Supplemental Material

Figure S1

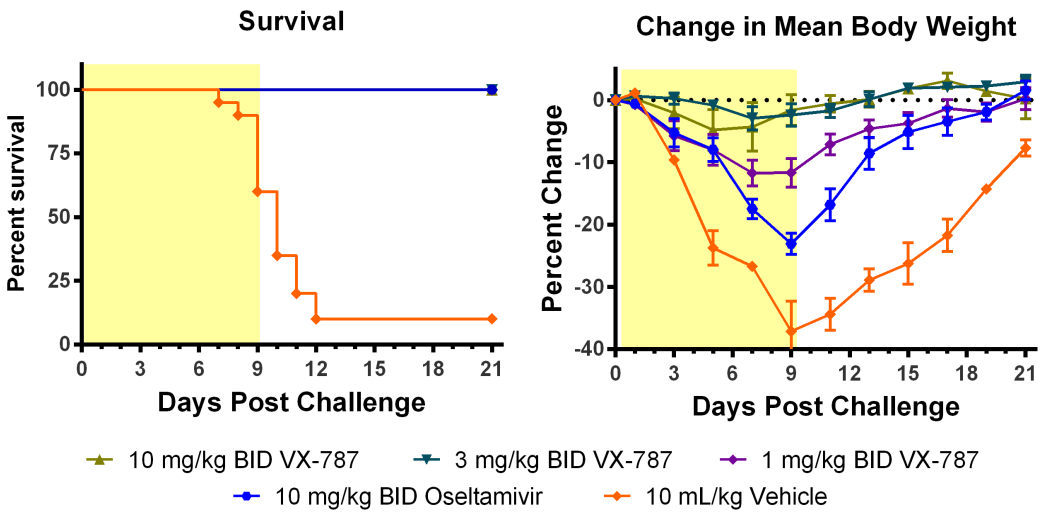

Figure S1. VX-787 demonstrates superior efficacy against 2009 pandemic influenza (H1N1pdm09) when compared to oseltamivir. The time course of morbidity/mortality, and bodyweight loss for BALB/c mice (10-20/group) challenged with A/California/04/2009 (H1N1pdm09) and treated with VX-787 or oseltamivir. Treatment with VX-787, oseltamivir or vehicle was initiated 2 h prior to infection, and continued BID for 10 days as denoted by the yellow boxes. Mice were monitored every day for morbidity/mortality and every other day for bodyweight loss (mean ± SEM) and plotted as Survival (Left panel) or percent bodyweight change (Right panel) for 21 days.

17 **Supplemental Tables**

18

Table S1. Isothermal titration calorimetry and surface plasmon resonance results for VX-787, and m^7GTP binding to PB2

	ITC					SPR Kinetics			SPR Steady State
	Stochiometry	K_D (M)	ΔH (cal/mol)	ΔG (cal/mol)	-TΔS (cal/mol)	K_D (M)	k_{on} ($M^{-1}sec^{-1}$)	k_{off} (sec^{-1})	K_D (M)
VRT-0761704[a]	0.93	4.02 E-07	-8145	-8731	-585	-	-	-	3.3-3.5E-07
SD[b]	0.08	6.30 E-08	532	97	590				
VX-787	0.84	2.38E-08	-8151	-10419	-2268	8.28E-09	2.61E+06	0.021	1.3-1.5E-08
SD	0.07	7.45E-09	289	192	481	2.80E-10	1.20E+06	0.009	
m7GTP	1.24	1.53E-06	-6247	-7914	-1667	-	-	-	-
SD	0.04	3.44E-07	353	192	479				

[a]Mean of 3 or more determinations
[b]Standard Deviation

19

20

Table S2. Summary of VX-787 and Oseltamivir prophylaxis and start to treatment efficacy experiments in a murine pulmonary influenza A model (A/Puerto Rico/8/34)

Treatment Start Time Relative to Infection (h)	VX-787 Dose (mg/kg; BID)	Oseltamivir Dose (mg/kg; BID)	Survival (Percent)	Bodyweight Loss on Study Day 9 (Percent±SEM)
-2	10	10	100	-2.8±1.9
	3		100	-8.7±1.1
	1		100	-16.8±1.1
	0.3		25	-30.4±1.8
	0.1		0	-31.9±2.0
	0		100	-19.1±1.1
			0	-32.2±0.9
+24	10	10	100	-6.2±1.4
	3		100	-14.2±1.5
	1		100	-23.4±1.5
	0		100	-28.9±1.6
			0	-33.8±0.9
+48	10	10	100	-7.1±1.2
	3		100	-10.9±1.6
	1		100	-22.5±1.5
	0		12.5	-31.1±1.3
			0	-34.4±0.8
+72	10	10	100	-17.4±1.3
	3		100	-23.2±1.4
	1		100	-29.4±1.5
	0		N.D.	N.D.
			0	-36.1±1.0
+96	10	10	100	-25.5±1.2
	3		100	-27.3±1.8
	0		N.D.	N.D.
			0	-34.6±1.1
+120	10	10	30	-34.4±1.6
	3		10	-32.6±1.5
	0		N.D.	N.D.
			0	-34.6±1.0

N.D., not determined due to health
N=8 mice/group

23

Table S3. Summary of VX-787 efficacy against pandemic influenza in a murine pulmonary influenza A prophylaxis model (A/California/04/2009)

Strain	Treatment Start Time Relative to Infection (h)	VX-787 Dose (mg/kg; BID)	Oseltamivir Dose (mg/kg; BID)	Survival (Percent)	Bodyweight Loss on Study Day 9 (Percent)	Lung Viral Titers Day 6 (Log Titer/ Lungs ± SD)	Log_{10} Reduction vs. Vehicle
A/California/ 04/2009(H1N1pdm09)	-2h	10		100	1.3	[a] <2.6***	>5.3
		3		100	2.4	3.1±0.9***	4.8
		1		100	11.5	5.5±1.2***	2.4
			10	100	22.8	7.9±0.2	0
		0		0	32.6	7.9±0.4	N/A

Two-way ANOVA with Bonferroni Post Test, *P<0.05, **P<0.01, ***P<0.001
N=5 Mice/group
[a]Level of detection

24
25

Table S4. Summary of VX-787 efficacy against avian influenza A/Viet Nam/1203/2004 (H5N1) in a murine pulmonary influenza A model

Treatment Start Time Relative to Infection (h)	VX-787 Dose (mg/kg; BID)	Oseltamivir Dose (mg/kg; BID)	Survival (Percent)	Bodyweight Loss on Study Day 7 (Percent ± SEM)
-2h	10	10	100	0.5±.01
	3		100	1.3±1.0
	1		100	1.6±1.1
			100	1.2±0.9
	0		0	15.6±1.4
+24	10	10	100	2.9±2.7
	3		100	6.5±3.4
			0	13.2±1.6
	0		0	16.0±1.8
+48	10	10	100	2.1±0.9
	3		100	4.9±0.8
			0	19.8±1.8
	0		0	16.0±1.8
+72	10	10	100	2.3±2.1
	3		70	3.3±1.1
			N.D.	N.D.
	0		0	16.0±1.8
+96	10	10	100	1.4±2.0
	3		30	16.6±7.2
			N.D.	N.D.
	0		0	16.0±1.8
+120	10	10	70	12.7±2.0
	3		0	18.4±3.7
			N.D.	N.D.
	0		0	16.0±1.8

N.D. Not determined

N=10 mice/group for treated and 20 mice/group for controls

Table S5. *In vitro* activity of VX-787, Oseltamivir carboxylate and Zanamivir against representative influenza type B strains

Virus	Mean EC_{50} (μM)		
	VX-787	**Oseltamivir carboxylate**	**Zanamivir**
B/Lee/40	> 10 ± ND, n=2	> 10 ± ND, n=4	> 10 ± ND, n=2
B/Maryland/1/59	> 10 ± ND, n=1	1.7 ± 0.42, n=2	7.5 ± ND, n=3
B/Taiwan/2/62	> 10 ± ND, n=1	0.40 ± 0.29, n=2	0.21 ± 0.16, n=2

Data shown represent mean ± standard deviation of "n" independent experiments.
EC_{50}: effective concentration at which ATP is half the maximum in the CPE-based assay.