

Abstract

Background: Several novel low molecular weight potent HIV-1 integrase inhibitors have been reported in the literature. These compounds possess a pharmacophore which is capable of chelating the divalent metals present in the catalytic site of the enzyme. Despite a common mechanism of action shared by these molecules, *in vitro* selection studies of HIV-1 variants with reduced susceptibilities to integrase inhibitors have revealed distinct pathways of resistance emergence. Our aim was to investigate the potential cross-resistance of these mutations with five structurally distinct integrase inhibitors.

Methods: Recombinant HIV-1 integrase mutants were generated by site-directed mutagenesis using HXB-2D proviral clone as starting material. Drug susceptibility was evaluated in MT-2 cells. Cells were infected at a multiplicity of infection of 0.5 for 3 hours, washed to remove any residual virus, resuspended in culture medium and seeded into 96-well plates in the presence of various concentrations of the test compound. Thereafter, infected cells were cultured for 3 days after which an aliquot of medium with cells was replaced with fresh medium containing the test compound. The level of HIV-1 replication was determined at day 5 post-infection by measuring the reverse transcriptase activity in the harvested supernatant fluid.

Results: The anti-HIV-1 activity of all compounds tested namely, L-731,988, GS-9137, MK-0518, L-870-810, and a novel Pyridone A was found to be significantly reduced when tested against HIV HXB-2D mutants carrying the F121Y, F121Y/T125K, V72I/F121Y/T125K, and V151I/V72I/F121Y/T125K mutations whereas the T66I, T66I/S153Y, T66I/M154I mutants were found to be associated with reduced susceptibility to L-731,988 and GS-9137.

Conclusions: It is well documented that *in vitro* resistance development to different chemical series of integrase inhibitors involved specific mutational pathways. In this study, phenotypic analysis of recombinant viruses harbouring selected mutations within the integrase gene has demonstrated significant cross-resistance with the compounds investigated.

Introduction

Integration of the proviral DNA into the host cell genome is an essential step in the HIV replication cycle. This process is mediated by the viral integrase.

Integrase is considered an attractive target for HIV therapeutics as there is no known human cellular homologue for this enzyme⁽¹⁾.

Two consecutive steps are catalyzed by the enzyme namely 3'-processing (3P) and a DNA strand transfer reaction. Both steps as well as the multimerization / assembly of the enzyme requires divalent metals⁽²⁻⁴⁾.

Integrase inhibitors can be classified as dual inhibitors of 3P and strand transfer, and selective strand transfer inhibitors⁽⁵⁾.

The most promising anti-HIV integrase class falls into the category of selective strand transfer inhibitors with representatives already in advanced clinical studies. Chemical structures of selected molecules investigated in this study are depicted in Figure 1.

Different patterns of mutations are selected under selective pressure despite a common mechanism of action shared by these inhibitors⁽⁶⁾.

A number of recombinant HIV-1 containing mutations known to confer resistance to selected integrase inhibitors were engineered and drug susceptibility testing performed for potential cross-resistance evaluation.

Results

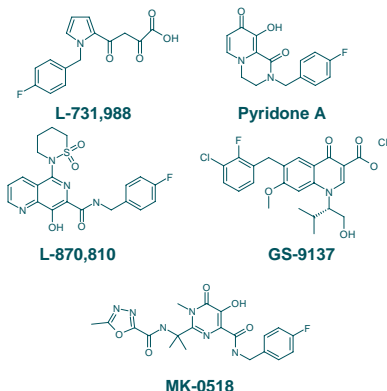


Figure 1 Structure of selected integrase inhibitors in this study.

When investigated for anti-HIV activity in the MT-2 cell line against HIV-1_{HXB-2D} harbouring the T66I, T66I/S153Y, or T66I/M154I amino acid changes (mutations known to be associated with diketo-acid resistance⁽⁶⁾), both L-731,988 and GS-9137 showed reduced sensitivity (7.7 to 28-fold) to the viruses whereas Pyridone A, MK-0518, and L-870,810 retained sensitivity (0.8 to 3.8-fold change) to the viruses (Table 1, Figure 2a).

All compounds were generally found to be associated with a reduced activity to the viruses (10 to 145-fold change) when investigated for anti-HIV activity in the MT-2 cell line against HIV-1_{HXB-2D} harbouring the F121Y, F121Y/T125K, V72I/F121Y/T125K, or V72I/F121Y/T125K/V151I amino acid changes (mutations known to be associated with naphthyridine carboxamide resistance⁽⁶⁾) (Table 1, Figure 2b).

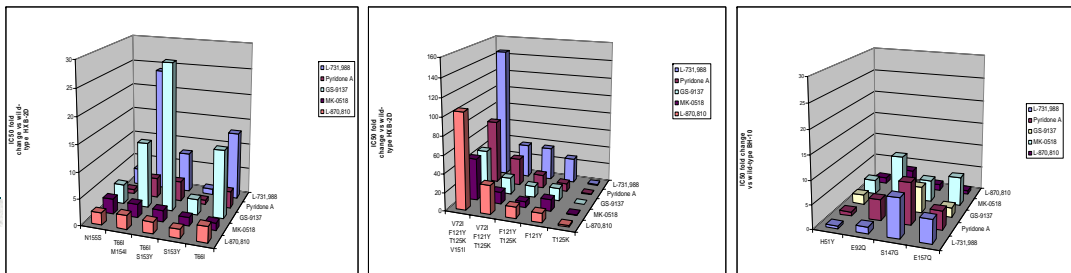


Figure 2a Susceptibility of HIV-1_{HXB-2D} with T66I, **Figure 2b** Susceptibility of HIV-1_{HXB-2D} with T125K, **Figure 2c** Susceptibility of HIV-1_{BH-10} with H51Y, T66I/S153Y, T66I/M154I, S153Y, or N155S mutations F121Y, F121Y/T125K, V72I/F121Y/T125K, or E92Q, S147G, or E157Q mutations in the IN gene to various integrase inhibitors.

HIV-1_{BH-10} harbouring the H51Y, E92Q, S147G, or E157Q amino acid changes (mutations known to be associated with GS-9137 resistance⁽⁷⁾) were found to exhibit a less than 10-fold change in activity vs the wild-type virus in the MT-2 cell line with all compounds examined with the exception of GS-9137 where a 48-fold reduction in potency against the HIV-1_{BH-10} harbouring the E92Q mutation was observed (Table 1, Figure 2c).

A 0.5-1.6-fold change in activity (mutants vs wild-type) was noticed with the negative control, 3TC.

HXB-2D		L-731,988	Pyridone A	GS-9137	MK-0518	L-870,810
T66I		13	3.2	3	1.4	3
S153Y		1.1	0.8	3	1.6	1.7
T66I/S153Y		7.7	3.8	28.1	2.2	2.2
T66I/M154I		2.4	3.8	12.6	2.6	2.6
N155S		3.2	0.8	3.7	2.9	2.3
HXB-2D		L-731,988	Pyridone A	GS-9137	MK-0518	L-870,810
T125K		1.2	0.7	0.2	0.6	1.6
F121Y		27.5	8.6	14	15	10
F121Y/T125K		36.4	14.6	12.3	6.3	12
V72I/F121Y/T125K		36.9	30	18	13	31
V72I/F121Y/T125K/V151I		135	70	45	46	105
BH-10		L-731,988	Pyridone A	GS-9137	MK-0518	L-870,810
H51Y		0.56	0.86	2	3	1.3
E92Q		1.4	4.4	48	9	3.7
S147G		8.2	8.8	5.5	4.5	1.3
E157Q		4.9	3.9	2	6	0.8

Table 1: Susceptibilities of recombinant integrase mutant HIV-1 to various integrase inhibitors (IC₅₀-fold change)

Conclusion

In this study, phenotypic analysis of recombinant viruses harbouring selected mutations within the integrase gene has demonstrated significant cross-resistance with the compounds investigated despite distinct pathways of emerging resistance associated with these compounds.

Mutation studies as described can provide new insights in the binding mode of these inhibitors and in the design of novel anti-integrase compounds with a favourable cross-resistance profile.

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