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UIC-02031: A Novel Nonpeptidic Protease Inhibitor Containing a Stereochemically Defined Fused Cyclopentanyltetrahydrofuran Potent Against Multi-PI-Resistant HIV-1 *In Vitro*

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Introduction

The rapid rise of protease inhibitor (PI)-resistant HIV-1 variants urges the development of new classes of PIs which are potent against existing resistant HIV-1 variants and do not allow or delay the emergence of resistance. We generated a novel non-peptidic PI, UIC-02031(Figure 1), by incorporating a stereochemically defined fused cyclopentanyltetrahydrofuran (Cp-THF), which exerted potent activity against a wide spectrum of HIV-1 including multi-drug-resistant HIV-1 variants (HIV_{MDR}).

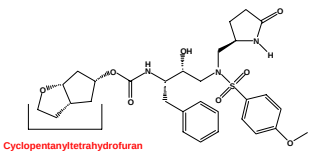
Methods

Each PI (saquinavir, amprenavir, indinavir, nelfinavir, or ritonavir)-resistant HIV-1 variants was selected *in vitro* by propagating HIV-1 in the presence of increasing concentrations of PIs using MT-4 cells. Anti-HIV-1 activity of the compound was determined using MTT assay employing MT-2 cells and p24 assay using PHA-stimulated PBM or MT-4 cells.

Results

UIC-02031 was highly potent against laboratory HIV-1 strains and primary clinical isolates including subtypes A, B, C, and E (IC_{50}: 0.015-0.038 μM) with minimal cytotoxicity (CC_{50}: >100 μM in $CD4^+$ MT-2 cells)(Tables 1 & 2), although it was less active to two HIV-2 strains ($HIV\text{-}2_{EHO}$ and $HIV\text{-}2_{ROD}$) (IC_{50}: ~0.60 μM)(Table 2). UIC-02031 at relatively low concentrations (IC_{50}: 0.036-0.14 μM) blocked the infection and replication of each of $HIV\text{-}1_{NL4\text{-}3}$ variants exposed to and selected by up to 5 μM of saquinavir, amprenavir, indinavir, nelfinavir, or ritonavir (Table 3). UIC-02031 was also potent against multi-PI-resistant clinical HIV-1 variants isolated from patients who had no response to existing antiviral regimens after having received a variety of antiviral agents with IC_{50} values ranging from 0.014-0.042 μM (<2-fold changes in IC_{50}s compared with that of wild-type HIV-1) (Table 4). Upon selection of $HIV\text{-}1_{NL4\text{-}3}$ in the presence of UIC-02031 (Figure 2), mutants carrying L10F, L33F, M46I, I47V, Q58E, V82I, I84V, and I85V in the protease-encoding region (Figure 3) and G62R (p17), L363M (p24/p2 cleavage site), R409K (p7), and I437T (p7/p1 cleavage site) in the *Gag*-encoding region emerged (Figure 4). UIC-02031 was also potent against various infectious PR clones and it was slightly less active against PR mutants containing M46I and I47V (Table 5).

Figure 1 **Structure of UIC-02031**



Cyclopentanyltetrahydrofuran (Cp-THF)

M.W.: 601.7

Table 1

Antiviral Activity against $HIV\text{-}1_{LAI}$ and Cytotoxicity of UIC-02031

Compound	IC_{50} (μM)	CC_{50} (μM)	SI
UIC-02031	**0.026**	**>100**	**>3800**
SQV	0.017	11.3	660
APV	0.033	>100	>2800
RTV	0.045	21.3	470

MT-2 cells (2x10⁴/ml) were exposed to 100 $TCID_{50}$ of $HIV\text{-}1_{LAI}$ and cultured in the presence of various concentrations of PIs, and the IC_{50}s were determined by using the MTT assay on day 7 of culture. All assays were conducted in duplicate. SI; selectivity index, CC_{50}/IC_{50}.

Table 2

Antiviral Activity of UIC-02031 against various HIV (IC_{50}: μM)

Virus	subtype	Cells	AZT	APV	IDV	NFV	RTV	UIC-02031
$HIV\text{-}1_{Ba\text{-}L}$	B	PBMC	0.012	0.023	0.031	0.018	0.044	**0.025**
$HIV\text{-}1_{JRFL}$	B	PBMC	0.009	0.035	0.019	0.014	0.034	**0.038**
$HIV\text{-}1_{92UG037}$	A	PBMC	0.012	0.015	0.016	0.020	0.048	**0.015**
$HIV\text{-}1_{97ZA003}$	C	PBMC	0.028	0.030	0.029	0.025	0.15	**0.028**
$HIV\text{-}1_{92TH019}$	E	PBMC	0.008	0.006	0.010	0.006	0.016	**0.016**
$HIV\text{-}2_{ROD}$	/	MT-2	0.017	**0.24**	0.012	0.021	**0.18**	**0.56**
$HIV\text{-}2_{EHO}$	/	MT-2	0.010	**0.15**	0.015	0.035	**0.27**	**0.60**

For $HIV\text{-}1_{Ba\text{-}L}$, $HIV\text{-}1_{JRFL}$, $HIV\text{-}1_{92UG037}$, $HIV\text{-}1_{97ZA003}$, and $HIV\text{-}1_{92TH019}$, the IC_{50} values were determined by employing PHA-PBM and using the inhibition of p24 *Gag* protein production by the drug as an endpoint. For $HIV\text{-}2_{ROD}$ and $HIV\text{-}2_{EHO}$, MT-2 cells were exposed to the virus and cultured, and the IC_{50} values were determined using the MTT assay. All assays were conducted in triplicate.

Table 3

Antiviral Activity of UIC-02031 against Laboratory PI-resistant HIV-1 (IC_{50}: μM)

	Amino acid substitutions in the protease	SQV	IDV	NFV	APV	RTV	LPV	UIC-02031
$HIV\text{-}1_{NL4\text{-}3}$	Wild-type	**0.008**	**0.014**	**0.018**	**0.028**	**0.021**	**0.018**	**0.020**
$HIV\text{-}1_{SQV5\mu M}$	L10I/G48V/I54V/L90M	>1 (>125)	>1 (>71)	0.32 (18)	0.21 (8)	>1 (>48)	0.70 (39)	**0.14 (7)**
$HIV\text{-}1_{IDV5\mu M}$	L10F/L24I/M46I/L63P/A71V/G73S/V82T	0.022 (3)	>1 (>71)	0.65 (36)	0.26 (9)	>1 (>48)	>1 (>56)	**0.047 (2)**
$HIV\text{-}1_{NFV5\mu M}$	L10F/D30N/K45I/A71V/T74S	0.028 (4)	0.27 (19)	>1 (>56)	0.078 (3)	0.080 (4)	0.29 (16)	**0.036 (1)**
$HIV\text{-}1_{APV5\mu M}$	L10F/V32I/M46I/I54M/A71V/I84V	0.016 (2)	0.22 (16)	0.17 (10)	>1 (>37)	>1 (>48)	0.14 (8)	**0.037 (2)**
$HIV\text{-}1_{RTV5\mu M}$	M46I/V82F/I84V	0.010 (1)	0.30 (21)	0.24 (13)	0.43 (15)	>1 (>48)	0.11(6)	**0.077 (3)**
$HIV\text{-}1_{UIC02031\text{-}5\mu M}$	L10F/L33F/M46I/I47V/Q58E/V82I/I84V/I85V	0.008 (1)	0.044 (3)	0.011 (1)	0.21 (8)	0.26 (12)	0.14 (8)	>1 (>50)

MT-4 cells (1x10⁶) were exposed to each HIV-1 (100 $TCID_{50}$s) and the inhibition of p24 *Gag* protein production by the drug was used as an endpoint. The numbers in parentheses represent fold changes of IC_{50} values against each isolate compared to IC_{50} values against $HIV\text{-}1_{NL4\text{-}3}$ (wild-type). All assays were conducted in triplicate.

Table 4

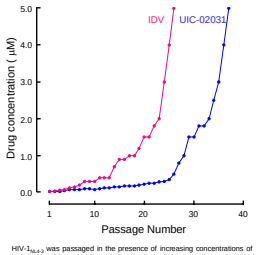
Antiviral Activity of UIC-02031 against Primary HIV-1 Isolates in PBM (IC_{50}: μM)

Virus	AZT	SQV	APV	IDV	NFV	RTV	LPV	UIC-02031
$HIV\text{-}1_{ERS104pre}$ (X4)	0.005	0.009	0.025	0.021	0.016	0.036	0.032	**0.027**
$HIV\text{-}1_{TM}$ (X4)	0.89 (178)	0.27 (30)	0.35 (14)	>1 (>48)	>1 (>63)	>1 (>30)	0.23 (7)	**0.026 (1)**
$HIV\text{-}1_{MM}$ (R5)	0.45 (90)	0.27 (30)	0.40 (16)	>1 (>48)	>1 (>63)	>1 (>30)	0.62 (19)	**0.041 (2)**
$HIV\text{-}1_{JSL}$ (R5)	0.18 (36)	0.32 (36)	0.66 (26)	>1 (>48)	>1 (>63)	>1 (>30)	0.74 (23)	**0.043 (2)**
$HIV\text{-}1_{A}$ (X4)	0.12 (24)	0.10 (11)	0.16 (6)	>1 (>48)	0.33 (21)	>1 (>30)	0.32 (10)	**0.014 (1)**
$HIV\text{-}1_{B}$ (X4)	>1 (200)	0.30 (33)	0.31 (12)	>1 (>48)	>1 (>63)	>1 (>30)	0.25 (8)	**0.029 (1)**
$HIV\text{-}1_{C}$ (X4)	0.28 (56)	0.033 (4)	0.22 (9)	>1 (>48)	0.36 (23)	>1 (>30)	0.46 (14)	**0.027 (1)**
$HIV\text{-}1_{G}$ (X4)	0.60 (120)	0.027 (3)	0.23 (9)	0.34 (16)	0.23 (14)	>1 (>30)	0.13 (4)	**0.028 (1)**

Amino acid substitutions identified in the protease-encoding region of $HIV\text{-}1_{ERS104pre}$, $HIV\text{-}1_{TM}$, $HIV\text{-}1_{MM}$, $HIV\text{-}1_{JSL}$, $HIV\text{-}1_{A}$, $HIV\text{-}1_{B}$, $HIV\text{-}1_{C}$, $HIV\text{-}1_{G}$ as compared to the consensus B sequence cited from the Los Alamos data base include L63P, L10I/K14R/R41K/M46L/I54V/L63P/A71V/V82A/L90M/I93L, L10I/K43T/M46L/I54V/L63P/A71V/V82A/L90M/Q92K, L10I/L24I/L33F/E35D/M36I/N37S/M46L/I54V/R57K/I62V/L63P/A71V/G73S/V82A, L10I/I15V/E35D/N37E/K45R/I54V/L63P/A71V/V82T/L90M/I93L/C95F, L10I/K14R/L33I/M36I/M46I/F53L/K55R/I62V/L63P/A71V/G73S/V82A/L90M/I93L, L10I/I15V/K20R/L24I/M36I/M46L/I54V/I62V/L63P/K70Q/V82A/L89M and L10I/V11I/T12E/I15V/L19I/R41K/M46L/L63P/A71T/V82A/L90M, respectively. The IC_{50} values were determined by employing PHA-PBM as target cells and the inhibition of p24 Gag protein production as an endpoint. Numbers in parentheses represent fold changes of the IC_{50} values against each isolate compared to the IC_{50} values against $HIV\text{-}1_{ERS104pre}$. All assays were conducted in triplicate.

Figure 2

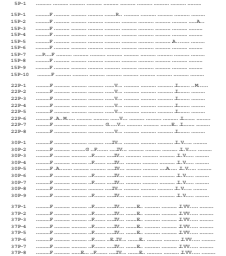
***In vitro* selection of HIV-1 variants resistant to UIC-02031**



$HIV\text{-}1_{NL4\text{-}3}$ was passaged in the presence of increasing concentrations of UIC-02031 (blue circle) or indinavir (red circle) in MT-4 cells. The selection was carried out in a cell-free manner for a total of 27 to 37 passages with drug concentrations escalating from 0.01 to 5 μM. Nucleotide sequences of proviral DNA were determined using cell lysates of HIV-1-infected MT-4 cells at the termination of each indicated passage.

Figure 3

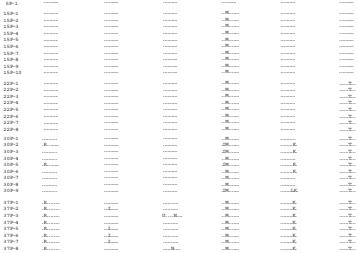
Sequence analysis of the protease-encoding region of HIV passaged in the presence of UIC-02031



The amino acid sequences of protease deduced from nucleotide sequences of the protease-encoding region of HIV clones determined at four different passages are illustrated. Identity of each amino acid with that from pNL4-3 (top) at individual amino acid position is indicated by dot.

Figure 4

Sequence analysis of the *Gag*-encoding region of HIV passaged in the presence of UIC-02031



The amino acid sequences of Gag deduced from nucleotide sequences of the Gag-encoding region of HIV clones determined at four different passages are illustrated. Identity of each amino acid with that from pNL4-3 (top) at individual amino acid position is indicated by dot.

Table 5

Sensitivity of infectious HIV PR mutants to UIC-02031

Infectious clone	IC_{50} (μM)	Fold change
pNL4-3 (wild-type)	0.026	/
L10F	0.038	1.5
L33F	0.025	1.0
M46I	0.034	1.3
I47V	0.042	1.6
Q58E	0.029	1.1
V82I	0.034	1.3
I84V	0.030	1.2
I85V	0.020	0.8
M46I/I47V	0.090	3.5
V82I/I85V	0.034	1.3

MT-4 cells (1x10⁵/ml) were exposed to 100 $TCID_{50}$ of each infectious molecular HIV clone and the inhibition of p24 *Gag* protein production by drug was used as an endpoint on day 7 in culture. All assays were performed in triplicate and the values shown are representative of three independent experiments.

Conclusions

The data warrant that UIC-02031 is further developed as a potential therapeutic agent for treatment of infection with primary and multi-drug-resistant HIV.