

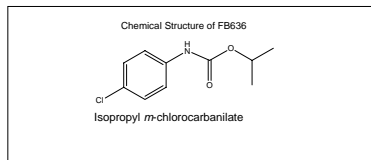
Inhibition of Late Stage Events in HIV Replication: Therapeutic Compounds with Effects on Viral RNA Synthesis

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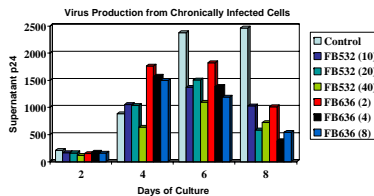
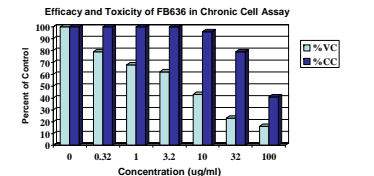
ABSTRACT

The effective use of highly active antiretroviral therapy results in the near complete suppression of virus production in infected individuals, but does not yield suppression of low level virus production in cells in sanctuary sites and does not result in the elimination of infection. Thus, the continued search for new antiretroviral agents with unique and different mechanisms of HIV inhibition remains critical, and compounds that can reduce the level of virus production from cells already infected with HIV, as opposed to preventing *de novo* infection, would be of great benefit. We have discovered and evaluated a series of compounds that suppress HIV replication in cells which are chronically infected with HIV and which constitutively produce HIV. These compounds were only identified in screening programs specifically designed to detect inhibitors of ongoing virus replication and were variably active in traditional anti-HIV assays which monitor inhibition of acute infection. Range of action studies demonstrated the ability of the compounds to inhibit the replication of diverse strains of HIV from chronically infected cells, including protease inhibitor-resistant strains. The compounds interacted in an additive fashion with all other tested anti-HIV agents in both acute and chronic *in vitro* antiviral assays and resistance to the compounds was not detected even after three years of passage of virus in the presence of the compounds. Mechanistic studies demonstrated the specific antiviral effect of the compound relative to host cell toxicity and have defined a mechanism of action for the compounds similar to that expected for a Rev inhibitor. The compounds yield a reduction in the quantity of singly spliced and unspliced HIV RNA transcripts and an increase in the quantity of multiply spliced RNA species. The antiviral profile of these compounds and their clinical potential will be presented.



Efficacy of FB636 in Acute Infection Systems

Cell Line	FB532		FB636	
	IC ₅₀	TC ₅₀	IC ₅₀	TC ₅₀
CEM-SS	>100 µg/ml	38.7 µg/ml	>25 µg/ml	10.7 µg/ml
AA5	>100 µg/ml	18.7 µg/ml	>10 µg/ml	>10 µg/ml
174xCEM	>100 µg/ml	25.3 µg/ml	>10 µg/ml	>10 µg/ml
H9	0.3 µg/ml	55.1 µg/ml	0.1 µg/ml	>25 µg/ml
PBMC	>100 µg/ml	59.5 µg/ml	>25 µg/ml	>25 µg/ml
Monocytes	>100 µg/ml	>100 µg/ml	>25 µg/ml	24 µg/ml



FB636 alters HIV RNA splice species expression in a manner suggestive of inhibition of HIV Rev action

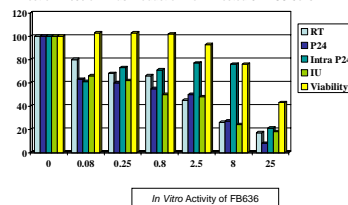
Cell Type (Model)	Quantitation of HIV RNA (% of Control)							
	Multi-spliced RNA				Singly Spliced/un-spliced RNA			
	2 ¹	4	8	16	2	4	8	16
CEM/SK-1 (Chronic)	ND	177	177	177	ND	25	15	22
U1 (Latent TNFα-induced)	214	214	71	ND	30	21	27	ND

¹Concentration of FB636 µg/ml

Mechanism of Action of FB636

- FB636 inhibits a step in HIV replication which occurs following the integration of the virus into the target cell genome.
- Evaluation of the effects of FB636 on viral RNA and protein synthesis indicates that FB636 specifically reduces the production of full length RNA species in the treated cell.
- FB636 has no effect on virus attachment or fusion, reverse transcriptase, protease, integrase or CD4 expression.
- Initial RNA expression data suggested that FB636 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.

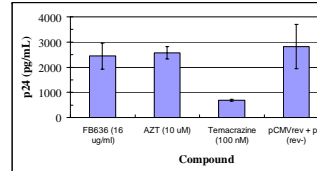
Effect of FB636 on Virus Production from Infected CEM-SS Cells



In Vitro Activity of FB636

1. In chronically infected cell systems, FB636 is active against all strains of HIV-1 and HIV-2 which have been tested.
2. Using various cell lines infected with HIV-1, FB636 inhibits replication independent of the cell type used (H9, CEM-SS, U937 and MT2).
3. FB636 inhibits the replication of drug resistant HIV strains, including protease inhibitor resistant viruses.
4. No efficacy of FB636 has been detected against SIV in acute or chronic infection assays.

FB636 does not reduce Rev-dependent virus expression in a Rev-dependant transient transfection model



Ratio of Unspliced:Multiply Spliced RNA in FB636 Treated CEM-SS Cells

Sample	Day 0	Day 3	Day 5	Day 11
No Drug	1000:1	1000:1	2500:1	1000:1
2 µg/ml	1000:1	4000:1	100:1	10:1
4 µg/ml	1000:1	400:1	100:1	10:1
8 µg/ml	1000:1	4000:1	10:1	10:1

Acute Infection Combination Assays

Compound	Interaction	Synergy Volume
AZT	Additive	4/38
DdC	Additive	26/32
DdI	Additive	37/7
3TC	Additive	1/33
Indinavir	Additive	19/37
Saquinavir	Additive	0/23

Preclinical Efficacy of FB636

1. Efficacy in acute infection assays: Inactive
 2. Acute infection combination assays: Additive
 3. Efficacy in chronic infection assays: Active
 4. Chronic infection combination assays: Additive
 5. Virucidal assays: Inactive
 6. Resistance selection assays: No resistance
 7. Super-infection: None
 8. Mechanism of action: Late stage RNA synthesis
9. Efficacy in latent infection assays: Active
 10. Infectability of pretreated cells: No effect
 11. Cellular macromolecular synthesis assays: No effect
 12. Cell cycle and apoptosis assays: No effect
 13. Serum/Plasma Protein: Little effect

Acknowledgements

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