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Pyridinone derivatives: Specific human immunodeficiency virus type 1 reverse transcriptase inhibitors with antiviral activity

(acquired immunodeficiency syndrome/antiviral agents/inhibition kinetics/synergy)

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ABSTRACT **Derivatives of pyridinones were found to inhibit human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) activity and prevent the spread of HIV-1 infection in cell culture without an appreciable effect on other retroviral or cellular polymerases. 3-{[(4,7-Dimethyl-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2(1*H*)-one (L-697,639) and 3-{[(4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2(1*H*)-one (L-697,661), two compounds within this series, had HIV-1 RT IC_{50} values in the range of 20–800 nM, depending upon the template–primer used. The most potent inhibition was obtained with rC·dG and dA·dT as template–primers. With rC·dG, reversible slow-binding noncompetitive inhibition was observed. [^{3}H]L-697,639 bound preferentially to enzyme–template–primer complexes. This binding was magnesium-dependent and saturable with a stoichiometry of 1 mol of [^{3}H]L-697,639 per mol of RT heterodimer. Displacement of [^{3}H]L-697,639 was seen with phosphonoformate. In human T-lymphoid-cell culture, L-697,639 and L-697,661 inhibited the spread of HIV-1 infection by at least 95% at concentrations of 12–200 nM. Synergism between 3′-azido-3′-deoxythymidine or dideoxyinosine and either of these compounds was also demonstrated in cell culture. Based upon their specificity for HIV-1 RT activity, template–primer dependence on potency and ability to displace [^{3}H]L-697,639; a tetrahydroimidazo[4,5,1-*jk*][1,4]-benzodiazepin-2(1*H*)-thione derivative R82150 and the dipyridodiazepinone BI-RG-587 appear to inhibit RT activity by the same mechanism as the pyridinones.**

Infection with the human immunodeficiency virus type 1 (HIV-1) causes progressive destruction of the immune system, which ultimately results in AIDS. An essential step in the life cycle of HIV-1 is reverse transcription of the viral RNA genome to produce a double-stranded DNA copy. This process is mediated by the virally encoded reverse transcriptase (RT). Thus, RT is a potential therapeutic target and, indeed, nucleoside analog inhibitors of RT, such as 3′-azido-3′-deoxythymidine (AZT) and dideoxyinosine (ddI), are clinically effective drugs for treating HIV-1 infection (1, 2). However, their effectiveness is limited by toxicities, which may reflect inhibition of cellular polymerases and/or alteration of nucleoside pools, given that the nucleoside analogs are phosphorylated (in competition with natural nucleosides) to their active form by cellular kinases (3, 4). The emergence of AZT-resistant virus (5) further emphasizes the need to develop selective RT inhibitors that can be used either alone or in combination with nucleoside analogs. The development of specific RT inhibitors is the subject of this communication.

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METHODS

Chemical Synthesis of RT Inhibitors. The aminomethylene derivatives 5-ethyl-6-methyl-3-[(phthalimidomethyl)amino]-pyridin-2(1*H*)-one (L-345,516), 3-{[(1,3-benzoxazol-2-yl)-methyl]amino}-5-ethyl-6-methylpyridin-2(1*H*)-one (L-696,040), 3-{[(4,7-dimethyl-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2(1*H*)-one (L-697,639), and 3-{[(4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino}-5-ethyl-6-methylpyridin-2(1*H*)-one (L-697,661) were synthesized by alkylation of 3-amino-5-ethyl-6-methylpyridin-2(1*H*)-one with either *N*-hydroxymethylphthalimide or the appropriate 2-halomethylbenzoxazole. Requisite aminopyridinone was obtained from condensation of 3-formyl-2-pentanone with nitroacetamide followed by catalytic reduction.

Synthesis of ethylene derivative 5-ethyl-6-methyl-3-(2-phthalimidoethyl)pyridin-2(1*H*)-one (L-693,593) began with the condensation of 3-formyl-2-pentanone and cyanoacetamide to give 3-cyano-5-ethyl-6-methylpyridin-2(1*H*)-one. Reaction of this cyanopyridinone with $POCl_3$ followed by methanolysis and reduction with diisobutylaluminum hydride led to 5-ethyl-2-methoxy-6-methylpyridine-3-carboxaldehyde. The aldehyde function was treated with trimethylsilyl cyanide, and the resulting cyanohydrin was reduced with lithium aluminum hydride to the amino alcohol. After conversion of the amino group to phthalimide, demethylation of the 2-methoxy group and alcohol dehydration was accomplished in one step by heating with pyridine hydrochloride. Catalytic reduction of the olefin formed yielded ethylene analog L-693,593. The structures of all pyridinones synthesized are consistent with their NMR spectra, and all compounds gave an acceptable combustion analysis (within 0.4%).

HIV-1 RT Assays. *rC·dG.* The HIV-1 RT assay was done in a reaction mixture (50 μl) containing 55 mM Tris·HCl (pH 8.2), 30 mM KCl, 30 mM $MgCl_2$, 1 mM dithiothreitol, bovine serum albumin at 1 mg/ml, $rC{\cdot}dG_{(12-18)}$ at 20 μg/ml (Pharmacia 27-7944), 50 μM EGTA, 8 μM [^{3}H]dGTP, 0.01% (vol/vol) Triton X-100, and 0.63 nM recombinant HIV-1 RT (90–95% pure heterodimer; ref. 6). The remainder of the procedure was done as described (7). The concentration that caused 50% inhibition (IC_{50}) is stated as the mean of at least three experiments.

Abbreviations: RT, reverse transcriptase; HIV-1, human immunodeficiency virus type 1; AZT, 3′-azido-3′-deoxythymidine; ddI, dideoxyinosine; SIV, simian immunodeficiency virus; AMV, avian myeloblastosis virus; Mo-MLV, Moloney murine leukemia virus; PFA, phosphonoformate; CIC_{90} and CIC_{95}, cell culture inhibitory concentration that inhibited the spread of HIV-1 infection 90% and 95%, respectively.

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dA·dT. Final conditions for the dA·dT assay were the same as for the rC·dG assay, except that 3 mM $MgCl_2$, 39 μM [^{3}H]TTP; 1.7 μM poly(dA)/0.17 μM oligo(dT) (Supertechs, Bethesda, MD), and 2.6 nM HIV-1 RT were used (KCl was omitted).

rA·dT. The final assay conditions for the rA·dT assay were the same as for the dA·dT assay, except that 80 mM KCl, 12 mM $MgCl_2$, 10 μM [^{3}H]TTP, 1 mM dithiothreitol, rA·dT$_{(12-18)}$ at 1.5 μg/ml (Pharmacia), and 0.063 nM HIV-1 RT were used.

Other Enzyme Assays. Recombinant HIV-2 RT assays were done by using the HIV-1/rC·dG conditions. Simian immunodeficiency virus (SIV) strain mac251 was lysed in a buffer containing 0.1% Triton X-100, sonicated, and added to assays under the HIV-1 RT/rA·dT assay conditions. Avian myeloblastosis virus (AMV) RT (Molecular Genetic Resources, Tampa, FL) assays were done under the HIV-1/rC·dG assay conditions. Moloney murine leukemia virus (Mo-MLV) was propagated in NIH 3T3 cells, and 10 μl of supernatant fluid was added to assays as described (8). Calf thymus DNA polymerase α was purified by immunoaffinity chromatography (9) and assayed as described (7). HeLa cell DNA polymerase assays were done under standard conditions with the following divalent cations/template–primers: (*i*) β, 0.5 mM $MnCl_2$/dA·dT; (*ii*) γ, 0.5 mM $MnCl_2$/rA·dT; and (*iii*) δ, 4 mM $MgCl_2$/dA·dT. The other polymerase assays (Klenow fragment, *Micrococcus luteus* DNA polymerase and *Escherichia coli* RNA polymerase) used 12 mM $MgCl_2$ and activated DNA. The HIV-1 RNase H assay used [^{3}H]rG·dC as substrate (10) and HIV-1 RT (see above).

HIV-1 RT Kinetic Assay. The 50-μl reaction mixture contained 50 mM Tris·HCl (pH 8.2), 100 mM KCl, 6 mM $MgCl_2$, 0.2% polyethylene glycol ($M_r \approx 8000$), 10 mM dithiothreitol, rC·dG at 14.7 μg/ml, and 20 μM [8,5′-^{3}H]dGTP (9 Ci/mmol; 1 Ci = 37 GBq). The reaction was initiated with 0.34–3.4 nM HIV-1 RT and incubated at 37°C. At the indicated times, reaction mixtures were made 4.8 mM in sodium pyrophosphate, and a 25-μl aliquot was applied onto Whatman 3 MM 25-mm filter disks. The disks were placed in 10% (vol/vol) trichloroacetic acid/50 mM sodium pyrophosphate (10 ml/filter) on ice and then washed four times with ice-cold 5% (vol/vol) trichloroacetic acid (≈8 min per wash), two times with cold ethanol (95%), and once with diethyl ether.

[^{3}H]L-697,639 Binding Studies. The 120-μl reaction mixture contained 50 mM Tris·HCl (pH 8.2), 100 mM KCl, 6 mM $MgCl_2$, 0.2% polyethylene glycol ($M_r \approx 8000$), 10 mM dithiothreitol, rC·dG at 14.7 μg/ml, 20 μM dGTP, 100 nM [^{3}H]L-697,639 (2.9 Ci/mmol). The reaction was initiated with 10 nM HIV-1 RT and incubated at 37°C for 20 min. After incubation, a 100-μl aliquot of the reaction mixture was withdrawn and applied to a 1-ml Sephadex G-50 spin column equilibrated in assay buffer and immediately centrifuged for 2 min at 2000 × *g* at ambient temperature (≈25°C). For columns that were not prespun, the elution volume was 600–650 μl (prespin had no effect on results). Aliquots were removed to determine total radioactivity as well as total enzyme activity (using rA·dT system; 2.4 sec^{-1} = turnover number) as an indicator of total RT recovery. Enzyme recovery ranged from 25–50% under these conditions in several experiments, and the results were normalized to individual enzyme recovery determinations.

Antiviral Assays. H9 human T-lymphoid cells (11) were grown in RPMI 1640 medium/10% heat-inactivated fetal bovine serum. In some experiments, cells were grown in the presence of serial 2-fold dilutions of inhibitor in 96-well culture dishes for 1 day before infection with HIV-1 strain IIIb (12). Cultures were infected at a low initial multiplicity of infection (≤1%) and were maintained in the continued presence of inhibitor for 13–14 days. At this time, culture supernatants were harvested, and the spread of infection through the culture was assessed by HIV-1 p24 gag antigen ELISA (Coulter). Indirect immunofluorescence assays with serum from an HIV-1-infected individual confirmed the direct relationship between p24 accumulation and virus spread. Control cultures in the absence of inhibitor were 100% infected within 7–10 days. Antiviral potencies were expressed as the concentration that inhibited the spread of HIV-1 infection by ≥95% (CIC_{95}).

In most experiments, H9 cell cultures were infected at a low multiplicity in the absence of inhibitor. Cultures were incubated overnight, and cells were then washed and distributed to 96-well culture dishes in the presence of inhibitor. H9 cell cultures were maintained for 10 days, and virus spread was assessed by HIV-1 p24 ELISA. Assays using MT4 human T-lymphoid cells (13) were similarly performed, except that virus spread was determined 4 days after infection. Human peripheral blood mononuclear cells were obtained from fresh plasmapharesis residues by centrifugation using Ficoll/Hypaque. Nonadherent cells were collected and incubated for 5 days in RPMI 1640 medium/10% heat-inactivated fetal bovine serum in the presence of phytohemagglutinin. Activated lymphocytes were subsequently maintained in medium containing recombinant human interleukin 2 (1000 units/ml; DuPont). Assay conditions with peripheral blood lymphocytes and HIV-1 strain IIIb were similar to those used with MT4 cells, except that virus spread was assessed 11 days after infection. In addition to HIV-1 strain IIIb, the following laboratory HIV-1 isolates of diverse origin were also tested: MN (14, 15); WMJ-2 (16); RF (17); and RUTZ. The antiviral potency against the patient isolates A018A/H112-2 and A018C/G910-6 (5) was determined in CEM cells (18). These assays were similar to those using MT4 cells.

Synergy. The antiviral potencies (MT4 cells/HIV-1 strain IIIb) of combinations of inhibitors were analyzed for potential synergism by the method of Elion *et al.* (19). Fractional inhibitory concentrations were derived and plotted, as described. Data were also analyzed using the median effect principle (20).

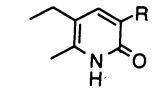
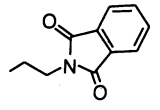
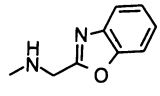
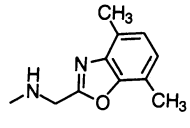
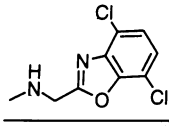
RESULTS AND DISCUSSION

Compound L-345,516 (Table 1) was discovered in a screening program designed to identify direct and specific inhibitors of HIV-1 RT. However, this aminomethylphthalimide derivative was hydrolytically unstable (at physiologic pH, $t_{1/2} \approx 2$ hr) and, therefore, not appropriate for further development. Initiation of a synthetic program based upon L-345,516 with the object of obtaining more stable analogs led to L-693,593 and L-696,040 (Table 1). Further structural modifications of the latter compound yielded the more potent derivatives, L-697,639 and L-697,661 (Table 1). The inhibitory potency toward HIV-1 RT (IC_{50}) of 18 stable pyridinone RT inhibitors, in addition to those listed in Table 1, correlated well (r^2 = 0.92, slope = 0.99) with their efficacy in inhibiting viral spread in H9 human T-lymphoid cell culture (Table 1, CIC_{95}), suggesting that the antiviral effect of these compounds is mediated via direct inhibition of RT.

Unlike nucleoside analogs, the pyridinone RT inhibitors showed unique HIV-1 RT specificity. In contrast to their nanomolar inhibitory potencies toward HIV-1 RT, L-697,639 and L-697,661 at 300 μM inhibited the following enzymes by <20%: SIV (SIV_{mac251}) RT; HIV-2 RT; AMV RT; Mo-MLV RT; HIV-1 RNase H; calf thymus DNA polymerase α; human DNA polymerases β, γ, and δ; Klenow fragment; *M. luteus* DNA polymerase, and *E. coli* RNA polymerase.

The inhibitory potencies of the pyridinones toward HIV-1 RT were dependent upon the template–primer substrates. For example, L-697,639 had similar potencies with rC·dG and dA·dT (IC_{50} = 20 ± 4 nM and 20 ± 2 nM, respectively) but was significantly weaker (IC_{50} = 600 ± 70 nM) with rA·dT. Likewise, L-697,661 exhibited IC_{50} values of 19 ± 4, 30 ± 8,

Table 1. Structure–activity relationships for inhibition of HIV-1 RT activity and inhibition of HIV-1 infection in cell culture

R =	Name	Potency, nM: HIV-1 RT, IC_{50}*	Potency, nM: HIV-1 spread activity, CIC_{95}†
	L-345,516	30	‡
	L-693,593	3700	40,000
	L-696,040	210	900
	L-697,639	20	150
	L-697,661	19	100

*The IC_{50} is stated as the mean of at least three experiments.
†Antiviral activity was determined using HIV-1 strain IIIb in H9 cell culture.
‡Hydrolytic instability precluded a reliable determination of this value.

and 830 ± 90 nM with the rC·dG, dA·dT, and rA·dT template–primers, respectively. These compounds, therefore, inhibit both RNA-directed and DNA-directed DNA synthesis. The observation that inhibitory potency was a function of the composition of the template–primer (at similar ratios of template–primer concentration to their respective K_m values) suggests that the pyridinones inhibit RT by binding to enzyme–template–primer complexes.

Studies of the inhibition of HIV-1 RT by L-697,639 with rC·dG revealed nonlinear progress curves (data not shown), consistent with that expected for a slow-binding, reversible inhibitor (21, 22). Preincubation of enzyme with rC·dG and L-697,639 for 5 min resulted in linear progress curves (data not shown). Reversible inhibition was directly demonstrated by the observation that removal of inhibitor (500 nM) from a reaction mixture (by dilution or Sephadex G-25 chromatography) resulted in total restoration of RT activity. Reverse-phase chromatography of a complete reaction mixture containing equimolar (500 nM) concentrations of enzyme and L-697,639 yielded only unchanged L-697,639, indicating that, unlike AZT triphosphate and dideoxynucleoside triphosphates, L-697,639 does not undergo an enzymatically catalyzed transformation. Lineweaver–Burk analysis of the limiting steady-state velocities as a function of dGTP at various L-697,639 concentrations indicated the inhibition was noncompetitive with respect to dGTP (apparent K_i = 10 nM) and was also noncompetitive with respect to rC·dG template–primer (data not shown). Moreover, using globin mRNA·dT as the template–primer, noncompetitive inhibition was observed with all four deoxynucleoside triphosphates. In contrast to the time-dependent inhibition observed with rC·dG, time-dependent inhibition was not apparent with rA·dT.

The direct binding of [^{3}H]L-697,639 to HIV-1 RT was studied. Maximal binding took place during catalysis in the presence of saturating concentrations of dGTP and rC·dG (Fig. 1), consistent with the noncompetitive patterns of inhibition (see above). The observation that [^{3}H]L-697,639 bound to enzyme–template–primer complexes and not to either template–primer alone or enzyme alone (under these conditions) directly confirmed the inferential evidence stated above that binding to enzyme–template–primer complexes was involved in RT inhibition. The binding of [^{3}H]L-697,639 to HIV-1 RT under steady-state conditions was found to be saturable (0.9 ± 0.2 mol/mol of heterodimer) and linearly dependent upon RT concentration; the approximate half-maximal binding concentration was 20 ± 10 nM. Direct observation of slow binding (Fig. 2*A*) yielded an apparent

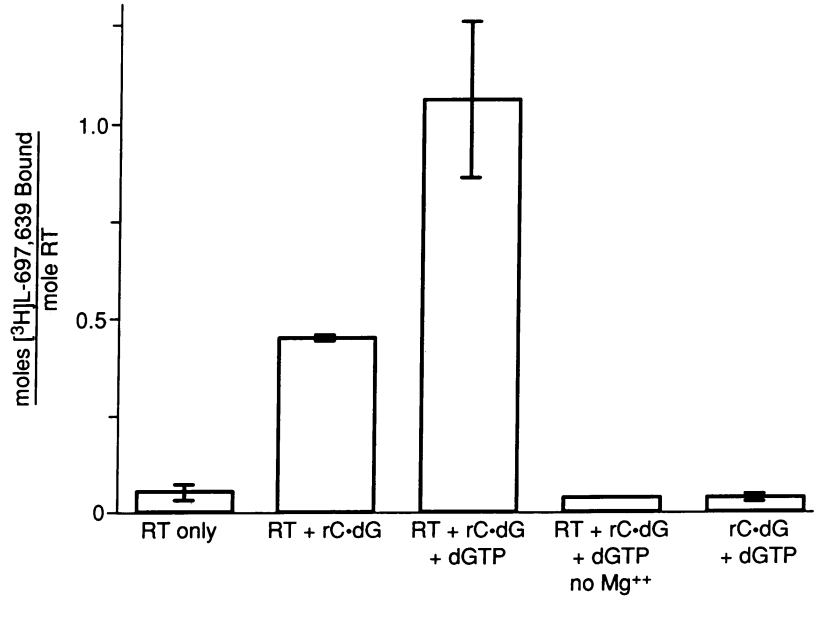


FIG. 1. Binding of [^{3}H]L-697,639 to HIV-1 RT in the presence and absence of substrates. Reactions were done as described. As indicated, under some conditions, enzyme, rC·dG, dGTP or Mg^{2+} (1 mM EDTA in no Mg^{2+} samples) was absent in the reaction. The range of duplicate experiments is indicated.

pseudo first-order rate constant, $k = 0.27 \pm 0.05$ min^{-1} at 50 nM L-697,639, consistent with the value of 0.19 ± 0.06 min^{-1} estimated for this rate constant for the approach of the steady-state velocity to its final value in the presence of 50 nM L-697,639. Studies of displacement of [^{3}H]L-697,639 by unlabeled inhibitor yielded a value of 0.08 ± 0.02 min^{-1} ($t_{1/2} \approx 9$ min) for the first-order rate constant for dissociation of L-697,639 from the enzyme–template–primer–inhibitor complex (Fig. 2*B*).

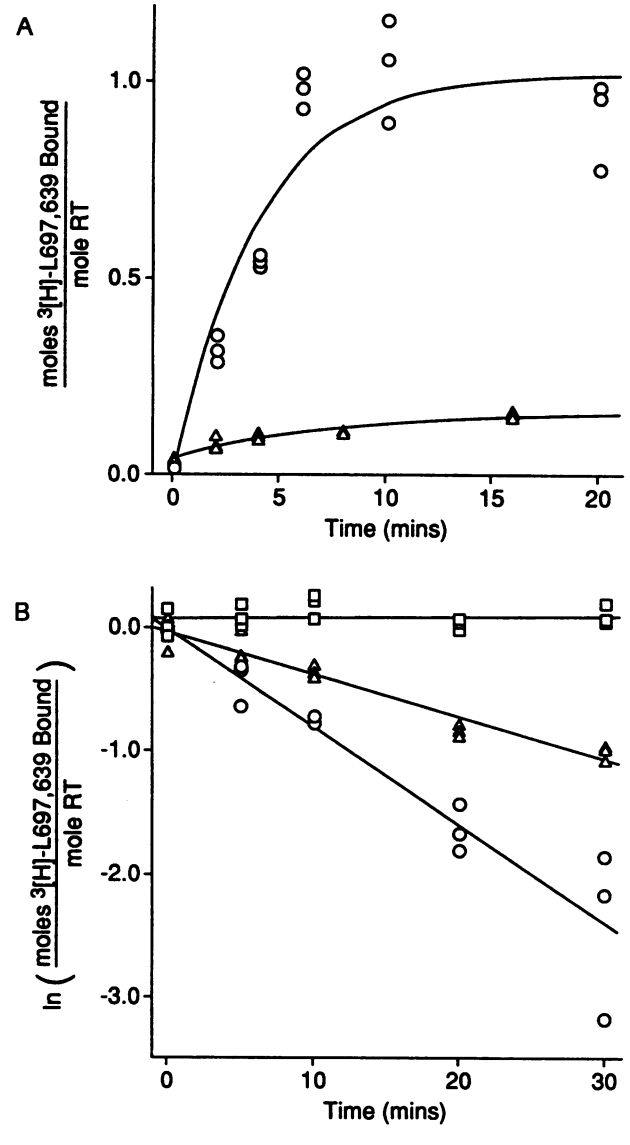


FIG. 2. The rates of formation (*A*) and dissociation (*B*) of [^{3}H]L-697,639–HIV-1 RT complexes during catalytic turnover. (*A*) Reaction mixtures contained a full complement of saturating levels of rC·dG and dGTP, and 50 nM [^{3}H]L-697,639 in the presence (△) or absence (○) of 30 μM PFA (Sigma). The reaction was initiated with enzyme (10 nM), and aliquots were withdrawn as a function of time, as indicated, and processed as described. Triplicate data points are shown and are fitted to an equation that describes the pseudo first-order association of ligand to enzyme. (*B*) Reaction mixtures contained a full complement of saturating levels of rC·dG and dGTP, and 100 nM [^{3}H]L-697,639. The reaction was initiated with enzyme and incubated for 20 min at 37°C. Either buffer (□) (10% dilution of total volume), unlabeled L-697,639 (○) (to a final concentration of 1 μM), or PFA (△) (200 μM final concentration) was then added. Aliquots were then withdrawn at the indicated times and processed as described. Triplicate data points are shown and fitted to an equation that describes the first-order dissociation of ligands from enzyme.

The putative pyrophosphate analog, phosphonoformate (PFA) displaced [^{3}H]L-697,639 from RT complexes when present in reaction mixtures initiated with RT (Fig. 2*A*) as well as when added to reaction mixtures containing preformed [^{3}H]L-697,639 RT complexes (Fig. 2*B*). PFA appeared to decelerate and not accelerate the dissociation rate of [^{3}H]L-697,639–RT complexes (Fig. 2*B*). The current data base is insufficient to determine whether the displacement of [^{3}H]L-697,639 from RT complexes, mediated by PFA, involves allosteric or direct (via partial overlap of the respective binding sites) interactions. Further studies are required to determine the role of Mg^{2+} (Fig. 1) in the binding of [^{3}H]L-697,639 to RT and the point in the reaction sequence where polymerization is inhibited by L-697,639 and related molecules.

In H9 human T-lymphoid cell culture, infected before treatment with inhibitor (Table 2), these compounds inhibited the spread of HIV-1 strain IIIb infection (CIC_{95}) at 100 and 200 nM, respectively. These potencies are comparable to those reported in Table 1, where inhibitor was added before infection. In MT4 human T-lymphoid cells, both compounds displayed CIC_{95} values of 25–50 nM (Table 2). L-697,639 and L-697,661 were also active in phytohemagglutinin-stimulated human peripheral blood lymphocytes maintained in interleukin 2, where CIC_{90} values of 100 nM and 50 nM, respectively, were obtained. Thus, these compounds are potent in the primary target cells of the virus. In contrast to AZT (23), the potency of the pyridinone inhibitors did not vary substantially among cell types. This difference may derive from the requirement that AZT, unlike L-697,639 or L-697,661, be activated by the host cell. In addition to the antiviral activity against HIV-1 strain IIIb, L-697,639 and L-697,661 were comparably potent against several diverse laboratory strains of HIV-1 and against two primary patient isolates (Table 2). The two clinical isolates, A018A/H112-2 and A018C/G910-6, are AZT-sensitive and AZT-resistant viruses, respectively, from an AZT-treated individual (5). These viruses remained sensitive to L-697,639 and L-697,661. Consistent with the specificity of the pyridinone inhibitors toward HIV-1 RT, these compounds did not inhibit SIV_{mac251} infection in cell culture, nor were they cytotoxic at concentrations as high as 60 μM.

Because the mode of action of the pyridinone RT inhibitors differs from that of nucleoside analogs, we were prompted to determine the potential for interactions between compounds of these classes. Combinations of L-697,639 and AZT or ddI were prepared, and the antiviral potencies of these mixtures were determined in MT4 cell culture with HIV-1 strain IIIb. These analyses indicated substantial synergism in the combined use of L-697,639 and either AZT or ddI (Fig. 3). The cooperativity indices (19) were each ≈0.5. Similar indices were obtained on

Table 2. Antiviral properties of L-697,639 and L-697,661 in cell culture: comparison with AZT

HIV-1	Cell type	CIC_{95}, nM		
		L-697,639	L-697,661	AZT
IIIb	H9	100	200	20,000
IIIb	MT4	25–50	25–50	12–25
IIIb	PBL	50	100	200
MN	MT4	100	50	100
WMJ-2	MT4	200	100	25
RF	MT4	50	50	ND*
RUTZ	MT4	100	50	ND*
A018A/H112-2	CEM	25	25	12
A018C/G910-6†	CEM	12	12	1600

PBL, human peripheral blood lymphocytes.
*Not determined.
†AZT-resistant.

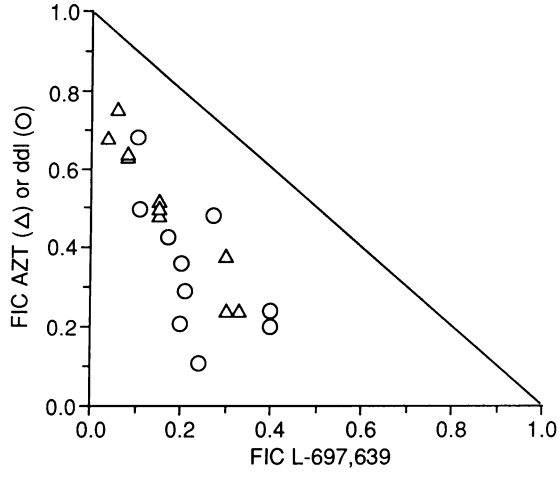


FIG. 3. Synergism in the combined use of L-697,639 and AZT or ddI. Fractional inhibitory concentrations (FICs) of combination of L-697,639 and AZT (Δ) or L-697,639 and ddI (○) are shown. The diagonal line represents additivity.

analysis by using the median effect principle of Chou and Talalay (20). Experiments using L-697,661 revealed comparable synergism with the two nucleoside analogs.

Recently, two other groups have identified submicromolar, HIV-1-specific, nonnucleoside RT inhibitors, R82150 (24) and BI-RG-587 (25), which are structurally distinct from those reported here. Using the HIV-1 RT assays with rC·dG, dA·dT, and rA·dT, we observed that BI-RG-587 had IC_{50} values of 73, 47, and 3920 nM, respectively, and R82150 had IC_{50} values of 70, 110, and 5100 nM, respectively. This pattern of template–primer selectivity is similar to that exhibited by the pyridinone compounds. In contrast to previous results showing that R82150 was not a potent inhibitor of polymerization with dC·dG (26), the present data with dA·dT demonstrate that all three chemical classes are potent inhibitors of DNA-directed DNA synthesis. BI-RG-587 and R82150 also displaced [^{3}H]L-697,639 from the HIV-1 RT–template–primer complex. These results suggest that the three groups of specific HIV-1 RT inhibitors may be structurally diverse members of a common pharmacological class. This conclusion was independently obtained from experiments demonstrating that RT from HIV-1 resistant to L-693,593 was resistant to L-697,639, L-697,661, BI-RG-587, and R82150 but not nucleoside analog triphosphates or PFA (27).

Safety and tolerability studies with L-697,639 and L-697,661 have been initiated in humans. In studies to determine whether the potential exhibited by these pyridinones will be realized clinically, the demonstration of synergism in cell culture with combinations of these compounds and nucleoside analogs is important. In addition to benefits that may accrue in increased efficacy and reduced toxicity, the concurrent use of these agents with nucleoside analogs may ultimately prove most useful in minimizing the likelihood of the emergence of drug-resistant virus variants.

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