Video Article

An *In Vitro* Enzymatic Assay to Measure Transcription Inhibition by Gallium(III) and H$_3$ 5,10,15-tris(pentafluorophenyl)corroles

Grace Y. Tang[1], Melanie A. Pribisko[1], Ryan K. Henning[1], Punnajit Lim[2], John Termini[2], Harry B. Gray[1], Robert H. Grubbs[1]

[1]Division of Chemistry and Chemical Engineering, California Institute of Technology

[2]Department of Molecular Medicine, Beckman Research Institute of the City of Hope

Correspondence to: Robert H. Grubbs at rhg@caltech.edu

Abstract

Chemotherapy often involves broad-spectrum cytotoxic agents with many side effects and limited targeting. Corroles are a class of tetrapyrrolic macrocycles that exhibit differential cytostatic and cytotoxic properties in specific cell lines, depending on the identities of the chelated metal and functional groups. The unique behavior of functionalized corroles towards specific cell lines introduces the possibility of targeted chemotherapy.

Many anticancer drugs are evaluated by their ability to inhibit RNA transcription. Here we present a step-by-step protocol for RNA transcription in the presence of known and potential inhibitors. The evaluation of the RNA products of the transcription reaction by gel electrophoresis and UV-Vis spectroscopy provides information on inhibitive properties of potential anticancer drug candidates and, with modifications to the assay, more about their mechanism of action.

Little is known about the molecular mechanism of action of corrole cytotoxicity. In this experiment, we consider two corrole compounds: gallium(III) 5,10,15-(tris)pentafluorophenylcorrole (Ga(tpfc)) and freebase analogue 5,10,15-(tris)pentafluorophenylcorrole (tpfc). An RNA transcription assay was used to examine the inhibitive properties of the corroles. Five transcription reactions were prepared: DNA treated with Actinomycin D, triptolide, Ga(tpfc), tpfc at a [complex]:[template DNA base] ratio of 0.01, respectively, and an untreated control.

The transcription reactions were analyzed after 4 hr using agarose gel electrophoresis and UV-Vis spectroscopy. There is clear inhibition by Ga(tpfc), Actinomycin D, and triptolide.

This RNA transcription assay can be modified to provide more mechanistic detail by varying the concentrations of the anticancer complex, DNA, or polymerase enzyme, or by incubating the DNA or polymerase with the complexes prior to RNA transcription; these modifications would differentiate between an inhibition mechanism involving the DNA or the enzyme. Adding the complex after RNA transcription can be used to test whether the complexes degrade or hydrolyze the RNA. This assay can also be used to study additional anticancer candidates.

Video Link

The video component of this article can be found at https://www.jove.com/video/52355/

Introduction

Chemotherapy often involves broad-spectrum cytotoxic agents with undesired side effects and limited targeting, yet with greater understanding of cancer biology, there is an ever increasing demand for anticancer agents with higher cancer-targeting efficacy and fewer side effects.[1] Human cancer cells frequently become dependent on a single activated or overexpressed oncogene for survival.[2] Thus, many anticancer drugs are evaluated by their ability to inhibit RNA transcription. Treatments that block the expression of these transforming genes are effective in eliminating cancer cells and lead to cell death.[3] Transformed cells are more sensitive to disruptions in RNA transcription than are corresponding normal cells.[4] Anticancer drugs that inhibit transcription are expected to selectively inhibit the expression of the oncogenes which are necessary for the cancer cell to survive.[5] Consequently, RNA transcription inhibition is a useful way to identify potential anticancer drug candidates and learn more about their mechanism of action. This protocol demonstrates that Ga(tpfc) inhibits RNA transcription on the same order as the chemotherapy drugs Actinomycin D and triptolide; similar comparisons can be made using this protocol with other anticancer drug candidates. Actinomycin D is a RNA transcription inhibitor commonly used to treat gestational trophoblastic cancer, testicular cancer, Wilm's tumor, rhabdomyosacoma, and Ewing's sarcoma[6]. Actinomycin D has been used in cancer therapy for nearly fifty years since it was first approved by the FDA in 1964.Triptolide is a selective transcription inhibitor that has been investigated *in vitro* and in various tumor-bearing animal models for 30 years.[7]

The amphiphilic macrocyclic nature of corroles imparts significant advantages over other drug classes such as small molecules or biologics.[8-14] The macrocyclic character allows for cellular permeability that is greater than expected for such large molecules, and they are large enough to interact with macromolecular surfaces, such as those of proteins.[8] Corroles are known to form tight noncovalent complexes with biomolecules

and drugs.[10] In addition to the inherent cytotoxicity of the corrole framework, we have demonstrated that a sulfonated corrole acts as a carrier molecule for chemotherapeutic agents, specifically the DNA-intercalating anthracycline drug doxorubicin. When the sulfonated corrole was coadministered with doxorubicin, a 3-fold enhancement in the IC_{50} of doxorubicin was observed for DU-145 cells.[9] The corrole framework is stable and has inherent absorbance and fluorescence properties that, when functionalized, undergo unique absorbance shifts that can be used for characterization.[10] Functionalization of the scaffold does not inherently affect the photophysical properties of the corrole,[9-15] but, as seen with a sulfonated corrole, selectively modifying the framework of the corrole can substantially change its biological properties.[16] We previously evaluated six metallocorroles against seven human cancer cell lines. The results indicate that toxicity toward human cancer cells is dependent on the specific metal ion, as well as functional group substitution. For instance, sulfonated gallium corroles experienced high cellular uptake and penetrated selectively into the nucleus of brain metastatic prostate cancer cells (DU-145); the same corrole, though it does not penetrate into the nucleus of other cell lines, exhibits greater cytotoxicity for breast (MDA-MB-231), melanoma (SK-MEL-28), and ovarian (OVCAR-3) cancer cells than for prostate cancer.[9]

Initial cell-based assays indicate that these compounds show promise as anti-cancer therapeutic agents, which merits further investigation into the mechanism of action. Transcription inhibition is observed with certain organometallic complexes[17-27] and we sought to examine this process as a possible mechanism for the cytotoxic behavior of the corrole family. This transcription assay provides a straightforward, inexpensive, and facile method for assessing transcription inhibition, which will lead to more detailed information about the effects of these molecules in live cells.

Here, the transcription inhibition of gallium(III) 5,10,15-(tris)pentafluorophenylcorrole (Ga(tpfc)) and its freebase analogue 5,10,15-(tris)pentafluorophenylcorrole (tpfc) (**Figure 1**) are tested. Unlike some transition metal complexes, gallium(III) is redox inactive and therefore is not directly involved in the redox process of redox-based metabolic pathways.[28] Regardless, gallium(III) does exhibit cytotoxic properties and has been investigated for therapeutic purposes. Gallium is the second most promising metal for anticancer therapeutics after platinum and has undergone many studies and investigations; nitrate and chloride gallium salts have been evaluated in clinical trials against hepatoma, lymphoma, bladder cancers, and other diseases.[29-34] Gallium(III) is therefore ideal for anticancer corrole studies. Initial data show Ga(tpfc) and tpfc have low GI_{50}, the drug concentration necessary to inhibit 50% of maximal cell proliferation, with various cancer cell lines (see **Figure 2**); this affirms the validity of further experiments on these two compounds to determine their inhibitive properties. We compare these compounds with the common anticancer drugs Actinomycin D and triptolide. Actinomycin D intercalates DNA, inhibits RNA elongation, and induces apoptosis in certain cell line at picomolar concentrations.[6,35-37] Triptolide has shown to inhibit tumor growth; it binds to human XPB/ERCC3, a subunit of transcription factor TFIIH, leading to inhibition of RNA polymerase II activity.[6-7,38-40]

While it is commonly known that corroles exhibit cytotoxic properties, there exists little information about the different mechanisms arising from functionalization. Corrole inhibition of RNA transcription would offer greater insight on their interactions with biomacromolecules. Other complexes known to bind to DNA, such as dirhodium(II,II) complexes, chromium(III) complexes, ruthenium(II) polypyridyl complexes, rhodium(III) complexes, and various others, were subjected to RNA transcription assays,[18-27] resulting in greater understanding of their interactions with biomacromolecules. This facile and widely available experiment is also a good initial test to assess the cytotoxicity properties of a given molecule and determine whether it merits further biological testing. The RNA transcription assay also allows for many modifications, such as varying the quantity of compound or enzymes used; varying the incubation period, reaction time and sample time points; and varying the DNA template length and sequence, among other variables of interest, thus potentially providing a large amount of data. This transcription assay is also readily available as affordable kits with all necessary reaction components provided, although components can be bought and prepared individually. In these experiments, we use a commercially available kit known to have high yield.[41]

To assess transcription inhibition, we use two methods: agarose gel electrophoresis and UV-Vis spectroscopy. Agarose gel electrophoresis is a simple and effective method for separating, identifying, and purifying 0.5- to 25-kb DNA and RNA fragments.[42] UV-Vis spectroscopy can be used to determine the concentration and purity of RNA.[43]

Protocol

NOTE: When working with RNA maintain a clean working environment to avoid contamination by DNase and RNase enzymes that degrade DNA and RNA. Ensure that pipette tips and tubes are DNase and RNase free. It is also helpful to wipe down lab surfaces and equipment such as pipettes, tube holders, etc. with a decontamination solution.

1. RNA Transcription with Corrole Treatment

1. Prepare the corrole and inhibitor compounds in a 0.01:1 molar ratio of [complex]:[DNA].
   NOTE: In our case, the ratio was 4.3 fmol complex: 0.43 pmol DNA, where the DNA template used was apET-28c vector with ligA gene from *E. coli* under control of the T7 promoter. Other DNA with the T7 promoter are also valid candidates for running the transcription assay.
   1. Dissolve Actinomycin D, triptolide, tpfc, and Ga(tpfc) in dimethyl sulfoxide (DMSO) in clean, separate containers. Obtain the final concentration of a 0.01:1 molar ratio of [complex]:[DNA] by using serial dilutions with nuclease-free water.
      1. Dissolve 1 mg Actinomycin D in 1.8 ml DMSO. Aliquot 10 µl to a separate container and dilute to 1 ml with nuclease-free water to create a 1/100 dilution. Aliquot 1 µl of the dilution to a separate container and dilute to 1 ml with nuclease-free water to create a 1/1,000 dilution.
         NOTE: The concentration of the final solution of Actinomycin D is 4.3 fmol.
      2. Dissolve 1 mg triptolide in 0.64 ml DMSO. Aliquot 1 µl to a separate container and dilute to 1 ml with nuclease-free water to create a 1/1,000 dilution. Aliquot 1 µl of the dilution to a separate container and dilute to 1 ml with nuclease-free water to create a second 1/1,000 dilution.
         NOTE: The concentration of the final solution of triptolide is 4.3 fmol.
      3. Dissolve 1 mg tpfc in 2.9 ml DMSO. Aliquot 10 µl to a separate container and dilute to 1 ml with nuclease-free water to create a 1/100 dilution. Aliquot 1 µl of the dilution to a separate container and dilute to 1 ml with nuclease-free water to create a 1/1,000 dilution.
         NOTE: The concentration of the final solution of tpfc is 4.3 fmol.

4. Dissolve 1 mg Ga(tpfc) in 2.6 ml DMSO. Aliquot 10 µl to a separate container and dilute to 1 ml with nuclease-free water to create a 1/100 dilution. Aliquot 1 µl of the dilution to a separate container and dilute to 1 ml with nuclease-free water to create a 1/1,000 dilution.
NOTE: The concentration of the final solution of Ga(tpfc) is 4.3 fmol.
NOTE: These corroles and inhibitors are not soluble in water and must be pre-dissolved in DMSO. Small amounts of DMSO (below 1%) do not induce cytotoxicity in the cells (unpublished data).

2. Prior to beginning the transcription reaction, prepare the necessary reagents individually or purchase them from a commercial vendor.
   1. Thaw on ice all frozen reagents (refer to **Table 1** for a list of frozen reagents).
   2. Allow the 10x Reaction Buffer and the 4 ribonucleotide (ATP, CTP, GTP, and UTP) solutions time to mix until they are completely dissolved in solution.
   3. Briefly centrifuge all reagents to prevent loss of material on the rim of the tube. Once thawed, store ribonucleotides on ice while keeping the 10x Reaction Buffer at RT.
   4. Combine reagents for transcription reaction at RT (refer to **Table 2** for a list of reagents).
   NOTE: The spermidine in the 10x Reaction Buffer can coprecipitate the template DNA if the reaction is assembled on ice rather than at RT.
   5. Mix thoroughly by flicking the tube or pipetting the mixture up and down gently. After mixing, centrifuge briefly to collect reaction mixture at the bottom of the tube.
   6. Incubate the reaction mixture at 37 °C for a total of 2-4 hr.
   NOTE: The necessary reaction times will vary with DNA template size and sequence. This step may require optimization for specific sequences. Shorter templates may require longer reaction times for a high yield of total RNA.
   7. Remove 4 µl aliquots from each reaction after each hour and store at -20 °C. Modify as needed for desired timepoints.

2. RNA Spin Column

1. Following the transcription reaction, purify the RNA using microcentrifuge-compatible chromatography columns.[44]
   NOTE: Use of RNA spin columns to purify RNA with greater than 20 bases results in greater than 80% recovery.[44]
   NOTE: Column chromatography is a common and convenient method to purify RNA. Other purification methods also exist such as Lithium Chloride precipitation, phenol:chloroform extraction, isopropanol precipitation, as well as other commercially available kits.
   1. Evenly resuspend the Sephadex matrix in the column buffer by vigorously inverting the column three or more times. Do this by flicking the column sharply.
   2. Remove the top cap from the column. There will be some residual Sephadex matrix in the cap; if the cap is filled with the matrix, replace the cap onto the column and repeat the previous step until the majority of the matrix is in the column.
   3. Snap off the bottom tip of the column.
   NOTE: Some liquid may escape from the column.
   4. Pack the column.
      1. Place column in a sterile (RNase and DNase free) 1.5 ml microcentrifuge tube.
      2. Centrifuge the tube in a tabletop centrifuge at 1,000 x g for 1 min.
   5. Discard the 1.5 ml microcentrifuge tube with eluted buffer from the column.
   6. Immediately place the column in a new sterile 1.5 ml microcentrifuge tube and pipette the sample to the center of the column bed. Use 20-50 µl of sample to avoid overloading the column.
   7. Centrifuge the tube in a tabletop centrifuge at 1,000 x g for 1 min.
   NOTE: The eluent is the purified RNA sample.

3. Agarose Gel Electrophoresis (1% Agarose Gel) [42]

NOTE: Ethidium bromide fluoresces upon binding to DNA and RNA, therefore these biomolecules can be visualized with UV light by incubating them with 0.5 µg/ml ethidium bromide solution.

1. Prepare a 1,000x stock solution of ethidium bromide (0.5 mg/ml) by dissolving 5 mg ethidium bromide in 10 ml of water.
2. Prepare Tris Acetate EDTA (TAE) running buffer for gel electrophoresis. To make a 50x TAE buffer combine 2.0 M Tris-acetate with 0.05 M Ethylenediaminetetraacetic acid (EDTA) and adjust the pH to 8.5.
3. Prepare a 1% (weight/volume) agarose gel by dissolving 10 g UltraPure Agarose in 1 L 1x TAE buffer and melting the agarose with a conventional microwave oven. Once it has cooled to 50 °C, add 1 ml of 1,000x ethidium bromide to the 1% agarose gel solution, mix by gently swirling or by inverting in a closed container, then pour the agarose solution into a gel-casting platform and allow the gel to solidify at RT.
   NOTE: Ethidium bromide is a mutagen and potential carcinogen. Wear gloves and handle solutions carefully. For proper handling procedures of ethidium bromide, please see: http://www.sciencelab.com/msds.php?msdsId=9927667.
4. After the gel has solidified, place the set gel in the electrophoresis tank. Add enough TAE buffer to cover the gel until the wells are submerged. Check that the level of the TAE buffer is approximately 1 mm above the level of the gel. Remove the gel comb.
   NOTE: Removing the comb after wells are submerged prevents air pockets from being trapped in the wells.
5. Prepare the RNA samples. Combine 1 µl of each purified sample aliquot with 1 µl Gel Loading Buffer (or with 1 µl 10x loading buffer and diluted to 10 µl with nuclease-free water) and mix thoroughly by pipetting up and down with a micropipette.
   NOTE: 10x loading buffer contains 20% Ficoll 400, 0.1 M Na_2EDTA, pH 8.0, 1.0% SDS, 0.25% bromophenol blue. 0.25% xylene cyanol may also be added to the loading buffer, as it runs ~50% as fast as bromophenol blue and can be useful for monitoring very long runs.[42]

6. Load the gel with each sample by carefully pipetting the solution into the bottom of each well. Take care not to leave any air bubbles or mix samples between wells.
   NOTE: A RNA ladder can also be used to determine the size of the transcript.
7. Attach the leads so that the DNA will migrate into the gel towards the positive lead. Set the voltage to the desired level, typically 1 to 10 V/cm of gel. Run the gel for however long is sufficient for significant separation between the RNA fragments, using the migration of dyes to monitor the progress of the separation.
   NOTE: A 23 cm x 25 cm gel at 250 V will take approximately 1 hr, while an 8 cm x 8 cm gel at 150 V will take approximately 20 min. Smaller RNA fragments have better resolution when run at higher voltages.
8. Turn off the power supply when the dye from the loading buffer has migrated a distance judged sufficient for separation between the RNA fragments.
9. Image the RNA under UV light as the ethidium bromide will fluoresce.
10. Take a picture of the image and compare fluorescence intensities of the purified RNA from each condition.

4. RNA Quantification via UV-Vis Spectroscopy

1. Place 2 µl H_2O on a NanoDrop 2000, or similar machine, and measure the Blank.
2. Next, place 2 µl of each RNA sample, following purification, on the spectrophotometer and measure UV-Vis from a wavelength range of 200 nm to 800 nm.
   NOTE: An A_{260} reading of 1.0 is equivalent to about 40 µg/ml of RNA, and pure RNA has an A_{260}/A_{280} ratio of 2.1.[45]
3. In the case that absorbance is above 1 O.D., dilute the samples in nuclease#free water to obtain an O.D. less than 1.
4. Use graphing software to plot the various samples and compare O.D. at 260 nm.

Representative Results

RNA Transcription Qualitatively Assessed by Agarose Gel Electrophoresis

Agarose gel electrophoresis is used to image the transcribed RNA. Ethidium bromide fluoresces upon binding (λ_{em} = 605 nm, λ_{ex} = 210 nm, 285 nm)[46] allowing imaging of RNA. Darker bands in the gel correspond to higher concentrations of RNA. If Actinomycin D, triptolide, or either corrole complex inhibits RNA transcription, the production of RNA is reduced and the band will appear lighter. Using this concept, relative inhibition can be assessed.

Figure 3 shows the ethidium bromide stained agarose gel (1%) of RNA transcription reactions pre-treated with no complex, Actinomycin D, triptolide, tpfc, or Ga(tpfc) at a [complex]:[template DNA base] ratio of 0.01:1. Actinomycin D and triptolide show a clear decrease of RNA compared to that of the control, as expected of these long-studied inhibitors. The Ga(tpfc) band also has a very low level of RNA, while the tpfc band shows little to no inhibition and exhibits the same relative intensity as the control.

**RNA Transcription Quantified by UV-Vis Spectroscopy**

UV-Vis measurements were taken for each sample after undergoing RNA transcription for 4 hr and purified with RNA spin column chromatography. The spectra of wavelengths 220 nm to 350 nm are shown in **Figure 4**, with the λ_{max} occurring at 260 nm, corresponding to RNA absorption, and an extinction coefficient of 0.025 $(µg/ml)^{-1}$ cm^{-1}. The absorbance at 260 nm and 280 nm are reported in **Table 3**. An A_{260} reading of 1.0 is equivalent to about 40 µg/ml of RNA, and pure RNA has an A_{260}/A_{280} ratio of 2.1, allowing for quantitative calculations of RNA produced during the transcription reaction and an assessment of the purity of the RNA after using the RNA spin column.[44] Three of the four inhibitor-treated transcription reactions yielded less RNA than the control, with Ga(tpfc)-treated DNA transcribing only 0.07 times as much RNA as the untreated DNA. tpfc-treated DNA showed no apparent inhibition. All samples have an A_{260}/A_{280} ratio of approximately 2.2, indicating relatively pure samples. Purification by RNA spin columns removes short RNA strands 20 or fewer nucleotides long. Residual DNA or strands longer than 20 nucleotides may be present in what is considered the purified samples. These slight impurities would result in an A_{260}/A_{280} ratio slightly greater than 2.1.
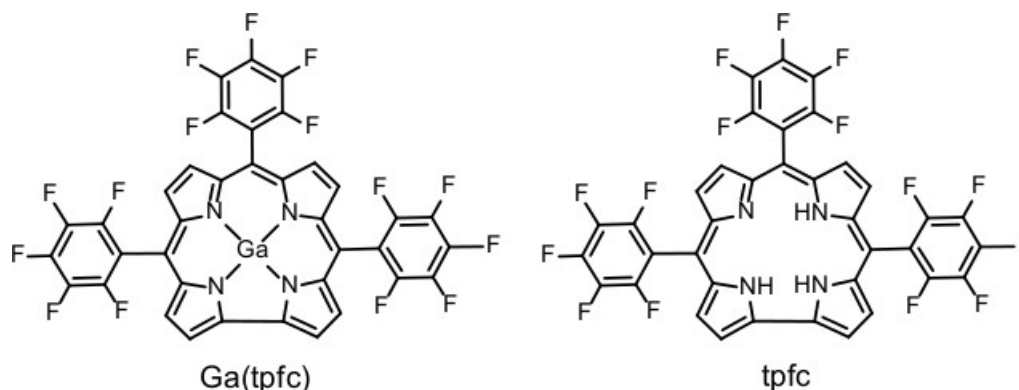

Figure 1. Molecular structures of Gallium(III) 5,10,15-(tris)pentafluorophenylcorrole (Ga(tpfc)) and the freebase analogue 5,10,15-(tris)pentafluorophenylcorrole (tpfc). Please click here to view a larger version of this figure.
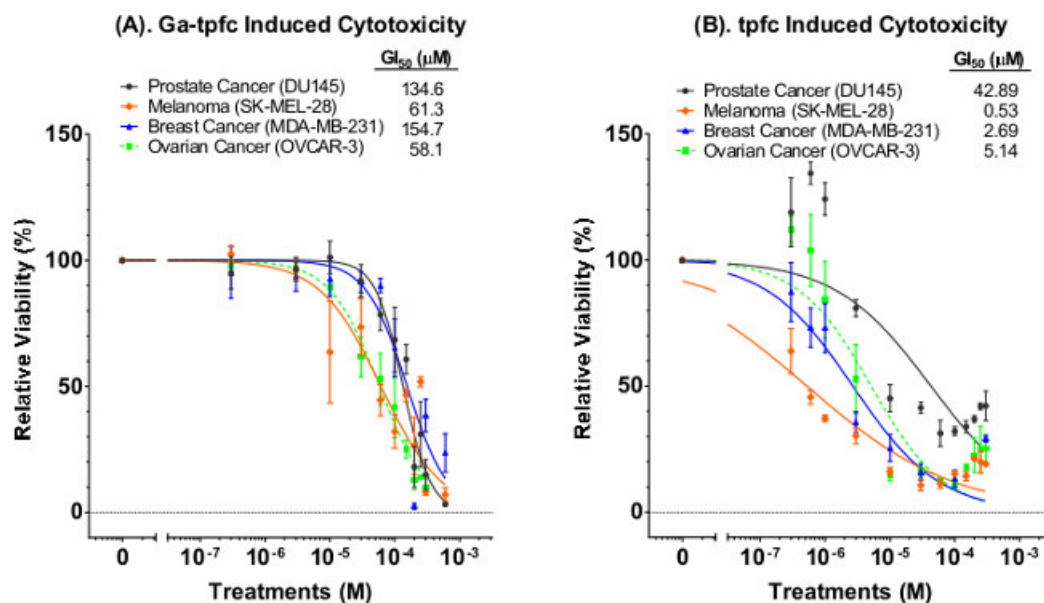
Figure 2. Cytotoxicity curves and GI$_{50}$ values of corroles in prostate (DU-145) (gray), breast (MDA-MB-231) (blue), skin (SK-MEL-28) (orange), and ovarian (OVCAR-3) (green) cancer cell lines. Cells were incubated with **(A)** Ga(tpfc) and **(B)** tpfc, followed by determination of viability using the MTS-based colorimetric cell viability assay according to the manufacturer's protocol. Please click here to view a larger version of this figure.
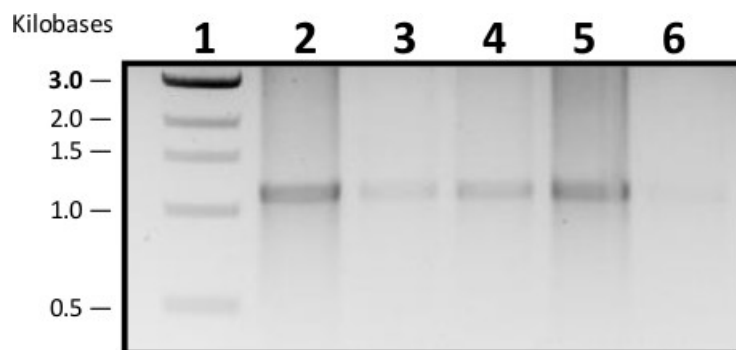
Figure 3. Ethidium bromide stained agarose gel (1%) of transcription reactions after 4 hr of incubation. Lane 1 is a 1,000 bp DNA ladder as a standard. Lanes 2-6 show the RNA transcribed from 0.43 pmol DNA and treated with 4.3 fmol of each inhibitor (a [complex]:[template DNA base] ratio of 0.01): the control (lane 2), Actinomycin D (lane 3), triptolide (lane 4), tpfc (lane 5), and Ga(tpfc) (lane 6).
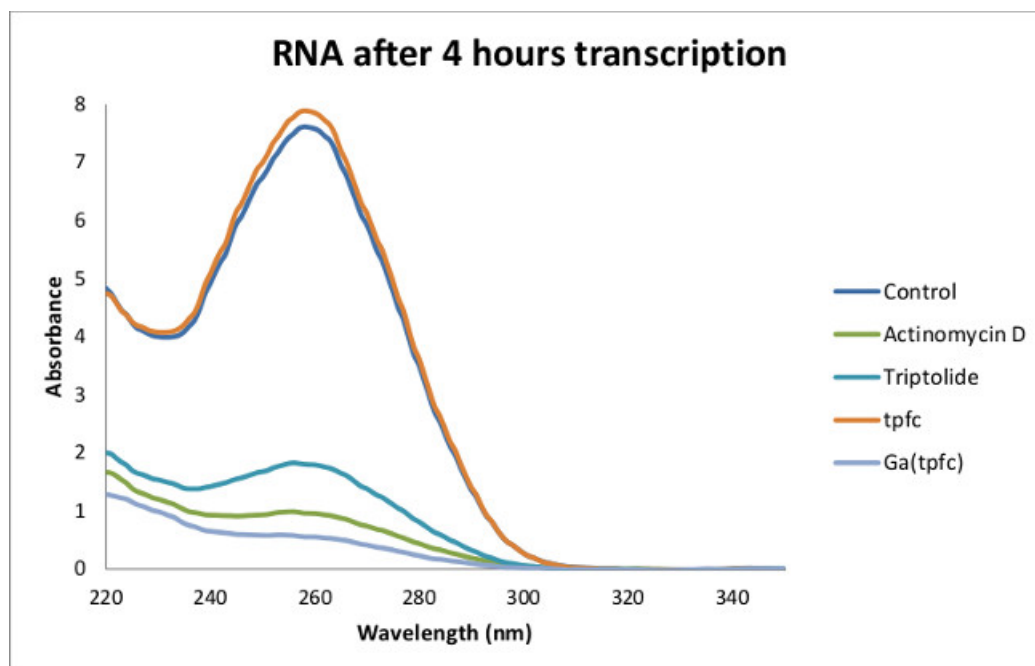
Figure 4. UV-Vis spectrum of 1:200 dilution of transcribed RNA at after 4 hr of incubation. The DNA template for the transcription reaction was treated with no complex, or with Actinomycin D, triptolide, tpfc, or Ga(tpfc) at a [complex]:[template DNA base] ratio of 0.01:1. The RNA concentration is measured by O.D. at 260 nm.

Thaw frozen reagents on ice:
75 mM solutions of adenosine triphosphate, guanosine triphosphate, cytidine triphosphate, and uridine triphosphate (ATP, CTP, GTP, UTP)
Nuclease-free Water
10x Reaction Buffer (1x Reaction Buffer: 40 mM Tris-HCl, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM dithiothreitol, pH 7.9)
Linearized template DNA (0.5 µg/ml, 1.85 kb)
RNA Polymerase Enzyme (20 U/µl)

Table 1. List of frozen reagents.

To begin the transcription reactions, add the following amount of each reagent in 0.2 ml thin-walled PCR tubes:
2 µl ATP solution (75 mM)
2 µl CTP solution (75 mM)
2 µl GTP solution (75 mM)
2 µl UTP solution (75 mM)
1 µl nuclease-free water
2 µl 10x Reaction Buffer
2 µl of 0.5 µg/µl linear template DNA
5 µl complex (nucleus-free water as the blank, actinomycin D, triptolide, tpfc, Ga(tpfc))
2 µl RNA Polymerase (20 U/µl)

Table 2. List of reagents for transcription reaction.

Time Point (hr)	Complex	A_{260}	A_{280}	A_{260}/A_{280}	Dilution Factor	[RNA]:A_{260} (µg/ml)	[RNA] (mg/ml)
4	Control	7.583	3.516	2.16	200	40	60.67
4	Actinomycin D	0.955	0.438	2.18	200	40	7.638
4	triptolide	1.794	0.816	2.2	200	40	14.35
4	tpfc	7.858	3.631	2.16	200	40	62.87
4	Ga(tpfc)	0.554	0.232	2.39	200	40	4.434

Table 3. Concentration and purity of transcribed RNA after 4 hr measured by UV-Vis Spectroscopy. The extinction coefficient of single-stranded RNA is 0.025 $(µg/ml)^{-1}$ cm^{-1}, an A_{260} reading of 1.0 is equivalent to about 40 µg/ml of RNA, and pure RNA has an A_{260}/A_{280} ratio of 2.1.[45]

Discussion

This assay demonstrates that the addition of Ga(tpfc) inhibits RNA transcription comparably to the known DNA-binding anticancer complexes Actinomycin D and triptolide. The cytotoxic behavior of Ga(tpfc) (GI_{50} = 58.1-154.7 µM) may due to its inhibitive properties. Since no transcription inhibition was observed in tpfc, the cytotoxicity of tpfc is not due to RNA transcription inhibition but is caused by other means not yet studied.

In the four transcription reactions performed, DNA was treated with Actinomycin D, triptolide, tpfc, or Ga(tpfc), at a [complex]:[template DNA base] ratio of 0.01, respectively, or an untreated control. The transcription reaction reagents were combined and the transcription reaction was allowed to proceed for 4 hr at 37 °C. The transcribed RNA was purified through a RNA spin column and the yield analyzed by UV-Vis and gel electrophoresis. The nature of transcription inhibition can be further investigated using this same technique with various modifications, or the compounds can be subjected to alternative *in vitro* and *in vivo* studies. Additional experiments which may help determine the molecular nature of the inhibition include X-ray crystallography of possible corrole-DNA adducts or computational modeling of the transcription reaction. The objective of this experiment was to determine whether the compounds inhibit transcription in order to collect information on their potential anticancer properties. That objective was achieved: tpfc exhibited no inhibition, while Ga(tpfc) exhibited clear inhibition and merits further study.

The RNA transcription assay can be modified to provide more mechanistic detail. Addition of the anticancer agents (*e.g.*, the corrole complexes) after the transcription reaction is complete can show whether the complexes degrade or hydrolyze the RNA. Another recommended experiment is to conduct the transcription reaction with all components with the exception of the RNA polymerase enzyme 10-fold lower to allow for differentiation between an inhibition mechanism involving the DNA or the enzyme. Finally, incubation of the DNA or the polymerase with the complexes prior to RNA transcription would allow for increased binding between the complex and the respective target, ascertaining whether the complex inhibitive qualities are involved in DNA or polymerase binding, respectively.

Disclosures

The authors have nothing to disclose.

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