

Supplementary Information

Identification of influenza polymerase inhibitors targeting C-terminal domain of PA through surface plasmon resonance screening

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Supplementary Figure

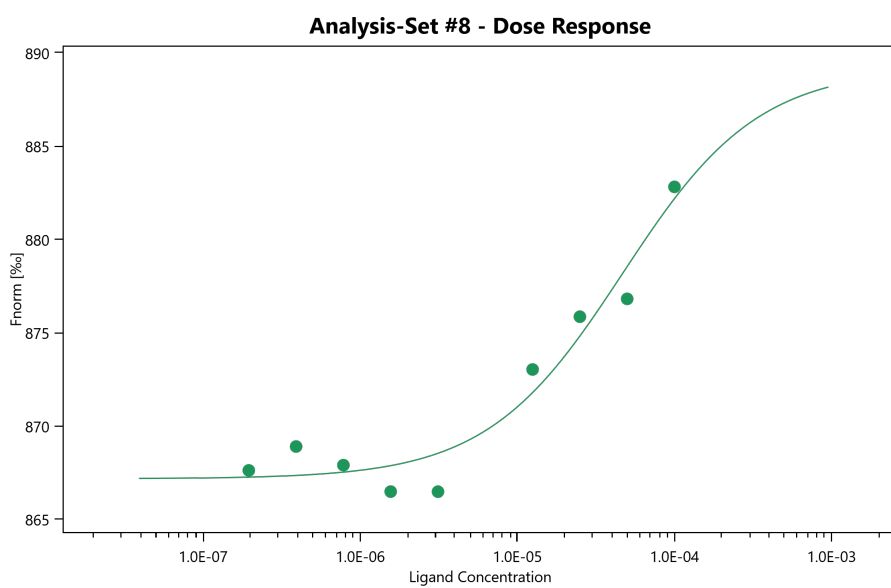


Figure S1. MST study of compound **387** with PAC.

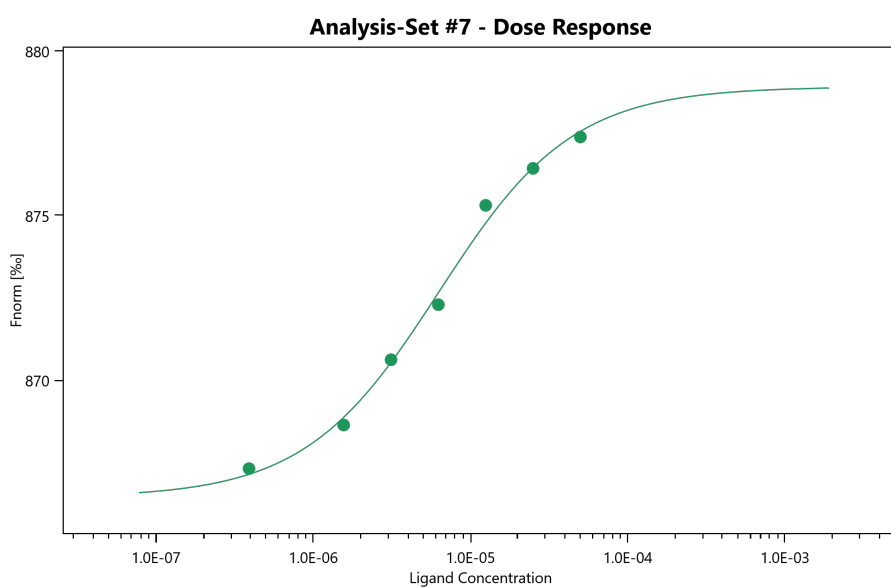


Figure S2. MST study of compound **392** with PAC.

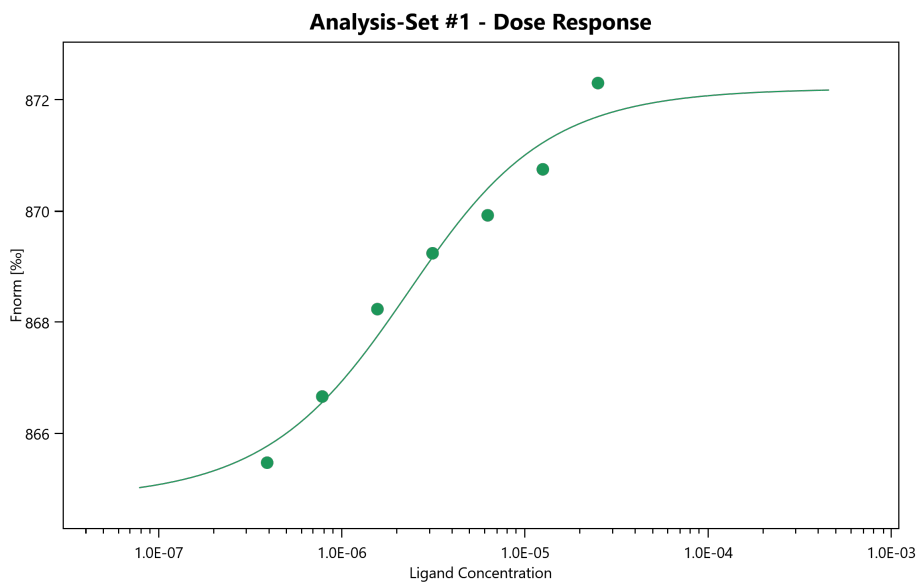


Figure S3. MST study of compound **S2a** with PAC.

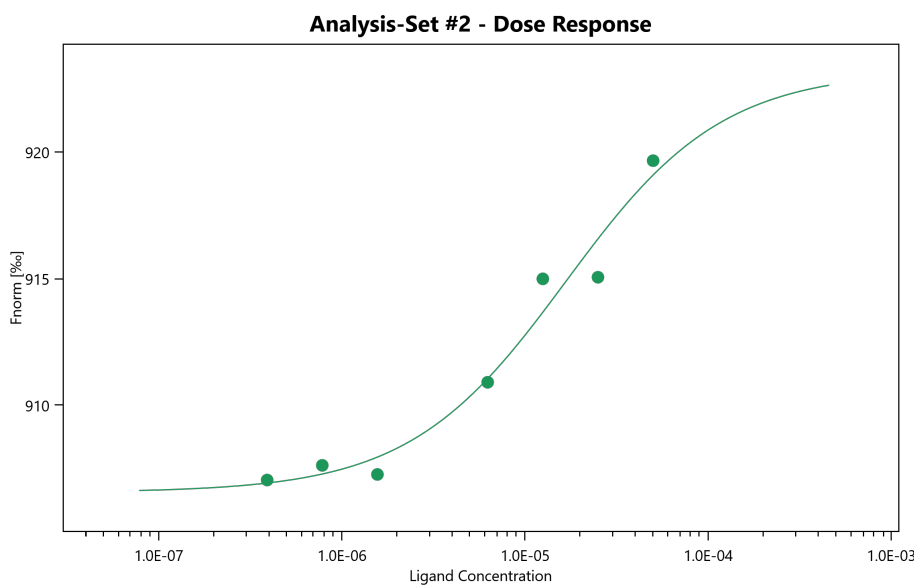


Figure S4. MST study of compound **S2b** with PAC.

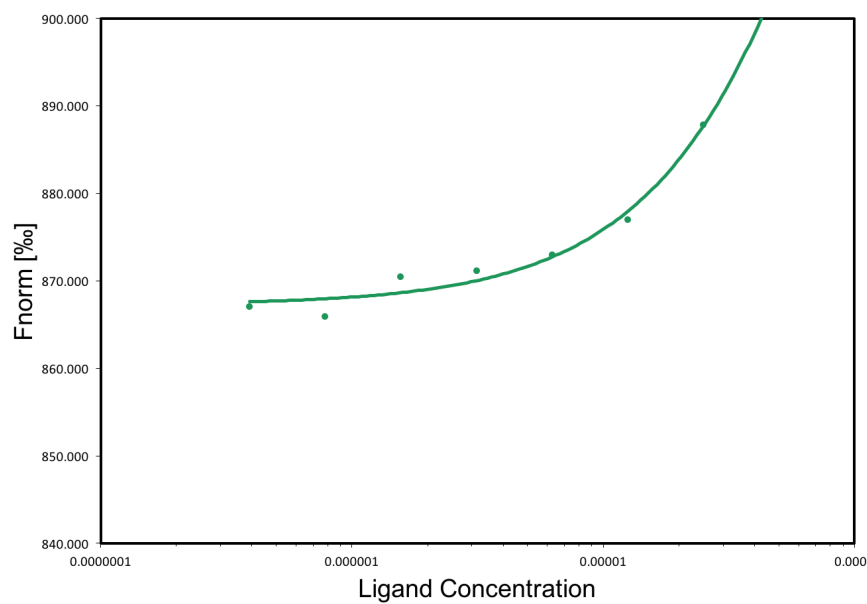


Figure S5. MST study of compound S2d with PAC.

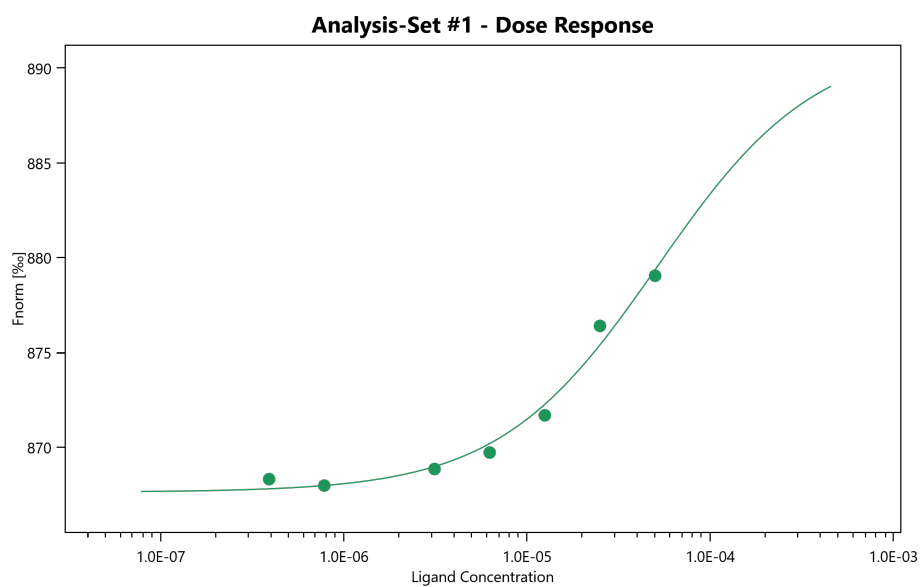


Figure S6. MST study of compound S2e with PAC.

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H5N1 257      IEPFLKTTPRPLRLPDGPPCSQRSKFLLMDALKLSIEDPSHEGE
H1N1          IEPFLKSTPRPLRLPDGPPCSQRSKFLLMDALKLSIEDPSHEGE
H2N2          IEPFLKTTPRPIRLPDGPPCSQRSKFLLMDALKLSIEDPSHEGE
H3N2          IEPFLKTTPRPIRLPDGPPCFQRSKFLLMDALKLSIEDPSHEGE
              *****:****:***** *****

H5N1 301      GIPLYDAIKCMRTFFGWKEPNIKPKHEKGINPNYLLWKQVLAELQDIENEKIPRTKNM
H1N1          GIPLYDAIKCMRTFFGWKEPNVVKPHEKGINPNYLLSWKQVLAELQDIENEKIPRTKNM
H2N2          GIPLYDAIKCMRTFFGWKEPYVVKPHEKGINPNYLLSWKQVLAELQDIENEKIPRTKNM
H3N2          GIPLYDAIKCMRTFFGWKEPYIVKPKHEKGINPNYLLSWKQVLAELQDIENEKIPRTKNM
              *****:****:***:*****:*****:*****:*****:*****

H5N1 361      KKTSQLKQWALGENMAPEKVDFFDCKDIDDLKQYDSDEPELRSLSWIQNEFNKACELTDS
H1N1          KKTSQLKQWALGENMAPEKVDFFDCKDIDDLKQYDSDEPELRSLSWIQNEFNKACELTDS
H2N2          KKTSQLKQWALGENMAPEKVDFFDCCRDTSDLKQYDSDEPELRSLSWIQNEFNKACELTDS
H3N2          KKTSQLKQWALGENMAPEKVDFFDNCRDVSDLKQYDSDEPELRSLSWIQNEFNKACELTDS
              ***** *****:***:***** ***** *****:*****

H5N1 421      SWIELDEIGEDVAPIEHIASMRNYFTAESHCRATEYIMKGVYINTALLNASCAAMDDF
H1N1          SWIELDEIGEDAAPIEHIASMRNYFTAESHCRATEYIMKGVYINTALLNASCAAMDDF
H2N2          IWIELDEIGEDVAPIEHIASMRNYFTAESHCRATEYIMKGVYINTALLNASCAAMDDF
H3N2          TWIELDEIGEDVAPIEYIASMRNYFTAESHCRATEYIMKGVYINTALLNASCAAMDDF
              *****:****:***** *****

H5N1 481      QLIPMISKRCRTKEGRRKTNLYGFIKGRSHLRNDTDVVNFVSMEFSLTDPRLPHKWEKY
H1N1          QLIPMISKRCRTKEGRRKTNLYGFIKGRSHLRNDTDVVNFVSMEFSLTDPRLPHKWEKY
H2N2          QLIPMISKRCRTKEGRRKTNLYGFIKGRSHLRNDTDVVNFVSMEFSLTDPRLPHKWEKY
H3N2          QLIPMISKRCRTKEGRRKTNLYGFIKGRSHLRNDTDVVNFVSMEFSLTDPRLPHKWEKY
              *****:*****:***** *****

H5N1 541      CVLEIGDMLLRSAIGQVSRPMPFLYVRTNGTSGIKMKWGMEMRCLLQSLQQIESMIEAES
H1N1          CVLEVGDMLLRSAIGHVSRPMPFLYVRTNGTSGIKMKWGMEMRCLLQSLQQIESMIEAES
H2N2          CVLEIGDMLLRSAIGQVSRPMPFLYVRTNGTSGIKMKWGMEMRCLLQSLQQIESMIEAES
H3N2          CVLEIGDMLLRSAIGQMSRPMPFLYVRTNGTSGIKMKWGMEMRCLLQSLQQIESMIEAES
              *****:****:****:****:***** *****

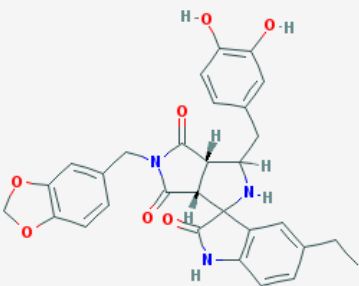
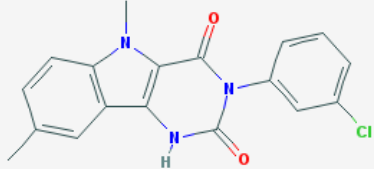
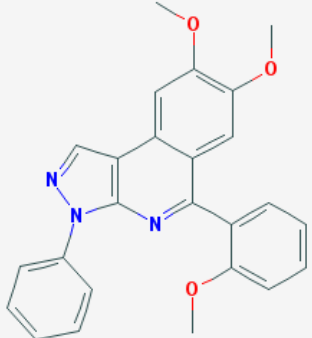
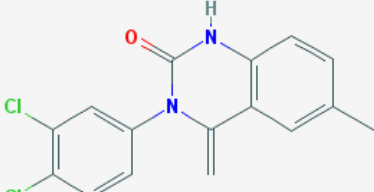
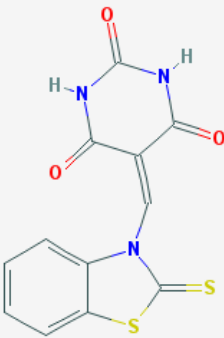
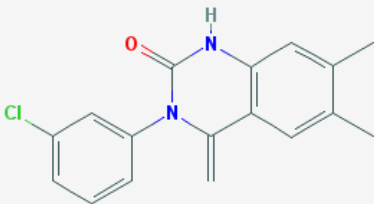
H5N1 601      SVKEKDMTKEFFENKSETWPIGESPKGVEEGSIGKVCRTLLAKSVFNLSYASPOLEGFSA
H1N1          SVKEKDMTKEFFENKSETWPIGESPKGVEEGSIGKVCRTLLAKSVFNLSYASPOLEGFSA
H2N2          SVKEKDMTKEFFENKSETWPIGESPKGVEEGSIGKVCRTLLAKSVFNLSYASPOLEGFSA
H3N2          SVKEKDMTKEFFENKSETWPIGESPKGVEDGSIGKVCRTLLAKSVFNLSYASPOLEGFSA
              *:*****:*****:*****:*****:***** *****

H5N1 661      ESRKLLLVQALRDNLEPGTFDLEGLYEAIIEECLINDPWVLLNASWFNSFLTHALR
H1N1          ESRKLLLVQALRDNLEPGTFDLGGLYEAIIEECLINDPWVLLNASWFNSFLTHALR
H2N2          ESRKLLLVQALRDNLEPGTFDLGGLYEAIIEECLINDPWVLLNASWFNSFLTHALR
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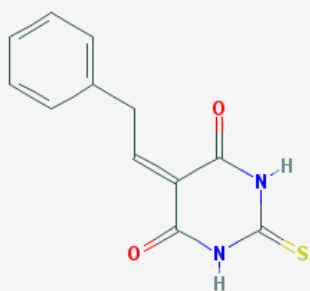
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Figure S7. Sequence alignment of A/WSN/33 (H1N1), A/Japan/305/1957 (H2N2), A/HK/1/68 (H3N2) and A/HK/156/97 (H5N1) PAC. Distinct residues of H5N1 PAC are highlighted in red.

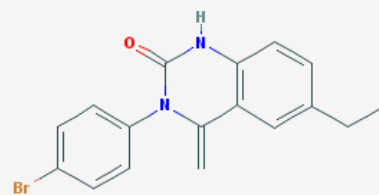
Supplementary Table 1. Chemical structure of hit compounds from SPR screening.

Compound ID.	Chemical Structure	Compound ID.	Chemical Structure
57		265	
95		270	
123		271	

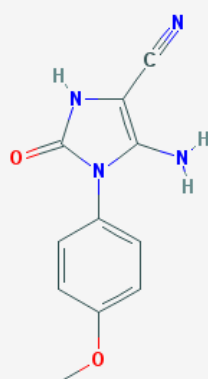
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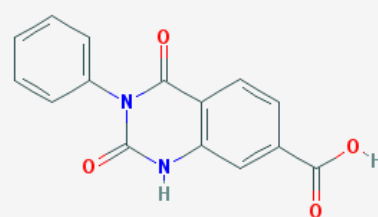
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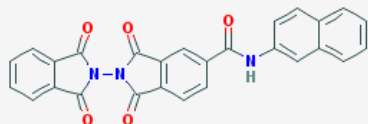
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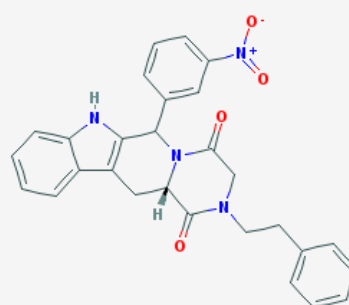
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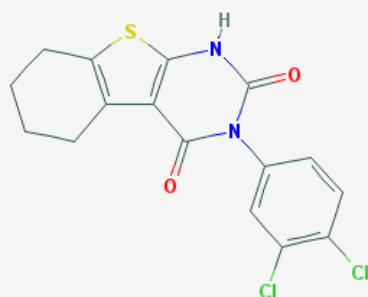
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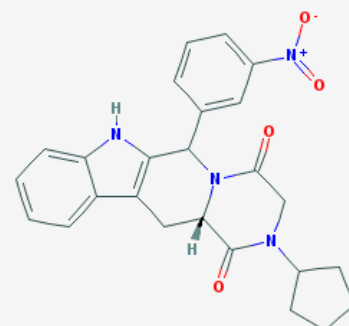
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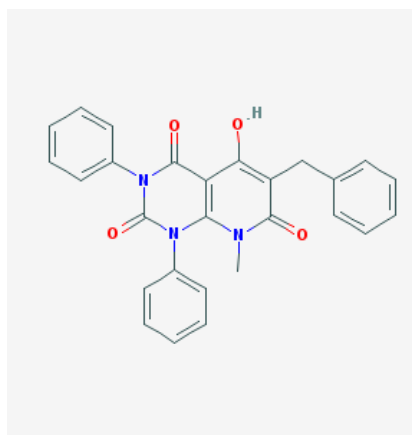
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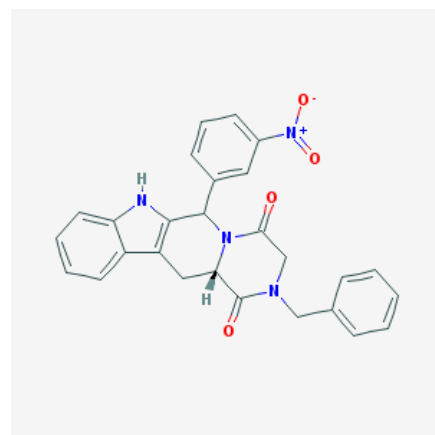
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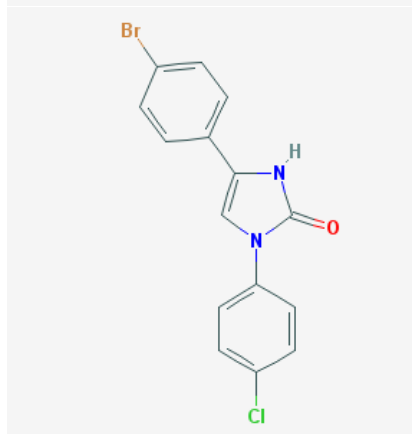
221



345



263



Supplementary Table2. Cytotoxicity of compounds

		CC ₅₀ ^a (μM)	
Compound		293Ta	MDCK
Hit compounds from SPR screening	57	>50	N.D.
	95	>25	N.D.
	123	>50	N.D.
	131	>50	N.D.
	190	>100	N.D.
	198	>5	N.D.
	203	>100	N.D.
	221	>100	>100
	263	>100	N.D.
	265	>100	N.D.
	270	82.0 ± 2.5	N.D.
	271	>100	N.D.
	272	>100	N.D.
	273	>100	N.D.
	280	>100	N.D.
	283	>10	>10
345	>100	N.D.	
Analogues obtained through chemical modification	S1a	>100	N.D.
	S1b	>100	>100
	S1c	>100	N.D.
	S1d	>100	N.D.
	S2a	84.6 ± 17.30	>100
	S2b	>100	42.8 ± 9.0
	S2c	97.1 ± 16.10	N.D.
	S2d	66.6 ± 6.09	35.6 ± 9.24
	S2e	>100	53.1 ± 9.25
S2f	>100	>100	
Commercially available analog	310	>100	>100
	312	>100	>100
	384	>100	>100
	385	>100	N.D.
	387	>100	>100
	389	>100	>100
	390	>100	N.D.
	391	>100	N.D.
	392	62.66 ± 18.7	87.03 ± 7.22
	394	>100	N.D.
	395	>100	N.D.
396	>100	N.D.	
397	>100	N.D.	

^a293T or MDCK cells were incubated with test compounds for 24 hrs; CC50 is the concentration of test compounds which produces 50% cytotoxicity as determined by MTT assays; N.D., not determined; reported values represent means \pm standard deviation of data from three independent experiments.