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Influence of Dimethylsulfoxide on RNA Structure and Ligand Binding

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Abstract

Dimethyl sulfoxide (DMSO) is widely used as a cosolvent to solubilize hydrophobic compounds in RNA-ligand binding assays. Although it is known that high concentrations of DMSO (>75%) can significantly affect RNA structure and folding energetics, a thorough analysis of how lower concentrations (<10%) of DMSO typically used in binding assays affects RNA structure and ligand binding has not been undertaken. Here, we use NMR and 2-aminopurine fluorescence spectroscopy to examine how DMSO affects the structure, dynamics, and ligand binding properties of two flexible hairpin RNAs; the transactivation response element from HIV-1 and bacterial ribosomal A-site. In both cases, 5%-10% DMSO decreased stacking interactions and increased local disorder in non-canonical residues within bulges and loops and resulted in 0.3–4 fold reduction in the measured binding affinities for different small molecules, with the greatest reduction observed for an intercalating compound that binds RNA non-specifically. Our results suggest that by competing for hydrophobic interactions, DMSO can have a small but significant effect on RNA structure and ligand binding. These effects should be considered when developing ligand binding assays and high throughput screens.

Introduction

Many regulatory RNA elements are emerging as new potential drug targets for treating a wide range of diseases¹⁻⁵. For example, riboswitches are RNA-based gene regulatory elements in bacteria that form complex 3D structures, and are being targeted in the development of antibiotics⁶⁻⁸. Many RNA hairpin structures, including bacterial^{9,10} and retroviral elements¹¹⁻¹⁵, human micro-RNAs¹⁶, various repeats^{17,18} as well as more complex pseudoknots¹⁹ are being targeted in the development of therapeutics against infectious diseases, diabetes, various genetic disorders, and cancer. There is growing interest in using experimental and computational high throughput screens (HTS) to identify small molecules that can target RNA²⁻⁵ and that overcome delivery limitations inherent to large molecular weight RNA-based therapeutics such as antisense²⁰ and small interfering RNAs²¹.

Dimethyl sulfoxide (DMSO) is widely used as a universal solvent in HTS due to its miscibility with water, non-reactivity, and ability to dissolve hydrophobic compounds²². Although it is well established that DMSO concentrations (<10%) typically used in HTS can have a significant effect on the stability of certain proteins²² and reduce ligand binding

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Supporting Information: Additional figures are as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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affinities by as much as 10-fold²³, few studies have examined the impact of DMSO (<10%) on RNA structure and ligand-binding. Such studies are needed given that many RNA targets are highly flexible^{24,25} and therefore potentially highly susceptible to perturbations by external chemical agents.²⁶ Indeed, high DMSO concentrations (>75%) have previously been shown to disrupt the structure and stability of RNA^{27,28} and DNA.²⁹⁻³¹ In particular, NMR studies of yeast tRNA^{phe} showed that high concentrations of DMSO (up to 83%) resulted in changes in the ¹H spectra that were consistent with disruption of base-stacking and increased flexibility²⁸. Moreover, in a recent virtual screen targeting the transactivation response element (TAR) RNA from the human immunodeficiency virus type I (HIV-1), we found that the vast majority of false positive hits were water insoluble compounds that required DMSO for dissolution.¹³ Therefore, it is important to verify that DMSO does not affect the structure of TAR and other RNAs or interfere with their ability to bind small molecules in ways that are not accounted for in the virtual screen.

Here we use a combination of nuclear magnetic resonance (NMR) and fluorescence spectroscopy to explore how DMSO affects the structure and ligand-binding properties of two flexible hairpin RNA structures; HIV-1 TAR^{32,33} and the ribosomal A-site^{34,35}. These two RNAs have served as model systems for understanding RNA-small molecule targeting and provide an excellent opportunity, to examine in depth, the impact of DMSO on RNA structure and ligand binding.

Experimental Section

Materials

2-aminopurine (2AP)-labeled TAR and A-site RNA were purchased from Dharmacon (Lafayette, CO). Uniformly ¹³C/¹⁵N labeled TAR RNA and A-site RNA (Figure 1) were prepared by *in vitro* transcription using double stranded DNA encoding the RNA sequence of interest and containing the T7 promoter at 5'-end (Integrated DNA Technologies). T7 RNA polymerase (Takara Mirus Bio, Inc.) was used to transcribe the DNA sequence in the presence of ¹³C/¹⁵N labeled nucleotide triphosphates (ISOTEC, Inc. and Cambridge Isotope Laboratories, Inc.). The RNA was purified using 20% (w/v) denaturing polyacrylamide gel electrophoresis (PAGE) in 8M urea and 1× TBE. The RNA was electroeluted in 20mM Tris (pH 8) buffer and then precipitated in ethanol. The purified RNA pellet was dissolved and exchanged into NMR buffer (15mM sodium phosphate, 25mM sodium chloride, 0.1mM EDTA, 10% (v/v) D₂O, and pH ~ 6.4) using a centricon ultracel YM-3 concentrator (Millipore Corp.). The compounds L-argininamide (A3913), mitoxantrone (M6545), kanamycin B (B5264), and paromomycin (P9297) were purchased from Sigma-Aldrich (St. Louis, MO). 2-aminopurine base (276560500) was purchased from Acros Organics (Geel, Belgium). DMSO was purchased from Amresco (Solon, OH).

NMR spectroscopy

All NMR experiments were performed at 298K on 600 MHz Avance Bruker or Agilent spectrometers equipped with 5mm triple-resonance cryogenic probe. 5% and 10% DMSO were added volume by volume (v/v). All NMR spectra were processed using NMRPipe³⁶ and SPARKY 3³⁷. The overall chemical shift perturbations ($\Delta\delta_{\text{overall}}$) upon incremental addition of DMSO in 2D-HSQC spectra of TAR and A-site were calculated using the following equation:

$$\Delta\delta_{\text{overall}} = \sqrt{(\Delta\delta_H)^2 + \left(\frac{\gamma_C}{\gamma_H}(\Delta\delta_C)\right)^2} \quad (1)$$

where $\Delta\delta_H$ and $\Delta\delta_C$ are the changes in 1H and ^{13}C chemical shift (in ppm), respectively, and γ_H and γ_C are gyromagnetic ratios for hydrogen and carbon, respectively. For N-H HSQC spectra, γ_C and $\Delta\delta_C$ are replaced with γ_N and $\Delta\delta_N$, respectively, which are gyromagnetic ratio for nitrogen and the changes in ^{15}N chemical shift (in ppm), respectively.

Fluorescence spectroscopy

The fluorescence-based binding assays employed TAR RNA with 2AP labeled at the bulge residue U25 (2AP-U25-TAR) and ribosomal A-site RNA with 2AP labeled at the internal loop residue A92 (2AP-A92-Asite). 10 μM of 2AP-labeled RNA was annealed by heating to 95°C for 5 minutes and then cooled on ice for an hour prior to use. The folded RNA was diluted to 20 nM concentration in assay buffer (10mM sodium phosphate, 50mM NaCl, 0.1mM EDTA, and pH ~6.8). The temperature of the cuvette holder was maintained at 25°C with a water-cooling system. DMSO was added volume by volume (v/v). Time-resolved fluorescence intensity measurements were collected using a Fluoromax-2 fluorimeter at 320 nm excitation and 370 nm emission wavelengths following incremental addition of small molecules in 1:1000 dilutions. Each titration point with small molecule was averaged over 15 seconds of fluorescence intensity measurement with 0.1 second time interval. The slit width was 10 nm. All measurements were triplicated. The fluorescence emission intensities were normalized with respect to the fluorescence emission intensity in the absence of small molecules. With the exception of kanamycin B binding to TAR, all dissociation constants were computed by fitting to the following equation using the Origin software (Origin Lab Corporation),

$$F = A \times [RNA]_T + B \times \left\{ \frac{([RNA]_T + [L]_T + K_d) - \sqrt{([RNA]_T + [L]_T + K_d)^2 - (4 \times [RNA]_T \times [L]_T)}}{2} \right\} \quad (2)$$

where $[RNA]_T$ is the total RNA concentration, $[L]_T$ is the total small molecule concentration, and A and B are fluorescence contribution factors that account for relative fluorescence intensities in the free and bound state, respectively.³⁸ The titration data for kanamycin B binding to TAR exhibited two inflection points characteristic of two-site binding. This data did not fit well to Equation 2 (reduced $\chi^2 \sim 44$) and was instead fitted to Equation 3 (reduced $\chi^2 \sim 0.2$) which assumes two independent site binding:

$$F = A \times [RNA]_T + B \times \left\{ \frac{([RNA]_T + [L]_T + K_{d,1}) - \sqrt{([RNA]_T + [L]_T + K_{d,1})^2 - (4 \times [RNA]_T \times [L]_T)}}{2} \right\} + C \times \left\{ \frac{([RNA]_T + [L]_T + K_{d,2}) - \sqrt{([RNA]_T + [L]_T + K_{d,2})^2 - (4 \times [RNA]_T \times [L]_T)}}{2} \right\} \quad (3)$$

The lower affinity binding site is not observed under higher ionic strength conditions (150mM NaCl or 3mM MgCl₂) and therefore likely reflects non-specific binding to the RNA.

The fluorescence emission spectra of 2AP-U25-TAR, 2AP-A92-Asite, and 2-aminopurine base were measured using Fluoromax-2 fluorimeter in the same assay buffer used in the binding assays. The fluorescence emission was measured from 350nm to 400nm with excitation at 320nm. All spectra were averaged over 3 scans.

Results and Discussion

Impact of DMSO on the structure and dynamics of RNA by NMR

We investigated how DMSO affects the structure of HIV-1 TAR (Figure 1A) and bacterial ribosomal A-site RNA (Figure 1B) using NMR chemical shift titrations, where we acquired 2D N-H and C-H HSQC spectra of uniformly $^{13}\text{C}/^{15}\text{N}$ labeled RNA following the incremental addition of DMSO up to 10% concentration, which corresponds to the higher end of DMSO concentrations typically used in ligand-binding assays.³⁹

Increasing concentrations of DMSO resulted in specific perturbations in the TAR chemical shifts at residues located in and around the flexible bulge and apical loop (Figure 2A). This data suggests that DMSO affected the TAR conformation and that the transition between free and “DMSO-bound” TAR occurs in fast exchange relative to the NMR timescale. The most significant ($\Delta\delta_{\text{overall}} > 0.1$ ppm) perturbations were observed for residues A22 and U23 in and around bulge and the apical loop residue A35 (Figure 2B), which are all flexible residues that adopt partially stacked conformations.^{40,41,42}

The downfield perturbations in the nucleobase (C6/C8) carbon resonances induced by DMSO at the bulge (A22(C8H8), U23(C6H6), and U25(C6H6)) suggest loss of stacking interactions⁴²⁻⁴⁴. Interestingly, all of the DMSO-induced chemical shift perturbations in and around the bulge, including for A22, U23, C24, U25 and U40 were similar to those induced by increasing Mg^{2+} and Na^{+} concentrations (Figure S-1, Supporting Information), which were previously shown to stabilize a co-axial TAR conformation in which all three bulge residues are flipped out and flexible.^{45,46} Indeed, DMSO induced a gradual increase in the normalized resonance intensities⁴⁷ in the bulge resonances (U23, C24, U25) (Figure S-2, Supporting Information), consistent with an increase in picosecond-to-nanosecond timescale motions due to loss of stacking interactions.

In contrast, the DMSO-induced perturbations at apical loop residue A35(C1'H1') were not observed with increasing Mg^{2+} (Figure S-1, Supporting Information) and there were many resonances which showed significant perturbations with Mg^{2+} that showed little to no perturbations with DMSO (Figure S-1, Supporting Information). The carbon perturbations for G34(C8H8), A35(C1'H1'), and A35(C8H8) suggest stabilization of A35 in a flipped out conformation and G34 in an intra-helical stacked conformation. Prior studies have shown that A35 and G34 exist in a dynamic equilibrium in which they inter-change stacking interactions, with one residue flipping in while the other flips out, with the dominant form being a conformation in which A35 is flipped out and G34 flipped in⁴². The NMR data suggests that DMSO favors this dominant conformation. This is also consistent with the gradual increase in measured normalized resonance intensities (Figure S-2, Supporting Information) seen for A35(C1'H1') and A35(C8H8) upon addition of DMSO.

In contrast, little to no chemical shift perturbations or changes in resonance intensities were observed for more stable helical residues. This is also consistent with the imino N1-H1 and N3-H3 HSQC spectra (Figure 2A), which showed little to no changes on addition of DMSO. Thus, the more stable helical residues seem to be more shielded from the effects of DMSO.

Addition of DMSO to A-site also resulted in significant chemical shift perturbations, which were localized in flexible internal loop (A08, A92 and A93) and nearby residues (G91 and G94), indicating that DMSO also induced changes in the structure of A-site (Figure 3A). Once again, the gradual changes in the NMR resonance positions with increasing DMSO suggests that any transition between free and “DMSO-bound” A-site occurred in fast exchange relative to the NMR timescale. The largest perturbations ($\Delta\delta_{\text{overall}} > 0.1$ ppm) were observed for the flexible and intra-helically stacked A92 (Figure 3B). The downfield

shifted ^{13}C chemical shift particularly for A92(C8H8) is consistent with the loss of stacking and flipping out of the adenosine base. Indeed, similar perturbations were observed for A92 upon the addition of the aminoglycoside paromomycin, which has been known to promote the flipping out of A92.^{34,48,49} This is also consistent with the gradual increase in measured normalized resonance intensities⁴⁷ (Figure S-3, Supporting Information) for A92 and A93 upon addition of DMSO, consistent with an increase in picosecond-to-nanosecond timescale motions due to loss of stacking interactions.

DMSO did not induce any significant chemical shift perturbations in the thermodynamically stable UUCG apical loop⁵⁰. However, in contrast to TAR, DMSO did have an effect on the 2D N-H HSQC spectrum of A-site. In particular, DMSO induced a significant downfield shift in the imino proton of G91-H1, suggesting that DMSO affects the C09-G91 base-pair, which is near the non-canonical residues. Once again, canonical base-pairs embedded within helices did not exhibit significant chemical shift perturbations with DMSO.

Impact of DMSO on stacking interactions using 2-AP fluorescence

We used 2-aminopurine (2AP) fluorescence to further examine the impact of DMSO on stacking interactions. 2AP is widely used as a fluorescent reporter of stacking interactions in nucleic acids since the fluorescence emission intensity is highly sensitive to the details of the stacking interactions, and generally increases upon transitioning from a stacked to an unstacked conformation.⁵¹⁻⁵³ For these studies, we used a TAR construct (2AP-U25-TAR)³⁸ in which the flipped out bulge residue U25 is labeled with 2-AP and an A-site construct (2AP-A92-A-site)⁵⁴ in which A92 is labeled with 2-AP (Figure 4). Both of these constructs have previously been used in ligand binding studies^{38,54}.

As a control, we first examined how DMSO affects the fluorescence intensity of the 2-AP base. Upon addition of 5% and 10% DMSO, we observed a small increase in the fluorescence intensity of 2-aminopurine base at maximum emission wavelength ($\lambda_{\text{max}} = 378\text{nm}$) of 5% and 11%, respectively (Figure 4A). This indicates that DMSO did not significantly alter the fluorescence properties of 2-AP. Moreover, addition of 10% DMSO did not shift the maximum emission wavelengths of 2-aminopurine base and 2AP-labeled RNA constructs.

Addition of 5%-10% DMSO increased the fluorescence intensity of 2AP-U25-TAR at maximum emission wavelength ($\lambda_{\text{max}} = 374\text{nm}$) by 44%-75% (Figure 4B). For A-site, 5%-10% DMSO increased the fluorescence intensity at maximum emission wavelength ($\lambda_{\text{max}} = 370\text{nm}$) by 19% and 32%, respectively (Figure 4C). The observed increase in the fluorescence intensities is consistent with the DMSO-induced loss of stacking interactions within the TAR bulge and A-site internal loop, as suggested independently by the NMR data. The smaller DMSO-induced increase in fluorescence intensity observed for A-site as compared to TAR is likely because the purine A92 retains stacking interactions with the flipped out A93 as observed in structures of A-site bound to aminoglycosides^{48,54}.

Impact of DMSO on ligand binding affinity

We used 2-AP fluorescence to examine the impact of DMSO on the binding affinity of small molecules that bind to TAR and A-site. We measured the dissociation constants (K_d) for argininamide (ARG) (Figure 5A), kanamycin B (Figure 5B), and mitoxantrone (Figure 5C) binding to TAR and for paromomycin (Figure 6A) and mitoxantrone (Figure 6B) binding to A-site. ARG is a ligand mimic of TAR's cognate protein target, the transactivator protein, which binds TAR with micromolar affinity and which has been shown to recapitulate many essential features of TAR-Tat recognition⁵⁵⁻⁵⁷. Kanamycin B and paromomycin are example aminoglycosides that bind RNA in the nanomolar range and in a manner strongly dependent

on electrostatic interactions.⁵⁸⁻⁶¹ Mitoxantrone is a newly identified intercalator that binds non-specifically to RNA with affinities on the nanomolar range¹³.

All of the molecules tested resulted in significant changes in fluorescence intensity upon binding to their RNA targets allowing the accurate determination of K_d s. In all cases, the addition of DMSO slightly weakened the RNA-ligand binding affinity and resulted in an increase in the measured K_d (Table 1). This increase was small for 5% DMSO but became significant at 10% DMSO. For example, while the addition of 5% DMSO increased the measured K_d for ARG, kanamycin B, and mitoxantrone by only 1.1, 1.2 and 1.7 fold, respectively, 10% DMSO resulted in much larger increases of 1.4, 1.5, and 2.8 fold, respectively. It should be noted that kanamycin B exhibited a second weaker and non-specific binding to TAR which was not observable in the presence of 10% DMSO. A more significant 1.7-2.8 fold increase in K_d was observed for the intercalator, mitoxantrone (Table 1). Similar results and trends were observed with A-site, where the addition of 5% DMSO increased the measured K_d for paromomycin and mitoxantrone by 1.5 and 1.8 fold respectively, whereas 10% DMSO resulted in much larger increases of 2.6 and 3.9 fold respectively (Table 1).

In general, the weakened binding affinity observed with DMSO was not as dramatic as that reported for some proteins²³. However, our study focused on hairpin structures; additional studies are required with more complex RNA structures, such as riboswitches, that have deeper and often more hydrophobic binding pockets that are more similar to typical protein binding sites.

Impact of DMSO on RNA ligand binding mode

To further examine whether DMSO affects the RNA-ligand binding kinetics and the RNA-ligand bound structure, we performed NMR chemical shift titrations in which spectra of TAR or A-site were recorded upon addition of a small molecule in the presence of 5% DMSO and compared the NMR titration profiles with counterparts observed in the absence of DMSO (Figure S-4, Supporting Information). In both cases, similar chemical shift perturbations were observed in the absence and presence of DMSO, indicating that the exchange kinetics between free and ligand-bound RNA structure, and the ligand-binding mode were not significantly affected by DMSO. Comparison of the spectra of TAR-ARG and A-site-paromomycin complexes with and without 5% DMSO (Figure S-5, Supporting Information) revealed even smaller differences than observed for the unbound RNA, indicating that DMSO has a smaller effect on the structures of RNA-ligand complexes.

Mechanism of DMSO perturbations and implications for experimental and virtual screens

Our results show that DMSO promotes the flipping out of the flexible non-canonical residues. This effect is likely due to the increased hydrophobicity of the solvent, which favors solvation of the hydrophobic nucleobase as compared to the polar water solvent, and possibly due to DMSO forming hydrogen bonds with nucleobases and other hydration effects. Prior studies showed that organic solvents, including N,N-dimethylformamide, DMSO, tetramethylurea, methanol, ethanol, and ethylene glycol denature duplex DNA in a manner strongly dependent on their degree of hydrophobicity³⁰. Another study has shown that replacement of the two methyl groups in DMSO with ethyl groups results in a more hydrophobic solvent (diethylsulfoxide or DESO) that is more effective at denaturing duplex DNA³¹. While the DMSO concentrations (5%-10%) used in binding assays are not sufficient to disrupt duplex structures, it can promote destacking and 'local melting' of flexible non-canonical residue, that are often the sites of small molecule binding. These effects are expected to become even more pronounced for more flexible RNAs containing single-strands. While the effects of DMSO on RNA observed here are smaller than those

reported for certain proteins²³, they warrant careful attention before proceeding with ligand binding assays and high throughput screening campaigns.

Our results suggest that DMSO has a smaller effect on the ligand binding affinities of polycationic aminoglycosides that bind TAR and A-site primarily through electrostatic interactions, as compared to the intercalating mitoxantrone that binds primarily via hydrophobic stacking interactions. One possibility would be that DMSO more strongly perturbs the RNA binding site for mitoxantrone as compared to the aminoglycosides. However, comparison of the chemical shift perturbations induced by DMSO and the small molecules does not provide any evidence that the DMSO more greatly affects the mitoxantrone binding site. Rather, this effect is likely due to the increase in the hydrophobicity of the solvent with DMSO, which favorably solvates the hydrophobic mitoxantrone. The slightly weakened K_d of paromomycin in 10% DMSO could also be explained by the weakened stacking interaction between ring I of paromomycin and G91 of A-site⁴⁸ due to increase in the hydrophobicity of the solvent and possibly due to competing h-bonding interactions among DMSO, water, and the A-site nucleobases.

Our study was motivated in large part by a recent virtual screen targeting HIV-1 TAR¹³. Among 58 top-scoring compounds tested, only 14 compounds were water-soluble and did not require DMSO for solubilization, and among these compounds, 7 were active in *in vitro* binding assays. In stark contrast, among 44 water insoluble compounds that required DMSO for solubilization, none were active in *in vitro* binding assays. The overall hit rate for the virtual screen was 12%, but improved to 50% when excluding compounds that required DMSO for their solubilization. Our results suggest that DMSO is unlikely to have affected TAR-ligand binding to such an extent as to completely abrogate binding. Rather, it is more likely that the docking scoring function used in the virtual screen generates false positives that are biased toward hydrophobic compounds. Indeed, preliminary analysis of a virtual screen using a distinct docking program and scoring function reveals a smaller level of enrichment with hydrophobic compounds among the top virtual screening hits (data not shown).

Conclusion

We have investigated how DMSO affects the structure and ligand binding properties of two well-studied RNA targets, HIV-1 TAR and ribosomal A-site. In both cases, typical concentrations (5%-10%) of DMSO used in ligand binding assays and in high throughput screens destabilized non-canonical residues within bulges and loops and resulted in a 0.3–4 fold reduction in the measured binding affinities for different small molecules, with the greatest reduction observed for an intercalating hydrophobic compound that binds RNA non-specifically. Our results suggest that by competing for hydrophobic interactions, DMSO can have a significant effect on RNA structure and ligand binding. These effects should be considered when developing ligand binding assays and high throughput screens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Secondary structure of RNA used in this study. Solid and dashed lines denote Watson-Crick and flexible base pairs, respectively. **A.** HIV-1 TAR. **B.** Bacterial ribosomal A-site. The open circle denotes a U-U wobble base pair.

Figure 2.

Examining impact of DMSO on HIV-1 TAR conformation by NMR. **A.** 2D C-H and N-H HSQC spectra of TAR in increasing DMSO concentration. **B.** Secondary structure of TAR highlighting residues (in red circles) that undergo the largest DMSO induced chemical shift perturbations ($\Delta\delta_{\text{overall}} > 0.1$ ppm).

Figure 3.

Examining impact of DMSO on bacterial A-site conformation by NMR. **A.** 2D C-H and N-H HSQC spectra of A-site in increasing DMSO concentration. **B.** Secondary structure of A-site highlighting residues (in red circles) that undergo the largest DMSO induced chemical shift perturbations ($\Delta\delta_{\text{overall}} > 0.1$ ppm).

Figure 4. Examining impact of DMSO on RNA stacking interactions using 2AP fluorescence. Shown is the emission spectrum of 2AP at max wavelength with increasing DMSO concentration **A.** 2-AP **B.** 2AP-U25-TAR. **C.** 2AP-A92-Asite.

Figure 5. Impact of DMSO on TAR-small molecule binding affinities. Shown are 2-AP fluorescence intensity titration curves for small molecule binding to 2AP-U25-TAR with varying DMSO concentration. **A.** Argininamide. **B.** Kanamycin. **C.** Mitoxantrone. Error bars are obtained from repeating the measurements in triplicate.

Figure 6. Impact of DMSO on A-site-small molecule binding affinities. Shown are 2-AP fluorescence intensity titration curves for small molecule binding to 2AP-A92-A-site with varying DMSO concentration. **A.** Paromomycin. **B.** Mitoxantrone. Error bars are obtained from repeating the measurements in triplicate.

Table 1

Dissociation constants (K_{ds}) for RNA-small molecule binding determined using 2-AP fluorescence at different DMSO concentrations.

2AP-U25-TAR			
Small Molecule	0% DMSO	5% DMSO	10% DMSO
Argininamide	63.7 $\pm$ 2.0 μ M	70.6 $\pm$ 1.5 μ M	87.5 $\pm$ 1.9 μ M
Kanamycin B	13.7 $\pm$ 0.4 nM 10.2 $\pm$ 4.1 μ M	16.8 $\pm$ 0.4 nM 5.5 $\pm$ 1.1 μ M	20.4 $\pm$ 0.3 nM N/A
Mitoxantrone	44.1 $\pm$ 1.8 nM	75.4 $\pm$ 4.4 nM	124.5 $\pm$ 4.7 nM
2AP-A92-Asite			
Small Molecule	0% DMSO	5% DMSO	10% DMSO
Paromomycin	8.3 $\pm$ 0.4 nM	12.3 $\pm$ 0.6 nM	21.3 $\pm$ 1.0 nM
Mitoxantrone	64.4 $\pm$ 2.0 nM	117.6 $\pm$ 2.5 nM	253.9 $\pm$ 11.2 nM