

JTK-303/GS-9137, a Novel Small Molecule Inhibitor of HIV-1 Integrase: Anti-HIV Activity Profile and Pharmacokinetics in Animals

Yuji Matsuzaki¹, Wataru Watanabe¹, Kazunobu Yamataka¹, Motohide Sato¹, Seiji Enya¹, Mitsuki Kano¹, Eiichi Kodama², Masao Matsuoka², and Satoru Ikeda¹

¹Japan Tobacco Inc., Osaka, Japan and ²Institute for Virus Research, Kyoto University, Japan



Japan Tobacco Inc., CPRI
1-1, Murasaki-cho, Takatsuki,
Osaka, Japan, JP 569-1125

Phone: 81-72-681-9700
Fax: 81-72-681-9725

E-mail: yuji.matsuzaki@ims.jti.co.jp

Introduction

Currently approved therapies for HIV-1 infection target the virus's reverse transcriptase or protease, or inhibit fusion of the virus with the cell membrane. Integrase inhibitors fill an unmet need to target the third viral enzyme; none is approved to date, although several inhibitors are under development. JTK-303/GS-9137 (chemical name: 6-(3-chloro-2-fluorobenzyl)-1-[(2S)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) was discovered at the Central Pharmaceutical Research Institute of Japan Tobacco Inc. It was found to be a low molecular weight HIV-1 integrase inhibitor through screening of inhibitory activity against recombinant HIV-1 integrase (based on the full-length sequence of HIV-1 NL4-3 gene).

Methods

HIV-1 Integrase Assay

Gel Electrophoresis: HIV-1 NL4-3 integrase (1.5 μM) and compounds were incubated with 50 nM of a ³²P-labeled 21 bp viral DNA substrate (derived from HIV-1 U5 LTR) for 60min. After electrophoresis of reaction products, 19-mer oligonucleotides (3'-processed products), >21-mer oligonucleotides (strand transfer products, STP) and total radioactivity present in the lane were quantified by measuring radioactivity of corresponding bands using an image analyzer.

Microtiter Plate Assay: Biotin-labeled donor DNA representing HIV-1 U5 LTR was immobilized onto streptavidin-coated microtiter plates. HIV-1 NL4-3 integrase (300 nM) was incubated with 3'-end processed donor DNA, followed by removal of unassociated enzyme, and the DNA strand transfer reaction was initiated by the addition of target DNA (5 nM) and compounds. The amount of target DNA ligated to the donor DNA was measured by detecting digoxigenin-labeled target DNA.

Antiviral Assay in Human PBMCs and Macrophages: Human PBMCs (PHA-stimulated) and macrophages were infected with each isolate, then treated with serially diluted compounds and cultured for seven days. Antiviral activity was determined using p24 antigen ELISA.

In Vitro Combination Study: CEM-SS cells were infected with HIV-1_{IIIB} strain at an MOI of approximately 0.01, then treated with serially diluted JTK-303/GS-9137 and approved anti-HIV-1 drugs. After cultivation for six days, antiviral activity was determined using MTT assay.

Pharmacokinetic Study in Animals: In rats and dogs, plasma concentrations of JTK-303/GS-9137 were quantified after a single oral dose of 3 mg/kg or intravenous dose of 1 mg/kg, and relevant pharmacokinetic parameters were calculated.

Figure 1. Chemical Structure of JTK-303/GS-9137

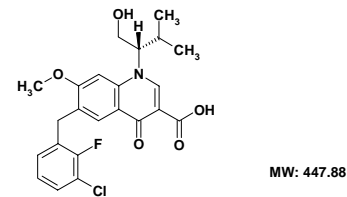
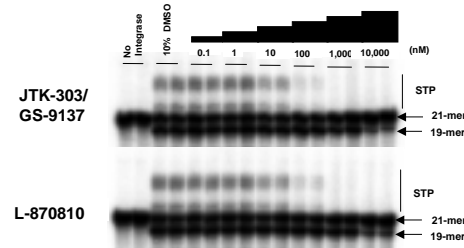
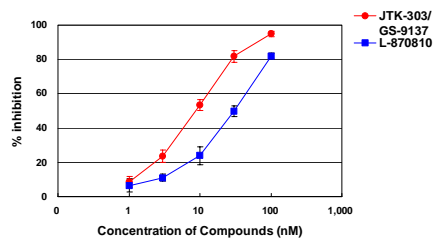


Figure 2. Inhibition of Formation of HIV-1 Integrase Strand Transfer Products by JTK-303/GS-9137



Gel electrophoresis showing 21-mer, 19-mer and STP, corresponding to DNA substrate, 3'-processed products and strand transfer products, respectively.

Figure 3. Inhibition of Recombinant HIV-1 Integrase Strand Transfer Reaction by JTK-303/GS-9137



IC₅₀s of JTK-303/GS-9137 and L-870810 on strand transfer reaction (microtiter plate assay) were 8.8 ± 0.9 nM and 30.2 ± 4.0 nM (mean ± SD of 3 experiments), respectively.

Results

Table 1. Antiviral Activity of JTK-303/GS-9137 against Laboratory Strains

Cells/virus and Parameter	JTK-303/GS-9137	Efavirenz	Nelfinavir
PBMC/HIV-1 IIIB^a			
EC ₅₀ (nM)	0.2 ± 0.1	0.2 ± 0.1	2.6 ± 1.7
EC ₉₀ (nM)	1.2 ± 0.4	1.2 ± 0.4	20.7 ± 4.1
CC ₅₀ (μM) ^c	9.7 ± 2.5	24.4 ± 11.4	10.9 ± 3.2
SI	48,500	122,000	4,192
Macrophage/HIV-1 Ba⁻L^b			
EC ₅₀ (nM)	0.7	2.1	36.3
CC ₅₀ (μM) ^d	> 0.5 ^e	> 10 ^e	> 10 ^e
SI	> 714	> 4,762	> 275
Macrophage/HIV-1 ADA^b			
EC ₅₀ (nM)	0.1	3.6	45.2
CC ₅₀ (μM) ^d	> 0.5 ^e	> 10 ^e	> 10 ^e
SI	> 5,000	> 2,778	> 221

a: Mean ± SD of 3 experiments
b: 1 experiment (triplicate)
c: Cytotoxicity was assessed by uptake of [³H]-thymidine into cells.
d: Cytotoxicity was determined using MTT assay.
e: CC₅₀s were higher than maximum concentrations of these compounds in each assay.

JTK-303/GS-9137 showed potent antiviral activity against the laboratory strains tested, with EC₅₀ ranging from 0.1 to 0.7 nM. The selectivity index (SI, CC₅₀/EC₅₀) also showed that the antiviral activity of JTK-303 was not the results of cytotoxicity. The potency of JTK-303 was greater than that of NFV and similar to that of EFV. The antiviral activity of JTK-303 was moderately reduced by the addition of 50% human serum.

Table 2. Antiviral Activity of JTK-303/GS-9137 against B and non-B Subtypes of HIV-1 and against HIV-2

Virus	Isolate	EC ₅₀ (nM)			
		JTK-303/GS-9137	AZT	Efavirenz	Nelfinavir
HIV-1					
Subtype A	RW/92/016	0.41	7.91	0.61	13.4
Subtype B	96USHIPS7	0.26	8.41	0.65	25.8
	BR/92/021	0.76	2.13	47.0	70.5
	BR/93/017	0.18	1.10	0.58	2.05
	BR/93/022	1.13	11.7	0.62	20.1
Subtype C	BR/92/025	0.10	2.84	0.30	< 0.10
Subtype D	UG/92/046	0.50	7.26	1.19	27.7
Subtype E	CMU02	1.26	9.07	1.82	22.7
Subtype F	BR/93/020	0.74	25.3	0.32	16.4
Subtype G	JV1083	0.35	11.1	0.65	14.0
Subtype O	BCF01	1.17	1.52	0.69	0.99
HIV-2					
	CDC 310319	0.53	1.14	> 1000	16.1

The experiments were conducted in human PBMCs (1 experiment, triplicate).

JTK-303/GS-9137 showed potent antiviral activity against all clinical isolates representing 4 subtype B and 7 non-B subtypes of HIV-1. The average EC₅₀ of JTK-303 was 0.62 nM for the 8 subtypes of HIV-1 (11 viruses), and was 0.53 nM for the single HIV-2 clinical isolate. JTK-303 showed comparable or greater potency than AZT, EFV and NFV against all the isolates tested.

Table 3. Antiviral Activity of JTK-303/GS-9137 against Drug-Resistant Clinical Isolates of HIV-1

Isolate	Genotypes of Drug-Resistant Isolates	EC ₅₀ (nM)				
		JTK-303/GS-9137	AZT	Efavirenz	Nelfinavir	
PI-resistant:						
1064-52	Protease	L101 I54V L63P A71T V82F L90M	0.63	13.6	0.29	838.5
	Protease	L101 M46I I54V L63P V82F L90M	0.13	6.72	0.21	> 1,000
052-52	Protease	L10R M46I L63P A71V V82T I84V	0.17	63.9	< 0.10	95
	Protease	V32I M46I L63P L90M	0.72	7.24	0.6	165.3
Multidrug-resistant:						
MDR 769	RT	M41L K65R D67N V75I F116Y Q151M Y181I L210W T215Y	0.18	720.7	0.54	> 1,000
	Protease	L101 M36M/V M46I I54V L63P A71V V82A I84V L90M				
MDR 807	RT	M41L D67N M184V L210W T215Y K219N	1.13	159.4	0.71	135
	Protease	L101 G48V I54T L63Q A71V V82A				
MDR 1385	Protease	L101 M36I M46I I54V L63P A71V V82T L90M	0.02	> 1,000	> 1,000	> 1,000
	Protease	L101 M46I I84V L63P A71L L90M	0.73	> 1,000	> 1,000	> 1,000

The experiments were conducted in human PBMCs (1 experiment, triplicate).

a: Mutation sites are represented as the position number in the RT- or protease-coding region, and the amino acid residue in the wild-type and drug-resistant viruses.

The average EC₅₀ of JTK-303/GS-9137 was 0.46 nM. Although two of the isolates (MDR 1385 and MDR 3761) were completely resistant to AZT, EFV, and NFV (EC₅₀s > 1,000 nM), they were highly sensitive to JTK-303 (EC₅₀s < 1 nM).

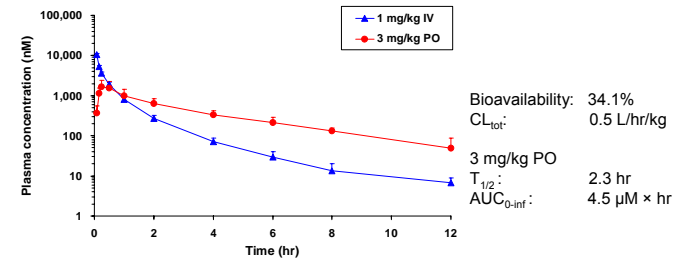
Table 4. Effects of JTK-303/GS-9137 in Combination with Other Anti-HIV Drugs

Drug Combination	Synergy/Antagonism Value (μM ² ; mean of 3 experiments)	Combination Effect
JTK-303/GS-9137 + NRTI		
JTK-303/GS-9137 + 3TC	67.7/-20.0	Slightly synergistic
JTK-303/GS-9137 + AZT/3TC	111.7/-13.0	Highly synergistic
JTK-303/GS-9137 + AZT	40.7/-29.3	Additive
JTK-303/GS-9137 + NNRTI		
JTK-303/GS-9137 + Efavirenz	35.3/-5.3	Additive
JTK-303/GS-9137 + PI		
JTK-303/GS-9137 + Indinavir	26.7/-18.7	Additive
JTK-303/GS-9137 + Nelfinavir	34.0/-17.3	Additive

The combination effects of JTK-303/GS-9137 were analyzed by the Prichard and Shipman method using MacSynergy II software. Results of the combination assays were expressed as the mean synergy/antagonism volume values (μM²) that were calculated at the 95% confidence interval from 3 separate experiments. Combination effects were defined as:

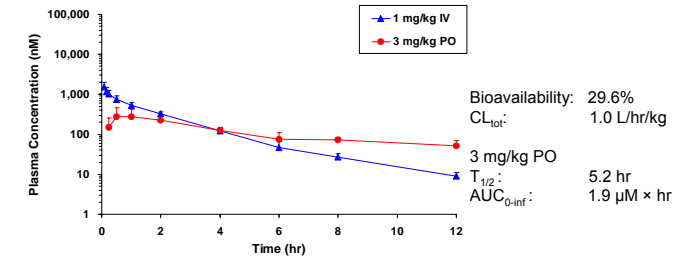
Highly synergistic: > 100 μM²
Slightly synergistic: > 50 and ≤ 100 μM²
Additive: ≤ 50 and > -50 μM²
Slightly antagonistic: ≤ -50 and > -100 μM²
Antagonistic: ≤ -100 μM²

Figure 4. Plasma Concentrations of the Parent Drug after a Single Dose of JTK-303/GS-9137 to Rats



Bioavailability: 34.1%
CL_{tot}: 0.5 L/hr/kg
T_{1/2}: 2.3 hr
AUC_{0-inf}: 4.5 μM × hr

Figure 5. Plasma Concentrations of the Parent Drug after a Single Dose of JTK-303/GS-9137 to Dogs



Bioavailability: 29.6%
CL_{tot}: 1.0 L/hr/kg
T_{1/2}: 5.2 hr
AUC_{0-inf}: 1.9 μM × hr

Conclusions

- JTK-303/GS-9137 is a highly selective HIV-1 integrase inhibitor with potent antiviral activity against both B and non-B subtypes of HIV-1.
- JTK-303/GS-9137 retains antiviral activity against drug-resistant HIV-1 carrying resistance mutations to multiple drug classes.
- JTK-303/GS-9137 has additive to highly synergistic antiviral activity with three currently approved anti-HIV-1 drug classes.
- In preclinical animal studies, JTK-303/GS-9137 has good oral bioavailability.
- JTK-303/GS-9137 has potential as a novel, orally bioavailable anti-HIV agent and has progressed to early clinical studies.