Supporting Information for:

Determining the Relative Binding Affinity of Ricin Toxin A (RTA) Inhibitors by Using Molecular Docking and Non-Equilibrium Work

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Table S1. SMD force profiles measured using 25 SMD trajectories and $\boldsymbol{k}$ = 2 kcal/mol/Å² in different pulling velocities ($\boldsymbol{v}$ = 0.05, 0.25 and 1.25 Å/ps).[a]

Inhibitor (PDB ID)	$\langle F \rangle_{max}$ **(rank)**		$\langle t \rangle_{max}$	$\langle F_{max} \rangle$ **(rank)**		$\langle W \rangle$ **(rank)**	
$\boldsymbol{k}$ = 2 kcal/mol/Å²; $\boldsymbol{v}$ = 0.05 Å/ps							
19M	852.96	(2)	157.56	979.70 ± 82.39	(2)	95.01 ± 9.10	(1)
RS8	884.57	(1)	170.82	1056.19 ± 129.92	(1)	91.98 ± 10.15	(2)
0RB	724.16	(3)	130.10	878.59 ± 89.13	(3)	73.51 ± 9.77	(3)
1MX	590.55	(4)	129.18	741.15 ± 93.04	(4)	57.34 ± 7.68	(4)
JP2	499.71	(5)	115.14	649.12 ± 57.32	(5)	48.52 ± 5.92	(5)
JP3	309.27	(6)	81.08	449.82 ± 61.08	(6)	31.50 ± 7.10	(6)
$\boldsymbol{k}$ = 2 kcal/mol/Å²; $\boldsymbol{v}$ = 0.25 Å/ps							
19M	1029.82	(2)	38.96	1167.41 ± 125.78	(2)	221.93 ± 18.87	(1)
RS8	1065.78	(1)	39.14	1213.66 ± 111.30	(1)	157.37 ± 14.77	(3)
0RB	920.13	(3)	34.66	1023.54 ± 88.68	(3)	162.68 ± 18.01	(2)
1MX	809.21	(4)	35.14	925.70 ± 94.35	(4)	141.48 ± 17.65	(4)
JP2	627.91	(5)	27.36	733.94 ± 64.78	(5)	106.02 ± 12.84	(5)
JP3	508.65	(6)	22.40	627.54 ± 62.99	(6)	105.08 ± 11.20	(6)
$\boldsymbol{k}$ = 2 kcal/mol/Å²; $\boldsymbol{v}$ = 1.25 Å/ps							
19M	1386.93	(1)	11.04	1459.87 ± 142.36	(1)	425.39 ± 22.98	(1)
RS8	1347.24	(2)	10.10	1437.74 ± 137.86	(2)	305.26 ± 20.54	(4)
0RB	1265.21	(3)	9.90	1368.14 ± 144.14	(3)	359.68 ± 25.44	(3)
1MX	1067.97	(4)	9.34	1142.91 ± 109.94	(4)	341.68 ± 14.42	(2)
JP2	865.02	(5)	7.46	944.45 ± 89.54	(5)	227.58 ± 15.15	(6)
JP3	848.27	(6)	8.28	926.80 ± 77.70	(6)	294.83 ± 17.27	(5)

[a] $\langle F \rangle_{max}$ and $\langle F_{max} \rangle$ are measured in pN, while $\langle W \rangle$ and $E_{docking}$ are in kcal/mol. $\langle t \rangle_{max}$ is in ps.

Table S2. SMD force profiles measured using 25 SMD trajectories and ***k*** = 10 kcal/mol/Å² in different pulling velocities (***v*** = 0.05, 0.25 and 1.25 Å/ps).[a]

Inhibitor (PDB ID)	$\langle F \rangle_{max}$ **(rank)**		$\langle t \rangle_{max}$	$\langle F_{max} \rangle$ **(rank)**		$\langle W \rangle$ **(rank)**	
k = 10 kcal/mol/Å²; ***v*** = 0.05 Å/ps							
19M	959.99	(2)	58.84	1315.48 ± 96.23	(2)	81.40 ± 6.69	(1)
RS8	1073.39	(1)	71.26	1419.30 ±107.48	(1)	71.39 ± 7.78	(2)
0RB	857.18	(3)	45.80	1195.42 ± 107.73	(3)	65.05 ± 8.05	(3)
1MX	705.84	(4)	65.98	1087.01 ± 93.15	(4)	48.67 ± 7.90	(4)
JP2	634.56	(5)	60.46	1023.45 ± 94.43	(5)	47.55 ± 5.57	(5)
JP3	428.74	(6)	39.24	836.88 ± 66.77	(6)	32.71 ± 4.77	(6)
k = 10 kcal/mol/Å²; ***v*** = 0.25 Å/ps							
19M	1179.53	(2)	13.76	1510.97 ± 105.39	(1)	213.36 ± 15.62	(1)
RS8	1214.61	(1)	14.00	1498.75 ± 140.96	(2)	133.82 ± 13.40	(3)
0RB	1033.68	(3)	11.90	1382.56 ± 140.37	(3)	144.84 ± 10.81	(2)
1MX	927.74	(4)	16.98	1233.56 ± 128.64	(4)	125.56 ± 9.31	(4)
JP2	711.74	(5)	12.88	1078.92 ± 112.86	(5)	94.53 ± 11.93	(6)
JP3	648.50	(6)	10.32	974.07 ± 111.79	(6)	99.68 ± 10.41	(5)
k = 10 kcal/mol/Å²; ***v*** = 1.25 Å/ps							
19M	1616.53	(1)	3.78	1879.34 ± 158.99	(1)	451.92 ± 25.67	(1)
RS8	1569.05	(2)	3.46	1778.43 ± 151.99	(2)	278.60 ± 16.06	(4)
0RB	1419.31	(3)	3.46	1632.58 ± 141.91	(3)	341.21 ± 17.15	(2)
1MX	1225.14	(4)	4.06	1501.44 ± 149.64	(4)	340.66 ± 19.45	(3)
JP2	932.68	(6)	2.80	1172.75 ± 125.97	(6)	221.27 ± 17.70	(6)
JP3	996.55	(5)	2.54	1276.72 ± 146.35	(5)	297.09 ± 20.16	(5)

[a] $\langle F \rangle_{max}$ and $\langle F_{max} \rangle$ are measured in pN, while $\langle W \rangle$ and $E_{docking}$ are in kcal/mol. $\langle t \rangle_{max}$ is in ps.

Table S3. Tests with different SMD settings. From the third column onwards are showed correlation coefficient (R^2) between theoretical results of $\langle F \rangle_{max}$, $\langle F_{max} \rangle$ and $\langle W \rangle$ and half-maximum inhibitory concentration (IC_{50}).

Spring Constant (*k*) (kcal/mol/Å²)	**Velocity (*v*)** (Å/ps)	**Steered MD Trajectories**								
		25			**15**			**5**		
		$\langle F \rangle_{max}$	$\langle F_{max} \rangle$	$\langle W \rangle$	$\langle F \rangle_{max}$	$\langle F_{max} \rangle$	$\langle W \rangle$	$\langle F \rangle_{max}$	$\langle F_{max} \rangle$	$\langle W \rangle$
2	0.05	0.978	0.969	0.961	0.959	0.969	0.967	0.981	0.976	0.955
2	0.25	0.942	0.925	0.690	0.903	0.900	0.698	0.983	0.962	0.631
2	1.25	0.899	0.902	0.325	0.886	0.901	0.332	0.820	0.863	0.297
10	0.05	0.956	0.940	0.954	0.978	0.951	0.965	0.926	0.952	0.975
10	0.25	0.905	0.954	0.558	0.900	0.933	0.574	0.893	0.904	0.570
10	1.25	0.832	0.773	0.216	0.798	0.725	0.184	0.822	0.641	0.171

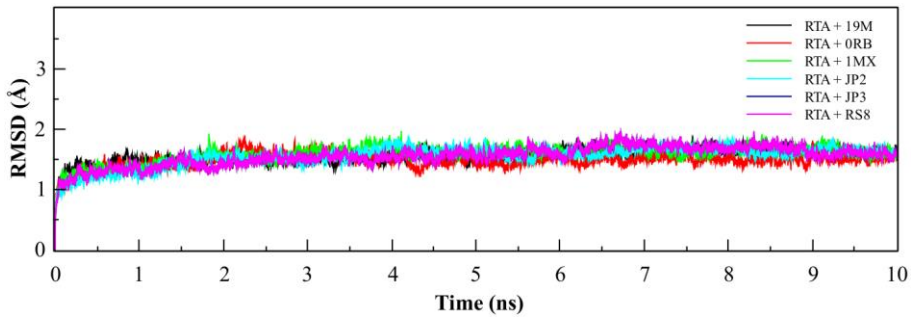

Figure S1. RMSDs of the molecular dynamics trajectories for all RTA-inhibitor complexes.

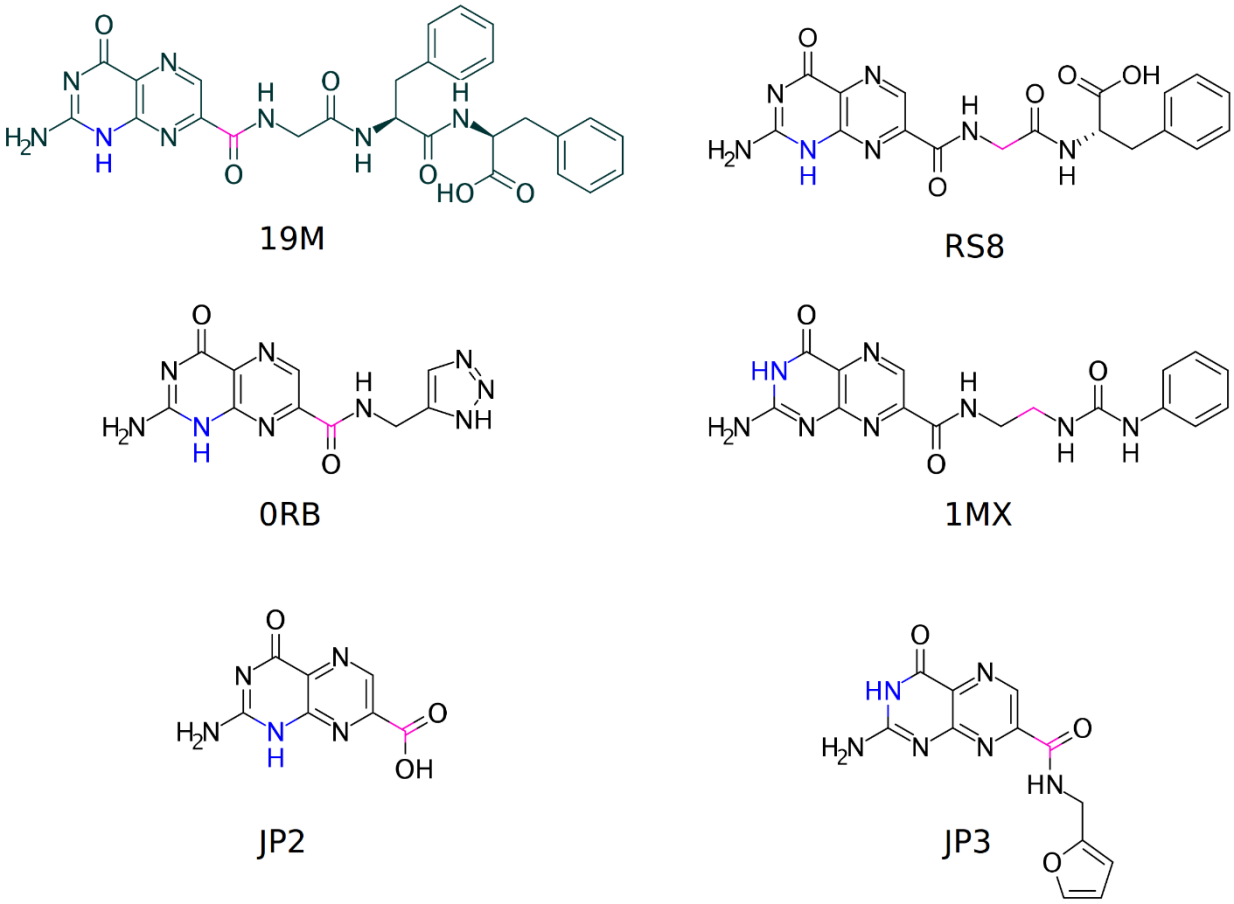

Figure S2. Chemical structures of the RTA inhibitors. The different protonations on the nitrogen atom of the pterine group are highlighted in blue. The SMDatoms are highlighted in magenta color.

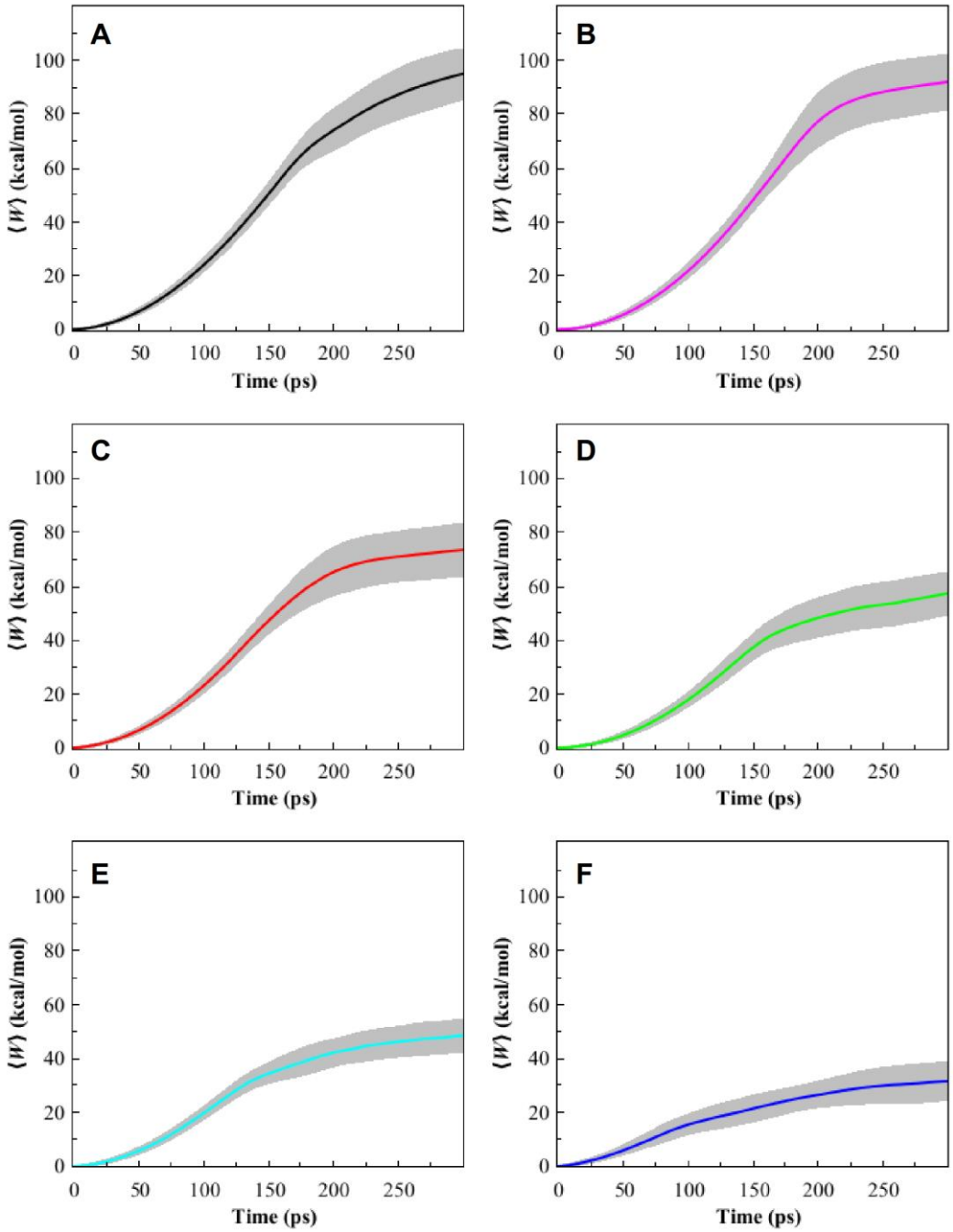

Figure S3. Work fluctuation by using twenty-five independent decoupling simulations and ***k*** = 2 kcal/mol/Å² and ***v*** = 0.05 Å/ps. The middle line corresponds to the mean non-equilibrium work profile $\langle W \rangle$. The work fluctuations for each simulation are depicted in gray and correspond to standard deviation considering the twenty-five SMD simulations. The plots correspond to the following ligands: 19M (**A**); 0RB (**B**); 1MX (**C**); JP2 (**D**); JP3 (**E**); RS8 (**F**).

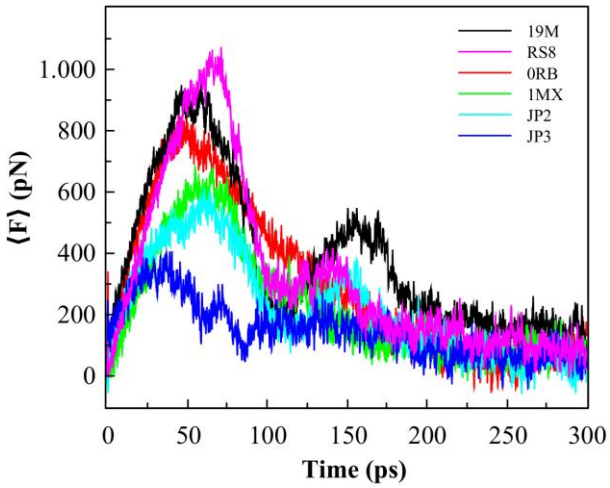

Figure S4. Mean force profiles using ***k*** = 10 kcal/mol/Å² and ***v*** = 0.05 Å/ps.