



京都大学 ウイルス研究所

Institute for Virus Research
Kyoto University

HIV-1 acquires resistance to new NNRTIs, thiazole derivatives, through steric hindrance with multiple mutations.

Eiichi Kodama¹, Naoyuki Masuda², Masaya Orita², Osamu Yamamoto², Masahiro Fujii²,
Shunji Kageyama², Mitsuaki Ohta², Toshifumi Hatta², Hiroshi Inoue², Hiroshi Suzuki²,
Kenji Sudo², Yasuaki Shimizu², and Masao Matsuoka¹.

¹*Institute for Virus Research, Kyoto University, Kyoto,*

²*Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., Tsukuba, Japan.*

Correspondence: E. Kodama, e-mail; ekodama@virus.kyoto-u.ac.jp, TEL&FAX; +81-75-751-3986

Abstract

Background: A thiazole derivative, RD4-2217, has anti-HIV activity to various NNRTI-resistant variants except for a Y181C variant. Here we identify 2 thiazole derivatives, YM-215389 and -228855, that show different resistance profiles.

Methods: Resistant variants were selected *in vitro* with the dose escalating. Antiviral activity was determined using HeLa CD4/LTR- β -Gal cells. Docking and molecular modeling studies were performed with the program GOLD and Sybyl.

Results: The variants resistant to YM-215389 showed modest cross resistance to nevirapine (NVP) and efavirenz (EFV) whereas that to YM-228855 showed strong cross resistance to NVP. Primary mutations were V106L (YM-215389) and Y181I (YM-228855), respectively. However, 4 amino acids substitutions (V106L/V108I/E138K/L214F) were needed for high resistance to YM-215389. Docking studies revealed that hydrophobic benzene and thiazole rings of YM-215389 and -228855 bind to at least 13 amino acids located in the NNRTI binding pocket. Steric hindrance by V106L for benzene and E138K/Y181I for thiazole ring is considered to be mechanism of the resistance.

Conclusions: Multiple interactions with the pocket and flexibility of YM-215389 require 4 mutations for the resistance. Our data may help the development of compounds to suppress drug resistant HIV replication without the cross resistance.

Introduction

- Nevirapine (NVP) containing regimen is widely used for prevention of the mother to child infection.
- Viral suppression by 2 NRTIs with efavirenz (EFV) is greater than that by 2 NRTIs with Indinavir.



NNRTIs are safe and effective agents for HIV chemotherapy. However, emergence of the resistant variants remains unsolved.

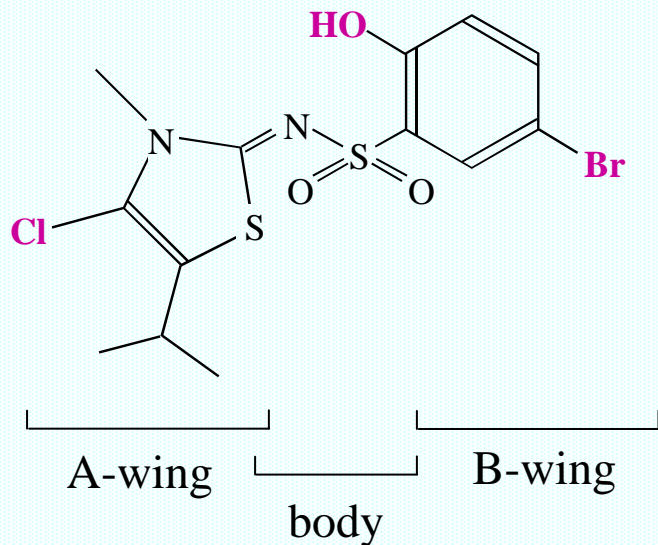


In the present study

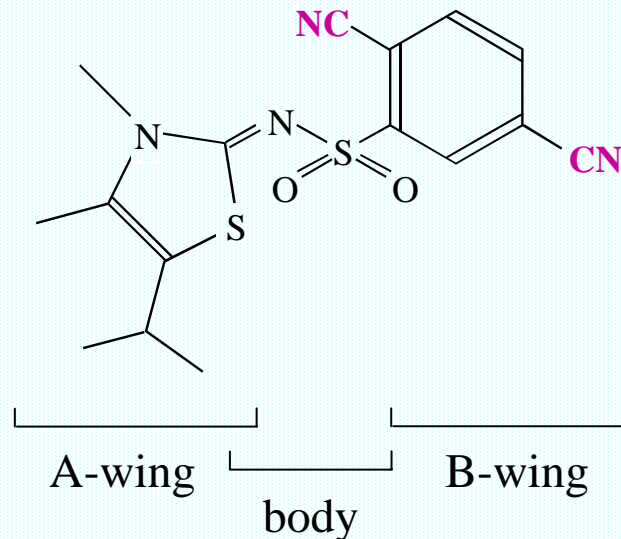
We identified novel NNRTIs that effectively inhibit NNRTI-resistant variants.

Chemical structure of thiazole derivatives

YM-215389



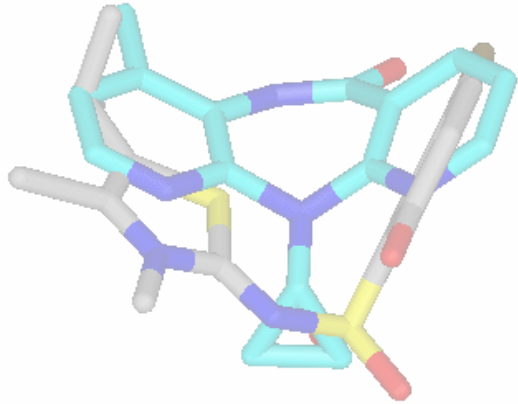
YM-228855



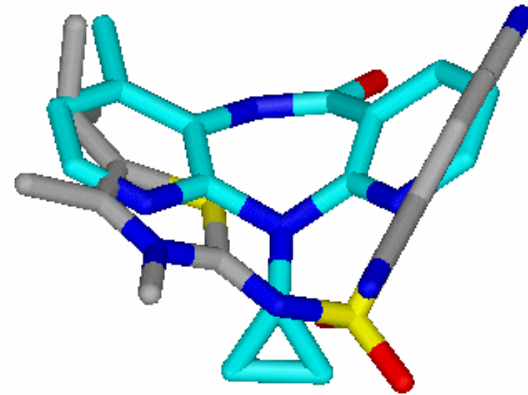
- Thiazole derivatives possess “Butterfly structure” like other NNRTIs, *e.g.*, nevirapine: thiazole ring (A-wing), sulfon-amide (body), and benzene ring (B-wing).
- Differences between YM-215389 and -228855 are chloro- and methyl- at thiazole ring, and hydroxy-/bromo- and cyano-/cyano- at benzene ring, respectively.

Superimposed with NVP (blue)

YM-215389

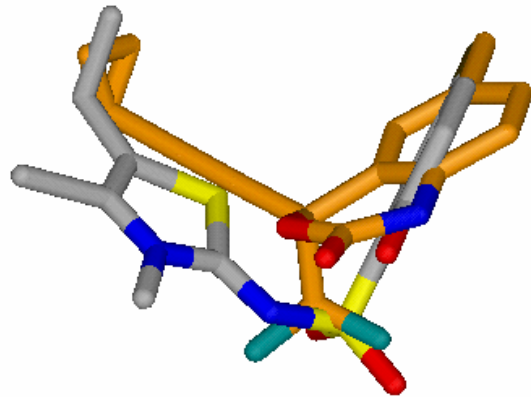


YM-228855

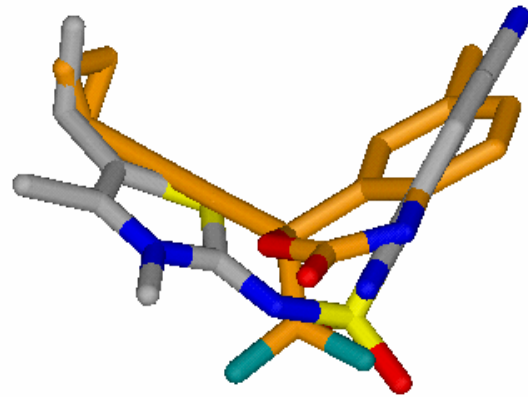


Superimposed with EFV (brown)

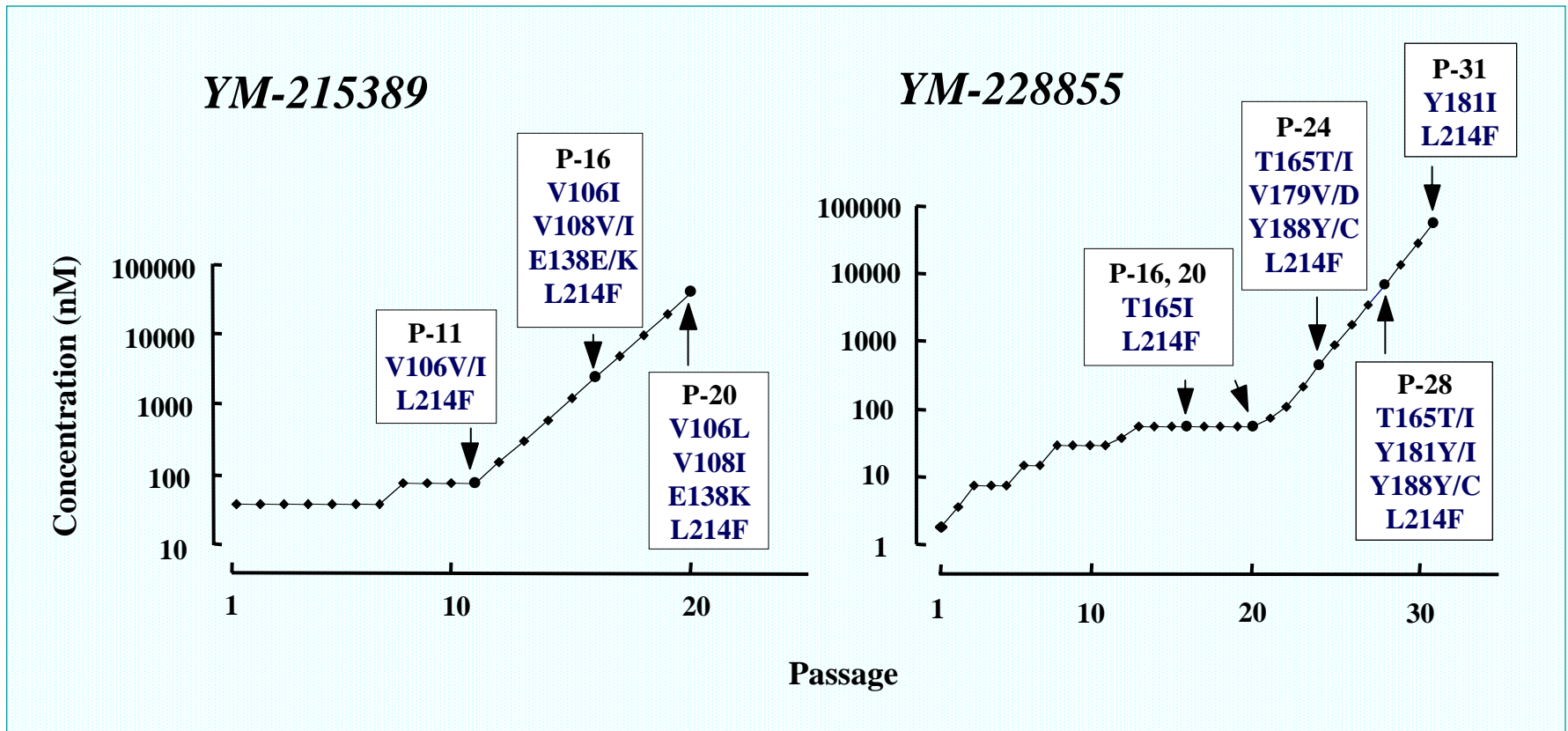
YM-215389



YM-228855



Induction of resistant variants



MT-2 cells were exposed to HIV-1_{IIIB} and cultured in the presence of YM-215389 and -228855 at an initial concentration of their EC₅₀ values. The culture supernatants were used for further passages in the presence of 2-fold increasing concentrations of compounds when massive cytopathic effect were seen in the earlier periods. Such dose-escalating culture was performed until resistant variants were obtained. At the indicated passages, the sequence of the RT region was determined by direct sequencing of the proviral DNA extracted from the infected MT-2 cells.

Profiles of the induced HIV variants

Virus	EC ₅₀ ^a (nM)				
	AZT	NVP	EFV	YM-215389	YM-228855
HIV-1 _{IIIB} ^b	21	40	0.7	13	2.3
HIV-1 _{YM-215389} ^r	6 (x 0.3) ^c	680 (x 17)	8 (x 10)	>10,000 (>x 770)	325 (x 141)
HIV-1 _{YM-228855} ^r	53 (x 2)	>10,000 (>x 250)	4.9 (x 7)	902 (x 70)	6,000 (x 2,600)

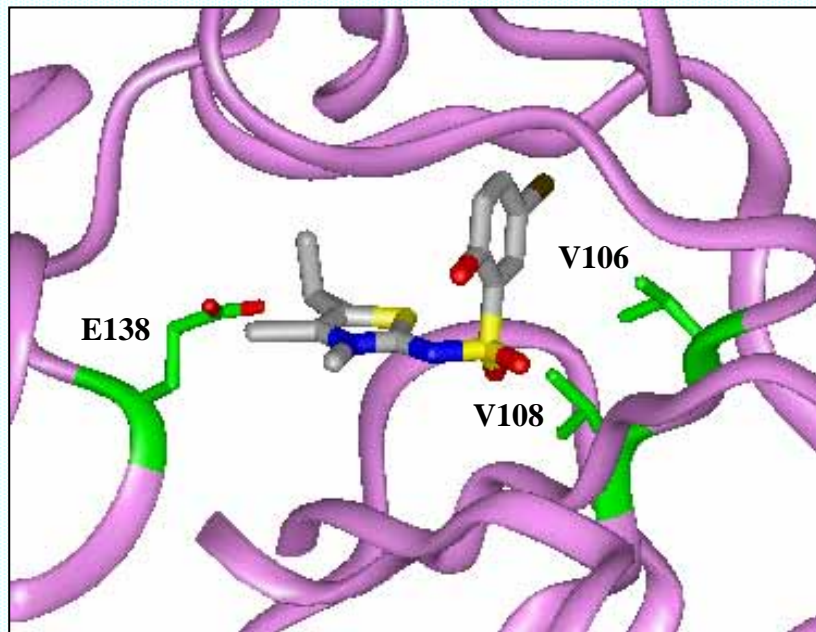
Anti-HIV activity was determined using the MAGI assay. ^aAnti-viral effective concentration that reduced viral replication by 50%. The data shown are mean values obtained from at least three independent experiments. ^bHIV-1_{LAI} was used as the wild type. ^cThe fold resistances in the EC₅₀ values for the induced viruses compared to that for HIV-1_{LAI} are shown in the parentheses.



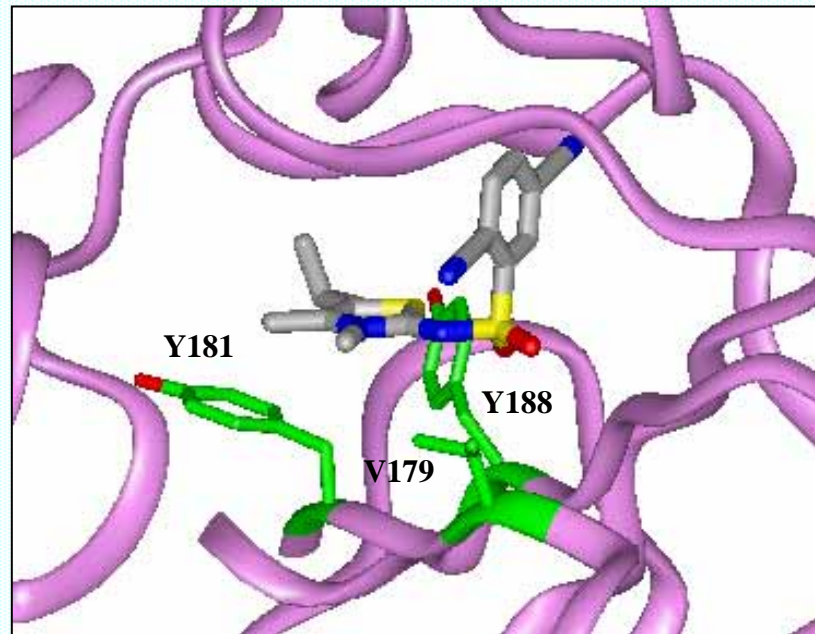
HIV-1_{YM-215389}^r and HIV-1_{YM-228855}^r shows distinct resistant profiles with marginal cross resistance.

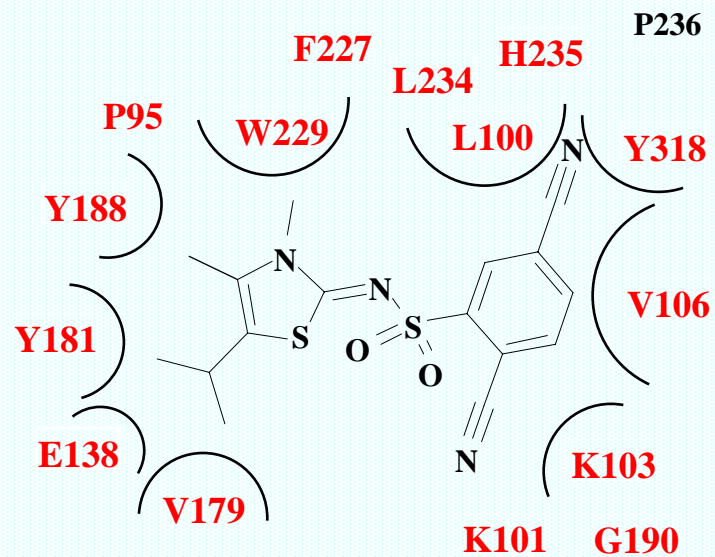
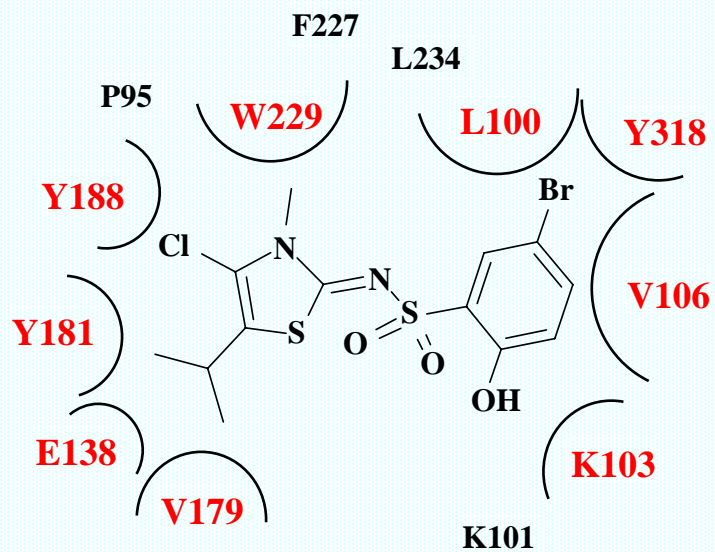
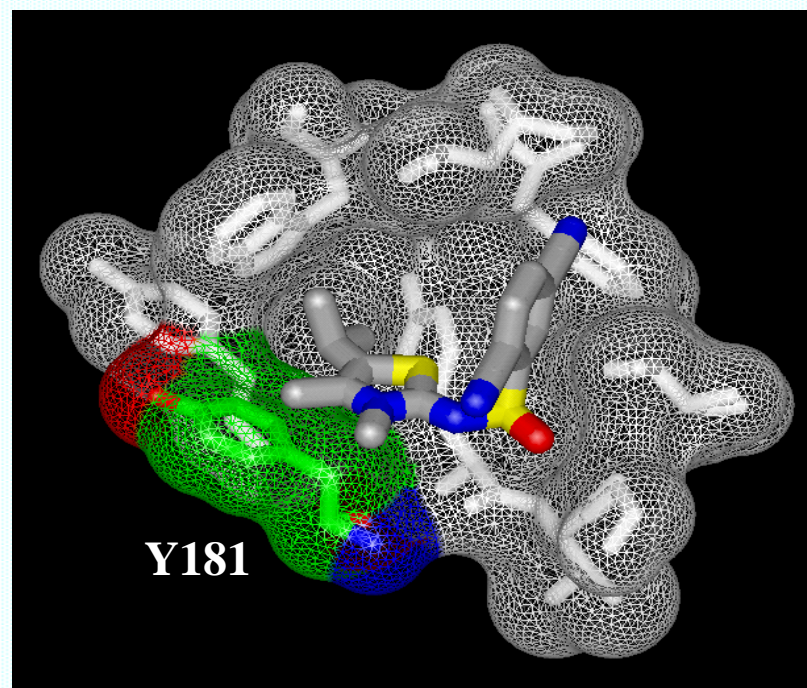
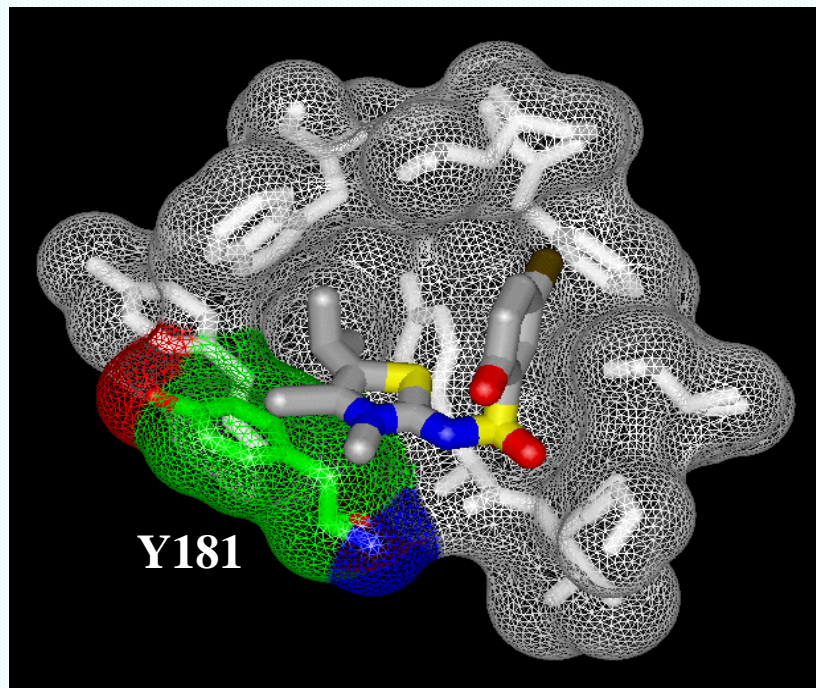
Thiazole derivatives in the NNRTI binding pocket

YM-215389



YM-228855





Amino acids shown in red and black locate within 3.5 and 4 Å to the compounds, respectively.

Efficacy to the NNRTI resistant variants

Virus	EC ₅₀ ^a (nM)									
	AZT		NVP		EFV		YM-215389		YM-228855	
HIV-1 _{NL-WT}	57		40		0.5		20		2	
HIV-1 _{A98G}	43	(x 0.7) ^b	192	(x 5)	2.7	(x 5)	184	(x 9)	34	(x 17)
HIV-1 _{K103N}	35	(x 0.6)	2,212	(x 55)	47	(x 94)	665	(x 33)	199	(x 87)
HIV-1 _{V106A}	36	(x 0.6)	2,723	(x 68)	2	(x 4)	22	(x 1)	0.4	(x 0.2)
HIV-1 _{Y181C}	35	(x 0.6)	6,470	(x 160)	0.7	(x 1)	46	(x 2)	107	(x 54)
HIV-1 _{V189I}	19	(x 0.3)	30	(x 1)	0.4	(x 1)	46	(x 2)	0.8	(x 0.4)
HIV-1 _{G190A}	30	(x 0.5)	2,600	(x 65)	4.5	(x 9)	145	(x 7)	12	(x 6)

Anti-HIV activity was determined using the MAGI assay. ^aAnti-viral effective concentration that reduced viral replication by 50%. The data shown are mean values obtained from at least three independent experiments. ^bHIV-1_{NL43} was used as the wild type. ^cThe fold resistances in the EC₅₀ values for the induced viruses compared to that for HIV-1_{WT} are shown in the parentheses.



YM-215389 remains its activity to the major NNRTI resistant variants.

Anti-HIV activity to the induced variants

Virus	Fold of Resistance ^a				
	AZT	NVP	EFV	YM-215389	YM-228855
HIV-1 _{NL-WT}	(57) ^b	(40)	(0.5)	(20)	(2)
	<i>Selection with YM-215389</i>				
HIV-1 _{V106I}	0.3 ^c	2	1	3	1
HIV-1 _{V106L}	0.4	1	1	16	15
HIV-1 _{V108I}	0.03	15	9	3	6
HIV-1 _{L214F}	0.1	2	2	2	2
HIV-1 _{V108I/E138K}	0.2	11	8	57	22
HIV-1 _{V106L/V108I/E138K}	0.1	2	8	330	53
HIV-1 _{V106L/V108I/E138K/L214F}	0.2	4	2	> 500	400
	<i>Selection with YM-228855</i>				
HIV-1 _{Y188C}	2	14	1	8	24
HIV-1 _{Y181I}	0.5	180	8	16	540
HIV-1 _{Y181I/L214F}	0.4	> 250	8	37	1,440

^aAnti-HIV activity was shown as fold of resistance compared to ^bEC₅₀ values for HIV-1WT were shown in the parentheses.

Docking scores for the resistant RT

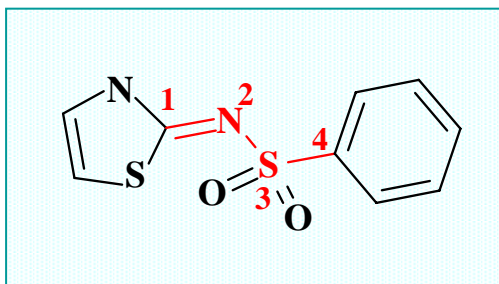
	NVP	EFV	YM-215389	YM-228855
WT	-22.2	-28.2	-8.2	-8.0
K103N	-22.2	-20.2	-2.2	0.34
V106I	> 0	> 0	> 0	> 0
V106L	> 0	> 0	> 0	> 0
V108I	-22.4	-20.3	-8.2	-8.0
E138K	-22.9	-23.1	> 0	> 0
Y181I	> 0	> 0	> 0	> 0
Y181C	-21	-21	-7.9	-7.8
V106L/V108I/E138K	> 0	> 0	> 0	> 0

The docking scores were determined with a program, Sybyl. Minus Score indicates good interaction or binding, whereas plus indicates steric hindrance or no interaction. The score indicated in Red does not co-relate with the susceptibility determined by the MAGI assay.

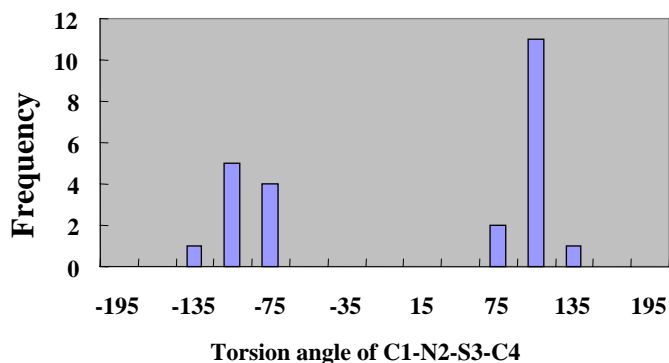


Docking score itself does not always co-relate with the resistance.

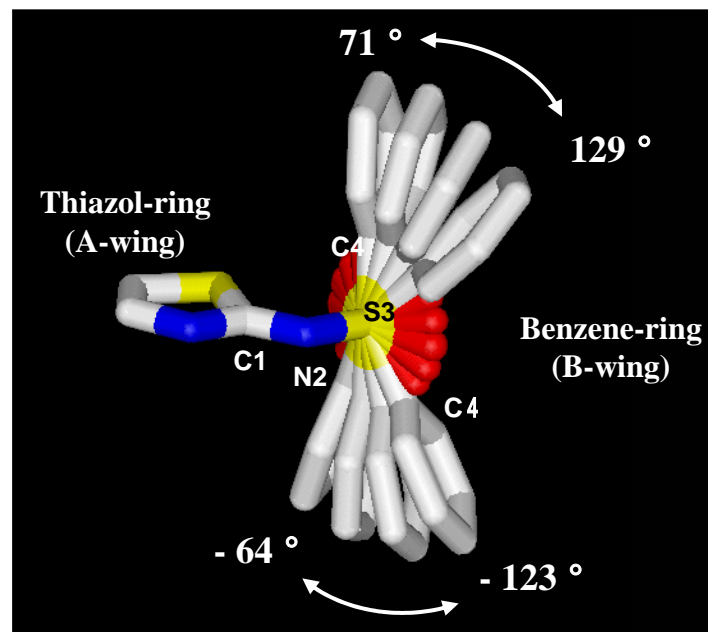
Flexibility of YM-215389



To estimate flexibility of YM-215389, we analyzed and compared the torsion angle of the body (C1-N2-S3-C4, indicated in red) using the Cambridge Structural Database (CSD). Twenty-four compounds that contain thiazol-sulfon-amide-benzene structure (left) are listed in the CSD.



Torsion angle of the 24 compounds are distributed within -123 to -64° and 71 to 129° .



YM-215389 seems to be highly flexible.

Conclusion

- **YM-215389 and -228855 interact with at least 9 (13 for 4.0 Å) and 15 amino acids in the binding pocket within 3.5 Å, respectively.**
- **Tight binding of YM-228855 contributes strong anti-HIV-1 activity, however, it is influenced by a single hindrance.**
- **Flexibility of NNRTI supports the interaction to the amino acid substituted binding pocket.**
- **Multiple steric hindrances are required for the resistance.**



These results suggest that multiple interactions and flexibility in the NNRTI play a key role to suppress the resistance.