

Supporting information

Table S1. The key docking parameters and RMSD of the predicted highest-ranked pose relative to the original pose in the crystal structure

Dock method	Binding site, Key docking parameters and Score function	RMSD (Å)
LibDock	Binding site: -48.6597, -5.33945, 4.06342, 9; Number of Hotspots:100; Docking Tolerance: 0.25; Docking Preferences: Fast Search; Minimization Algorithm: Smart Minimizer; Score function: LibDockScore.	0.65
LigandFit	Binding site: site1: 3148 points, 393.500 Å ³ , partition level 1; energy grid: dreiding; Number of Monte Carlo Trials: "2 500 120, 4 1200 300,6 1500 350,10 2000 500,25 3000 750"; Minimization Algorithm: Smart Minimizer; Minimization Forcefield:CHARMm; Scoring Functions: DOCK SCORE.	0.61
GOLD	Binding site: all atoms within 9Å of VX-787; Water configure: HOH141 and HOH146, toggle and spin; GA settings: Automatic; search efficiency: 100%; Scoring Functions: Gold Score.	0.34

Figure S1. Comparison between the highest ranked pose of VX-787 and the original pose in the crystal structure (Upper left: Libdock docking result comparison; Upper right: Ligandfit docking result comparison; Bottom: Gold docking result comparison.) The original pose of VX-787 is presented as green orange sticks, the docking poses of VX-787 are presented as orange sticks.

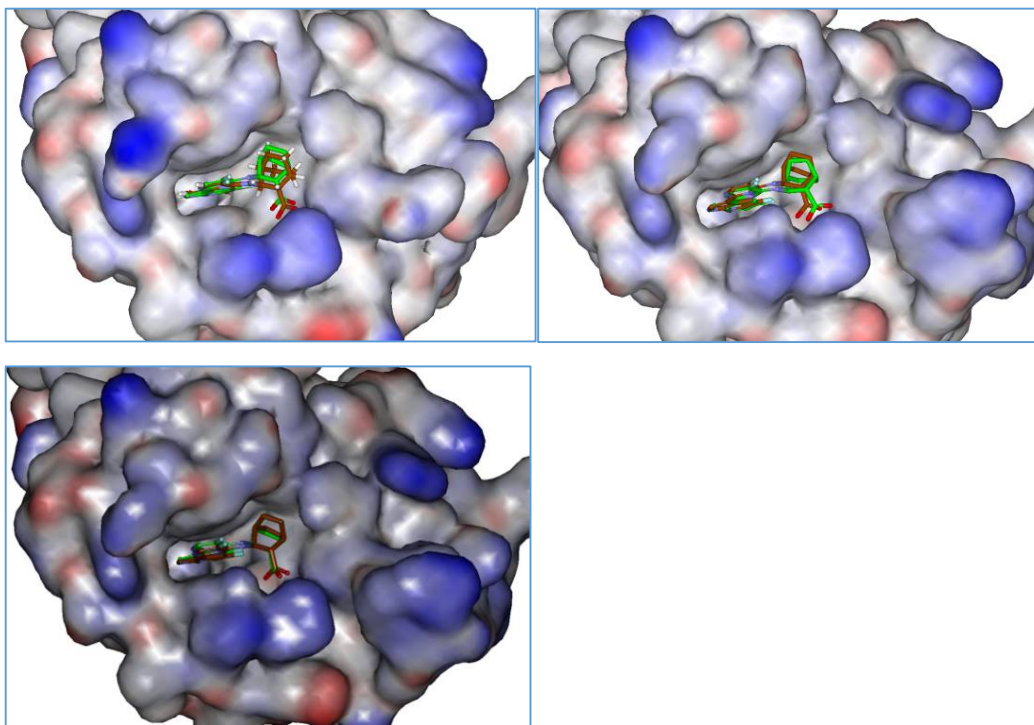


Figure S2. The structure, docking scores, ID and internal serial number of obtained by virtual screening

Figure S2-A

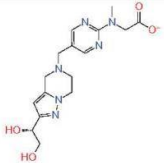
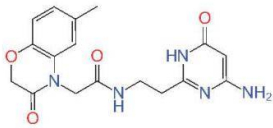
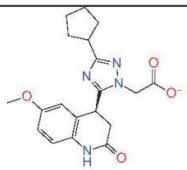
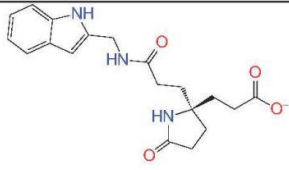
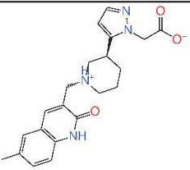
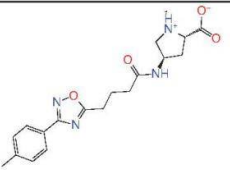
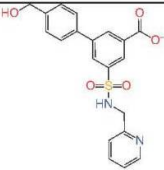
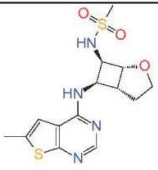
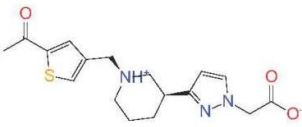
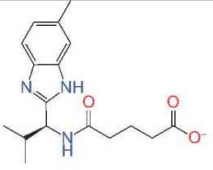
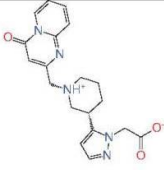
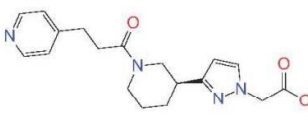
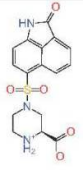
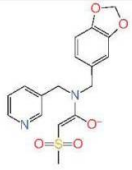

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Figure S2-B

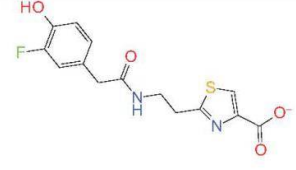
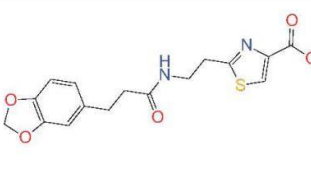
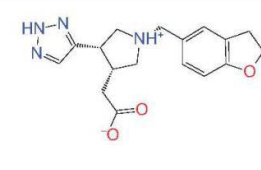
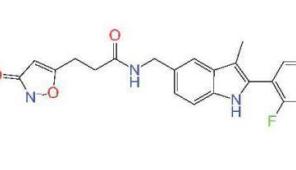
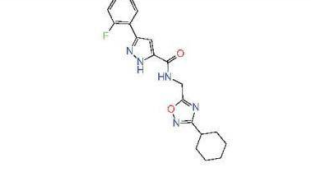
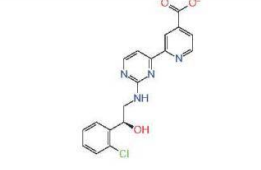
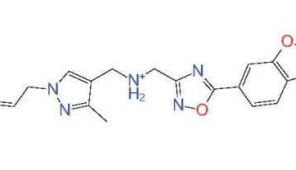
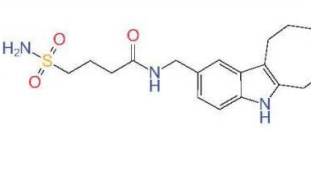
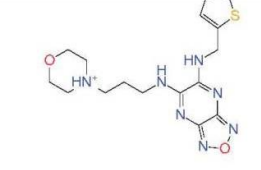
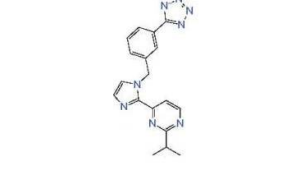

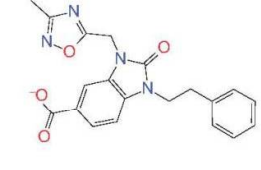
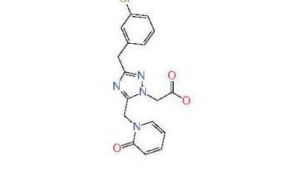
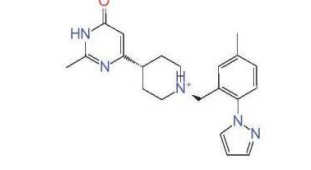
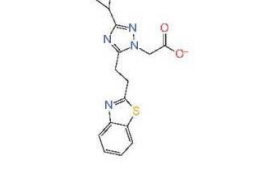
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Figure S2-C

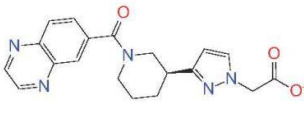
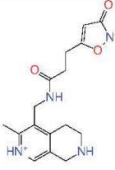
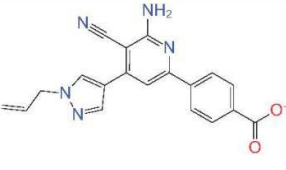
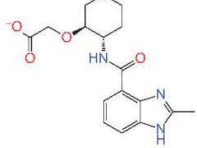
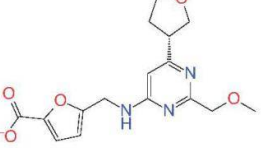
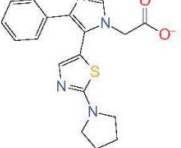
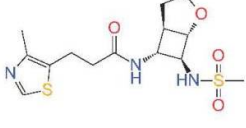
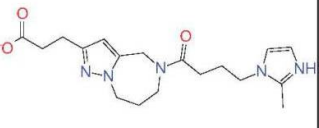
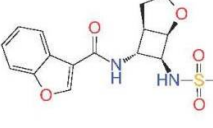
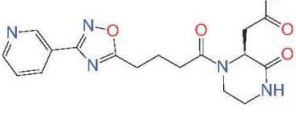
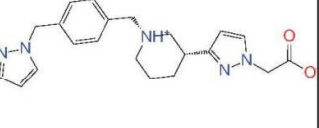
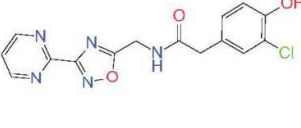
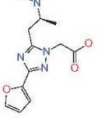
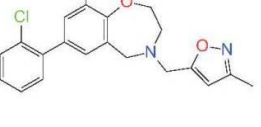
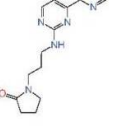
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Figure S2-D

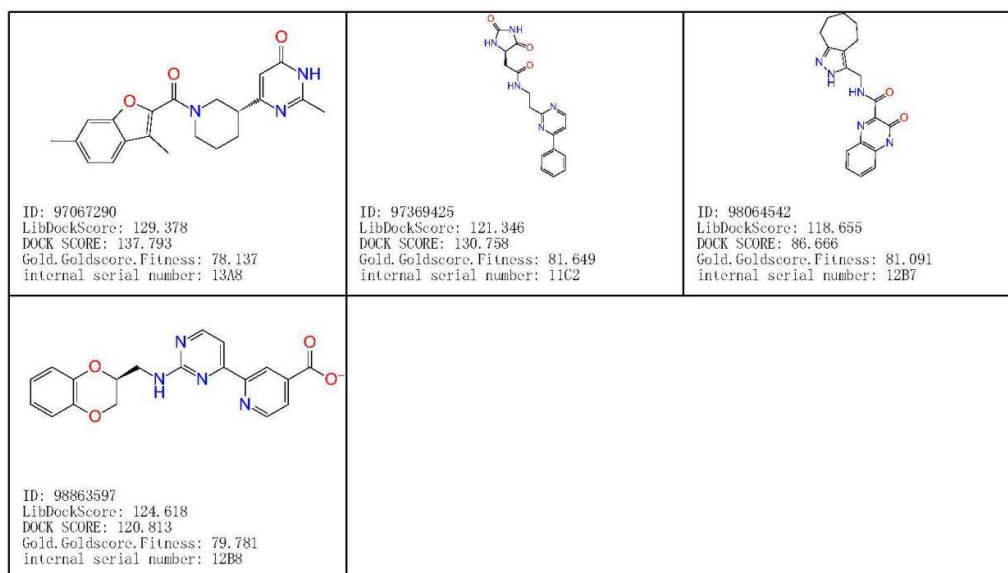


Figure S2-E

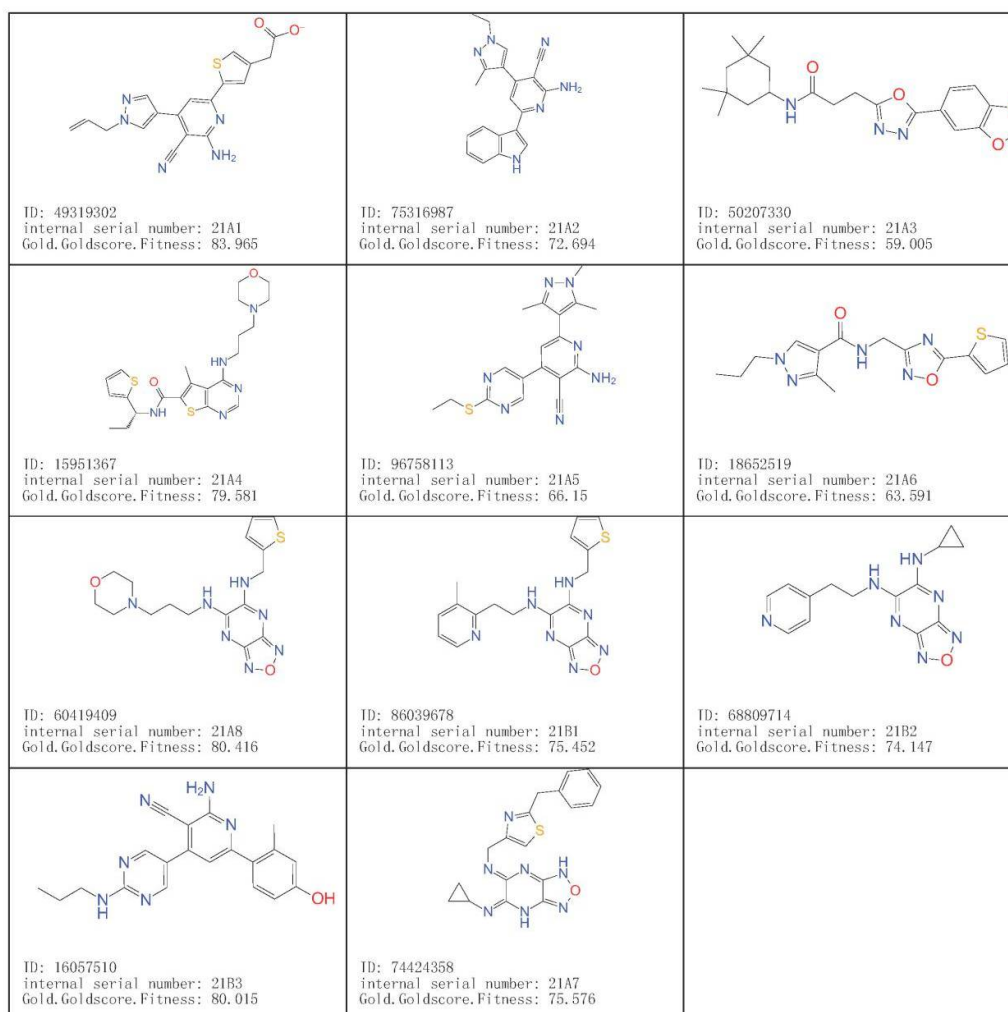


Figure S3. The hydrophobic amino acid residues interacting with the ligand found by Interaction fingerprints analysis and the hydrophobic interaction diagram.

Figure S3-A The hydrophobic amino acid residues interacting with the compound 11D4

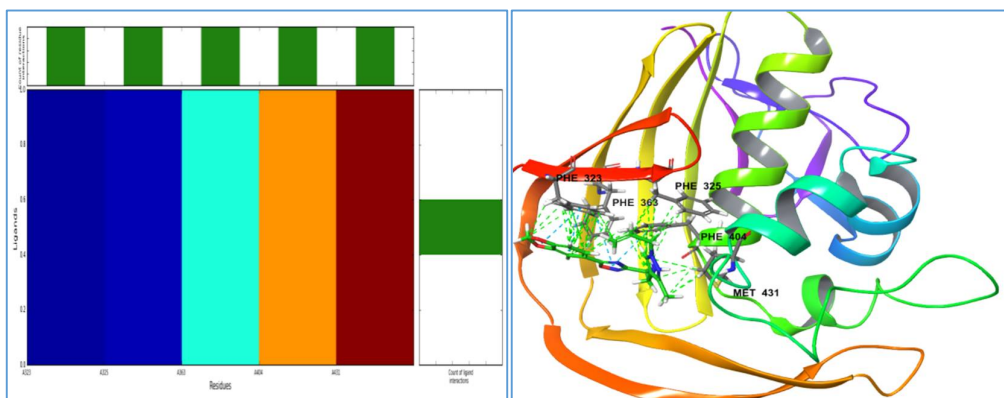


Figure S3-B The hydrophobic amino acid residues interacting with the compound 12C5

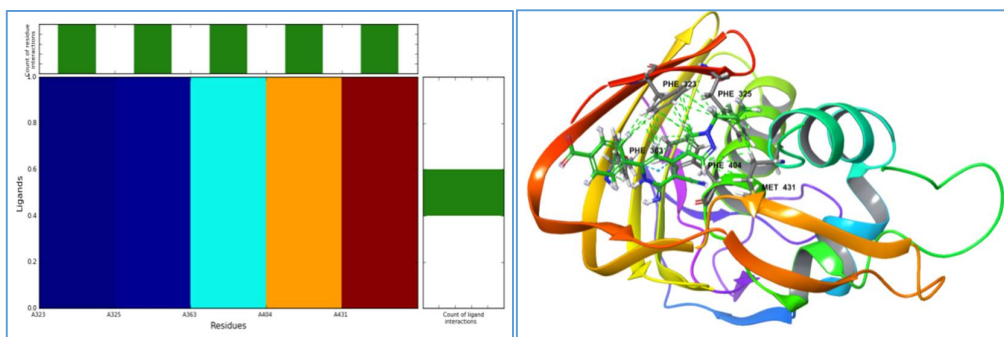


Figure S3-C The hydrophobic amino acid residues interacting with the compound 21A5

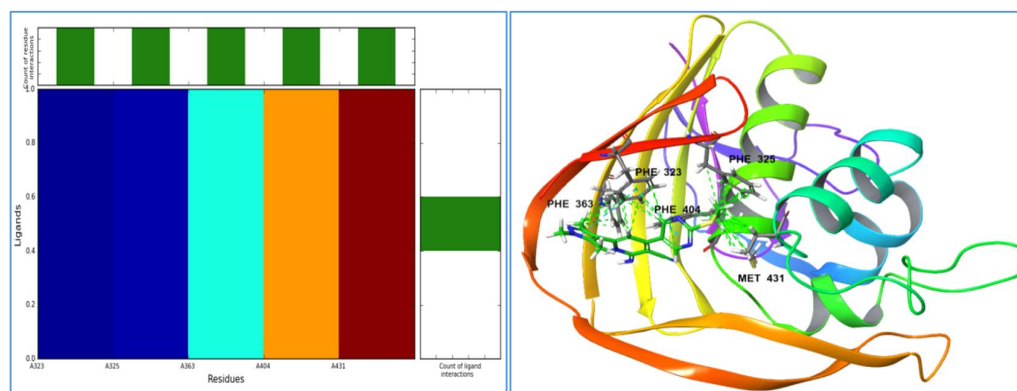


Figure S3-D The hydrophobic amino acid residues interacting with the compound 21B1

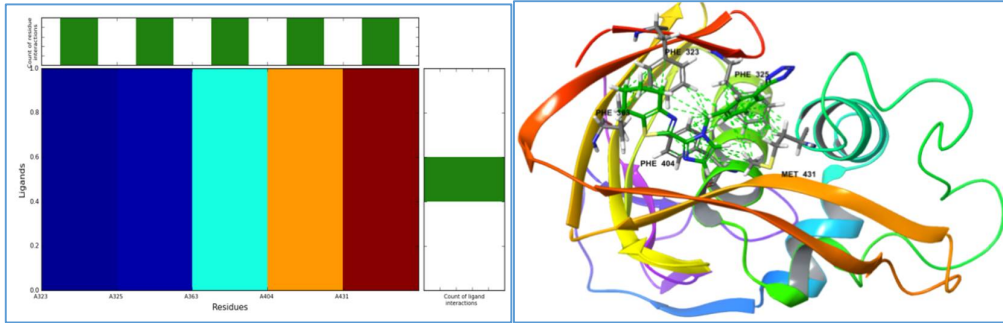


Figure S3-E The hydrophobic amino acid residues interacting with the VX-787

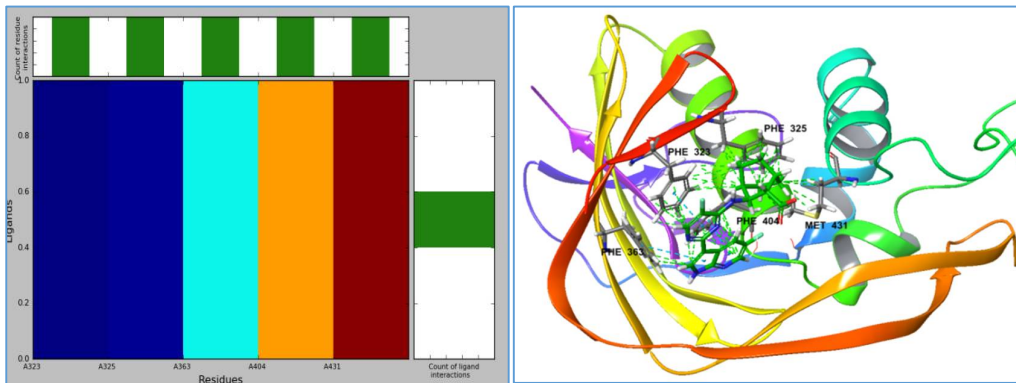


Figure S4. PB2 Cap-Binding Domain Sequence alignment of Influenza A/Victor/3/1975 (H3N2) and Influenza B Lee

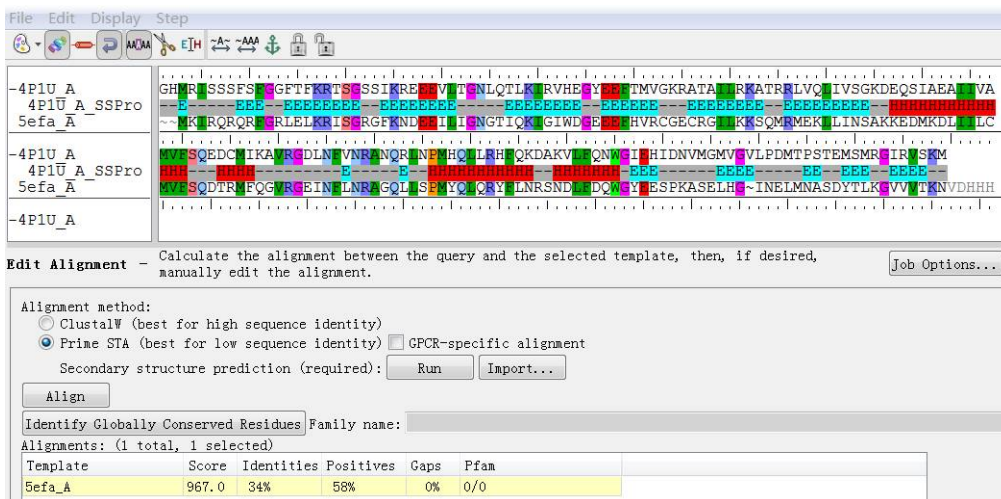


Figure S5. The sequence alignment results of the CBD site of influenza A virus and influenza B (partial) and the key amino acids that interact with active compounds.

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A/Hong Kong/1968 (H3N2)	S S F S F G G F T F K R T S G S S I K R E E E L L T G N L Q T L K I R V H E G Y			
A/Liaoning/2010 (H1N1)	S S F S F G G F T F K R T S G S S V K R E E E V L T G N L Q T L K L T V H E G Y			
A/WSN/1933 (H1N1)	S S F S F G G F T F K R T S G S S V K R E E E V L T G N L Q T L K I R V H E G Y			
B/Lee/40	Q R Q R F G R L E L K R I S G R G F K N D E E I L I G N G T I Q K I G I W D G E			
Compound 12C5	◆ ◆ ◆	◆		◆ ◆
Compound 11D4	▲ ▲			▲ ▲
Compound 21A5	● ● ●			● ●
Compound 21B1	● ● ●	●		● ●
Compound VX-787	★ ★			★ ★
	370	380	390	400
A/Victoria/1975 (H3N2)	E E F T M V G K R A T A I L R K A T R R L V Q L I V S G K D E Q S I A E A I I V			
A/Hong Kong/1968 (H3N2)	E E F T M V G K R A T A I L R K A T R R L V Q L I V S G R D E Q S V A E A I I V			
A/Liaoning/2010 (H1N1)	E E F T M V G K R A T A I L R K A T R R L I Q L I V S G R D E Q S I V E A I I V			
A/WSN/1933 (H1N1)	E E F T M V G R R A T A I L R K A T R R L I Q L I V S G R D E Q S I A E A I I V			
B/Lee/40	E E F H V R C G E C R G I L K K S K M R M E R L L I N S A K K E D M K D L I I L			
Compound 12C5	◆ ◆			
Compound 11D4	▲			
Compound 21A5	● ●	●		
Compound 21B1	● ●	●		
Compound VX-787	★ ★	★		
	410	420	430	440
A/Victoria/1975 (H3N2)	A M V F S Q E D C M I K A V R G D L N F V N R A N Q R L N P M H Q L L R H F Q K			
A/Hong Kong/1968 (H3N2)	A M V F S Q E D C M I K A V R G D L N F V N R A N Q R L N P M H Q L L R H F Q K			
A/Liaoning/2010 (H1N1)	A M V F S Q E D C M I K A V R G D L N F V N R A N Q R L N P M H Q L L R H F Q K			
A/WSN/1933 (H1N1)	A M V F S Q E D C M I K A V R G D L N F V N R A N Q R L N P M H Q L L R H F Q K			
B/Lee/40	C M V F S Q D T R M F Q G V R G E I N F L N R A G Q L L S P M Y Q L Q R Y F L N			
Compound 12C5	◆ ◆			◆ ◆
Compound 11D4	▲			▲ ▲
Compound 21A5	● ●			● ●
Compound 21B1	● ●			● ●
Compound VX-787	★			★ ★

Table S2 The sequences of the primers

Primers	Sequence
190822-R1	GTGCTCGAGCTATCATTTAATCCACGCCCATCTTGCTTATAC
190822-F2	CTGGAAGTCCTGTTTCAGGGACCCCGTATTAGCAGCTCCTTTAGCT
190822-F3	GGGAACAGATTGGAGGACTGGAAGTCCTGTTTCAGGGAC

