Anti-viral Characterization *in vitro* of a Novel Maturation Inhibitor, MPC-9055

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Abstract #: J-105

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Abstract

Background: There is a continuing need for anti-HIV drugs with novel mechanisms because of development of resistance to existing therapies. MPC-9055 was discovered in a medicinal chemistry program as a potent, broad-acting small molecule inhibitor of HIV-1 maturation and is now in Phase I clinical development.

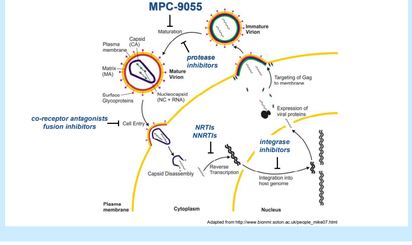
Methods: Antiviral replication assays in human PBMCs were used to determine the potency, range of action and activity of MPC-9055 against clinical drug-resistant isolates. Cytoprotection assays were used to analyze the mode of action and to isolate virus resistant to MPC-9055. Western blot with a p24 antibody was used to investigate effects of MPC-9055 on Gag processing.

Results: MPC-9055 had potent antiviral activity against the IIIB strain of HIV in a PBMC assay (IC_{50} 7 nM) and against the NL4-3 and RF strains in cytoprotection assays with MT-4 and CEM-SS cells, respectively (EC_{50} 10 and 13 nM). MPC-9055 exhibited efficacy against a broad range of clinical isolates from groups M, N and O, subtypes A to G, and receptor tropisms (X4, R5 and R5/X4). Importantly, it was also active against RT– and PI-resistant isolates and MDR strains with IC_{50} values ranging from 8 to 170 nM. Mode of action studies indicate that MPC-9055 acts at a late step in the viral life cycle with selective inhibition of Gag processing and HIV maturation. MPC-9055 specifically blocked processing of CA-SP1 Gag intermediate to mature CA and reduced infectivity of virions produced from 293T cells transfected with a proviral genome. Treatment of latently-infected ACH-2 cells with MPC-9055 resulted in production of non-infectious virus. Engineered mutations at the CA-SP1cleavage sites L363F and A364V were 60 and 395-fold less active, respectively, against MPC-9055 in a cytoprotection assay in MT-4 cells. In an analogous manner, virus selected for resistance to MPC-9055 by serial passage *in vitro* had the single amino acid change A364V at the CA-SP1 junction.

Conclusions: MPC-9055 is a potent anti-HIV agent with a broad range of action. It has a novel mechanism of action and targets the last step in Gag processing, cleavage of CA-SP1 to CA. MPC-9055 is also active against RT and PI-resistant strains. Based upon this efficacy profile and novel mechanism of action, MPC-9055 is a promising new HIV therapeutic.

Background

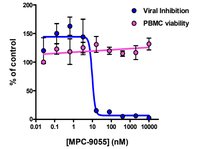
MPC-9055 discovered in a medicinal chemistry program is a small molecule inhibitor of HIV-1 maturation that is in clinical development (Abstract #: K-119)



In vitro Antiviral Activity

MPC-9055 has potent antiviral activity in cytoprotection and PBMC assays with laboratory adapted strains of HIV-1.

Activity in human PBMCs

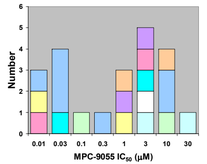


PBMC (IIIB)	MT-4 (NL4-3)	CEM-SS (RF)
IC_{50} = 7 nM	EC_{50} = 10 nM	EC_{50} = 13 nM
TC_{50} = 14,000 nM	TC_{50} > 10,000 nM	TC_{50} = 7,600 nM

Range of Action

MPC-9055 has a broad range of activity against clinical isolates including RT-resistant, PI-resistant and MDR strains.

Activity Against Clinical Isolates



Potency	IC_{50} (μM)
Max	0.007
Median	0.8
Min	12

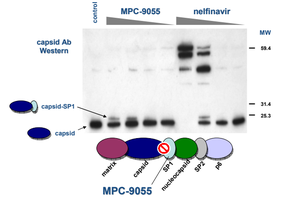
Twenty-two clinical isolates with representatives from clades A through G and from groups O and N were tested in a PBMC antiviral assay.

Activity Against Drug-resistant Isolates

Isolate	Mutant Gene	Resistant to:	PBMC IC_{50} (nM)
G910-6	RT	AZT	11
5705-72	RT	AZT, 3TC	15
1064-52	PR	Intermediate: APV, ATV, LPV High: IDV, NFV, RTV, SQV	42
1002-60	PR	Intermediate: APV, LPV High: ATV, IDV, NFV, RTV, SQV	107
MDR 769	RT	AZT, ddI, 3TC, d4T, PFA, NVP, IDV, SQV, NFV	8
	PR		
MDR 3761	RT	AZT, ddI, 3TC, d4T, NVP, IDV, SQV, NFV	31
	PR		
MDR 807	RT	AZT, ddI, 3TC, d4T, IDV, SQV, NFV	170
	PR		

Mechanism of Action

Selective Inhibition of Gag Processing



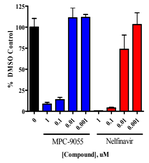
Virions were isolated from 293T cells transfected with a NL4-3 proviral construct and exposed to 1000, 100, 10 or 1 nM of MPC-9055 or nelfinavir. Gag processing was monitored with an anti-Capsid (p24) antibody in a Western blot.

Activity in Latently Infected ACH-2 Cells

	IC_{50} (nM)		TC_{50} (nM)	
	MPC-9055	Temacrazine	MPC-9055	Temacrazine
RT endpoint	10,100	5	12,900	> 500
Infectious Virus production	4	2.4	> 25,000	> 500

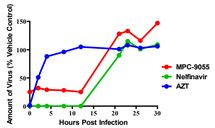
ACH-2 cells, which are latently infected with HIV IIIB, were stimulated with 1 ng/ml TNF α for 3 days in the presence of varying concentrations of MPC-9055 or Temacrazine, an inhibitor of HIV transcription. Virion production was measured by determining reverse transcriptase activity in the supernatant and infectious virus generated was determined by a MAGI assay. MPC-9055 inhibited infectious virus production with an IC_{50} value of 4 nM but had no effect on virus particle production (IC_{50} = 10,100 nM).

Inhibition of Infectious Virus Production



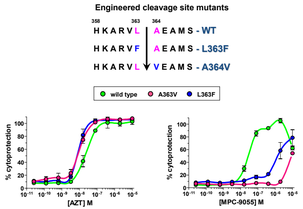
Virus isolated from 293T cells transfected with a NL4-3 proviral construct and exposed to MPC-9055 or nelfinavir was assayed for infectivity with a P4/MAGI assay.

Activity in Time of Addition Assay



The phase of HIV-1 life cycle targeted by MPC-9055 was determined by a time of addition experiment with NL4-3 virus. MPC-9055, nelfinavir or AZT were added at a final concentration of 1 μM to MT-4 cells infected with a high multiplicity of infection (0.5 m.o.i.) at 2, 4, 8, 12, 21, 23, 26 or 30h post-infection. Cell supernatants were assayed 30h p.i. for virus production by a MAGI assay for cells incubated with MPC-9055 and nelfinavir or with a p24 ELISA for cells incubated with AZT. MPC-9055 inhibited infectious virus production when added 12h post-infectionbut not when added 21h p.i.

Effect of CA-SP1 Cleavage Site Mutations



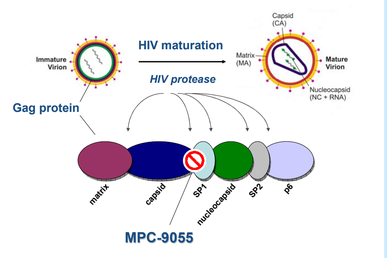
EC_{50} of MPC-9055 for NL4-3 virus with CA-SP1 cleavage site mutations L363F or A364V is increased by 60 and 395 fold respectively in a MT-4 cytoprotection assay.

Summary

- Inhibits virus maturation by blocking processing of CA-SP1 to CA
- Engineered mutations at the CA-SP1 cleavage site yield virus resistant to MPC-9055
- Serial passage in increasing concentrations of MPC-9055 yields resistant virus with CA-SP1 cleavage site A364V mutation
- Inhibits production of infectious virus from a latently infected T-cell line
- Acts late in the viral life cycle based on time of addition assays

Mechanism of Action Model

MPC-9055 is a maturation inhibitor that blocks processing of the CA-SP1 Gag intermediate to CA.



Conclusions

- MPC-9055 potently inhibits HIV replication *in vitro*
- Broad range of activity against clinical isolates including drug resistant strains
- Inhibits virus maturation by blocking Gag cleavage at the CA-SP1 site
- Mechanism of action is distinct from current HIV therapies
- In clinical development (Abstract #: K-119)

Poster available from
http://www.myriad.com/research/MPC-9055.php