

Kinetic and Thermodynamic Parameters for Binding of the Non-nucleoside Inhibitors 678248 and 695634 to Wild Type and 12 Mutants of HIV-1 Reverse Transcriptase

Introduction

Reverse transcriptase (RT) is required for HIV-1 replication and is a clinically validated target for Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). NNRTIs are not substrates for RT and, although chemically diverse, all bind in the same well-defined site distinct from the catalytic region of the enzyme. NNRTIs non-competitively inhibit enzymatic activity by inducing a conformational change in the enzyme.

Analogues in a benzophenone compound series were synthesized and assayed for anti-HIV-1 activity with particular emphasis on the potency against NNRTI-resistant HIV-1 strains. 678248 was modified into a prodrug form to improve solubility and bioavailability.

The NNRTI candidate 695634, in phase I clinical development, is the prodrug of the active compound, 678248. Antiviral activity of this prodrug was examined. The interactions of 695634 and 678248 with HIV-1 RT were investigated by spectroscopic techniques. Inhibition of primer unblocking was also investigated.

Methods

Virus assays were performed in HeLa CD4, MT4, and PBMCs. The kinetics of inhibition of RT activity were determined with a continuous time-resolved fluorescence energy transfer assay in which Cy5-dUMP was incorporated into 5' europium-labelled primer template.¹ The values of the dissociation constants in the absence of catalysis were determined by monitoring fluorescence of the hexachlorofluorescence moiety of the primer:template, HF15X:20 (PrTp). Wild-type and mutant enzymes examined were RT(wt), RT(L100I), RT(K103N), RT(V106A), RT(V106I), RT(V106A/Y181C), RT(V108I/Y181C), RT(V108I), RT(E138K), RT(Y181C), RT(Y188C), RT(P236L), and RT(D67N/K70R/T215Y/K219Q). Primer unblocking activity was measured by following loss of ³H-AZT-MP from chain-terminated primer template in a filter paper binding assay.

Results

Table 1 • Anti-HIV-1 Activity of 678248 and 695634 Against Wild Type HIV-1 Laboratory Strains in Designated Cell Types and Purified Recombinant HIV-1 RT

Cell/Virus	Active Parent 678248 IC ₅₀ [nM]	Prodrug 695634 IC ₅₀ [nM]
	IC ₅₀ Avg (SE)	IC ₅₀ Avg (SE)
HeLa-CD4 / HXB2	0.6 ± 0.1	240 ± 30
MT4 / IIB	1.0 ± 0.3	49 ± 2
PBL / IIB	0.4 ± 0.1	18 ± 3
PBL / Ba-L	0.7 ± 0.1	18 ± 2
Enz Inhib (WT)	1.8 ± 0.6*	5.7 ± 2*
Enz Binding (WT), K ₄ nM	4.7	21

* Std Dev

Figure 1 • Concentration Dependencies for the Binding of 695634 and 678248

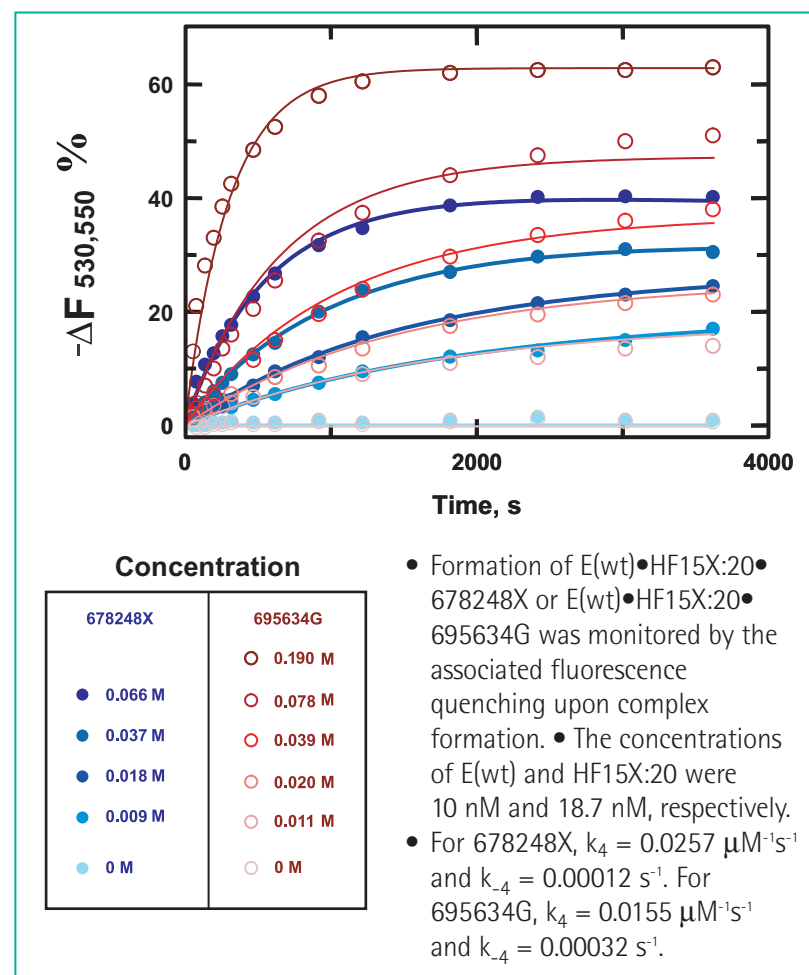
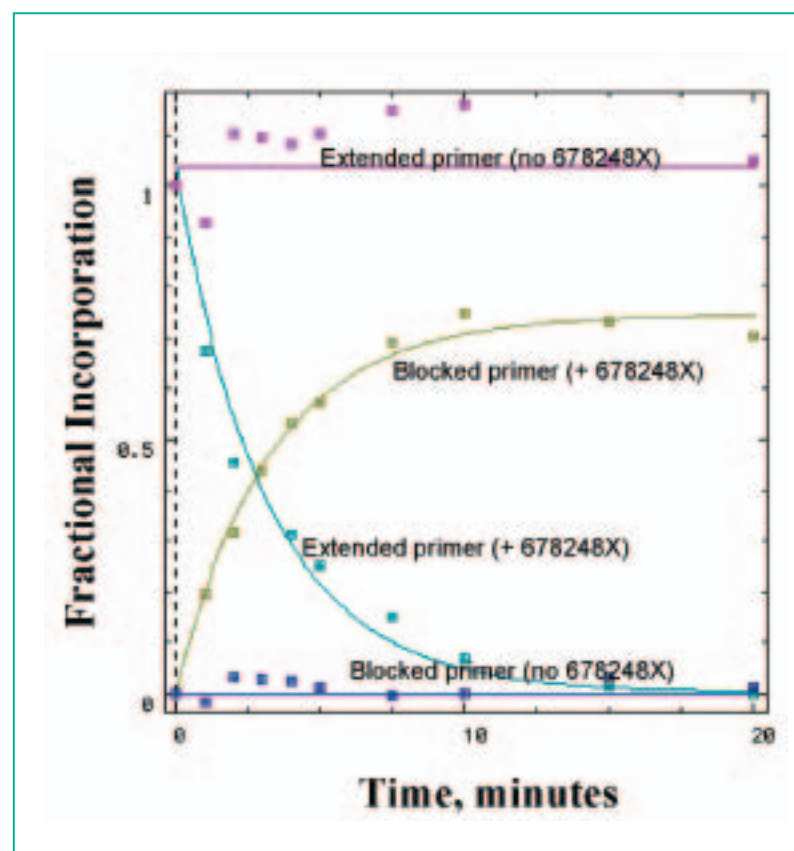


Figure 2 • Comparison of the Time-courses for Inhibition of Synthesis and of Primer Unblocking by 695634



- Residual primer extension and unblocking activity were determined after incubating 20 nM RT(D67N/K70R/T215Y/K219Q)•PrTp with 185 nM 678248X for the indicated times
- These data were analysed to yield values for the second order rate constant for binding of 678248X to RT(D67N/K70R/T215Y/K219Q)•PrTp of $0.030 \mu\text{M}^{-1}\text{s}^{-1}$, for binding of 678248X to RT(D67N/K70R/T215Y/K219Q)•PrTp terminated with AZTMP of $0.028 \mu\text{M}^{-1}\text{s}^{-1}$
- 72% of the unblocking activity was inhibited by 678248

Figure 3 • Binding of 678248 or 695634 (I) to E in the Presence or Absence of Nucleic Acid (HF15X:20)

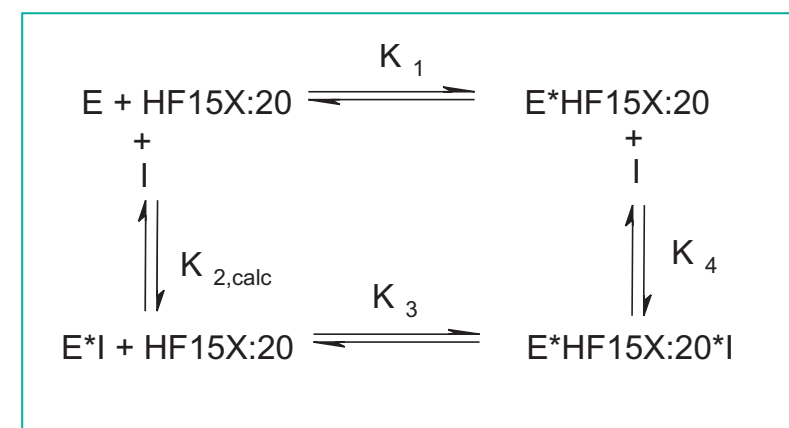


Table 2 • Antiviral Activity in HeLa Magi Cells, Activity in Fluorescence Enzyme Assay and Binding Constants of 678248

HIV-RT Genotype	Antiviral Activity IC ₅₀ , nM	Enzyme Inhibition IC ₅₀ , nM	Binding	
			K ₂ , nM	K ₄ , nM
Wild Type	0.5	1.8	4.7	4.7
Y188C	0.1	3.1	30	17
L100I	0.5	0.8	46	13
Y181C	0.7	2.3	<0.4	<0.8
V108I	0.9	2.0	33	17
V106A/Y181C	0.9	3.5	52	29
K103N	1.0	1.9	28	8.5
V106I	1.1	2.7	8.3	12
P236L	1.1	6.8	100	24
E138K	1.3	4.2	66	33
V106A	3.4	4.0	120	35
V108I/Y181C	4.9	4.8	23	7.8

- Data demonstrated that 678248 binds tightly to free RT and RT bound to nucleic acid
- The rate of dissociation of 678248 from RT•PrTp•678248 was very slow with values ranging between $<0.00002 \text{ s}^{-1}$ and 0.005 s^{-1}

Discussion

- 678248 inhibits NNRTI-resistant mutant RT enzyme in biochemical assays
- The range of activities against mutants in the enzyme assay was 0.8 nM to 6.8 nM
- IC₅₀ values of 11 mutants tested did not exceed 5-fold higher than WT (1.8 nM) (Table 2), in general agreement with the cell-based antiviral data

Conclusions

- Comparison of antiviral activity indicates that the active parent 678248 is 25- to 400-fold more potent than the prodrug 695634
- 678248 bound tightly to mutant RTs that are resistant to other NNRTIs
- 678248 inhibited the primer unblocking reaction that has been proposed to contribute to ZDV resistance associated with the D67N/K70R/T215Y/K219Q quad mutant

Reference

- Chan, et al. *J Med Chem.* 2001;44:1866-1882.

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