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U-90152, a Potent Inhibitor of Human Immunodeficiency Virus Type 1 Replication

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Bisheteroarylpiperazines are potent inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT). We describe a novel bisheteroarylpiperazine, U-90152 {1-(5-methanesulfonamido-1*H*-indol-2-yl-carbonyl)-4-[3-(1-methylethyl-amino)pyridinyl]piperazine}, which inhibited recombinant HIV-1 RT at a 50% inhibitory concentration (IC_{50}) of 0.26 μM (compared with IC_{50}s of >440 μM for DNA polymerases α and δ). U-90152 blocked the replication in peripheral blood lymphocytes of 25 primary HIV-1 isolates, including variants that were highly resistant to 3′-azido-2′,3′-dideoxythymidine (AZT) or 2′,3′-dideoxyinosine, with a mean 50% effective dose of 0.066 ± 0.137 μM. U-90152 had low cellular cytotoxicity, causing less than 8% reduction in peripheral blood lymphocyte viability at 100 μM. In experiments assessing inhibition of the spread of HIV-1_{IIIB} in cell cultures, U-90152 was much more effective than AZT. When approximately 500 HIV-1_{IIIB}-infected MT-4 cells were mixed 1:1,000 with uninfected cells, 3 μM AZT delayed the evidence of rapid viral growth for 7 days. In contrast, 3 μM U-90152 totally prevented the spread of HIV-1, and death and/or dilution of the original inoculum of infected cells prevented renewed viral growth after U-90152 was removed at day 24. The combination of U-90152 and AZT, each at 0.5 μM, also totally prevented viral spread. Finally, although the RT amino acid substitutions K103N (lysine 103 to asparagine) and Y181C (tyrosine 181 to cysteine), which confer cross-resistance to several nonnucleoside inhibitors, also decrease the potency of U-90152, this drug retains significant activity against these mutant RTs in vitro (IC_{50}s, approximately 8 μM).

Bisheteroarylpiperazine (BHAP) derivatives inhibit human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) and block HIV-1 replication in vitro (1, 3, 6, 20, 27). The BHAPs are nonnucleosides and thus differ from RT inhibitors, such as 3′-azido-2′,3′-dideoxythymidine (AZT) or 2′,3′-dideoxyinosine (ddI), which mimic the normal deoxynucleotide triphosphate substrate of RT and act as chain terminators during proviral synthesis (25). In this respect, the BHAPs are similar to several other recently described HIV-1 RT inhibitors, including nevirapine (15), the pyridinones (9), and the tetrahydroimidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1H)-thione (TIBO) compounds (18). Studies have shown that the BHAPs and these other nonnucleoside RT inhibitors all bind to a common region of RT (6, 9, 27). The similarity extends to the kinetic characterization of their mode of inhibition (1, 8, 9, 28) and also to their common susceptibility to several RT amino acid substitutions that confer resistance (22). U-87201E, the first BHAP RT inhibitor, has entered clinical trials and has been described most widely (20). This report identifies a novel BHAP derivative, U-90152 (Fig. 1), that is structurally similar to U-87201E but has significantly higher antiviral potency.

MATERIALS AND METHODS

Compounds. U-90152, U-87201E, and [^{3}H]U-88204E were synthesized by Upjohn Laboratories as described by Romero et al. (20, 21). Nevirapine (6,11-dihydro-11-cyclopropyl-4-methyldipyrido[2,3-*b*:2′,3′-*e*]-[1,4]diazepin-6-one) was synthesized as described by Hargrave et al. (11). L-697,661 (3-{[(4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2(1*H*)-one) was synthesized as described by Goldman et al. (9).

HIV-1 RT assays. In vitro inhibition of recombinant heterodimeric RT derived from HIV-1_{IIIB} by U-87201E, U-90152, AZT triphosphate, and 2′,3′-dideoxythymidine was assayed as previously described (5, 20) but with highly purified RT (4). Inhibition of DNA polymerases α and δ by these compounds was assayed (26) with poly(dA) · oligo(dT) as the template-primer and 1.6 μg of proliferating cell nuclear antigen per ml in the DNA polymerase δ assay. Drug-resistant mutants of HIV-1_{IIIB} RT were prepared by site-directed mutagenesis (Amersham, Arlington Heights, Ill.). Mutant RT genes were cloned into plasmid DE 5.2 (23), which expresses RT with an amino-terminal hexahistidine linker. Mutant RTs were purified by metal affinity chromatography (23) and used as a p66 homodimer. Drug inhibition of the RNA-dependent DNA polymerase activity of the mutant RTs was assayed with 40 nM enzyme as described previously (7).

Antiviral drug susceptibility assays. The antiviral activity of U-90152, U-87201E, and AZT against HIV-1_{IIIB} was assayed in MT-2 cells (10) and primary peripheral blood lymphocytes (PBL) as follows. MT-2 cells were infected with HIV-1_{IIIB} at 0.001 50% tissue culture infective dose per cell for 1 to 2 h at 37°C, extensively washed to remove virus, and cultured in the presence of drug for 4 days, the time needed for a maximal viral cytopathic effect. The 50% effective dose (ED_{50}) was determined by counting syncytia. PBL were similarly infected and cultured in the presence of

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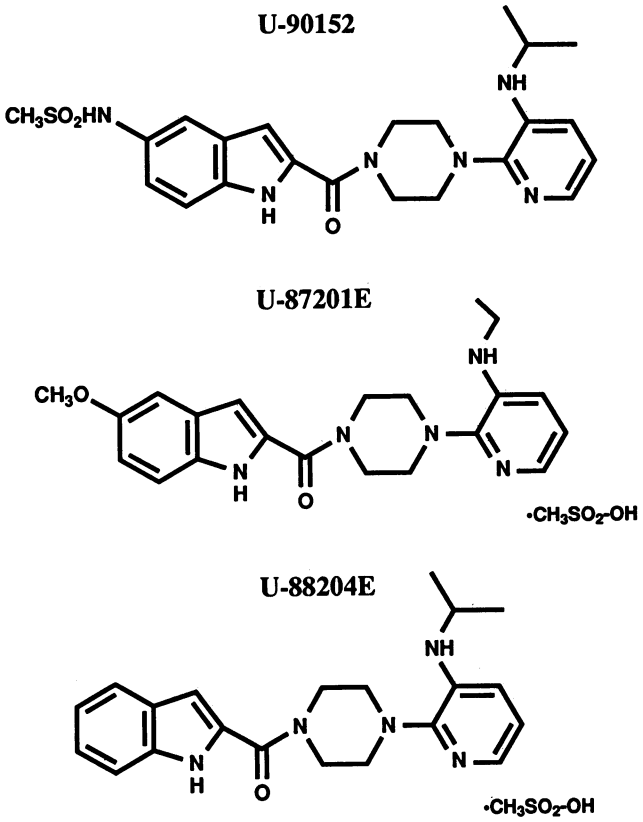

FIG. 1. Structures of U-90152 and related BHAPs.

drug for 6 days, when the extent of HIV-1 replication was assessed by determining supernatant p24 antigen levels (Coulter Immunology, Hialeah, Fla.). The activity of U-90152 against 25 clinical HIV-1 isolates cultured in PBL was directly compared with that of AZT, ddI, and 2',3'-dideoxycytidine by use of the AIDS Clinical Trials Group-U.S. Department of Defense standardized protocol for HIV-1 drug susceptibility testing (12). Cytotoxicity was assayed by culturing 2×10^5 phytohemagglutinin-stimulated normal PBL in 200 μl of medium containing 0 to 100 μM U-90152 for 7 days, when cells were counted and viability was determined by trypan blue exclusion. The anti-HIV activity of U-90152 against monocytotropic HIV-1_{JR-FL} was determined with a primary monocyte-macrophage culture by previously described methods (2, 13).

Inhibition of viral spread in cell cultures. A human T-cell line, MT-4 (10), was infected with HIV-1_{IIIB} at a multiplicity of 0.005 50% tissue culture infective dose per cell and cultured for 7 to 8 days, when abundant syncytia were evident. The infected cells were washed and mixed with fresh, uninfected MT-4 cells at a ratio of 1:1,000; 5×10^5 cells were used to seed 1-ml cultures. The cultures were diluted 1:6 into fresh medium containing drug every 3 or 4 days to maintain logarithmic cell growth. Prior to dilution, cell viability was assessed by trypan blue exclusion, supernatant p24 antigen levels were assayed, and some cells were frozen for later analysis. Beginning at day 24 postcocultivation, portions of cultures with low p24 antigen levels were also diluted into medium lacking drug to determine whether HIV-1 was still present.

PCR of proviral DNA. Total cellular DNA from cultures that lacked evidence of viral growth at day 85 was isolated by use of approximately 10^6 MT-4 cells. DNA samples were also isolated from infected and uninfected cultures to be used as controls. A series of dilutions of DNA (0 to 250 ng) from the infected control culture were amplified by the polymerase chain reaction (PCR) with 60 cycles (95°C, 1 min; 60°C, 1 min) and primers with the sequence of the *gag* gene region of HIV-1 ($SK_{38/39}$ [17]). In each amplification, DNA isolated from the uninfected cells was added so that all reaction mixtures contained a total of 250 ng of DNA. PCR products were resolved on an 8% acrylamide gel and stained with ethidium bromide to visualize the 115-bp target band. The limit of detection of the 115-bp PCR product corresponded to the amplification of 0.025 ng of infected-cell DNA, or about five human cell genomes (24). The absence of HIV-1 proviral DNA in cultures treated with U-90152 was confirmed by subjecting 250 ng of DNA to the PCR as described above.

TABLE 1. In vitro inhibition of HIV-1 RT and normal cellular polymerases

Compound	IC_{50} (μM) for[a]:		
	HIV-1 RT	Polymerase α	Polymerase δ
U-90152	0.26	440	>550
U-87201E	1.3	260	1,976
AZT triphosphate	0.15	60	140
2',3'-Dideoxythymidine triphosphate	0.025	100	100

[a] Determined from the slopes of median effect plots of combined data from a minimum of two independent determinations. The correlation coefficients for the plots were ≥0.98.

RESULTS AND DISCUSSION

Inhibition of HIV-1 RT activity. U-90152 inhibited HIV-1 RT activity at an 50% inhibitory concentration (IC_{50}) of 0.26 μM (Table 1). This inhibitory concentration was roughly comparable to that of AZT triphosphate and about fivefold lower than that of U-87201E. The concentration of U-90152 required to inhibit HIV-1 RT was significantly lower than that required to inhibit cellular DNA polymerase α and DNA polymerase δ (IC_{50}s of 440 and >550 μM, respectively), resulting in a selectivity index of $>10^3$. When compared with AZT triphosphate and 2',3'-dideoxythymidine triphosphate, U-90152 was considerably less inhibitory to either of the cellular DNA polymerases (Table 1).

Our previous work indicated that the inhibition of RT activity by BHAPs is mediated by a specific interaction with RT (6). The binding of radiolabeled U-88204E (Fig. 1), an analog of U-90152, to heterodimeric RT was quantitated by equilibrium dialysis. U-88204E specifically interacted with RT in the presence or absence of saturating concentrations of the poly(rC) · oligo(dG) template-primer, with a K_d of about 60 nM and a stoichiometry of 1 mol of drug per mol of RT heterodimer. U-90152 and U-87201E competed with [^{3}H]U-88204E for binding to HIV-1 RT (data not shown), indicating that these BHAPs bind to RT at the same site. U-90152 bound to RT with a high apparent affinity (≅80 nM), consistent with its potent inhibition of both recombinant HIV-1 RT (Table 1) and viral replication in cell cultures (Fig. 2 and Table 2). U-87201E bound to RT with an apparent affinity of approximately 700 nM.

U-90152 prevents HIV-1 spread in human lymphocytes in

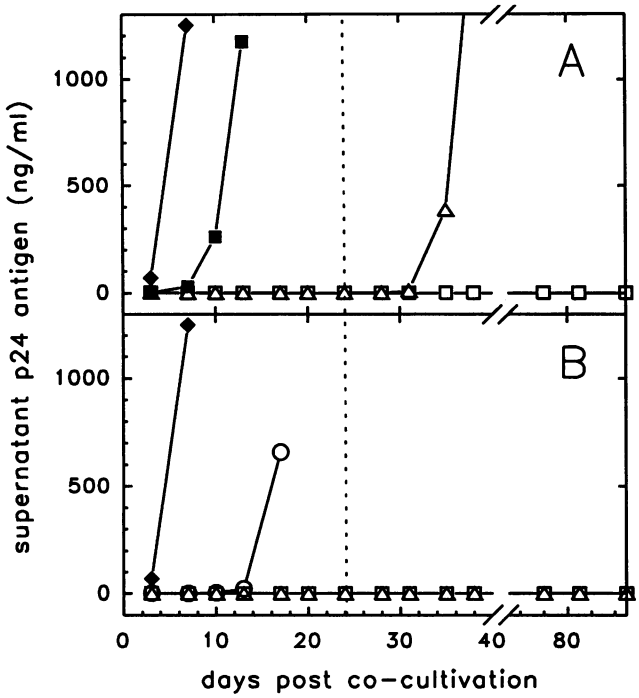

FIG. 2. Prevention of HIV-1 spread in vitro by U-90152, AZT, and the two combined. Cell-to-cell spread of HIV-1_{IIIB} in the presence of RT inhibitors was monitored by measuring supernatant p24 antigen levels as described in Materials and Methods. A viral replication burst accompanied by an abrupt increase in p24 levels (to >500 ng/ml) caused cell death, and cultures were not maintained further. The vertical broken line indicates day 24 postcocultivation, when drugs were no longer added to the cultures and were thus allowed to be diluted out over time. (A) Cultures grown in the presence of no drug (◆), 3 μM AZT (■), 1 μM U-90152 (△), and 3 μM U-90152 (□). (B) Cultures grown in the presence of U-90152 and AZT combined at a 1:1 ratio: ◆, no drug; ○, 0.3 μM total drug (i.e., 0.15 μM each U-90152 and AZT); △, 1 μM total drug; □, 3 μM total drug.

vitro. For determination of whether U-90152 blocked the spread of virus from infected to uninfected lymphocytes, approximately 500 MT-4 cells that had been infected with HIV-1_{IIIB} for 7 days were cocultivated with a 1,000-fold excess of uninfected cells in the presence or absence of U-90152 alone or in combination with AZT. Cultures were diluted every 3 to 4 days with fresh medium containing drug to maintain exponential growth of the infected control cultures. Under these conditions in the absence of drug, HIV-1 spreads rapidly, as indicated by the formation of multinucleated syncytia and an abrupt increase in p24 levels after day 3 of cocultivation (Fig. 2). At a concentration of 3 μM, AZT prolonged the interval to the rapid burst of viral replication to about 7 days. In contrast, U-90152 at 3 μM completely prevented the spread of HIV-1 (Fig. 2). U-90152 at 1 μM delayed viral spread until 42 days (data not shown). U-90152 was also very effective in combination with AZT (Fig. 2B); the combination of 0.5 μM U-90152 and 0.5 μM AZT totally prevented viral spread. This effect with the drugs in combination is not unexpected, because the BHAPs and AZT inhibit RT by different mechanisms and because previous work had indicated that AZT and BHAP U-87201E inhibit HIV-1 synergistically (3). In all cases in which viral growth was suppressed, cell viability was indistinguishable from

TABLE 2. Antiviral activity of U-90152 and nucleoside analog RT inhibitors against clinical HIV-1 isolates

Isolate	ED_{50} (μM) of[a]:			
	AZT	ddI	2',3'-Dideoxycytidine	U-90152[b]
1	4.2	3.9	0.02	<0.005
2	0.005	0.3	0.02	0.005
3	0.003	2.6	0.003	0.006
4	0.008	0.2	0.01	0.007
5	0.2	1.9	0.02	0.008
6	0.2	0.6	0.03	0.01
7	4.3	1.4	0.03	0.01
8	7.4	2.2	0.02	0.01
9	0.05	9.8	0.08	0.02
10	6.6	2.8	0.03	0.02
11	0.5	2.1	0.02	0.02
12	0.02	4.3	0.02	0.03
13	>5.0	15.5	0.05	0.03
14	3.5	16.7	0.15	0.04
15	1.0	2.5	0.01	0.04
16	0.1	6.0	0.02	0.05
17	0.02	3.1	0.04	0.06
18	2.9	3.1	0.03	0.08
19	2.1	6.5	0.05	0.10
20	0.1	2.7	0.06	0.11
21	0.05	9.5	0.12	0.18
22	0.6	15.4	0.07	0.16
23	3.1	5.8	0.03	0.69
24	0.02	0.4	<0.01	<0.005
25	0.005	0.05	<0.01	<0.005

[a] Determined with the median effect equation and triplicate wells at each of six drug concentrations.
[b] U-90152 at 100 μM caused less than 8% reduction in cell viability.

that in uninfected control cultures. At day 24 postcocultivation, cultures lacking viral growth were split, and drug was allowed to be diluted out of one of the cultures to monitor any renewed viral growth in the absence of drug (vertical broken line in Fig. 2). When drug was allowed to be diluted out of the culture previously treated with 1 μM U-90152, a rapid viral replication burst occurred within 10 more days (Fig. 2). The culture previously exposed to 3 μM U-90152 or the combination of 0.5 μM U-90152 and 0.5 μM AZT continued to grow without any evidence of infection after drug was removed. At day 85, cells from these two cultures were assayed for the presence of proviral DNA by PCR and reinfected to confirm their continued sensitivity to infection. Total cellular DNA was isolated and subjected to PCR amplification with primers with the sequence of the HIV-1 *gag* gene region as described in Materials and Methods. With a sensitivity of detection equal to approximately five copies of proviral HIV-1 DNA, no evidence of the HIV-1 genome was observed (data not shown).

These data suggest that the effective prevention of viral spread, coupled with death and/or dilution of the initially infected cells, removed all detectable virus from the cultures. Reinfection of the cultures lacking virus after drug treatment led to a rapid burst of supernatant p24 antigen production by day 7, so the cells remained sensitive to HIV-1 infection (data not shown). In a similar experiment (27), BHAP U-87201E prevented HIV-1 spread to an extent equal to that observed with AZT.

U-90152 potently blocks the replication of multiple laboratory-adapted or primary isolates of HIV-1 in vitro. The anti-HIV activity of U-90152 against multiple isolates of HIV-1 after acute infection of cells was also quantitated.

TABLE 3. Inhibition of nonnucleoside-resistant HIV-1 RT

RT mutant	IC_{50} (μM) of[a]:		
	U-90152	Nevirapine	L-697,661
None (wild type)	0.26 ± 0.04	2.7 ± 0.2	0.67 ± 0.19
Y181C	8.32 ± 0.70	>60[b]	>60[b]
K103N	7.70 ± 0.60	>60[b]	15.0 ± 4.1

[a] Determined from best fit of a competitive inhibition curve to triplicate datum points at each of 12 drug concentrations (± asymptotic standard deviation).
[b] Highest concentration tested.

U-90152 consistently showed a high potency against HIV-1_{IIIB}, typically 10- to 100-fold higher than that of AZT or U-87201E. For example, in HIV-1_{IIIB}-infected PBL, the ED_{50}s of U-90152, AZT, and U-87201E were approximately 0.1 to 1 nM, 1 nM, and 1 to 10 nM, respectively. Similarly, in MT-2 cells, the ED_{50}s of U-90152 and AZT for inhibiting HIV-1-induced cellular syncytia were about 10 nM and about 100 nM, respectively. U-90152 also blocked HIV-1 replication in primary monocyte-macrophage cultures, with an ED_{50} of approximately 20 nM against monocytotropic HIV-1_{JR-FL}.

The antiviral activity of U-90152 against a panel of clinical isolates was also evaluated and compared with that of three nucleoside RT inhibitors (AZT, ddI, and 2′,3′-dideoxycytidine) by methods described by Japour et al. (12). As shown in Table 2, U-90152 was a potent inhibitor of all the HIV-1 strains tested (ED_{50}s, <0.005 to 0.69 μM; mean, 0.066 ± 0.137 μM). U-90152 at 100 μM caused less than 8% decrease in PBL viability under similar conditions. U-90152 also potently blocked the replication of several HIV-1 isolates with high-level resistance to AZT, ddI, or both. On the basis of these data, U-90152 should potently inhibit most HIV-1 variants, including resistant isolates that arise upon treatment with nucleoside RT inhibitors.

U-90152 inhibits the RT activity of drug-resistant enzymes. The acquisition of HIV-1 resistance to U-90152, nevirapine, and the pyridinones occurs rapidly in vitro (7, 14, 16, 19). HIV-1 resistance to nevirapine and L-697,661 was found to be conferred mainly by substitutions at amino acids 103, 181, and 188 of RT, but these substitutions may not be the predominant means of the development of resistance to U-90152 (7). Since the development of drug resistance by HIV-1 will likely limit the clinical utility of these compounds, it is important to determine whether RT resistant to these compounds is also cross-resistant to U-90152. To investigate this question, we quantitated the drug sensitivities of recombinant HIV-1_{IIIB} RTs carrying the amino acid substitutions described above. Specifically, RTs carrying previously described substitutions conferring resistance to nevirapine or L-697,661 (tyrosine 181 to cysteine [Y181C]) and lysine 103 to asparagine [K103N]) were prepared. The sensitivities of mutant RTs to various inhibitors were determined in vitro with the poly(rA) · oligo(dT) template-primer (Table 3). The substitutions Y181C and K103N conferred some resistance to all the nonnucleoside inhibitors tested, suggesting that these amino acid changes define areas of drug-RT interactions common to U-90152, nevirapine, and L-697,661. U-90152 inhibited Y181C-substituted RT at an IC_{50} of 8.3 μM, while nevirapine and L-697,661 failed to achieve 50% inhibition at 60 μM, the highest concentration tested.

In summary, we have described a novel BHAP HIV-1 RT inhibitor. The potent antiviral activity of U-90152 against multiple primary HIV-1 isolates suggests that this compound may be useful in treating HIV-1 infection in vivo. The ability of U-90152 to completely suppress the spread of HIV-1_{IIIB} in cell cultures emphasizes this potential, particularly when U-90152 is used in combination with AZT. As with all other RT inhibitors described to date, the emergence of HIV-1 variants resistant to U-90152 is a concern. However, because U-90152 has significant inhibitory activity against the known nonnucleoside-resistant forms of RT, it may have utility despite the emergence of resistant HIV-1 carrying the RT substitution Y181C or K103N. Moreover, in laboratory animals, U-90152 exhibits good oral bioavailability, and serum drug levels well in excess of those required for in vitro antiviral activity can be safely maintained for prolonged periods (data not shown). Clinical trials with the mesylate salt of U-90152 have been initiated.

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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. **Baldwin, G. C., J. Fleischmann, Y. Chung, Y. Koyanagi, I. S. Y. Chen, and D. W. Golde.** 1990. Human immunodeficiency virus causes mononuclear phagocyte dysfunction. Proc. Natl. Acad. Sci. USA **87**:3933–3937.
3. **Campbell, T. B., R. K. Young, J. J. Eron, R. T. D'Aquila, W. G. Tarpley, and D. R. Kuritzkes.** Evaluation of the bisheteroarylpiperazine U-87201E in combination with zidovudine or didanosine against human immunodeficiency virus type 1 infection. J. Infect. Dis., in press.
4. **Chattopadhyay, D., D. B. Evans, M. R. J. Deibel, A. F. Vosters, F. M. Eckenrode, H. M. Einspahr, J. O. Hui, A. G. Tomasselli, H. A. Zurcher-Neely, R. L. Heinrikson, and S. K. Sharma.** 1992. Purification and characterization of heterodimeric human immunodeficiency virus type 1 (HIV-1) reverse transcriptase produced by *in vitro* processing of p66 with recombinant HIV-1 protease. J. Biol. Chem. **267**:14227–14232.
5. **Deibel, M. R., T. J. McQuade, D. P. Brunner, and W. G. Tarpley.** 1990. Denaturation/refolding of purified recombinant HIV reverse transcriptase yields monomeric enzyme with high enzymatic activity. AIDS Res. Hum. Retroviruses **6**:329–340.
6. **Dueweke, T. J., F. J. Kezdy, G. A. Waszak, M. R. Deibel, and W. G. Tarpley.** 1992. The binding of a novel bisheteroarylpiperazine mediates inhibition of human immunodeficiency virus type 1 reverse transcriptase. J. Biol. Chem. **267**:27–30.
7. **Dueweke, T. J., T. Pushkarskaya, S. M. Poppe, S. M. Swaney, J. Q. Zhao, I. S. Y. Chen, M. Stevenson, and W. G. Tarpley.** A novel mutation in bisheteroarylpiperazine-resistant HIV-1 reverse transcriptase confers increased sensitivity to other nonnucleoside inhibitors. Proc. Natl. Acad. Sci. USA, in press.
8. **Frank, K. B., G. J. Noll, E. V. Connell, and I. S. Sim.** 1991. Kinetic interaction of 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.
9. **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.

10. **Harada, S., Y. Koyanagi, and N. Yamamoto.** 1985. Infection of HTLV-III/LAV in HTLV-1-carrying MT-2 and MT-4 and application in a plaque assay. Science **229**:563–566.
11. **Hargrave, K. D., J. R. Proudfoot, K. G. Grozinger, E. Cullen, S. R. Kapadia, U. R. Patel, V. U. Fuchs, S. C. Mauldin, J. Vitous, M. L. Behnke, J. M. Klunder, K. Pal, J. W. Skiles, D. W. McNeil, J. M. Rose, G. Chow, M. T. Skoog, J. C. Wu, G. Schmidt, W. W. Engel, W. G. Eberlein, T. D. Saboe, S. J. Campbell, A. S. Rosenthal, and J. Adams.** 1991. Novel non-nucleoside inhibitors of HIV-1 reverse transcriptase. 1. Tricyclic pyridobenzo- and dipyridodiazepinones. J. Med. Chem. **34**:2231–2241.
12. **Japour, A. J., D. L. Mayers, V. A. Johnson, D. R. Kuritzkes, L. A. Beckett, J.-M. Arduino, J. Lane, R. J. Black, P. S. Reichelderfer, R. T. D'Aquila, C. S. Crumpacker, the RV-43 Study Group, and the AIDS Clinical Trials Group Virology Committee Resistance Working Group.** 1993. Standardized peripheral blood mononuclear cell culture assay for determination of drug susceptibilities of clinical human immunodeficiency virus type 1 isolates. Antimicrob. Agents and Chemother. **37**:1095–1101.
13. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merril, H. V. Vinters, and I. S. Y. Chen.** 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science **236**:819–822.
14. **Mellors, J. W., G. E. Dutschman, G.-J. Im, E. Tramontano, S. R. Winkler, and Y.-C. Cheng.** 1992. *In vitro* selection and molecular characterization of human immunodeficiency virus-1 resistant to non-nucleoside inhibitors of reverse transcriptase. Mol. Pharmacol. **41**:446–451.
15. **Merluzzi, V. J., K. D. Hargrave, M. Labadia, K. Grozinger, M. Skoog, J. C. Wu, C.-K. Shih, K. Eckner, S. Hattox, J. Adams, A. S. Rosenthal, R. Faanes, R. J. Eckner, R. A. Koup, and J. L. Sullivan.** 1990. Inhibition of HIV-1 replication by a nonnucleoside reverse transcriptase inhibitor. Science **250**:1411–1413.
16. **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.
17. **Ou, C.-Y., S. Kwok, S. W. Mitchell, D. H. Mack, J. J. Sninsky, J. W. Krebs, P. Feorino, D. Warfield, and G. Schochetman.** 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science **239**:295–297.
18. **Pauwels, R., K. Andries, J. Desmyter, D. Schols, M. J. Kukla, H. J. Breslin, A. Raeymaeckers, J. VanGelder, R. Woestenborghs, J. Heykants, K. Schellelens, M. A. C. Janssen, E. DeClerq, and P. A. J. Janssen.** 1990. Potent and selective inhibition of HIV-1 replication *in vitro* by a novel series of TIBO derivatives. Nature (London) **343**:470–474.
19. **Richman, D., C.-K. Shih, I. Lowy, J. Rose, P. Prodanovich, S. Goff, and J. Griffin.** 1991. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. Proc. Natl. Acad. Sci. USA **88**:11241–11245.
20. **Romero, D. L., M. Busso, C.-K. Tan, F. Reusser, J. R. Palmer, S. M. Poppe, P. A. Aristoff, K. M. Downey, A. G. So, L. Resnick, and W. G. Tarpley.** 1991. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. Proc. Natl. Acad. Sci. USA **88**:8806–8810.
21. **Romero, D. L., R. A. Morge, M. J. Genin, C. Biles, M. Busso, L. Resnick, I. Althaus, F. Reusser, R. C. Thomas, and W. G. Tarpley.** Bisheteroarylpiperazine (BHAP) reverse transcriptase inhibitors: structure activity relationships of novel substituted indole analogues and the identification of U-901525, a second generation clinical candidate. Submitted for publication.
22. **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.
23. **Sharma, S. K., D. B. Evans, A. F. Vosters, T. J. McQuade, and W. G. Tarpley.** 1991. Metal affinity chromatography of recombinant HIV-1 reverse transcriptase containing a human renin cleavable metal binding domain. Biotechnol. Appl. Biochem. **14**:69–81.
24. **Starchich, B. B., B. H. Hahn, G. M. Shaw, P. D. McNeely, S. Modrow, H. Wolf, E. S. Parks, W. P. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal.** 1986. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell **45**:637–648.
25. **St. Clair, M. H., C. A. Richards, T. Spector, K. J. Weinhold, W. H. Miller, A. J. Langlois, and P. A. Furman.** 1987. 3′-Azido-3′-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. Antimicrob. Agents Chemother. **31**:1972–1977.
26. **Tan, C.-K., C. Castillo, A. G. So, and K. M. Downey.** 1986. An auxiliary protein for DNA polymerase δ from fetal calf thymus. J. Biol. Chem. **261**:12310–12316.
27. **Vasudevachari, M. B., C. Battista, H. C. Lane, M. C. Psallidopoulos, B. Zhao, J. Cook, J. R. Palmer, D. L. Romero, W. G. Tarpley, and N. P. Salzman.** 1992. Prevention of the spread of HIV-1 infection with nonnucleoside reverse transcriptase inhibitors. Virology **190**:269–277.
28. **Wu, J. C., T. C. Warren, J. Adams, J. Proudfoot, J. Skiles, P. Raghavan, C. Perry, I. Potocki, P. R. 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.