Supplementary Information

Identification of Biologically Active, HIV TAR RNA-Binding Small Molecules Using Small Molecule Microarrays

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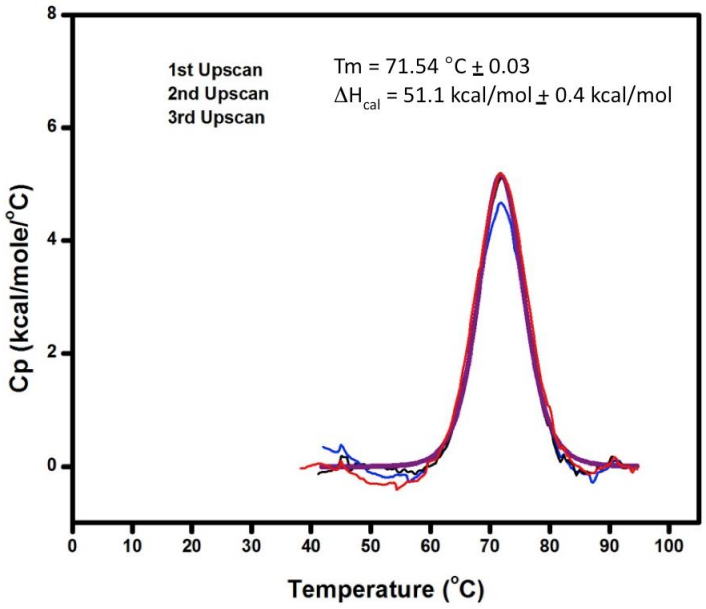
General Information:

Nucleic acids were purchased from Dharmacon (ThermoFisher), with the following sequences:

TAR RNA: 5'-GGCAGAUCUGAGCCUGGGAGCUCUCUGCC-3'
Cy5-labeled TAR RNA: 5'-Cy5-GCAGAUCUGAGCCUGGGAGCUCUCUGCC-3'
2-AP-labeled TAR RNA: 5'-GGCAGAUC(2AP)GAGCCUGGGAGCUCUCUGCC-3'
Cy5-labeled miR-21 RNA:5'-Cy5-GGGUUGACUGUUGAAUCUCAUGGCAACCC-3'

Small molecules were purchased from commercial vendors, assessed for purity with LC/MS analysis and ^{1}H NMR, dissolved in DMSO at a concentration of 10 mM, and used without further purification. See Figure SI-4 for compound supplier/product ID information.

Figure SI-1: Reversibility of TAR RNA hairpin folding by differential scanning calorimetry.



Differential Scanning Calorimetry (DSC) – DSC experiments were carried out on a Microcal VP-DSC microcalorimeter (GE Healthcare, originally Microcal, Northampton, MA). Prior to running the experiments, all surfaces were treated with RNase Zap Wipes (Ambion®) to minimize RNase contamination. The calorimeter, syringes, degassing tubes and stir bars were cleaned with 70% ethanol, followed by DEPC treated water (Ambion®). A 50 μM solution of TAR RNA in PBS pH 7.4 was heated to 95 °C in a hot water bath for 5 minutes and was allowed to cool to room temperature (over a 2 hour period). Annealed RNA was frozen in 1 mL aliquots at -20 °C. Per routine protocol, buffer (1X PBS, pH 7.4) was introduced to both the reference and sample cells and the calorimeter was allowed to ramp through one heat-cool cycle (10 °C to 95 °C) at a rate of 60 °C/hr. During the down scan at 25° C, the buffer solution from the sample cell was quickly replaced with a degassed solution of 50 μM TAR RNA. The entire system was re-pressurized to approximately 30 psi of positive pressure to prevent evaporation at higher temperatures, and the experiment was allowed to continue. A total of 8 alternating up-down scans (10 C to 95 C) was performed to measure reversibility of folding/unfolding of the RNA. According to Origin DSC Anaylsis software, buffer effects were corrected and integration of the unfolding transition was performed. Thermograms were fitted to a two-state melting model and the calorimetric transition enthalpy (ΔH_{unf}) was obtained from the area under the excess heat capacity peak, the midpoint of the transition calculated as the melting temperature (T_m).

Minus strand strong stop assay performed in the presence of 4

RNA-dependent DNA polymerase activity was determined on a viral RNA corresponding to nt 1–365 of the HIV-1 NL4-3 (+) strand genome. A fluorescently labeled DNA primer (5'-Cy5 GTC CCT GTT CGG GCG CCA-3') was combined with template RNA at a ratio of 1:1.2 in 10 mM Tris/HCl, pH 7.6, 25 mM KCl and heated in a thermal cycler at 85 °C for 3 min and then cooled to 4 °C at 0.2 °C/s. **4** was added at final concentrations of 0, 1, 5, 10 μM and incubated for 10 min at room temperature. DNA polymerase reactions were performed at 37 °C and contained 10 mM Tris/HCl, pH 7.8, 50 mM $MgCl_2$, 80 mM KCl, 1 mM DTT, 0.2 mM dNTPs, 25 nM template/ primer, and 12.5 nM RT. Aliquots were removed at 0, 5, and 20 min time points and combined with an equal volume of 8 M urea in 1x Tris/borate/EDTA. Before loading, samples were heated to 95 °C for 3 min and immediately placed on ice. Nucleic acids were fractionated by denaturing 8% polyacrylamide gel electrophoresis. Gels were scanned with a GE Health- care Typhoon Trio + and analyzed with Image Quant Total Lab software.

Figure SI-2.

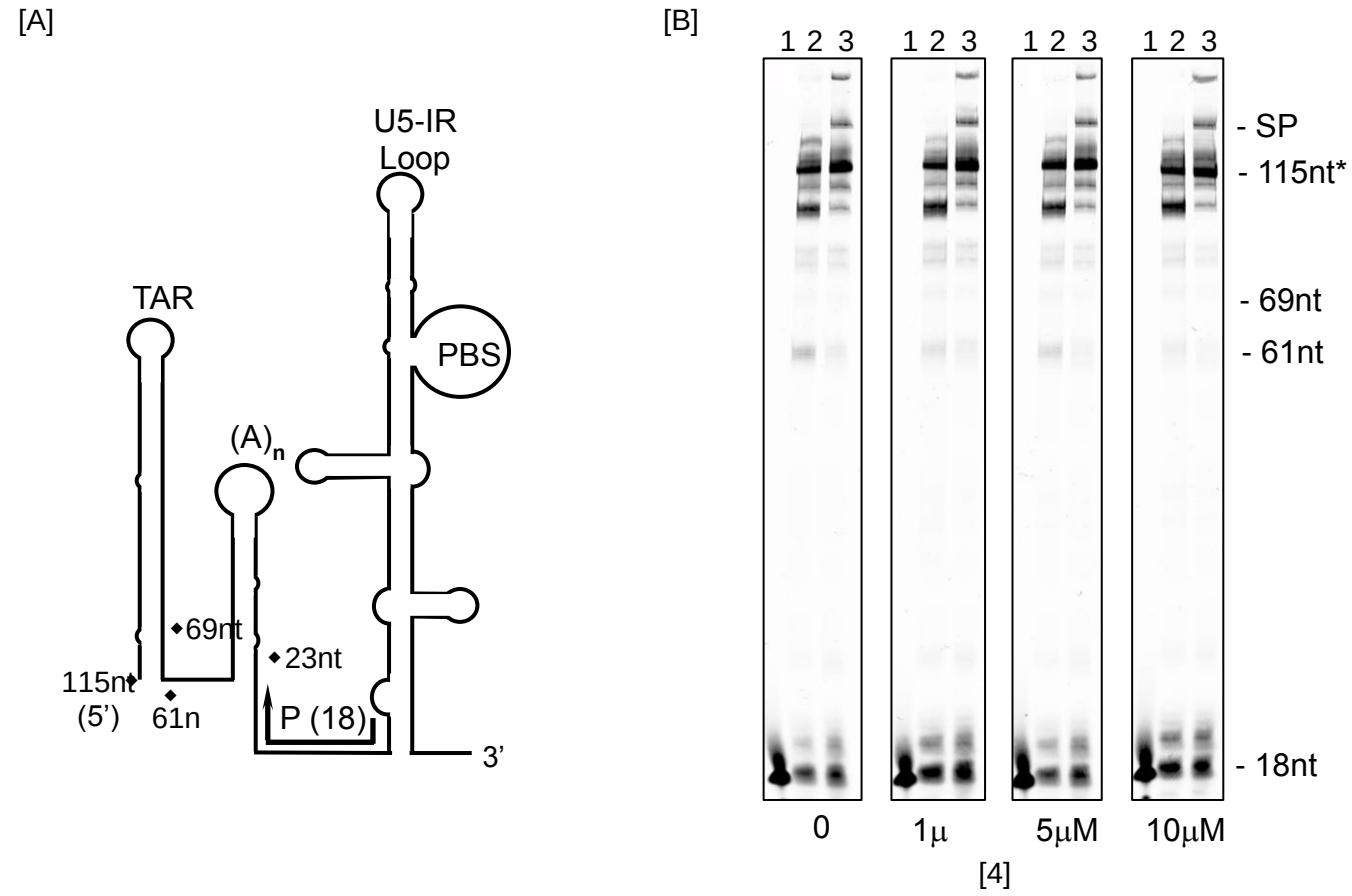


Figure SI-2. Minus strand strong stop assay performed in the presence of **4**.

A schematic representation of the HIV-1 genomic RNA substrate is presented in panel [A]. PBS, primer binding site; Poly(A), poly(A) hairpin; TAR, transactivation hairpin. The position to which the synthetic primer was hybridized is indicated via arrow. In the RNA-dependent DNA synthesis assay of panel [B], the asterisk indicates the expected DNA synthesis product (~115 nt), and SP reflects the results of a self-priming event. Lanes 1, 2 and 3 represent 0, 5 and 20 min time points, respectively, performed in the presence of 4 compound at different final concentrations: 0, 1, 5, 10 uM.

Figure SI-3.

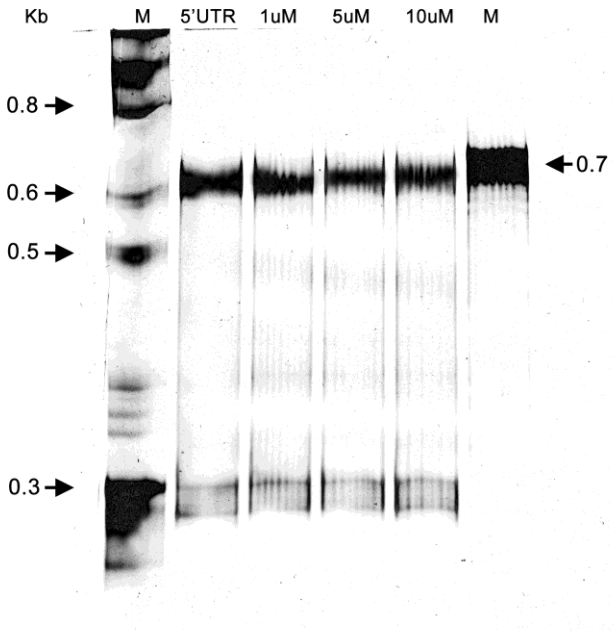
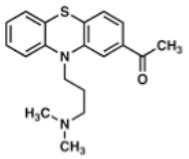


Figure SI-3. Verification of homogenous conformation of the HIV-1 RNA 5' UTR region. Increasing concentrations of the compound **4** (indicated on top) incubated with HIV-1 RNA 5' UTR region were added prior the non-denaturing polyacrylamide gel electrophoresis. The leftmost lane provides molecular weight markers.

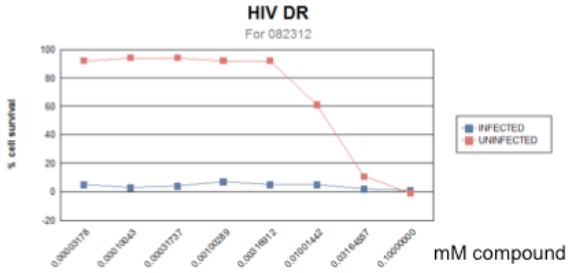
Figure SI-4. Cell-based Anti-HIV activity of **1, 4**, and related compounds. Compounds were purchased from commercial vendors, analyzed for purity by LC/MS, and used without further purification. For each compound, vendor name (below, in parenthesis) and product ID are indicated. Assays were performed as described in the main text.

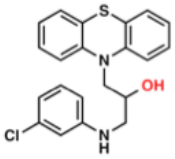
Compound	EC_{50} (μM)	CC_{50} (μM)	Comments
ST4133609 (Compound **4**)	28	ND	
4478-7480 (Compound **1**)	123	ND	
ST50055849	> 100	ND	
AKOS001656555 (Compound **2**)	> 10	9	
5251219	> 20	40	
4340-1594 (Compound **3**)	> 30	ND	
9233834	> 63	ND	
5277323	> 63	ND	
7878578	> 63	ND	
7928037	> 63	ND	
7852383	> 63	ND	
7911696	> 63	ND	
Acepromazine maleate	> 16	18	
ST4119563	> 330	190	
ST4133739	> 316	ND	
7746407	> 3	ND	Abnormal curves
ST014353 (Compound **5**)	ND	ND	Abnormal curves

ND = Not Determined.



Acepromazine maleate





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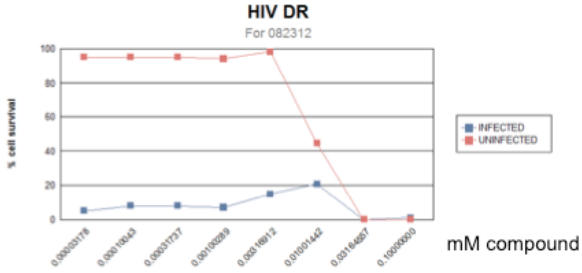

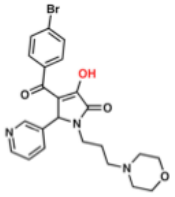

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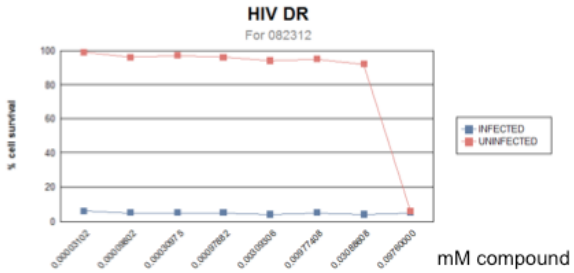

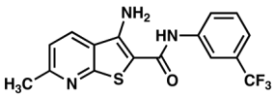

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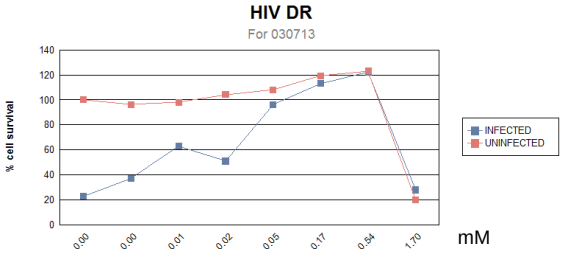

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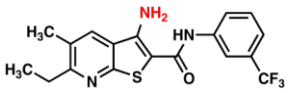

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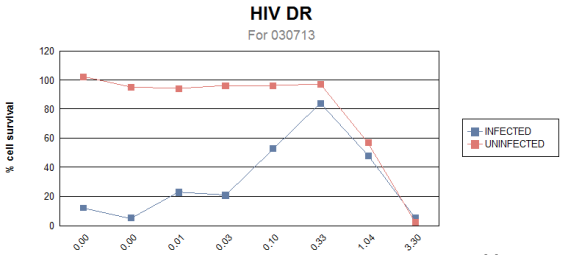

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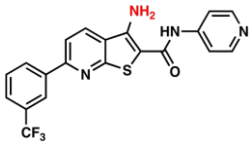

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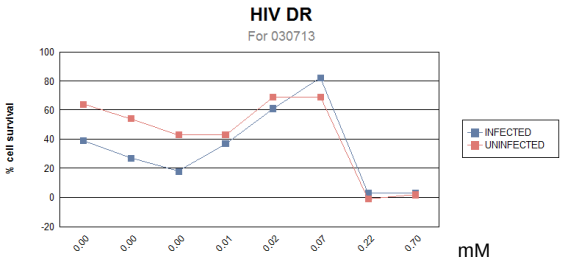

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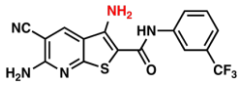

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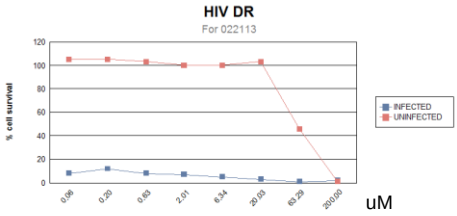

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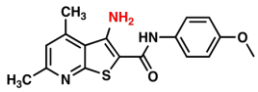

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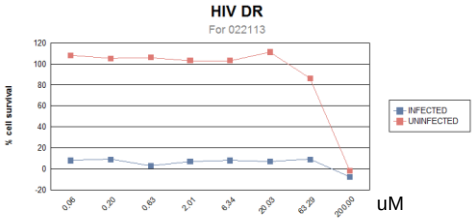

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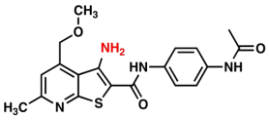

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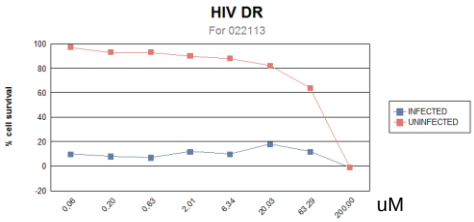

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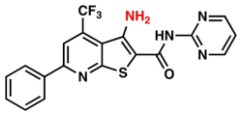

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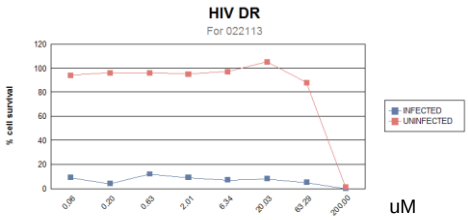

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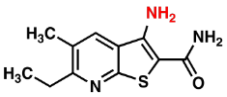

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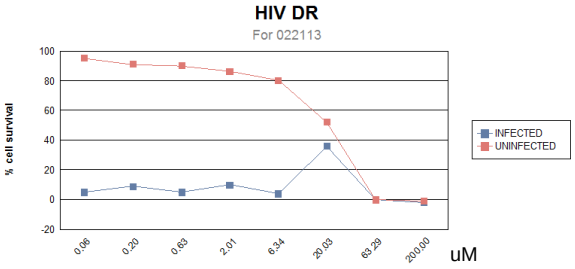

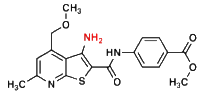

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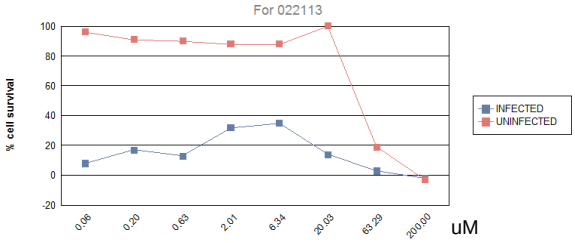

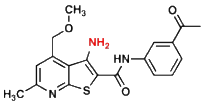

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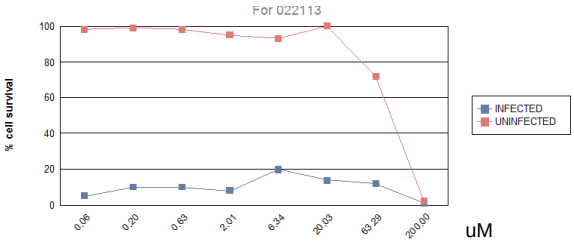

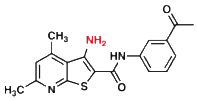

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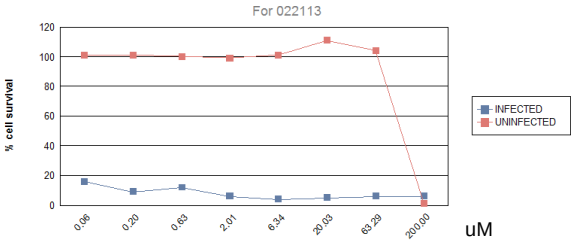

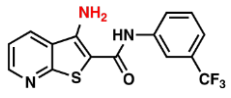

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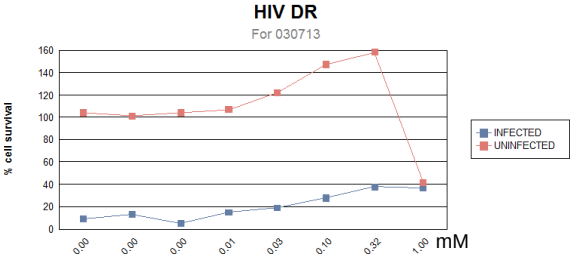

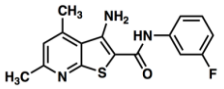

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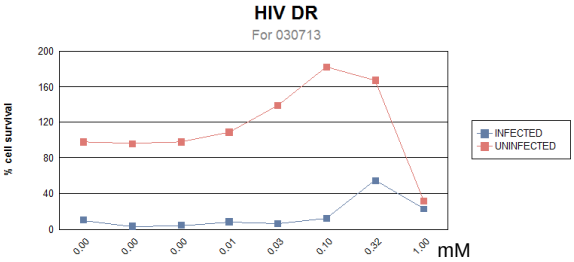


Figure SI-5. K_d measurement with compound **5** using a 2-aminopurine titration. The titration was carried out as described in the main text.

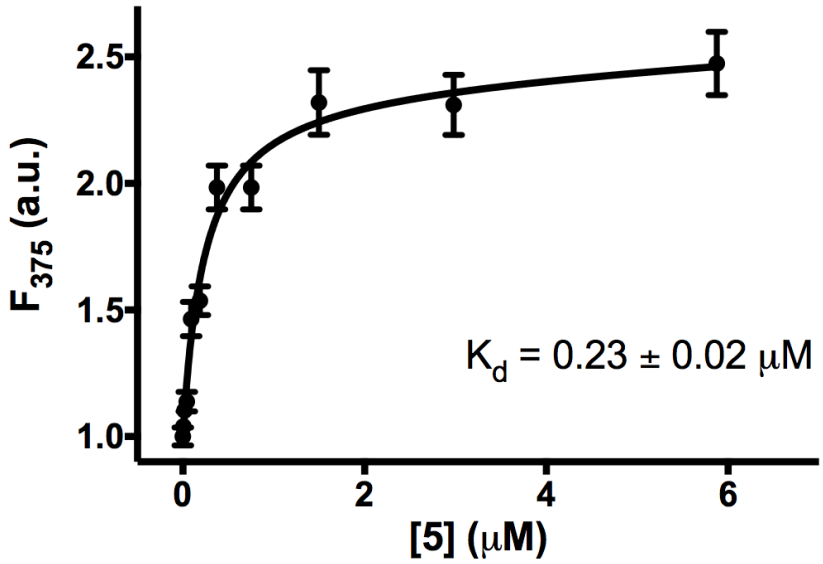
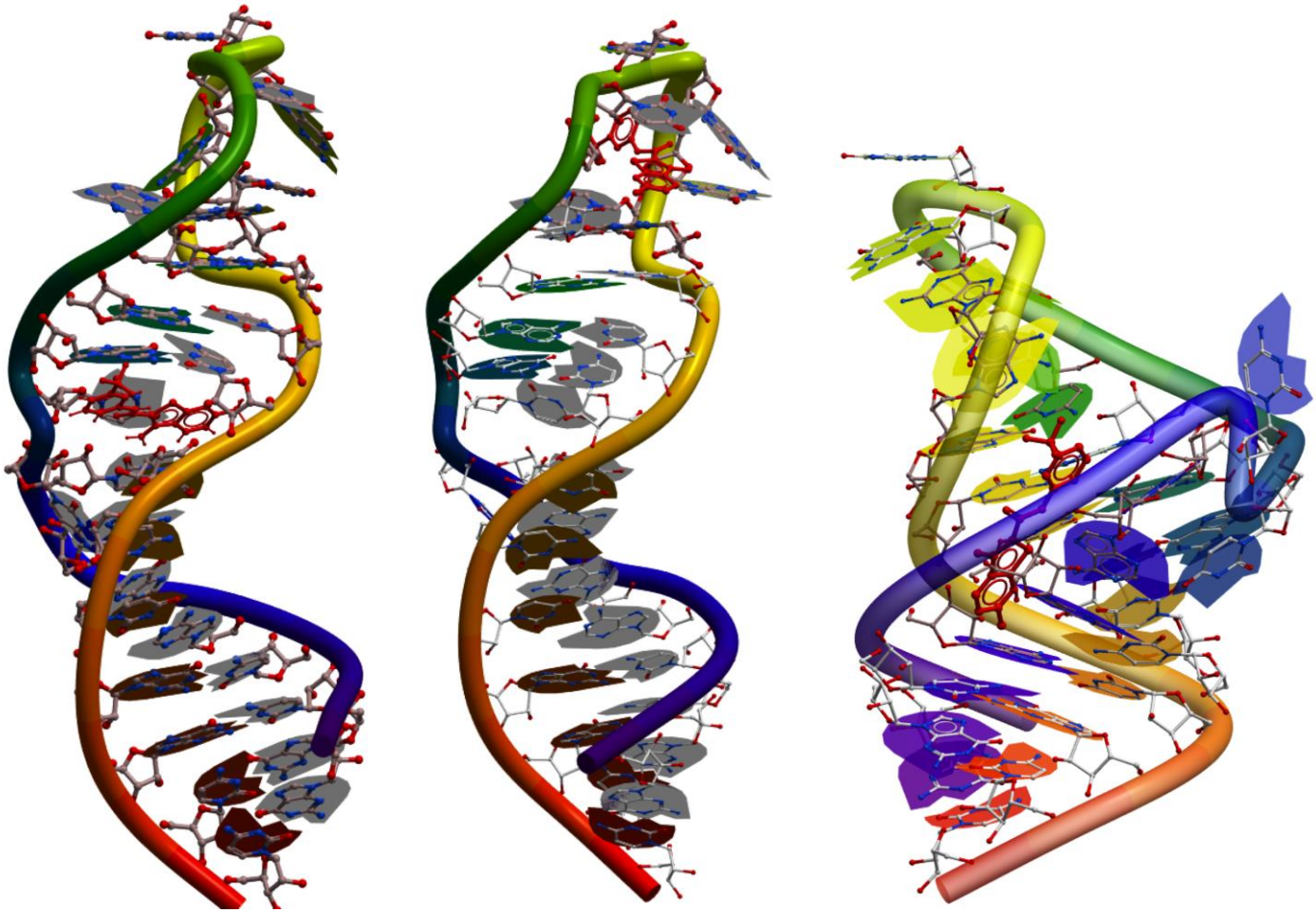


Figure SI-6. Docking compound **4** to multiple known TAR conformations



	1LVJ (Bulge)	1LVJ (Loop)	2L8H (Bulge)
ICM Score:	**-28.46**	**-22.02**	**-24.18**
Other forces: (van der Waals, hydrogen bonding, hydrophobic forces)	**-39.06**	**-41.33**	**-38.31**
Electrostatics:	**6.21**	**14.21**	**14.6**

Docking studies were performed with ICM (Molsoft) using TAR structures from the PDB (listed below) and compound **4**. Binding pockets on TAR were defined both using the ICM PocketFInder module as well as by docking **4** to known small molecule binding sites. For each of the structures, compound **4** was docked both to all identified binding sites. Docking simulations were carried out at a thoroughness of 10 (the maximum number of Monte Carlo iterations). Each simulation provided ~15-20 poses, each of which was visually inspected after completion of the simulations. The three best scoring poses are illustrated above, with compound **4** drawn in red. In each case, the relative contribution of non-electrostatic forces (van der Waals, hydrophobic interactions, hydrogen bonding interactions) and electrostatics to the binding score are indicated.

Structures Used in Docking Simulations:

1LVJ (acetylpromazine-bound)
1QD3 (neomycin-bound)
1UTS (synthetic compound-bound)
1UUD (synthetic compound-bound)
1UUI (synthetic compound-bound)
2L8H (synthetic compound-bound)