Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information for

Fluorescent peptide displacement as a general assay for screening small molecule libraries against RNA

Neeraj N. Patwardhan, Zhengguo Cai, Colby N. Newson, and Amanda E. Hargrove*.

Index

General Experimental Methods:

RNA Preparation: RNA with the following sequences were purchased from Integrated DNA Technologies, dissolved in water at 50 µM conc., and annealed in water by heating to 95 ºC and cooling on ice for 30 min.

RNA Name	Sequence
HIV-1-TAR	5'-GGCAGAUCUGAGCCUGGGAGCUCUCUGCC-3'
HIV-2-TAR	5'-GGCAGAUUGAGCCCUGGGAGGUUCUCUGCC-3'
A-Site	5'- CGGCGUCACACCUCGGGGUGAAGUCGCCG-3'
RRE-IIB	5'-GGUCUGGGCGCAGCGCAAGCUGACGGUACAGGCC-3'

Purity of RNA samples was monitored periodically by gel electrophoresis. Concentrations of RNA were determined using Beer's law with the following extinction coefficients (ε)$_{260}$ of HIV-1-TAR: 268900 M^{-1} cm^{-1}; HIV-2-TAR: 283900 M^{-1} cm^{-1}; A-Site: 270600 M^{-1} cm^{-1}; RRE-IIB: 322900 M^{-1} cm^{-1}

Ligands: Aminoglycoside and known RNA binding small molecule ligands were purchased from commercial suppliers and used without further purification. Amiloride derivatives were synthesized in-house.[1] Small molecule stock solutions (50 mM) were prepared by dissolving them in either water or DMSO and diluted to appropriate stock concentrations in the assay buffer containing 15% DMSO.

Binding assays for Tat peptide to different RNA: Tat peptide [N-(5-FAM)-AAARKKRRQRRRAAAK(TAMRA)- C] was purchased from Lifetein and dissolved at a concentration of 100 nM in the appropriate assay buffer (See Table S1). RNA stock solutions were serially diluted in assay buffer from 0 - 1 µM concentrations. The Tat peptide solution (10 µL) and RNA solution (10 µL) were combined in a Corning™ low volume round-bottom black 384 well-plates, shaken on a platform stirrer for 5 min, centrifuged at 4000 rpm for 1 min, and allowed to incubate for 30 min (Final Tat concentration = 50 nM, final RNA concentrations = 0 - 500 nM). The 384 well-plates were then read on Spectramax-i3™ (Molecular Devices), or Clariostar™ monochromator (BMG Labtech) microplate readers using the excitation and emission wavelengths of 485 nm (FAM) and 590 nm (TAMRA) respectively. The fluorescence intensities at each RNA concentrations were normalized to the Tat-only control wells. Dissociation constants (K_d) were calculated by fitting the observed relative fluorescence intensity values at each concentration to equation (1) using the GraphPad

Prism curve fitting software. Assays were minimally conducted in triplicate and each replicate contained three technical replicates.

$$Y = Bmax * X/(Kd + X) + NS * X + Background \quad \text{(Equation S1)}$$

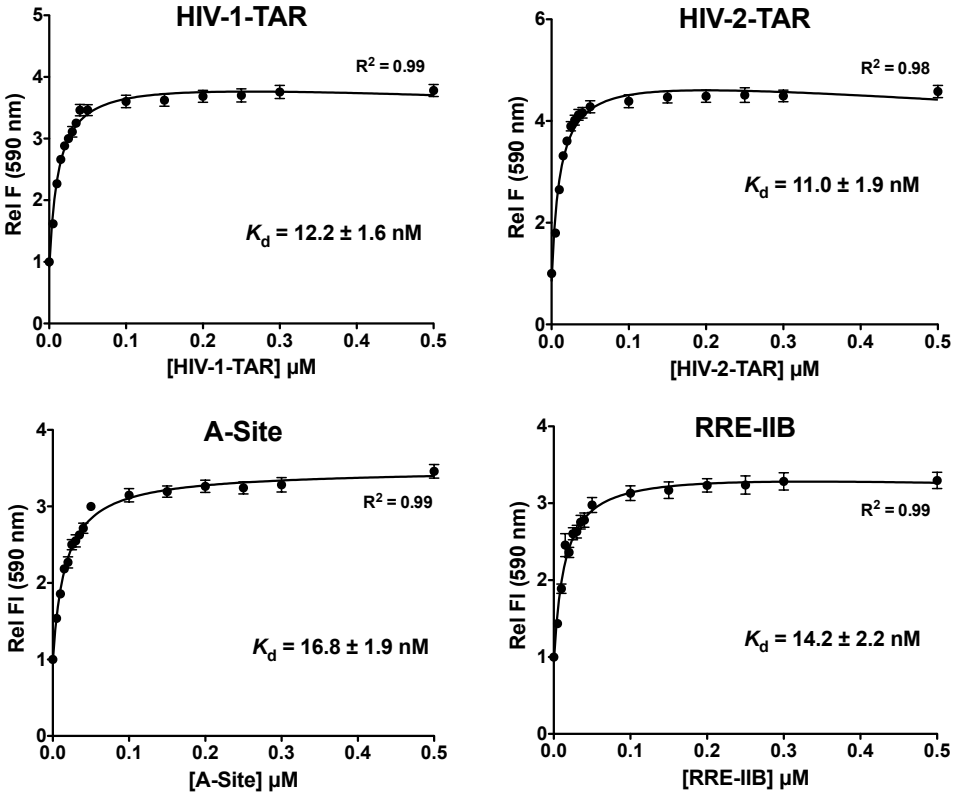
Where, Y = Total binding; X = Conc. of added ligand; Bmax = Maximum binding; K_d = Equilibrium dissociation constant; NS: Slope of Nonlinear regression; Background: Measured binding without the ligand.

Table S1: Binding constants (K_d) for interaction of Tat peptide against 4 RNA under different buffer conditions. Errors presented are the standard errors of mean calculated from three replicates.

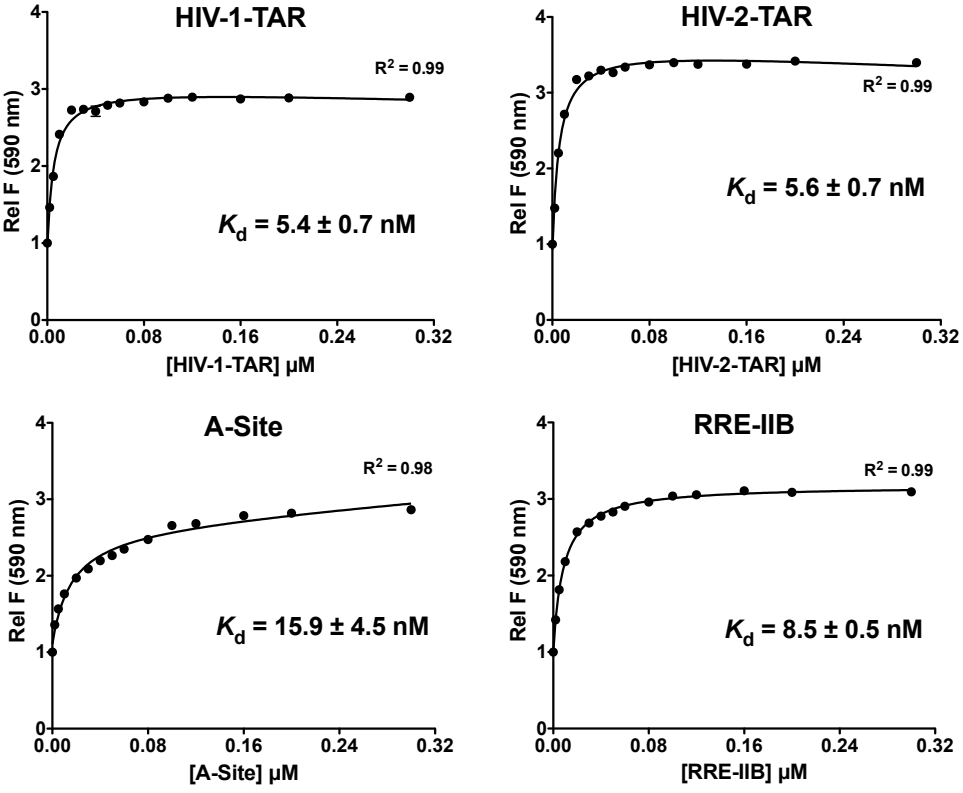
Entry	Buffer	K_d (nM)			
		HIV-1-TAR	**HIV-2-TAR**	**A-Site**	**RRE-IIB**
1	50 mM Tris, **50 mM KCl,** 0.01% Triton-X-100, pH = 7.4	22.1 ± 6.9	24.9 ± 8.1	57.1 ± 12.2	32.7 ± 6.6
2	50 mM Tris, **10 mM KCl,** 0.01% Triton-X-100, pH = 7.4	12.2 ± 1.6	11.0 ± 1.9	16.8 ± 1.9	14.2 ± 2.2
3	50 mM Tris, **20 mM KCl,** 0.01% Triton-X-100, pH = 7.4	5.4 ± 0.7	5.6 ± 0.7	15.9 ± 4.5	8.5 ± 0.5
4	50 mM Tris, 50 mM KCl, **0.5 mM $MgCl_2$,** 0.01% Triton-X-100, pH = 7.4	34.8 ± 3.8	27.0 ± 3.1	499 ± 398	109 ± 25.2
5	50 mM Tris, 50 mM KCl, **1.0 mM $MgCl_2$,** 0.01% Triton-X-100, pH = 7.4	63.9 ± 6.3	35.4 ± 2.9	Poor Fit	211 ± 75.2
6	50 mM Tris, 50 mM KCl, **4 mM $MgCl_2$,** 0.01% Triton-X-100, pH = 7.4	Poor Fit	287 ± 232	Poor Fit	Poor Fit
7	10 mM **NaH_2PO_4**, 25 mM NaCl, 4mM $MgCl_2$, 0.5 mM EDTA, 0.01% Triton-X-100, pH = 7.4	254 ± 269	100 ± 42	Poor Fit	Poor Fit
8	10 mM **NaH_2PO_4**, 25 mM NaCl, 0.5 mM EDTA, 0.01% Triton-X-100, pH = 7.4	10.3 ± 1.2	8.1 ± 1.0	15.0 ± 3.0	12.3 ± 3.2
9	**PBS:** 10 mM NaH_2PO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.01% Triton-X-100, pH = 7.4	133.3 ± 51.2	77.6 ± 12.3	49.6 ± 67.4	80.0 ± 73.3

Binding Curves for all results listed in Table S1

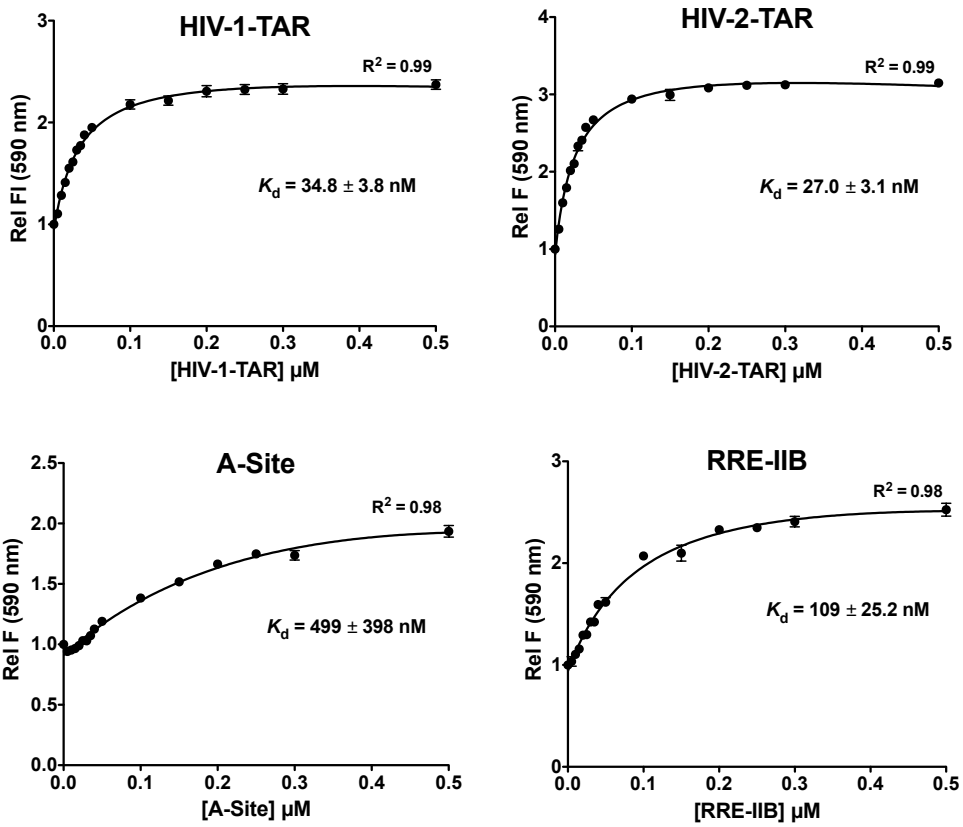
i. 50 mM Tris, 10 mM KCl, 0.01% Triton-X-100, pH = 7.4



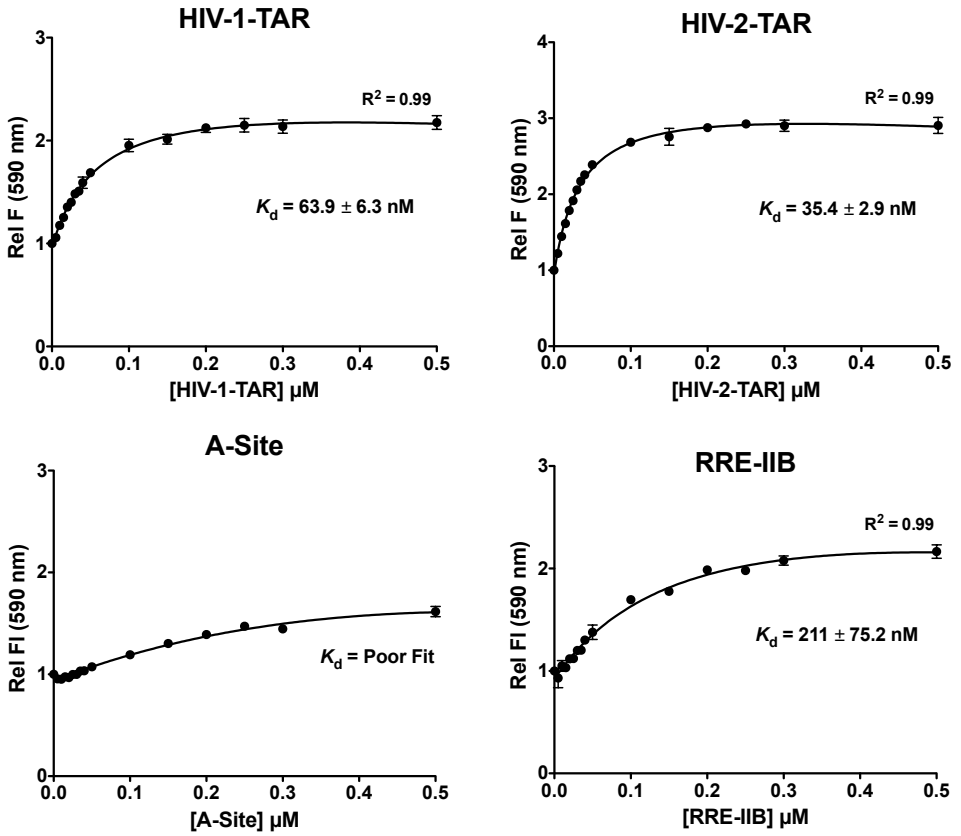
ii. 50 mM Tris, 20 mM KCl, 0.01% Triton-X-100, pH = 7.4



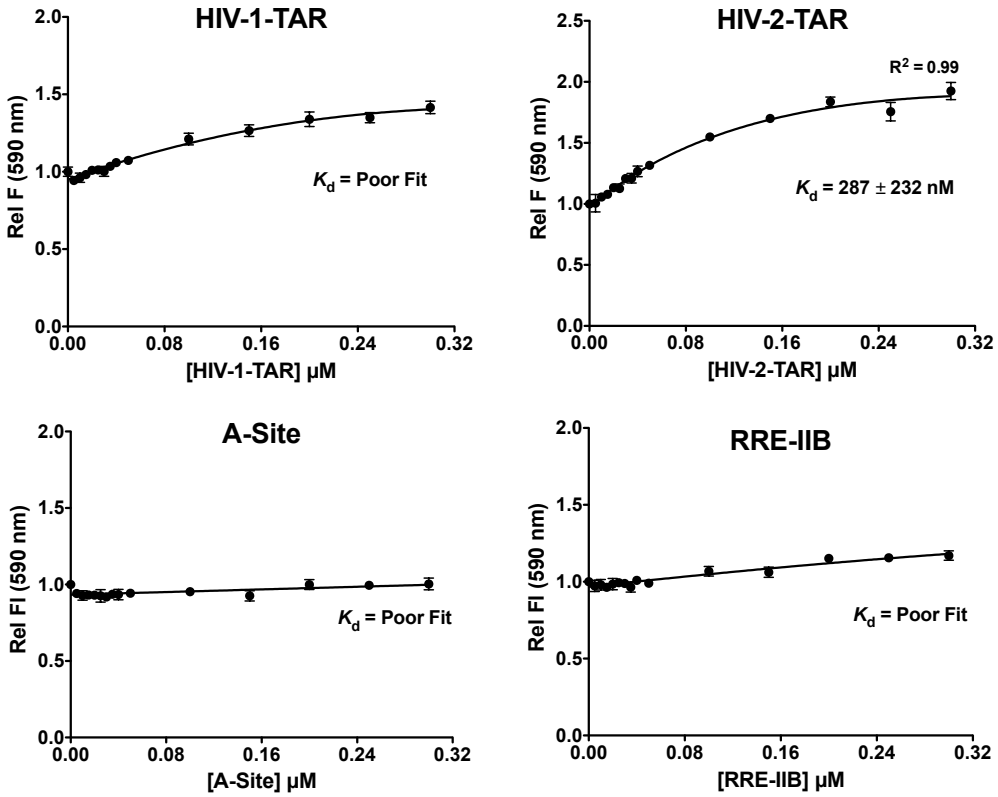
iii. 50 mM Tris, 50 mM KCl, 0.5 mM $MgCl_2$, 0.01% Triton-X-100, pH = 7.4



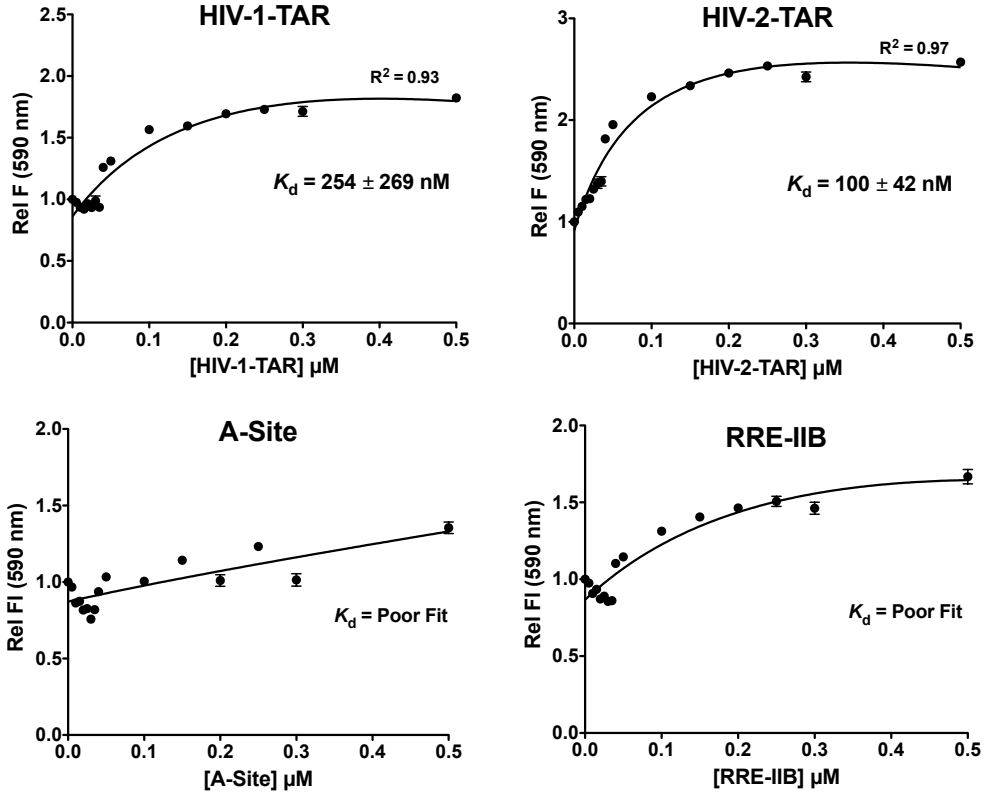
iv. 50 mM Tris, 50 mM KCl, 1.0 mM $MgCl_2$, 0.01% Triton-X-100, pH = 7.4



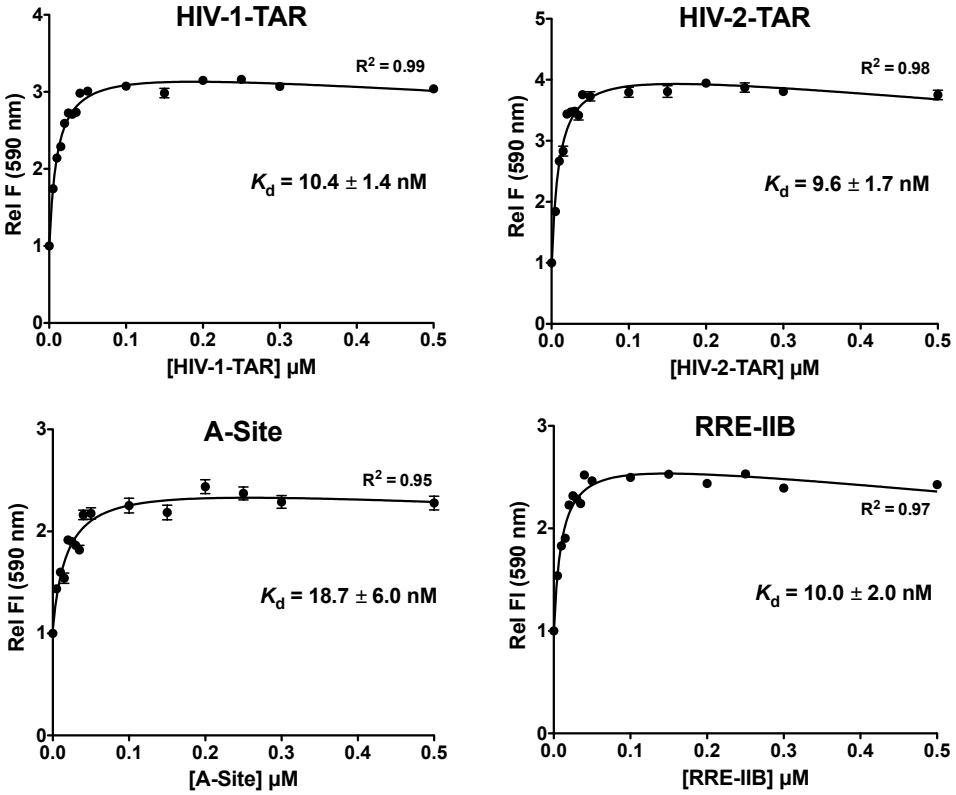
v. 50 mM Tris, 50 mM KCl, 4 mM $MgCl_2$, 0.01% Triton-X-100, pH = 7.4



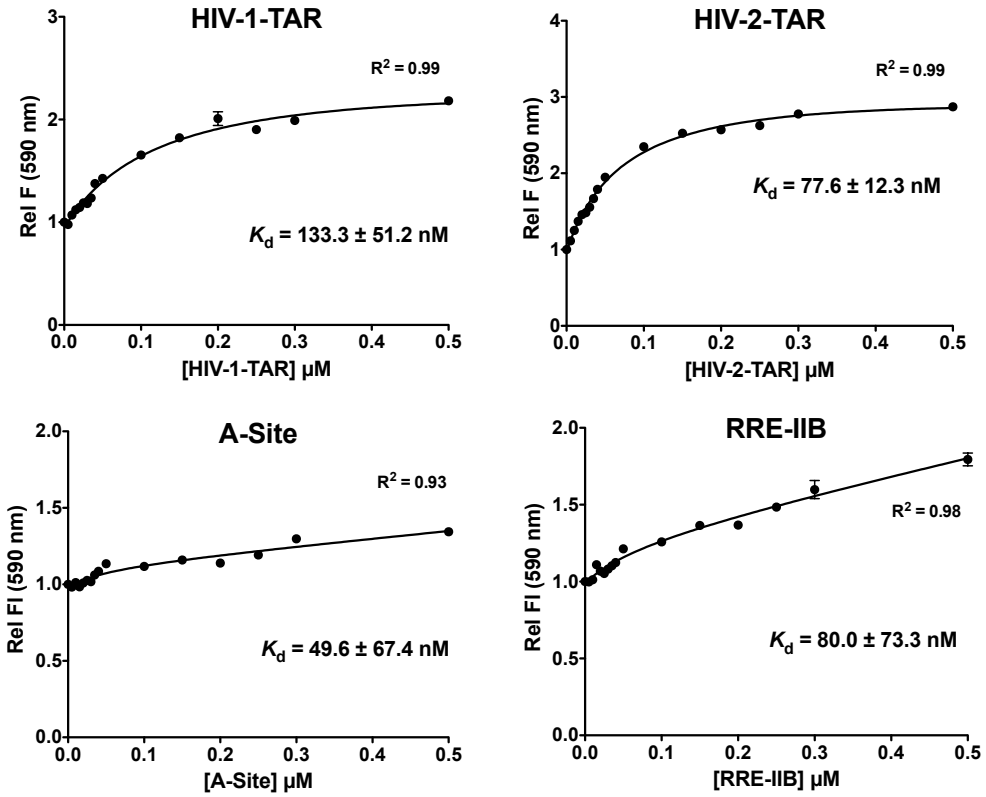
vi. 10 mM NaH_2PO_4, 25 mM NaCl, 4mM $MgCl_2$, 0.5 mM EDTA, 0.01% Triton-X-100, pH = 7.4



vii. 10 mM NaH_2PO_4, 25 mM NaCl, 0 mM $MgCl_2$, 0.5 mM EDTA, 0.01% Triton-X-100, pH = 7.4



viii. **PBS**: 10 mM NaH_2PO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.01% Triton-X-100, pH = 7.4



Tat peptide displacement based screening of small molecule libraries: Screening assays were performed in a 50 mM Tris buffer containing 50 mM KCl, 5% DMSO, and 0.01% v/v of Triton-X-100 at pH of 7.4. Tat peptide was diluted at a concentration of 150 nM in the assay buffer. RNA solutions were prepared by diluting stocks in the assay buffer at the following concentrations - HIV-1-TAR: 120 nM, HIV-2-TAR: 120 nM, A-Site: 360 nM, and RRE-IIB: 225 nM. These concentrations were chosen to provide best fluorescence intensities possible in the bound state [See Equation 2 and Table S2 for calculations of the fraction of Tat bound to RNA (Fb^0) at these concentrations]. Small molecules were diluted to concentrations of 30 µM, and 150 µM. Tat peptide (6 µL), RNA (6 µL), and small molecule solution (6 µL) were combined in a 384 well-plate. Final concentrations in each assay well were as follows- Tat peptide: 50 nM; RNA conc: 40 nM (HIV-1 and 2-TAR), 120 nM (A-Site), 75 nM (RRE-IIB); small molecule: 0 µM, 10 µM, and 50 µM, and 5% DMSO. The well-plate was shaken on a platform stirrer for 5 min, centrifuged at 4000 rpm for 1 min, and allowed to incubate in dark for 30 min and read using the excitation and emission wavelengths of 485 nm (FAM) and 590 nm (TAMRA) respectively. % Displacement of Tat peptide from RNA was calculated using the equation (2). Each screening experiment was performed in triplicate and each replicate contains a technical triplicate.

$$\%\mathrm{FID} = 100 - \left(100 \times \frac{F}{F0}\right) \qquad \text{(Equation 2)}$$

$F = F_{(\text{ligand + RNA + Tat})} - F_{(\text{Buffer + Tat})} - F_{(\text{RNA+Ligand})}$

$F_0 = F_{(\text{RNA+Tat})} - F_{(\text{Buffer+Tat})}$

Molecules that show >25% FID at both tested concentrations of 10 µM, or 50 µM were labeled as hits of the screening assay.

Fraction of Tat Bound to RNA (Fb_0): Total fraction of the Tat peptide bound as a function of the binding constant of each RNA was calculated using reported methods.[2]

Table S2: Fraction of Tat bound to RNA.

RNA	K_d (nM)	RNA Conc nM	Fb_0
HIV-1-TAR	23.6 ± 3.6	40	0.48
HIV-2-TAR	19.5 ± 4.0	40	0.46
A-Site	51.3 ± 3.4	120	0.67
RRE-IIB	35.1 ± 5.2	75	0.56

Z' Scores: Z' Scores for each RNA: Tat system were calculated using the equation shown below, using 48 data points for each RNA:Tat complex –

$$Z' = 1 - \left(\frac{3\,(\sigma_{(positive)} + \sigma_{(negative)})}{|\,\mu_{(positive)} - \mu_{(negative)}|} \right)$$

$\mu_{(positive)}$ = Mean Fluorescence Intensity for Tat + RNA
$\mu_{(negative)}$ = Mean Fluorescence Intensity for Tat alone
$\sigma_{(positive)}$ = Standard deviation for positive
$\sigma_{(negative)}$ = Standard deviation for negative

Table S3: Z'-Scores for each RNA-Tat combination.

RNA	K_d (nM)	RNA Conc nM	Fb_0	Z' Score
HIV-1-TAR	23.6 ± 3.6	40	0.48	0.54
HIV-2-TAR	19.5 ± 4.0	40	0.46	0.65
A-Site	51.3 ± 3.4	120	0.67	0.35
A-Site (20 mM KCl Buffer)	51.3 ± 3.4	120	0.67	0.47
RRE-IIB	35.1 ± 5.2	75	0.56	0.48

Raw Data for Screening against four RNA Targets.

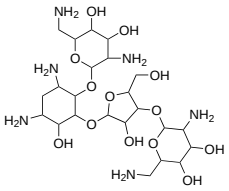
Table S4: Data at 10 µM Small Molecule Concentration.

Molecule	HIV-1-TAR		HIV-2-TAR		A-Site		RRE-IIB	
	% FID	Stdev	% FID	Stdev	% FID	Stdev	% FID	Stdev
Neomycin	40.56	13.15	39.86	8.14	31.13	4.69	36.51	19.00
Apramycin	-0.20	12.05	0.89	12.14	22.68	4.60	12.89	4.87
Paromomycin	5.51	9.44	8.19	6.47	33.85	0.46	24.43	1.42
Tobramycin	19.75	5.01	27.90	13.18	34.50	2.26	32.14	20.75
Kanamycin	-8.50	6.74	-3.23	18.06	23.77	1.53	5.93	3.81
Gentamycin	17.21	7.37	24.95	11.86	33.88	2.60	42.89	3.04
Streptomycin	9.77	8.38	-4.14	10.35	5.61	3.69	1.91	6.40
Sisomycin	9.27	2.08	15.29	14.72	31.79	2.66	31.48	2.40
Amikacin	6.89	6.85	1.80	6.03	19.37	4.10	20.16	18.35
Neamine	11.97	0.35	22.56	1.73	18.05	3.74	11.25	2.56
2-DOS	-8.01	1.48	-2.48	0.89	2.17	0.94	-2.67	1.59
Mitoxantrone	38.09	1.53	56.65	0.05	41.11	1.35	42.79	0.82
Hoechst 33258	33.04	1.30	34.81	0.43	48.17	5.75	39.74	0.82
Arginamide	-10.48	0.93	-0.71	0.83	-0.19	1.30	-1.56	0.38
Tetracycline	-8.10	5.72	-4.73	1.65	0.64	3.36	-2.07	0.44
Hoechst 33342	24.88	6.05	32.68	1.06	31.04	2.09	33.71	2.84
(+) Usnic Acid	-6.51	1.75	-2.68	3.49	-4.59	3.22	3.60	0.72
Yohimbine	-2.61	0.43	-17.02	0.30	-4.53	0.91	1.01	0.00
Theophilline	0.12	0.02	-2.22	0.05	1.50	1.30	-0.85	0.00
L-Citrulline	0.64	0.90	-4.05	1.87	0.42	4.21	-1.48	0.49
DMA-001	0.83	2.48	1.47	2.58	2.90	3.59	5.35	3.80
DMA-019	1.69	2.84	1.10	5.95	0.11	2.50	7.36	3.05
DMA-096	4.94	2.19	-5.53	17.62	8.72	13.68	4.56	6.21
DMA-097	-8.66	5.94	-0.23	5.55	12.24	4.56	-2.29	7.54
DMA-101	-0.43	8.69	5.38	7.69	5.55	5.40	1.41	0.64
DMA-132	10.06	12.03	3.84	6.43	4.53	0.96	-0.35	1.67
DMA-135	17.40	2.48	23.16	2.56	19.25	3.71	20.87	1.19
DMA-155	0.68	1.12	0.52	3.39	4.12	2.96	5.69	5.21
DMA-165	7.38	2.15	17.76	22.29	5.91	1.39	6.56	4.15
DMA-169	43.86	5.99	30.25	1.34	1.34	5.31	-0.78	7.30

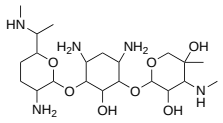
Table S5: Data at 50 µM Small Molecule Concentration.

Molecule	HIV-1-TAR		HIV-2-TAR		A-Site		RRE-IIB	
	% FID	Stdev	% FID	Stdev	% FID	Stdev	% FID	Stdev
Neomycin	54.19	2.32	55.39	2.50	27.98	2.47	58.12	1.95
Apramycin	8.16	8.23	7.70	10.37	30.17	1.01	37.45	12.34
Paromomycin	19.74	8.63	22.67	3.23	31.99	3.00	47.46	9.47
Tobramycin	34.92	14.75	37.47	10.46	37.28	1.51	55.34	11.06
Kanamycin	-3.52	11.36	3.19	5.38	32.35	0.63	38.43	12.93
Gentamycin	39.26	8.22	45.31	4.21	33.81	1.91	53.08	17.00
Streptomycin	25.55	7.44	24.44	13.06	17.69	0.03	29.36	16.79
Sisomycin	21.61	9.24	23.32	15.84	34.10	1.81	51.00	5.27
Amikacin	19.76	13.28	11.29	4.60	30.28	2.29	34.27	5.74
Neamine	-9.00	0.49	37.24	1.19	32.65	1.96	31.38	1.82
2-DOS	0.87	1.81	1.50	0.73	0.78	2.21	4.53	1.85
Mitoxantrone	42.20	0.49	60.19	0.37	42.84	2.10	48.59	0.59
Hoechst 33258	86.79	0.56	89.78	0.08	86.64	0.73	85.75	0.64
Arginamide	-2.24	1.20	18.54	0.46	0.88	4.16	-3.23	1.53
Tetracycline	-11.70	0.15	-2.01	2.54	0.28	3.21	-4.04	3.69
Hoechst 33342	84.91	0.83	90.18	1.84	83.54	0.43	83.14	0.24
(+) Usnic Acid	-3.90	2.26	-2.49	0.57	-7.75	3.40	8.22	2.56
Yohimbine	-4.50	0.11	-16.36	0.27	-3.24	6.61	3.42	0.00
Theophilline	-6.79	0.07	1.30	0.04	2.45	1.52	1.03	0.00
L-Citrulline	0.19	4.27	-0.05	3.19	1.28	6.06	-1.69	2.36
DMA-001	2.88	10.95	7.61	5.20	7.72	4.06	18.05	7.42
DMA-019	5.38	5.42	9.90	2.78	3.47	1.95	14.84	4.09
DMA-096	7.14	3.65	4.83	19.91	9.11	0.91	12.71	6.00
DMA-097	2.28	2.07	0.98	3.35	25.42	12.07	4.82	12.26
DMA-101	11.99	2.60	23.29	1.44	17.13	1.36	16.49	5.56
DMA-132	22.68	8.54	13.47	4.71	15.88	5.90	7.55	2.55
DMA-135	50.84	2.98	60.22	2.16	62.98	3.24	59.86	6.44
DMA-155	44.08	24.75	44.53	21.21	81.07	2.56	51.78	33.21
DMA-165	38.72	4.11	34.28	3.00	31.74	0.67	33.27	7.93
DMA-169	62.80	3.04	64.62	1.92	51.08	4.00	41.38	8.82

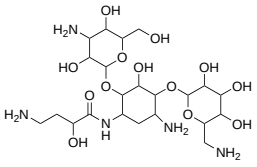
Figure S1: Structures of small molecules tested in displacement assays.



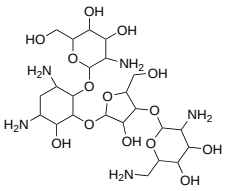
Neomycin B



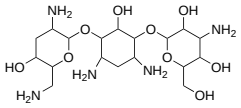
Gentamicin



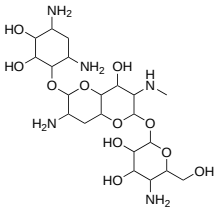
Amikacin



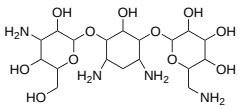
Paromomycin



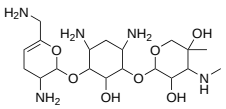
Tobramycin



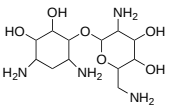
Apramycin



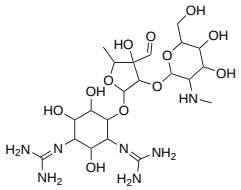
Kanamycin



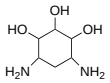
Sisomicin



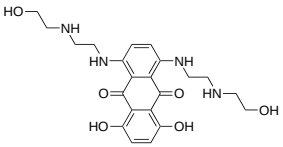
Neamine



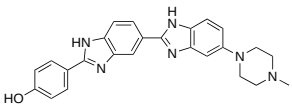
Streptomycin



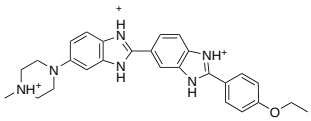
2-Deoxystreptomycin



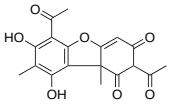
Mitoxantrone



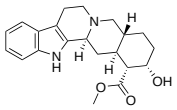
Hoechst 33258



Hoechst 33342

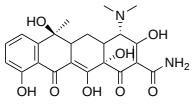


Usnic Acid

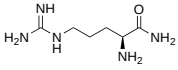


Yohimbine

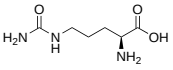
Theophylline



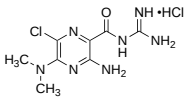
Tetracycline



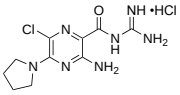
Argininamide



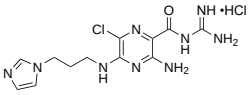
L-Citrulline



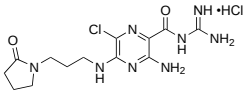
DMA-001



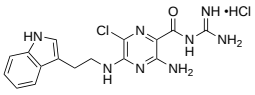
DMA-019



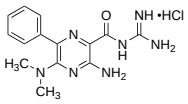
DMA-096



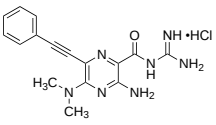
DMA-097



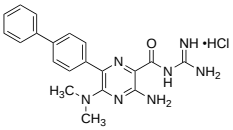
DMA-101



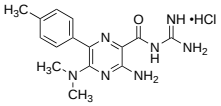
DMA-132



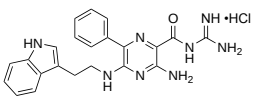
DMA-135



DMA-155



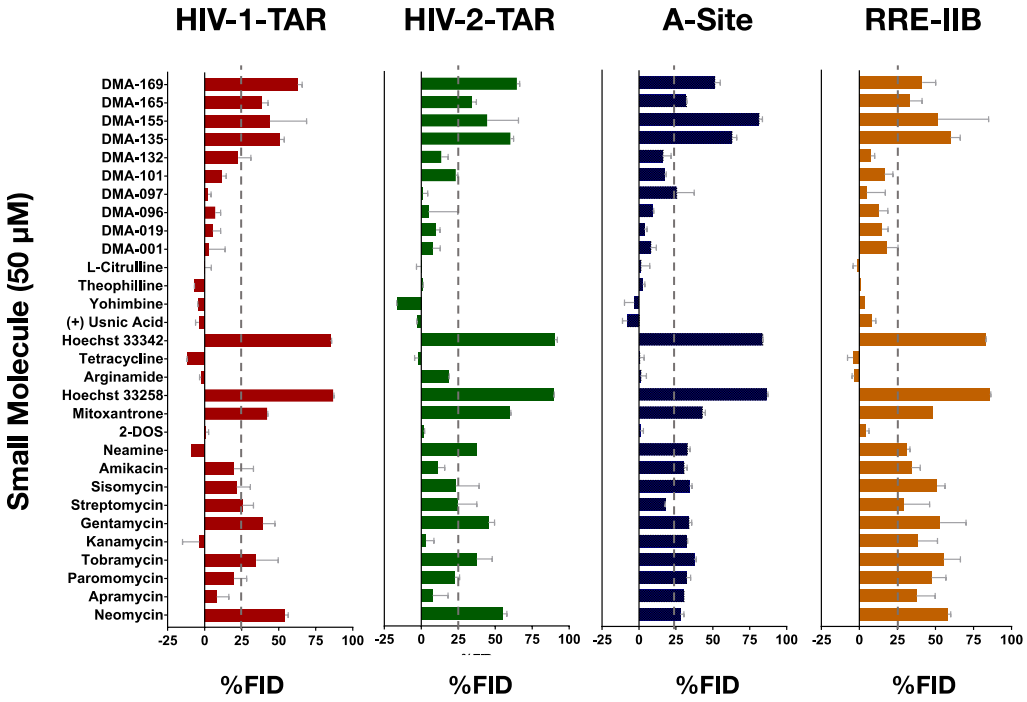
DMA-165



DMA-169

Screening small molecule probes against 4 RNA targets –

Figure S2: Screening at 50 µM SM concentration.



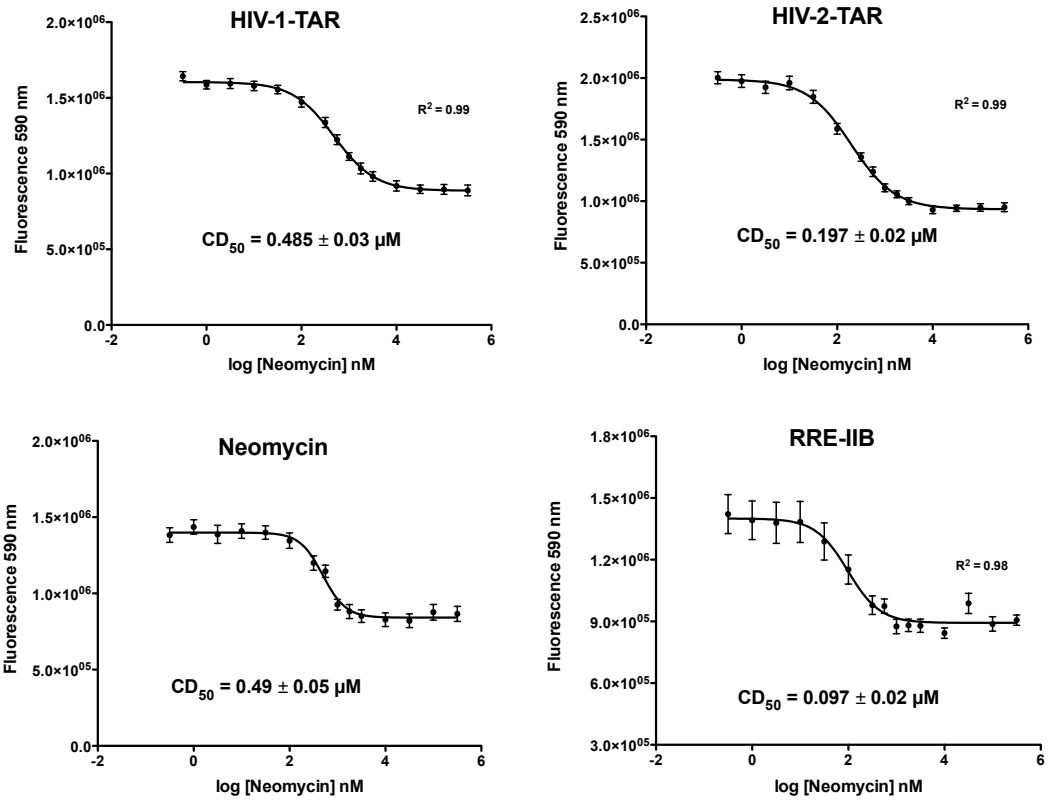
Tat peptide displacement assay for small molecule hits: Displacement assays to measure the activity of small molecule against each RNA studied in the same 50 mM Tris buffer containing 50 mM KCl, 5% DMSO, and 0.01% Triton-X-100 at pH 7.4. RNA and Tat peptide concentrations used are same as the screening assay. Small molecules were diluted in the assay buffer to achieve final assay concentrations between 0-334 µM. Tat peptide (6 µL), RNA (6 µL), and small molecule solution (6 µL) were combined in a 384 well-plate, shaken on a platform stirrer for 5 min, centrifuged at 4000 rpm for 1 min, and allowed to incubate for 30 min before being read using the excitation and emission wavelengths of 485 nm (FAM) and 590 nm (TAMRA) respectively. Observed fluorescence at each small molecule concentration was normalized to the fluorescence of Tat peptide: RNA complex. Competitive displacement of 50% of peptide from RNA was calculated by fitting the normalized data to equation (3) and (4) below using the GraphPad Prism curve fitting software. Assays were minimally conducted in triplicate and each replicate contained three technical replicates. Errors presented are the standard errors of mean.

$$y = A1 + (A2 - A1)(1 + 10(Logx0 - xp)) \quad \text{(Equation 3)}$$

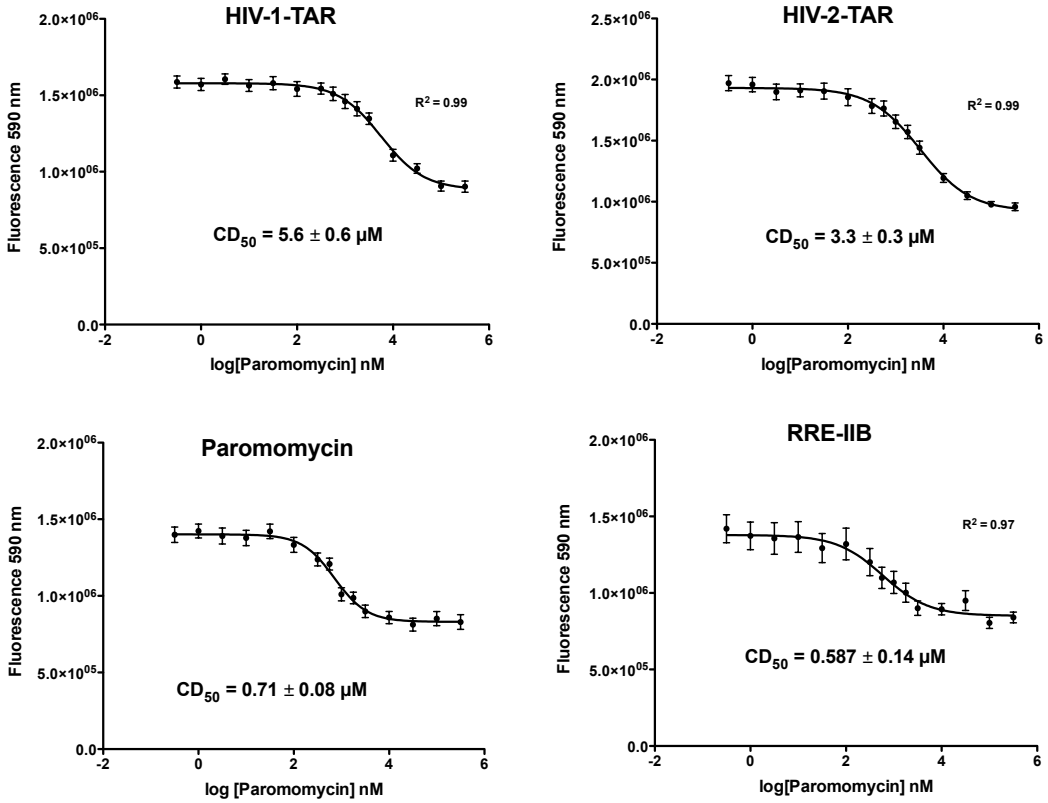
$$CD_{50} = 10^{Logx0} \quad \text{(Equation 4)}$$

Displacement assay curves for small molecules.

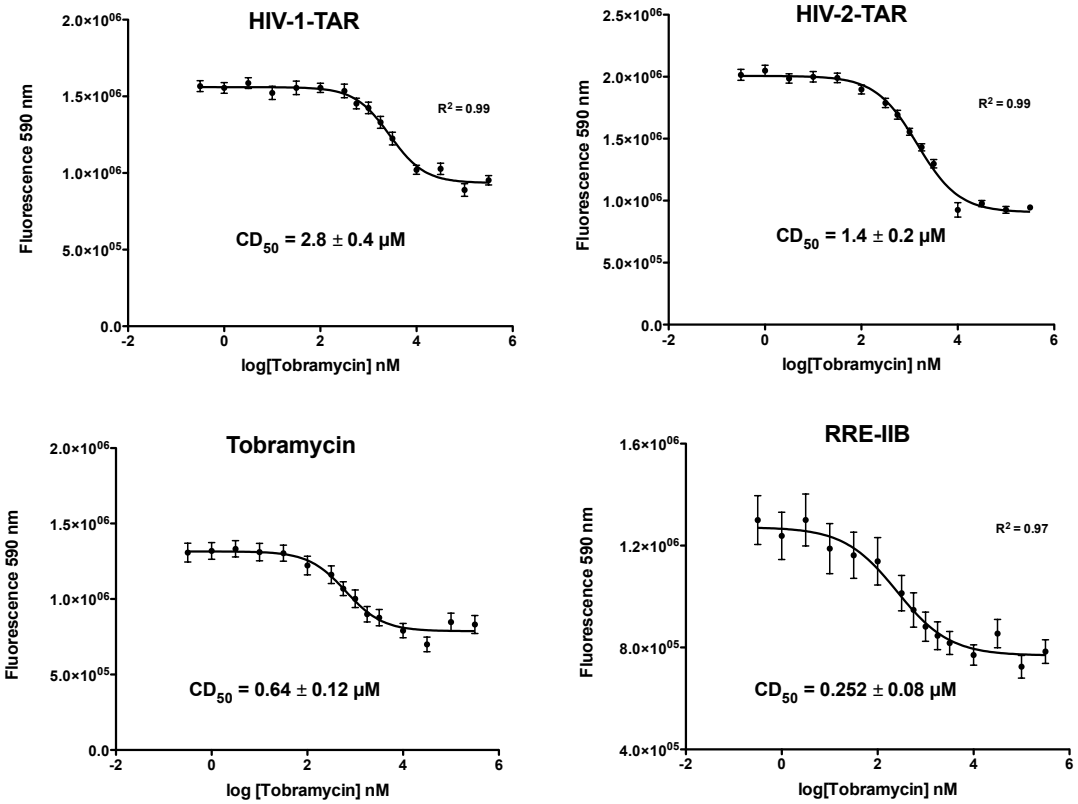
1. Neomycin



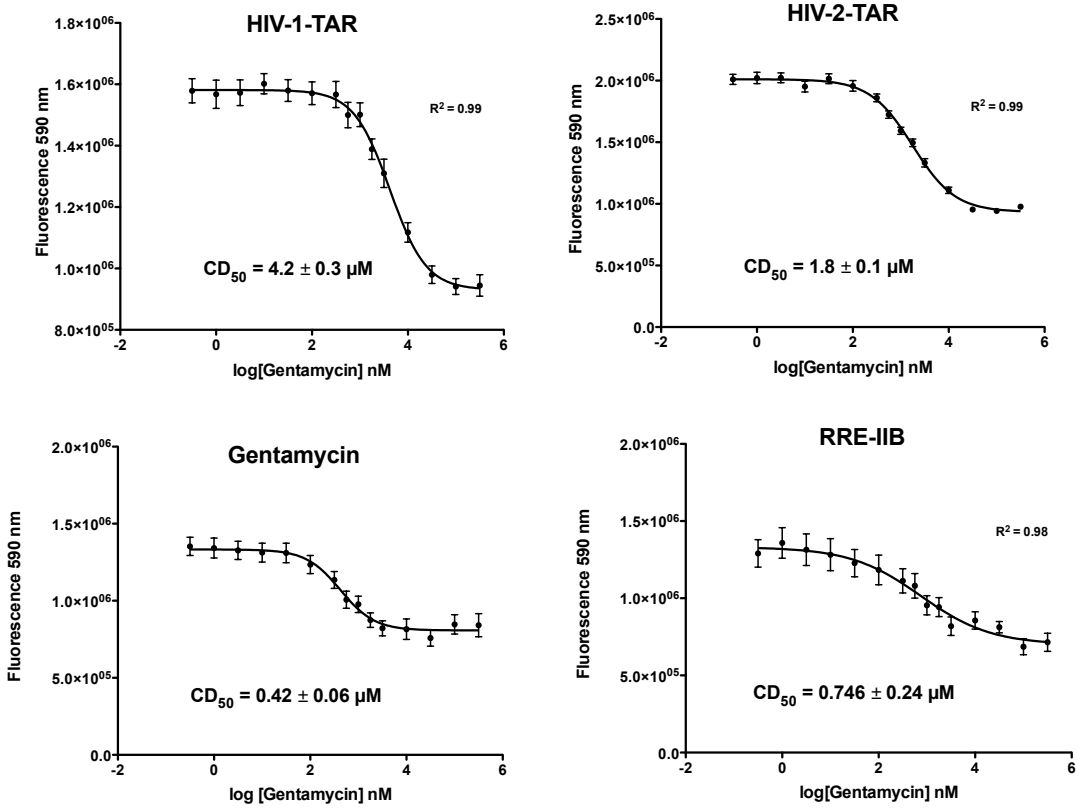
2. Paromomycin



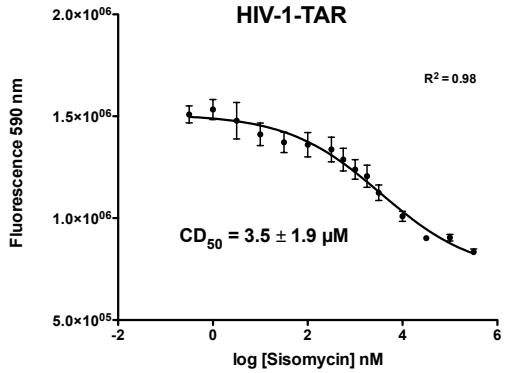
3. Tobramycin

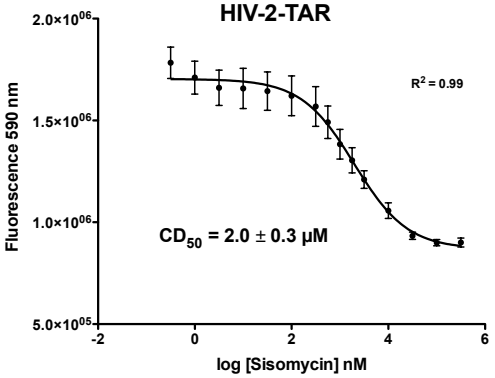


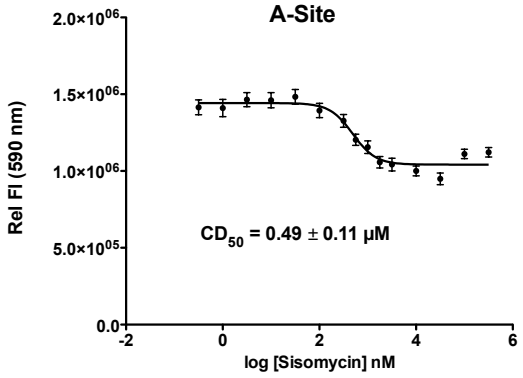
4. Gentamycin

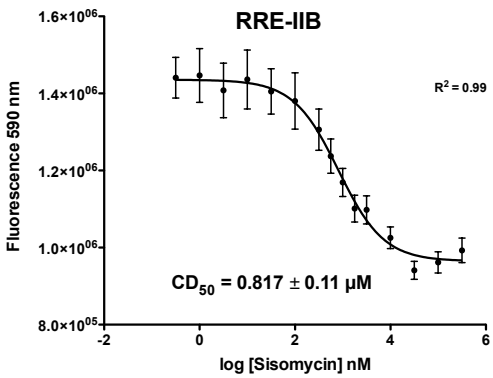


5. Sisomycin

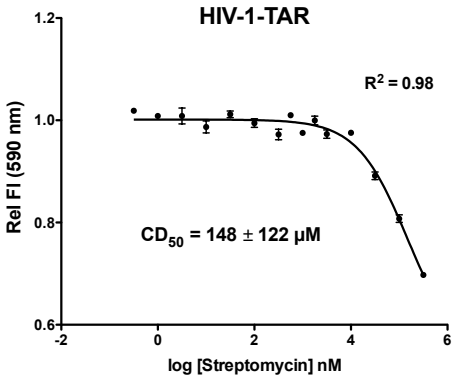


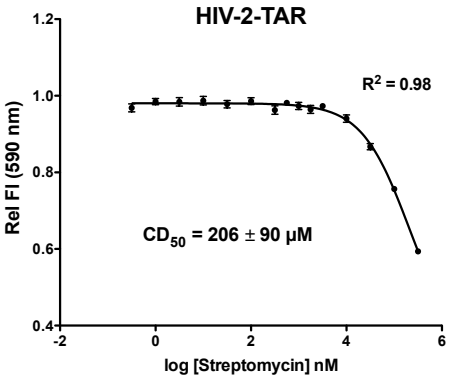


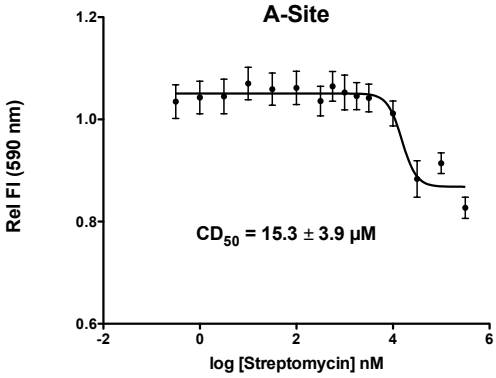


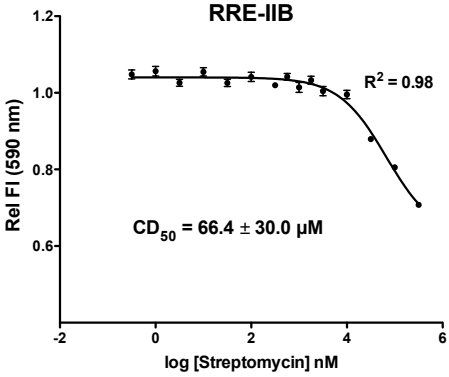


6. Streptomycin

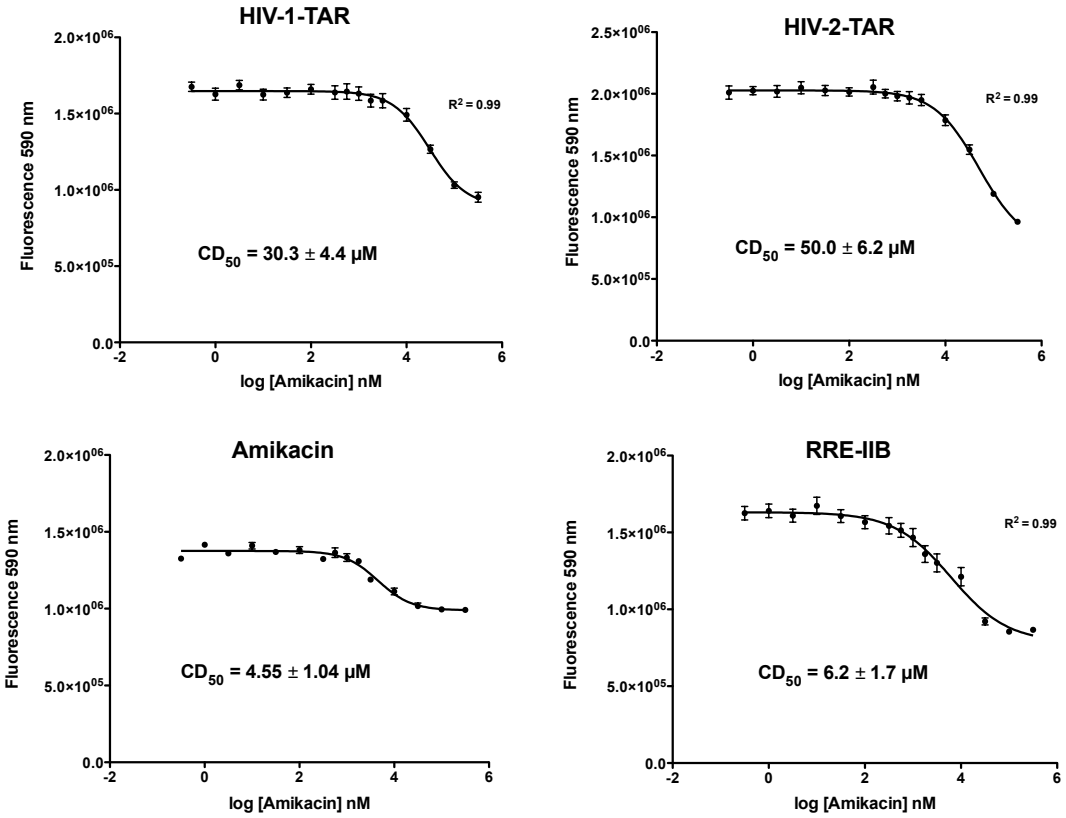




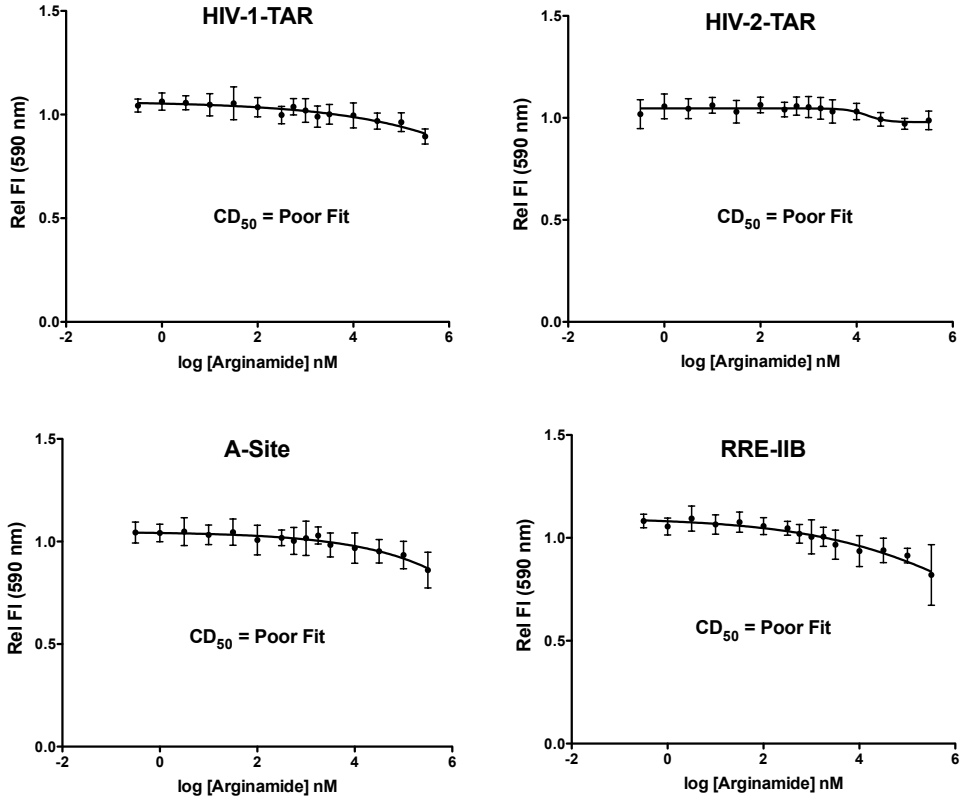




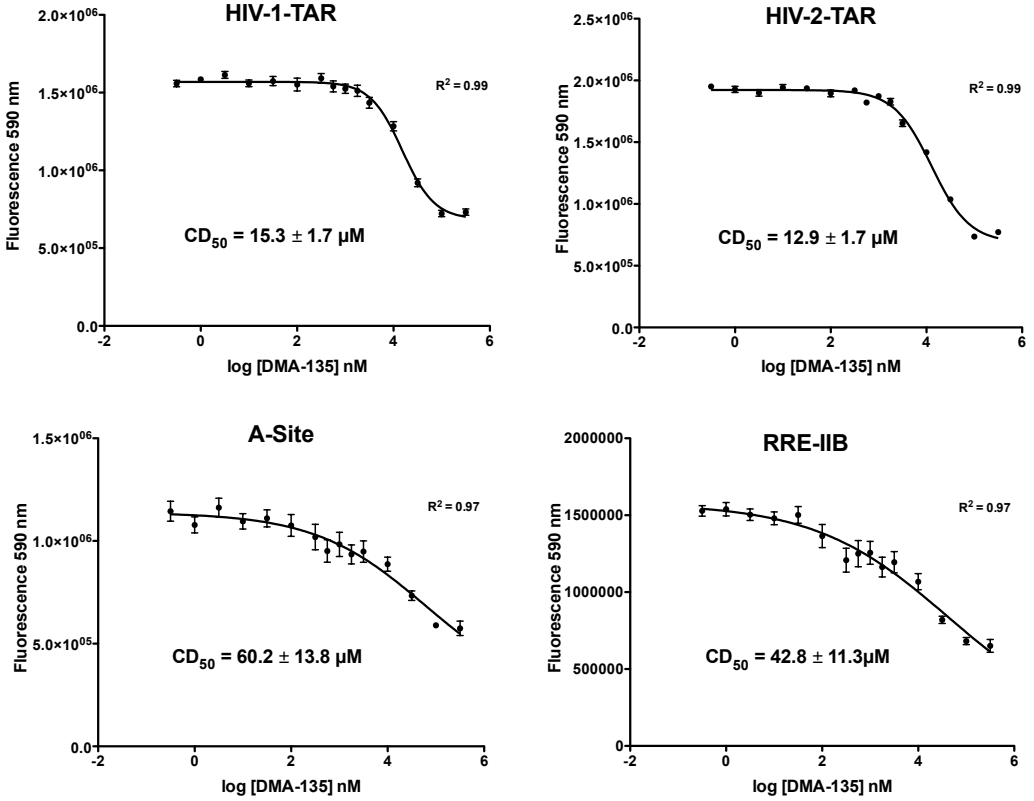
7. Amikacin



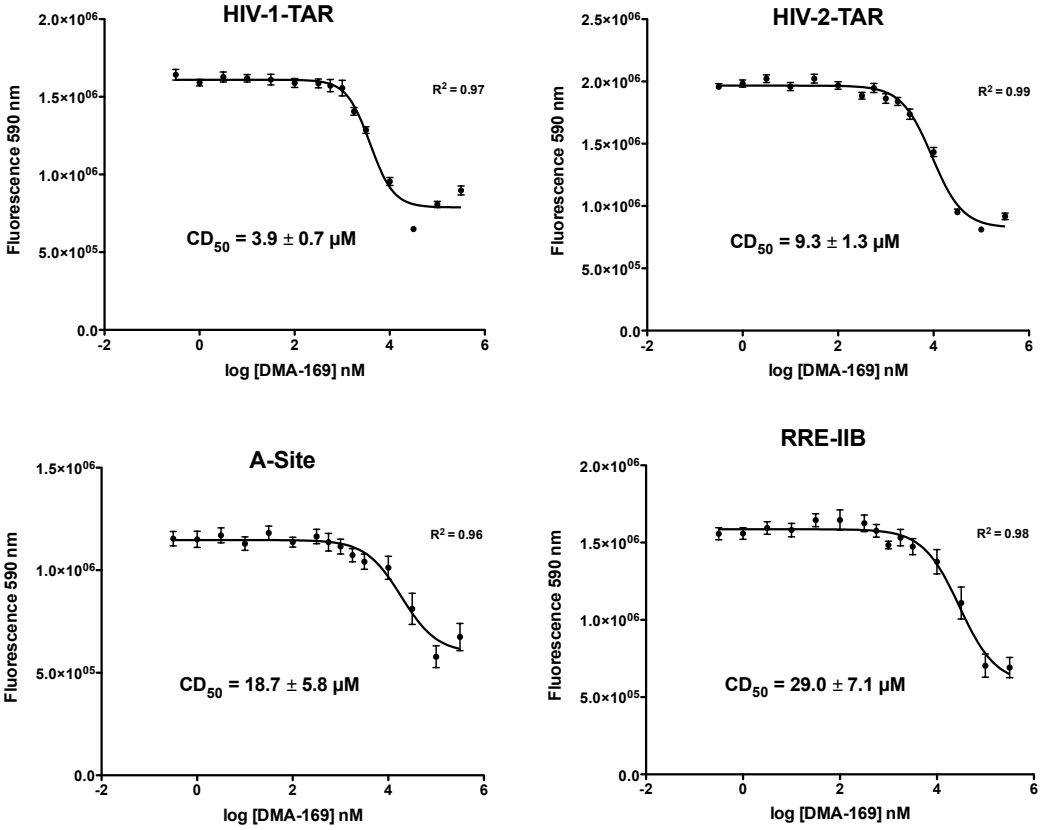
8. Argininamide



9. DMA-135

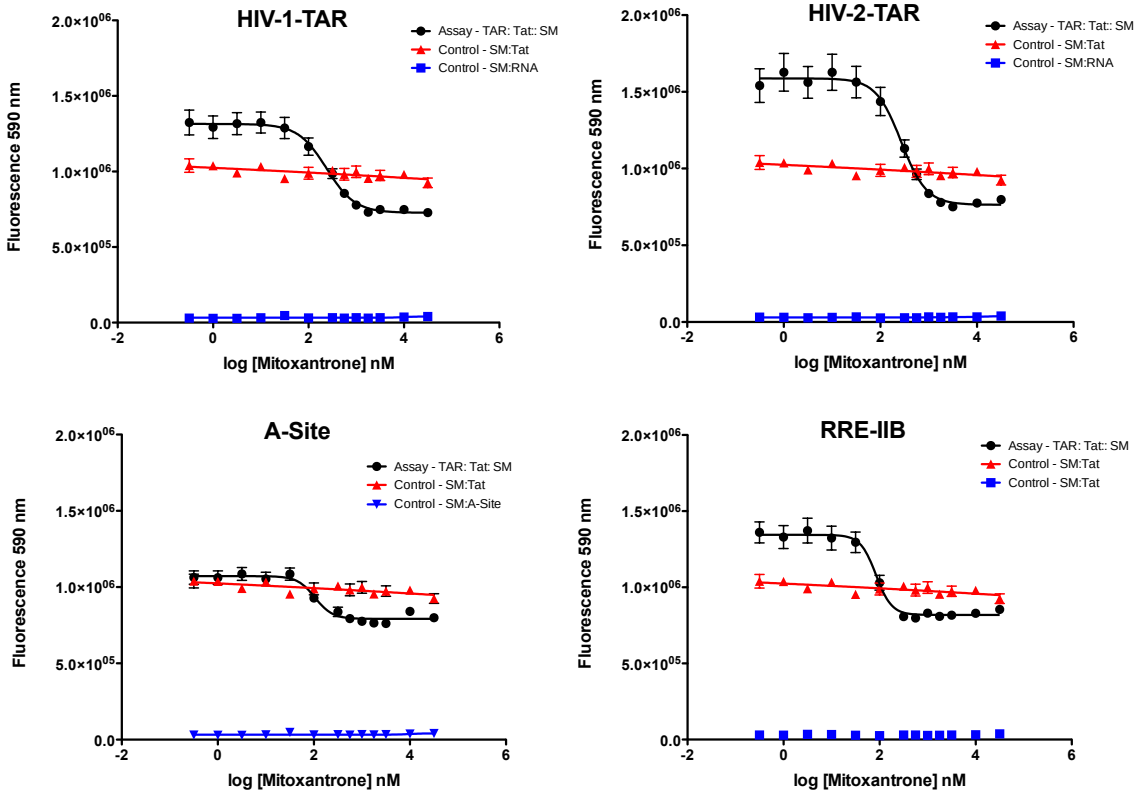


10. DMA-169

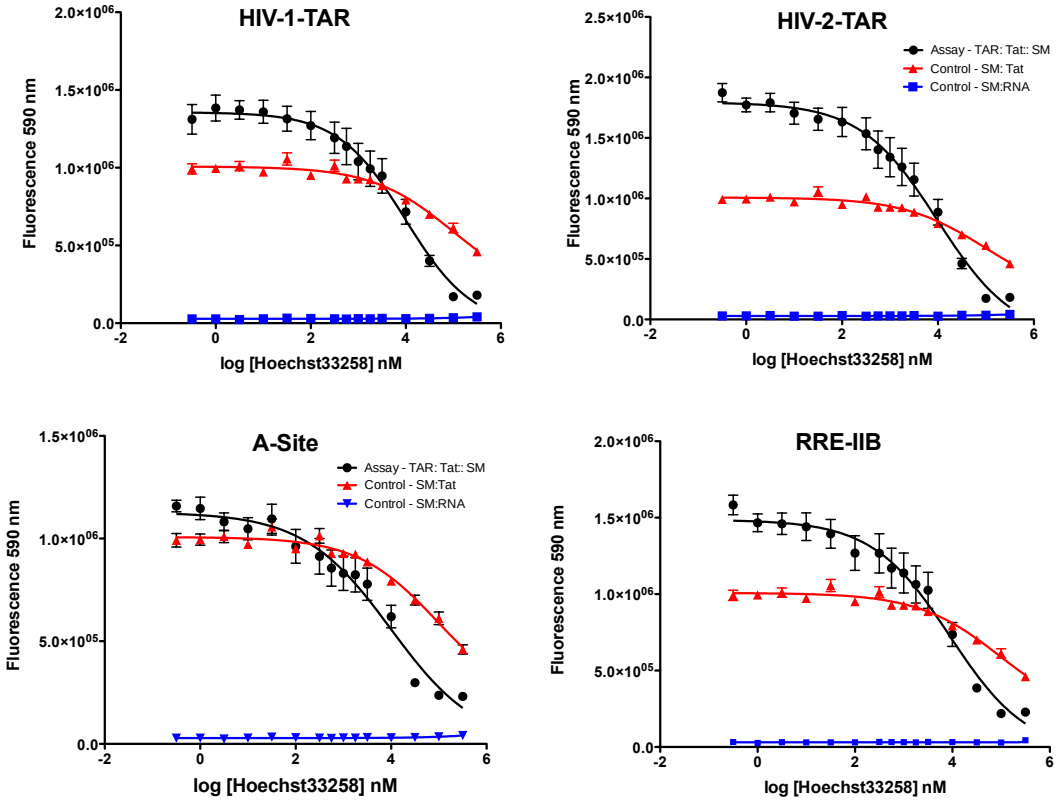


Interference in displacement assay of Mitoxantrone and Hoechst 33258

1. Mitoxantrone



2. Hoechst 33258



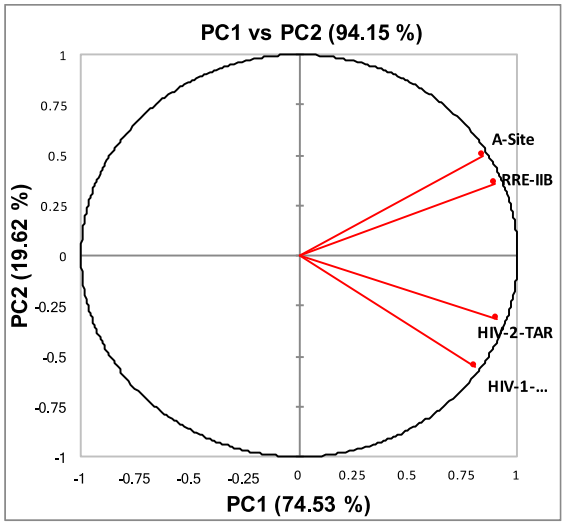
Statistical Analysis of %FID data:

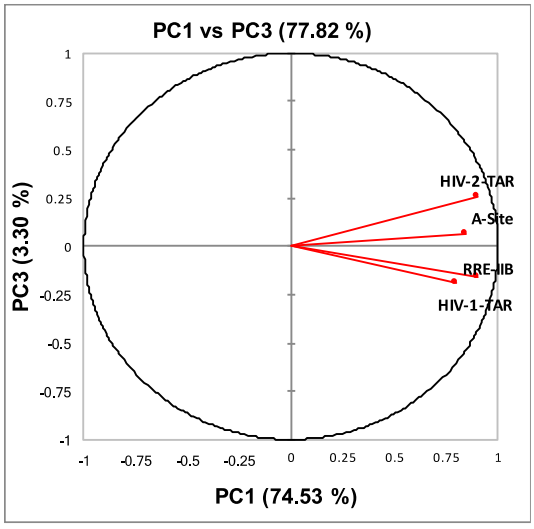
Principal Components Analysis of %FID data at 10 μM SM concentration.

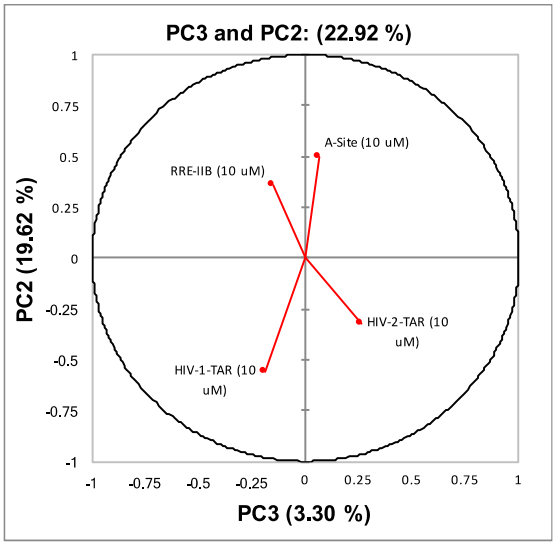
Factor scores -

SM	F1	F2	F3
Neomycin	4.342	-0.881	-0.195
Apramycin	0.114	1.018	-0.013
Paromomycin	1.490	1.394	-0.067
Tobramycin	3.096	0.448	0.180
Kanamycin	-0.575	1.359	0.337
Gentamycin	3.296	0.952	-0.237
Streptomycin	-0.824	-0.410	-0.562
Sisomycin	2.103	1.149	-0.097
Amikacin	0.563	0.732	-0.531
Neamine	1.149	-0.401	0.650
2-DOS	-1.699	0.102	0.331
Arginamide	-1.763	0.102	0.453
Tetracycline	-1.826	0.118	0.173
(+) Usnic Acid	-1.663	-0.070	-0.041
Yohimbine	-2.189	0.054	-0.876
Theophilline	-1.356	-0.267	-0.046
L-Citrulline	-1.476	-0.310	-0.160
DMA-001	-0.886	-0.149	-0.060
DMA-019	-0.896	-0.241	-0.219
DMA-096	-0.824	0.075	-0.487
DMA-097	-1.233	0.527	0.608
DMA-101	-0.833	-0.198	0.366
DMA-132	-0.633	-0.756	-0.089
DMA-135	1.794	-0.328	0.170
DMA-155	-0.868	-0.052	-0.098
DMA-165	0.147	-0.731	0.555
DMA-169	1.451	-3.235	-0.046

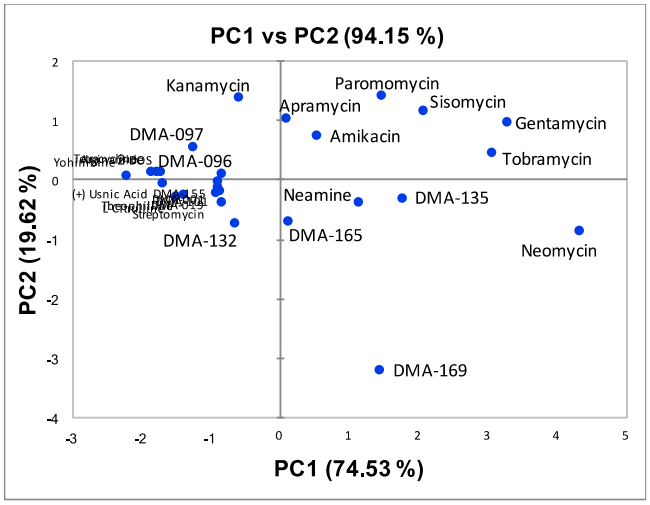
Loading Plots –

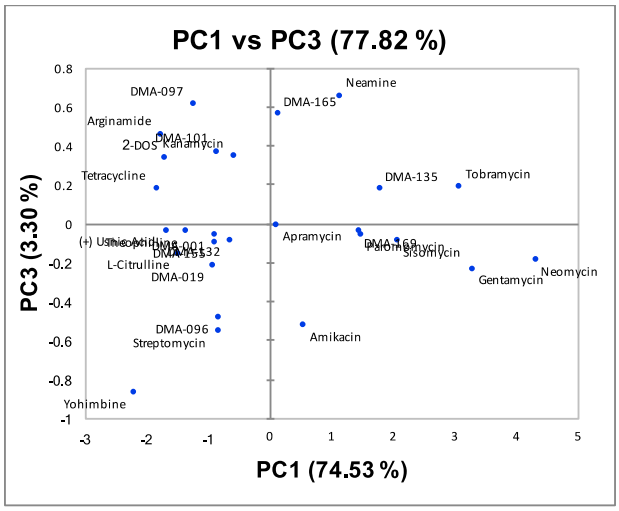


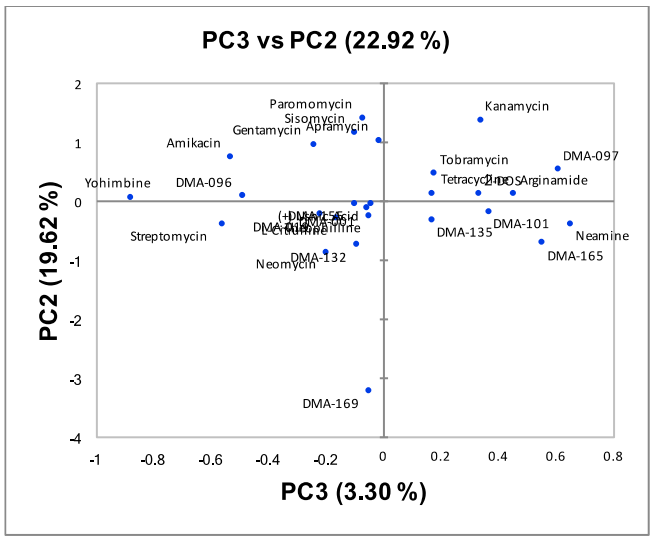




Scatter Plots:



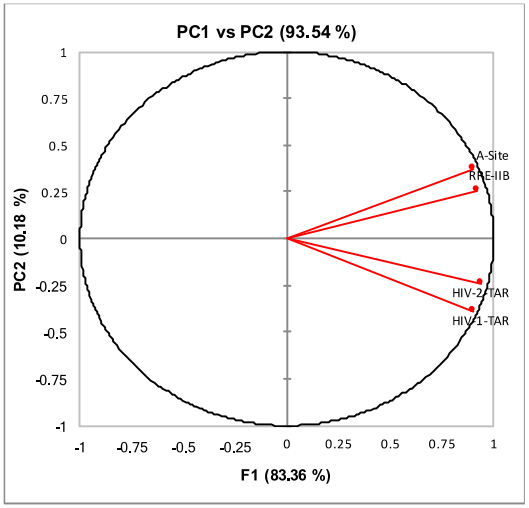


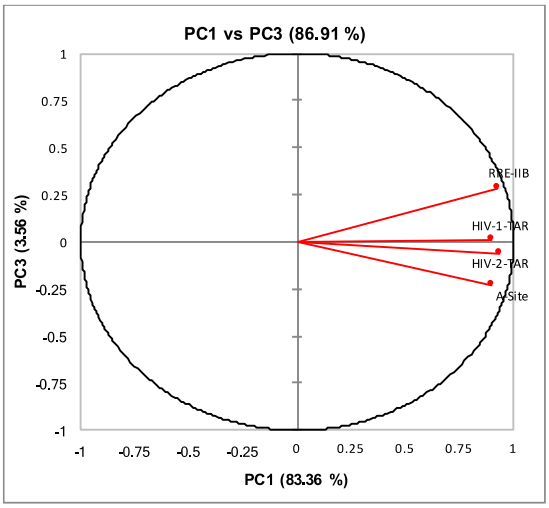


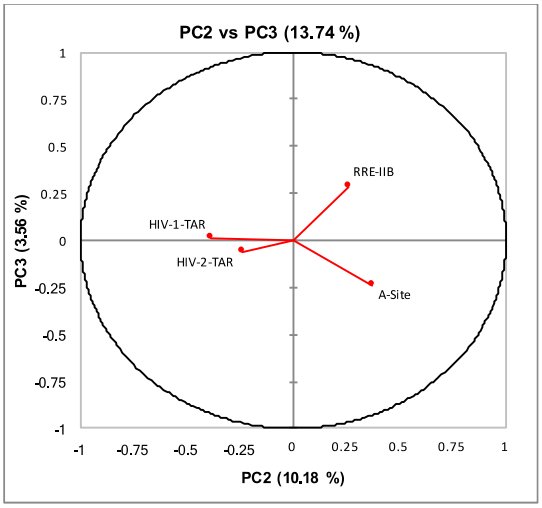
Principal Components Analysis of %FID data at both 10 μM and 50 μM SM concentrations

	F1	F2	F3
Neomycin	4.983	0.086	-1.852
Apramycin	0.004	1.170	0.731
Paromomycin	1.606	1.647	0.394
Tobramycin	3.506	1.107	-0.470
Kanamycin	-0.711	1.407	1.309
Gentamycin	3.733	1.391	-0.590
Streptomycin	-0.301	-0.799	0.187
Sisomycin	2.176	1.675	0.064
Amikacin	0.530	0.865	0.329
Neamine	0.943	0.253	-0.348
2-DOS	-2.498	-0.095	-0.150
Arginamide	-2.430	-0.392	-0.232
Tetracycline	-3.036	0.065	-0.264
(+) Usnic Acid	-2.691	0.019	-0.567
Yohimbine	-3.334	0.071	-0.171
Theophilline	-2.423	-0.146	-0.493
L-Citrulline	-2.475	-0.331	-0.507
DMA-001	-1.427	-0.026	-0.281
DMA-019	-1.473	-0.171	-0.516
DMA-096	-1.450	0.096	-0.215
DMA-097	-1.790	0.230	0.668
DMA-101	-0.822	-0.512	0.054
DMA-132	-0.837	-0.940	-0.389
DMA-135	3.848	-0.740	0.897
DMA-155	1.765	-1.408	2.844
DMA-165	1.079	-1.106	0.141
DMA-169	3.524	-3.414	-0.572

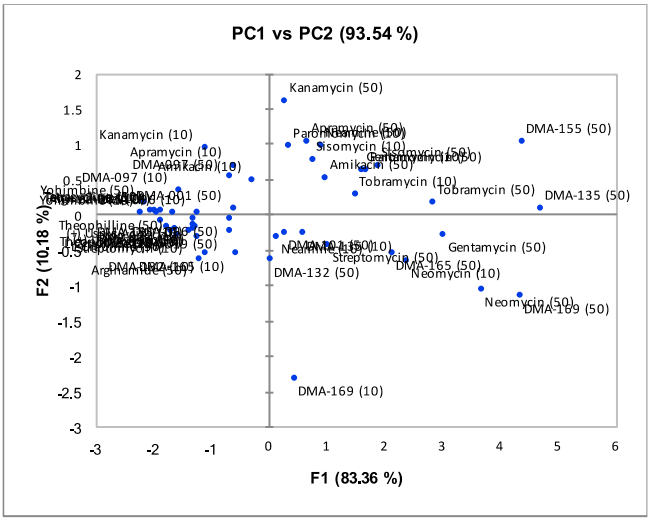
Loading Plots:

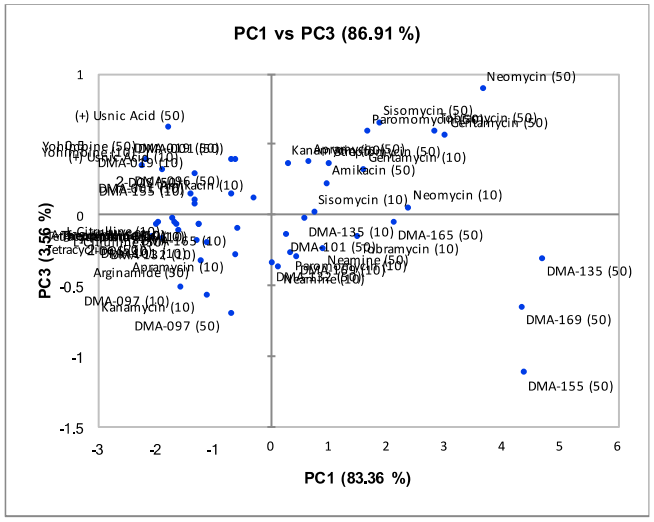


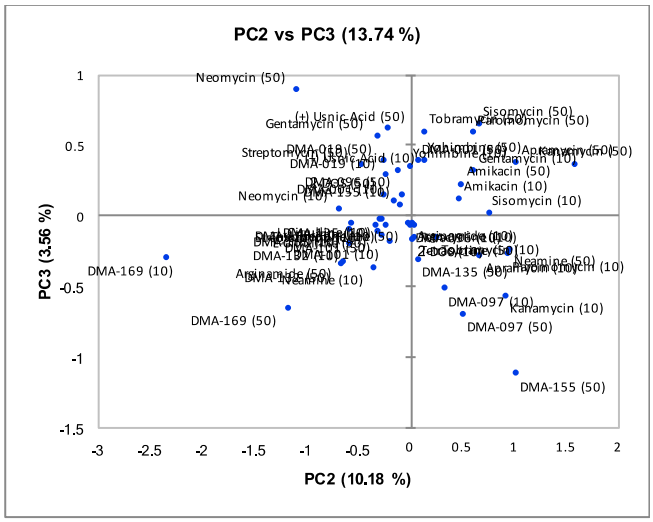




Scatter Plots:







References:

1. Patwardhan, N. N.; Ganser, L. R.; Kapral, G. J.; Eubanks, C. S.; Lee, J.; Sathyamoorthy, B.; Al-Hashimi, H. M.; Hargrove, A. E., Amiloride as a new RNA-binding scaffold with activity against HIV-1 TAR. *MedChemComm* **2017,** *8* (5), 1022-1036.
2. del Villar-Guerra, R.; Gray, R. D.; Trent, J. O.; Chaires, J. B., A rapid fluorescent indicator displacement assay and principal component/cluster data analysis for determination of ligand–nucleic acid structural selectivity. *Nucleic Acids Res.* **2018,** *46* (7), e41-e41.