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L-743,726 (DMP-266): a Novel, Highly Potent Nonnucleoside Inhibitor of the Human Immunodeficiency Virus Type 1 Reverse Transcriptase

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The clinical benefit of the human immunodeficiency virus type 1 (HIV-1) nonnucleoside reverse transcriptase (RT) inhibitors (NNRTIs) is limited by the rapid selection of inhibitor-resistant viral variants. However, it may be possible to enhance the clinical utility of this inhibitor class by deriving compounds that express both high levels of antiviral activity and an augmented pharmacokinetic profile. Accordingly, we developed a new class of NNRTIs, the 1,4-dihydro-2*H*-3,1-benzoxazin-2-ones. L-743,726 (DMP-266), a member of this class, was chosen for clinical evaluation because of its in vitro properties. The compound was a potent inhibitor of the wild-type HIV-1 RT (K_i = 2.93 nM) and exhibited a 95% inhibitory concentration of 1.5 nM for the inhibition of HIV-1 replicative spread in cell culture. In addition, L-743,726 was found to be capable of inhibiting, with 95% inhibitory concentrations of ≤1.5 μM, a panel of NNRTI-resistant mutant viruses, each of which expressed a single RT amino acid substitution. Derivation of virus with notably reduced susceptibility to the inhibitor required prolonged cell culture selection and was mediated by a combination of at least two RT amino acid substitutions. Studies of L-743,726 in rats, monkeys, and a chimpanzee demonstrated the compound's potential for good oral bioavailability and pharmacokinetics in humans.

The nonnucleoside reverse transcriptase (RT) inhibitors (NNRTIs) of human immunodeficiency virus type 1 (HIV-1) have been studied extensively in recent years in both laboratory and clinical settings. The NNRTIs comprise a structurally diverse series of compounds that are highly specific for inhibition of the HIV-1 RT (22). The compounds bind to a common site on the RT heterodimer that is distinct from the enzyme's active site (5, 8, 15, 18, 20). Kinetically, the NNRTIs' inhibitory activities are generally noncompetitive with respect to the template-primer and nucleotide substrate (1, 3, 6, 9). The compounds appear to function by mediating a noted decrease in the enzyme's polymerizing activity (16).

The clinical utility of the nonnucleoside inhibitors has been limited by the rapid selection of highly resistant viral variants (11). However, the usefulness of this inhibitor class may be enhanced by derivation of a compound whose pharmacokinetics and intrinsic antiviral potency in humans are such that it will contribute significantly toward prolonging a combination therapy's in vivo antiviral effects.

Here we report the development of a new class of NNRTIs, the 1,4-dihydro-2*H*-3,1-benzoxazin-2-ones. The discovery of the benzoxazin-2-one NNRTIs is an outgrowth of our previous work on the 3,4-dihydroquinazolin-2(1*H*)-one-based RT inhibitors (19). The leading member of this class, L-743,726 (DMP-266), was found to be a potent inhibitor of wild-type HIV-1 in cell culture. It also exhibited noted antiviral activity against HIV-1 variants that expressed a series of NNRTI resistance-associated amino acid substitutions. In addition, animal pharmacokinetic analyses of L-743,726 in three species demonstrated superior oral bioavailability and plasma half-life characteristics for the compound.

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MATERIALS AND METHODS

Compounds. L-743,726 (DMP-266), (−)-6-chloro-4-cyclopropylethynyl-4-trifluoromethyl-1,4-dihydro-2*H*-3,1-benzoxazin-2-one (Chemical Abstracts Registry number 154598-52-4) and L-697,661 (3-[[[4,7-dichloro-1,3-benzoxazol-2-yl]methyl]amino]-5-ethyl-6-methylpyridin-2(1*H*)-one) (7, 12) were synthesized at Merck Research Laboratories, West Point, Pa. The synthetic sequence for L-743,726 is shown in Fig. 1. The aqueous solubility of L-743,726 is below our limits of detectability (<0.00002 mg ml^{-1} at pH 7.8). Therefore, measurement of certain physical parameters such as protein binding in aqueous buffer were not possible. A detailed description of the synthesis as well as complete structure-activity data will be published elsewhere.

Absolute configuration of L-743,726. The absolute configuration of L-743,726 was determined from a single crystal X-ray diffraction analysis of the (−)-camphanate imide derivative (Fig. 1, compound IV). Solving the structure so that the absolute configuration of the camphanate moiety is in the known *S* configuration results in the determination of the chiral site on the benzoxazin-2-one ring to be *S* also. (The *R* enantiomer, prepared in a similar fashion, was inactive in the in vitro RT inhibition assay.)

For compound IV, $C_{24}H_{21}ClF_3NO_5$, M_r = 495.887, orthorhombic, $P2_12_12_1$, a = 1.0603 (2) nm, b = 2.5403 (3) nm, c = 0.8794 (3) nm, V = 2,369 (1) nm^3 Z = 4, D_x = 1.391 g cm^{-3}, monochromatized radiation λ(Cu K_α) = 0.1541838 nm, m = 1.96 mm^{-1}, $F(000)$ = 1,024, T = 294 K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 70°, which yielded 2,474 unique reflections. There are 1,130 unique, observed reflections [with $I \geq 3\sigma(I)$ as the criterion for being observed] of the total number of reflections measured. The structure was solved by direct methods (SHELXS-86) (14) and was refined by using full-matrix least-squares on F (SDP-PLUS; Structure Determination Package version 3, 1985; Enraf-Nonius, Delft, The Netherlands). Given the large thermal parameters associated with the three-membered ring and its somewhat distorted geometry, it is probable that this group is disordered. No attempt was made, other than anisotropic refinement, to further model this disorder. The final model was refined by using 232 parameters and the observed data. The non-hydrogen atoms were refined with a mixture of isotropic and anisotropic thermal displacement parameters. Hydrogen atoms were included by using a "riding-atom" model. The final agreement statistics are as follows: R = 0.054, wR = 0.045, and S = 1.72 with

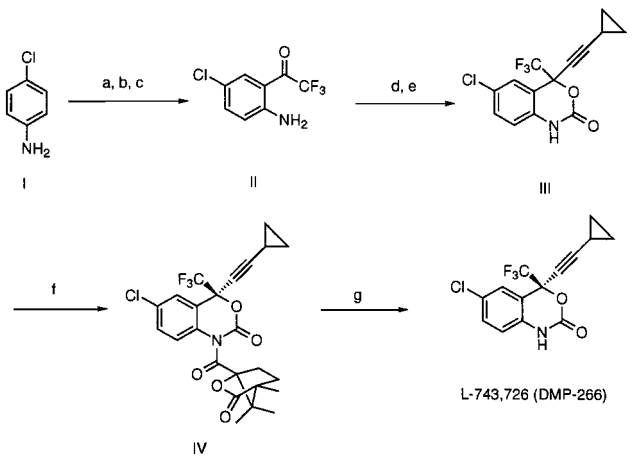

FIG. 1. Synthesis and chemical structure of the nonnucleoside inhibitor L-743,726 (DMP-266). Reagents: a, pivaloyl chloride; triethylamine; b, *n*-butyllithium, tetrahydrofuran, and then ethyl trifluoroacetate; c, 3 N HCl; d, 1-lithio-2-cyclopropylacetylene, e, tetrahydrofuran; 1,1′-carbonyldiimidazole, tetrahydrofuran; f, (−)-camphanoyl chloride, 4-(dimethylamino)pyridine, and then recrystallization from hexane; g, 1 N HCl-ethanol.

$(\Delta/\sigma)_{max}$ = 0.35. The least-squares weights were defined by using $1/\sigma^2(F)$. The maximum peak height in a final difference Fourier map is 230 e/nm^3, and this peak is located near the CF_3 group. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, United Kingdom.

In vitro RT inhibition assay. Recombinant RT enzymes were expressed, purified, and assessed for inhibition by L-743,726 as described previously (2, 13, 17). K_i and K_{ii} values were determined for each enzyme tested. The wild-type RT exhibited exclusively noncompetitive inhibition kinetics (data not shown), and, therefore, the K_i and K_{ii} values are identical (see Table 1). Pure noncompetitive inhibition was not assumed for the mutant enzymes, and, hence, the values of both K_i and K_{ii} were obtained from the linear mixed-type inhibition equation. The two- to threefold differences between the K_i and K_{ii} values probably reflect a small contribution of competitive inhibition with the mutant RTs.

Virus infection inhibition assays. Assays with T-lymphoid cell line-adapted wild-type and mutant HIV-1 variants were performed in human MT-4 T-lymphoid cell cultures as described previously (2, 10). Primary HIV-1 isolates were tested either in human peripheral blood mononuclear cell cultures or in cultures of primary human monocytes (7). Compound-mediated cytotoxicity was assessed by tetrazolium dye staining.

Animal pharmacokinetic studies. Studies were performed in rats, rhesus monkeys, and a single chimpanzee. For analyses of the drug given to rats intravenously (i.v.), a group (n = 4 or 5) of fasted male Sprague-Dawley rats (weight, 250 to 450 g) received a bolus (at a volume of 1.0 ml/kg of body weight) of the compound in dimethyl sulfoxide (DMSO) via a cannula implanted in the right jugular vein. For oral studies, rats were dosed by gavage by using a suspension of L-743,726 prepared in 0.5% aqueous methylcellulose (Methocel). Similarly, four monkeys received either an i.v. bolus of the compound in DMSO via the saphenous vein at a volume of 0.1 ml/kg or were administered the compound orally in suspension by using a nasogastric tube. Monkeys were fasted for 18 h prior to dosing. One nonanesthetized, nonfasted male chimpanzee (weight, approximately 60 kg) was dosed orally by voluntary ingestion by using an aqueous suspension of the compound. In all studies, heparinized blood was obtained at appropriate times. Plasma was separated immediately by centrifugation and was stored at −20°C until analysis. Plasma samples were extracted with methylene chloride; this was followed by analysis by high-performance liquid chromatography.

RESULTS

In vitro anti-RT activity. L-743,726 was tested for its in vitro inhibitory activity against purified wild-type HIV-1 RT and a panel of mutant RTs expressing amino acid substitutions known to engender the loss of susceptibility to various other NNRTIs. The data (Table 1) demonstrated that the compound is a potent inhibitor of all of the enzymes tested. The use of no single mutant RT in the library resulted in a K_i greater than 17.6 nM, and the K_is obtained by using the two double-mutant enzymes were well below 100 nM.

L-743,726 was also tested for its activity against a variety of polymerase enzymes and was found to be inactive (50% inhibitory concentration [IC_{50}], >300 μM). These included Moloney murine leukemia virus RT, avian myeloblastosis virus RT, human DNA polymerases α, β, and γ, *Escherichia coli* RNA polymerase, and the Klenow fragment.

Synergistic inhibition of RT activity by combinations of the nucleoside analog RT inhibitors zidovudine triphosphate, dideoxyinosine triphosphate, and dideoxycytidine triphosphate with L-743,726 was examined by the method of Yonetani and Theorell (21). The combinations gave detectable levels of synergistic inhibition, as evidenced by nonparallel lines in the Yonetani-Theorell plot (data not shown). Synergy was observed at levels as low as 60% inhibition of RT, which is comparable to those observed previously for the quinazolinone L-738,372 (4). In contrast, synergy between zidovudine triphosphate and L-697,661 was observed only when the degree of RT inhibition was greater than 95%.

In vitro antiviral activity and selection for resistant virus. L-743,726 was tested for its ability to inhibit virus spread in an acute infection assay. The results (Table 2) showed that the compound effectively inhibited several wild-type T-lymphoid cell line-adapted variants. Identical activity (95% cell culture inhibition concentrations [IC_{95}], 1.5 to 3.0 nM) was seen with wild-type primary isolates of the virus in both primary lymphoid and monocytoid cell cultures. The lowest concentration of inhibitor that yielded evidence of cytotoxicity, both in primary cells and in a T-cell line, was 80 μM (selectivity index, approximately 80,000). The compound also effectively inhibited HIV-1 variants that expressed RT amino acid substitutions which confer the loss of susceptibility to other NNRTIs. For purposes of comparison, Table 2 also presents data obtained from concurrent assays with the prototypic pyridinone NNRTI L-697,661 (7, 12). These data are in agreement with those reported previously for this inhibitor (2).

In contrast to the pyridinone, no single RT substitution yielded a mutant virus for which the IC_{95} for inhibition by L-743,726 was >1.5 μM. Most mutants were inhibited by L-743,726 at IC_{95}s of 50 nM or less. Also, several mutants expressing double RT substitutions were suppressed in cell culture at concentrations of L-743,726 that are potentially achievable in vivo (see below).

TABLE 1. Inhibition of wild-type and mutant HIV-1 RT by L-743,726[a]

Position of amino acid substitution	K_i (nM)	K_{ii} (nM)
Wild type	2.93 ± 0.17	2.93 ± 0.17
98 (Ala→Gly)	3.85 ± 0.60	4.48 ± 0.63
100 (Leu→Ile)	17.13 ± 3.57	25.30 ± 8.95
101 (Lys→Glu)	7.27 ± 1.08	4.17 ± 0.04
103 (Lys→Asn)	17.60 ± 2.31	31.24 ± 4.02
106 (Val→Ala)	6.89 ± 3.37	6.75 ± 1.96
108 (Val→Ile)	2.97 ± 0.46	3.22 ± 0.22
179 (Val→Asp)	3.60 ± 1.51	3.68 ± 1.65
179 (Val→Glu)	9.68 ± 1.57	3.51 ± 0.13
181 (Tyr→Cys)	7.62 ± 1.12	3.74 ± 0.68
101 (Lys→Glu) + 103 (Lys→Asn)	56.50 ± 27.60	92.94 ± 0.68
103 (Lys→Asn) + 181 (Tyr→Cys)	26.05 ± 1.28	40.41 ± 10.94

[a] K_i and K_{ii} values were determined from two or more experiments. Data are presented as means ± standard errors of the means (see Materials and Methods).

[b] Each mutant enzyme expressed the noted amino acid substitution at the indicated RT residue. Mutants were constructed as described by Byrnes et al. (2). L-743,726 was found to be essentially inactive against HIV-2 RT.

TABLE 2. Inhibition of wild-type and mutant HIV-1 infection in cell culture by L-743,726[a]

Virus or position of amino acid substitution[b]	IC_{95} (nM)[c]	
	L-743,726	L-697,661
Wild type (strain IIIb)	1.5	100.0
Wild type (strain MN)	3.0	50.0
Wild type (strain RFII)	3.0	50.0
98 (Ala→Gly)	12.0	800.0
100 (Leu→Ile)	100.0	200.0
101 (Lys→Glu)	25.0	800.0
103 (Lys→Asn)	100.0	800.0
106 (Val→Ala)	12.0	100.0
108 (Val→Ile)	3.0	400.0
179 (Val→Asp)	3.0	400.0
181 (Tyr→Cys)	6.0	>3,000.0
188 (Tyr→Leu)	1,500.0	>3,000.0
101 (Lys→Asp) + 103 (Lys→Asn)	1,500.0	>3,000.0
101 (Lys→Asp) + 100 (Leu→Ile)	1,500.0	>3,000.0
103 (Lys→Asn) + 181 (Tyr→Cys)	400.0	>3,000.0
100 (Leu→Ile) + 103 (Lys→Asn)[d]	25,000.0	1,500.0

[a] Comparative values are presented for L-743,726 and the unrelated prototypic NNRTI L-697,661 (see text).

[b] Each mutant virus expressed the noted amino acid substitution at the indicated RT residue. Mutants were constructed and derived as described by Byrnes et al. (2).

[c] The IC_{95} was defined as the concentration of test compound that inhibited virus expression by at least 95% relative to virus expression in untreated control cultures. Assays were performed in MT-4 human T-lymphoid cells (see Materials and Methods). The reported values are representative of multiple determinations.

[d] This variant was selected by passage of the HIV-1 IIIb strain in increasing concentrations of L-743,726 (see text).

The variant virus expressing the double substitution at RT residue positions 100 (Leu→Ile) and 103 (Lys→Asn) was selected in cell culture following serial passage in increasing concentrations of L-743,726 by previously described methods (10). Ten passages were required for selection of this highly resistant variant (data not shown). Similarly, virus resistant to L-697,661 was obtained after only several passages (10).

Pharmacokinetic characteristics. The pharmacokinetic profile of L-743,726 in both rats and rhesus monkeys is summarized in Table 3. After i.v. administration, the compound was cleared rapidly from rats, but it was cleared considerably more slowly from monkeys. The large volume of distribution (two to four times the amount of body water) in both species indicated extensive tissue binding. The oral bioavailability in rats was 16%. In monkeys, the half-life of L-743,726 after administration of a 1.0-mg/kg i.v. dose exceeded 2.5 h. L-743,726 was well absorbed orally. Administration to monkeys of oral doses as fine suspensions in 0.5% aqueous methylcellulose yielded consistently high levels in plasma. A 2.0-mg/kg dose produced peak levels of 0.5 μM at approximately 3.0 h (Table 3). The absolute bioavailability was estimated to be 42%. A 10-mg/kg dose yielded a peak level in plasma of 3.22 μM (Fig. 2). A 10-mg/kg oral dose given to a single chimpanzee gave concentrations in plasma of 4.12, 2.95, and 2.69 μM at 2, 8, and 24 h after dosing, respectively.

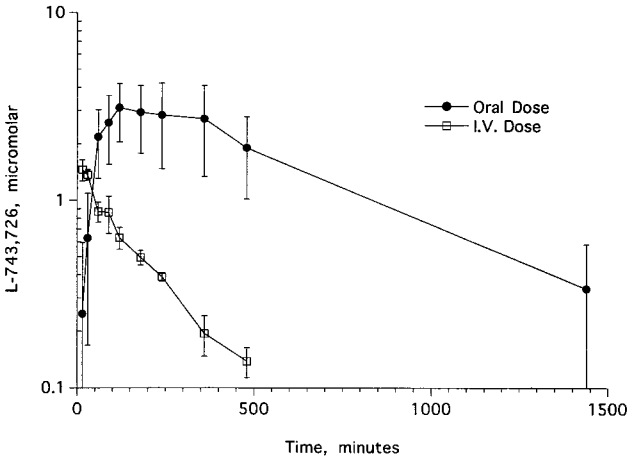

FIG. 2. Concentrations of L-743,726 in the plasma of rhesus monkeys following the administration of either an i.v. (1.0 mg/kg) or an oral (10 mg/kg) dose.

DISCUSSION

The clinical promise of the NNRTI inhibitors of HIV-1 has remained largely unfulfilled because of the rapid selection of highly resistant viral variants in infected individuals. However, it remains possible that the NNRTIs can be effective when they are used in combination with additional antiretroviral agents such as the nucleoside analog RT inhibitors and/or the HIV protease inhibitors. In theory, such combination therapy may result in a greater degree of initial viral suppression than that which can be mediated by a single agent. More importantly, combination therapy may notably prolong the time to selection of viral variants that would express multiple patterns of resistance.

We previously reported our clinical experiences with the pyridinone NNRTI L-697,661 and showed that the compound's utility was limited, even when it was coadministered with the nucleoside analog zidovudine (11). The NNRTI's antiviral potency was not sufficient to overcome its apparently restricted pharmacokinetics, so that its ability to contribute to zidovudine's anti-HIV-1 effects was rather poor. Given this, we embarked on the development of structurally novel NNRTIs that would exhibit significantly enhanced anti-HIV-1 activity and demonstrate the potential for improved pharmacokinetic profiles. The 1,4-dihydro-2*H*-3,1-benzoxazin-2-ones proved to generally display such characteristics. L-743,726 (Fig. 1) presented the best properties.

TABLE 3. Pharmacokinetic profiles of L-743,726 in animals[a]

Species	i.v. administration				Oral administration			
	Dose (mg/kg)	CL (ml/min/kg)	V_{ss} (liters/kg)	$t_{1/2}$ (min)	Dose (mg/kg)	C_{max} (μM)	T_{max} (min)	F (%)
Rat	2	68.2	4.4	50	10	0.82	38	16
Monkey	1	11.6	2.4	158	2	0.50	176	42

[a] Studies were performed as described in Materials and Methods. CL, clearance from plasma; V_{ss}, volume of distribution; $t_{1/2}$, half-life; C_{max}, maximum concentration of drug in plasma; T_{max}, time to maximum concentration of drug in plasma; F, oral bioavailability, determined by comparing the mean areas under the plasma concentration-time curves following i.v. and oral dosing.

This compound was found to be a potent inhibitor of purified wild-type HIV-1 RT in vitro and of HIV-1 replication in cell culture. In the latter respect, its activity was at least 10-fold greater than that of L-697,661. In addition, L-743,726 exhibited noted antiviral activity against an extensive panel of HIV-1 mutants constructed so that they expressed single and multiple RT substitutions which mediate resistance to many of the described NNRTIs. Analysis of similar data obtained with purified mutant RT enzymes (Table 1) suggested that the primary interaction between the inhibitor and the RT was probably mediated by the RT amino acid residues at positions 100 and 103. Both residues contribute to the defined NNRTI RT binding site (15, 18). Alterations at each of these sites had striking adverse effects on L-743,726's activity. Cell culture selection of mutant virus with greatly reduced susceptibility to the inhibitor resulted in a variant that expressed a combination of substitutions at both residues (Table 2). In contrast to the relative ease with which L-697,661-resistant virus was previously derived in culture (10), derivation of L-743,726-resistant virus required an extended number of passages and an extended selection period, suggesting that the resistant mutant was not significantly represented in the initial wild-type virus population.

The compound's pharmacokinetic characteristics in several test animal species were also encouraging. Oral bioavailability was good, as was the maintenance of reasonably high systemic levels following the oral administration of an aqueous suspension. In a chimpanzee, a 10-mg/kg oral dose resulted in concentrations greater than 2.5 μM at 24 h. This concentration of compound would, in theory, be sufficient to suppress replication of all of the NNRTI-resistant mutants that express a single RT amino acid substitution and some that express multiple substitutions.

Of course, it is likely that monotherapy with L-743,726 would rapidly select for a highly resistant variant with amino acid alterations at both residues 100 and 103. However, the usefulness of the compound in combination therapy will as likely be a reflection of its high degree of potency against wild-type HIV-1 and its excellent bioavailability. In this regard, L-743,726 represents a reasonable candidate for inclusion in such a therapeutic regimen to determine whether the NNRTIs will contribute significantly to the treatment of HIV-1-infected persons.

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REFERENCES

1. **Althaus, I. W., J. J. Chou, A. J. Gonzales, M. R. Deibel, K. C. Chou, F. J. Kezdy, D. L. Romero, P. A. Aristoff, W. G. Tarpley, and F. Reusser.** 1993. Steady-state kinetic studies with the non-nucleoside HIV-1 reverse transcriptase inhibitor U-87201E. J. Biol. Chem. **268:**6119–6124.
2. **Byrnes, V. W., V. V. Sardana, W. A. Schleif, J. H. Condra, J. A. Waterbury, J. A. Wolfgang, W. J. Long, C. L. Schneider, A. J. Schlabach, B. S. Wolanski, D. J. Graham, L. Gotlib, A. Rhodes, D. L. Titus, E. Roth, O. M. Blahy, J. C. Quintero, S. Staszewski, and E. A. Emini.** 1993. Comprehensive mutant enzyme and viral variant assessment of human immunodeficiency virus type 1 reverse transcriptase resistance to the nonnucleoside inhibitors. Antimicrob. Agents Chemother. **37:**1576–1579.
3. **Carroll, S. S., D. B. Olsen, C. D. Bennett, L. Gotlib, D. J. Graham, J. H. Condra, A. M. Stern, J. A. Shafer, and L. C. Kuo.** 1993. Inhibition of HIV-1 reverse transcriptase by pyridinone derivatives. J. Biol. Chem. **268:**276–281.
4. **Carroll, S. S., M. Stahlhut, J. Geib, and D. B. Olsen.** 1994. Inhibition of HIV-1 reverse transcriptase by a quinazolinone and comparison with inhibition by pyridinones. J. Biol. Chem. **269:**32351–32357.
5. **Dueweke, T. J., F. J. Kezdy, G. A. Waszak, M. R. Deibel, and W. G. Tarpley.** 1992. The binding of a novel bisheteroarypiperazine mediates inhibition of human immunodeficiency virus type 1 reverse transcriptase. J. Biol. Chem. **267:**27–30.
6. **Frank, K. B., G. J. Noll, E. V. Connell, and I. S. Sim.** 1991. Kinetic interaction of the human immunodeficiency virus type 1 reverse transcriptase with the antiviral tetrahydroimidazo [4,5,1,jk]-[1,4]-benzodiazepine-2-(1*H*)-thione compound, R82150. J. Biol. Chem. **266:**14232–14236.
7. **Goldman, M. E., J. H. Nunberg, J. A. O'Brien, J. C. Quintero, W. A. Schleif, K. F. Freund, S. L. Gaul, W. S. Saari, J. S. Wai, J. M. Hoffman, P. S. Anderson, D. J. Hupe, E. A. Emini, and A. M. Stern.** 1991. Pyridinone derivatives: specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity. Proc. Natl. Acad. Sci. USA **88:** 6863–6867.
8. **Kohlsteadt, L. A., J. Wang, J. M. Friedman, P. A. Rice, and T. A. Steitz.** 1992. Crystal structure at 3.5Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science **256:**1783–1790.
9. **Kopp, E. B., J. J. Miglietta, A. G. Shrutkowski, C. K. Shih, P. M. Grob, and M. T. Skoog.** 1991. Steady state kinetics and inhibition of HIV-1 reverse transcriptase by a non-nucleoside dipyridodiazepinone, BI-RG-587, using a heteropolymeric template. Nucleic Acids Res. **19:**3035–3039.
10. **Nunberg, J. H., W. A. Schleif, E. J. Boots, J. A. O'Brien, J. C. Quintero, J. M. Hoffman, E. A. Emini, and M. E. Goldman.** 1991. Viral resistance to human immunodeficiency virus type 1-specific pyridinone reverse transcriptase inhibitors. J. Virol. **65:**4887–4892.
11. **Saag, M. S., E. A. Emini, O. L. Laskin, J. Douglas, W. I. Lapidus, W. A. Schleif, R. J. Whitley, V. W. Byrnes, C. Hildebrand, J. C. Kappes, K. W. Anderson, F. E. Massari, G. M. Shaw, and the L-697,661 Working Group.** 1993. A short clinical trial of L-697,661, a nonnucleoside inhibitor of HIV-1 reverse transcriptase. N. Engl. J. Med. **329:**1065–1072.
12. **Saari, W. S., J. S. Wai, T. E. Fisher, C. M. Thomas, J. M. Hoffman, C. S. Rooney, A. M. Smith, J. H. Jones, D. L. Bamberger, M. E. Goldman, J. A. O'Brien, J. H. Nunberg, J. C. Quintero, W. A. Schleif, E. A. Emini, and P. A. Anderson.** 1992. Synthesis and evaluation of 2-pyridinone derivatives as HIV-1 specific reverse transcriptase inhibitors. 2. Analogs of 3-aminopyridin-2(1*H*)-one. J. Med. Chem. **35:**3792–3802.
13. **Sardana, V. V., E. A. Emini, L. Gotlib, D. J. Graham, D. W. Lineberger, W. J. Long, A. J. Schlabach, J. A. Wolfgang, and J. H. Condra.** 1992. Functional analysis of HIV-1 reverse transcriptase amino acids involved in resistance to multiple nonnucleoside inhibitors. J. Biol. Chem. **267:**17526–17530.
14. **Sheldrick, G. M.** 1990. Phase annealing in SHELX-90: direct methods for larger structures. Acta Crystallogr. **A46:**467–473.
15. **Smerdon, S. J., J. Jager, J. Wang, L. A. Kohlstaedt, A. S. Chirino, A. J. Friedman, P. A. Rice, and T. A. Steitz.** 1994. Structure of the binding site for nonnucleoside inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **91:**3911–3915.
16. **Spence, R. A., W. M. Kati, K. S. Anderson, and K. A. Johnson.** 1995. Mechanism of inhibition of HIV-1 reverse transcriptase by nonnucleoside inhibitors. Science **267:**988–993.
17. **Stahlhut, M., Y. Li, J. H. Condra, J. Fu, L. Gotlib, D. J. Graham, and D. B. Olsen.** 1994. Purification and characterization of HIV-1 reverse transcriptase having a 1:1 ration of p66 and p51 subunits. Protein Expr. Purif. **5:**614–621.
18. **Tantillo, C., J. Ding, A. Jacobo-Molina, R. G. Nanni, P. L. Boyer, S. H. Hughes, R. Paulwels, K. Andries, P. A. J. Janssen, and E. Arnold.** 1994. Location of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. J. Mol. Biol. **243:**369–387.
19. **Tucker, T. J., T. A. Lyle, C. M. Wiscount, S. F. Britcher, S. D. Young, W. M. Sanders, W. C. Lumma, M. E. Goldman, J. A. O'Brien, R. G. Ball, C. F. Homnick, W. A. Schleif, E. A. Emini, J. R. Huff, and P. S. Anderson.** 1994. Synthesis of a series of 4-(arylethynyl)-6-chloro-4-cyclopropyl-3,4-dihydroquinazolin-2(1*H*)-ones as novel non-nucleoside HIV-1 reverse transcriptase inhibitors. J. Med. Chem. **37:**2437–2444.
20. **Wu, J. C., T. C. Warren, J. Adams, J. Proudfoot, J. Skiles, P. Raghavan, C. Perry, I. Potocki, P. Farina, and P. M. Grob.** 1991. A novel dipyridodiazepinone inhibitor of HIV-1 reverse transcriptase acts through a nonsubstrate binding site. Biochemistry **30:**2022–2026.
21. **Yonetani, T., and H. Theorell.** 1964. Studies on liver alcohol dehydrogenase complexes. III. Multiple inhibition kinetics in the presence of two competitive inhibitors. Arch. Biochem. Biophys. **106:**243–251.
22. **Young, S. D.** 1993. Non-nucleoside inhibitors of HIV-1 reverse transcriptase. Perspect. Drug Discov. Des. **1:**181–192.