

Viral Resistance to the HIV-1 Integrase Inhibitor GS-9160

GS Jones, F Yu, A Zeynalzadegan, J Hesselgesser, X Chen, J Chen, H Jin, CU Kim, M Wright, R Geleziunas and M Tsiang*

Gilead Sciences, Inc., Foster City, CA, USA

Introduction

- Integrase is essential for HIV-1 replication and inhibitors of the integrase strand transfer reaction have been validated clinically
- The integrase inhibitor raltegravir (MK-0518) was approved for clinical use in 2007 and is dosed twice daily, while elvitegravir (GS-9137) is in late-stage clinical development and is dosed once daily with ritonavir
- L-870,810 is an efficacious HIV-1 integrase inhibitor.^{1,2,3} L-870,810 can exist as two different conformers; the higher energy conformer is active against integrase
- A pre-organized tricyclic pharmacophore was designed to lock the structure into the active conformation to increase binding affinity (Fig. 1)⁴
- GS-9160 which emerged from this effort inhibits the integrase strand transfer reaction and displays potent anti-HIV-1 activity^{5,6,7}
- Here, we describe the biological characterization of GS-9160 and the identification of a novel pattern of viral resistance mutations to GS-9160

Methods

- Antiviral and Cytotoxicity Assays.** 5-fold serially diluted compound was added to each well of a 96-well plate. HIV-1 IIIb infected MT-2 or MT-4 cells were added to each well containing compound. After 5 days, CellTiter-Glo™ Reagent was added to each well. After cell lysis, chemiluminescence was read. For compound cytotoxicity assessment, the protocol was identical except that uninfected cells were used and compounds were serially diluted 3-fold
- To study the effect of serum proteins on the antiviral activity of integrase inhibitors, compounds were tested in the presence of either 35 mg/ml human serum albumin (HSA) or 1.5 mg/ml α1-acid glycoprotein (α1-AGP). To assess the effect of human serum on compound potency, the assay was performed in the presence of 10%, 20%, 35% and 50% human serum from clotted whole blood at three different multiplicities of infection (m.o.i.): 0.01, 0.02 and 0.04. The result was extrapolated to 100% human serum
- Drug Combination Studies.** The effect of combining any two drugs in the MT-2 antiviral assay was analyzed by two different methods: the Prichard and Shipman method using MacSynergy™ II software and the combination index method using CalcuSyn software
- 2-LTR Circle Accumulation Assay.** SupT1 cells were infected with HIV-1 IIIb at a multiplicity of infection of 10. 2-LTR junction (2-LTR circles) and the host globin gene (used to normalize for cell number) were quantified using real-time Q-PCR
- Alu-PCR Assays.** SupT1 cells were infected with pseudo-typed HIV-1 at an MOI of 10. Late-RT products were quantified by PCR at 12 hours and Alu-PCR products at 48 hours. These products were normalized to the level of globin gene
- Viral Resistance Selections.** MT-2 cells were seeded in 6-well tissue culture plates at a density of 0.5 x 10⁶ cells per well in 5 ml of culture medium. Compounds were added at final concentrations corresponding to their antiviral EC₅₀ or twice the EC₅₀. The cultures were split 1/2 – 1/3 once or twice a week depending on the growth of the cells. Virus-induced syncytia formation was used to follow progression of infections. Virus was harvested and transferred to fresh MT-2 cells in the presence of the same compound but at a 2-fold higher concentration. Successive viral passages were obtained by repeating this procedure. The duration of each passage ranged from 10 – 15 days

Figure 1. The GS-9160 Tricyclic Pharmacophore Locks the Structure into an Active Conformation

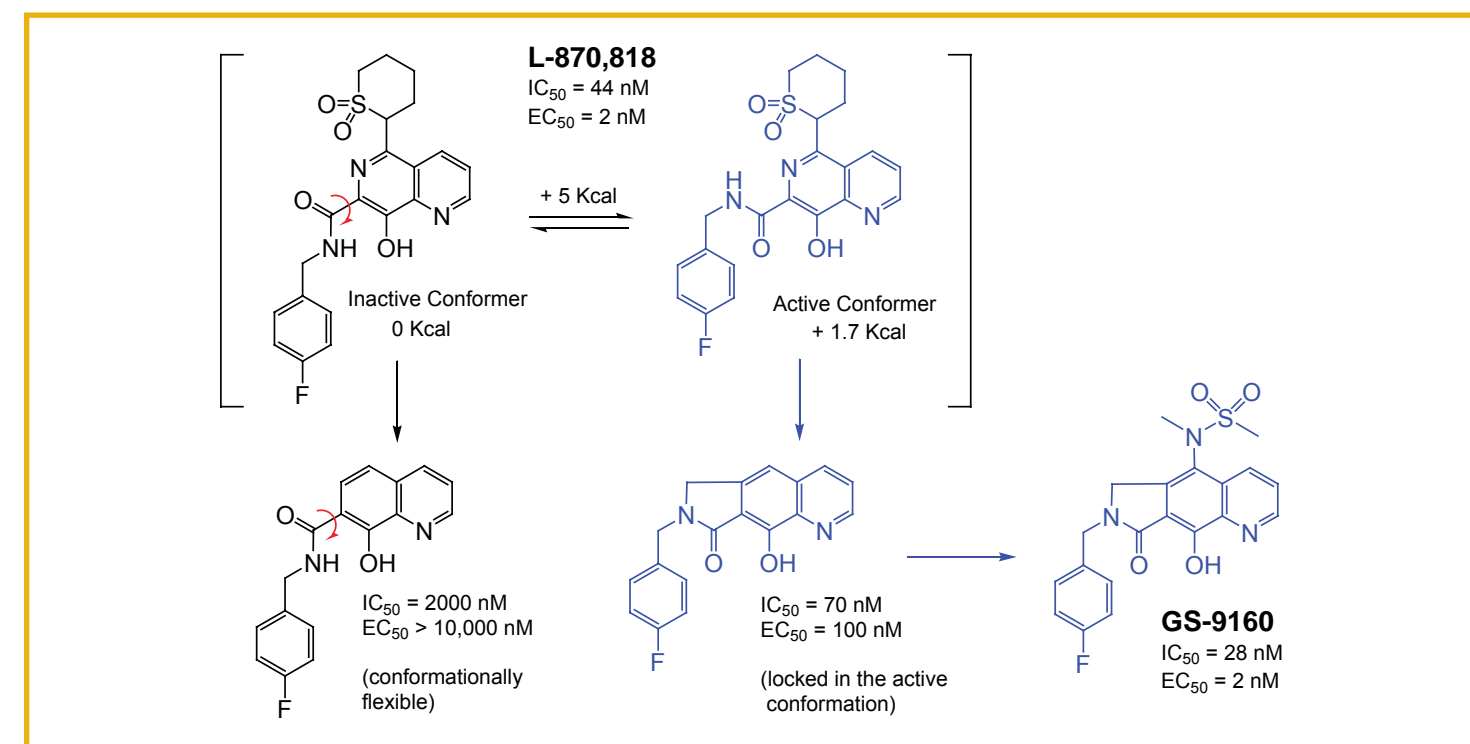


Table 1. GS-9160 Inhibits Integrase Strand Transfer Activity and Integration in HIV-1 Infected Cells

Compound	Structure	Strand Transfer IC ₅₀ (nM)	2-LTR Circles Accumulation Assay		Determination of Integration Junctions		
			2-LTR Circles in SupT1	Maximum Fold-Increase	Cytotoxicity in SupT1	Late-RT Signal 12 h Post-Infection	Alu-PCR Signal 48 h Post-Infection
GS-9160		28 ± 5	1.9 ↑	600	> 1000	0.9	
Elvitegravir		40 ± 5	2.3 ↑	5300	> 1000	0.3	
L-870,810		44 ± 4	3.0 ↑	1900	> 1000	1.3	
Raltegravir		34 ± 5	-	-	-	-	
Efavirenz		-	0.17 ↓	15500	5.0	0.5	
Amprenavir		-	1 ↔	96000	> 1000	> 1000	

Dash (-) = Not done

Table 2. Antiviral Activity of GS-9160 in MT-2, MT-4 and Human T-Lymphocytes

Compound	Anti-HIV in MT-2			Cytotoxicity in MT-2		Anti-HIV in MT-4		Cytotoxicity in MT-4		Anti-HIV in Hu T-Cell		Cytotoxicity in Hu T-Cell	
	10% FBS	10% FBS + HSA + α1-AGP	100% HS	10% FBS	10% FBS	10% FBS	10% FBS	10% FBS	10% FBS	10% FBS + HSA + α1-AGP	10% FBS	10% FBS	
	EC ₅₀ (nM)	EC ₅₀ (nM)	EC ₅₀ (nM)	CC ₅₀ (nM)	EC ₅₀ (nM)	CC ₅₀ (nM)	EC ₅₀ (nM)	CC ₅₀ (nM)	EC ₅₀ (nM)	EC ₅₀ (nM)	CC ₅₀ (nM)		
GS-9160	2.1 ± 0.7	15 ± 5	12	4000 ± 1000	0.7 ± 0.5	320 ± 20	1.5 ± 0.8	15 ± 16	4000 ± 5000				
Elvitegravir	0.6 ± 0.1	24 ± 6	83	8000 ± 1000	0.17 ± 0.03	4000 ± 2000	0.2 ± 0.1	16 ± 18	40000 ± 18000				
L-870,810	2 ± 1	43 ± 17	41	3000 ± 2000	4 ± 3	1600 ± 400	3 ± 2	60 ± 76	2100 ± 800				
Raltegravir	3 ± 1	18 ± 6	-	>100,000	8 ± 2	58200 ± 7300	-	-	-				

Dash (-) = Not done

Table 3. GS-9160 is Synergistic when Combined with HIV-1 Antiviral Drugs from Other Classes

Drug Combination	MacSynergy™ II		Description	CalcuSyn		
	Synergy/Antagonism Volumes (nM ² %)	Description		Combination Index	SD	Score
EFV + TDF	266.9/-13.2 (n=2)	Highly Synergistic	0.551 (n=2)	0.159	3	Synergism
TDF + FTC	150.6/-15.8 (n=2)	Highly Synergistic	0.605 (n=2)	0.205	3	Synergism
AZT + 3TC	412.7/-1.2 (n=2)	Highly Synergistic	0.313 (n=2)	0.048	3	Synergism
AZT + d4T	44.7/-47.0 (n=2)	Additive	0.985 (n=2)	0.329	0	Nearly Additive
RBV + d4T	0.6/-255.5 (n=2)	Highly Antagonistic	1.716 (n=2)	0.424	-3	Antagonism
GS-9160 + LPV	190.2/-0.7 (n=2)	Highly Synergistic	0.438 (n=2)	0.119	3	Synergism
GS-9160 + ATV	240.0/-12.9 (n=2)	Highly Synergistic	0.751 (n=2)	0.254	2	Moderate Synergism
GS-9160 + NFV	153.7/-14.6 (n=2)	Highly Synergistic	0.478 (n=2)	0.112	3	Synergism
GS-9160 + EFV	221.0/-12.3 (n=2)	Highly Synergistic	0.600 (n=2)	0.098	3	Synergism
GS-9160 + TDF	410.5/-20.8 (n=2)	Highly Synergistic	0.373 (n=2)	0.069	3	Synergism
GS-9160 + AZT	276.6/-23.2 (n=2)	Highly Synergistic	0.537 (n=2)	0.065	3	Synergism
GS-9160 + FTC	365.7/-18.2 (n=2)	Highly Synergistic	0.580 (n=2)	0.119	3	Synergism
GS-9160 + 3TC	284.6/-12.8 (n=2)	Highly Synergistic	0.547 (n=2)	0.111	3	Synergism

- The yellow shaded drug combinations are known examples for synergism, additivity or antagonism and are used as controls
- Using the MacSynergy™ II analysis, synergy volumes of -50 to 50 are considered additive, 50 to 100 are slightly synergistic, -100 to -50 are slightly antagonistic, > 100 are highly synergistic and < -100 are highly antagonistic
- Using the CalcuSyn analysis, combination indices around 0 are considered nearly additive, 1 to 5 are moderately to strongly synergistic and -1 to -5 are moderately to strongly antagonistic

Results

Table 4. GS-9160 is Active Against Drug-Resistant Mutants of HIV-1

Compounds	EC ₅₀ (nM) against HIV-1 IIIb	Fold-Change in EC ₅₀ using virus with indicated mutations							
		NRTI Resistant			NNRTI Resistant		PI Resistant		
		K65R	M184V	6TAMs*	K103N	Y181C	I84V/ L90M	G48V/V82A/ L90M	
TDF	6.8	4	1	9	1	1	1	1	
FTC	323	26	> 55	23	1	0.4	1	1	
EFV	0.2	1	1	> 530	61	3	1	1	
CPV ^b	0.2	0.4	1	762	0	89	1	1	
LPV	4.8	3	3	5	1	1	29	9	
L-870,810	2.0	1	1	1	1	1	1	1	
Elvitegravir	0.6	2	1	2	1	0.5	1	1	
GS-9160	2.1	1	1	2	1	1	1	1	

The color shadings subdivide the resistance fold-change into four levels as follows:

>3-fold: >10-fold: >50-fold: >500-fold

a. Clinical isolate containing 6 thymidine analog resistant mutations and derived from a highly treatment experienced patient with additional NNRTI and PI resistance mutations

b. CPV = Capravirine

Table 5. Resistance Selections with GS-9160 and Other HIV Antiviral Drugs

Selected Virus	Duration of Selection (day)	[Drug] reached (nM)	Fold-Change in EC ₅₀ using the indicated viral passage							
			3TC	EFV	APV	GS-9160	L-870,810	Elvitegravir	Raltegravir	
HIV-1 IIIb	-	-	1 (3200 nM)	1 (0.3 nM)	1 (23 nM)	1 (2.6 nM)	1 (5 nM)	1 (0.8 nM)	1 (3.3 nM)	
3TC P4 virus	33	64,000	>272	1.1	1.6	0.5	1.3	2.6	1.0	
3TC P8 virus	59	1,024,000	>272	0.8	0.9	0.5	0.8	1.9	0.8	
EFV P3 virus	31	4	2	35	1.2	0.9	1.2	1.5	1.7	
EFV P7 virus	74	64	1.0	281	1.6	0.6	1.3	2.1	0.8	
APV P2 virus	25	60	7	13	3.4	1.0	2.5	4.5	1.8	
APV P4 virus	48	120	9	2.5	8.7	1.6	1.9	4.8	1.9	
GS-9160 P5 virus	56	64	1.8	2.2	1.0	4.3	2.7	8	3.9	
GS-9160 P6 virus	76	128	4.5	3.7	1.5	26	29	130	38	
GS-9160 P8 virus	99	256	5.7	4.3	4.0	60	65	410	59	
GS-9160 P9 virus	115	512	1.4	2.2	1.5	51	49	292	26	

- The yellow shading highlights the viral passages in which phenotypic resistance became detectable. The light blue shading highlights the development of cross resistance in GS-9160 selected viral pools against L-870,810, elvitegravir and raltegravir
- Viral passages are denoted as P, e.g passage 4 is P4

Table 6. GS-9160 Selected a Novel Pattern of Resistance Mutations

GS-9160 Selected Virus	Duration of Selection (day)	[Drug] reached (nM)	Mutation in Integrase	
			(% clones with mutation(s) indicated)	
P5 virus	56	64	E92V (33%)	
P6 virus	76	128	L74M (100%), E92V (100%)	
P8 virus	99	256	L74M (100%), E92V (100%)	
P9 virus	115	512	L74M (100%), E92V (100%)	

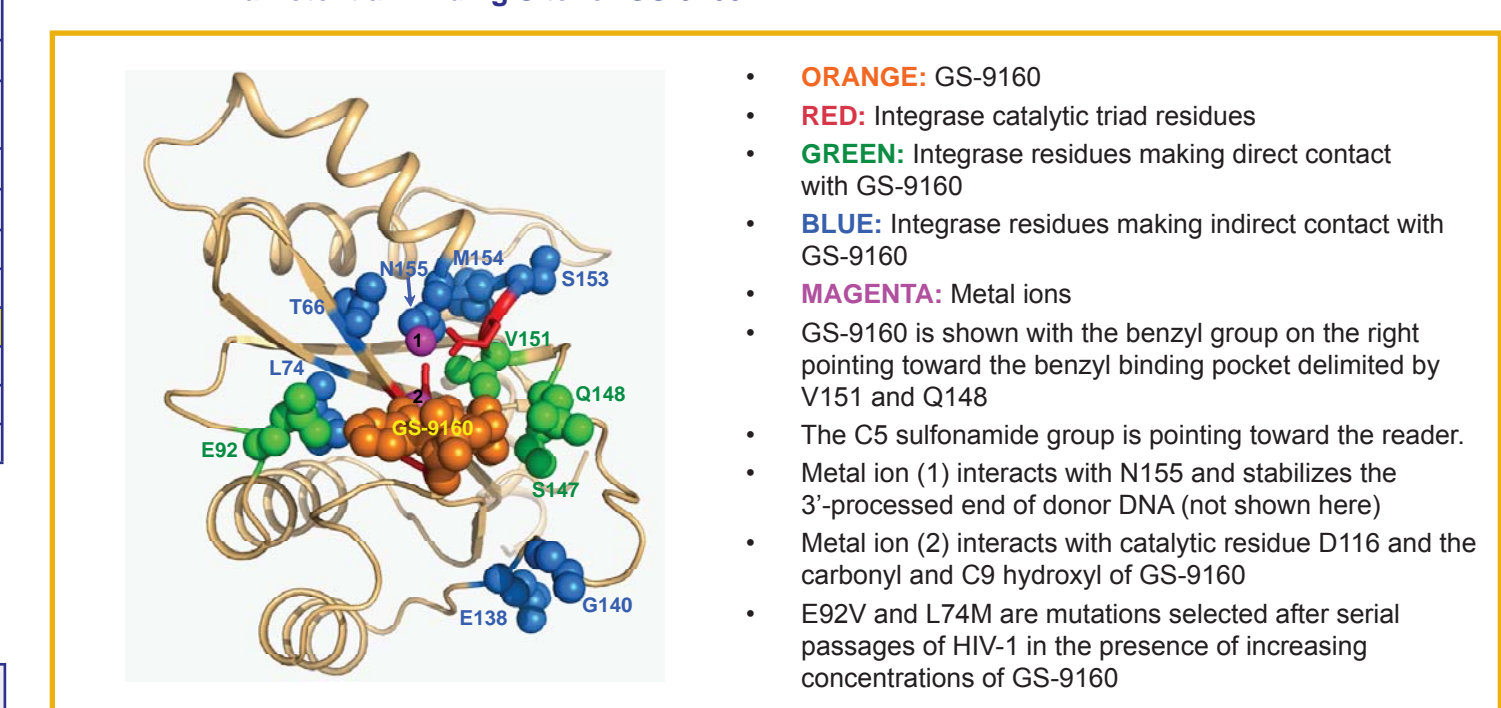
Table 7. Resistance Profile of GS-9160

Compounds	EC ₅₀ (nM) against HIV-1 IIIb	Fold-Change in EC ₅₀ using virus with indicated mutation(s)														
		T66I	L74M	E92Q	E92V	E138K	G140S	Q148K	V151A	N155H	N155S	L74M/ E92V	E92V/ V151A	E138K/ Q148K	E92V/ G140S/ V151A	G140S/ Q148H
TDF	6.8	1	1	1	2	1	1	1	2	2	2	1	2	2	2	0.3
FTC	323	1	1	2	1	0.4	1	2	3	2	3	2	4	2	2	0.8
EFV	0.2	2	1	2	2	2	2	4	4	5	6	2	4	2	4	0.4
CPV	0.2	2	1	1	3	2	2	1	4	6	6	3	2	3	3	0.2
LPV	4.8	4	1	1	1	5	1	1	1	2	2	1	1	1	2	1
L-870,810	2.0	2	1	42	8	1	2	35	5	65	21	39	111	314	371	478
Raltegravir	3.3	2	2	7	6	1	3	152	4	33	61	12	28	1034	166	1653
Elvitegravir	0.6	28	2	153	44	0.2	0.2	600	12	100	133	123	300	1833	2667	7780
GS-9160	2.1	1	1	32	12	1	2	23	5	50	12	67	104	250	583	1132

The color shadings subdivide the resistance fold-change into four levels as follows:

>2-fold: >10-fold: >50-fold: >500-fold

Figure 2. Location of Resistance Mutations in the Integrase Catalytic Core Domain and a Potential Binding Site for GS-9160



- ORANGE:** GS-9160
- RED:** Integrase catalytic triad residues
- GREEN:** Integrase residues making direct contact with GS-9160
- BLUE:** Integrase residues making indirect contact with GS-9160
- MAGENTA:** Metal ions
- GS-9160 is shown with the benzyl group on the right pointing toward the benzyl binding pocket delimited by V151 and Q148
- The C5 sulfonamide group is pointing toward the reader.
- Metal ion (1) interacts with N155 and stabilizes the 3'-processed end of donor DNA (not shown here)
- Metal ion (2) interacts with catalytic residue D116 and the carbonyl and C9 hydroxyl of GS-9160
- E92V and L74M are mutations selected after serial passages of HIV-1 in the presence of increasing concentrations of GS-9160

Conclusions

- GS-9160 is an integrase strand-transfer inhibitor with potent antiviral activity – Novel structural class: N-Benzyl-pyrrolidinone-hydroxyquinoline
- GS-9160 is an authentic inhibitor of HIV-1 integrase – Causes elevation of 2-LTR circles and decreases integration junctions
- GS-9160 is active against NRTI-, NNRTI- and PI-resistant HIV-1 mutants and is synergistic with clinically approved anti-HIV-1 drugs
- GS-9160 selected resistance mutations E92V and L74M in the integrase catalytic core – These mutations confer cross-resistance to the structurally distinct strand-transfer inhibitors L-870,810, raltegravir and elvitegravir – L74M which appeared later potentiates E92V resistance to GS-9160 – Modeling suggests:
 - Carboxylic side chain of residue E92 interacts with the quinoline nitrogen of GS-9160 through a water molecule
 - Mutation E92V would eliminate this interaction and weaken the binding of GS-9160
- Clinical development of GS-9160 was discontinued because pharmacokinetics in healthy human volunteers revealed an apparent half-life of ~2 hours which does not adequately support once daily dosing

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