Synthesis and Biological Evaluation of Geminal Disulfones as HIV-1 Integrase Inhibitors

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Integration of HIV-1 viral DNA into the host genome is carried out by HIV-integrase (IN) and is a critical step in viral replication. Although several classes of compounds have been reported to inhibit IN in enzymatic assays, inhibition is not always correlated with antiviral activity. Moreover, potent antiviral IN inhibitors such as the chicoric acids do not act upon the intended enzymatic target but behave as entry inhibitors instead. The charged nature of the chicoric acids contributes to poor cellular uptake, and these compounds are further plagued by rapid ester hydrolysis in vivo. To address these critical deficiencies, we designed neutral, nonhydrolyzable analogues of the chicoric acids. Herein, we report the synthesis, enzyme inhibition studies, and cellular antiviral data for a series of geminal disulfones. Of the 10 compounds evaluated, 8 showed moderate to high inhibition of IN in purified enzyme assays. The purified enzyme data correlated with antiviral assays for all but two compounds, suggesting alternative modes of inhibition. Time-of-addition studies were performed on these analogues, and the results indicate that they inhibit an early stage in the replication process, perhaps entry. In contrast, the most potent member of the correlative group shows behavior consistent with IN being the cellular target.

Introduction

There are currently four classes of antiretroviral drugs that are approved for use in AIDS therapy. Three of these classes have been extensively used in highly active antiretroviral therapy (HAART): nucleoside analogues that inhibit reverse transcriptase (RT), nonnucleoside inhibitors of reverse transcriptase (NNRTI), and protease inhibitors (PI).[1] A fourth class is represented by T20 (Fuzeon), a fusion/entry inhibitor that was recently approved for use in humans. Current HAART is based on using potent combinations of these drugs, usually three or more drugs from two or more classes. Major forces leading to development of combination therapy for AIDS were the inability of individual drugs (monotherapy) to adequately reduce virus loads and the emergence of drug-resistant mutants that was usually rapid with any single drug. Viral drug resistance was considered the major limitation of antiretroviral drugs in the pre-HAART era.[2−4] The development of HAART enabled suppression of virus load to undetectable levels for prolonged periods in many patients but has not eliminated problems from viral drug resistance. The potent combinations used in HAART, when successful, decrease the rate of emergence of resistant variants because of greatly decreased viral load. Nevertheless, treatment failure is usually accompanied by emergence of HIV-1 variants that contain multiple drug-resistance mutations.[5] These multi-drug-resistant variants of HIV-1 can also be transmitted.[6] The problem of drug resistance is not limited to inhibitors of reverse transcriptase and protease. In vitro studies have also identified mutations that confer resistance to drugs targeted against HIV-1 IN and entry inhibitors,[7] suggesting that resistance is likely to occur with any new agent regardless of the viral target.

HIV integrase (IN) is required for integration of viral DNA into host chromosomes. It is an especially attractive chemotherapeutic target because no mammalian counterpart to this enzyme has been identified, and the region of the HIV pol gene encoding IN shows more conservation than RT- and PR-encoding regions.[8] IN is active in both the cytoplasm and nucleus of the host cell. While in the cytoplasm, IN forms a preintegration complex with viral DNA and selectively cleaves 3′-terminal dinucleotides from each end.[9] This so-called 3′-processing event occurs prior to nuclear translocation of the complex. In the nucleus, IN catalyzes strand transfer, which leads to integration of viral DNA into the host DNA.[10] The latter process is essential for viral replication and thus has become a primary target for drug discovery.[11] Because IN is actively involved in viral replication for a limited period of time, its viability as a target was long debated. Skeptics felt the chances were low that sufficient concentrations of drug would be available to inhibit strand transfer for the relatively short time IN is activated. Recent findings by Hazuda et al. have essentially disproved this argument by demonstrating sustained suppression of viremia in

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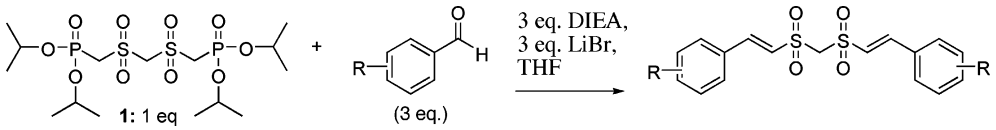
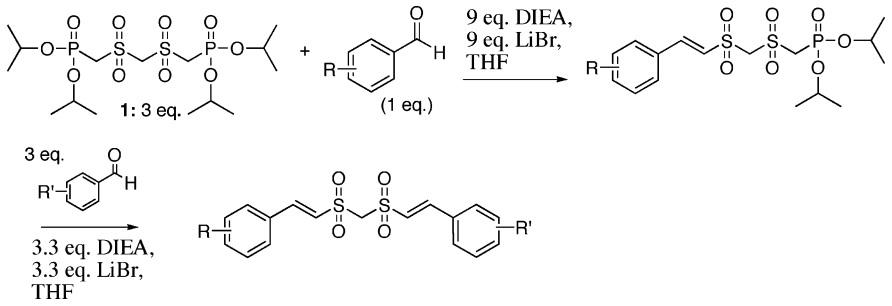
Scheme 1. Synthesis of Symmetrical Geminal Disulfones



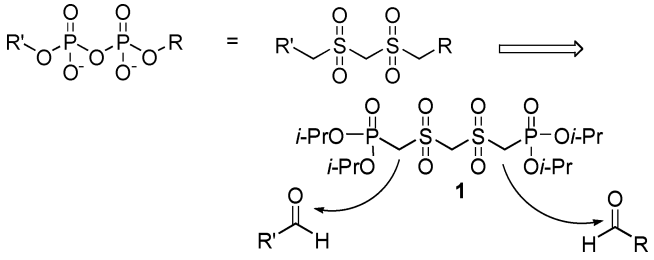
Scheme 2. Synthesis of Unsymmetrical Geminal Disulfones



simian−human immunodeficiency virus (SHIV) infected rhesus macaques treated with IN inhibitors.[12] In follow-up studies, these researchers showed that several known IN inhibitors have unique resistance profiles highlighting the importance of continual identification of new classes of inhibitors that are active at all levels of viral replication.[13]

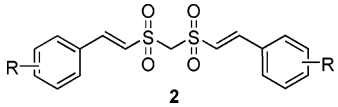
Although several compounds have been reported to inhibit IN,[14,15] enzyme activity is not always correlated with antiviral activity.[16] Even compounds showing antiviral activity may not act upon the intended intracellular enzymatic target but serve to block viral entry instead.[17,18] Compounds possessing charge at biological pH appear to be particularly susceptible to this alternative pathway of viral inhibition, prompting us to develop synthetic methods for the preparation of neutral IN inhibitors. In particular, we were inspired by developments in the Robinson labs indicating that chicoric acids are potent IN inhibitors.[19] Unfortunately, these inhibitors are plagued by poor cellular uptake and rapid hydrolysis in vivo, but they present an excellent starting point for drug discovery.

We have been involved in studies targeting phosphate mimics for several years with studies centered on using sulfones as neutral phosphate analogues.[20,21] Critical to the success of these endeavors was the discovery that highly functionalized geminal disulfones could be efficiently prepared from simple bis-phosphonate reagents **1** invented in our laboratory.[22] The basic design elements include maintaining the bond distance between functional groups by substituting a carbon atom for the oxygen atoms in the phosphate backbone. Similarly, the phosphorus atoms are substituted with sulfur, which is oxidized to the sulfone to increase metallophilicity.



The pK_a of the methylene proton between the two sulfones is 12.5, indicating that it would not be deprotonated at biological pH.[23] Given that IN is a metalloenzyme that makes and breaks phosphate bonds,[24] we felt that metallophillic phosphate isosteres would be well-suited as structural cores for IN inhibitors.

Previously, we reported the synthesis of **1** and its utility in synthesizing geminal disulfones via Horner−Emmons−Wadsworth reaction with a variety of aliphatic and aromatic aldehydes.[25] In this report, we describe the synthesis and biological evaluation of a collection of geminal disulfones having the general structure **2** as potential HIV-1 IN inhibitors. Neamati



2

and co-workers have reported using monosulfones for this purpose,[26] but to our knowledge geminal disulfone analogues have not been previously evaluated.

Results and Discussion

Synthesis of the biaryl/disulfone library was accomplished by coupling a variety of aromatic aldehydes to disulfone/diphosphonate reagent **1** in a Horner−Emmons−Wadsworth olefination reaction using either LiO*t*Bu or Hünig's base in the presence of LiBr in dry THF (Scheme 1). This strategy allows for the synthesis of both symmetric and asymmetric compounds dependent upon reactant stoichiometry. For symmetric compounds, 1 equiv of **1** is reacted with 3 equiv of aldehyde. Asymmetric compounds are prepared using 3 equiv of **1** with 1 equiv of aldehyde followed by workup to give the monocoupled product. This adduct (1 equiv) can then be reacted under the same conditions using 3 equiv of a different aldehyde (Scheme 2). Table 1 lists the compounds prepared with the corresponding aldehydes reacted according to Schemes 1 and 2. Aldehydes containing phenols or phenols masked as esters were primarily chosen because catechols are known to be important functionalities in many IN inhibitors.[27−29]

Biological Assays with Purified Enzyme. The compounds shown in Table 1 were first tested against purified enzyme to determine whether 3′-processing or

Table 1. Geminal Disulfones Prepared According to Schemes 1 and 2

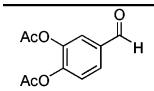
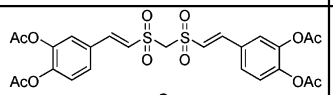
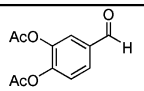
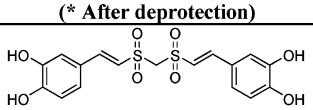
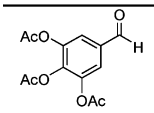
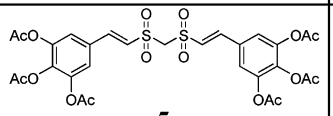
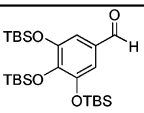
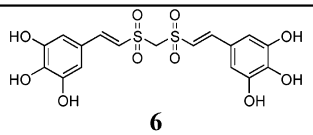
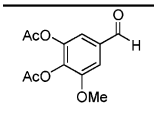
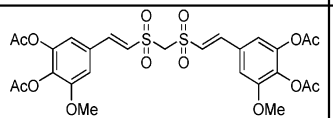
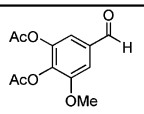
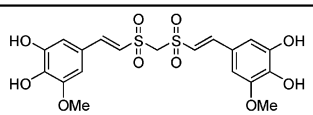
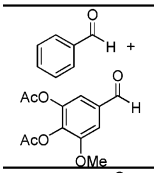
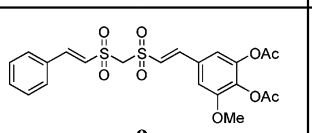
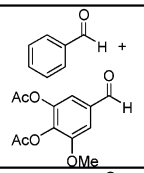
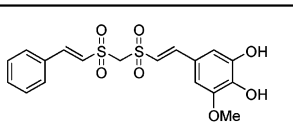
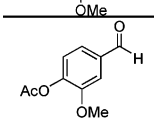
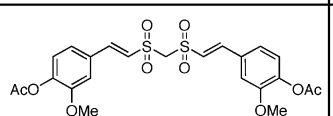
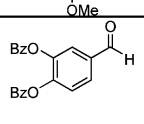
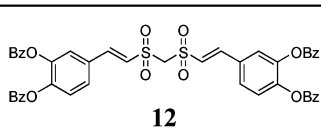
Aldehyde	Coupled Product	Aldehyde(s)	Coupled Product (* After deprotection)
	 **3**		 **4***
	 **5**		 **6**
	 **7**		 **8***
	 **9**		 **10***
	 **11**		 **12**

Table 2. HIV-IN Inhibition Data

compd	IC_{50} (μM) 3′-processing	IC_{50} (μM) integration
3	70 ± 5	70 ± 5
4	4 ± 1	5 ± 1
5	50 ± 6	5 ± 2
6	1.8 ± 0.3	0.9 ± 0.1
7	30 ± 3	30 ± 2
8	50 ± 10	50 ± 10
9	80 ±10	80 ± 10
10	80 ± 11	80 ± 11
11	>1000	>1000
12	700 ± 40	700 ± 70
L-708,906	>1000	0.48 ± 0.08[30]

integration would be inhibited. As can be seen in Table 2, five of the compounds, **3**, **7**, **8**, **9**, and **10**, exhibited moderate integration activity, while three compounds, **4**, **5**, and **6** displayed potent integration inhibition. Replacing a *m*-acetoxy (as in **3**) with an electron-donating methoxy (as in **11**) has deleterious effects, which can be overcome by substituting the other meta position with an acetoxy functionality (as in **7**). Similarly, substituting acetates with benzoates (compare **3** and **12**) results in loss of activity. It is noteworthy that only **5** shows any selectivity for 3′-processing over integration while all other compounds show comparable inhibition for both catalytic functions.

Cell-Based Assays. After the geminal disulfones were demonstrated to be capable of inhibiting purified HIV-1 IN, the next step was to evaluate these compounds for antiviral activity. The North Laboratory has used a focal infectivity assay (FIA) extensively for studies of antiviral drugs and viral drug resistance, with feline immunodeficiency virus (FIV), simian immunodeficiency virus (SIV),[31−33] and HIV-1. The assay we currently use for HIV-1 has recently been described.[31,32] It is similar to the FIA that was originally developed by Chesebro[34] except that indicator cells are HeLa H1-JC.37 cells. These are HeLa CD4 cells expressing human CCR5 (these cells naturally express CXCR4)[35] and are permissive for infection by T-cell tropic and macrophage tropic isolates of HIV-1, SIV, or Env-SHIVs. With this assay, we have determined that a known IN inhibitor, L-708,906, inhibits HIV-1 with an EC_{50} of 0.78 μM, which is consistent with previous reports of this compound.[36] These data validate use of the FIA to evaluate IN inhibitors (Table 3). As shown in Table 3, the most potent inhibitor of HIV-1 is compound **6**. Representative dose response curves for compounds **3**−**6** and are shown in Figure 1.

Table 3. Antiviral Activity, Cytotoxicity, and Antiviral Selectivity Data for the IN Inhibitors

integrase inhibitor	antiviral activity[a] EC_{50} ± SE (μM)	cytotoxicity[b] IC_{50} (μM)	antiviral selectivity IC_{50}/EC_{50}
3	8.0 ± 2.5	148 ± 2.7	19 ± 6.0
4	2.4 ± 0.3	187 ± 7.6	78 ± 6.7
5	0.4 ± 0.1	27 ± 0.8	69 ± 16
6	0.3 ± 0.03	169 ± 6.4	563 ± 35
7	1.5 ± 0.02	18 ± 1.2	12 ± 0.7
8	1.3 ± 0.09	29 ± 1.5	22 ± 0.4
9	3.9 ± 1.2	21 ± 5.6	5.3 ± 0.24
10	4.5 ± 0.86	19 ± 1.9	4.3 ± 0.4
L-708,906	0.78	ND[c]	ND[c]

[a] EC_{50} values are the mean ± SE from three separate determinations except for L-708,906, which is from a single experiment. [b] IC_{50} values are the mean ± SE from two separate determinations. [c] ND: not determined.

Comparison of the data in Tables 2 and 3 shows that diacetate **3** is less active than its hydroxy analogue **4** in both the IN and FIA assays, whereas there is little difference between **7** and **8** and between **9** and **10**, suggesting that acetate substitution has no effect in the latter cases. Furthermore, antiviral activities for these three pairs of compounds strongly correlate with puri-

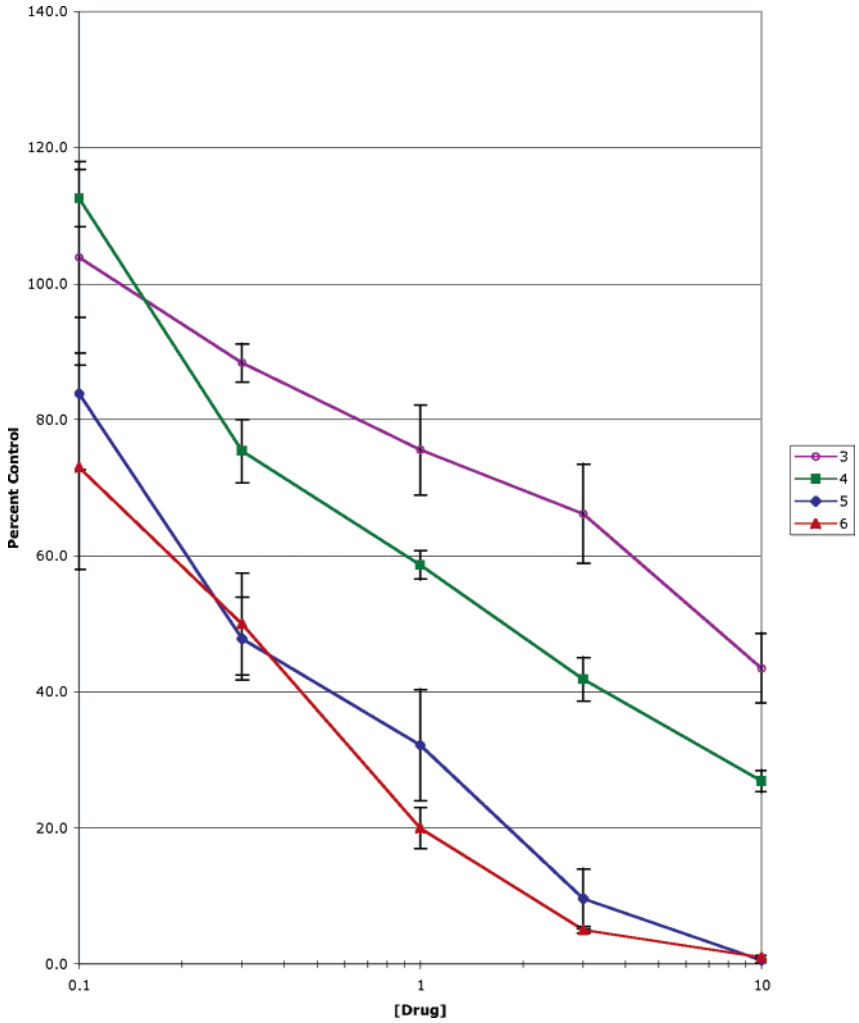


Figure 1. Dose response curves for inhibitors **3**−**6**. Reported data represent the mean ± SE for three independent experiments.

fied enzyme studies, consistent with the hypothesis that they are targeting IN. To ensure that the inhibitors are capable of crossing the cell membrane, cells were incubated for 6 days with **4**. On the sixth day, the supernatant was removed and the cells were lysed via multiple freeze/thaw cycles. The lysate was removed and purified on HPLC detecting at the absorption maximum for **4**, which gave a single peak corresponding to the molecular ion of **4** as determined by mass spectrometry. These combined studies suggest that the inhibitors are crossing the cell membrane and entering the cytoplasm of the cell.

The fact that antiviral activities are generally higher than purified enzyme activity is not particularly alarming because similar observations have been reported for viral neuraminadase activity.[37] However, we did take note of the discrepancy between antiviral activity of **5** and **6** and its lack of correlation with purified enzyme activity because this result could be indicative of an alternative mode of action such as entry. To address this critical issue, we next looked for evidence of early stage inhibition.

Time-of-Addition Studies. Time-of-addition studies are performed to determine how long after infection the addition of a drug can be delayed and still retain its activity. The earlier in the replication cycle the drug inhibits, the shorter the time its addition can be delayed and still retain activity. For example, entry inhibitors can be delayed only 1 h, RT inhibitors for 4 h, and protease inhibitors for 18−19 h.[38] In our study, we used dextran sulfate, a known entry inhibitor, T20, a fusion inhibitor, and 3TC, a reverse transcriptase inhibitor and compared their activity to compounds **4**−**6**. The inhibitors were each used at 10 times their respective EC_{50} values and were added at six different time points: −1, 0, 2, 4, 6, and 10 h. The cells were incubated for 4 days, after which they were fixed and stained and foci were counted and plotted against a no-drug control. As can be seen from Figure 2, **5** and **6**, like dextran sulfate and T20, exhibit a sharp decrease in activity if added more than 2 h after infection. This behavior strongly implicates an early event, such as viral entry, as the primary mode of action for these compounds. In contrast, **4** shows behavior similar to that of 3TC, suggesting that its mode of action is at a later stage in the replication cycle. Given that **4** is a potent IN inhibitor, it is likely that IN is the cellular target, but further studies including selecting for mutant viruses will be required to prove this hypothesis.

We have used a standard cell proliferation assay (MTS assay) to evaluate cytotoxicity of these compounds to HeLa H1-JC.37 cells. Cytotoxicity of all the inhibitors we tested required concentrations substantially higher than required for antiviral activity (Table 3). The most selective inhibitors were compounds **4**−**6**, which had 78-, 69-, and 570-fold selectivity, respectively (Table 3).

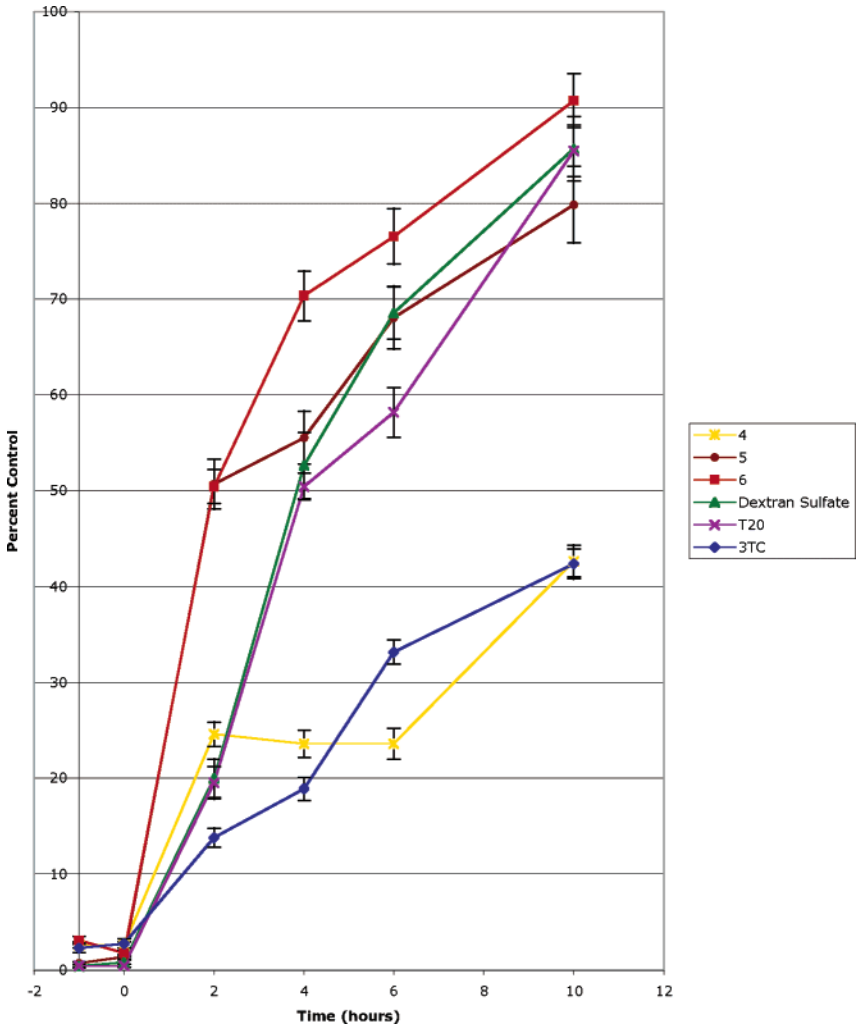


Figure 2. Time-of-addition studies for **4**−**6**. The data represent mean values ± SE from five independent experiments.

To rule out the possibility of conjugate addition contributing to toxicity, active compounds were screened for their ability to act as Michael acceptors of glutathione. Briefly, 1.5 mL of a 0.5 M solution of glutathione was prepared in PBS, and to this was added 40 μL of a 0.75 mM solution of the vinyl sulfone in DMSO (a large excess of the nucleophile is used to ensure first-order kinetics). This mixture was incubated for 4 days at 37 °C and then subjected to ESI mass spectrometry. The spectrum of the mixture was then compared to spectra of glutathione monoaddition and double-addition compounds prepared synthetically. The spectrum of the mixture showed only molecular ion peaks corresponding to unreacted vinyl sulfone, glutathione, and oxidized glutathione, indicating that under the assay conditions the vinyl sulfones are not reactive toward biological nucleophiles.

Conclusion

We have prepared a series of neutral nonhydrolyzable geminal disulfone analogues of the chicoric acids using a Horner−Wadsworth−Emmons condensation reaction between aromatic aldehydes and a geminal disulfone bisphosphonate reagent developed in our laboratory. Biological evaluation of these compounds reveals potent activity in purified HIV-IN enzyme assays that is correlated with antiviral activity using focal infectivity assays. We have also demonstrated that the compounds are cell-permeable and therefore capable of targeting IN in vitro. For the most part, there is little difference in antiviral activity between pairs of substituted analogues differing only in the acetylation pattern, while replacement of an acetate with either a benzoate or a methyl ether completely obliterates activity. A trihydroxy analogue (**6**) (EC_{50} = 0.3 μM) is the most potent compound we have identified thus far, and it has >500-fold antiviral selectivity. However, the purified enzyme activity of **6** compared to its triacetoxy analogue **5** did not correlate well with cell-based assays, raising the possibility of an alternative mode of action. To probe this possibility, time-of-addition studies were conducted, and the results indicate that **5** and **6** target an earlier event in viral replication. This activity contrasts with the dihydroxy analogue **4**, which inhibits at a later stage, consistent with IN being the cellular target. It is possible that these compounds actually have dual modes of inhibition acting both at entry and at viral integration. Continuing investigations in our laboratory focus on identifying lead candidates for viral resistance studies to identify the molecular targets of inhibition.

Experimental Section

Elemental analyses were obtained from Desert Analytics Laboratory, Tucson, AZ. All materials were obtained from commercial sources and used without additional purification.

The aromatic aldehydes used were bought from Aldrich Chemical Co., Inc. The THF used for reaction was 99.9% anhydrous, inhibitor-free in sureseal bottles also obtained from Aldrich Chemical Co., Inc. All glassware for reactions under anhydrous conditions was flame-dried prior to use. Flash chromatography was performed on silica gel Geduran (40−63 um) from Merck and using EM Science ACS grade solvents. For TLC, silica gel 60 F_{254} plates from Merck were used with detection by UV light and/or iodine chamber. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-500, Varian Mercury 300 MHz, or Varian Inova 400 MHz spectrometers at 25 °C. Chemical shifts in ppm were referenced to $CDCl_3$ (7.26 ppm, 77.16 ppm), DMSO-d_6 (2.50 ppm, 39.52 ppm), and acetone-d_6 (2.05 ppm, 29.84 ppm) as internal standards. IR data were recorded on a Galaxy series FT-IR 3000 instrument at 25 °C. Melting points were determined on a Fisher-Johns melting point apparatus.

General Procedure for the Synthesis of Symmetrical Geminal Disulfones. In a flame-dried flask, 1.0 equiv of the disulfone reagent (**1**), 3.0 equiv of aldehyde, and 3.3 equiv of LiBr were dissolved in 4 mL of dry THF. Once in solution, 3.3 equiv of Hunig's base was added. The mixture was stirred overnight before the reaction was quenched by the addition of 5% HCl until pH 3−4 was attained. The solution was partitioned between ethyl acetate (80 mL) and water (50 mL) and extracted three times (50 mL). The organic phase was collected and dried over sodium sulfate. The solvent was evaporated to yield a solid material, which was purified as indicated for the individual compounds.

(Diisopropoxyphosphorylmethanesulfonylmethanesulfonylmethyl)phosphonic Acid Diisopropyl Ester (1). To commercially available diisopropyl bromomethylphosphonate (Lancaster) (5.24 g, 20.2 mmol) in 15 mL of DMF, 3.46 g (30.3 mmol) of potassium thioacetate and 373 mg of tetrabutylamonium iodide were added, and the mixture was heated to 80 °C with stirring for 2 h. The solution was cooled and then partitioned between water and ethyl acetate. The ethyl acetate layer was collected and dried over sodium sulfate and then evaporated to dryness. To the crude oil was added acetonitrile (15 mL), 3 M NaOH (7.4 mL), and methanol (7.4 mL), and the reaction mixture was stirred for 30 min. The flask was then cooled to 0 °C. Diiodomethane (797 μL, 9.90 mmol) was then added, and the reaction mixture was stirred and allowed to warm to room temperature overnight. The reaction mixture was then partitioned between water and ethyl acetate, and the ethyl acetate layer was collected and dried over sodium sulfate. After evaporation, the crude oil was oxidized using 24.86 g (40.44 mmol) of oxone in methanol/water (~100 mL 1:1) overnight to give a crude solid after ether/bicarbonate extraction. The crude solid was dissolved in ether and hexanes were added dropwise until crystals formed, yielding 3.15 g of **1** (69% yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.33 (d, 24H, J = 6.2 Hz), 3.91 (d, 4H, J = 16.0 Hz), 4.80 (m, 4H), 5.58 (s, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 23.7, 24.1, 50.7, 51.8, 68.1, 73.2, 73.3. HRFABMS calcd for $C_{15}H_{35}O_{10}P_2S_2$ (M + H): 501.1147. Found: 501.1151 (M + H).

Bis(*trans*-β-3,4-diacetoxystyrenesulfonyl)methane (3). To a solution of disulfone **1** (98 mg, 0.20 mmol) in 3 mL of THF was added 0.59 mL of a 1 M solution of lithium *tert*-butoxide in THF. After 10 min 165 mg (0.74 mmol) of 3,4-diacetoxybenzaldehyde in 1 mL of THF was added and the reaction proceeded for an additional 30 min. Approximately 100 μL of acetic acid was then added, and the solvents were removed to yield a crude oil that was subjected to column chromatography (1:1 hexanes/ethyl acetate) to yield a mixture of the monosubstituted and bis-substitutued products. This mixture is resolved by letting the mixture sit in ether in the freezer overnight to give 83 mg (60%) of the disubstituted as a white powder and 14% monosubstituted product in solution. ^{1}H NMR (acetone-d_6, 250 MHz) δ 2.29 (s, 12H), 5.21 (s, 2H), 7.36 (m, 4H), 7.62 (m, 6H); ^{13}C NMR (acetone-d_6, 62.5 MHz) δ 20.4, 20.5, 73.3, 124.7, 125.3, 127.7, 128.2, 131.8, 143.9, 144.7, 145.9, 168.4, 168.6. HRFABMS calcd for $C_{25}H_{25}O_{12}S_2$ (M + H): 581.0787. Found: 581.0795 (M + H). Anal. ($C_{25}H_{24}O_{12}S_2$) C, H.

Bis(trans-β-3,4-dihydroxystyrenesulfonyl)methane (4). To a solution of **3** in 3 mL of methanol was added a catalytic amount of sodium methoxide, and the solution was stirred for 20 min. Dowex 50WX8-100 strongly acidic resin was then added, and the solution was stirred for 5 min. The solution was filtered and the solvent was removed in vacuo to give a quantitative yield (NMR analysis) of the target compound. ^{1}H NMR (acetone-d_6, 250 MHz) δ 5.07 (s, 2H), 6.93 (d, 2H, J = 8 Hz), 7.10 (m, 4H), 7.20 (d, 2H, J = 2 Hz), 7.47 (d, 2H, J = 15 Hz), 8.39 (s, 2H), 8.71 (s, 2H); ^{13}C NMR (acetone-d_6, 62.5 MHz) δ 73.9, 115.8, 116.5, 123.2, 123.8, 146.5. Electrospray (−) MS calcd for $C_{17}H_{15}O_8S_2$: 411.0 (M − H). Found: 411.0 (M − H). Anal. Calcd for $C_{17}H_{16}O_8S_2$ (2 H_2O): C, 45.53; H, 4.50. Found: C, 46.00; H, 3.70.

Acetic Acid 2,6-Diacetoxy-4-{2-[2-(3,4,5-triacetoxyphenyl)-ethenesulfonylmethanesulfonyl]vinyl}phenyl Ester (5). To a solution of **1** (144 mg, 0.28 mmol) in THF (2 mL) was added LiBr (88 mg, 1 mmol) followed by DIEA (175 μL, 1 mmol). 3,4,5-Tri-*O*-acetylbenzaldehyde (202 mg, 7.2 mmol) was then added, and the reaction mixture was allowed to sit for 7 days. Then approximately 100 mL of acetic acid was added, the solvent was removed in vacuo, and the resulting oil was subjected to column chromatography (6:4 hexanes/ethyl acetate) to yield 70 mg (35% yield) of the title compound. ^{1}H NMR (acetone-d_6, 600 MHz) δ 2.30 (s, 12H), 2.32 (s, 6H), 5.61 (s, 2H), 7.44 (d, 2H, J = 15.5 Hz), 7.58 (d, 2H, J = 15.5 Hz), 7.66 (s, 4H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 19.8, 20.3, 71.5, 121.6, 128.2, 130.5, 136.8, 142.4, 143.6, 167.0, 168.0. HRFABMS (+) calcd $C_{29}H_{29}O_{16}S_2$ (M + H): 697.0897. Found: 697.0906 (M + H). Anal. ($C_{29}H_{29}O_{16}S_2$) C, H.

Bis(*trans*-β-3,4,5-trihydroxystyrenesulfonyl)methane (6). The disulfone reagent **1** (153 mg, 0.31 mmol) and 3,4,5-tri(*tert*-butyldimethylsilyloxy)benzaldehyde (530 mg, 1.07 mmol) were reacted according to general procedure A. Following removal of solvent, 350 mg of the crude coupled product was isolated (55%). The crude product (356 mg, 0.32 mmol) was dissolved in 4 mL of THF to which was added acetic acid (120 μL, 2.08 mmol) and a 1 M solution of tetrabutylammonium fluoride (2.08 mL, 2.08 mmol). This was allowed to stir for 1 h, at which time the solvent was removed. The crude oil was extracted with ethyl acetate (3 × 100 mL), and the organic phases were collected and dried over sodium sulfate. The solvent was removed and the crude oil was run through a short plug of silica, eluting with 15% methanol/85% ethyl acetate (R_f = 0.41) to give 142 mg of pure title compound (56%). ^{1}H NMR (acetone-d_6, 400 MHz) δ 5.05 (s, 2H), 6.78 (s, 4H), 7.03 (d, 2H, J = 15.2 Hz), 7.39 (d, 2H, J = 15.2 Hz), 8.27 (br s, 6H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 74.0, 109.3, 123.4, 124.4, 137.8, 146.7, 146.8; FT-IR (KBr) ν 3526 (OH str), 1651 (conj alkene), 1323, 1152 (SO_2). Anal. Calcd for $C_{17}H_{16}O_{10}S_2$: C, 45.94; H, 3.63. Found: C, 46.72; H, 3.40.

Bis(*trans*-β-3,4-diacetoxy-5-methoxy-styrenesulfonyl)-methane (7). The disulfone reagent **1** (245 mg, 0.49 mmol) was reacted with 3,4-diacetoxy-5-methoxybenzaldehyde (407 mg, 1.61 mmol) according to general procedure A. After removal of solvent, the crude product was subjected to column chromatography (hexanes/ethyl acetate 1:1, R_f = 0.33) to yield 207 mg (66%) of the title compound. ^{1}H NMR (acetone-d_6, 250 MHz) δ 2.29 (s, 6H), 2.30 (s, 6H), 3.92 (s, 6H) 5.22 (s, 2H), 7.24 (d, 2H, J = 1.9 Hz), 7.36−7.44 (m, 4H), 7.60 (d, 2H, J = 15.5 Hz); ^{13}C NMR (acetone-d_6, 125 MHz) δ 20.2, 20.5, 57.1, 73.5, 111.2, 117.1, 128.0, 131.7, 135.7, 145.1, 145.4, 154.1, 167.9, 168.7. HRFABMS calcd for $C_{27}H_{29}O_{14}S_2$ (M + H): 641.0999. Found: 641.1013 (M + H). Anal. ($C_{27}H_{28}O_{14}S_2$) C, H.

Bis(*trans*-β-3,4-hydroxy-5-methoxystyrenesulfonyl)-methane (8). To a solution of **7** (20 mg, 0.031 mmol) in DMSO-d_6 (1 mL) was added 10 μL of hydrazine hydrate. After approximately 10 min the solution was subjected to reverse-phase HPLC (C_{18}) to yield 7.5 mg (51%) of the title compound. ^{1}H NMR (acetone-d_6, 500 MHz) δ 3.87 (s, 6H), 5.04 (s, 2H),

6.90 ($_{app}$dd, 4H, J = 13.3, 1.9 Hz), 7.10 (d, 2H, J = 15.3 Hz), 7.45 (d, 2H, J = 15.3 Hz); ^{13}C NMR (acetone-d_6, 125 MHz) δ 56.7, 74.1, 105.6, 111.2, 123.7, 124.4, 138.7, 146.6, 147.2, 149.3. HRFABMS (+) calcd $C_{19}H_{21}O_{10}S_2$ (M + H): 473.0576. Found: 473.0573 (M + H). Anal. ($C_{19}H_{21}O_{10}S_2$) C, H.

Acetic Acid 2-Acetoxy-6-methoxy-4-[2-(2-*trans*-phenylethenesulfonylmethanesulfonyl)-*trans*-vinyl]phenyl Ester (9). To a solution of **1** (443 mg, 0.88 mmol) in 3 mL of THF are added DIEA (513 μL, 2.95 mmol) and LiBr (256 mg), and the solution is agitated until solvation of the LiBr occurs. Then benzaldehyde is added (30 μL, 0.29 mmol) and the solution is allowed to sit overnight. Approximately 500 μL of acetic acid is then added, and the solution was diluted with ethyl acetate and washed with water. The organic layer is then dried over sodium sulfate and then evaporated. The resulting oily solid is then subjected to HPLC (7:3 up to 1:2, hexanes/ethyl acetate) to give 104.3 mg (83%) of the desired monosulfone as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.38 (d, 12H, J = 6.2 Hz), 4.03 (d, 2H, J = 15.9 Hz), 4.86 (m, 2H), 5.12 (s, 2H), 7.17 (d, 1H, J = 15.5 Hz), 7.41−7.47 (m, 3H), 7.54−7.55 (m, 2H), 7.69 (d, 1H, J = 15.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 23.6 (d, $J_{(C-P)}$ = 5 Hz), 24.1, 51.4 (d, $J_{(C-P)}$ = 138 Hz), 76.9 (d, $J_{(C-P)}$ = 32 Hz), 77.2, 124.4, 128.3, 129.0, 129.2, 131.7, 146.8. HRFABMS calcd for $C_{16}H_{26}O_7PS_2$ (M + H): 425.0858. Found: 425.0862 (M + H).

To a solution of the monosubstituted disulfone (37.7 mg, 0.088 mmol) in 1 mL of THF are added DIEA (47 μL, 0.26 mmol) and LiBr (23 mg, 0.26 mmol), and the solution was agitated until solvation of the LiBr occurs. Then 82 mg (0.34 mmol) of crude 3,4-*O*-acetoxy-5-methoxybenzaldehyde in 1 mL of THF is added, and the solution continues overnight. The reaction is quenched by the addition of 250 μL of acetic acid followed by dilution with ethyl acetate. The resulting mixture is washed with water and the organic layer is dried over sodium sulfate to give a crude oil that is subjected to HPLC (2:1 up to 1:3, hexanes/ethyl acetate) to give 34.5 mg (80%) of the desired product. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.31 (s, 3H), 2.32 (s, 3H), 3.88 (s, 3H), 4.64 (s, 2H), 6.99 (d, 1H, J = 1.8 Hz), 7.03 (d, 1H, J = 1.8 Hz), 7.18 (d, 1H, J = 12.9 Hz), 7.21 (d, 1H, J = 12.9 Hz), 7.26 (s, 2H), 7.43−7.50 (m, 3H), 7.55−7.57 (m, 2H), 7.61 (d, 1H, J = 15.4 Hz), 7.70 (d, 1H, J = 15.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.2, 20.6, 56.5, 74.2, 109.7, 116.3, 124.1, 125.5, 128.3, 129.1, 129.3, 130.0, 131.6, 132.1, 144.0, 145.5, 147.1, 153.0, 167.3, 167.9. HRFABMS calcd for $C_{22}H_{23}O_9S_2$ (M + H): 495.0784. Found: 495.0789 (M + H). Anal. ($C_{22}H_{22}O_9S_2$) C, H.

3-Methoxy-5-[2-(2-phenylethenesulfonylmethanesulfonyl)vinyl]benzene-1,2-diol (10). To a solution of **9** (12.4 mg, 0.025 mmol) in approximately 1 mL of methanol is added 240 μL of 2 M NH_3 in methanol, and the reaction proceeded for 1 h. The solvent was removed and the resulting oil was subjected to reverse-phase (C_{18}) HPLC (40% gradient acetonitrile in water (1%TFA) up to 70% acetonitrile over 40 min) to give the 3.0 mg (29%) of **10** as a white powder. ^{1}H NMR (acetone-d_6, 500 MHz) δ 3.87 (s, 3H), 5.17 (s, 2H), 6.91 (d, 2H, J = 15.4 Hz), 7.11 (d, 1H, J = 15.4 Hz), 7.35 (d, 1H, J = 15.0 Hz), 7.45−7.51 (m, 4H), 7.62 (d, 1H, J = 15.7 Hz), 7.70 (d, 2H, J = 7.3 Hz), 8.06 (broad s, 1H), 8.23 (broad s, 1H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 56.7, 73.8, 105.6, 111.4, 123.5, 127.2, 129.9, 130.1, 132.4, 138.3, 146.2, 147.4. HRFABMS (−) calcd $C_{18}H_{17}O_7S_2$ (M − H): 409.0416. Found: 409.0418 (M − H). Anal. Calcd for $C_{18}H_{18}O_7S_2$ (1 H_2O): C, 50.46; H, 4.70. Found: C, 50.08; H, 4.12.

Bis(*trans*-acetic acid 4-(2-methanesulfonyl-vinyl)-2-methoxyphenyl ester) (11). The disulfone reagent **1** (212 mg, 0.42 mmol) and 4-acetoxy-3-methoxybenzaldehyde (271 mg, 1.40 mmol) were used according to general procedure A. Following removal of the solvent, 5 mL of acetone was added, from which a white solid precipitated. This was filtered off and rinsed with cold acetone to give 145 mg (66%) of the title compound (mp 202−203 °C). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.27 (s, 6H), 3.81 (s, 6H), 5.54 (s, 2H), 7.15 (d, 2H, J = 7.8 Hz), 7.27 (d, 2H, J = 8.1 Hz), 7.43 (d, 2H, J = 15.6 Hz), 7.49 (s, 2H), 7.55 (d, 2H, J = 15.3 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 20.4, 56.0, 71.1, 112.8, 122.3, 123.5, 126.6, 131.0, 141.8, 144.3, 151.3, 168.3; FT-IR (KBr) ν 3074, 1618, 1514 (C=C str), 1763 (C=O str), 1466 (−CH_2− str), 1329, 1126 (SO_2 str), 1273, 1028 (Ph−O−C str), 1207 (C−O str), 970 (trans C=C str). Anal. ($C_{23}H_{24}O_{10}S_2$) C, H.

Bis(*trans*-β-3,4-di-*O*-benzoyl-styrenesulfonyl)methane (12). To a solution of **1** (150 mg, 0.3 mmol) in THF (2 mL) was added LiBr (78 mg, 0.9 mmol) followed by DIEA (157 μL, 0.9 mmol). 3,4-Di-*O*-benzoylbenzaldehyde (387 mg, 0.9 mmol) was then added, and the reaction mixture was allowed to sit overnight. Then approximately 100 μL of acetic acid was added followed by water and ethyl acetate. The whole solution is then filtered to give 185 mg (93% yield) of a crude product. An analytical sample was acquired by reverse-phase (C_{18}) HPLC (40% acetonitrile in water (1%TFA) up to 100% acetonitrile). ^{1}H NMR (DMSO-d_6, 600 MHz) δ 5.59 (s,2H), 7.42 (d, 4H, J = 8.1 Hz), 7.45 (d, 4H, J = 8.1 Hz), 7.48 (d, 2H, J = 15.5 Hz), 7.64−7.65 (m, 6H), 7.74 (dd, 2H, J = 8.5, 1.9 Hz), 7.91−7.96 (m, 5H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 124.0, 124.4, 127.3, 127.7, 128.0, 128.91, 128.94, 129.6, 131.1, 134.3, 142.4, 143.2, 144.3, 163.2, 163.4. HRFABMS (+) calcd $C_{45}H_{33}O_{12}S_2$ (M + H): 829.1413. Found: 829.1418 (M + H). Anal. ($C_{45}H_{33}O_{12}S_2$) C, H.

Purified Enzyme Assays. Biological Materials, Chemicals, and Enzymes. All compounds were dissolved in DMSO, and the stock solutions were stored at −20 °C. The γ-[^{32}P]-ATP was purchased from either Amersham Biosciences or ICN. The expression systems for the wild-type IN and soluble mutant IN^{F185KC280S} were generous gifts of Dr. Robert Craigie, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD.

Preparation of Oligonucleotide Substrates. The oligonucleotides 21top, 5′-GTGTGGAAAATCTCTAGCAGT-3′, and 21bot, 5′-ACTGCTAGAGATTTTCCACAC-3′, were purchased from Norris Cancer Center Microsequencing Core Facility (University of Southern California) and purified by UV shadowing on polyacrylamide gel. To analyze the extent of 3′-processing and strand transfer using 5′-end labeled substrates, 21top was 5′-end labeled using T_4 polynucleotide kinase (Epicentre, Madison, WI) and γ-[^{32}P]-ATP (Amersham Biosciences or ICN). The kinase was heat-inactivated, and 21bot was added in 1.5 molar excess. The mixture was heated at 95 °C, allowed to cool slowly to room temperature, and run through a spin 25 minicolumn (USA Scientific) to separate annealed double-stranded oligonucleotide from unincorporated material.

Integrase Assays. To determine the extent of 3′-processing and strand transfer, wild-type IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% DMSO, and 25 mM MOPS, pH 7.2) at 30 °C for 30 min. Then, 20 nM of the 5′-end ^{32}P-labeled linear oligonucleotide substrate was added, and incubation was continued for an additional 1 h. Reactions were quenched by the addition of an equal volume (16 μL) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue). An aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris-borate, pH 8.3, 2 mM EDTA, 20% acrylamide, 8 M urea). Gels were dried, exposed in a phosphorimager cassette, analyzed using a Typhoon 8610 variable mode imager (Amersham Biosciences), and quantitated using ImageQuant 5.2. Percent inhibition (% I) was calculated using the following equation:

$$\% I = 100 \times \frac{1 - (D - C)}{N - C}$$

where C, N, and D are the fractions of a 21-mer substrate converted to a 19-mer (3′-processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The IC_{50} values were determined by plotting the logarithm of drug concentration versus percent inhibition to obtain concentration that produced 50% inhibition.

Cell-Based Assays. Drug susceptibility of HIV-1 to IN inhibitors was determined with a focal infectivity assay (FIA) as previously described.[31,32] Immunostaining was performed using the monoclonal antibody 22-6[13] at a $^1/_{800}$ dilution. Foci were counted under a dissecting microscope at 30× to 100× magnification. Data for drug-susceptibility assays were plotted as a percentage of control foci (no drug) versus inhibitor concentrations. The concentrations required to inhibit focus formation by 50% (EC_{50}) were obtained from a best-fit line of the linear portions of those plots. EC_{50} values for each drug were determined from at least three separate experiments.

Cytotoxicity was determined with the Promega CellTiter 96 Aqueous One Solution Cell Proliferation Assay (MTS assay) using the manufacturer's recommended conditions. Data for cell proliferation assays were plotted as a percentage of control foci (no drug) versus inhibitor concentrations. The concentrations required to inhibit cell proliferation by 50% (IC_{50}) were obtained from a best-fit line of the linear portions of those plots. IC_{50} values for each drug were determined from at least two separate experiments.

Time of Addition Assays. The time of addition studies were performed similarly to the focal infectivity assays except that the virus was incubated with the cells for 2 h at 37 °C, after which unadsorbed virus was removed from the wells and replaced with fresh virus-free medium. Dextran sulfate, T20, 3TC, and test compounds were used at 10 times their respective EC_{50} values and were added at seven different time points before or after infection: −1, 0, 2, 4, 6, 10, and 24 h. The cells were incubated for 4 days at 37 °C when they were fixed and stained, and foci were counted and plotted against a no-drug control.

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Supporting Information Available: Results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Richman, D. D. HIV Chemotherapy. *Nature* **2001**, *410*, 995−1001.
(2) Richman, D. D. Resistance of clinical isolates of human immunodeficiency virus to antiretroviral agents. *Antimicrob. Agents Chemother.* **1993**, *37*, 1207−1213.
(3) D'Aquila, R. T.; Johnson, V. A.; Welles, S. L.; et al. Zidovudine resistance and HIV-1 disease progression during antiretroviral therapy. AIDS Clinical Trials Group Protocol 116B/117 Team and the Virology Committee Resistance Working Group. *Ann. Intern. Med.* **1995**, *122*, 401−408.
(4) Arts, E. J.; Wainberg, M. A. Mechanisms of nucleoside analog antiviral activity and resistance during human immunodeficiency virus reverse transcription. *Antimicrob. Agents Chemother.* **1996**, *40*, 527−540.
(5) Fauci, A. S. The AIDS epidemic. *N. Engl. J. Med.* **1999**, *341*, 1046−1050.
(6) Yerly, S.; Kaiser, L.; Race, E.; Bru, J.-P.; Clavel, F.; Perrin, L. Transmission of antiretroviral-drug-resistant HIV-1 variants. *Lancet* **1999**, *354*, 729−733.
(7) Schinazi, R. F.; Larder, B. A.; Mellors, J. W. Mutations in retroviral genes associated with drug resistance: 1999−2000 update. *Int. Antiviral News* **1999**, *7*, 46−69.
(8) Kuiken, C. L.; Foley, B.; Hahn, B.; Marx, P. A.; McCutchan, F.; Mellors, J.; Mullins, J.; Wolinsky, S.; Korber, B. *HIV Sequence Compendium 2000*; Theoretical Biology and Biophysics Group, Los Alamos National Labatory: Los Alamos, NM, 2000.
(9) Brown, P. O. *Integration*; Cold Spring Harbor Press: Cold Spring Harbor, Woodbury, NY, 1999.
(10) Asante-Appiah, E.; Skalka, A. M. HIV-1 integrase: structural organization, conformational changes, and catalysis. *Adv. Virus Res.* **1999**, *52*, 351−369.
(11) Sakai, H.; Kawamura, M.; Sakugari, J.; Sakugari, S.; Shibata, R.; Ishimoto, A.; Ono, N.; Ueda, S.; Adachi, A. Integration is essential for efficient gene expression of human immunodeficiency virus type 1. *J. Virol.* **1993**, *67*, 1169−1174.
(12) Hazuda, D. J.; Young, S. D.; Guare, J. P.; Anthony, N. J.; Gomez, R. P.; Wai, J. S.; Vacca, J. P.; Handt, L.; Motzel, S. L.; Klein, H. J.; Dornadula, G.; Danovich, R. M.; Witmer, M. V.; Wilson, K. A. A.; Tussey, L.; Schleif, W. A.; Gabryelski, L. S.; Jin, L.; Miller, M. D.; Casimiro, D. R.; Emini, E. A.; Shiver, J. W. Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. *Science* **2004**, *305,* 528−532.
(13) Hazuda, D. J.; Anthony, N. J.; Gomez, R. P.; Jolly, S. M.; Wai, J. S.; Zhuang, L.; Fisher, T. E.; Embrey, M.; Guare, J. P., Jr.; Egbertson, M. S.; Vacca, J. P.; Huff, J. R.; Felock, P. J.; Witmer, M. V.; Stillmock, K. A.; Danovich, R.; Grobler, J.; Miller, M. D.; Espeseth, A. S.; Jin, L.; Chen, I.-W.; Lin, J. H.; Kassahun, K.; Ellis, J. D.; Wong, B. K.; Xu, W.; Pearson, P. G.; Schleif, W. A.; Cortese, R.; Emini, E.; Summa, V.; Holloway, M. K.; Young, S. D. A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101* (31), 11233−11238.
(14) Neamati, N. Patented small molecule inhibitors of HIV-1 integrase: a ten year saga. *Expert Opin. Ther. Pat.* **2002**, *12*, 709−724.
(15) Dayam, R.; Neamati, N. Small-molecule HIV-1 integrase inhibitors: the 2001−2002 Update. *Curr. Pharm. Des*. **2003**, *9*, 1789−1802.
(16) Pluymers, W.; Pais, G.; Van Maele, B.; Pannecouque, C.; Fikkert, V.; Burke, T., Jr.; De Clercq, E.; Witvrouw, M.; Neamati, N.; Debyser, Z. Inhibibition of human immunodeficiency virus type 1 integration by diketo derivatives. *Antimicrob. Agents Chemother.* **2002**, *46*, 3292−3297.
(17) Este, J. A.; Cabrera, C.; Schols, D.; Cherepanov, P.; Gutierrez, A.; Witvrouw, M.; Pannecouque, C.; Debyser, Z.; Rando, R. F.; Clotet, B.; Desmyter, J.; De Clercq, E. Human immunodeficiency virus glycoprotein gp120 as the primary target for the antiviral action of AR177 (Zintevir). *Mol. Pharmacol*. **1998**, *53*, 340−345.
(18) Pluymers, W.; Neamati, N.; Pannecouque, C.; Fikkert, V.; Marchand, C.; Burke, T. R., Jr.; Pommier, Y.; Schols, D.; De Clercq, E.; Debyser, Z.; Witvrouw, M. Viral entry as the primary target for the anti-HIV activity of chicoric acid and its tetraacetyl esters. *Mol. Pharmacol.* **2000**, *58*, 641−648.
(19) Robinson, W. E., Jr.; Reinecke, M. G.; Abdel-Malek, S.; Jia, Q.; Chow, S. A. Inhibitors of HIV-1 replication that inhibit HIV integrase. *Proc. Natl. Acad. Sci. U.S.A.* **1996***,* *93*, 6326−6331.
(20) Gervay, J.; Flaherty, T. M.; Holmes, D. Studies on the stereoselective synthesis of *C*-glycoside sulfones as potential glycosyl transferase inhibitors. *Tetrahedron* **1997**, 16355−64.
(21) Hadd, M. J.; Smith, M. A.; Gervay-Hague, J. A Novel reagent for the synthesis of geminal disulfones. *Tetrahedron Lett*. **2001**, *42*, 5137−5140.
(22) Gervay-Hague, J.; Hadd, M. J. Divisional application based on U.S. 09/595,048. Filed Nov. 2002.
(23) (a) Hine, J.; Philips, J. C.; Maxwell, J. I. Rate and equilibrium in carbanion formation by bis(methylsulfonyl)methane. *J. Org. Chem.* **1970**, *35*, 3943−3945. (b) Castro, A.; Spencer, T. A. Formation and alkylation of anions of bis(methylsulfonyl)-methane. *J. Org. Chem.* **1992**, *57*, 3496−3499.
(24) Esposito, D.; Craigie, R. HIV integrase structure and function. *Adv. Virus Res.* **1999**, *52*, 319−333.
(25) Gervay-Hague, J.; Hadd, M. J. U.S. Patent 60/138,986.
(26) Neamati, N.; Mazumder, A.; Zhao, H.; Sunder, S.; Burke, T.R., Jr.; Schultz, R. J.; Pommier, Y. Diarylsulfones, a novel class of human immunodeficiency virus type 1 integrase inhibitors. *Antimicrob. Agents Chemother.* **1997**, *41*, 385−393.
(27) Farnet, C. M.; Wang, B.; Lipford, J. R.; Bushman, F. D. Differential inhibition of HIV-1 preintegration complexes and purified integrase protein by small molecules. *Proc Natl Acad Sci U.S.A.* **1996**, *93*, 9742−9747.
(28) Molteni, V.; Rhodes, D.; Rubins, K.; Hansen, M.; Bushman, F. D.; Siegel, J. S. A new class of HIV-1 inhibitors. The 3,3,3′,3′-tetramethyl-1,1′-spirobi(indan)-5,5′,6,6′-tetrol family. *J. Med. Chem*. **2000**, 43, 2031−2039.
(29) Lin, Z.; Neamati, N.; Zhao, H.; Kiryu, Y.; Turpin, J. A.; Aberham, C.; Strebel, K.; Kohn, K.; Witvrouw, M.; Pannecouque, C.; Debyser, Z.; De Clercq, E.; Rice, W. G.; Pommier, Y.; Burke, T. R., Jr. Chicoric acid analogues as HIV-1 integrase inhibitors. *J. Med. Chem*. **1999**, *42*, 1401−1414.
(30) Pais, G. C. G.; Zhang, X.; Marchand, C.; Neamati, N.; Cowansage, K.; Svarovskaia, E. S.; Pathak, V. K.; Tang, Y.; Nicklaus, M.; Pommier, Y.; Burke, T. R., Jr. Structure activity of 3-Aryl-

1,3-diketo-containing compounds as HIV-1 integrase inhibitors. *J. Med. Chem.* **2002**, *45*, 3184−3194.
(31) Murry, J. P.; Higgins, J.; Matthews, T. B.; Huang, V. Y.; Van Rompay, K. K. A.; Pedersen, N. C.; North, T. W. Reversion of the M184V mutation in simian immunodeficiency virus reverse transcriptase is selected by tenofovir, even in the presence of lamivudine. *J. Virol.* **2003**, *77*, 1120−1130.
(32) Giuffre, A. C.; Higgins, J.; Buckheit, R. W., Jr.; North, T. W. Susceptibilities of simian immunodeficiency virus to protease inhibitors. *Antimicrob. Agents Chemother.* **2003**, *47*, 1756−1759.
(33) Hofman, M. J.; Higgins, J.; Matthews, T. B.; Pedersen, N. C.; Tan, C.; Schinazi, R. F.; North, T. W. Efavirenz therapy in rhesus macaques infected with a chimera of simian immunodeficiency virus containing reverse transcriptase from human immunodeficiency virus type-1. *Antimicrob. Agents Chemother.* **2004**, *48,* 3483−3490.
(34) Chesebro, B.; Wehrly, K. Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. *J. Virol.* **1988**, *62*, 3779−3788.
(35) Leutenegger, C. M.; Higgins, J.; Matthews, T. B.; Tarantal, A. F.; Luciw, P. A.; Pedersen, N. C.; North, T. W. Real-time TaqMan PCR as a specific and more sensitive alternative to the branched-chain DNA assay for quantitation of simian immunodeficiency virus RNA. *AIDS Res. Hum. Retroviruses* **2001**, *17*, 243−251.
(36) Espeseth, A. S.; Felock, P.; Wolfe, A.; Witmer, M.; Grobler, J.; Anthony, N.; Egbertson, M.; Melamed, J. Y.; Young, S.; Hamill, T.; Cole, J. L.; Hazuda, D. J. HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. *Proc. Natl. Acad. Sci, U.S.A.* **2000**, *97*, 11244−11249.
(37) Masuda, T.; Shibuya, S.; Arai, M.; Yoshida, S. Tomozawa, T.; Ohno, A.; Yamashita, M.; Honda, T. *Bioorg. Med. Chem. Lett.* **2003**, 669−673.
(38) Pluymers, W.; Pais, G.; Van Maele, B.; Pannecouque, C.; Fikkert, V.; Burke, T. R., Jr.; De Clercq, E.; Witvrouw, M.; Neamati, N.; Debyser, Z. Inhibition of HIV-1 integration by diketo derivatives. *Antimicrob. Agents Chermother*. **2002**, *46*, 3292−3297.

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