Identification & Early Lead Optimization of A Chemical Series With Activity Against The Dengue Virus

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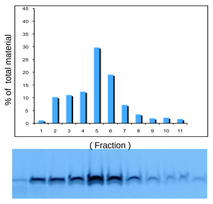
Abstract

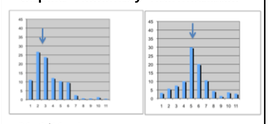
Dengue virus, a member of the *Flaviviridae* family, reportedly infects 40 to 100 million people each year and is the biggest arbovirus problem in the world today, with over half the world's population at risk. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. South-east Asia and the Western Pacific are the most seriously affected. There are four serotypes of Dengue (DEN-1, DEN-2, DEN-3 and DEN-4). The severity of the infection ranges from self-limiting to the more serious forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) with mortality ranging from 1 to 5% to 10 to 30% respectively. Currently, there is no specific treatment for the dengue virus. Prosetta has developed a proprietary, broad-spectrum platform (CFP) for the identification of potential antiviral compounds. The innovative platform developed by Prosetta is a broad-spectrum technology which can survey the entire viral capsid assembly pathway and identify small molecules which disrupt key steps involved in capsid assembly. The stage of the viral life cycle that the platform targets – capsid synthesis and assembly – has been largely ignored by antiviral developers, potentially enabling identification of novel modes of therapy. The compounds identified using this platform provide broad-spectrum protection against members of the same virus family, demonstrating the highly conserved nature of the viral capsid across the viral family and making it an excellent target for therapeutic intervention The protein synthesis-linked capsid assembly system was converted into an ELISA-based screening platform for identification of small molecules that interfere with proper Dengue virus capsid formation. This screen has identify multiple distinct classes of small molecules capable of modulating the host factors involved in the viral capsid assembly pathway. All of the small molecules hits conform to Lipinski's Rule of Five. When tested against live Dengue virus in cell culture, a number of these compounds were found to be robustly active, resulting in multi-log drop in plaque forming unit (pfu) titer in the nanomolar to low micromolar range. These active molecules were sorted by chemical class, activity, and toxicity. A total of 11 chemical classes (pharmacophores) were found to be potent (EC 50<7.5uM) and non-toxic (TI>10) against the dengue virus. These findings provide strong support for the hypothesis that critical steps in Dengue virus capsid formation are faithfully re-created in the cell-free system. One class of inhibitors was selected for early lead optimization and will be presented briefly.

Cell-free Translation of DENV Core Reveals an Apparent Assembled Complex by ssg



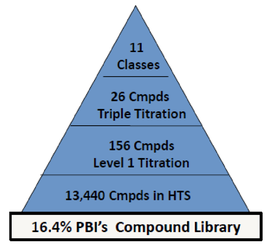
Legend to Figure 1. Cell-free translation of DENV core transcript reveals the expected 12kDa protein in a discrete complex in fraction (f) 5 on sucrose step density gradients (ssg).

Time Course Reveals Putative DENV Capsid Assembly Intermediates



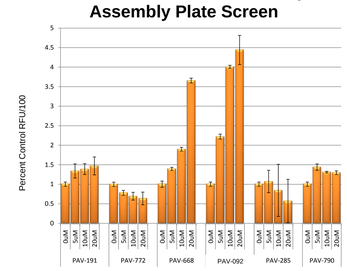
Legend to Figure 2. Translation products of DENV core polypeptide were analysed by sucrose step gradient velocity sedimentation (ssg), polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS PAGE), with autoradiography and quantitative densitometry at various times after start of protein synthesis. The parameters expected to affect an enzymatic process (time, temperature, substrate and host factor concentration) were varied and the consequences for flux of putative intermediates assessed.

From HTS to Antiviral Hits



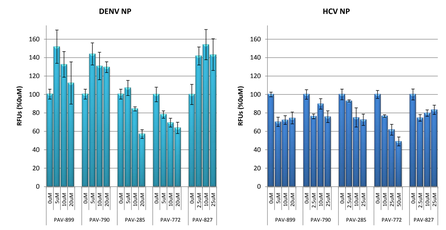
Legend to Figure 3. Approximately 16% of the Prosetta compound library was screened in a HTS format to yield 156 positives. These hits were subjected to a single concentration and triple point titration, to identify 11 classes of inhibitors of the dengue viral capsid pathway.

A Diversity of Phenotypes of DENV Core Fluorescence Readout in the Capsid Assembly Plate Screen



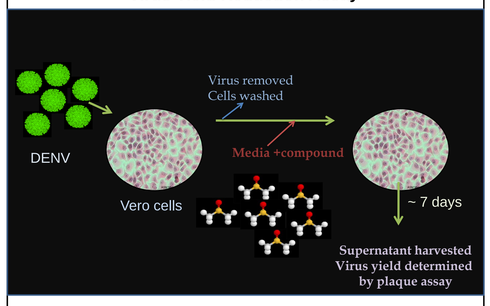
Legend to figure 4. Fluorescence output of compounds titrated in Prosetta's DENV capsid assembly screen over the indicated dose ranges. Error bars refer to standard deviation.

DENV vs. HCV Cell-Free Screen



Legend to fFigure 5. Fluorescence output of compounds titrated in both Prosetta's DENV and HCV capsid assembly screens over the indicated dose ranges. Error bars refer to standard deviation.

Virus Yield Reduction Assay



Legend to Figure 6. Prosetta compounds were tested in standard plague assays for a measure of virus yield reduction. The live virus cell cultures were performed at USAMRIID.

Profile of Small Molecule Dengue Virus Inhibitors

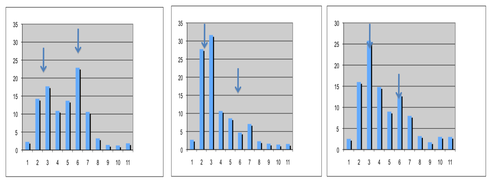
- 11 Chemical Series Identified from proprietary screen
  - 8 Chemical Series with EC_{50} < 10 μM in live virus assay
  - 5 Chemical Series with EC_{50} < 1.0 μM in live virus assay
    - 6 Chemical Series with EC_{99} < 10 μM in live virus assay
    - 2 Chemical Series with EC_{99} < 1.0 μM in live virus assay
- Promising Series Selected For Lead Optimization
  - SAR Optimization of Efficacy, PK and Tox profiles on-going
  - Several compounds with Selectivity Index (SI) 50 - 100
  - Animal Studies Underway (Tox, PK, etc…)
- Potential Back-Up Series with multiple chemically distinct classes

Efficacy of Prosetta Drugs on Live DENV in Cell Culture

	DENV		WNV		HCV		VEEV	
PAV #	**EC50**	**EC99**	**EC50**	**EC99**	**EC50**	**EC99**	**EC50**	**EC99**
PAV-899	<0.05uM	<0.05uM	<0.05uM	<0.05uM	<1uM	1uM	1uM	10uM
PAV-790	<7.5uM	10uM	>20uM	>20uM	ND	ND	>20uM	>20uM
PAV-285	<1uM	7.5uM	1uM	>20uM	ND	ND	<25uM	25uM
PAV-772	<20uM	20uM	<10uM	>10uM	<2uM	10uM	10uM	>10uM
PAV-827	<10uM	<10uM	10uM	>10uM	ND	ND	ND	ND

Legend to Figure 7. Compounds representing several pharmacophores identified through the Prosetta plate screen were compared for their efficacy against live DENV in cell culture and against other members of family *Flaviviridae* (WNV and HCV). In addition, as we have observed a strong correlation between activity against the *Flaviviridae* and the *Togaviridae*, their efficacy against VEEV, a member of the latter family was assessed as well. Some remarkable observations are that 1) PAV-899 and PAV-285 shows strong efficacy against all viruses tested, illustrating the propensity of some of our compounds to be pan family and more in its anti-viral spectrum; 2) PAV-790 is active *only* on DENV and not on WNV or VEEV, illustrating that a degree of viral specificity can be observed within this pharmacophore as well; 3) PAV-772 appears potent against all of the *Flaviviridae*, but without strong effect on VEEV; 4) PAV-827, the structure being disclosed, may be similar to PAV-790 in its relative DENV specificity.

Blockade of DENV Capsid Assembly by Small Molecules in the Prosetta Cell-Free System



Legend to Figure 8. Prosetta compounds PAV-790 and PAV-092 appear to cause a block in DENV capsid formation such that the putative early assembly intermediate seen in fraction 2/3 accumulates upon drug treatment in the cell-free system.

Early Optimization of A Dengue Inhibitor Lead Series

- Lead series PAV-8668 shows robust SAR in live virus assay
- Lead series PAV-8668 has excellent drug-like properties
- Series optimization is underway to improve potency, PK and tox profiles

Other Pre-Lead Series Active Against DENV

Chemical Series	Cell Culture Live Virus EC_{50}
PAV-1065	EC_{50} < 1.0 μM, EC_{99} < 1.0 μM
PAV-8899	EC_{50} < 1.0 μM, EC_{99} = 5 μM
PAV-7285	EC_{50} < 1.0 μM, EC_{99} = 7.5 μM
PAV-1185	EC_{50} = 1.0 μM, EC_{99} = 10 μM

Legend to Figure 9. Lead series PAV-8668 has been subjected to initial optimization to improve the potency and selectivity profiles. The compounds above clearly demonstrate an improvement in the Selectivity Index (SI) from 10 to greater than 50. The tables to the right illustrate the ability of compounds in this series to selectively inhibit either the DENV or VEEV. Prosetta has also identified a number of compounds in this series that inhibit both DENV and VEEV.

Compounds From PAV-8668 Series Active on VEEV & DENV

PAV#	CC_{50} (μM)	Conc (μM)	VEEV % control	VEEV log reduction	Conc (μM)	DENV % control	DENV log reduction
PAV-071	>25	1	99.91	2	1	96.74	1
		7.5	100.00	8	7.5	100.00	5
		20	100.00	8	20	100.00	5
PAV-212	2.5-10	1	70.43	1	1	76.26	1
		7.5	100.00	5	7.5	100.00	5
		20	100.00	7	20	100.00	5
PAV-216	10 - 25	1	90.71	1	1	73.68	1
		7.5	99.86	3	7.5	100.00	5
		20	100.00	8	20	100.00	5
PAV-065	25-10	1	97.18	2	1	99.14	2
		5	100.00	5	5	100.00	4
		10	100.00	6	10	100.00	5
PAV-873	1 – 2.5	1	99.27	2	1	97.24	2
		5	100.00	5	5	100.00	5
		10	100.00	7	10	100.00	5
PAV-866	2.5-10	1	100.00	5	1	100.00	4
		7.5	100.00	8	7.5	100.00	5
		20	100.00	8	20	100.00	5
PAV-865	1	1	100.00	7	1	100.00	5
		5	100.00	8	5	100.00	5
		7.5	100.00	8	7.5	100.00	5

VEEV Selective Compounds From PAV-8668 Series

PAV#	CC_{50} (μM)	Conc (μM)	VEEV % control	VEEV log reduction	Conc (μM)	DENV % control	DENV log reduction
PAV-060	>25	1	0.55	0.9	1		
		7.5	92.07	1	7.5		
		20	99.98	4	20	18.32	1
PAV-057	2.5-10	1	100.00	6	1		
		7.5	100.00	8	7.5		
		20	100.00	8	20	15.70	1
PAV-896	2.5-10	1	100.00	6	1		
		7.5	100.00	8	7.5		
		20	100.00	8	20	0.03	4

DENV Selective Compounds From PAV-8668 Series

PAV#	CC_{50} (μM)	Conc (μM)	VEEV % control	VEEV log reduction	Conc (μM)	DENV % control	DENV log reduction
PAV-219	10-25	1	31.90	1	1	79.60	1
		5	11.30	1	5	98.84	2
		10	0.34	2	10	100.00	4
PAV-064	> 25	1			1	79.06	1
		7.5			7.5	99.99	4
		20	1.03	2	20	100.00	5
PAV-084	1 – 2.5	1			1	93.42	1
		7.5			7.5	100.00	5
		20	80.23	1	20	100.00	5
PAV-820	1	0.5			0.5	99.61	2
		1			1	99.99	4
		2	0.29	2	2	100.00	5

Summary

- Prosetta has developed a broad-spectrum screen which can survey the entire viral capsid assembly pathway and identify small molecules which disrupt key steps involved in capsid assembly.
- Prosetta's proprietary biochemical assay is capable of detecting the modulation of key protein protein interactions involved in the capsid assembly pathway.
- Multiple distinct chemical series were identified in proprietary Cell Free Screening System (CFSS).
- Each novel chemical series identified through the Prosetta platform represents a promising early stage Pre-Lead for an anti-DENV therapeutic drug discovery program.
- These molecules most likely target host protein since fractionation of extracts on drug affinity columns and reconstitution with dialyzed free drug eluates has been achieved. This occurs *prior* to viral capsid protein expression.
- These molecules are likely to exert their anti-viral effect through generation of aberrant capsids that are non-infectious due to misfiring of the machinery of catalytic capsid assembly.
- Direct evidence that compounds block the capsid assembly pathway suggests a higher barrier to resistance development due to the highly conserved capsid protein.
- Validation of hits from the biochemical assay were performed in cell culture live virus assays conducted by third party collaborators (academic/government facilities)
- Early lead optimization of 'Hits to Leads' for this series is underway to simultaneously improve potency, pharmacokinetic and toxicity profiles.

These guidelines denote the edge of you poster. Do not exceed them.