Supporting Information

A novel small-molecule inhibitor of the avian influenza H5N1 virus determined through computational screening against the neuraminidase

Jianghong An[1], Davy C. W. Lee[2], Anna H.Y. Law[2], Cindy L.H. Yang[2], Leo L.M. Poon[2], Allan S.Y. Lau[2], Steven J.M. Jones[1*]*

[1] British Columbia Cancer Agency Genome Sciences Centre, 675 West 10th Avenue, Vancouver, British Columbia V5Z 4S6, Canada.

[2] Immunology Research Laboratory, Department of Paediatrics and Adolescent Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, Special Administrative Region, China**.**

* Corresponding authors

Allan S.Y. Lau, LKS Faculty of Medicine Building, 21 Sassoon Road, Pokfulam, Hong Kong, China. Tel 852-28554269. Fax 852-28198142. E-mail asylau@hkucc.hku.hk

Steven J.M. Jones, 675 West 10th Avenue, Vancouver, British Columbia V5Z 4S6, Canada. Tel 604-877-6083. Fax 604-876-3561. E-mail sjones@bcgsc.ca

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Table S1. Test of toxicity of compounds in primary macrophages and MDCK cells using MTT assay.

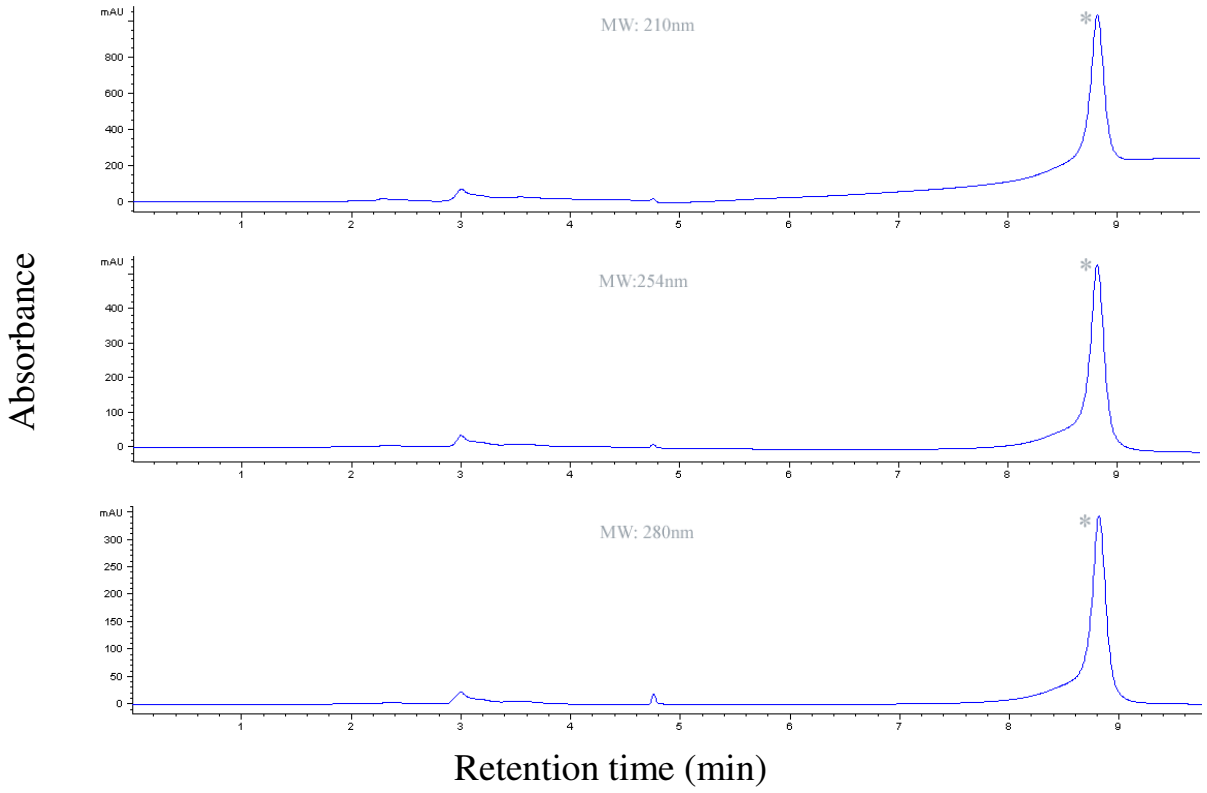
ASL no.	Macrophage	MDCK
#1	1.1	2.2
#2	1.1	1.5
#3	1.1	2.2
#4	1.0	1.2
#5	1.0	2.3
#6	*1.0	1.1
#7	0.9	1.7
#8	*1.1	1.1
#9	*1.1	1.0
#10	0.9	1.2
#11	0.9	1.3
#12	0.9	1.5
#13	*1.1	0.7
#14	1.0	1.4
#15	0.7	
#16	0.9	
#17	1.0	
#18	0.8	
#19	0.1	
#20	0.8	
DMSO	1.0	1.0

(Concentration of tested compounds, 100uM; *10uM)

Table S2. The 20 novel compounds selected for testing within mammalian cell-lines.

No	NSC#	ICM Score (kcal/mol)	Protein structure	PDB code	Testing Result
1	89853	-47.25	N1(apo)	2hty	Inhibitory
2	637682	-47.05	N1(complex with oseltamivir)	2hu4	
3	332542	-44.80	N1(complex with oseltamivir)	2hu4	
4	119437	-43.78	N1(complex with oseltamivir)	2hu4	
5	210268	-43.25	N1(complex with oseltamivir)	2hu4	
6	56681	-42.20	N1(apo)	2hty	
7	161061	-42.04	N1(complex with oseltamivir)	2hu4	
8	289523	-41.75	N4(apo)	2htv	
9	112915	-41.62	N1(complex with oseltamivir)	2hu4	
10	289517	-41.28	N1(apo)	2hty	Toxic
11	317609	-41.25	N1(apo)	2hty	
12	92812	-40.79	N1(complex with oseltamivir)	2hu4	
13	97424	-40.61	N1(apo)	2hty	
14	15153	-40.18	N1(complex with oseltamivir)	2hu4	
15	174493	-39.97	N1(complex with oseltamivir)	2hu4	
16	333447	-38.19	N4(apo)	2htv	
17	343727	-36.64	N1(complex with oseltamivir)	2hu4	
18	614938	-36.36	N1(complex with oseltamivir)	2hu4	Toxic
19	32200	-35.80	N1(complex with oseltamivir)	2hu4	
20	43129	-34.70	N1(complex with oseltamivir)	2hu4	

Figure S3. Analysis of compound **1** was performed by an Agilent 1200 series system interfaced to a G1315c photodiode array detector equipped with a Waters Atlantis® HILIC silica column. After calculation, the purity of compound **1** (with *) at absorbance 210, 254, and 280nm was determined to be higher than 95%.



Methods

Cell viability test. Primary human blood macrophages or MDCK cells were seeded at $2x10^6$/ml in a 96-well plate for 16-24 hours. Various concentrations including 1uM, 10uM and 100uM of the indicated compounds were added to the cells. Same concentrations of DMSO were added to the cells as controls. Cells were incubated at 37°C and 5%CO_2 for 72 hours, and then Thiazolyl Blue Tetrazolium Bromide (MTT) was added to a final concentration of 0.1mg/ml. After two hours, the culture supernatant was removed and each well was replenished with isopropanol. The MTT metabolic product, formazan, was dissolved in isopropanol for five minutes with shaking. The optical densities of formazan and background were measured at 560nm and 670nm, respectively.