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Inhibition of human immunodeficiency virus type 1 wild-type and mutant reverse transcriptases by the phenyl ethyl thiazolyl thiourea derivatives trovirdine and MSC-127

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Abstract

A new class of very potent and selective non-nucleoside inhibitors of HIV reverse transcriptase (RT) has recently been identified. The prototype compound trovirdine (LY 300046 HCl) and one analogue, MSC-127, have been studied with respect to inhibition of wild-type HIV-1 RT and RT with various mutations known to give rise to resistance to other non-nucleoside RT inhibitors, namely $Leu^{100} \rightarrow Ile$ (Ile^{100}), $Glu^{138} \rightarrow Arg$ (Arg^{138}), $Tyr^{181} \rightarrow Cys$ (Cys^{181}) and $Tyr^{188} \rightarrow His$ (His^{188}). The inhibition of HIV-1 RT by trovirdine and MSC-127 was reversible and template dependent. Trovirdine inhibited HIV-1 RT with an IC_{50} of 0.007 μM when employing heteropolymeric primer/template (oligo-DNA/ribosomal RNA) and dGTP as substrate. Enzyme kinetic studies showed that inhibition of RT by trovirdine was non-competitive with regard to deoxynucleoside triphosphates and uncompetitive with respect to varied primer/template under steady-state conditions. The amino acid changes Leu^{100}, Tyr^{181} and Tyr^{188} gave rise to 25-, 147- and 12-fold decrease in inhibition by trovirdine. Enzyme-kinetic studies on trovirdine have been carried out using various RT mutants and compared to the properties of the earlier reported non-nucleoside RT inhibitors 9-Cl-TIBO, nevirapine and L-697,661.

Keywords: Human immunodeficiency virus (HIV) reverse transcriptase; Mutant; Enzyme kinetics; Inhibitor; Trovirdine

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1. Introduction

The AIDS pandemic has brought about extensive research on antiviral drugs. HIV reverse transcriptase (RT) has a key role in the early stages of HIV replication and is a major specific target for chemotherapy of HIV infections (for reviews, De Clercq 1991, 1992; Sandström and Öberg 1993a,b).

A new class of non-nucleoside HIV reverse transcriptase inhibitors – phenyl ethyl thiazolyl thiourea derivatives (PETT) (Ågren et al., 1995) has recently been discovered and a large number of derivatives have been synthesized and evaluated in vitro.

In the present study, the inhibitory mechanisms of trovirdine (LY 300046 HCl) and one analogue, MSC-127, on HIV-1 RT have been evaluated by using both homopolymeric and heteropolymeric primer/templates. The inhibitory effects have also been compared to those of other non-nucleoside RT inhibitors such as 9-Cl-TIBO (Pauwels et al., 1990; Debyser et al., 1991), L-697,661 (Goldman et al., 1991) and nevirapine (Merluzzi et al., 1990). In addition, we have determined the interactions between trovirdine and MSC-127 using RT with different point mutations known to cause resistance to non-nucleoside RT inhibitors, namely RT ($Leu^{100} \rightarrow Ile$), RT ($Glu^{138} \rightarrow Arg$), RT ($Tyr^{181} \rightarrow Cys$) and RT ($Tyr^{188} \rightarrow His$) (Zhang et al., 1993; 1994).

2. Materials and methods

2.1. Materials

The synthetic template-primers $(rA)_n(dT)_{12-18}$, $(rC)_n(dG)_{12-18}$ and $(dC)_n(dG)_{12-18}$ were purchased from Pharmacia LKB Biotechnology AB, Uppsala, Sweden. 16*S* and 23*S* ribosomal RNA templates and a specific 15-mer deoxyprimer (5′-TAACCTTGCG-GCCGT-3′) (rRNA/oligo DNA) were generous gifts from Dr. Richard Jaskunas, Eli Lilly Co. (Indianapolis, IN, USA) and the hybrid was prepared by the procedure of White et al., (1991). Activated calf thymus (CT) DNA was prepared by treating calf thymus DNA with pancreatic DNase at room temperature for 15 min using the procedure of Baril et al. (1977). All template-primer preparations were diluted to 1 mg/ml as stock solutions in 10 mM Tris HCl, pH 7.9 and 100 mM NaCl, and stored in aliquots at −20°C.

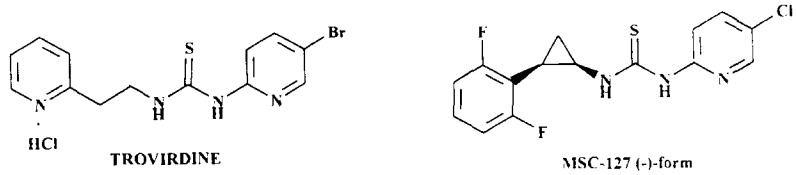

Fig. 1. Structures of the PETT compounds trovirdine and MSC-127.

Deoxy[1′,2′-^{3}H]guanosine 5′-triphosphate and [methyl,1′,2′-^{3}H]thymidine 5′-triphosphate, were obtained from Amersham, UK. The specific activities were 35 and 107 Ci/mmol, respectively. The 4 deoxyribonucleoside triphosphates (dATP, dCTP, dTTP and dGTP) were purchased from Sigma Chemicals.

The PETT compounds trovirdine (LY 300046 HCl) and MSC-127 were synthesized according to methods described elsewhere (Ågren et al., 1995 and Noreén et al., The VIth International Antiviral Symposium, Abstract 69, 1993) and the structures are shown in Fig. 1. Stock solutions of 10 mg/ml were made in dimethylsulphoxide (DMSO).

9-Cl-TIBO was purchased from Pharmatech Int. Inc., NJ, USA. L-697,661 (3-{[(4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2(1*H*)-one) was kindly supplied by Dr. M. E. Goldman at Merck, Sharp and Dohme Research Laboratories (Rahway, NY, USA). Nevirapine was synthesized according to published methods (Hargrave et al., 1991). Stock solutions of 9-Cl-TIBO, L-697,661 and nevirapine were made in DMSO.

2.2. Enzymes

Construction, expression system and preparation of HIV-1_{IIIB} RT have been described by Studier et al. (1990) and Unge et al. (1990). The mutants made were HIV-1 RT ($Leu^{100} \rightarrow Ile$), ($Glu^{138} \rightarrow Arg$), ($Tyr^{181} \rightarrow Cys$) and ($Tyr^{188} \rightarrow His$). The RT preparations were > 90% pure as judged by SDS polyacrylamide gel electrophoresis after a sequence of 3 chromatographic steps: Q-Sepharose, heparin Sepharose and S-Sepharose.

HIV-2 (LAV_{II}) reverse transcriptase was prepared as virus disrupted particles and the inhibitory effects were determined using a reaction directed by homopolymeric primer/template.

2.3. Enzyme kinetics

The RT-assay has been described by Zhang et al. (1993, 1994).

Enzyme kinetic studies were carried out under steady-state conditions. The initial velocities were expressed as function of substrate concentrations or function of template/primer concentrations that follow Michaelis–Menten kinetics. Inhibitory constants (k_{is} and k_{ii}) were determined by replots of Lineweaver–Burk plots and expressed as functions of inhibitor concentrations against slopes (k_{is}) and intercepts (k_{ii}).

The RT-catalyzed reaction was considered to be ordered, first forming the enzyme–template complexes (Majumdar et al., 1988) and followed by the binding of deoxynucleoside triphosphate. The inhibition constant k_{is} is defined as the ratio of $[E][I]/[EI]$ and k_{ii} is defined as the ratio of $[ES][I]/[ESI]$ where $[E]$ is the concentration of enzyme, $[I]$ the inhibitor concentration and $[S]$ the substrate concentration. The enzyme-kinetic analysis was applied to the interaction of enzyme-inhibitor $[EI]$ and ternary complex of enzyme–substrate–inhibitor $[ESI]$. k_{is} is equal to k_{ii} for linear non-competitive inhibition. When $k_{ii} \neq k_{is}$ this reflects different distribution of competitive with non-competitive or non-competitive with uncompetitive characters.

3. Results

3.1. Inhibitory effects by trovirdine and MSC-127 on HIV-1 RT

The PETT compounds trovirdine and MSC-127 (Fig. 1), as well as 9-Cl-TIBO, L-697,661 and nevirapine, were evaluated for inhibition of the activity of purified recombinant HIV-1 RT. Trovirdine and MSC-127 inhibited both the RNA- and DNA-directed DNA polymerase activity of HIV-1 RT (wt) with IC_{50} values of 0.017 and 0.00044 μM, respectively, when using $(rC)_n(dG)_{12-18}$ as template/primer (Table 1). By contrast, no inhibition was observed of CT DNA polymerase α, human DNA polymerase β and calf liver DNA polymerase γ at concentrations up to 500 μM (data not shown). Trovirdine and MSC-127 did not have any detectable inhibitions on the activity of HIV-2(LAV_{II}) RT at concentrations up to 270 μM when the disrupted virus (LAV_{II}) particles were used together with $(rC)_n(dG)_{12-18}$ (data not shown). For MSC-127, the selectivity index was about 10^6. Inhibition of HIV-1 RT by the other non-nucleoside RT inhibitors is shown for comparison in Table 1.

The dependence of enzyme concentration on the inhibition of HIV-1 RT (wt) by trovirdine, L-697,661 and 9-Cl-TIBO were investigated using the $(rC)_n(dG)_{12-18}$-directed reaction. The same inhibitory effect was observed at RT concentrations from 22, 66, 200, and 600–1800 ng/ml. These concentrations give RT (wt)-inhibitions of 90, 80, 84, 84 and 83%, respectively, using 0.13 μM trovirdine. This indicates a reversible inhibition.

3.2. Dependence of inhibitory effect on primer / template

The inhibitory effects of trovirdine and MSC-127 on HIV-1 RT (wt) were evaluated using different homo- and heteropolymeric DNA-primed RNA and DNA templates and compared to the effects of 9-Cl-TIBO, L-697,661 and nevirapine. The results are summarized in Table 1.

Table 1
Dependence for inhibition of HIV-1 RT (wt) by non-nucleoside RT inhibitors

	Homopolymeric template/primer			Heteropolymeric template/primer	
	rAdT	rCdG	dCdG	rRNA/oligo-DNA	Activated CT DNA
Tritium-labelled substrate					
	dTTP [a]	dGTP [a]	dGTP [a]	dGTP [a]	dGTP [a]
k_{cat}/k_m (s^{-1} μM^{-1})	0.82	0.38 ± 0.06	0.45 ± 0.04	0.19- ± 0.06	0.45 ± 0.07
Compound	IC_{50} (μM)				
Trovirdine	0.260 ± 0.09	0.017 ± 0.007	0.070 ± 0.012	0.007 ± 0.0016	0.029 ± 0.008
MSC-127	0.0040 ± 0.001	0.0004 ± 0.00005	0.0060 ± 0.002	0.0060 ± 0.0001	0.0040 ± 0.001
9-Cl-TIBO	1.10	0.22 ± 0.05	0.70 ± 0.23	0.30 ± 0.02	0.62 ± 0.34
L-697,661	0.230	0.063 ± 0.023	0.190 ± 0.05	0.060 ± 0.002	0.280 ± 0.04
Nevirapine	0.42	0.15 ± 0.04	0.11	1.50	1.30 ± 0.30

[a] Tritium-labelled substrate.

The RT activity was dependent on template/primer. The highest rate of incorporation of radiolabelled deoxynucleoside triphosphates was achieved by a $(rA)_n(dT)_{12-18}$-directed DNA synthesis and the lowest by a $(rC)_n(dG)_{12-18}$-directed reaction. When heteropolymeric template/primers were used the highest activity was seen for activated CT DNA and the lowest for rRNA/oligo DNA.

Inhibition of HIV-1 RT (wt) by trovirdine and MSC-127 displayed primer/template dependence. For trovirdine the lowest IC_{50} value (0.017 μM) was found using $(rC)_n(dG)_{12-18}$ and the highest (0.26 μM) using $(rA)_n(dT)_{12-18}$. A similar inhibitory dependency pattern was observed for MSC-127, which gave a 50% inhibition at 0.00044 μM when using a $(rC)_n(dG)_{12-18}$ and at 0.004 μM when using $(rA)_n(dT)_{12-18}$.

When using heteropolymeric template/primers, trovirdine was a better inhibitor of rRNA-directed DNA synthesis ($IC_{50} = 0.007$ μM) than of the activated CT. DNA-directed synthesis ($IC_{50} = 0.029$ μM) when dGTP was used as substrate.

The inhibition by trovirdine and MSC-127 of HIV-1 RT (wt) activity directed by $(rC)_n(dG)_{12-18}$ was found to be more potent than that of other non-nucleoside RT inhibitors. A similar result was also demonstrated when HIV-1 RT (wt) was used in a rRNA-directed DNA synthesis with dGTP as substrate, where trovirdine was 40-fold, 8.5-fold and 87-fold more active than 9-Cl-TIBO, L-697,661 and nevirapine, respectively.

With the exception of nevirapine, all non-nucleoside inhibitors were more inhibitory to HIV-1 RT (wt) when using $(rC)_n(dG)_{12-18}$ as template/primer than when using $(dC)_n(dG)_{12-18}$.

3.3. Enzyme kinetic studies of trovirdine

Enzyme kinetic studies were carried out under steady-state conditions. The initial velocities of enzyme activity were measured as functions of various substrate and template/primer concentrations according to Michaelis–Menten kinetics.

Inhibition of the RNA- and DNA-dependent DNA polymerase activity of HIV-1 RT (wt) by trovirdine was studied using rRNA/oligo DNA and activated CT DNA. Trovirdine caused a non-competitive inhibition with respect to deoxynucleoside triphosphates, $k_{is} = k_{ii} = 0.09$ μM for variable dGTP (Fig. 2A) and $k_{ii} = 0.11$ μM and $k_{is} = 0.13$ μM for variable dTTP (not shown) in the presence of saturated rRNA/oligo-DNA template/primer. The same non-competitive inhibitory mechanism was found when activated CT DNA was used at saturated concentration resulting in $k_{is} = 0.09$ μM and $k_{ii} = 0.10$ μM for varied dGTP (not shown) and $k_{is} = 0.07$ μM and $k_{ii} = 0.06$ μM for varied dTTP.

When rRNA/oligo-DNA (Fig. 2B) and activated CT DNA (not shown) were varied at saturated dGTP concentration (20 times the k_m), an uncompetitive type of inhibition was found with $k_{ii} = 0.013$ μg/ml and $k_{ii} = 0.07$ μg/ml, respectively. This pattern was also seen when dTTP was at saturated concentration (not shown). The enzyme kinetic constants are shown in Table 2.

The kinetic properties were also determined for the $(dG)_{12-18}$ $(rC)_n$-directed reaction. Linear non-competitive inhibition by trovirdine ($k_{is} = k_{ii} = 0.015$ μM) and by MSC-127

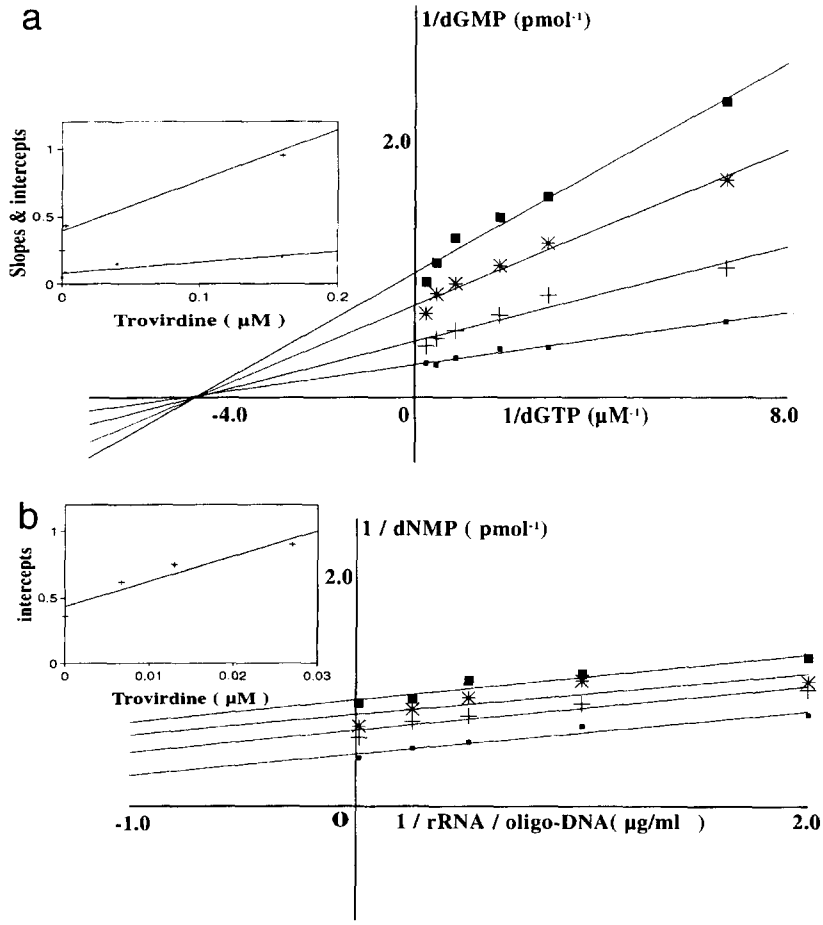

Fig. 2. A: double-reciprocal plot of the inhibition of HIV-1 RT (wt) by trovirdine at 0.0 μM (■), 0.03 μM (+), 0.04 μM (*) and 0.16 μM (■) using saturated ribosomal RNA template with oligo DNA-primer (15-mer) in the presence of various dGTP concentrations, $k_m = 0.21$ μM for dGTP. The inset replot of slope (■) and intercept (+) versus trovirdine gave $k_{ii} = k_{is} = 0.09$ μM. B: double-reciprocal plot of the inhibition of HIV-1 RT (wt) by trovirdine at 0.0 μM (■), 0.0025 μM (+), 0.005 μM (*) and 0.01 μM (■) using various concentrations of rRNA template/oligo-DNA in the presence of saturated dGTP (5 μM), $k_m = 0.77$ μg/ml for rRNA-template. The inset replot of intercept (+) versus trovirdine gave $k_{ii} = 0.013$ μg/ml.

($k_{is} = 0.00044$ μM and $k_{is} = 0.00056$ μM) were obtained with regard to varied dGTP concentration (Table 3).

3.4. Inhibition of HIV-1 RT (wt) and RT mutants

The inhibition of HIV-1 RT mutants by trovirdine and MSC-127 were characterized by a $(rC)_n(dG)_{12-18}$-directed reaction in the presence of various concentrations of dGTP. The results are summarized in Table 3.

Table 2
Enzyme kinetic constants for wild-type reverse transcriptase using the heteropolymeric template primer

	Template/primer			
	rRNA/oligo-DNA		Activated CT DNA	
Tritium-labelled substrate	dGTP [a]	dTTP [a]	dGTP [a]	dTTP [a]
Variable substrate [b]				
k_m (μM)	0.25 ± 0.05	0.12 ± 0.07	0.17 ± 0.03	0.31 ± 0.06
k_{is} (μM)	0.09	0.13	0.09	0.07
k_{ii} (μM)	0.09	0.11	0.10	0.06
Variable template / primer [c]				
k_m (μg/ml)	0.77	5.80	0.43	0.44
k_{ii} (μg/ml)	0.013	0.032	0.070	0.067

[a] Tritium-labelled substrate.
[b] Non-competitive inhibition.
[c] Uncompetitive inhibition.

RT (Ile^{100}), RT (Arg^{138}), RT (Cys^{181}) and RT (His^{188}) were 25-, 13-, 147- and 12-fold less susceptible than RT (wt) to trovirdine when the IC_{50}-values were compared. The same comparison of IC_{50}-values for MSC-127 gave ratios of 3.9, 2.5, 6.0 and 1.5, respectively. Thus, MSC-127 was a very potent inhibitor not only of HIV-1 RT (wt) but also of RT (Ile^{100}), RT (Arg^{138}), RT (Cys^{181}) and RT (His^{188}).

Enzyme kinetic studies showed trovirdine to inhibit HIV-1 RT (wt), RT (Arg^{138}) and RT (His^{188}) non-competitively and RT (Ile^{100}) and RT (Cys^{181}) in a mixed fashion with respect to dGTP. A different inhibitory pattern was obtained for MSC-127 which

Table 3
Inhibition of HIV-1 RT (wt) using the homopolymeric template primer $(rC)_n(dG)_{12-18}$

Compound	k_{is} (μM)	k_{ii} (μM)	IC_{50} (μM)	Mode of inhibition
HIV-1 RT (wt)				
Trovirdine	0.015	0.015	0.017 ± 0.007	Non-competitive
MSC-127	0.00044	0.00056	0.00044 ± 0.00005	Non-competitive
HIV-1 RT (Ile^{100})				
Trovirdine	0.30	0.78	0.43 ± 0.09	Mixed
MSC-127	0.0010	0.0009	0.0017 ± 0.0004	Non-competitive
HIV-1 RT (Arg^{138})				
Trovirdine	0.18	0.18	0.22 ± 0.05	Non-competitive
MSC-127	0.0050	0.0084	0.0011 ± 0.0003	Mixed
HIV-1 RT (Cys^{181})				
Trovirdine	1.54	2.60	2.50 ± 1.10	Mixed
MSC-127	0.0004	0.0016	0.0012 ± 0.00005	Mixed
*HIV-1 RT (His^{188})*Trovirdine	0.19	0.21	0.20 ± 0.07	Non-competitive
MSC-127	0.0006	0.0007	0.0007 ± 0.0002	Non-competitive

displayed a mixed inhibition of RT (Arg^{138}) and RT (Cys^{181}) and a non-competitive inhibition of other RT's.

For other non-nucleoside inhibitors, 9-Cl-TIBO, L-697,661 and nevirapine, the inhibitory mechanism was altered from non-competitive inhibition for wild-type RT to a mixed type for different RT mutants with the exception of the inhibition of RT (His^{188}) by nevirapine which was non-competitive (Zhang et al., 1994).

4. Discussion

The PETT compound trovirdine (LY 300046 HCl) (Ågren et al., 1995) as well as 9-Cl-TIBO (Pauwels et al., 1990; Debyser et al., 1991), L-697,661 (Goldman et al., 1991) and nevirapine (Merluzzi et al., 1990) have been shown to inhibit HIV-1 RT activity and to have antiviral effects against HIV-1 in cell cultures. A rapid development of resistance is a major problem for several non-nucleoside RT inhibitors and the resistance patterns have been studied extensively (Mellors et al., 1991; Richman et al., 1991; Condra et al., 1992; Boyer et al., 1993; De Clercq et al., 1993; Goldman et al., 1993; Hizi et al., 1993). The structurally unrelated non-nucleoside inhibitors have displayed unique resistance profiles to different RT mutants (Byrnes et al., 1993). Selection of virus resistant to trovirdine, 9-Cl-TIBO and nevirapine has been studied in T-cell lines with subsequent DNA sequencing analysis of the *pol* gene showed amino acid changes in the Leu^{100}, Tyr^{181} and Tyr^{188} codons (Dr. L. Vrang, personal communication). Thus, It is important to evaluate the RT with those amino acid changes in cell-free RT assay.

Trovirdine has some features in common with other non-nucleoside RT inhibitors. It specifically inhibits both the RNA- and DNA-dependent DNA polymerase activities of HIV-1 RT and it does not inhibit HIV-2 RT or DNA polymerase α, β and γ (Ågren et al., 1995). The inhibitory effects of trovirdine and MSC-127 were dependent on template/primer. There was a more than 10-fold difference in inhibition when $(rA)_n(dT)_{12-18}$ and $(rC)_n(dG)_{12-18}$-directed DNA synthesis were compared (Table 1). This pattern of template/primer dependence has been observed earlier for other non-nucleoside RT inhibitors (Debyser et al., 1991; Goldman et al., 1991; Tramontano et al., 1992) and a dependence on the sequence of primer/template has been observed for inhibition by L-696,229 (Carroll et al., 1993).

Reardon and Miller (1990) found that the rate of synthesis seems to influence the inhibition of HIV-1 RT activity by AZT-TP. Our results in Table 1 indicate that a low catalytic efficiency (k_{cat}/k_m), which reflects slower incorporation of deoxynucleoside monophosphates, was associated with a high degree of inhibition by trovirdine. Different primer/templates will possibly induce slightly different enzyme conformational changes influencing the rate of synthesis (Dr. B. Lindborg, personal communication) and the rate of DNA synthesis can be influenced by different lengths of template and ratio of enzyme/primer (Goody et al., 1991).

Trovirdine inhibited the RT activity non-competitively with respect to dGTP and dTTP (Table 2). This kinetic pattern of inhibition is similar to those of other non-nucleoside RT inhibitors (Merluzzi et al., 1990; Frank et al., 1991; Kopp et al., 1991;

Carroll et al., 1993). The reversible inhibition of RT by trovirdine is comparable to the reversible inhibition by L-696,661 and 9-Cl-TIBO (data not shown) and the reversible inhibition by 9-Cl-TIBO also agrees with results from Debyser et al. (1991).

The RT-catalyzed reaction pathway for DNA synthesis is considered to be ordered, the first complex being enzyme and template/primer (Majumdar et al., 1988) and this is followed by binding of deoxynucleoside triphosphate. The non-competitive inhibition by trovirdine indicates an allosteric binding site on RT with equal affinities towards enzyme (k_{is}) and enzyme–substrate (k_{ii}) complexes (Table 3) and this results in $k_{is} = k_{ii}$. For instance, the inhibition of RNA-directed DNA synthesis in the presence of various concentrations of dGTP depended on trovirdine interacting with the enzyme–template/primer complex giving $k_{is} = 90$ nM which is the same as the interaction between trovirdine and enzyme–template/primer–substrate ternary complex giving $k_{ii} = 90$ nM. This inhibition seemed to be independent of incoming deoxynucleoside triphosphate and similar inhibitory constants (k_{is} and k_{ii}) were observed for incorporation of dGTP and dTTP.

The inhibitory effect was also measured as a function of heteropolymeric template/primer concentration in the presence of saturated dGTP concentration (Fig. 2B). Trovirdine displayed an uncompetitive type of inhibition indicating a binding of trovirdine on RT only after binding of primer/template to enzyme. This observation is in agreement with results with 9-Cl-TIBO and HEPT which were inhibited uncompetitively with respect to varied primer/template (Debyser et al., 1991, 1992).

The change of amino acid 181 in RT from tyrosine to cysteine resulted in a more than 100-fold (for k_{is}) reduction in sensitivity to trovirdine (Table 3). This indicates a physical interaction between trovirdine and Tyr^{181} in RT. The same comparison for 9-Cl-TIBO, L-697,661 and nevirapine showed reductions of 61-, 300- and 330-fold, respectively (Zhang et al., 1994). This reduced susceptibility of the RT mutant indicates that trovirdine and other non-nucleoside RT inhibitors have overlapping binding sites on RT. Kohlstaedt et al. (1992) have shown nevirapine to bind in a pocket on HIV RT under the active site. The side chains of Tyr^{181} and Tyr^{188} have displayed a significant role in the binding of this class of inhibitors (Sardana et al., 1992) and a proximity of the trovirdine binding site is implicated.

A mixed type of inhibition by trovirdine was found against RT (Ile^{100}) and RT (Cys^{181}). This was reflected by increased inhibitory constants (k_{is} and k_{ii} values) and resulted in $k_{ii} > k_{is}$. The difference between k_{is} and k_{ii} was 2-5 fold. This phenomenon was observed for the other non-nucleoside RT inhibitors, but not for nevirapine, when acting on RT (His^{188}). These results are in good agreement with those of Byrnes et al. (1994). The altered inhibitory mechanism, from non-competitive to mixed type, implies a change in binding affinities on HIV RT. The binding of trovirdine to RT (Ile^{100}) and RT (Cys^{181}) showed more than 2- to 5-fold increased affinities as compared to binding to enzyme–primer/template–substrate ternary complexes. The mixed type of RT inhibition by trovirdine also involved a competitive character with respect to competition with substrate, possibly implying indirect influences on the enzyme conformation at the deoxynucleoside triphosphate binding site. Spence et al. (1995) indicated that the nevirapine binding site is conformationally connected to the binding of dNTP. In agreement with this consideration is the result that substitutions of tyrosine at positions

181 and 188 by cysteine and histidine on HIV-1 RT, give lower catalytic efficiency as reflected by decreased substrate affinity (Zhang et al., 1993, 1994). The inhibitory mechanism of trovirdine was dependent on inhibitor concentration and when the concentration of trovirdine increased the mechanism of inhibition of RT (Ile^{100}) and RT (Cys^{181}) changed from non-competitive ($k_{ii} = k_{is}$) to mixed inhibition ($k_{ii} > k_{is}$).

In conclusion, the PETT compounds trovirdine and MSC-127 have been found to be potent non-competitive inhibitors of HIV-1 RT (wt). A decreased sensitivity to PETT compounds was observed for HIV RT mutants resistant against 9-Cl-TIBO, nevirapine and L-697,661, indicating an interaction at a similar binding site. However, with all the mutants tested trovirdine and MSC-127 were more effective inhibitors than the other non-nucleoside RT inhibitors tested.

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