Pre-clinical characterization of AV5080, a new oral influenza neuraminidase inhibitor, active against Oseltamivir-resistant virus

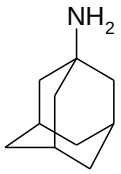
Vadim Bichko

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[1]ASAVI LLC, Hallandale Beach, FL, USA; [2]Chemical Diversity Research Institute, Khimki, Moscow Reg., Russia; [3]ChemDiv, San Diego, CA, USA; [4]The Moscow Institute of Physics and Technology, Dolgoprudny, Moscow Reg., Russia; [5]I. Mechnikov Research Institute of Vaccines and Sera of Russian Academy of Medical Science, Moscow, Russia
*Corresponding author. Tel: +1-858-794-4860; Fax: +1-858-794-4931; E-mail: av@asavillc.com

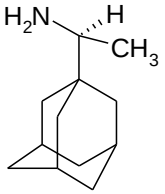
ASAVI, LLC
1835 E. Hallandale Beach Blvd, #442
Hallandale Beach, Fl 33009

Influenza Inhibitors

M2 channel blockers

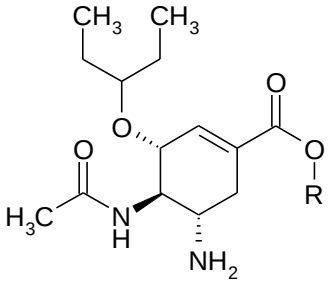


1. Amantadine

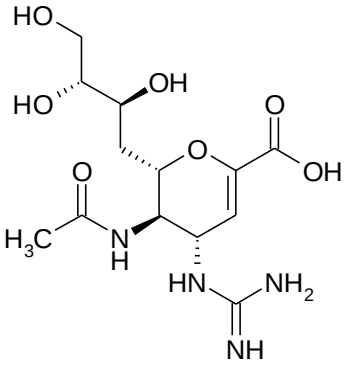


2. Rimantadinum

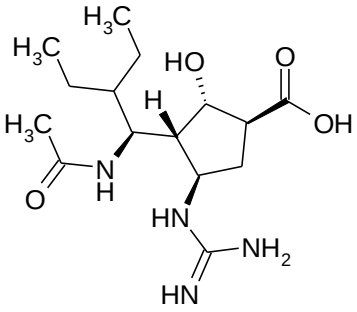
NA inhibitors



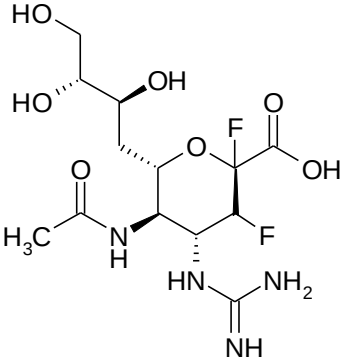
3. Oseltamivir carboxylate: R = H
4. Oseltamivir: R = C_2H_5



5. Zanamivir

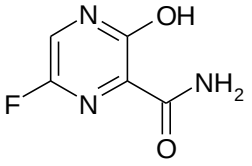


6. Peramivir



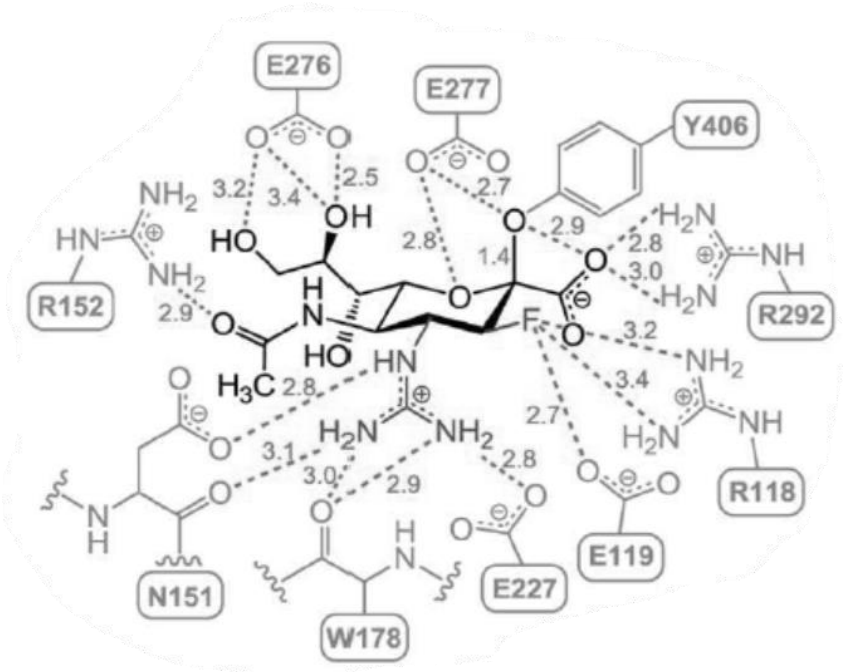
Compound 7

Pol inhibitor



8. Favipiravir

Covalent NA Inhibitor

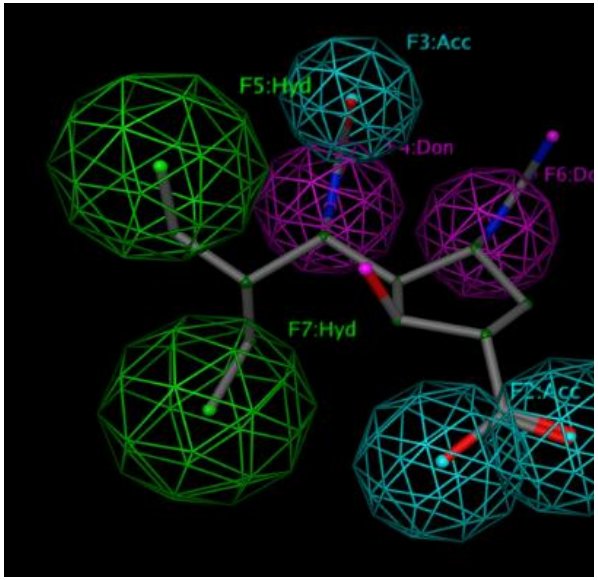


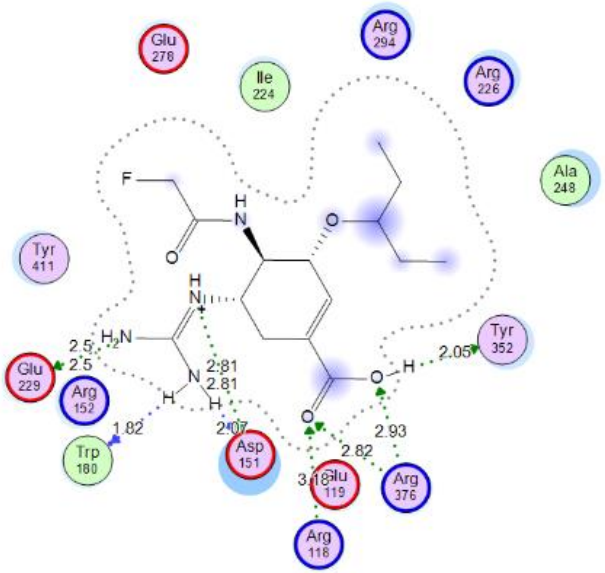
Binding interface (Å) for Compound **7** in the active site of Neuraminidase.
The covalent link between the inhibitor's C-2 atom and the OH group of Y406 is shown

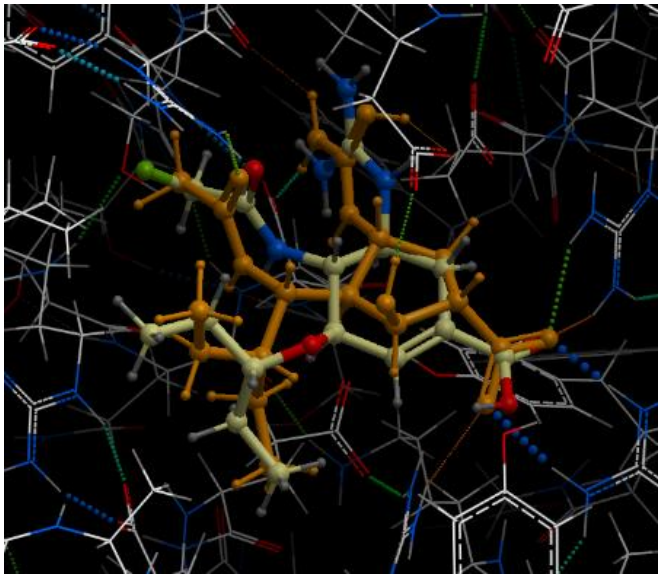
Kim et al,. Science 2013; 340: 71–5

Molecular Modeling of NA Inhibitors

A B C







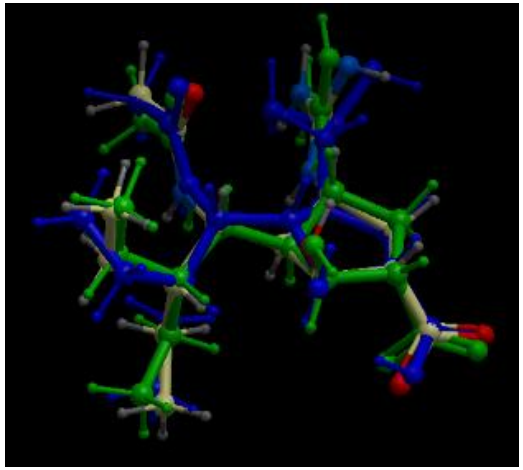
A: 3D Pharmacophore model for the active binding conformation of Peramivir;

B: 2D supramolecular interface predicted for AV5080 (docking);

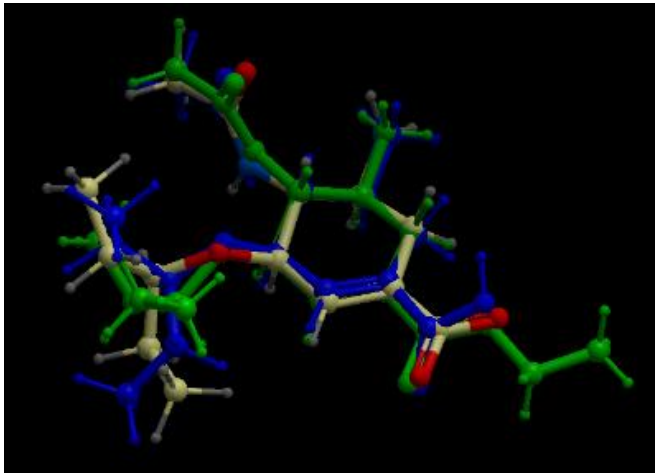
C: 3D binding mode of AV5080 (white. *docking study*) and Peramivir (grey. *RSA*)

Molecular Modeling of NA Inhibitors (cont.)

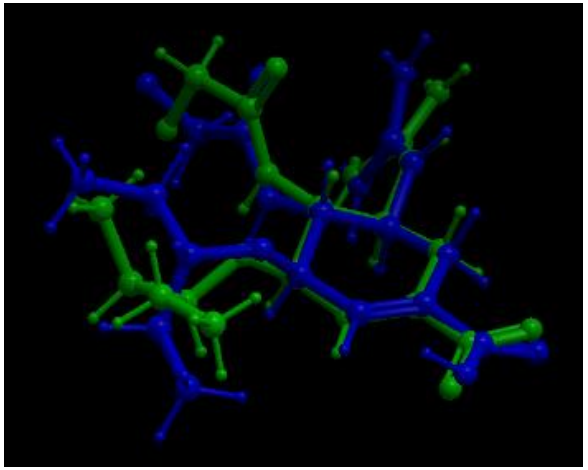
Peramivir



Oseltamivir

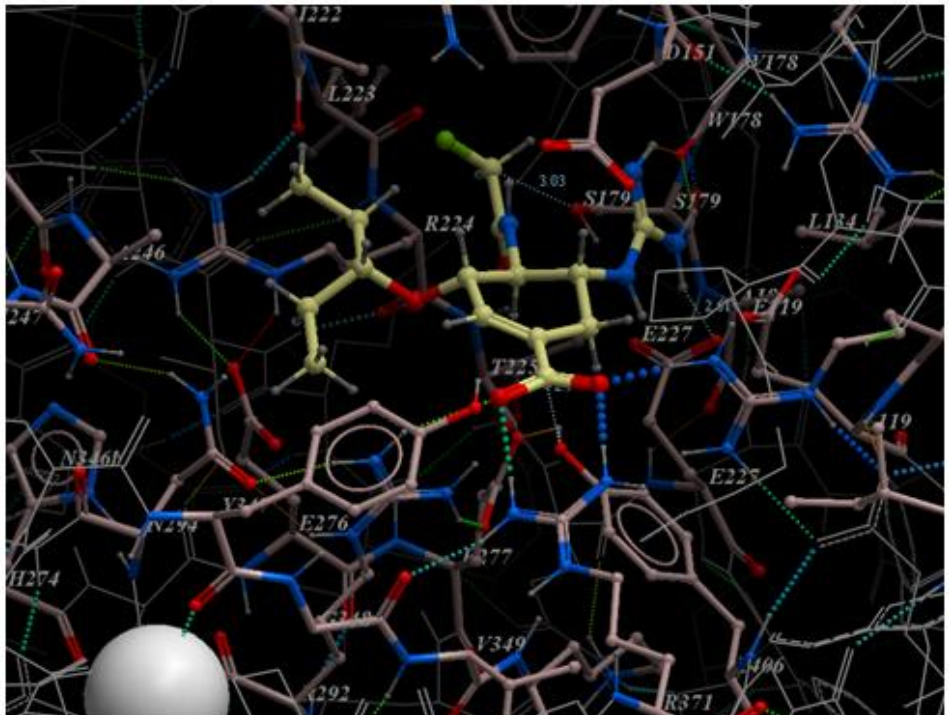


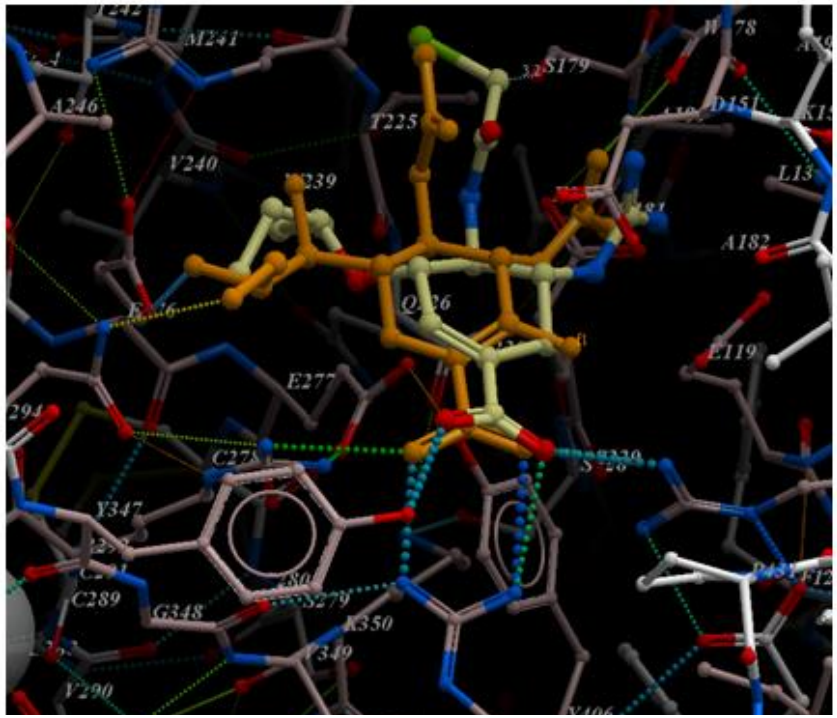
AV5080



Green: docking;
Blue: cristals;
White: Roentgen stereogrammatic analysis with NA

Molecular Modeling of NA Inhibitors (cont.)

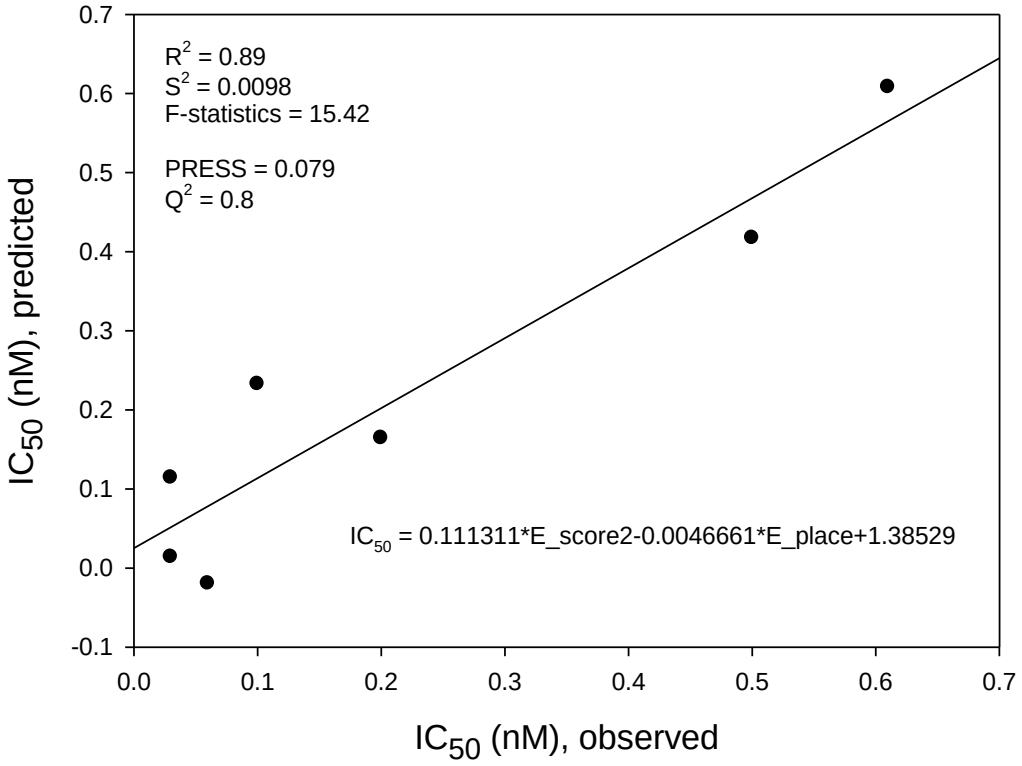




Left: 3D Pharmacophore model for the active binding conformation of AV5080;

Right: 3D binding mode of AV5080 (yellow, docking) and Zanamivir (grey. *RSA*)

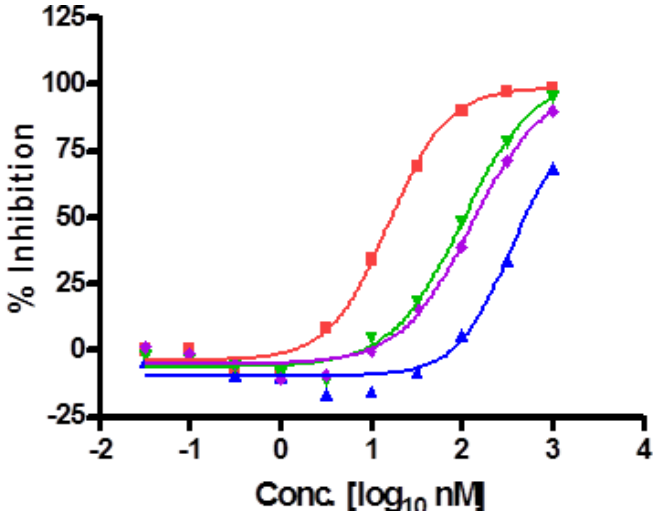
Linear regression model, constructed for seven NA inhibitors

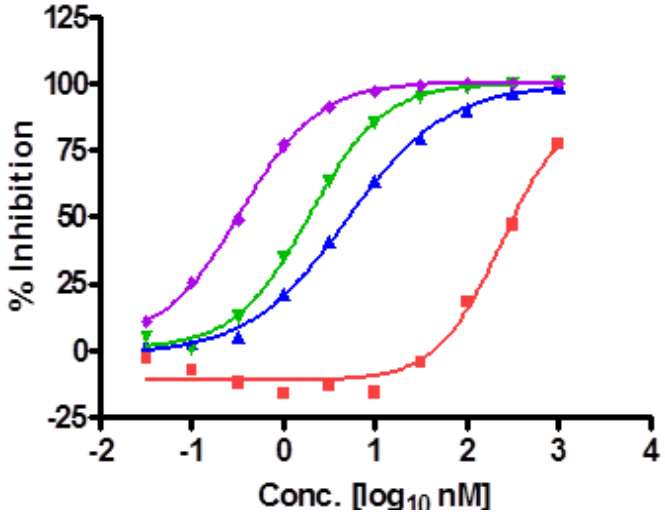


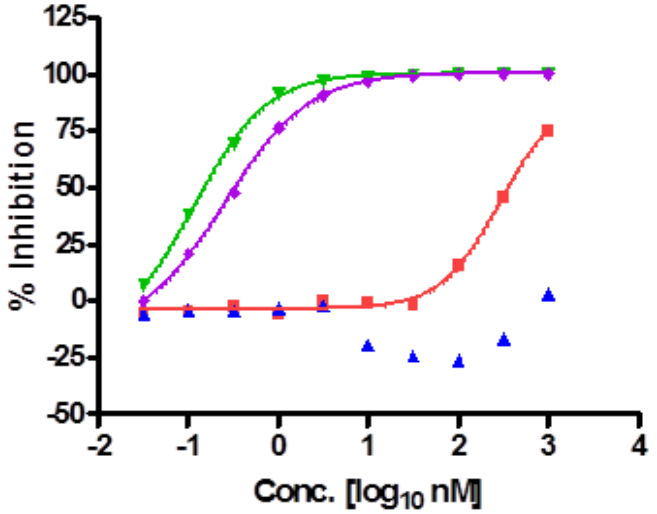
PRESS: predicted sum-of-squares error;
Q^2: cross-validated multiple determination coefficient

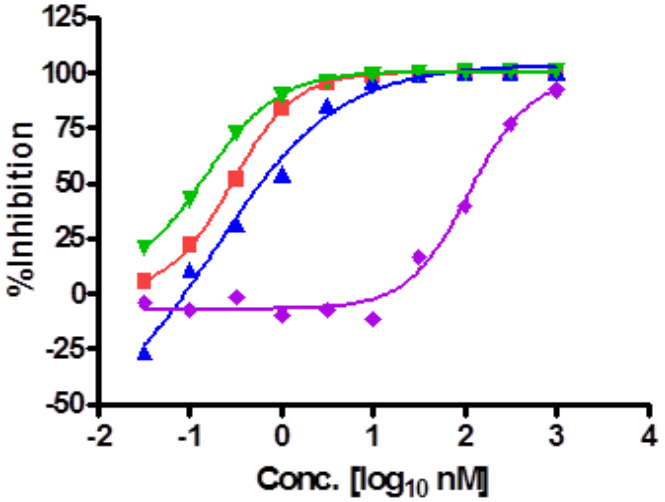
NA inhibition chemiluminescence assay

Influenza A/Duck/ /1525/1981/H5N1









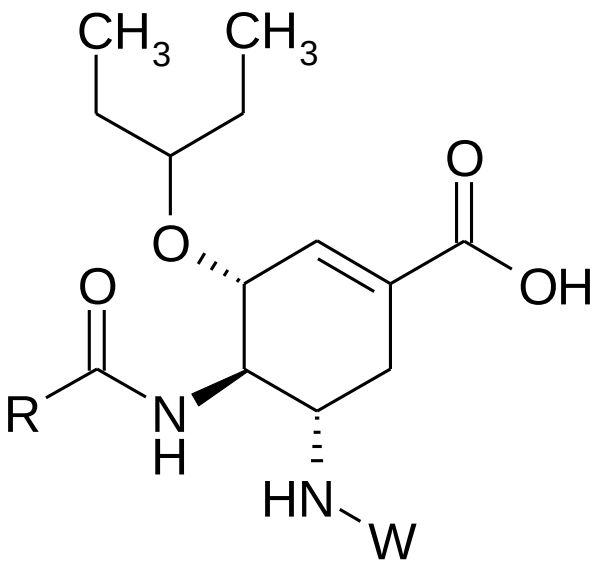
MOE scores and NA inhibition (IC_{50} values)

Influenza A/Duck/ /1525/1981/H5N1 (*chemiluminescence assay*)

Compound	IC_{50}	SD	E_score2*	E_place**
AV5080	0.03	0.00	-18.86	-155.906
AV5082	0.61	0.17	-13.27	-149.918
AV5085	0.50	0.05	-13.28	-109.36
AV5063	0.06	0.01	-17.97	-127.538
Oseltamivir	0.10	0.01	-15.49	-122.485
Zanamivir	0.20	0.03	-15.61	-110.623
Peramivir	0.03	0.01	-16.30	-116.423
Outliers				
AV5068	48.67	7.41	-14.00	-132.87
AV5070	12.57	3.18	-13.93	-133.076

* - score from the rescoring stage(s) / docking stage
** - score from the placement stage / pharmacophore stage

Selected NA inhibitors, designed in this study



W=C(NH)NH_2.
R: CH≡C (AV5063). FCH_2 (AV5080). F_2CH (AV5068). F_3C (AV5070);

W=4.5-dihydro-1*H*-imidaz-2-yl.
R: CH_3 (AV5085). FCH_2 (AV5082). F_2CH (AV5084). F_3C (AV5078)

NA inhibition activity

Compound	A, H1N1, California/07/2009		A, H5N1, Duck/MN/1525/1981		B, Florida/4/2006	
	IC_{50} (nM)	SD	IC_{50} (nM)	SD	IC_{50} (nM)	SD
AV5070	18.17	7.15	12.57	3.18	66.67	5.31
AV5082	0.22	0.02	0.61	0.17	2.93	1.37
AV5080	0.06	0.00	0.03	0.00	0.61	0.16
AV5085	0.13	0.00	0.50	0.05	126.67	17.00
AV5063	0.12	0.01	0.06	0.01	2.93	0.33
AV5068	51.00	4.32	48.67	7.41	356.67	60.18
Oseltamivir	0.07	0.00	0.10	0.01	0.54	0.14
Zanamivir	0.24	0.02	0.20	0.03	1.13	0.19
Peramivir	0.03	0.01	0.03	0.01	0.21	0.13

NA inhibition activity: Antiviral spectrum

Virus strain	AV5080	Oseltamivir carboxylate	Zanamivir	AV5080	Oseltamivir carboxylate	Zanamivir
	IC_{50} ± SD, nM (n)			IC_{90} ± SD, nM (n)		
A/California/07/2009 (H1N1)	0.19±0.08 (6)	0.76±0.43 (6)	0.2606±0.3241 (6)	0.71±0.24 (6)	19.85±7.11 (6)	2.96±1.81 (6)
A/Aichi/2/1969 (H3N2)	0.69±0.19 (6)	0.43±0.24 (6)	0.5953±0.4474 (6)	3.35±0.51 (6)	3.50±1.64 (6)	7.04±2.28 (6)
A/Vladivostok/16/2009 (H1N1) Oselt.-resistant	4.45±0.85 (7)	844.4±110.0 (7)	0.6035±0.2509 (7)	70.36±27.96 (7)	7504±2524 (7)	7.69±2.44 (7)
A/Perth/16/2009 (H3N2) Rimantadine-resistant	0.46±0.018 (2)	0.92±0.01 (2)	0.04±0.00 (2)	2.04±0.07 (2)	3.31±0.20 (2)	5.10±0.32 (2)
A/Chicken/Rostov-on-Don/35/2007 (H5N1)	0.20±0.00 (2)	1.78±0.09 (2)	0.59±0.07 (2)	0.73±0.02 (2)	147.4±1.4 (2)	10.45±0.56 (2)
B/Brisbane/60/2008	**1.27±0.14 (2)**	**25.42±0.51 (2)**	1.87±0.02 (2)	6.04±1.36 (2)	649.8±182.3 (2)	49.16±7.30 (2)
A/Perth/265/2009 (H1N1; 275H), Oselt.-resistant	0.07±0.05 (4)	1.002±0.068 (4)	0.46±0.07 (4)	0.70±0.07 (4)	12.49±4.41 (4)	3.64±0.68 (4)
A/Perth/265/2009 (H1N1; 275Y), Oselt.-resistant	1.39±0.38 (4)	359.9±87.2 (4)	0.72±0.16 (4)	38.15±18.13 (4)	3886±745 (4)	5.34±0.83 (4)
B/Perth/211/2001 (179D)	**2.08±0.71 (4)**	**39.24±2.436 (4)**	3.67±0.20 (4)	13.49±1.26 (4)	719.5±124.5 (4)	70.88±9.42 (4)
B/Perth/211/2001 (179E), Oseltamivir-resistant	6.65±0.90 (4)	230.3±62.6 (4)	21.98±5.88 (4)	99.15±34.12 (4)	3536±413 (4)	642.5±192.6 (4)

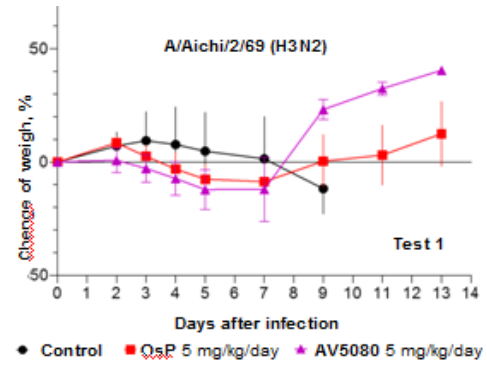
Antiviral activity and cytotoxicity of selected compounds in MDCK cells, infected with influenza virus

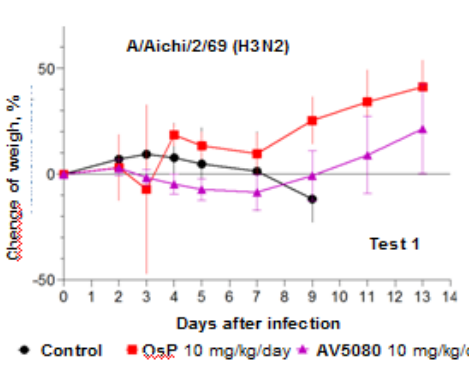
Compound	A/California/07/2009 (H1N1)			A/Duck/Minnesota/1525/1981 (H5N1)		
	$EC_{50,}$ nM	CC_{50}, μM	SI	$EC_{50,}$ nM	CC_{50}, μM	SI
AV5080	0.75	>690	>925926	2.50	>568	>227273
Zanamivir	15.95	>30	>1887	51	>30	>588
Oseltamivir carboxylate	9.85	>35	>3571	77	>35	>455
T-705	1530	>796	>521	6370	>64000	>10

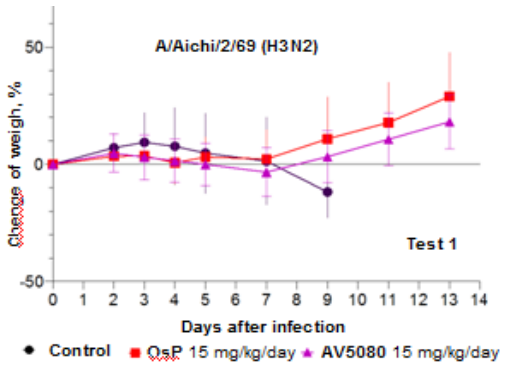
Antiviral efficacy of AV5080 and Oseltamivir phosphate (OsP) after oral administration in infected mice

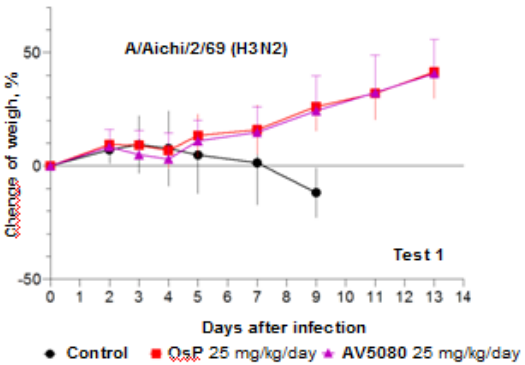
Virus	Compound	Dose, mg/kg/day	Survival	Mean day to death ± SD* (n)
A/Aichi/2/1969 (H3N2)	Control		0/12	7.58 ± 2.19 (12)
	OsP	5	5/10**	10.40 ± 3.92 (10)
	AV5080	5	2/10	9.60 ± 2.41 (10)
	OsP	10	7/9***	11.78 ± 4.41 (9)
	AV5080	10	4/9*	10.00 ± 4.00 (9)
	OsP	15	9/10***	13.00 ± 3.16 (10)
	AV5080	15	6/10**	11.10 ± 3.93 (10)
	OsP	25	10/10***	14.00 ± 0.00 (10)
	AV5080	25	9/10***	13.10 ± 2.85 (10)
	OsP	35	8/10***	12.20 ± 3.82 (10)
	AV5080	35	7/10***	12.00 ± 3.97 (10)
	OsP	40	8/10***	12.70 ± 2.75 (10)
	AV5080	40	9/10***	13.00 ± 3.16 (10)
	OsP	50	9/10***	13.30 ± 2.21 (10)
	AV5080	50	8/10***	12.40 ± 3.50 (10)

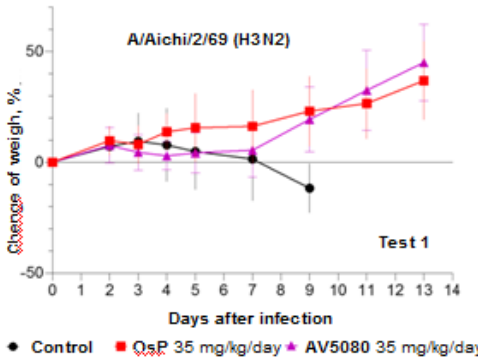
Body weight change in infected mice after oral administration Of AV5080 and Oseltamivir phosphate (OsP)

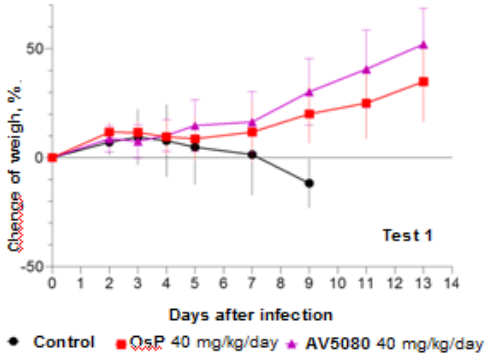


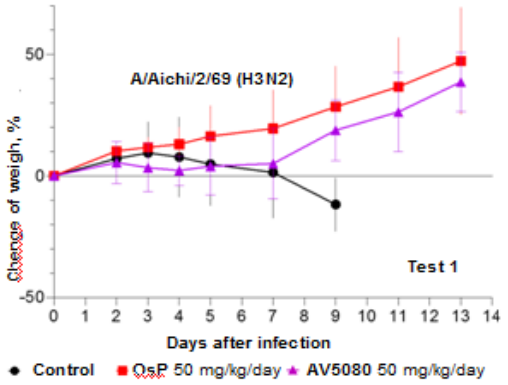


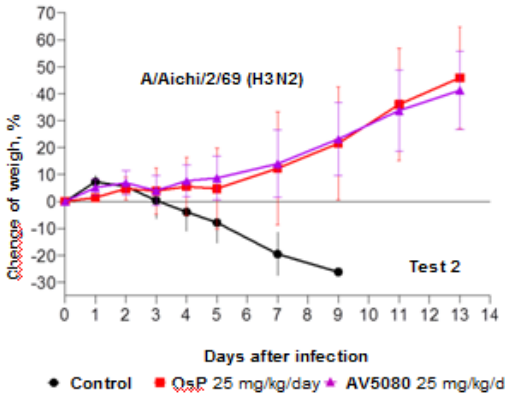


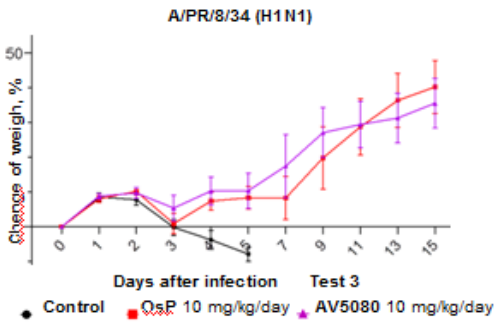




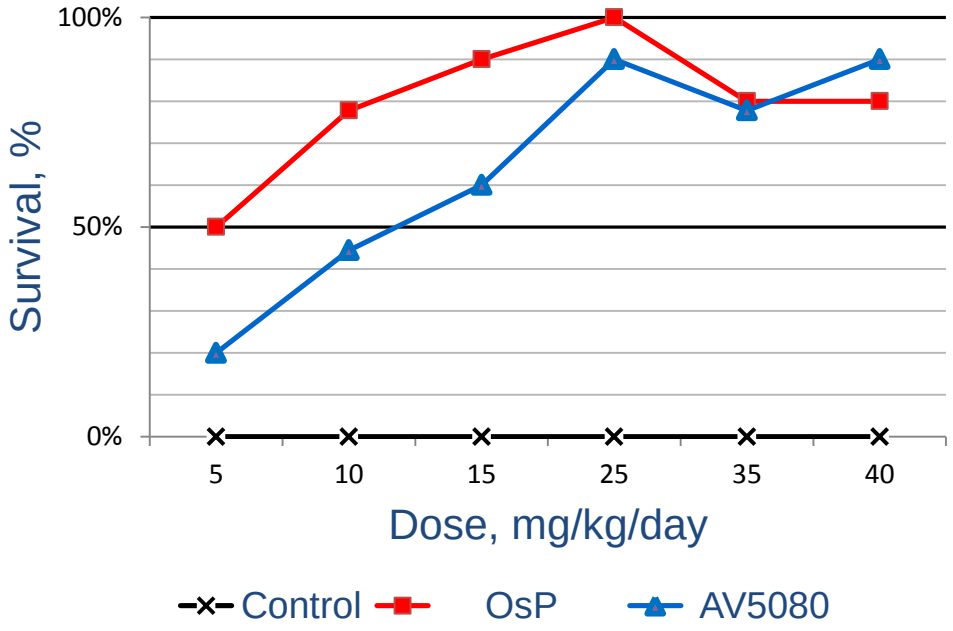








Mouse pneumonia model: survival rates



- Strain A/Puerto Rico/8/1934 (H1N1)
- Oral drug administration @ 5-50 mg/kg/day

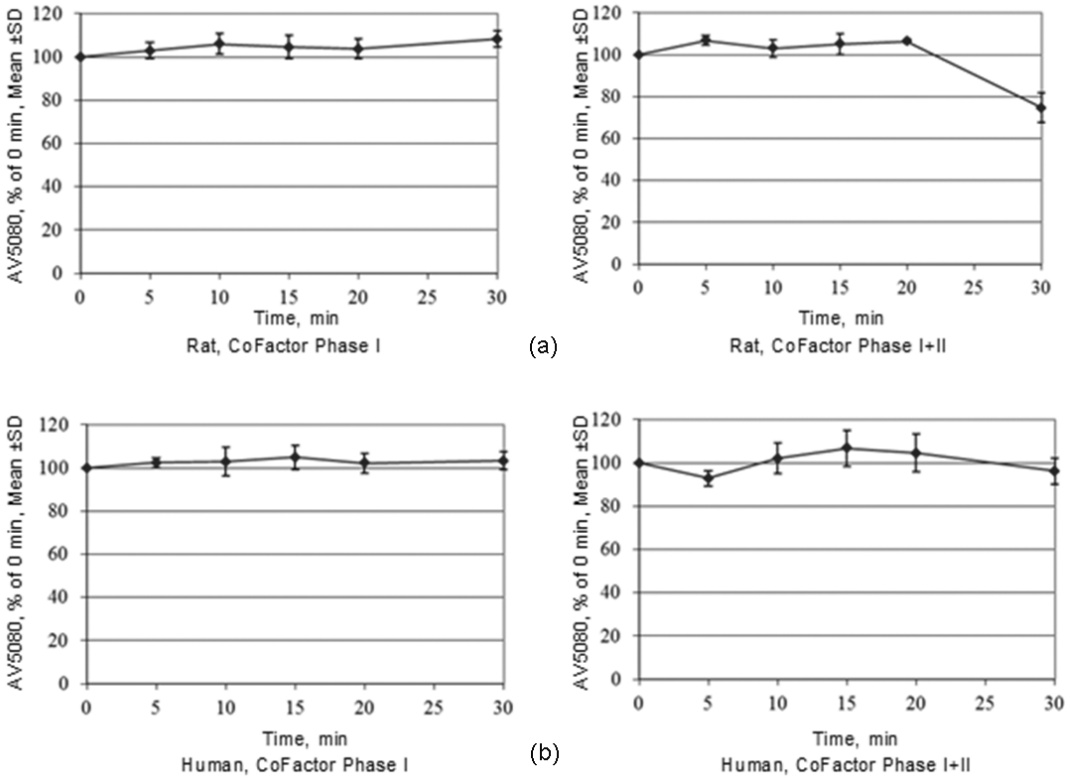
AV5080 Pharmacology

- Low plasma protein binding
  - Rat: 31.3%
  - Human: 35.0%
- *In vitro* metabolism
  - Good microsomal stability (rat, dog, human)
- Genotox: Non-mutagenic
  - AIMS test
  - *In vivo* mutagenesis
- hERG assay: IC50>20 μM
- No CYP inhibition: IC50>10 μM
  - 1A2, 2C9, 2C19, 3A4, 2D6

Stability of AV5080 in the presence of liver microsomes

CoFactors Phase I

CoFactors Phase I+II



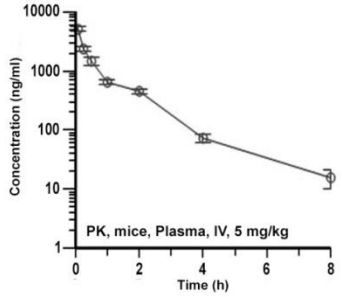
rat

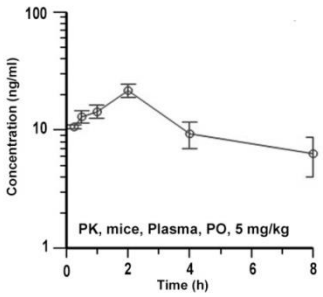
human

rat			dog			human		
0 h	4 h	24 h	0 h	4 h	24 h	0 h	4 h	24 h
AV5080 (% intact compound remaining)								
100	82±4.1	93±5.3	100	93±4.5	99±6.7	100	82±4.1	93±5.3

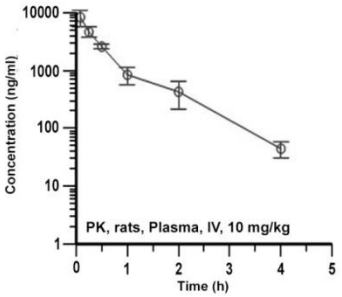
Preclinical pharmacokinetics

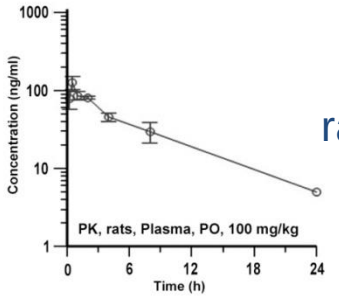
I.V. P.O.



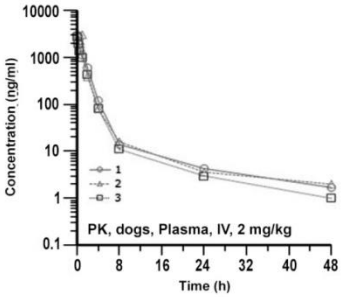


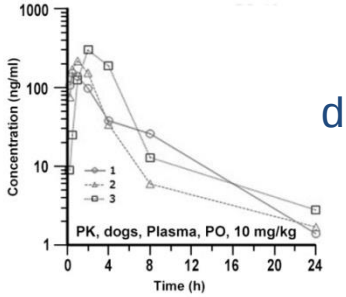
mouse





rat





dog

Bioavailability: Rat 2.5%, dog 4.3%

Dog, 2 mg/kg I.V.

Animal #	$t_{1/2}$	C_0	AUC_{0-t}	$AUC_{0-\infty}$	Kel	Vd	Vss	CL	MRT last
Units	h	Ng/ml	h*ng/ml	h*ng/ml	1/h	l/kg	l/kg	l/h/kg	h
1	13.4	3185	4083	4116	0.052	9.4	1.3	0.5	2.1
2	13.9	2270	5039	5079	0.050	7.9	0.9	0.4	1.8
3	11.8	3082	3211	3228	0.059	10.5	1.4	0.6	2.0
Mean	**13.0**	**2846**	**4111**	**4141**	**0.053**	**9.3**	**1.2**	**0.5**	**2.0**
SD	1.12	501.2	914.2	925.7	0.005	1.3	0.26	0.11	0.17
SEM	0.65	289.4	527.8	534.5	0.003	0.8	0.15	0.07	0.10

Dog, 10 mg/kg P.O.

Animal #	$t_{1/2}$	C_{max}	T_{max}	AUC_{0-t}	$AUC_{0-\infty}$	Kel	MRT last
Units	h	Ng/ml	h*ng/ml	h*ng/ml	h*ng/ml	1/h	h
1	4.1	152	0.5	720	728	0.17	4.50
2	8.8	218	1.0	653	674	0.08	2.87
3	7.2	300	2.0	1272	1301	0.10	3.80
Mean	**6.7**	**223**	**1.2**	**882**	**901**	**0.11**	**3.7**
SD	2.40	74.1	0.8	339.6	347.3	0.048	0.81
SEM	1.39	42.8	0.4	196.1	200.5	0.028	0.47

A 6-week tox study in rats

- 30-day administration, 15-day follow up
- Doses of 0 (vehicle), 15, 50 or 150 mg/kg PO once daily
- A total of 80 animals (10 animals per dose per sex)
- body weight, food consumption, hematological, or serum chemistry parameters, and postmortem examinations: organ weights, macroscopic and microscopic evaluation of tissues

- Results
  - Generally safe and well tolerated
  - Mild signs of gonadotoxicity in males (not confirmed by a follow-om reproductive tox study)

A 6-week tox study in dogs

- 30-day administration, 15-day follow up
- Doses of 0 (vehicle), 7 and 35 mg/kg PO once daily
- A total of 36 animals (6 animals per dose per sex)
- body weight, food consumption, hematological, or serum chemistry parameters, ECG and postmortem examinations: organ weights, macroscopic and microscopic evaluation of tissues

- Results
  - Generally safe and well tolerated
  - Signs of reversible hepato- and renal toxicity at 35 mg/kg/day
  - NOAEL for dogs was 7.5 mg/kg/day

Animal toxicology studies with AV5080

- 1. Single ascending dose and repeat-dose (7 day PO) toxicity and PK study in mice and rats
- 2. Immunotoxicity and allergenisity study
- 3. Acute toxicity study in rats (oral and intraperitoneal administration)
- 4. A 6 week toxicity study in rats
- 5. A 6-week toxicity study in dogs
- 6. Gonadotoxicity study in female rats (oral administration)
- 7. Study of embryotoxicity and teratogenic effects in female rats (oral administration)
- 8. Reproductive toxicity study in male rats (oral administration)
- 9. Reproductive toxicity study in female rats (oral administration)

Phase Ia clinical study planned

- Double-blind, randomized, placebo controlled study to assess safety and tolerability of increasing doses of AV5080 over single and following multiple oral dosing in healthy volunteers
- Study design:
  - Cohort 1– 30 mg or placebo in 4:2 ratio (1 40 mg capsule or placebo)
  - Cohort 2– 80 mg or placebo in 4:2 ratio (1 80 mg capsule or placebo)
  - Cohort 3– 160 mg or placebo in 8:2 ratio (1 80 mg capsule or placebo twice daily)
  - Cohort 4– 320 mg or placebo in 8:2 ratio (2 80 mg capsule or placebo twice daily)
- Objectives:
  - safety,
  - tolerability
  - PK
- To begin Q3 2015

Summary

- AV5080 is a new NA inhibitor for treatment of influenza infections
- IP position established
- Robust antiviral activity *in vitro* and *in vivo* demonstrated
- Efficacious upon oral administration in mouse pneumonia model
- Active against Tamiflu-resistant virus
- Metabolically stable
- Good *in vitro* safety pharmacology and preclinical PK profiles
- Safe and well tolerated in rodents and dogs
- Preclinical studies completed
- Clinical studies to begin Q3 2015