Preclinical Development of Second Generation HIV-1 Maturation Inhibitors

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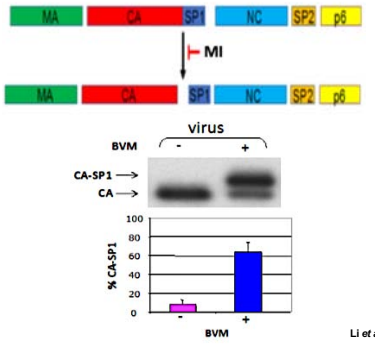
Abstract

Background: Maturation inhibitors (MIs) block HIV replication by disrupting conversion of CA-SP1 to mature CA, resulting in the formation of non-infectious viral particles. MIs bind inside a six-helix bundle that assembles at the junction of CA and SP1 in the immature Gag lattice. Proof-of-concept for MIs was established with bevirimat (BVM), which was found safe and effective in reducing viral load in infected individuals. However, a single amino acid polymorphism in the SP1 region of Gag (V7A) reduced susceptibility to BVM.

Methods: We identified three C-28 alkyl amine BVM derivatives (compounds A-C) that exhibited potent activity against BVM-resistant polymorphs. These compounds were characterized for in vitro activity against i) a panel of primary HIV-1 clinical isolates representing subtypes A-G and ii) a set of viruses resistant to the approved classes of HIV-1 drugs. In vitro metabolic stability was characterized in human, rat, mouse, dog and monkey liver S9 fractions. Binding to human plasma proteins was determined using i) equilibrium dialysis and ii) in vitro activity assays employing human serum concentrations of 0-40%. In vivo pharmacokinetic studies were carried out in mice and rats. Resistance selection experiments were carried out using both subtype B and C isolates.

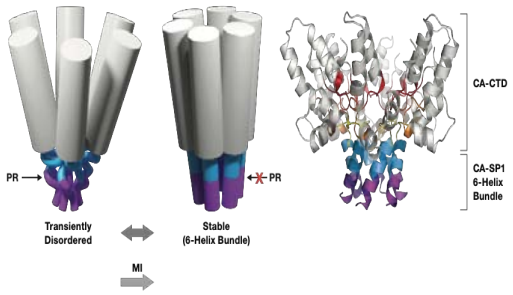
Results: All compounds exhibit potent antiviral activity. Compound A exhibited an average IC_{50} value of 7.3nM against a panel of 12 primary isolates including those with the BVM-resistant SP1 V7A genotype (n=5). Compounds B and C inhibit HIV-1 with average IC_{50} values against V7 virus of 7.9 and 14.9nM and against A7 isolates of 48.2 and 138.7nM, respectively. A, B and C inhibit drug resistant virus with average IC_{50} values of 3.1, 1.0 and 1.2nM, respectively (n=6). All compounds were metabolically stable in liver S9 fractions across species, demonstrated plasma protein binding of >99% (equilibrium dialysis) or ~90% (40% HS) and were orally bioavailable in rodents. By using low concentrations of inhibitor, resistance-conferring mutations were identified.

Mechanism of Action of Maturation Inhibitors



Li *et al.*, PNAS, 2003

MIs Stabilize the CA-SP1 6-Helix Bundle



Several of our MI-resistant mutants off-set the stabilizing effect of MI by destabilizing the 6-helix bundle (Waki et al., PLoS Pathog 2012)

Polymorphisms in SP1 Reduce HIV-1 Susceptibility to BVM and Are Associated with a Lack of Response in Patients

	CA										SP1								
								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
A1	-	-	-	G	-	-	-	-	I	-	-	-	-	-	-	-	-	Q	H
A2	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	Q	-
B	-	-	-	G	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	A	N	-
D	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	A	-	-
F1	-	-	-	G	-	-	-	-	I	-	-	-	-	-	-	-	A	-	-
G	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	A	S	G
H	-	-	-	-	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-
K	-	-	-	G	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-

Antiviral Activity Against a Diverse Set of HIV-1 Isolates

DFH COMPOUND	NR_1R_2	NL4-3	V7 Clinical Isolates	A7 Clinical Isolates	YBF30 Group N	BCF02 Group O	Drug Resistant Isolate	All Group M
A		0.8	7.9	48.2	8.4	1.7	1	33.1
B		0.8	14.9	138.7	3.6	2.7	1.2	92.3
C		1.3	7.4	7.2	132	3.8	3.1	7.3
BMS-955176		0.8	7.2	6.7	24.6	2.9	2.4	6.9
BVM		23.1	4106	7095	9068	4451	56	5974

*IC_{50} values in nM

Activity Against BVM-Resistant SP1 Polymorphs

SP1 Genotype: AEAMS$Q^6V^7T^8$NPATIM

Compounds	WT	V7M	V7I	T8Q	T8S	T8N	$Q^6V^7T^8$-HV^7Q	$Q^6V^7T^8$-KV^7N
A	17.9	37.1	20.1	18.5	>407	204	27.1	>1377
B	73.5	330	>52.3	>722	>407	20.6	771	>1377
C	13.9	294	4.8	100	15.7	34.3	334	529

*Fold Increase in antiviral activity

Metabolic Stability in Liver S9 Fraction

Test Article	Species	Half-life (min)	Clint (mL/min/mg protein)
	Human	>60	<0.0116
	Rat	>60	<0.0116
Compound A	Mouse	>60 (87.7)	<0.0116 (0.00791)
	Dog	>60	<0.0116
	Monkey	>60 (68.7)	<0.0116 (0.0101)
	Human	>60	<0.0116
	Rat	>60	<0.0116
Compound B	Mouse	>60	<0.0116
	Dog	>60	<0.0116
	Monkey	>60	<0.0116
	Human	>60 (66.1)	<0.0116 (0.0105)
	Rat	>60 (67.0)	<0.0116 (0.0103)
Compound C	Mouse	45.1	0.0154
	Dog	>60 (83.9)	<0.0116 (0.00826)
	Monkey	>60 (96.3)	<0.0116 (0.00720)

Average Pharmacokinetic Parameters in Male CD-1 Mice

PK Parameters	Mouse Oral (30 mg/kg)		
	Compound A	Compound B	Compound C
C_{max} (ng/mL)	279	258	31.4
t_{max} (hr)	4	4.7	4
$t_{1/2}$ (hr)	ND	ND	26
MRT_{last} (hr)	6.2	4.49	17.2
AUC_{last} (hr.ng/mL)	2757	1766	513
AUC_{∞} (hr.ng/mL)	ND	ND	ND
Dose-normalized Values			
AUC_{last} (hr.kg.ng/mL/mg)	91.9	58.9	17.1
AUC_{∞} (hr.kg.ng/mL/mg)	ND	ND	ND

Average Pharmacokinetic Parameters in Male Sprague-Dawley Rats

PK Parameters	Rats Oral (30 mg/kg)		
	Compound A	Compound B	Compound C
C_{max} (ng/mL)	27.5	67.0	12.4
t_{max} (hr)	2.7	4.7	11
$t_{1/2}$ (hr)	ND	ND	ND
MRT_{last} (hr)	7.48	10.2	18.4
AUC_{last} (hr.ng/mL)	265	784	277
AUC_{∞} (hr.ng/mL)	ND	ND	ND
Dose-normalized Values			
AUC_{last} (hr.kg.ng/mL/mg)	8.82	26.1	9.27
AUC_{∞} (hr.kg.ng/mL/mg)	ND	ND	ND

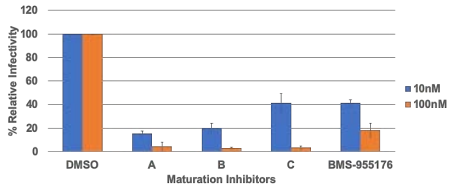
Serum Protein Binding

Compound	IC_{50} (nM) 0% HSA	IC_{50} (nM) 40% HSA	Fold Change IC_{50} (nM) 40% / IC_{50} 0%
A	3.7	30.4	8.4
B	7.0	87.5	12.6
C	6.2	43.8	7.0

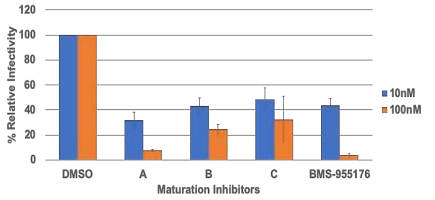
Sequence Alignment of the CA-SP1 Region of HIV-1 clade B and C

	CA										SP1										
NL4-3	G	G	P	G	H	K	A	R	V	L	A	E	A	M	S	Q	V	T	N	P	A	T	I	M
ZM247	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	N	S	.	T	N	-	-
IndieC1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	N	S	.	T	.	-	L
K3016	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	N	-	.	G	N	-	-

MIs Reduce the Infectivity of HIV-1 subtype B



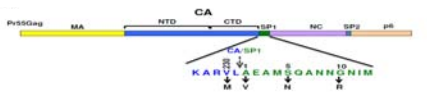
MIs Reduce the Infectivity of HIV-1 subtype C virus K3016



IC50 Values Against HIV-1 subtype C K3016

Compound	IC50 (nM)
A	3.60 +/- 0.22
B	2.27 +/- 0.56
C	3.89 +/- 0.18
BMS-955176	1.88 +/- 0.63

Mutations Identified in the Presence of Other BVM Analogs



Compound	Concentration (nM)	Mutations Selected
7m	100	CA-T187I + SP1-S5N + SP1-G10R
	200	SP1-A1V
	500	SP1-A1V
7r	100	-
	200	CA-V230M
	500	SP1-A1V
7s	100	SP1-A1V
	200	SP1-A1V
	500	SP1-A1V

Urano E *, Timilsina U* *et al., Journal of Virology,* 2018

Conclusions

As resistance to currently approved HIV therapies develops new drugs will be needed. MIs employ a novel mechanism of action to block HIV replication and could replace drugs that are no longer effective due to resistance. Compounds A-C exhibit pre-clinical development profiles similar or superior to MI drug candidates that have advanced to the clinic. Based on these results, we plan to continue drug development activities for each compound.

Acknowledgements

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