

# Novel Small Molecule Transcriptional Inhibitors of HIV-1 which Suppress Virus Production from Infected Cells

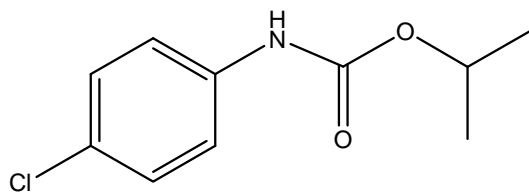
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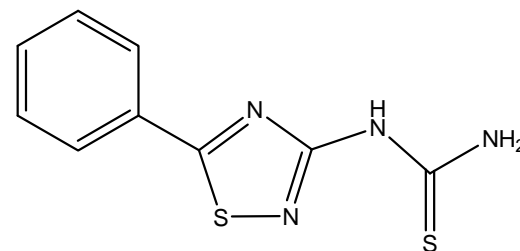
# Overview of Transcriptional Inhibitors of HIV-1

1. Marginally active (FB 532 and FB 636) to highly active (FB 642) in acute infection assays (compound dependent).
2. Active in chronic and latent infection assays and activity sustained after removal of compound from chronically infected cell cultures (memory effect).
3. Additive interactions with other ARVs in combination antiviral assays employing acute or chronic infection assays.
4. Not directly virucidal to HIV-1.
5. No resistant viruses observed upon culture (acute and chronic models).
6. No HIV-1 super-infection observed in treated chronically infected cells.
7. Inhibitors of late stage step in virus replication involving RNA synthesis.
8. Cells pretreated with inhibitors remain susceptible to infection.
9. No effect on cellular macromolecular synthesis at inhibitory concentrations.
10. No effect on cell cycle distribution; do not induce apoptosis.
11. Little protein binding effect on antiviral activity (~2-fold).

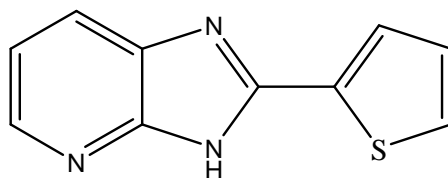
# Structures of Lead Inhibitors



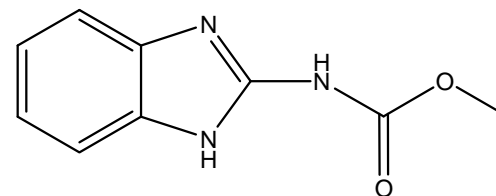
FB-636



PG-301029



PG-300995

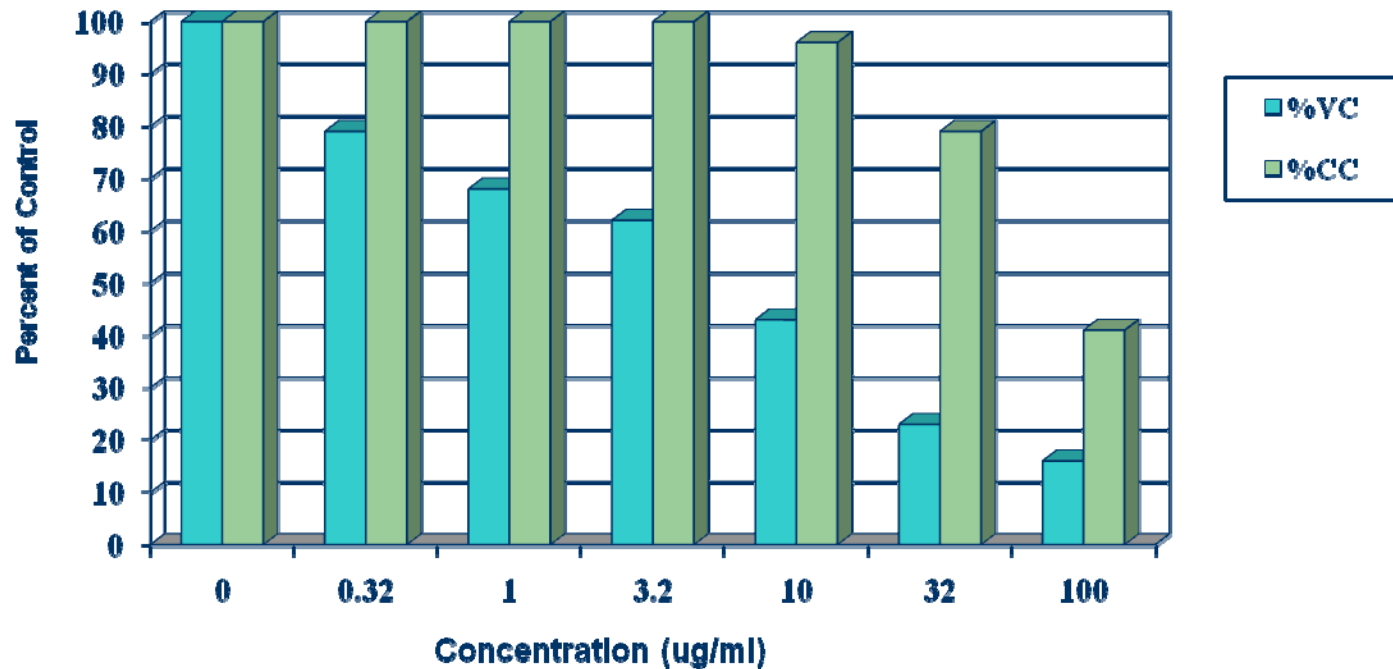


FB-642

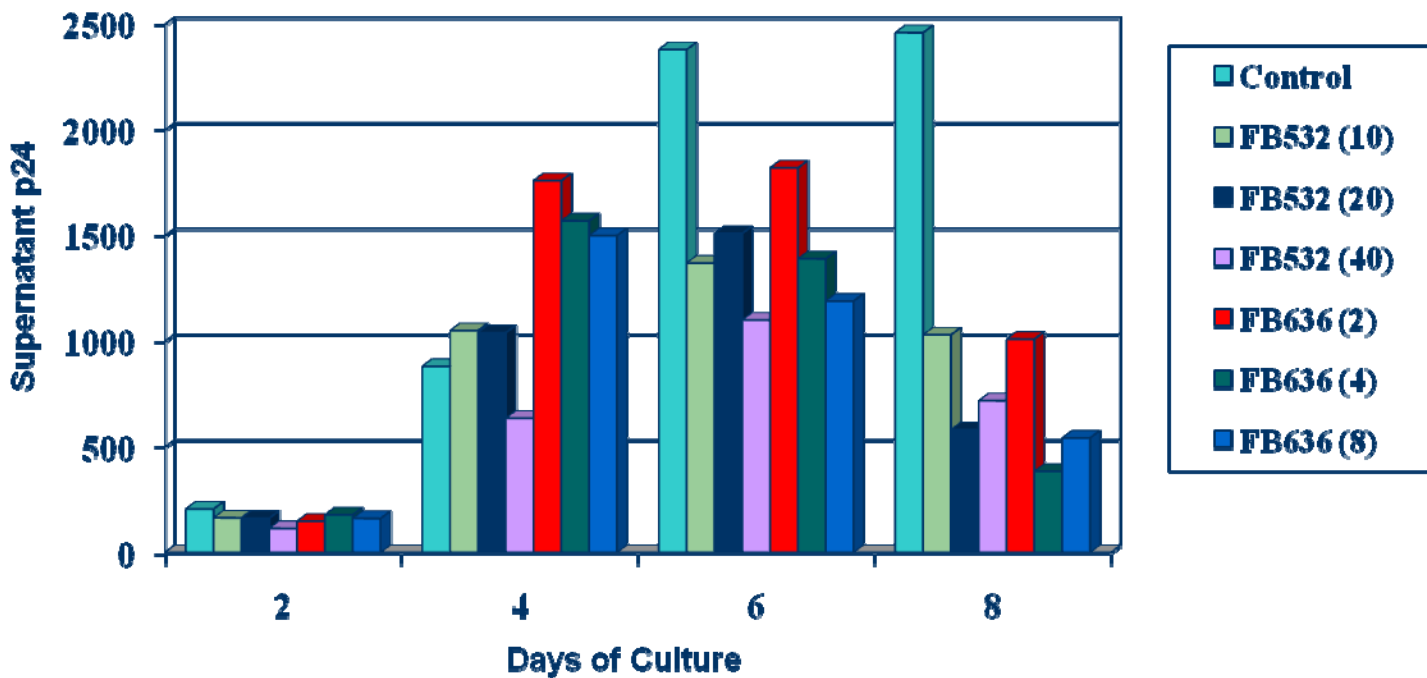
# Antiviral Profile of FB 636

- Active in chronic and latent infection models against wild type and drug resistant strains.
- Slightly active in short term acute infection assays.
- Additive in combination with other anti-HIV agents in both acute and chronic assays.
- Long term selection assays have not resulted in the isolation of drug resistant strains of virus.
- Effectively suppresses the production of singly spliced and unspliced viral RNA. Biological data suggests inhibition of Rev function, though a direct effect on Rev-RRE is not observed.

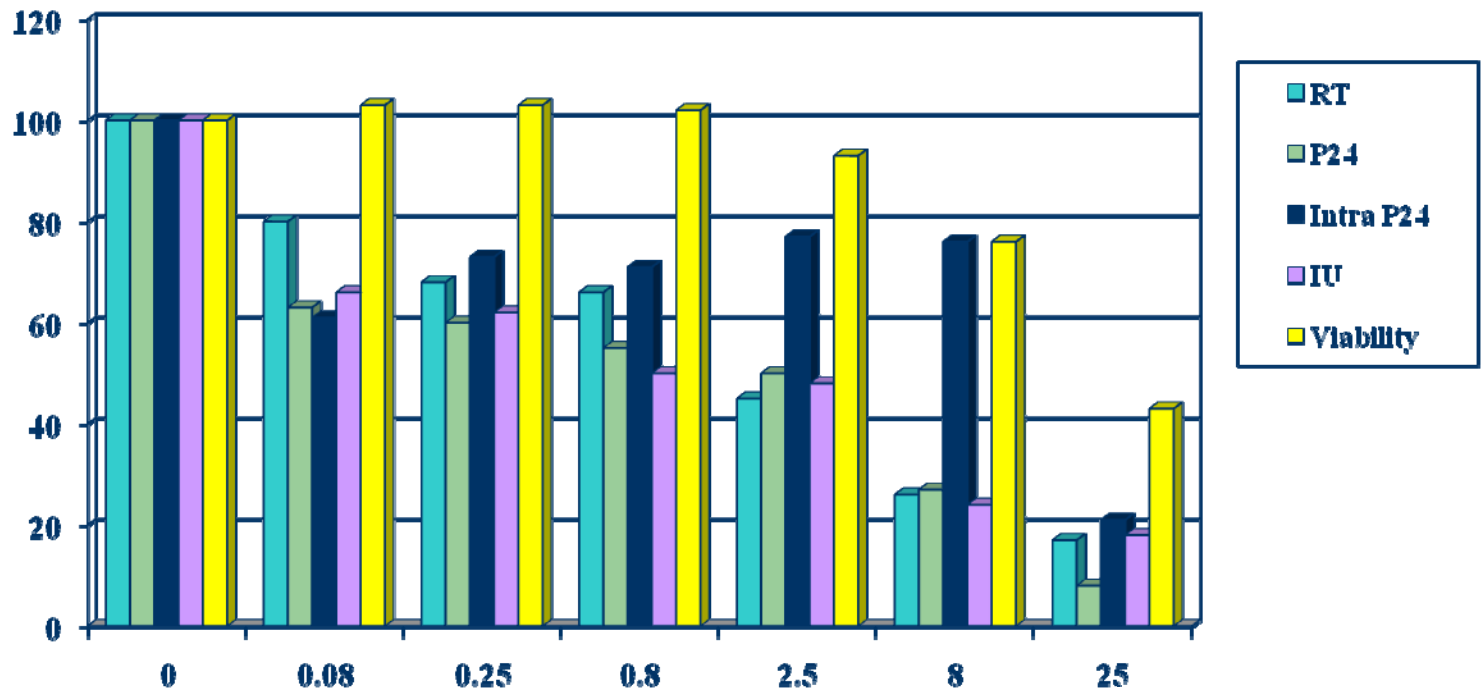
# Inhibition of Virus Replication from Chronically Infected Cells by FB 636



# Virus Replication from Treated Chronically Infected Cells



# Virus Replication from Treated Chronically Infected Cells



# Range of Action of FB 636

- In chronically infected cell systems, FB 636 is active against all strains of HIV-1 and HIV-2 which have been tested.
- Using various cell lines infected with HIV-1, FB-636 inhibits replication independent of the cell type used (H9, CEM-SS, U937 and MT2).
- FB 636 inhibits HIV replication in chronically infected fresh human PBMCs and monocyte-macrophages.
- FB 636 inhibits the replication of drug resistant HIV strains, including nucleoside and nonnucleoside RT and protease inhibitor resistant viruses.
- No efficacy of FB 636 has been detected against SIV.

# Mechanism of Action of FB 636

- FB 636 inhibits a step in HIV replication which occurs following the integration of the virus into the target cell genome.
- Evaluation of the effects of FB 636 on viral RNA and protein synthesis indicates that FB 636 reduces the production of full length viral RNA in the treated cell.
- FB 636 has no effect on virus attachment or fusion, reverse transcriptase, protease, integrase, or cell surface CD4 expression.
- Initial RNA expression data suggested that FB 636 inhibited the function of the HIV regulatory protein Rev or acted on cellular moieties to inhibit full length RNA synthesis through a mechanism similar to that employed by Rev.

# Mechanism of Action of FB 636

- In kinetic studies, singly spliced and unspliced viral RNA decreased significantly when measured by two different RNA PCR assays and the branched chain assay.
- The decrease in viral RNA preceded and paralleled the decrease observed in the expression of p24, RT and infectious virus.
- The ratio of unspliced to multiply spliced RNA decreased by several logs.
- In latently infected U1 cells induced with TNF-alpha or PMA, marked inhibition of p24 and RT was observed with no appreciable change in multiply spliced viral RNAs. The decrease in viral RNA preceded and paralleled the decrease in viral proteins.
- There was no affect on latently infected ACH2 cells. Previous data has shown that induced U1 cells shift from a Rev-independent to a Rev-dependent mode of viral expression, whereas ACH2 cells demonstrate less Rev dominant mode of expression when induced.

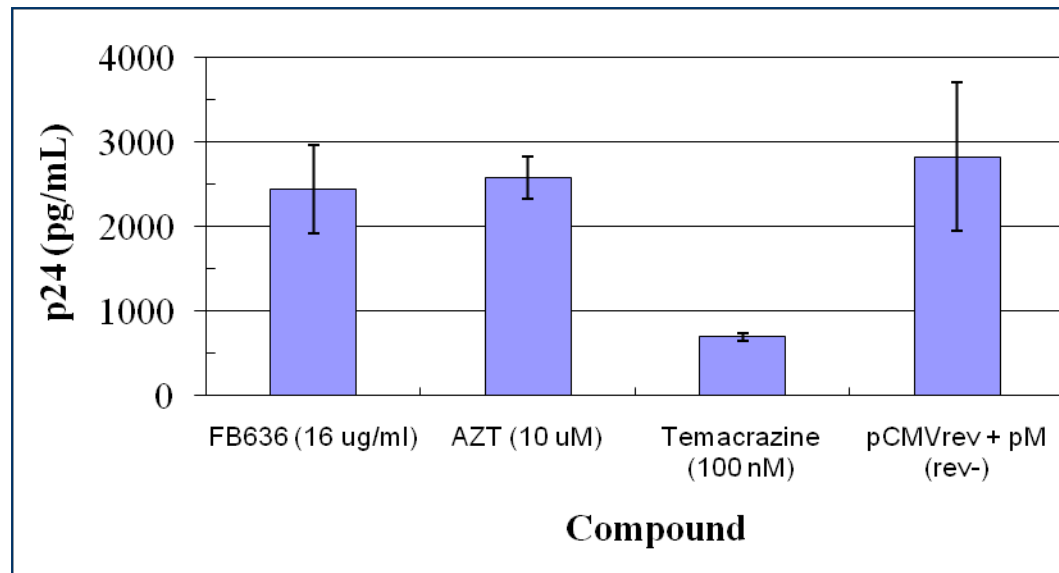
# Mechanism of Action of FB 636

## A. FB 636 reduces singly and unspliced viral RNAs

Cell Type (Model)	Quantitation of HIV RNA (% of Control)							
	Multi-spliced RNA				Singly Spliced/un-spliced RNA			
	2 <sup>1</sup>	4	8	16	2	4	8	16
CEM/SK-1 (Chronic)	ND	177	177	177	ND	25	15	22
U1 (Latent TNF $\alpha$ -induced)	214	214	71	ND	30	21	27	ND

<sup>1</sup>Concentration of FB636  $\mu\text{g/ml}$

## B. FB 636 does not inhibit Rev-dependent HIV expression



# Mechanism of Action of FB 636

## Ratio of Unspliced:Multiplly Spliced RNA in FB 636 Treated CEM-SS Cells

<b>Sample</b>	<b>Day 0</b>	<b>Day 3</b>	<b>Day 5</b>	<b>Day 11</b>
<b>No Drug</b>	1000:1	1000:1	2500:1	1000:1
<b>2 <math>\mu\text{g/ml}</math></b>	1000:1	4000:1	100:1	10:1
<b>4 <math>\mu\text{g/ml}</math></b>	1000:1	400:1	100:1	10:1
<b>8 <math>\mu\text{g/ml}</math></b>	1000:1	4000:1	10:1	10:1

# Mechanism of Action of FB 636

- There is no evidence that FB 636 directly inhibits REV/RRE or alters basal levels of transcription.
- Our hypothesis is that FB 636 is interfering with a virus specific RNA expression regulatory pathway which alters the pattern of HIV RNA expression.
- FB 636 may specifically inhibit TAT/TAR and or REV/RRE interactions or may have an effect on the host cell such that these virally encoded interactions are muted, resulting in alterations in viral RNA expression.
- Candidate targets?
  - TAT/TAR
  - REV/RRE
  - REV/nuclear pore interactions
  - cellular chaperone or RNA regulatory pathways

# Toxicity of FB 636 to Human Target Cells

- FB 636 is relatively non-toxic to fresh human cells (PBMCs, monocyte-macrophage, dendritic cells, hepatocytes). Toxicity is observed to established cell lines.
- Short or long term treatment of cells with FB 636 does not alter the cell surface expression of CD4.
- Treated cells remain fully infectable by HIV.
- FB 636 does not induce cellular apoptosis.
- FB 636 does not alter the distribution of cells in the cell cycle.
- FB 636 toxicity is reversible and appears to involve a slowing of cellular replication (cytostasis).

# Antiviral Profile of PG 300995

- Active in fresh human PBMC cultures against low passage wild type and multi-drug resistant strains.
- Active in chronic infection models against wild type and drug resistant strains.
- Additive in combination with other anti-HIV agents in both acute and chronic assays.
- Long term selection assays did not result in the isolation of drug resistant strains of virus.
- Effectively suppresses the production of multi-spliced and full length, unspliced viral RNA. PG 300995 may be a more general transcriptional inhibitor or may inhibit the same anti-viral target better than FB 636.

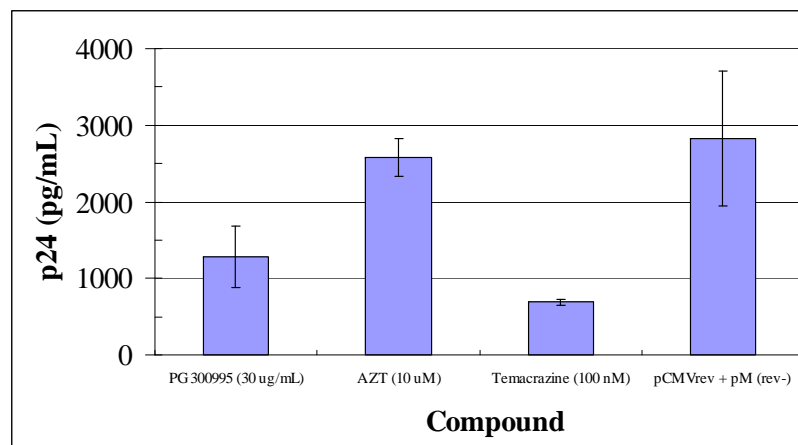
# Mechanism of Action of PG 300995

## A. PG 300995 down regulates all RNA splice species

Cell Type (Model)	Quantitation of HIV RNA (% of Control)					
	Multi-spliced RNA			Singly Spliced RNA		
	7 <sup>1</sup>	15	30	7	15	30
U1 (Latent TNF $\alpha$ -induced)	8	3	3	1	1	1

<sup>1</sup> PG300995  $\mu\text{g/ml}$

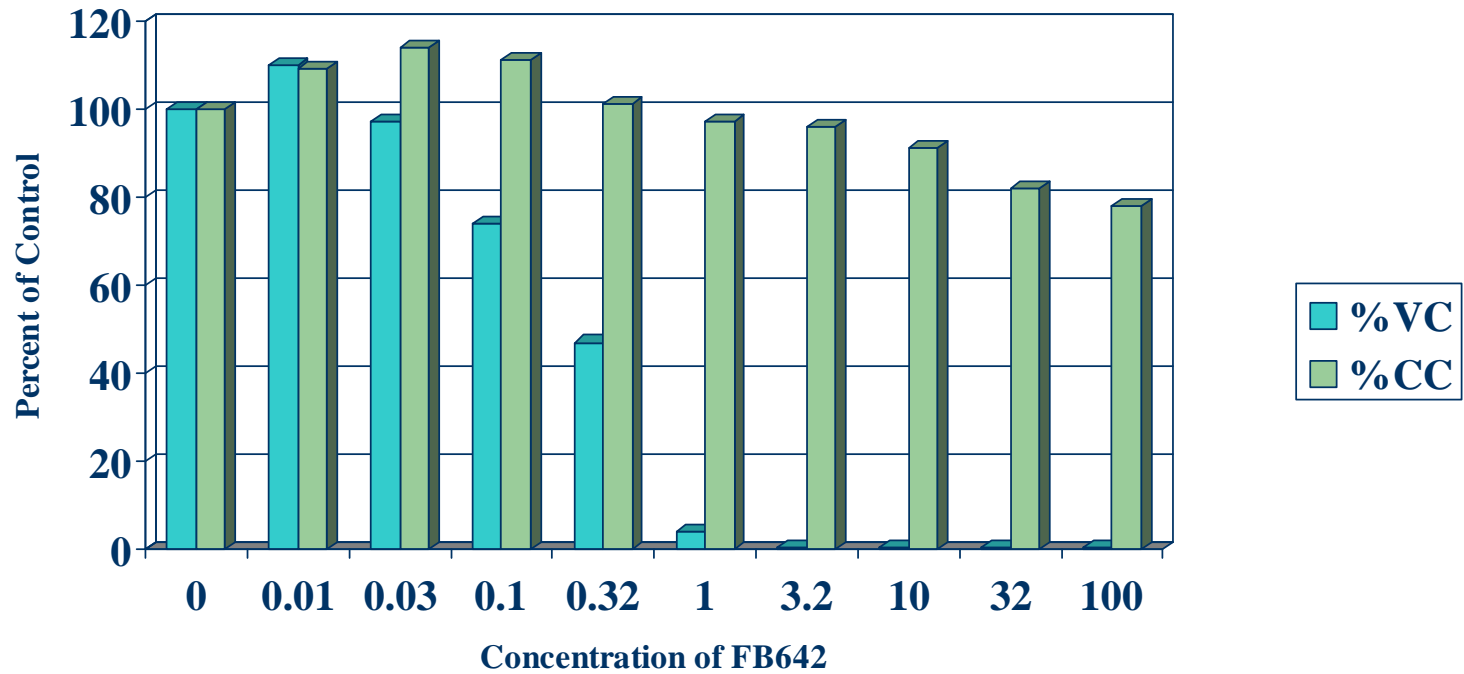
## B. PG300995 inhibits Rev-dependent HIV expression



# Antiviral Profile of FB 642

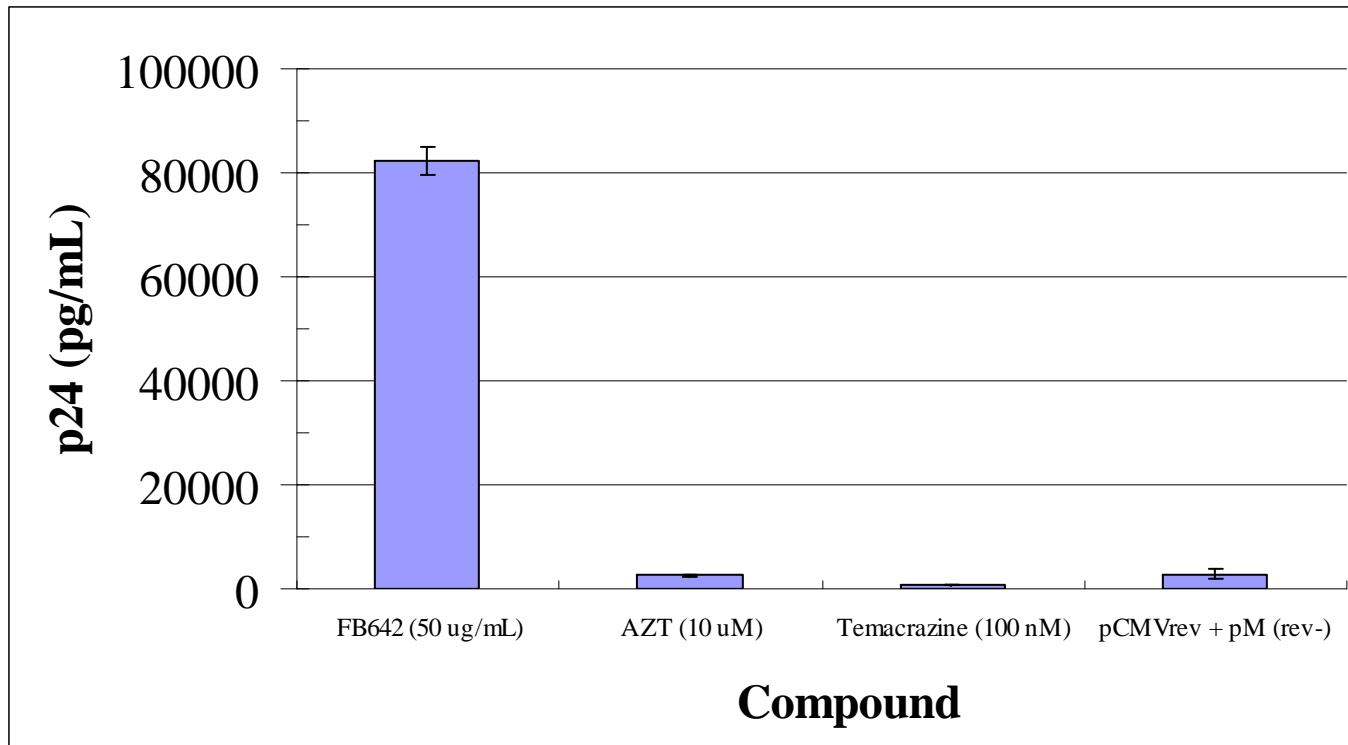
- Highly active in fresh human PBMC cultures against low passage wild type and multi-drug resistant strains with a significant therapeutic index (1,000-10,000).
- Difficult/impossible to evaluate activity in chronically infected cells due to its anti-tumor activity.
- In combination with protease inhibitors, synergistically inhibits HIV-1. Additive interactions in combination with RT inhibitors.
- Evaluations of mechanism of action are in progress but suggest a transcriptional inhibitory mechanism

# Activity of FB 642 in Acutely Infected Fresh Human PBMCs



# Mechanism of Action of FB 642

## FB 642 Stimulates Rev-Dependent HIV Replication



# Current Status of Development

- FB 636 has progressed to a Phase 1 human clinical trial. Results suggests compound was safe and well tolerated but target blood concentrations were not obtained likely due to solubility of the compound. Compound is being re-formulated as a nanoparticle suspension and alternative delivery mechanisms explored.
- FB 642 was evaluated in Phase 1 study as anti-cancer agent. PK data suggest potential for use as an anti-HIV agent in light of broad therapeutic index and safety at higher concentrations used in trial.
- Preclinical development of new analogs.
- Mechanism of action evaluations to define molecular target.

# Acknowledgements

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