sients associated with the recovery of tension from a quick release, then it may have a bearing on the interpretation of such transients described by some models of cross-bridge behavior (7).

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3. Lattice spacing, a, refers to the center-to-center distance between adjacent myosin filaments. This value may be calculated from the separation of the 10 and 11 reflections from the center of the pattern, according to: $a = 2\ell/d_{11} = 2\ell/d_{10}\sqrt{3}$, where d_{10} is the 10 separation, d_{11} is the 11 separation, and ℓ is the camera constant for the experiment, typically 5.9×10^{-10} m^2.
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5. It could be suggested that the anomalous behavior of the recovery phase of the lattice spacing with respect to the constant volume theory might be due to the relatively small length of fiber sampled by the laser diffractometer (250 μm) compared to the 4-mm region sampled by x-ray diffraction. We do not believe this to be the explanation because (i) lattice expansion during the recovery from a stretch or compression during recovery from a release was observed in all 27 experiments conducted and is therefore unlikely to have arisen from the random selection of a fiber region in which sarcomere length changes behaved anomalously in each case; and (ii) typical fiber lengths were 6 to 7 mm, so that the x-ray beam sampled more than 50% of the total fiber length. Since in the case of a release, for example, the discharge of isometric tension would be expected to cause relaxation of any series compliance in the system, then the recovery of tension to a new isometric plateau after the release was completed should be accompanied by a reextension of such series compliance with a consequent reduction of total fiber length. The accompanying compression of the filament lattice that we recorded could only be accounted for by a constant volume theory if more than 50% of the fiber length were behaving anomalously, which seems highly improbable. A similar argument may also be applied in the case of a stretch.
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Inhibition of HIV-1 Replication by a Nonnucleoside Reverse Transcriptase Inhibitor

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A series of dipyridodiazepinones have been shown to be potent inhibitors of human immunodeficiency virus–1 (HIV-1) reverse transcriptase (RT). One compound, BI-RG-587, had a K_i of 200 nanomolar for inhibition of HIV-1 RT that was noncompetitive with respect to deoxyguanosine triphosphate. BI-RG-587 was specific for HIV-1 RT, having no effect on feline and simian RT or any mammalian DNA polymerases. BI-RG-587 inhibited HIV-1 replication in vitro as demonstrated by in situ hybridization, inhibition of protein p24 production, and the lack of syncytia formation in cultured human T cell lines and freshly isolated human peripheral blood lymphocytes. Cytotoxicity studies of BI-RG-587 on human cells showed a high therapeutic index (>8000) in culture.

THE REVERSE TRANSCRIPTASE (RT) of HIV-1 is required for early proviral DNA synthesis and is thus a prime target for antiviral therapy against acquired immunodeficiency syndrome (AIDS) (*1*). Inhibition of the RT-catalyzed polymerization of DNA from viral RNA inhibits virus replication. In most cases, effective inhibitors are nucleoside analogs that are converted to triphosphates by cellular enzymes and act as chain terminators (*2, 3*). The first approved drug for use in HIV-1 infection was the nucleoside analog azidothymidine (AZT) (*4*). Although this compound has been shown to be of benefit in HIV-1–infected individuals, there are toxic side effects associated with its use (*5*), and complete inhibition of viral replication is usually not achieved (*6*). In addition, emergence of AZT-resistant strains may complicate its use in long-term therapy (*7*).

From a previous program involving the synthesis of muscarinic receptor antagonists, we identified a series of dipyridodiazepinone inhibitors of HIV-1 RT polymerase. Compounds were optimized on the basis of potency against HIV-1 RT with a favorable pharmacokinetic profile and lack of ancillary pharmacologic activities. The compound BI-RG-587 is a potent inhibitor of HIV-1 RT and does not have muscarinic or benzodiazepine (peripheral and central) activities (*8*). Further analysis has shown similar potency of BI-RG-587 on the inhibition of HIV-1 in cell culture. Since BI-RG-587 is not a nucleoside analog structure, it is hoped that the clinical side effects observed in AIDS patients treated with nucleoside-based chain terminators of RT such as AZT and 2′,3′-dideoxyinosine (ddI) will not be observed.

BI-RG-587 was synthesized as described in the legend to Fig. 1. The K_i value for RT inhibition by BI-RG-587 was 200 nM, and inhibition was noncompetitive with respect to deoxyguanosine triphosphate (dGTP) (Fig. 2). The noncompetitive character of this inhibition was consistent with, but not proof for, an allosteric binding site on the binary (RT:template-primer) or ternary (RT:template-primer:dGTP) complex of the enzyme. Template-primer binds before deoxynucleotide (*9*), therefore a compound that binds when dGTP is bound apparently occupies a site distinct from the template-primer site. In addition, BI-RG-587 inhibits RT regardless of whether RT was assayed with poly(rA):oligo(dT) [median inhibition concentration (IC_{50}) = 100 nM] or a het-

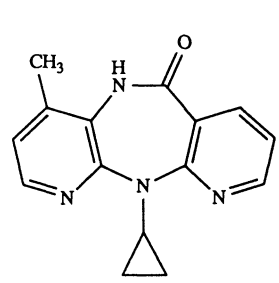

Fig. 1. BI-RG-587. BI-RG-587 was synthesized in four steps starting from 2-chloro-4-methyl-3-nitropyridine. This was reduced to 3-amino-2-chloro-4-methylpyridine, which was then condensed with 2-chloronicotinic acid chloride to form the amide. Reaction with cyclopropylamine followed by cyclization provided the desired compound, BI-RG-587.

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eropolymer template-primer (*8*).

The structure activity of the series of tricyclic diazepinones correlated with regard to RT inhibition potency as follows: dibenzo- < monopyrido- < dipyrido-diazepinones. Furthermore, the positional requirement for the nitrogen atoms in the rings was found to be absolute. A detailed analysis of the structure activity will be published elsewhere. In addition, there was direct correlation with the ability of compounds in this series to inhibit HIV-1 RT and HIV-1 replication in vitro. This is highly suggestive of RT as the target of BI-RG-587 and analogs in this series.

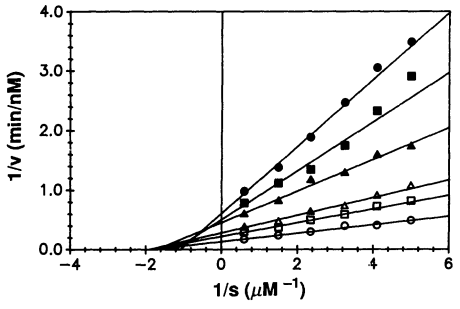

Fig. 2. Noncompetitive inhibition was determined from a double reciprocol plot for inhibition of RT by BI-RG-587 with dGTP as variable substrate [poly(rC):oligo(dG) was fixed and saturating]. At the two highest concentrations of BI-RG-587, the intercept on the 1/s axis appears to increase. Although these changes are not statistically significant, they might suggest an additional low-affinity BI-RG-587 site on RT. Inhibition, however, is complete before saturation of this site. A replot of ordinal intercepts versus concentration of BI-RG-587 is linear and yields a K_i of 200 nM. For dGTP, Michales constant (K_m) is 570 nM and for RT polymerase, k_{cat} is 15 min^{-1}. The enzyme was assayed with 40 nM primer sites on poly(rC):oligo(dG) and variable concentrations of [^{3}H]dGTP as substrates in a reaction mixture consisting of 50 mM tris, 1 mM dithiothreitol (DTT), 2 mM $MgCl_2$, 50 mM glutamic acid, and 0.02% (v/v) 3-[(3-chloamidopropyl)-diethylammonio]-1-propanesulfonic acid (CHAPS) at pH 7.8.

BI-RG-587 was remarkably specific for the HIV-1 RT. It did not inhibit the RT from simian immunodeficiency virus or feline leukemia virus. In addition, BI-RG-587 did not inhibit calf thymus DNA polymerase α or human DNA polymerase α, β, γ, or δ (Table 1). Several other enzymes, including HIV-1 protease, human plasma renin, and HSV-1 ribonucleotide reductase, were also unaffected by this compound (*8*). BI-RG-587 caused partial inhibition of HIV-1 ribonuclease (RNase) H activity. This partial inhibition of RNase H may reflect binding of BI-RG-587 to a site distant from the active site. These observations suggest that a conformational change caused by the binding of BI-RG-587 may influence the polymerase site of RT to a greater degree than at the RNase H site. BI-RG-587 inhibited HIV-1 replication in c8166 (*10, 11*) T cell cultures (Fig. 3). The IC_{50} against HIV-1 strain IIIb was 40 nM with a maximum inhibition (I_{max}) of 100% as determined by inhibition of cytopathic effect (CPE) and 10 nM with an I_{max} of 100% as determined by inhibition of p24 production. Viability of c8166 cells was determined by means of a tetrazolium salt (MTT) metabolic assay (*12*). This assay shows 50% cytotoxicity of BI-RG-587 at 321,000 nM providing a selectivity ratio in vitro of 8025.

In a separate set of experiments, the IC_{50} of BI-RG-587 was determined against HIV-1_{IIIb} and two other HIV-1 isolates, the Haitian isolate, HIV-1_{RF} (*10, 13*), and a clinical isolate from an asymptomatic hemophiliac patient, HIV-1_{UMGL}. All three viruses were inhibited equally well by BI-RG-587 (IC_{50} = 32.0 ± 2.6 to 42 ± 3.8 nM with an I_{max} ranging from 100.7 to 104.0%). Despite the similar sensitivities of HIV-1 isolates to BI-RG-587, this compound has no activity against HIV-2_{ROD} at concentrations up to 100,000 nM (*8*). BI-RG-587 did not inhibit cytopathogenicity in HeLa cells infected with rhinovirus 54, poliovirus I, influenza A virus–induced CPE on bovine kidney cells, or vaccinia virus–induced CPE on human lymphoid cells. BI-RG-587 inhibited the replication of HIV-1 primary isolates from four patients undergoing AZT therapy. We did p24 and in situ hybridization studies (*14*) on one of the four clinical isolates. In the absence of

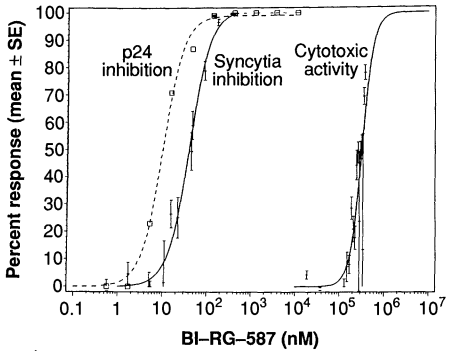

Fig. 3. The effect of BI-RG-587 on HIV-1_{IIIb} replication in c8166 cells. BI-RG-587 was dissolved in dimethyl sulfoxide (DMSO) and RPMI 1640 culture medium to a final 10× concentration. Stock compound solution was serially diluted, and 20 μl of each dilution was added to eight replicate wells of a 96-well flat-bottomed microtiter plate before addition of 5 × 10^4 c8166 cells. Cells were inoculated with 50 tissue culture infectious doses (TCID) of HIV-1. After incubation at 37°C for 72 hours (at 5% CO_2 and humidified conditions), centers of syncytia were enumerated in each well. Control wells received vehicle DMSO solutions without added compound. In addition, cells and media were collected from eight replicate wells containing serialized compound dilutions and pooled. Samples were inactivated with 0.5% Triton X-100 and quantified for HIV-1 Gag antigen by means of the p24 core profile ELISA test (Du Pont Biotechnology Systems). The percent inhibition associated with BI-RG-587 was calculated as follows: percent inhibition = [(control) − (BI-RG-587) ÷ control] × 100. Compound toxicity for c8166 cells was determined with a tetrazolium salt MTT assay (*12*). BI-RG-587 concentration mediating 50% inhibition for all assays was determined by application of the Sigmoid E_{max} model (*22*) for the analysis of concentration-effect data.

Table 1. The effect of BI-RG-587 on HIV-1 RT and RNase H, other RTs, and DNA polymerases. HIV-1 RT was assayed in a mixture of 40 nM primer on poly(rC):oligo(dG), 330 nM [^{3}H]dGTP, 50 mM tris, 1 mM DTT, 2 mM $MgCl_2$, 50 mM glutamic acid, and 0.02% (v/v) CHAPS at pH 7.8. Simian immunodeficiency virus (SIV) RT was assayed in a mixture of poly(rA):oligo(dT) (2.2 μg/ml:0.55 μg/ml), 2.4 μM [^{3}H]deoxythymidine triphosphate ([^{3}H]dTTP), 100 mM tris, 2.5 mM DTT, 2 mM $MgCl_2$, 50 mM KCl, and bovine serum albumin (BSA) (50 μg/ml) at pH 7.8. RT from feline leukemia virus was isolated by centrifugation from lysed virus particles produced in lymphoma cell line FeLV-3281 (*18, 19*) and assayed in poly(rA):oligo(dT) (2 μg/ml), 1 μM [^{3}H]dTTP, 50 mM tris, 60 mM NaCl, 0.05% (v/v) NP-40, 0.2 mM $MnCl_2$, and 20 mM DTT at pH 8. Human DNA polymerases α, β, γ, and δ purified from the cell line Hep G2 and gapped calf thymus DNA used as template were assayed in 50 mM tris, 2 mM DTT, 8 mM $MgCl_2$, BSA (250 μg/ml), gapped calf thymus DNA (150 μg/ml), 100 μM each of deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), and dTTP, and 10 μ*M* [^{3}H]dGTP at pH 8. Polymerase β was further supplemented with 100 mM KCl. Calf thymus DNA polymerase α was assayed with activated calf thymus DNA (0.2 mg/ml), 50 μM each of dATP, dGTP, and dCTP, 2.5 μM [^{3}H]dTTP, 10 mM potassium phosphate, 50 μM EDTA, 2 mM DTT, 5 mM $MgCl_2$, and 125 μM BSA at pH 7.2. Acid-precipitable products were quantified by liquid scintillation counting. HIV-1 RNase H was assayed from preparations of HIV RT cloned and expressed in *Escherichia coli* and purified by the method of Mizrahi *et al.* (*20*). Substrate was constructed and the assay was conducted by the method of Starnes and Cheng (*21*). IC_{50} is stated as means ± SE.

Enzyme	Enzyme specificity of BI-RG-587	
	Concentration (μM)	IC_{50} (nM)
HIV-1 RT polymerase	160*	84 ± 4
HIV-1 RT RNase H	160†	50 ± 6
Simian RT	>>185‡	
Feline RT	>>185‡	
Human DNA polymerase α	>> 24‡	
Human DNA polymerase β	>> 24‡	
Human DNA polymerase γ	>> 24‡	
Human DNA polymerase δ	>> 24‡	
Calf thymus DNA polymerase α	>>185‡	

*Maximal achievable inhibition by BI-RG-587 at 160 μM (I_{max}) = 100%. †I_{max} = 54%. ‡Insignificant inhibition at these concentrations.

BI-RG-587, many HIV-1–infected cells stained blackish-purple. In the presence of BI-RG-587, no HIV-1–infected cells were seen. These results (*15*) were significant in that these isolates have never been adapted to grow in cells other than peripheral blood mononuclear cells (PBMC). They were tested in these experiments after the third PBMC passage.

We have described a novel nonnucleoside inhibitor of HIV-1 RT. BI-RG-587 acts as a noncompetitive enzyme inhibitor with exquisite specificity against HIV-1 RT. BI-RG-587 has potent antiviral activity against HIV-1 in vitro with extremely low cytotoxicity in uninfected human cells. Antiviral activity has been demonstrated against HIV-1 isolates from patients receiving AZT therapy. Like BI-RG-587, compounds reported by Pauwels *et al.* (*16*) have specificity for HIV-1 but not HIV-2. BI-RG-587 has shown no cytotoxic effects on human bone marrow colonies including erythroid burst-forming units and colony-forming units of granulocyte, erythroid, macrophage megakaryocyte and granulocyte macrophage at concentrations up to 37,500 nM (*17*). In cynomolgus monkeys, plasma levels remained between 35 and 140 times the IC_{50} (cell culture) during an 8-hour period after a single oral dose of 20 mg per kilogram of body weight. In chimpanzees, plasma levels of over 600 times the IC_{50} were observed after a single similar dose. In rodent and primate, tissue distribution studies after oral administration indicate a plasma:brain ratio of 0.8 to 1.0. Taken together, these results are encouraging for the development of BI-RG-587 as an antiviral for the treatment of HIV-1 infection in humans. The nonnucleoside nature of this compound may circumvent the associated toxicities of nucleoside chain terminators. Subsequent reports from our laboratories will delineate structure-activity relationships of dipyridodiazepinone inhibitors of RT, their metabolites and pharmacokinetics, effects on human bone marrow progenitors, and mechanism of action as RT inhibitors.

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gCap39, a Calcium Ion– and Polyphosphoinositide-Regulated Actin Capping Protein

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The polymerization of actin filaments is involved in growth, movement, and cell division. It has been shown that actin polymerization is controlled by gelsolin, whose interactions with actin are activated by calcium ion (Ca^{2+}) and inhibited by membrane polyphosphoinositides (PPI). A smaller Ca^{2+}- and PPI-regulated protein, gCap39, which has 49% sequence identity with gelsolin, has been identified by cDNA cloning and protein purification. Like gelsolin, gCap39 binds to the fast-growing (+) end of actin filaments. However, gCap39 does not sever actin filaments and can respond to Ca^{2+} and PPI transients independently, under conditions in which gelsolin is ineffective. The coexistence of gCap39 with gelsolin should allow precise regulation of actin assembly at the leading edge of the cell.

AGONIST STIMULATION INDUCES rapid actin polymerization in the cortical cytoplasm, a process that can be explained by an increase in the amount of polymerization-competent actin molecules and an increase in the number of actin-nucleating sites (*1*). The latter could be achieved rapidly by uncapping of existing actin oligomers that were blocked at the (+) ends by end-capping proteins. It has been suggested that membrane polyphosphoinositides promote nucleated actin filament growth by coordinated regulation of profilin (*2*), a protein that sequesters actin monomer, and gelsolin (*3*), a protein that caps, severs, and nucleates actin filament (*4*). A family of gelsolin-like, actin filament–severing proteins has been identified, and they have a highly conserved and internally repeated primary sequence (*5*). These include vertebrate villin (*6*), which is similar in size to gelsolin (*7–9*) (80 to 90 kD), and *Dictyostelium* severin (*10*) and *Physarum* fragmin (*11*), which are half as large and resemble the NH_2-terminal half of gelsolin. We report here the identification of a member of this family, gCap39, which caps actin filaments but does not sever them and dissociates from filament ends readily, either by decreasing Ca^{2+} to submicromolar concentrations or by increasing phosphatidylinositol 4,5-bisphosphate (PIP_2) without necessarily lowering Ca^{2+} concentration. Since gelsolin uncapping requires both an increase in PIP_2 and a decrease in Ca^{2+} concentration, gCap39 can generate actin nuclei by dissociating from filament ends under conditions in which gelsolin and other capping proteins remain associated.

The gCap39 cDNA was isolated during

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