Styrylquinoline Derivatives: A New Class of Potent HIV-1 Integrase Inhibitors That Block HIV-1 Replication in CEM Cells

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Received January 21, 1998

On the basis of the fact that several polynucleotidyl transferases, related to HIV integrase, contain in their active site two divalent metal cations, separated by ca. 4 Å, new potential HIV integrase inhibitors were designed, in which a quinoline substructure is linked to an aryl nucleus possessing various hydroxy substitution patterns, by means of an ethylenic spacer. Although the most active compounds contain the catechol structure, this group is not essential for the activity, since compound **21** that lacks such a moiety is a potent drug, implicating the presence of a different pharmacophore. The most promising styrylquinolines thus synthesized inhibit HIV-1 integrase in vitro at micromolar or submicromolar concentrations and block HIV replication in CEM cells, with no significant cellular toxicity in a 5-day period assay. These inhibitors are active against integrase core domain-mediated disintegration, suggesting that fragment 50−212 is their actual target. These new styrylquinolines may provide lead compounds for the development of novel antiretroviral agents for AIDS therapeutics, based upon inhibition of HIV integrase. They might also be used in the elucidation of the mechanism of inhibition of this enzyme; e.g., they could serve as candidates for cocrystallization studies with HIV integrase.

Introduction

AIDS is essentially a viral disease and should be treated by antiretroviral agents.[1] From this standpoint, HIV DNA integration into genomic DNA of the host cell, a crucial step in the replicative life cycle of the virus, constitutes a particularly attractive target for AIDS chemotherapeutics, including potential synergy with currently available HIV reverse transcriptase and protease inhibitors.[2] The integration step, which is mediated by a viral protein, integrase (IN), is required for the production of progeny viruses.[3] IN directs two distinct reactions: terminal cleavage at each 3′ end of the proviral DNA removing a pair of bases and strand transfer which results in the joining of each processed 3′ end to 5′-phosphates in the target DNA.[4] The terminal cleavage and strand-transfer reactions can be modeled in vitro, by using purified recombinant IN protein.[5] Under simple reaction conditions, purified IN requires a double-stranded DNA substrate mimicking the viral LTRs extremity. In the absence of any other nucleic species, homologous integration is observed, whereas addition of a nonspecific target leads to heterologous integration. Moreover, integrase is capable of catalyzing the reverse reaction termed "disintegration".[6] The disintegration assay involves a short oligonucleotide, the structure of which resembles the reaction intermediate.[7] Systematic screening of potential inhibitors has been undertaken using those three activity assays.

Several families of IN inhibitors have now been identified. Most of them can be classified into three groups: DNA ligands, C-terminal domain ligands, and compounds that interfere with the catalytic domain of the protein. The first family contains nonspecific intercalating agents,[8,9] as well as more specific oligonucleotides targeting integrase binding sites on both LTRs.[10,11] The second class includes polyanionic drugs, such as suramin which interacts with the highly basic C-terminal domain.[12] Finally, the third group embodies hydroxylated aromatic compounds including aurintricarboxylic acids,[13] bis-catechols,[14] CAPE, flavones and flavonoids,[15] curcumin,[16] tyrphostins,[17] lignanolides,[18] coumarin derivatives,[19] cosalanes,[20] hydrazide derivatives,[21] and depsides and depsidones.[22] Systematic screening or more rational studies[23−27] of these families allowed to identify possible pharmacophores,[23] such as the catechol structure.[16] Finally, peptides obtained by rational[28] or combinatorial research strategies[29] were also described. Overall, while many integrase inhibitors have now been identified, only a handfull displayed antiviral activity in cell culture. This poorly populated group comprises lignanolides,[18] curcumin,[16] aurintricarboxylic acids,[13] dicaffeoylquinic acids and analogues,[30,31] diaryl sulfones,[23] and finally G-rich oligonucleotides.[32] To date, only one serious candidate has entered clinical assay. Surprisingly, it is a G-rich oligonucleotide that was shown to inhibit integrase activity both in vitro and in vivo.[32−34] However, despite its remarkable antiviral properties, the clinical use of such an oligonucleotide still rests on the capacity to produce amounts of the compound compatible with therapeutic exigencies.[35]

Consequently, we set out to synthesize a new class of compound based upon the quinoline structure, with the

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S0022-2623(98)00043-0 CCC: $15.00
Published on Web 06/25/1998

view to identify new structurally simple inhibitors of HIV integrase. In this report, we describe new styrylquinoline derivatives *that inhibit HIV integrase in vitro at micromolar or submicromolar concentrations, block HIV replication in CEM cells, and are devoid of cellular toxicity.*

Results and Discussion

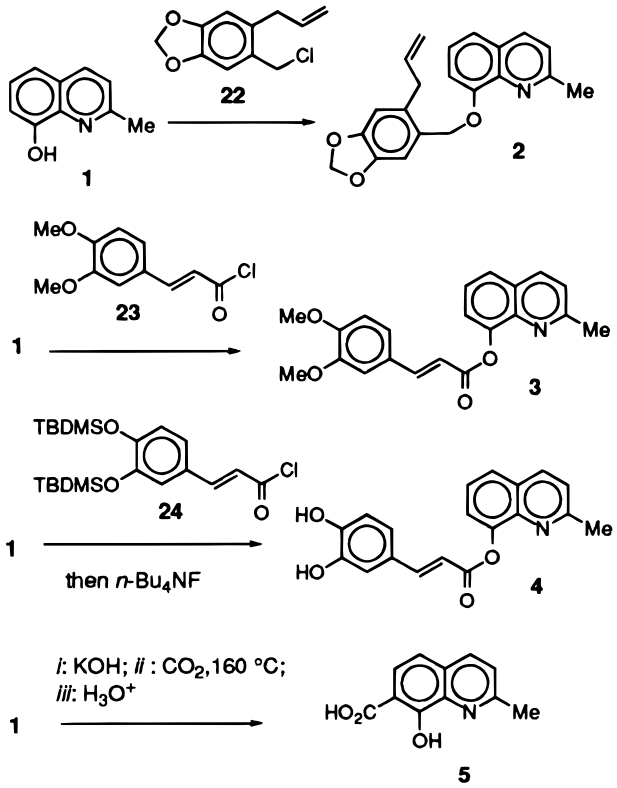
Chemistry. It is manifest that the presence of divalent cations is essential for the catalytic activity of HIV integrase.[36−38] Today it is not clear whether cations are directly involved in catalysis, in integrase activation through a metal-induced conformational change,[39,40] or in both. However, several lines of evidence support the proposal that a metal cofactor is coordinated by the acidic residues of the D,D(35)E motive that constitutes the active site of the enzyme. In view of the fact that several polynucleotidyl transferases, related to HIV integrase, contain in their active site two divalent metal cations, separated by ca. 4 Å,[41−46] we planed to design new potential HIV integrase inhibitors, in which two metal-chelating subunits are linked by means of an appropriate central spacer. We reasoned that 8-hydroxyquinoline ("oxine"), a well-known reagent used in analytical chemistry for the determination of numerous divalent metals, including manganese, zinc, and magnesium, might constitute the basic structure of one of the chelating systems. An aryl nucleus possessing various hydroxy substitution patterns was chosen for the second chelating subunit, on the basis of the observation that the most potent HIV integrase inhibitors known to date generally contain an ortho-dihydroxylated (catechol-type) aromatic moiety. In support of this endeavor, a series of quinoline-containing compounds were synthesized and tested in vitro against HIV integrase and ex vivo against CEM cells infected with HIV-1.

Etherification of commercially available 8-hydroxyquinaldine (**1**) with (chloromethyl)safrole (**22**)[47] gave **2**. Condensation of **1** with acid chlorides **23** and **24** led to the corresponding esters **3** and **4**, respectively. Carboxylation of **1** under the Kolbe−Schmitt conditions furnished the known acid **5**[48] (Scheme 1).

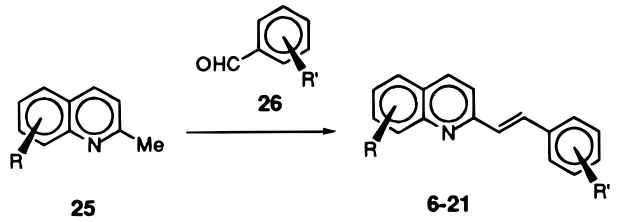
Basically, the elaboration of the central ethylenic linker, of pure *E* geometry, in styrylquinolines **6**−**21** was secured through the Perkin condensation between quinaldines **25** and aromatic aldehydes **26** (Scheme 2).[49]

Syntheses of substrates **8**, **11**, **14**, and **16** employed the following peculiar protocols. The aldehyde partner in the preparation of the symmetrical substrate **8** was the known aldehyde **27**,[50] prepared from **1**. The starting material in the preparation of nitrile **16** was the known aldehyde **28**,[51] which was converted into oxime **29**. Concomitant dehydration of the oxime function of **29** took place during the next Perkin condensation, which thus delivered nitrile **16**. Addition of aldehyde **30**, obtained from **28**, to Wittig reagent prepared from phosphonium salt **31** gave after removal of the protecting groups triol **32**, the precursor through Perkin condensation of the bis-ethylenic derivative **14**. Amino derivative **11** was prepared from commercially available 8-nitroquinaldine (**33**) by a three-step sequence involving Perkin condensation into **34**, tin(II) chloride reduction, and finally hydrolysis of the acetoxy groups (Scheme 3).

Scheme 1



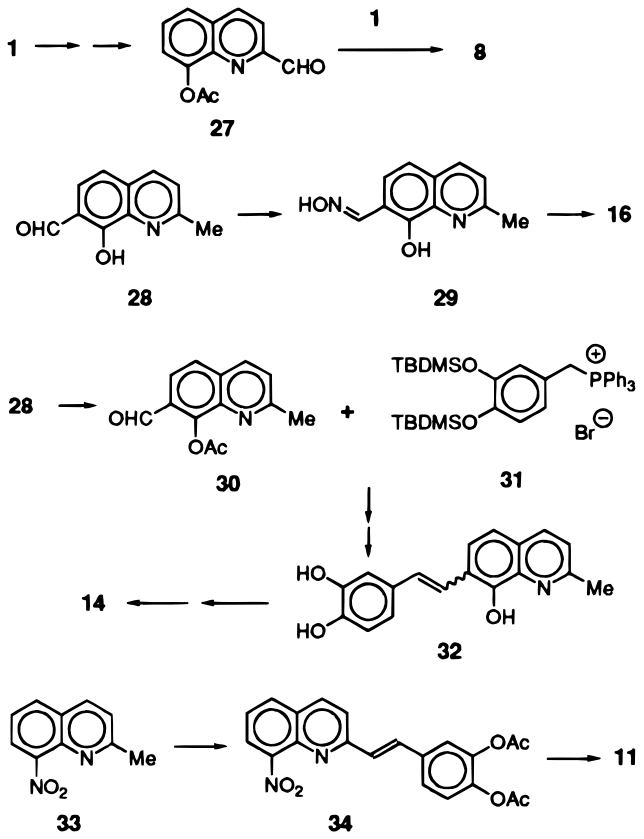
Scheme 2



Anti-Integrase Activities. First, styrylquinoline derivatives were tested against 3′-processing and strand-transfer activities. Strand-transfer experiments were performed using a plasmid as a target in order to simplify the quantitative calculation[52] (see the Experimental Section). The in vitro anti-HIV integrase activity of the molecules synthesized is summarized in Table 1.

These compounds can be roughly classified as inactive, **1**−**12**, and active, **13**−**21**. In turn, the latter class of molecules can be subdivided into three families: moderately potent drugs **13** and **14** fail to show complete inhibition at 100 μM; potent compound **15** has very little effect at 1 μM, although it shows IC_{50} below 10 μM; and highly potent drugs **16**−**21** gave rise to a marked effect at 1 μM. A complete lack of inhibitory potency was observed with the parent 8-hydroxyquinaldine (**1**) and with its O-alkylated and O-acylated derivatives **2** (data not shown), **3**, and **4**. However, a promising observation was made in this series: quinaldine **5** bearing a hydroxyl group at C-8 and a carboxyl function at C-7 exhibited a discrete but significant activity. Styrylquinolines **6**−**12**, in which a quinoline moiety is linked to an aryl nucleus by means of an ethylenic spacer, proved to be completely devoid of activity, despite the presence of a catechol center in some of these compounds (**9**−

Scheme 3



11). When an additional hydroxyl group was introduced at the C-8 position in the quinoline half of inactive styrylquinoline **9**, a moderate inhibitory potency was gained (compare **13** or **14** with **9**). However, the replacement of the three hydroxyl groups of **13** by acetoxy substituents completely abolished the activity (compare **12** with **13**); thus the inhibitory potency is clearly associated with the presence of " free" phenolic hydroxyl groups. When a carboxyl or nitrile function and a hydroxyl group were both introduced in the quinoline subunit, at the summits C-7 and C-8, respectively, potent to highly potent HIV integrase inhibitors were designed (compounds **15**−**21**). Styrylquinoline **15**, in which the phenyl half possesses the 2,4-*m*-dihydroxy substitution pattern, showed good potency. High inhibitory activities were finally obtained with molecules **16**−**20**, where the phenyl nucleus is now 3,4-ortho-dihydroxylated or 3,4,5-trihydroxylated. Note in this respect that in the lower-energy conformer of **13** (compound having the minimun requirement for activity), the pattern of the oxygen atom triad (interatomic distances of 9.46, 7.67, and 2.72 Å) satisfactorily matches one of the putative three-point pharmacophores previously identified by Pommier and co-workers[22,25] (for a recent indentification of a four-point pharmacophore, see ref 53). It is noteworthy that, compared with the catechol-containing compound **17**, the same level of activity was gained with substrate **21**, in which the phenyl subunit now carries a carboxyl function at C-3 and a hydroxyl group at C-4. *Thus, the latter example clearly demonstrates that the design of potent HIV integrase inhibitors does not necessarily require the presence of an ortho-dihydroxylated (catechol-type) aromatic moiety.*

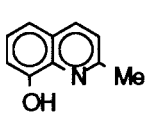
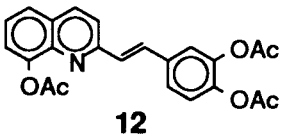
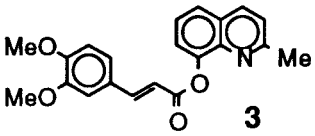
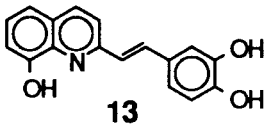
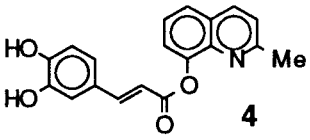
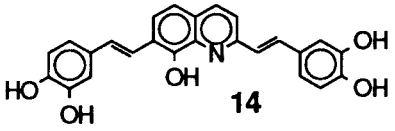
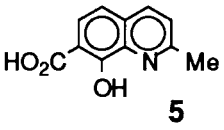
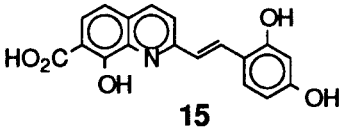
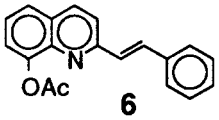
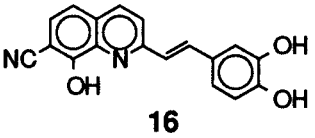
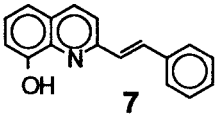
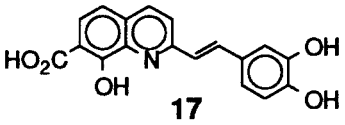
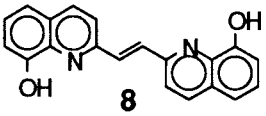
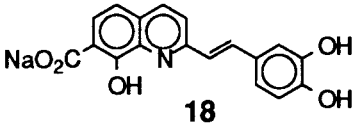
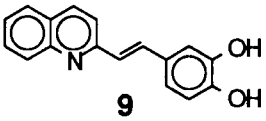
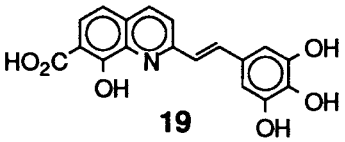
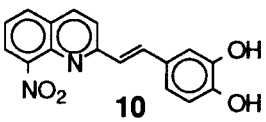
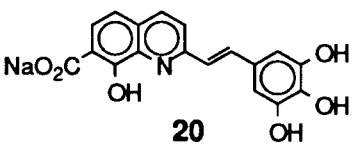
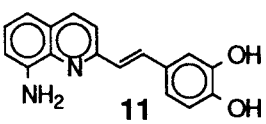
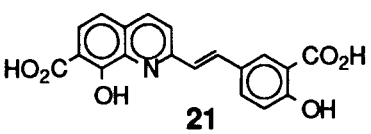
Interaction with the Catalytic Core Domain. Integrase can catalyze a reverse reaction called "disintegration" reaction.[6] Unlike processing and strand transfer, this reaction may be carried out by the core domain of integrase lacking both N- and C-terminal domains.[54] Therefore, inhibition of integrase-mediated disintegration activity is good evidence for a mechanism involving direct interaction with the catalytic domain. To determine whether styrylquinoline derivatives act at the core domain of integrase, disintegration using the soluble 50−212 core domain of HIV integrase was performed.[55] Table 2 shows the activity of active styrylquinoline derivatives against the reaction catalyzed by this domain.

All compounds **13**−**21** that were active against integration inhibited disintegration as well. Compounds **18**−**21** which displayed IC_{50} values below 1 μM are highly active, the 3,4,5-trihydroxylated **19** being the most potent, in agreement with results of strand-transfer assay. Again, salts **18** and **20** were as active as parent compounds **17** and **19**. This result strongly supports the hypothesis that the target site of styrylquinoline derivatives is located in the catalytic core of IN. To reinforce this conclusion, another set of experiments was undertaken. As a matter of fact, integrase belongs to the superfamily of polynucleotidyl transferases.[45] Structural resemblances between polynucleotidyl transferases and type II restriction enzyme catalytic domains have been reported,[56] suggesting similar chemical mechanisms for reaction pathways in both families. Consequently, a common effect against integrase and a member of the type II restriction enzyme family should provide another evidence for an interaction with their structurally related catalytic domain. To support this hypothesis, we tested the lead compound **17** against the *Eco*RI-mediated cleavage of a linearized plasmid (see the Experimental Section). Results are shown in Figure 1.

It can be observed that **17** is actually an inhibitor of *Eco*RI. However, the IC_{50} for *Eco*RI inhibition, roughly equal to 50 μM, was markedly higher than the one calculated for integrase inhibition (ca.. 1 μM, see Table 1). Since concentrations of *Eco*RI and integrase in either assays were roughly equal, the IC_{50} discrepancy likely results from specificity of the drug toward integrase. From this standpoint, it is important to note that neither **17** nor **19** was active against HIV reverse transcriptase in vitro (data not shown) for concentrations ranking up to 100 μM. In conclusion, styrylquinoline derivatives most likely interact with the IN core domain. It is in agreement with previous reports which showed that hydroxylated compounds interfere with the HIV IN core domain. However, despite the fact that catechol-containing styrylquinolines were the most effective derivatives, it is important to note that derivatives **15** and **21**, lacking the catechol structure, were active at concentrations lower than 10 μM, *suggesting the presence of a different pharmacophore.*

Although IN displays more activity in vitro with Mn^{2+} as the cationic cofactor, it is generally admitted that Mg^{2+} is the actual cofactor in vivo.[56] Since styrylquinoline derivatives were designed to interfere with the active site of integrase, it was of interest to investigate their properties against Mg^{2+}-dependent activity. How-

Table 1. HIV-1 Integrase Inhibitory Potencies Determined as Described in the Experimental Section

Compounds	IC_{50} Values (μM)		Compounds	IC_{50} Values (μM)	
	3'-Processing	Integration		3'-Processing	Integration
 **1**	>100	>100	 **12**	>100	>100
 **3**	>100	>100	 **13**	7.4	2.4
 **4**	>100	>100	 **14**	2.2	3.4
 **5**	>100	114	 **15**	3.7	2.8
 **6**	>100	>100	 **16**	3.0	nd
 **7**	>100	>100	 **17**	2.4	1.0
 **8**	>100	nd	 **18**	0.8	nd
 **9**	>100	nd	 **19**	0.3	0.4
 **10**	>100	>100	 **20**	0.26	nd
 **11**	>100	>100	 **21**	2.7	0.6

ever, under our standard conditions, the strand-transfer activity of IN was virtually undetectable. To adress that issue, we designed a different assay in which the full-length IN was first incubated on ice in the presence of 10 mM of Mg^{2+}. The 500-bp viral substrate was subsequently added with or without the potential inhibitor, and the reaction mixture was incubated for 10 min at 4 °C. The target DNA was eventually added,

Table 2. Effect of Active Styrylquinolines Derivatives upon Disintegration Catalyzed by the Deletion Mutant (50−212)

compd	IC_{50} (μM)
13	2.5
15	2.5
16	3.6
17	0.9
18	0.7
19	0.24
21	0.6

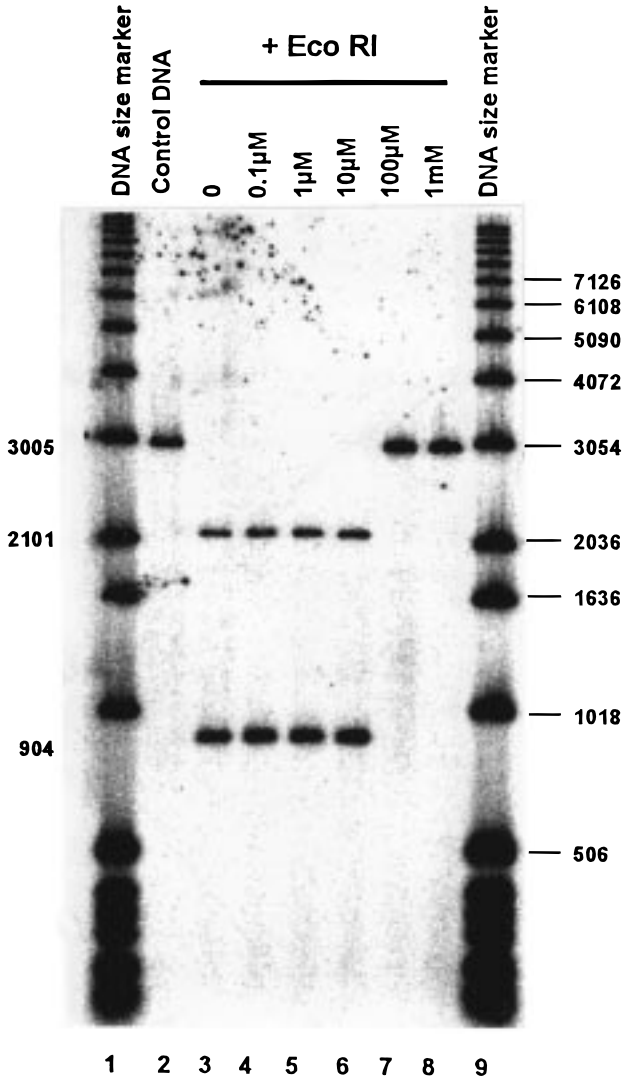


Figure 1. Phosphorimager picture of *Eco*RI activity assay. Plasmid pSP65 was linearized using *Ssp*I restriction enzyme and radiolabeled with T4 kinase. Incubation of the resulting 30005-bp DNA fragment with 0.1 U of *Eco*RI gave rise to 2101- and 904-bp DNA fragments. Lanes 1 and 9: linear DNA size marker. Lanes 2−8: concentration-dependent inhibition of *Eco*RI activity by compound **17.** Drug concentrations are indicated above each lane.

and the reaction was allowed to proceed for 60 mn at 37 °C. Preincubation with Mg^{2+} gave rise to a significant increase in strand-transfer activity allowing us to test the compounds with these new conditions. As shown in Figure 2 for compound **19**, inhibition of strand-transfer reaction (IC_{50} = 1.9 mM) was readily observed with Mg^{2+} as cofactor.

Ex Vivo Activity of Styrylquinoline Derivatives. Compounds **13**−**21** were evaluated for their antiviral

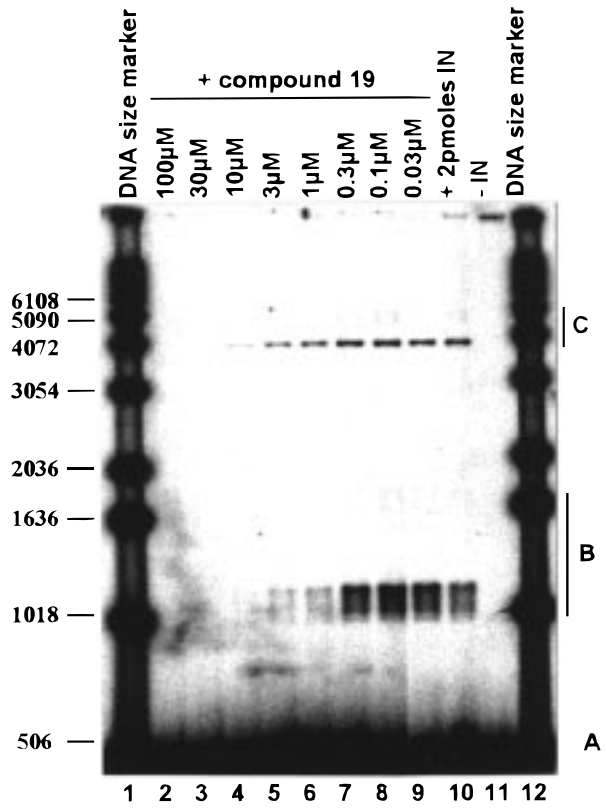


Figure 2. Phosphorimager picture of Mg^{2+}-dependent activity of HIV IN in the presence of compound **19**; 10 ng of 500-bp viral substrate and 40 ng of pSP70 were incubated in the presence of 2 pmol of HIV-1 IN and increasing concentrations of compound **19**, in a buffer containing 10 mM $MgCl_2$. Lanes 1 and 12: DNA size marker. Lanes 2−10: concentration-dependent inhibition of IN activity by compound **19** in the presence of Mg^{2+}. Drug concentrations are indicated above each lane. Lane 11: incubation of viral substrate and 2 pmol of HIV-1 IN without drug. Lane 12: viral substrate without IN. Reaction products are shown on the right; (A) 500-bp viral DNA, (B) homologous integration products, (C) heterologous integration products.

activities against HIV-1 replication in CEM cells. They were tested for their ability to lower the viral charge in culture supernatants. CEM cells were infected with HIV-1 and subsequently treated with increasing concentrations of styrylquinoline derivatives. Viral load was estimated 72 h after infection. The amount of virus was assayed by β-galactosidase assay, with HeLaCD4-β-gal cells as reporting cells. Antiviral assays were confirmed by virion-associated p24 antigen assay in supernatants. More interestingly, several compounds were active against viral replication as shown in Table 4. Toxicity was estimated by MTT transformation assay. As shown in Table 3, most compounds were devoid of cellular toxicity up to 100 μM, which was the highest concentration tested. Under our standard conditions, antiviral activity was tested after 72 h postinfection. To determine wether some cytoxicity could build up over a longer period of time, toxicity of compounds **17**, **15**, and **21** was also tested 120 h after infection. Again, very moderate or no toxicity at all was detected with active compounds **17**, **15**, and **21** (see Table 4).

In vitro active compound **15** exhibited a moderate activity ex vivo with IC_{50} between 50 and 100 μM and

Table 3. Cytotoxicity of Quinoline Derivatives

compd	TC_{50} (μM)	TC_{90} (μM)
5	>100	>100
7	50.1	>100
9	12.3	100
13	8.1	71
15	>100	>100
16	6.2	56.3
17	>100	>100
18	>100	>100
19	10.7	93.3
21	>100	>100

Table 4. Inhibition of HIV-1 Replication in CEM Cells by Quinoline Derivatives

	antiviral activity (μM) on CEM cells			toxicity at 100 μM (%)	
compd	IC_{25}	IC_{50}	IC_{90}	72 h	120 h
5	>100	>100	>100	0	
13	1.2	3.6		99	
15	26.3	79.8	>100	0	<10
17	0.46	1.3[a]	12.3[a]	0	<10
		20[b]			
18		20[b]	>100[b]	0	
19	0.4	1.2	10.7	100	
21	20.9	62	>100	0	0

[a] As measured by P4 infection. [b] As measured by P24 ELISA test.

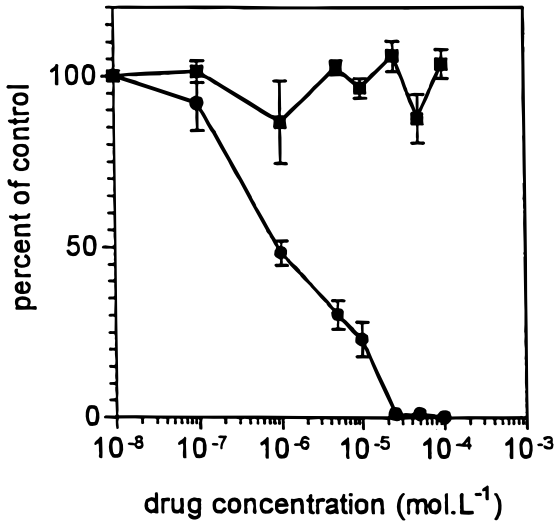


Figure 3. Antiviral activity (●) and cytotoxicity (■) of compound **17**. CEM cells were infected with cell-free virus; 2 h after virus addition, cells were washed to remove unbound virus, and fresh medium containing increasing concentration of compound **17** dissolved in DMSO was added. At 72 h postinfection, supernatants were collected and used to infect HeLaCD4$^+$-β-gal cells. Cultures were then incubated for 24 h before lysis and subsequent incubation with CPRG for 1 h. The red-staining intensity was quantified on a multiscan photometer at 570 nm. After 72 h, cell viability was estimated by the MTT assay. Results are the average of three experiments.

was devoid of cytotoxicity. Styrylquinolines **13** and **19** were also active but proved to be dramatically cytotoxic. However, it is worthy to note that the most effective drug **19** (IC_{50} = 0.4 μM) ex vivo was the best lead in vitro. Finally, compound **17** yielded the most promising results as shown in Figure 3.

This compound gave rise to a strong antiviral effect as measured by β-gal assay, whereas no toxicity on either infected or noninfected cells was observed. In-

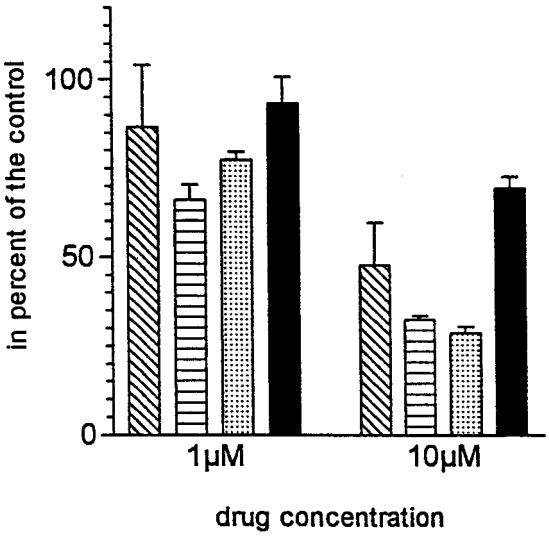


Figure 4. Effect of time of drug addition on HIV replication inhibition by compound **17**. CEM cells infected with HIV-1_{PLN4-3} were treated at 2 h (oblique bars), 4 h (horizontal bars), 8 h (dotted bars), and 24 h (filled bars) after virus infection with compound **17** at 1 and 10 mM. Viral load in supernatants was monitored after 72 h by HeLaβ-gal cell infection assay. SEM of triplicate samples are shown as error bars.

terestingly, viral replication could be followed visually, since it gave rise to syncytia. As a matter of fact, the antiviral effect led to a marked decrease of the amount of these syncytia, which became virtually undetectable above 50 μM. This anti-HIV activity of compound **17** was confirmed by measuring p24. Sodium salt **18** led to the same antiviral activity. It has been previously suggested that polyhydroxycarboxylates such as dicaffeoylquinic acids could interfere with virus penetration.[22] Consequently, to investigate further the antiintegrase activity of **17**, ex vivo, kinetic experiments were performed. Compound **17** was added respectively 2, 4, 8, and 24 h after infection. Cells were washed after virus treatment in order to eliminate virus that had not penetrated into cells. Again, a marked antiviral effect was observed (Figure 4).

However, percent of inhibition was dependent upon the time of addition. Inhibition was maximized when the drug was added after 4 and 8 h. Clearance and/or degradation of the drug can account for the lower effect when the drug was added only 2 h after infection. Adding the drug after 24 h gave rise to a markedly decrease of the drug activity. One can infer from this experiment that drug was effective during the first round of viral replication. Since interference with virus penetration was not possible, drugs must be active against an early postpenetration step. Reverse transcription is generally believed to begin in HIV virions and to continue during the first hour of infection. Reverse transcription leads to the synthesis of viral DNA, which enters a nucleoprotein complex containing at least integrase and possibly other viral and cellular proteins. This preintegration complex, often called PIC, migrates into the cell nucleus where it is covalently inserted into host genome between 8 and 16 h postinfection. Recent reports[57] suggested that polyhydroxylated inhibitors of HIV IN are most likely active against the assembly of preintegration complexes. Accordingly, some compounds that are potent inhibitors of recombi-

nant IN were found to be poorly active against integration mediated by purified preintegration complexes.[58] These results indicate that such inhibitors must be present in cells at the time of PIC formation to be fully active. Our data are consistent with such a model. It can be argued that kinetic results are compatible with inhibition of reverse transcription as well. To rule out such a possibility, compounds **17** and **19** were tested against HIV reverse transcriptase in vitro. No effect against the enzyme was observed in the range of concentrations tested (1 nM−100 μM).

Conclusion

In summary, the present styrylquinoline derivatives constitute a new class of HIV integrase inhibitors that display a marked effect against HIV replication ex vivo. Since most of these compounds are devoid of cytotoxicity at concentrations up to 100 μM, they represent a new lead for the development of anti-HIV drugs. Furthermore, seeing that several of the these inhibitors exhibit a strong activity, they might be used in the elucidation of the mechanism of inhibition of HIV integrase; e.g., they could serve as candidates for cocrystallization studies with this enzyme.

Experimental Section

Melting points were recorded on a capillary tube melting point apparatus and are uncorrected or by microthermal analysis on a Mettler FP5 apparatus. Infrared (IR) spectra were obtained as neat films between NaCl plates or KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, unless otherwise stated; when diffuse, easily exchangeable protons were not listed. Recognition of methyl, methylene, methine, and quaternary carbon nuclei in ^{13}C NMR spectra rests on the *J*-modulated spin−echo sequence. Analytical thin-layer chromatography was performed on Merck silica gel $60F_{254}$ glass precoated plates (0.25-mm layer). All liquid chromatography separations were performed using Merck silica gel 60 (230−400 mesh ASTM). Ether and tetrahydrofuran (THF) were distilled from sodium benzophenone ketyl. Methanol was dried over magnesium and distilled. Benzene and CH_2Cl_2 were distilled from calcium hydride, under a nitrogen atmosphere. All reactions involving air- or water-sensitive compounds were routinely conducted in glassware which was flame-dried under a positive pressure of nitrogen. Organic layers were dried over anhydrous $MgSO_4$. Chemicals obtained from commercial suppliers were used without further purification. Elemental analyses were obtained from the Service de microanalyse, Centre d'Etudes Pharmaceutiques, Châtenay-Malabry, France.

8-[[(2-Allyl-4,5-(methylenedioxy)phenyl)methyl]oxy]-quinaldine (2). To a mixture of 8-hydroxyquinaldine (**1**) (2.21 g, 13.9 mmol), potassium carbonate (3.80 g, 27.8 mmol) and potassium iodide (4.60 g, 27.8 mmol), in dry acetonitrile (50 mL) was added dropwise a solution of (chloromethyl)safrole[47] (3.60 g, 18.0 mmol) in acetonitrile (20 mL). After being stirred for 18 h at 20 °C, the reaction mixture was poured into water and extracted with three 50-mL portions of CH_2Cl_2. The organic layer was dried and concentrated in vacuo to leave a solid which was recrystallized from isopropyl ether to provide colorless crystals of **2** (3.0 g, 65%): mp 118−119 °C; IR (KBr) 3061, 1638, 1617, 1603; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.96 (d, J = 8.4 Hz, 1H), 7.40−7.20 (m, 3H), 7.03 (s, 1H), 6.94 (dd, J = 6.4, 2.4 Hz, 1H), 6.68 (s, 1H), 6.10−5.80 (m, 1H), 5.86 (s, 2H), 5.27 (s, 2H), 5.10−4.90 (m, 3H), 3.41 (d, J = 6.4 Hz, 1H), 2.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.2, 154.0, 147.3, 146.3, 136.9, 136.1, 131.3, 128.4, 127.8, 125.6, 122.6, 120.1, 116.0, 110.9, 110.1, 108.9, 103.3, 101.0, 69.0, 37.0, 25.8. Anal. ($C_{21}H_{19}NO_3$) C, H, N.

(*E*)-Quinaldin-8-yl 3,4-Dimethoxycinnamate (3). To a mixture of 8-hydroxyquinaldine (**1**) (1.17 g, 7.41 mmol) and potassium carbonate (2.10 g, 15 mmol) in dry acetone (15 mL) was added dropwise a solution of dimethoxycaffeoyl chloride (1.60 g, 7.41 mmol) in acetone (10 mL). After stirring for 4 h at 20 °C, the reaction mixture was filtered and the filtrate concentrated in vacuo. The oily residue was chromatographed on silica gel (AcOEt:cyclohexane = 1:2) to provide a solid which was recrystallized from ethyl acetate to afford **3** (1.4 g, 54%) as colorless crystals: mp 119−121 °C; IR (neat) 1723, 1633, 1600; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.04 (d, J = 8.6 Hz, 1H), 7.91 (d, J = 15.8 Hz, 1H), 7.70−7.60 (m, 1H), 7.51−7.44 (m, 2H), 7.28 (d, J = 8.6 Hz, 1H), 7.25−7.16 (m, 2H), 6.89 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 16.0 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 2.70 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.9, 159.4, 151.3, 149.2, 147.0, 146.4, 135.9, 127.7, 127.3, 125.4, 125.1, 123.0, 122.5, 121.4, 115.0, 110.9, 109.6, 55.9, 55.8, 25.7. Anal. ($C_{21}H_{19}NO_4$) C, H, N.

3,4-Bis[[[(1,1-dimethylethyl)dimethyl]silyl]oxy]cinnamyl Chloride (24). To a solution of caffeic acid (3.5 g, 19.4 mmol) and imidazole (3.94 g, 58 mmol) in anhydrous DMF (20 mL) was added *tert*-butyldimethylchlorosilane (8.7 g, 58 mmol) at 0 °C. The resulting mixture was stirred at 20 °C for 16 h. Water (20 mL) was then added, and the reaction mixture stirred for 10 min. The mixture was extracted with ether. The combined organic extracts were dried and concentrated in vacuo. CH_2Cl_2 (100 mL) and DMF (0.5 mL) were added to the oily residue. The solution was cooled to 0 °C, and oxalyl chloride (3.43 g, 27 mmol) was added dropwise. After stirring for 1 h at 20 °C, the reaction mixture was concentrated under vaccum to leave crude acid chloride **24**, which was used directly in the next step without further purification.

(*E*)-Quinaldin-8-yl 3,4-Dihydroxycinnamate (4). To a solution of 8-hydroxyquinaldine (**1**) (3.34 g, 21 mmol) and potassium carbonate (5.80 g, 42 mmol) in dry acetone (50 mL) was added dropwise a solution of crude acid chloride **24** (9.0 g, 21 mmol) in acetone (30 mL). After stirring for 16 h at 20 °C, the reaction mixture was filtered and the filtrate concentrated in vacuo. The oily residue was taken into dry THF (10 mL), and a 1.1 M solution of *tetra*butylammonium fluoride in THF (11.6 mL, 126 mmol) was added. After the mixture stirred at 20 °C for 1 h, acetic acid (5 mL) and water (20 mL) were added. The mixture was diluted with ethyl acetate, the organic layer separated, and the aqueous phase extracted with two 20-mL portions of ethyl acetate. The combined organic extracts were dried and concentrated. The solid residue was recrystallized from 2-propanol to give colorless crystals of **4** (2.28 g, 33%): mp 201−203 °C; IR (KBr) 3600−2400, 1694, 1634, 1605; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.28 (d, J = 8.4 Hz, 1H), 7.82 (dd, J = 6.4, 3.0 Hz, 1H), 7.72 (d, J = 16.0 Hz, 1H), 7.60−7.46 (m, 2H), 7.42 (d, J = 8.4 Hz, 1H), 7.20−7.10 (m, 2H), 6.81 (d, J = 8.0 Hz, 1H), 6.65 (d, J = 16.0 Hz, 1H), 2.50 (s, 3H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 165.4, 159.0, 148.9, 147.0, 146.8, 145.7, 140.4, 136.3, 127.5, 125.7, 125.6, 125.5, 122.8, 121.9, 121.7, 115.9, 115.2, 113.2, 25.3. Anal. ($C_{19}H_{15}NO_4$) C, H, N.

8-Hydroxy-7-quinaldic Acid (5).[48] To a suspension of 8-hydroxyquinaldine (**1**) (29 g, 0.18 mol) in toluene (130 mL) was added potassium hydroxide (11.3 g, 0.20 mol). The stirred mixture was heated under reflux for 24 h, collecting the water of the reaction in a Dean−Stark trap. After the mixture cooled at 20 °C, DMF (100 mL) was added, and the Dean−Stark trap was replaced with a distillation column. The reaction mixture was progressively heated until most of the toluene had been distilled. When the temperature reached 140 °C, a stream of CO_2 was passed into the solution and continued throughout the reaction. The distillation of the solvents was continued while the temperature was gradualy raised to 160 °C. The reaction mixture was heated for 2 h at this temperature and then cooled to 20 °C. The stream of CO_2 was stopped, and water (250 mL) was added. The solution was acidified to pH 7 with concentrated HCl, and the mixture was extracted with ethyl acetate. The aqueous phase was acidified to pH 4.2, and the precipitate was filtered, washed with water, and dried in vacuo. The crude acid was recrystallized in 2-propanol to give acid **5** (8.0 g, 22%) as yellow crystals: mp 206−208 °C (lit.[48]

mp 207−215 °C); IR (neat) 3433, 3200−2400, 1700, 1633; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.54 (d, J = 8.5 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.20 (d, J = 8.6 Hz, 1H), 2.80 (s, 3H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 170.7 (C), 160.8 (C), 156.2 (C), 140.9 (CH), 135.0 (C), 130.4 (C), 127.5 (CH), 125.0 (CH), 112.8 (CH), 112.6 (C), 22.4 (CH_3). Anal. ($C_{11}H_9NO_3 \cdot {}^1/_4H_2O$) C, H, N.

(*E*)-2-(2-Phenylethenyl)-8-acetoxyquinoline (6). To a solution of 8-hydroxyquinaldine (**1**) (0.60 g, 3.7 mmol) in acetic anhydride (6 mL) was added benzaldehyde (0.79 g, 7.5 mmol). The mixture was heated under reflux for 16 h and concentrated in vacuo. The oily residue was chromatographed on silica gel (AcOEt:cyclohexane = 1:4) to provide a solid which was recrystallized from di-*n*-butyl ether to afford **6** (0.6 g, 56%) as colorless crystals: mp 113−114 °C; IR (neat) 1769, 1619, 1598; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.12 (d, J = 8.6 Hz, 1H), 7.80−7.58 (m, 5H), 7.55−7.30 (m, 5H), 7.28 (d, J = 8.0 Hz, 1H), 2.50 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.9, 155.9, 147.5, 141.1, 136.5, 136.4, 134.8, 129.0, 128.9 (2C), 128.8, 128.6, 127.4 (2C), 125.7, 125.6, 121.7, 120.3, 21.1. Anal. ($C_{19}H_{15}NO_2$) C, H, N.

(*E*)-2-(2-Phenylethenyl)-8-hydroxyquinoline (7). A solution of acetoxyquinoline **6** (1.2 g, 4.1 mmol) in pyridine (20 mL) was heated under reflux, water (8 mL) was then added, and the reaction mixture refluxed for 3 h. After the mixture cooled, water (20 mL) and CH_2Cl_2 were added to the mixture. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated. The solid residue was recrystallized from di-*n*-butyl ether to afford yellow crystals of **7** (0.95 g, 92%): mp 96−100 °C; IR (KBr) 3402, 3060, 1641, 1602; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.97 (d, J = 8.6 Hz, 1H), 7.59 (d, J = 16.2 Hz, 1H), 7.50 (d, J = 8.4 Hz, 1H), 7.50 (m, 1H), 7.35−7.10 (m, 6H), 7.03 (dd, J = 8.8, 1.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.5, 152.0, 137.9, 136.3 (2C), 134.2, 128.7 (2C), 128.6, 128.0, 127.6, 127.2 (3C), 120.2, 117.6, 110.0. Anal. ($C_{17}H_{13}NO$) C, H, N.

(*E*)-1,2-Bis[2-(8-hydroxyquinolyl)]ethylene (8). A solution of aldehyde **27**[50] (9.40 g, 43 mmol) and 8-acetoxyquinaldine (8.78 g, 43 mmol) in acetic anhydride (100 mL) and acetic acid (50 mL) was heated under reflux for 8 h and concentrated in vacuo. Aqueous sodium bicarbonate was added, and the mixture was extracted with CH_2Cl_2. The organic extracts were dried and concentrated to leave a yellow solid, which was dissolved in refluxing pyridine (40 mL). Water (10 mL) was then added, and the reaction mixture refluxed for 6 h. After standing at 0 °C for 8 h, the reaction mixture was filtered. The solid washed with ether and dried in vacuo to give compound **8** (2.50 g, 18%) as yellow crystals: mp >220 °C; IR (KBr) 3362, 1633, 1596; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 9.30 (broad s, 2H), 8.28 (d, J = 8.6 Hz, 2H), 8.20 (s, 2H), 7.85 (d, J = 8.6 Hz, 2H), 7.35−7.25 (m, 4H), 7.06 (dd, J = 7.0, 2.0 Hz, 2H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 153.2, 153.0, 138.5, 136.8, 133.7, 128.1, 127.6, 121.6, 117.7, 111.5. Anal. ($C_{20}H_{14}N_2O_2$) C, H, N.

(*E*)-2-[2-(3,4-Dihydroxyphenyl)ethenyl]quinoline (9). To a solution of quinaldine (0.9 g, 6.3 mmol) in acetic anhydride (10 mL) was added 3,4-diacetoxybenzaldehyde (1.48 g, 7.0 mmol). The mixture was heated under reflux for 12 h and concentrated in vacuo. The residue was dissolved in pyridine (20 mL), and the resulting solution was heated under reflux. Water (8 mL) was then added and the reaction mixture refluxed for 3 h. After the mixture cooled, water (20 mL) and CH_2Cl_2 (20 mL) were added to the mixture. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated. The solid residue was recrystallized from 2-propanol to give yellow crystals of **9** (1.51 g, 91%): mp 246−251 °C; IR (KBr) 3536, 3050−2200, 1602; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 9.30 (broad s, 2H), 8.35 (d, J = 8.8 Hz, 1H), 8.10−7.70 (m, 5H), 7.60 (t, J = 6.8 Hz, 1H), 7.20 (m, 2H), 7.10 (dd, J = 8.0, 1.0 Hz, 1H), 6.85 (d, J = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 156.3, 147.8, 146.9, 145.7, 136.4, 134.8, 129.8, 128.6, 127.9 (2C), 126.9, 125.9, 125.4, 119.9 (2C), 116.0, 114.1. Anal. ($C_{17}H_{13}NO_2 \cdot {}^1/_4H_2O$) C, H, N.

(*E*)-2-[2-(3,4-Dihydroxyphenyl)ethenyl]-8-nitroquinoline (10). To a solution of 8-nitroquinaldine (3.0 g, 16 mmol) in acetic anhydride (30 mL) was added 3,4-diacetoxybenzaldehyde (3.86 g, 18.3 mmol). The mixture was heated under reflux for 36 h and concentrated in vacuo. The residue was dissolved in pyridine (36 mL), and the resulting solution was heated under reflux. Water (12 mL) was then added and the reaction mixture refluxed for 3 h. After the mixture cooled, water (100 mL) and CH_2Cl_2 (100 mL) were added to the mixture. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated. The solid residue residue was chromatographed on silica gel (AcOEt:cyclohexane = 3:1) to provide a solid which was recrystallized from 2-propanol to afford **10** (2.7 g, 55%) as brown crystals: mp 198−199 °C; IR (KBr) 3526, 3200−2400, 1638, 1600; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.47 (d, J = 8.7 Hz, 1H), 8.19 (d, J = 8.2 Hz, 2H), 7,99 (d, J = 8.7 Hz, 1H), 7.75−7.60 (m, 2H), 7.16−7.05 (m, 2H), 7.01 (dd, J = 8.4, 2.0 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 158.2, 147.5, 147.3, 145.7, 138.5, 137.0, 136.7, 131.7, 127.6, 127.4, 124.7, 124.4, 123.5, 121.5, 120.3, 115.9, 114.3. Anal. ($C_{17}H_{12}N_2O_4$) C, H, N.

(*E*)-2-[2-(3,4-Dihydroxyphenyl)ethenyl]-8-aminoquinoline (11). To a solution of 8-nitroquinaldine (3.0 g, 16 mmol) in acetic anhydride (30 mL) was added 3,4-diacetoxybenzaldehyde (3.86 g, 18.3 mmol). The mixture was heated under reflux for 36 h and concentrated in vacuo. The residue was dissolved in ethanol (30 mL); tin(II) chloride (7.2 g, 38 mmol) was then added, and the reaction mixture stirred for 1 h. The solution was cooled to 0 °C; water (10 mL) was added. The mixture was basified to pH 8 with aqueous sodium carbonate and extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated to leave crude 2-[2-(3,4-diacetoxyphenyl)ethenyl]-8-aminoquinoline which was directly hydrolyzed in the next step. The obtained product (2.0 g, 5.5 mmol) was dissolved in refluxing pyridine (36 mL), water (12 mL) was added, and the reaction mixture refluxed for 3 h. After the mixture cooled, water (40 mL) and CH_2Cl_2 (50 mL) were added to the mixture. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated. The solid residue was chromatographed on silica gel (CH_2Cl_2:MeOH = 13:1) to provide amine **11** (0,18 g, 5%) as a brown amorphous solid: IR (KBr) 3600−2600, 1633, 1597; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 9.20 (broad s, 2H), 8.07 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 16.4 Hz, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.25−6.90 (m, 5H), 6.90−6.70 (m, 2H), 5.93 (s, 2H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 152.6, 146.5, 145.6, 145.0, 137.1, 136.1, 133.6, 128.2, 127.3, 127.0, 125.4, 119.9, 119.6, 115.9, 113.8, 113.6, 108.9. Anal. ($C_{17}H_{14}N_2O_2 \cdot {}^1/_4H_2O$) C, H, N.

(*E*)-2-[2-(3,4-Diacetoxyphenyl)ethenyl]-8-acetoxyquinoline (12). To a solution of 8-acetoxyquinaldine (2.8 g, 14 mmol) in acetic anhydride (30 mL) was added 3,4-diacetoxybenzaldehyde (3.86 g, 18.3 mmol). The mixture was heated under reflux for 24 h and concentrated in vacuo. The solid residue was taken into ether and filtered on a sintered glass funnel. The solid was thoroughly washed with ether to leave compound **12** (3.0 g, 53%) as a red solid: mp 163−165 °C; IR (KBr) 1777, 1596; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.13 (d, J = 8.8 Hz, 1H), 7.80−7.56 (m, 3H), 7.54−7.40 (m, 4H), 7.26−7.19 (m, 2H), 2.55 (s, 3H), 2.33 (s, 3H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 168.1 (3C), 155.3, 147.4, 142.1, 140.1, 136.4, 135.5, 132.8, 130.1, 128.6, 125.8, 125.5 (2C), 125.3, 123.7, 121.8, 121.7, 120.2, 21.0, 20.7 (2C). Anal. ($C_{23}H_{19}NO_6$) C, H, N.

(*E*)-2-[2-(3,4-Hydroxyphenyl)ethenyl]-8-hydroxyquinoline (13). A solution of compound **12** (1.37 g, 3.4 mmol) in pyridine (10 mL) was heated under reflux. Water (5 mL) was then added, and the reaction mixture refluxed for 3 h. After the mixture cooled, water (30 mL) was added and the reaction mixture was stored at 0 °C overnight. The crystals were collected by filtration and washed with water, ethanol, and finally ether to give quinoline **13** (0.53 g, 56%) as bright-red crystals: mp 232−235 °C; IR (KBr) 3600−2400, 1589; ^{1}H NMR

(acetone-d_6, 200 MHz) δ 8.23 (d, J = 8.7 Hz, 1H), 7.91 (d, J = 16.2 Hz, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.40−7.30 (m, 2H), 7.22 (s large, 1H), 7.21 (d, J = 16.2 Hz, 1H), 7.10−7.05 (m, 2H), 6.87 (d, J = 8.1 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 154.1, 152.9, 146.8, 145.7, 138.2, 136.4, 135.1, 128.2, 127.5, 126.7, 124.7, 120.8, 119.8, 117.7, 116.0, 114.0, 111.2. Anal. ($C_{17}H_{13}NO_3 \cdot {}^1/_4H_2O$) C, H, N.

7-[2-(3,4-Dihydroxyphenyl)ethenyl]-8-hydroxyquinaldine (32). To a suspension of phosphonium salt **31** (6.9 g, 10 mmol) in anhydrous THF (35 mL) was added at − 78 °C a 1.5 M hexane solution of *n*-BuLi (6.9 mL, 10.3 mmol). The temperature was gradually raised to room temperature, and a solution of 8-acetoxyquinaldine-7-carbaldehyde (**31**) (1.7 g, 7.4 mmol) in THF (15 mL) was added dropwise. After the mixture stirred for 2 h at room temperature, water (10 mL) was added and the mixture was filtered. The filtrate was extracted with three 50-mL portions of CH_2Cl_2. The combined organic extracts were dried and concentrated in vacuo. The residue was taken up in THF (10 mL) and treated with a 1 M THF solution of *n*-Bu_4NF (16 mL, 16 mmol). After the mixture stirred for 1 h at 20 °C, 10% aqueous acetic acid (30 mL) was added and the mixture was extracted with AcOEt. The combined organic extracts were dried and concentrated to give 0.60 g of **32**, as a yellow solid which was directly used in the next step without further purification.

(*E*,*E*)-2,7-Bis[2-(3,4-hydroxyphenyl)ethenyl]-8-hydroxyquinoline (14). To a solution of crude compound **32** (0,6 g, 2 mmol) in dry acetone (10 mL) were added at 0 °C potassium carbonate (1.7 g, 12 mmol) and acetyl chloride (0,94 g, 12 mmol). After being stirred for 2 h at 20 °C, the reaction mixture was filtered and concentrated in vacuo. Water was added, and the mixture was extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated. The residue was taken up into acetic anhydride (8 mL), and 3,4-diacetoxybenzaldehyde (0.28 g, 1.3 mmol) was added. The mixture was heated under reflux for 4 days, and concentrated in vacuo. The obtained product was dissolved in refluxing pyridine (10 mL), water (4 mL) was added, and the reaction mixture refluxed for 3 h. After the mixture cooled, water (40 mL) and CH_2Cl_2 (50 mL) were added to the mixture. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated. The solid residue was chromatographed on silica gel (CH_2Cl_2:MeOH = 13:1) to give compound **14** as a red solid (0.07 g, 2% overall yield from **31**): mp >220 °C; IR (KBr) 3600−2600, 1597; ^{1}H NMR (acetone-d_6, 200 MHz) δ 8.20 (d, J = 8.7 Hz, 1H), 7.91 (d, J = 16.4 Hz, 1H), 7.76 (d, J = 9.1 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 16.4 Hz, 1H), 7.32−7.15 (m, 5H), 7.08 (d, J = 8.9 Hz, 1H), 6.98 (d, J = 7.8 Hz, 1H), 6.85 (m, 2H). Anal. ($C_{25}H_{19}NO_5 \cdot H_2O$) C, H, N.

General Procedure A. Condensation of 8-Hydroxy-7-quinaldic Acid (5) with Aromatic Aldehydes. To a solution of 1.0 equiv of acid **5** in acetic anhydride (3 mL/mmol) was added 1.3 equiv of aldehyde. The mixture was heated under reflux for 24 h and concentrated in vacuo. The residue was dissolved in pyridine (4 mL/mmol), the resulting solution was heated under reflux, water (1 mL/mmol) was then added, and the reaction mixture refluxed for 3 h. After the mixture cooled, water and CH_2Cl_2 were added with stirring. After standing at 0 °C for 8 h the reaction mixture was filtered. The solid was washed with methanol, CH_2Cl_2, and finally ether and dried in vacuo to provide acids **15**, **17**, **19**, and **21**.

(*E*)-8-Hydroxy-2-[2-(2,4-dihydroxyphenyl)ethenyl]-7-quinolinecarboxylic Acid (15). Method A using 2,4-diacetoxybenzaldehyde afforded acid **15** in 39% overall yield as red crystals: mp >220 °C; IR (KBr) 3600−2400, 1587; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.46 (d, J = 8.9 Hz, 1H), 8.13 (d, J = 8.9 Hz, 1H), 8.03 (d, J = 16.4 Hz, 1H), 7.81 (d, J = 8.6 Hz, 1H), 7.65 (d, J = 16.4 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.13 (d, J = 8.5 Hz, 1H), 6.39 (s, 1H), 6.35 (d, J = 8.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 170.7, 161.1, 160.9, 158.5, 153.3, 140.7, 135.6, 134.5, 130.3, 129.9, 127.7, 120.3, 118.8, 114.3, 113.5, 112.6, 108.2, 102.7. Anal. ($C_{18}H_{13}NO_5 \cdot H_2O$) C, H, N.

(*E*)-8-Hydroxy-2-[2-(3,4-dihydroxyphenyl)ethenyl]-7-quinolinecarboxylic Acid (17). Method A using 3,4-diacetoxybenzaldehyde afforded acid **17** in 50% overall yield as red crystals: mp ≥230 °C; IR (KBr) 3400−2600, 1667, 1590; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.48 (d, J = 8.8 Hz, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 16.2 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 16.2 Hz, 1H), 7.16 (d, J = 8.6 Hz, 1H), 7.09 (d, J = 1.5 Hz, 1H), 7.04 (dd, J = 8.2; 1.5 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 170.6, 160.8, 152.8, 148.0, 145.7, 140.0, 139.2, 135.2, 130.5, 127.5, 127.3, 120.9, 120.7, 120.2, 116.0, 114.2, 113.2, 112.8. Anal. ($C_{18}H_{13}NO_5 \cdot {}^1/_4H_2O$) C, H, N.

(*E*)-8-Hydroxy-2-[2-(3,4,5-trihydroxyphenyl)ethenyl]-7-quinolinecarboxylic Acid (19). Method A using 3,4,5-triacetoxybenzaldehyde afforded acid **19** in 40% overall yield as red crystals: mp ≥230 °C; IR (KBr) 3600−2400, 1630, 1585; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.51 (d, J = 8,8 Hz, 1H), 8.21 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.80 (d, J = 16.1 Hz, 1H), 7.44 (d, J = 16.1 Hz, 1H), 7.16 (d, J = 8.4 Hz, 1H), 6.68 (s, 2H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 170.6, 161.0, 152.7, 146.4 (2C), 140.4, 140.1, 136.3, 134.9, 130.5, 127.8, 126.2, 120.8, 120.0, 113.2, 112.9, 107.4 (2C). Anal. ($C_{18}H_{13}NO_6 \cdot {}^1/_2H_2O$) C, H, N.

(*E*)-8-Hydroxy-2-[2-(3-carboxyl-4-hydroxyphenyl)-ethenyl]-7-quinoline carboxylic Acid (21). Method A using 5-formylsalicylic acid afforded diacid **21** in 38% overall yield as red crystals: mp >220 °C; IR (KBr) 3600−2200, 1665, 1610, 1590; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.47 (d, J = 8.8 Hz, 1H), 8.17−8.07 (m, 2H), 7.98 (d, J = 16.4 Hz, 1H), 7.90 (dd, J = 8.8, 2.0 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 16.4 Hz, 1H), 7.23 (d, J = 8.6 Hz, 1H), 7.04 (d, J = 8.6 Hz, 1H). Anal. ($C_{19}H_{13}NO_6 \cdot H_2O$) C, H, N.

8-Hydroxy-7-quinaldinecarbaldehyde Oxime (29). To a solution of 8-hydroxy-7-quinaldinecarbaldehyde (**28**)[51] (1.0 g, 5.3 mmol) in acetic acid (20 mL) were added hydroxylamine hydrochloride (0,69 g, 10 mmol) and sodium acetate (0.82 g, 10 mmol). The resulting mixture was heated at 100 °C for 1 h. After cooling the reaction mixture was concentrated under reduced pressure. The residue was poured into water and extracted with three 50-mL portions of CH_2Cl_2. The combined organic extracts were dried and concentrated to give oxime **29** (0.70 g, 65%) as a yellow solid: mp 171−175 °C; IR (KBr) 3400−2400, 1643, 1614, 1563; ^{1}H NMR (DMSO-d_6, 200 MHz) (only the major anti isomer is described) δ 8.50 (s, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.32 (d, J = 8.6 Hz, 1H), 2.68 (s, 3H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 157.4, 150.8, 144.7, 138.1, 136.2, 127.2, 123.2, 122.9, 117.9, 115.2, 24.7.

(*E*)-7-Cyano-8-hydroxy-2-[2-(2,4-dihydroxyphenyl) ethenyl]-7-quinoline (16). A mixture of oxime **29** (0.60 g, 2.9 mmol) and 3,4-diacetoxybenzaldehyde (0.72 g, 3.4 mmol) in acetic anhydride (10 mL) was heated under reflux for 24 h and concentrated in vacuo. After cooling the reaction mixture was concentrated under reduced pressure. The residue was dissolved in pyridine (10 mL), and the solution was heated under reflux. Water (8 mL) was then added, and the reaction mixture refluxed for 3 h. After the mixture cooled, water (20 mL) and CH_2Cl_2 were added to the mixture. The organic phase was separated and the aqueous phase extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated. The solid residue was recrystallized from 2-propanol to afford dark-red crystals of **16** (0.63 g, 71%): mp 239−244 °C; IR (KBr) 3600−2600, 2194, 1630, 1603; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.32 (d, J = 8.6 Hz, 1H), 8.04 (d, J = 16.2 Hz, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.16 (d, J = 16.2 Hz, 1H), 7.10 (s, 1H), 7.00 (d, J = 8.4 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 158.2, 155.7, 149.6, 147.0, 145.6, 137.6, 136.7, 129.6, 127.8, 126.5, 123.9, 123.7, 123.3, 119.9, 118.5, 117.1, 115.9, 114.0. Anal. ($C_{18}H_{12}N_2O_3 \cdot {}^1/_4H_2O$) C, H, N.

General Procedure B. Synthesis of Sodium Salts 18 and 20. To a suspension of the acid (0.3 mmol) in degassed water was added, a 0.1 M solution of sodium hydroxide

dropwise (3 mL, 0.3 mmol) under nitrogen atmosphere. The obtained solution was lyophilized (Christ Lyophilizer, D) over 24 h (−40 °C/+40 °C) to give the expected salt as a brown solid (quantitative).

(*E*)-8-Hydroxy-2-[2-(3,4-dihydroxyphenyl)ethenyl]-7-quinolinecarboxylic Acid Sodium Salt (18). Method B starting from acid **17** gave salt **18** as a brown amorphous solid: IR (KBr) 3600−2200, 1630, 1598; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.08 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 16.4 Hz, 1H), 7.24 (d, J = 16.4 Hz, 1H), 7.13 (s, 1H), 6.95 (m, 2H), 6.74 (d, J = 7.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 171.6, 163.7, 154.0, 147.7, 146.2, 141.1, 136.0, 133.6, 129.9, 127.5, 127.0, 126.0, 119.5, 119.2, 115.8, 114.9, 113.7, 111.9.

(*E*)-8-Hydroxy-2-[2-(3,4,5-trihydroxyphenyl)ethenyl]-7-quinolinecarboxylic Acid Sodium Salt (20). Method B starting from acid **18** gave salt **20** as a brown amorphous solid: mp ≥300 °C; IR (KBr) 3700−2400, 1596; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.06 (d, J = 8.6 Hz, 1H), 7.84 (d, J = 8.6 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 16.4 Hz, 1H), 7.16 (d, J = 16.4 Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.65 (s, 2H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 171.6, 163.5, 154.0, 146.5, 146.2 (2C), 141.0, 136.0, 134.2, 129.7, 126.8, 125.7, 125.6, 119.2, 115.0, 112.0, 106.2 (2C).

Preparation of DNA Substrates. Oligonucleotides U5B 5′-GTGTGGAAAATCTCTAGCA, U5A ACTGCTAGAGATTTTC-CACAC, and D38U3 TGCTAGTTCTAGCAGGCCCTTGGGC-CGGCG-CTTGCGCC[6] were purchased from Eurogentec and further purified by denaturing 18% acrylamide gel. For processing and disintegration assays, 100 pmol of U5A and D30U5 oligonucleotides were radiolabeled respectively using T4 polynucleotide kinase (Biolabs) and 50 μCi of [γ-^{32}P]ATP (sp. act. 3000 Ci/mmol). Kinase was heat-inactivated, and unincorporated nucleotides were removed by passage through Sephadex G-10 (Pharmacia). NaCl was added to the final concentration of 0.1 M, and complementary unlabeled strand U5B was added to U5A. The mixture was heated to 90 °C for 2 min, and the DNA was annealed by slow cooling. For the integration experiment (strand transfer), a 492-bp DNA substrate which contains LTR termini at each end to mimic the unintegrated proviral DNA genome was used as donor substrate. Plasmid pSP70 was used as acceptor substrate. The 492-bp DNA donor substrate was prepared from plasmid pU3U5.[59]

HIV-1 Integrase Assay. Purified recombinant full-size HIV-1 integrase was a generous gift of Rhône-Poulenc-Rorer. Plasmid encoding the His-tagged soluble deletion mutant F(185)K corresponding to the (50−212) core domain of HIV-1 integrase was generously provided by Dr. R. Craigie. The protein was expressed and purified as described in ref 52. Processing assay was performed using 0.5 pmol of U5A/U5B substrate in the presence of 1 pmol of integrase in buffer containing 20 mM Tris (pH 7.2), 30 mM NaCl, 10% (w/v) glycerol, 10 mM DTT, 0.01% NP40, supplemented with 10 mM $MnCl_2$. Disintegration assays were performed in the presence of 0.5 pmol of D38U3 substrate and 2 pmol of integrase core domain. Finally, integration reactions were performed using 10 ng of donor and 40 ng of target DNA, in the presence of 1 pmol of IN. Gels were analyzed using a STORM Molecular Dynamics phosphorimager. Inhibition in the presence of drugs is expressed as percent of fractional product compared with the control.

***Eco*RI Cleavage Assay.** *Eco*RI enzyme and pSP70 were from Biolabs; 1-kb DNA size marker (Pharmacia) was labeled using T4 polynucleotide kinase-exchange reaction. pSP70 was linearized with restriction enzyme *Ssp*I and labeled using T4 polynucleotide-exchange reaction in the presence of 50 μCi of [γ-^{32}P]ATP (sp. act. 3000 Ci/mmol); 2 nM of the labeled fragment was incubated at 37 °C with 0.1 unit of *Eco*RI in the presence of increasing concentrations of drugs dissolved in DMSO, in a final volume of 10 μL. Final concentration of DMSO was 10%. An aliquot of the reaction was loaded on a 0.8% agarose gel. Results were analyzed using a STORM 840 Molecular Dynamics phosphorimager.

Antiviral Assays. The lymphocyte cell line CEM was maintained in RPMI-1640 medium (GIBCO Laboratories) supplemented with 10% fetal calf serum. HeLaCD4$^+$-β-gal cells, kindly provided by P. Charneau (Institut Pasteur, Paris), were grown in DMEM with 10% fetal calf serum and 0.5 mg/mL Geneticin. Cell-free viral supernatants were obtained by transfection of CEM cells with HIV-1 PLN4-3 genomic clone. Cells were plated in triplicate on 96-well plates (100 μL) and infected with cell-free virus. Viral supernatants were removed 2 h after infection, and drugs dissolved in DMSO were added in fresh medium; 72 h later, supernatants were used to infect HeLaCD4$^+$-β-gal cells. The P4 cultures were subsequently incubated for 24 h and subsequently lysed in a phosphate buffer containing 50 mM 2-mercaptoethanol, 10 mM $MgSO_4$, 25 mM EDTA, and 0.125% NP40; 20 μL of lysate was incubated with 100 μL of CPRG containing buffer.[58] The red staining intensity was quantified on a multiscan photometer at 570 nm. Cell viability was estimated by the MTT (Sigma) assay; 20 μL of a solution of MTT (7.5 mg/mL) in phosphate buffer was added to each well of the microtiter trays. The plates were further incubated at 37 °C in a CO_2 incubator for 4 h. Solubilization of the formazan crystals was achieved by adding 100 mL of 10% SDS, 10 mM HCl. Absorbances were read in a multiscan photometer at 570 nm. In some cases, antiviral activity was measured by p24 antigen ELISA.

Acknowledgment. A fellowship from the Fondation pour la Recherche Médicale (Sidaction) to K.M. is acknowledged. This work was in part supported by funds from the Agence Nationale de Recherche sur le Sida (ANRS). We thank also Professor B. Legendre (Faculté de Pharmacie de Châtenay-Malabry) for microthermal analyses, Mrs. S. Mairesse-Lebrun for performing elemental analyses, and M. E. Franque for skillfull help.

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JM980043E