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Resistance to Potent and Broadly Active HIV-1 Maturation Inhibitors

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Abstract

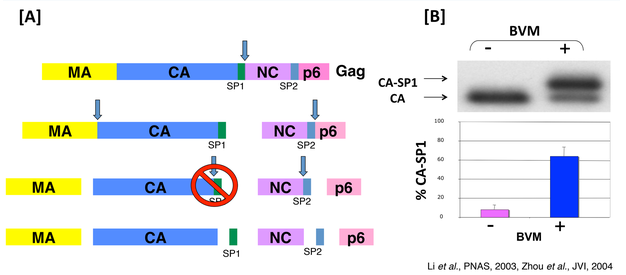
Background: Maturation Inhibitors (MI), a new class of anti-HIV-1 compounds, act by blocking the maturation of virions into infectious particles. Bevirimat (BVM), a first-in-class betulinic acid-based compound, acts by blocking a late step in protease-mediated Gag processing: the cleavage of the capsid-spacer peptide 1 (CA-SP1) intermediate into mature CA. BVM was shown to be safe and effective in reducing viral loads in patients. However, polymorphisms in the SP1 region of Gag reduced HIV-1 susceptibility to BVM in patients, effectively halting BVM's clinical development.

Methods: We carried out extensive screening to identify BVM derivatives with both increased potency against multiple HIV-1 clades and activity against primary isolates containing polymorphisms in SP1. Compound activity was tested in biochemical and biological assays measuring CA-SP1 processing and virus replication kinetics. Selection experiments with both clade B and clade C isolates were performed to identify mutations that confer resistance. Virological, structural, and molecular approaches were applied to elucidate the mechanism of resistance for each mutant. Further, combination assays with MIs alongside protease or integrase inhibitors were performed to determine inhibitor synergy or antagonism.

Results: We identified a set of BVM derivatives that are markedly more potent than BVM against clade B HIV-1 and show robust activity against SP1 polymorphic strains, clinical isolates, and some BVM-resistant mutants. Selection experiments with a clade B isolate identified an SP1-A1V mutation, and a CA-P157A mutation located in the major homology region (MHR) of CA. Selections with a clade C isolate identified several mutations in SP1. The P157A mutant was resistant to not only BVM and the second-generation BVM analogs but also to the structurally distinct maturation inhibitor PF-46396. Analysis of the HIV-1 database reveals that Ala1 of SP1 and Pro157 of CA are conserved in ~99.95% of available sequences. To date, combination assays with potent MI candidates have shown no antagonism with protease or integrase inhibitors.

Conclusions: This study identifies a panel of BVM derivatives that display improvements upon BVM in antiviral potency and breadth of activity. The characterization of resistant mutants provides insights into the structure of the maturation inhibitor binding site and the role of SP1 and the CA MHR in virus assembly and maturation. This study supports ongoing clinical development of this class of inhibitors.

Bevirimat: First-in-Class HIV-1 Maturation Inhibitor



Li *et al.*, PNAS, 2003, Zhou *et al.*, JVI, 2004

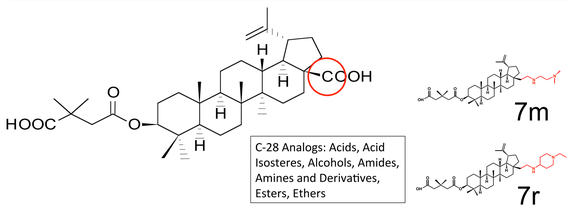
A) BVM blocks the final Gag cleavage step. B) Gel and graph depicting effects of BVM on CA-SP1 cleavage. % CA-SP1 was determined by calculating the ratio of accumulated CA-SP1 to total amounts of CA + CA-SP1.

Polymorphisms in SP1 (e.g. V7A) Reduce HIV-1 Susceptibility to BVM

	CA										SP1									
	G	G	P	S	H	K	A	R	V	L	A	E	A	M	S	Q	V	T	N	
									230								7	8		
>M GROUP CONSENSUS	G	G	P	S	H	K	A	R	V	L	A	E	A	M	S	Q	V	T	N	
>CONSENSUS_A1	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	Q	H	
>CONSENSUS_A2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Q	-	
>CONSENSUS_B	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
>CONSENSUS_C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	N	-	
>CONSENSUS_D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	
>CONSENSUS_F1	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	
>CONSENSUS_G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	S	G	
>CONSENSUS_H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
>CONSENSUS_K	-	-	-	G	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	

Sequences showing common polymorphisms found in the CA-SP1 region. These polymorphisms were associated with a lack of response both *in vivo* and *in vitro* (e.g., Adamson et al., Retrovirology 2010).

Developing BVM Analogs Effective Against SP1 Polymorphs



Structure of BVM with the C-28 carbon circled in red. Around 500 BVM-derived analogs were synthesized (Left). Structures of two BVM analogs 7m and 7r with C-28 side chains in red (Right).

Second-Generation BVM Analogs Show Low-nM Antiviral Activity

Compound	pNL4-3 WT IC_{50}	SP1-V7A IC_{50}
BVM	136 ± 30 nM	>500
7k	3 ± 1	145 ± 29
7l	14 ± 7	201 ± 44
7m	3 ± 0.3	9 ± 2
7n	7 ± 3	14 ± 4
7o	9 ± 3	22 ± 5
7p	3 ± 0.4	26 ± 6
7q	5 ± 1	22 ± 7
7r	5 ± 3	10 ± 3
7s	21 ± 19	8 ± 2
7t	2 ± 0.5	19 ± 6

IC50s were calculated in MT4 cell line. HIV-infected MT-4 cells were incubated with a serial dilution of compounds. Four days postinfection, culture supernatants were subjected to RT assay to measure viral replication. The 50% inhibitory concentrations were defined as compound concentrations that reduced RT levels by 50% relative to no inhibitor (DMSO alone) controls.

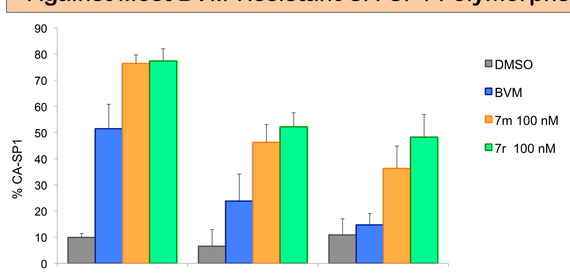
Second-Generation BVM Analogs Are Active Against Primary Isolates in PBMC

		IC_{50}: PBMC BVM	Compound 7r
NL4-3	Subtype B	4.8 nM	5.2 nM
92UG031	Subtype A	2829.0	>1000.0
93IN101	Subtype C	2651.0	66.6
99UGA07412MI	Subtype D	15.4	14.5
CMU02	Subtype E	3327.0	95.6
93BR029	Subtype F	6.9	12.9
JV1083	Subtype G	4188.0	221.0
YBF30	Group N	1489.0	43.4
BCF02	Group O	444.0	6.9

		CA	SP1
NL4-3 WT	Subtype B	HKARVLAEAMS	QVTN--PATIM
92UG031	Subtype A	****I*******	AQH**T-N**

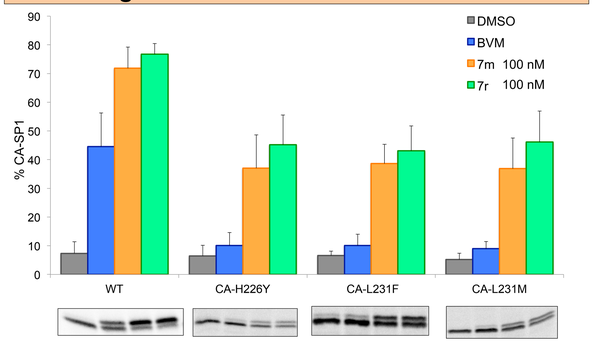
IC50s were calculated using PBMC with multi-clade HIV-isolates. HIV-infected cells were incubated with a serial dilution of compounds. At 6 days postinfection, culture supernatants were subjected to p24 ELISA assay to measure viral replication. The 50% inhibitory concentrations were defined as compound concentrations that reduced p24 antigen values by 50% relative to no inhibitor (DMSO) control.

Second-Generation BVM Analogs Are Active Against Most BVM-Resistant CA-SP1 Polymorphs



HeLa or 293T cells were transfected with WT or CA/SP1-polymorphic viruses. At 24 hour posttransfection, cells were labeled with [^{35}S]Met/Cys. Maturation inhibitors were maintained throughout the transfection and labeling. Virion-associated CA-SP1 was analyzed.

Second-Generation BVM Analogs Are Active Against BVM-Resistant CA Mutants



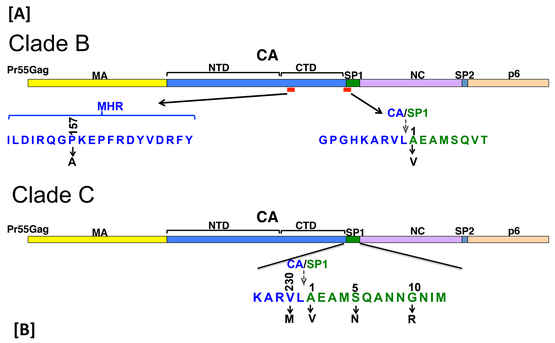
HeLa cells were transfected with pNL4-3 WT or BVM-resistant mutants. At 24 hour posttransfection, cells were labeled with [^{35}S]Met/Cys for 2-3 h. Maturation inhibitors were maintained throughout the transfection and labeling.

Second-Generation BVM Analogs Show No Significant Antagonism With Atazanavir

Inhibitor Combination	Synergy (μM²%)	Antagonism (μM²%)	Combination Effect
7m + Atazanavir	216.64	-22.89	strong synergy/ insignificant antagonism
7r + Atazanavir	221.36	-18.75	strong synergy/ insignificant antagonism

Synergy and antagonism volumes calculated using MacSynergy™ II software. Reported volumes are at 95% confidence, derived from 8X8 Matrix data sets from three independent replicates.

Mutations in CA and SP1 Were Acquired During Selection in Second-Generation BVM Analogs

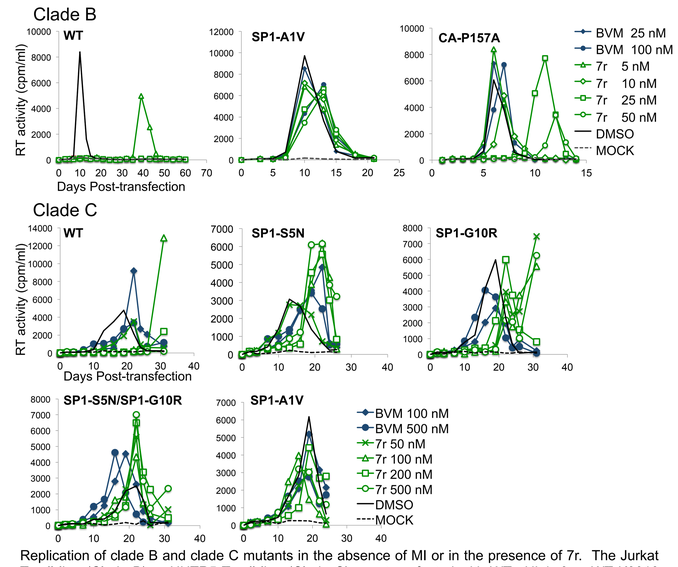


[B]

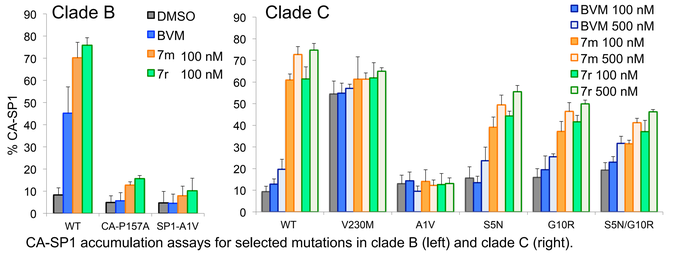
	All Clades	Clade B	Clade C
CA Residue 157	P: 99.96% (7976) other: 0.04% (3)	P: 99.97% (3049) L: 0.03% (1)	P: 100.00% (1778)
CA Residue 230	V: 91.88% (7330) I: 7.35% (586) other: 0.78% (62)	V: 84.62% (2581) I: 13.84% (422) other: 1.54% (47)	V: 98.48% (1751) other: 1.52% (27)
SP1 Residue 1	A: 99.69% (7953) other: 0.31% (25)	A: 99.64% (3039) other: 0.36% (11)	A: 99.66% (1772) other: 0.34% (6)
SP1 Residue 5	S: 96.89% (7728) other: 3.11% (248)	S: 97.31% (2968) other: 2.69% (82)	S: 98.82% (1757) other: 1.18% (21)

A) Positions of the amino acid changes in Gag. B) Table showing amino acid conservation at sites of selected mutations. Conservation data derived from Los Alamos Database, using AnalyzeAlign tool. SP1 Residue 10 was omitted due to its highly polymorphic nature.

Selected Mutations Confer Partial or Full Resistance to BVM Analogs

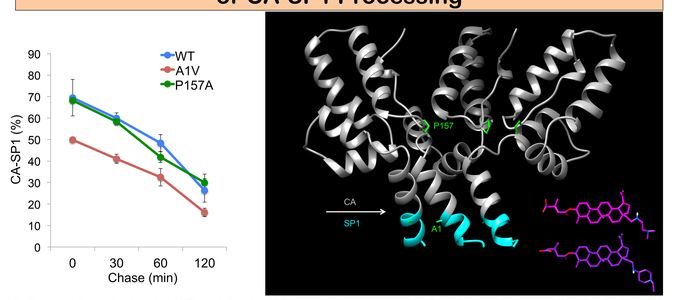


Replication of clade B and clade C mutants in the absence of MI or in the presence of 7r. The Jurkat T-cell line (Clade B) or HUTR5 T-cell line (Clade C) was transfected with WT pNL4- 3 or WT K3016 molecular clone or the indicated mutant derivatives. Virus replication was monitored by RT assay.



CA-SP1 accumulation assays for selected mutations in clade B (left) and clade C (right).

The CA-P157A Mutant Does Not Show An Increased Rate of CA-SP1 Processing



Cells transfected with the WT or HIV CA/SP1 mutants were pulse labeled and chased for the indicated times. Cell lysates were immunoprecipitated with HIV-Ig, and processing of CA-SP1 to CA was analyzed (Left). Structural model for the CA-SP1 six-helix bundle and location of residues at which mutations arose during selection experiments with compounds 7m and 7r (Right) (Wagner et al., eLife, 2016).

Conclusions

- A set of BVM derivatives have been identified that exhibit low-nM activity across major clades of HIV-1; these compounds are active against SP1 polymorphic viruses that display reduced susceptibility to BVM.
- These second-generation maturation inhibitors also retain some activity against HIV-1 isolates selected for resistance to BVM.
- Selection for resistance to the second-generation BVM analogs in the context of subtype B and C clones gave rise to a mutation in a highly conserved position in the CA major homology region (MHR) and mutations near the CA-SP1 cleavage site and downstream in SP1.
- The level of CA-SP1 accumulation was significantly reduced in the presence of BVM and BVM analogs against CA-P157A mutant; however, the rate of CA-SP1 processing of CA-P157A in the absence of inhibitor was similar to that of WT.
- Efforts are underway to define the structure of the maturation inhibitor binding pocket to facilitate the rational design of additional maturation inhibitors and to better understand the function of the CA-SP1 region of Gag in virus maturation.

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