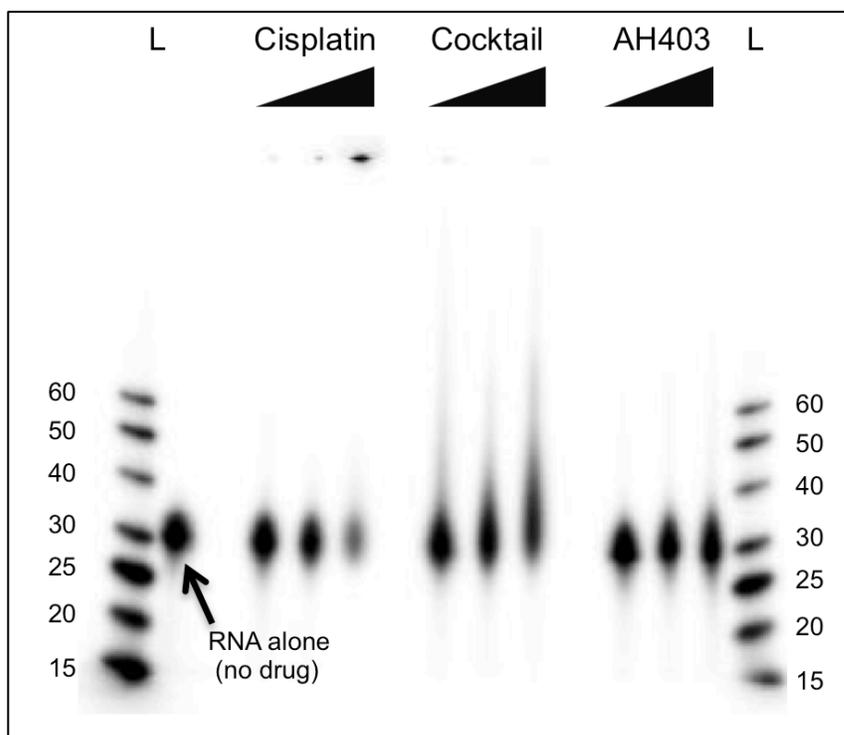


Figure S1: Electrophoretic mobility shift assay of Cisplatin, AH403, and a cocktail combination of Cisplatin + AH403 bound to the 29-base SRL RNA. RNA was incubated with metal complexes at 250, 500, and 1000 μM concentrations prior to gel electrophoresis. In the case of the cocktail, a 250 μM concentration, for example, is 250 μM in both Cisplatin and AH403 for a total metal concentration of 500 μM . Lanes marked L contain a single-stranded ladder with the size (in bases) indicated next to each band. As described in the Main Text, IT127, which contains both a Pt(II) and a Ru(III) metal center, has shown a markedly greater effect on the mobility of SRL RNA than either Cisplatin, a mononuclear Pt(II) complex, or AH403, a mononuclear Ru(III) complex. Cisplatin alone has a slight effect on mobility in comparison to IT127 (indicated by retention of RNA in the well and fainter RNA bands at higher drug concentrations) and AH403 alone has a minimal effect (displayed in slight band streaking), as we have previously shown in Figure 1 (Main Text). We have now carried out a Cisplatin+AH403 cocktail experiment in which, even at the 1000 μM concentration, the effect shown by the Cisplatin+AH403 combination is nominal in comparison to the IT127 effect displayed in Figure 1, main text file. Thus the presence of two transition metals in the dinuclear transition metal IT127 complex appears to have a synergistic rather than additive effect.

Effect of IT127 on purified DHFR enzyme activity

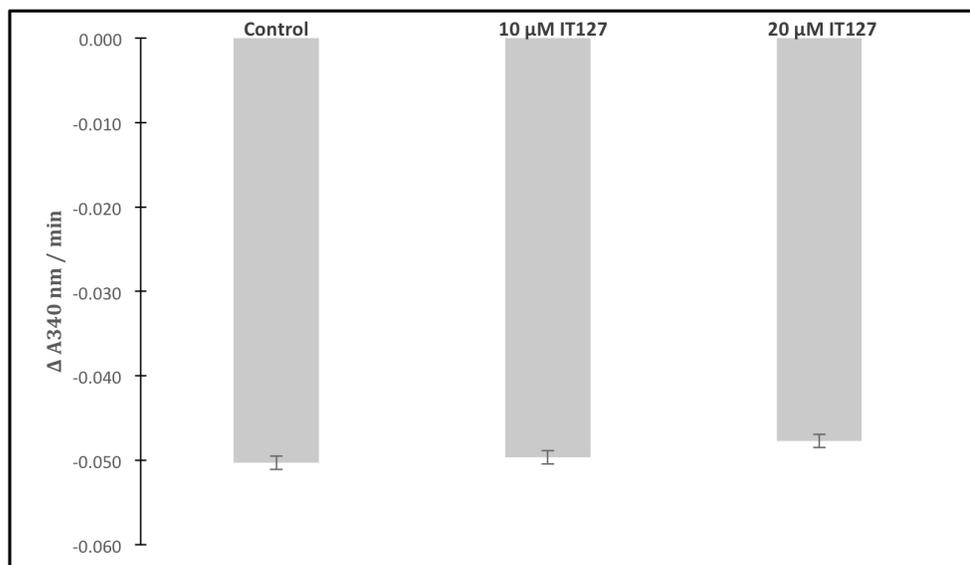


Figure S2: Translation inhibition experiments were carried out by incubating DHFR mRNA with metal complexes. Drug-bound mRNA was column purified to remove any unbound drug so as to avoid interactions with the translated DHFR enzyme present downstream. However, if some of the unbound drug were to remain in solution after purification; the interaction of the drug could reduce DHFR enzyme activity. To address this concern, an activity assay similar to Figure 3 in the main text was carried out. IT127 at 10 μM and 20 μM concentrations was incubated with pure DHFR enzyme. The rationale for using these concentrations of IT127 is as follows: The concentration of IT127 would be in this range in the DHFR assay, after accounting for dilutions in subsequent steps, if the purification was not carried out (false) or had somehow failed completely (highly unlikely). Our results show that we observe a 5% decline in DHFR activity at 20 μM IT127 and negligible loss in activity at 10 μM IT127 whereas the decline in enzyme activity when IT127 was incubated with DHFR mRNA is greater than 90%. Even if free IT127 was present in the solution after purification, its effect on the activity of the enzyme is negligible compared to the significantly greater effect on the translation of the enzyme by the drug-bound mRNA.

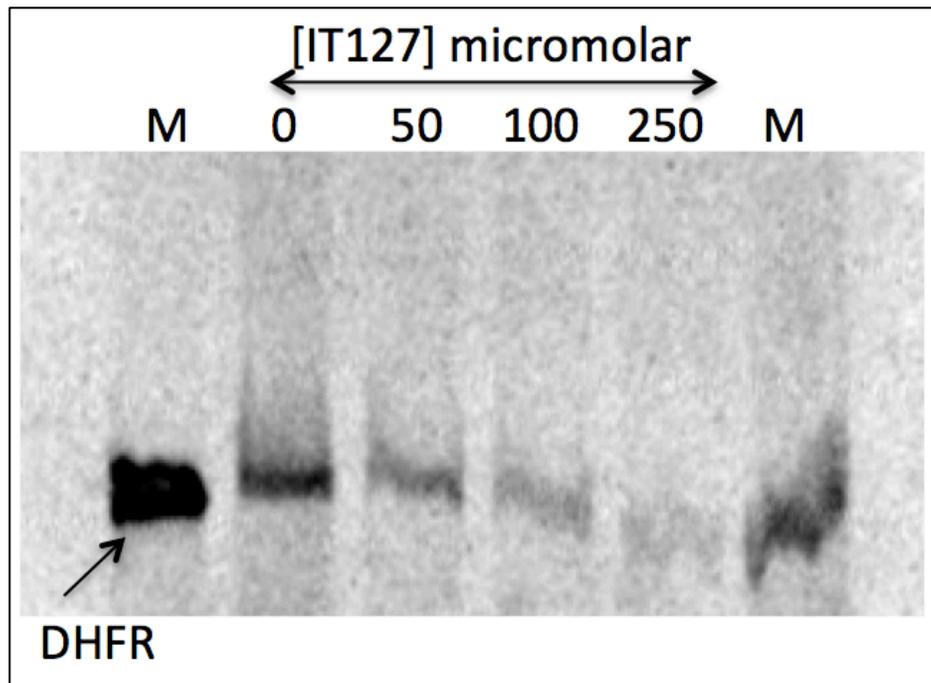


Figure S3: SDS-PAGE gel (12% acrylamide) of S-35 labeled dihydrofolate reductase enzyme. Briefly, 350 picomoles of DHFR mRNA was incubated with varying concentrations of IT127 and subsequently column purified to remove unbound IT127. Bound mRNA was used as a template to carry out protein translation using an *in vivo* translation kit (New England BioLabs). Radioactively-labeled methionine (S-35) was supplemented in the translation mixture. Lane marked as M contains the standard DHFR enzyme (25 kDa). Trials with IT127 show a reduction in the amount of translated DHFR enzyme as a function of increasing IT127 concentration. This indicates that the binding of IT127 to DHFR mRNA leads to a lower amount of DHFR enzyme production, which is likely due to the inhibition of translation initiation process.

EXPERIMENTAL DETAILS

Metal Complexes

Cisplatin (CDDP; cis-diammine-dichloro-platinum) was purchased from Sigma-Aldrich (Cat No. P4394) and used without further purification. The ruthenium (III) complex NAMI-A was prepared according to previously published protocols.¹ IT127 and AH403 were prepared as described respectively by Anderson et. al.(2012) and Iengo et. al. (1999).^{2,3} Stock solutions of each metal complex were prepared in deionized water immediately prior to use.

Nucleic Acid Sequences

Mobility shift and reverse transcription experiments were carried out using a 29-nucleotide RNA oligomer (shown below) representing the entire sequence of the Sarcin Ricin Loop from rat 28S rRNA. SRL RNA was transcribed using T7 RNA Polymerase from a 50-base synthetic DNA template and corresponding 46-base promoter strand containing a T7 promoter sequence (IDT). Synthesized RNA was purified using gel electrophoresis and stored at – 20°C until further use.

Promoter Strand:

5'-AATTTAATACGACTCACTATAGGGTGCTCAGTACGAGAGGAACCGCACCC-3'

Template Strand:

3'-ATTATGCTGAGTGATATCCCACGAGTCATGCTCTCCTTGGCGTGGG-5'

SRL RNA Sequence:

5'-GGGUGCUCAGUACGAGAGGAACCGCACCC-3'

RNA Mobility Shift Assay

Purified RNA was dephosphorylated at the 5'-end using Antarctic Phosphatase enzyme (NEB). Subsequent to the removal of 5' phosphate, RNA was labeled at the 5'-end using ATP γ ³²P (Perkin Elmer) and T4 polynucleotide kinase (NEB). Radiolabeled RNA was incubated in 10 mM phosphate buffer (pH 7.0) at a final concentration of 40 μ M for 2 hours at 37°C in the presence of varying concentrations of Cisplatin, AH403, and IT127 (0, 50, 100, 250, 500 μ M). RNA

samples were resolved on 15% denaturing polyacrylamide gels containing 8 M urea. After electrophoresis, gels were exposed overnight to a Phosphor screen and visualized using STORM 825 scanner (GE Healthcare) equipped with ImageQuant TL software.

RNA Site Specificity using Reverse Transcription

A deprotected synthetic 10-mer DNA primer (3'-TTGGCGTGGG-5') purchased from Integrated DNA Technologies was radiolabeled using ATP γ 32P (Perkin Elmer) and T4 polynucleotide kinase (NEB). Purified, label free SRL RNA was incubated in 10 mM phosphate buffer (pH 7.0) at a final concentration of 20 μ M for 4 hours at 37°C with varying concentrations of Cisplatin and IT127 (0, 50, 100 μ M). Labeled DNA primer was annealed in a 1:7 ratio (50:350 picomoles) to the RNA-drug adduct template by heating to 70°C for 90 seconds and cooling to room temperature over 25 min. AMV reverse transcription kit (Promega) was used to generate cDNA strands by extending the DNA primer on the RNA template. Reverse transcription reactions were run for 3 hours at 42°C. Full extension of this primer yields a product that is 29 bases in length. The extended cDNA strands were precipitated using sodium acetate and cold ethanol. Approximately 1 μ L of 1 mg/ μ L yeast RNA (Ambion) was added to the samples to enhance cDNA isolation during ethanol precipitation. The pellet was washed twice with 70% ethanol and resuspended in 10 μ L of diH₂O and 10 μ L of loading buffer (Promega). Samples were resolved on 15% sequencing denaturing polyacrylamide gels (0.4 mm thickness) containing 8M urea. After sequencing gel electrophoresis, gels were dried and exposed to Phosphor Screen and visualized using STORM 825 scanner (GE Healthcare) equipped with ImageQuant TL software. Using ImageQuant TL software, band intensity was calculated (with background subtraction).

Translation Inhibition

A single colony of *Escherichia coli* containing a plasmid that overexpresses DHFR was inoculated in LB medium overnight. Plasmid extraction and purification was performed using the QIAGEN Plasmid Midi Kit. Restriction digestion of isolated

DHFR DNA was conducted using the Hind-III High-Fidelity (HF) Restriction Endonuclease from New England Biolabs. Cut and linearized DHFR DNA was characterized by a 1.3% agarose gel before being transcribed by T7 RNA Polymerase (manufactured in house). Synthesized RNA (3000 μ M in RNA base) was then incubated with metal complexes (Cisplatin, NAMI-A, IT127, and a cocktail mixture of Cisplatin + NAMI-A) at 500 μ M in nuclease-free phosphate buffered saline (10 mM, pH 7.0) for 2 hours at 37°C. Drug-bound RNA was purified from unbound drug using Illustra MicroSpin G-50 columns (GE Healthcare Bio-Sciences). After column purification, drug-bound DHFR mRNA was used as a template to carry out protein translation using a New England BioLabs *PURExpress In Vitro* Protein Synthesis Kit. Following translation, the activity of synthesized DHFR enzyme was determined using the Sigma Aldrich Dihydrofolate Reductase Assay Kit and a Thermo Scientific NanoDrop One UV/Vis Spectrophotometer with a customized kinetics program.

References

1. Mestroni, G., Alessio, E., and Sava, G. New salts of anionic complexes of Ru(III), as antimetastatic and antineoplastic agents, World Intellectual Property Organization, I. B., 1998, WO 98/00431.
2. C. M. Anderson, I. R. Taylor, M. F. Tibbetts, J. Philpott, Y. Hu, J. M. Tanski, *Inorganic Chemistry* 2012, 51, 12917–12924.
3. E. Iengo, G. Mestroni, S. Geremia, M. Calligaris, E. Alessio, *J. Chem. Soc., Dalton Trans.* 1999, 3361–3371.