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Diarylsulfones, a Novel Class of Human Immunodeficiency Virus Type 1 Integrase Inhibitors

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A majority of reported human immunodeficiency virus type 1 integrase (HIV-1 IN) inhibitors are polyhydroxylated aromatic compounds containing two phenyl rings separated by aliphatic or aromatic linkers. Most inhibitors possessing a catechol moiety exhibit considerable toxicity in cellular assays. In an effort to identify nonhydroxylated analogs, a series of aromatic sulfones were tested for their ability to inhibit the 3′ processing and strand transfer steps that are necessary for HIV replication. Several aromatic sulfones have previously been shown to have moderate activity against HIV-1 reverse transcriptase in cellular assays; however, their inhibitory potencies against IN have not been explored. In the present study, the inhibitory effect of a series of sulfones and sulfonamides against IN was determined. Among 52 diaryl sulfones tested, 4 were determined to be highly potent (50% inhibitory concentration [IC_{50}], 0.8 to 10 μg/ml), 5 had good potencies (IC_{50}, 11 to 50 μg/ml), 10 showed moderate potencies (IC_{50}, 51 to 100 μg/ml), and 33 were inactive (IC_{50}, >100 μg/ml) against IN. All of the active compounds exhibited similar potencies against HIV-2 IN. Sulfa drugs, used extensively in treating *Pneumocystis carinii* pneumonia, a leading cause of morbidity and mortality in AIDS patients, were also examined. Among 19 sulfonamides tested, sulfasalazine (IC_{50}, 50 μg/ml) was the most potent. We conclude that potent inhibitors of IN can be designed based on the results presented in this study.

Of the three *pol* gene products, human immunodeficiency virus type 1 integrase (HIV-1 IN) is the only protein responsible for an efficient insertion or integration of a DNA copy of the viral RNA genome into the host cell DNA (for recent reviews, see references 11 and 26). During viral infection, IN catalyzes two consecutive reactions. Initially, IN processes linear viral DNA by removing two nucleotides from each 3′ end, leaving the recessed 3′-OH termini. This is followed by transesterification of phosphodiester bonds in which a host DNA strand is cut and the 5′ end of the cut is joined to a processed viral 3′ terminus. These two steps, known as 3′ processing and DNA strand transfer, can be easily measured in an in vitro assay employing purified recombinant IN and a 21-mer duplex oligonucleotide corresponding to the U5 end of the HIV long terminal repeat sequence. IN also catalyzes an apparent reversal of the strand transfer reaction, a process known as disintegration. In this reaction, a branched (Y) oligonucleotide substrate consisting of viral and target DNA components is resolved into its constituent duplexes (11, 26). These in vitro assays are ideal for screening large batteries of compounds.

Recent advances in the molecular and cellular biology of HIV-1 have identified several novel targets for potential chemotherapeutic intervention (8). IN is one such target, and for the past several years our laboratory has been involved in developing inhibitors of this enzyme. To date, several classes of IN inhibitors have been reported (2, 3, 5, 9, 12, 16–19, 22). Although a selective IN inhibitor has not emerged from these studies, much has been learned from several lead compounds. For example, a large number of compounds of different chemical classes containing phenolic moieties, such as caffeic acid phenethyl ester (CAPE) (9), curcumin (16), tyrphostins (17), CAPE amides (2), lignanolides (5), and quercetagetin (9), have good inhibitory potency against IN (Table 1). Whether these phenolic moieties are directly involved in divalent metal ion binding or act as hydrogen bond donors in the active site of the IN remains to be determined. Several active compounds contain two reactive catechol centers separated by aromatic or aliphatic linkers, and the nature or the length of the linkers generally does not significantly contribute to potency. A critical requirement for activity, however, is the presence of at least one catechol unit. While the catechol moiety is essential for potent activity against IN, it also potentially contributes to increased cytotoxicity in cellular assays. The mechanism of toxicity may stem from the formation of oxidized species, such as semiquinones or orthoquinones, which form protein or possibly DNA adducts (13, 28). In a separate study, we have recently designed and synthesized a series of monohydroxylated arylamides and determined their inhibitory activities against IN (30). Unfortunately, none of the monohydroxyl derivatives exhibited significant inhibitory potency against IN. We thus sought a unique way of circumventing formation of reactive quinone species while preserving the required pharmacophore for activity.

In the search for IN inhibitors which are superior to polyhydroxylated aromatics, our recent efforts have focused on the development of sulfones. Previously, McMahon et al. reported the inhibition of HIV-1 reverse transcription by diarylsulfones and identified 2-nitrophenyl phenyl sulfone as a potent inhibitor of this enzyme (20). Recently, Artico et al. also reported on the anti-HIV-1 activities of a series of pyrrolyl aryl sulfones (1). Although several of these compounds were potent inhibitors of HIV-1 recombinant reverse transcriptase, others were inactive against this enzyme even though they showed considerable activities in tissue culture. Taken together, these data

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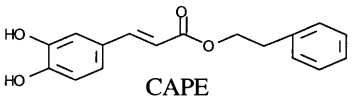
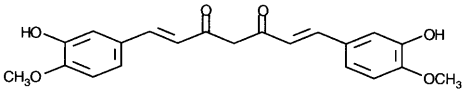
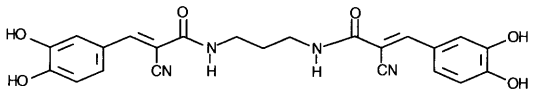
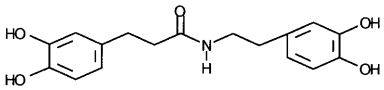
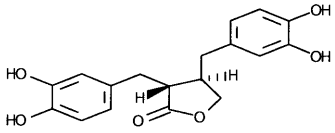
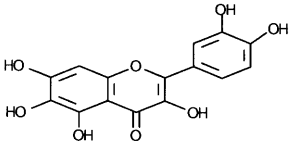
TABLE 1. Structures and anti-HIV-1 IN activities of several classes of inhibitors

Compound	Activity (μM)		Reference
	3′ processing	Strand transfer	
 CAPE	7	19	9
 Curcumin	95	40	16
 Tyrphostin 537	1.9	0.8	17
 a "CAPE amide"	3	3	2
 a "Lignanolide"	21.4	5.4	5
 Quercetagetin	0.8	0.1	9

support the notion that the sulfone class of compounds can target other steps in the HIV-1 life cycle.

We were interested in diarylsulfones for several reasons. Firstly, several diarylsulfones had previously been found to confer inhibitory potency against HIV-1 (1, 20). Secondly, numerous analogs were already available in the National Cancer Institute (NCI) drug repository and no further synthesis was needed. And thirdly, several sulfa drugs have shown great efficacy against *Pneumocystis carinii* pneumonia, a leading cause of morbidity and mortality in AIDS patients (10). Although a plethora of sulfones and sulfa drugs is available, an attempt was not made to undertake an exhaustive screen of these agents. At its inception, this study was focused on compounds that were confirmed as active in the NCI antiviral screen. Additionally, however, in order to establish a structure-activity relationship among these compounds several related analogs were also tested. In this fashion we sought to identify novel sulfones as IN inhibitors, thereby taking advantage of a structural class having a well-established safety profile. Herein, we present the inhibitory activities of several sulfones and sulfanilamides against purified recombinant HIV-1 IN.

MATERIALS AND METHODS

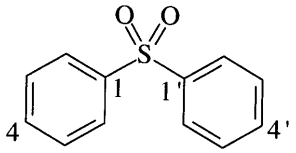
Preparation of oligonucleotide substrates. The high-performance liquid chromatography-purified oligonucleotides AE117 (5′-ACTGCTAGAGATTTTCCACAC-3′), AE118 (5′-GTGTGGAAAATCTCTAGCAGT-3′), AE157 (5′-GAAAGCGACCGCGCC-3′), AE146 (5′-GGACGCCATAGCCCCGGCGCGGTCGCTTTC-3′), and AE156, (5′-GTGTGGAAAATCTCTAGCAGGGGCTATGGCGTCC-3′) were purchased from Midland Certified Reagent Company (Midland, Tex.). The purified recombinant HIV-1 IN deletion mutant IN^{50-212} was a generous gift of T. Jenkins and R. Craigie, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Disorders, National Institutes of Health, Bethesda, Md. R. Craigie also provided the expression system for the wild-type HIV-1 IN. To analyze the extents of 3′ processing and strand transfer using 5′-end-labeled substrates, AE118 was 5′ end labeled with T4 polynucleotide kinase (Gibco BRL, Gaithersburg, Md.) and [γ-^{32}P]ATP (Dupont-NEN). To determine the extent of 30-mer target strand generation during disintegration, AE157 was 5′ end labeled and annealed to AE156, AE146, and AE117. The kinase was heat inactivated, and AE117 was added to the same final concentration. The mixture was heated at 95°C, allowed to cool slowly to room temperature, and run on a G-25 Sephadex quick spin column (Boehringer-Mannheim, Indianapolis, Ind.) to separate annealed double-stranded oligonucleotide from unincorporated label.

IN assay. 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 [wt/vol], 7.5 mM $MnCl_2$, 0.1 mg of bovine serum albumin per ml, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide (DMSO), and 25 mM MOPS [morpholinepropanesulfonic acid] [pH 7.2]) at 30°C for 30 min. Then, the 5′-end ^{32}P-labeled linear oligonucleotide substrate (20 nM) 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, 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).

Disintegration reactions (4) were performed with a Y oligonucleotide (i.e., the branched substrate in which the U5 end was integrated into target DNA) as described above.

Gels were dried, exposed in a PhosphorImager cassette (Molecular Dynamics, Sunnyvale, Calif.), and analyzed with a Molecular Dynamics PhosphorImager. Percent inhibition was calculated by using the following equation: 100 × [1 −

TABLE 2. Inhibition of HIV-1 replication in CEM cells and inhibition of HIV-1 IN catalytic activities by a series of diarylsulfones containing electron-withdrawing substituents[a]



Compound no.	Compound designation	Substituent	IC_{50} (μg/ml)[b,c] in assay			Cellular anti-HIV-1 data (μg/ml)[c]	
			3′ processing	Strand transfer	Disintegration	IC_{50}	EC_{50}
1	NSC 14153	3,3′-$(NO_2)_2$, 4,4′-$(F)_2$	51.0 ± 8.8	59.0 ± 8.9	63.5	>100	>100
2	NSC 122653	2,2′,4,4′-$(NO_2)_4$	60.0 (77.0)	76.5 (93.0)		0.97 ± 0.32	NR[d]
3	NSC 633001	2,2′-$(NO_2)_2$	>100	>100		>44.7	0.26 ± 0.05
4	NSC 642592	2,6-$(NO_2)_2$	>100	>100		2.6 ± 0.6	NR
5	NSC 20608	3,3′-$(NO_2)_2$	>100	>100		>100	>100
6	NSC 20609	4,4′-$(NO_2)_2$	>100	>100		>100	>100
7	NSC 87584	4,4′-$(F)_2$	>100	>100			
8	NSC 99885	4,4′-$(I)_2$	>100	90		>100	>100
9	NSC 91569	4,4′-$(CN)_2$	>100	>100		>100	>100
10	NSC 18285	3,3′-$(NO_2)_2$, 4-F	>100	>100			
11	NSC 156750	2-NO_2,4-Cl	87.5	82.5		8.7 ± 1.4	NR
12	NSC 85651	2-NO_2,4′-Cl	>100	>100		15.6 ± 1.5	3.4 ± 0.8
13	NSC 186261	3,3′-$(NO_2)_2$, 4,4′-$(COOH)_2$	55	83			
14	NSC 186265	3,3′,5-$(NO_2)_3$, 4,4′-$(Cl)_2$	67.5 ± 25.0	65.6 ± 19.6		2.5 ± 0.93	NR
15	NSC 646122	2,6-$(NO_2)_2$, 4-Me[e]	>100	>100		1.2 ± 0.14	NR
16	NSC 85609	3,3′,4,4′-$(Cl)_4$	>100	>100		>100	>100
17	NSC 39136	2,2′-$(CF_3)_2$, 4,4′-$(NO_2)_2$	>100	>100		>100	>100
18	NSC 642590	2-NO_2,4-CF_3	>100	>100		0.93 ± 0.43	NR
19	NSC 107159	4-C(O)N_3, 4′-F	90	90		16.6	NR
20	NSC 40768	3,3′-$(NCO)_2$	100	90		>100	>100
21	NSC 40769	3,3′-$(NCO)_2$, 4,4′-$(Cl)_2$	100	90		>100	>100
22	NSC 291628	4-$(NO_2)_2$, 4′-$NHCONHCH_2CH_2Cl$	>100	>100		7.0 ± 0.81	NR
23	NSC 291629	4-$(NO_2)_2$, 4′-$NHCONHCH(C_2H_5)CH_2Cl$	>100	>100		1.3 ± 0.7	NR
24	NSC 291630	4-$(NO_2)_2$, 4′-$NHCONHCH(C_2H_5)CH_2OH$	>100	>100		48.6 ± 31.0	NR
25	NSC 86042	3,3′-$(NO_2)_2$, 4,4′-$(NH_2)_2$	>100	100			
26	NSC 40765	3,3′-$(NO_2)_2$, 4,4′-[bis-$N(CH_2)_2OH$]	>100	>100		18.0	NR
27	NSC 40764	3,3′-$(NO_2)_2$, 4,4′-$(SH)_2$	2.9 ± 0.7 (2.8)	2.5 ± 0.5 (2.2)	13.0	10.9	NR
28	NSC 85597	3,3′-$(NO_2)_2$, 4,4′-$(OCH_2COOH)_2$	70.0 ± 3.2	64.2 ± 14.5		>100	>100
29	NSC 159281	2,2′,4,4′,6,6′-$(Me)_6$, 3,3′,5,5′-$(NO_2)_4$	>100	>100		>100	>100
30	NSC 116966	2,4,6-$(Me)_3$, 4′-Me	>100	>100		3.3	NR

[a] A diagram of the basic structure of the compounds listed is shown above the table.
[b] The values in parentheses refer to HIV-2 IN inhibition.
[c] Values with standard deviations are means from three independent experiments.
[d] NR, not reached due to cytotoxicity.
[e] Me, methyl.

$(D - C)/(N - C)$], where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′ processing product) or strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. The 50% inhibitory concentrations (IC_{50}s) were determined by plotting the drug concentration versus percent inhibition and identifying the concentration which produced an inhibition of 50%.

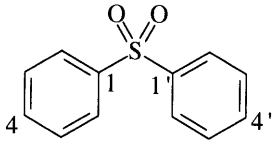
Topoisomerase reactions. Reactions were performed in 10 μl of reaction buffer (0.01 M Tris-HCl [pH 7.5], 150 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 mg of bovine serum albumin per ml) with the following duplex oligonucleotide substrate labeled (asterisk) with α-^{32}P-cordycepin at the 3′ end of the upper strand:

5′-GATCTAAAAGACTT^GGAAAAATTTTTAAAAAA*
ATTTTCTGAA–CCTTTTTAAAAATTTTTTCTAG-5′

This oligonucleotide contains a single topoisomerase I cleavage site (caret on the upper strand) (24). Approximately 50 fmol of oligonucleotide per reaction were incubated with 10 U of calf thymus DNA topoisomerase I (Gibco BRL). Reactions were stopped by adding sodium dodecyl sulfate (0.5% final concentration). Proteolysis was halted by the addition of 36 μl of 2.5× loading buffer (98% formamide, 0.01 M EDTA, 1 mg of xylene cyanol per ml, and 1 mg of bromophenol blue per ml.

Anti-HIV assays in cultured cell lines. The anti-HIV drug testing performed at the NCI is based on a protocol described by Weislow et al. (29). In brief, all compounds were dissolved in DMSO and diluted 1:100 in cell culture medium. Exponentially growing T4 lymphocytes (CEM cell line) were added at 5,000 cells per well. Frozen virus stock solutions were thawed immediately before use, suspended in complete medium to yield the desired multiplicity of infection (≈0.1), and added to the microtiter wells, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls. Cultures were incubated at 37°C in a 5% CO_2 atmosphere for 6 days. The tetrazolium salt XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production, and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity.

TABLE 3. Inhibition of HIV-1 replication in CEM cells and inhibition of HIV-1 IN catalytic activities by a series of diarylsulfones containing *meta* and/or *para* substituents[a]



Compound no.	Compound designation	Substituent	IC_{50} (μg/ml)[b,c] in assay			Cellular anti-HIV-1 data (μg/ml)[c]	
			3′ processing	Strand transfer	Disintegration	IC_{50}	EC_{50}
31	NSC 374413	4,4′-(N═NOH)$_2$ · 2Na	6.5 ± 3.0 (20.0)	6.1 ± 0.9 (12.0)		>100	>100
32	NSC 6091	4,4′-(NH$_2$)$_2$	>100	>100		>100	>100
33	NSC 155173	3,3′-(NH2)$_2$, 4,4′-(OH)$_2$	4.5 ± 3.0	4.9 ± 3.0			
34	NSC 14622	4,4′-[(NHC(NH$_2$)═NH]$_2$	>100	>100			
35	NSC 20635	4,4′-[NHC(═NH)NHC(NH$_2$)═NH]$_2$	>100	>100			
36	NSC 30176	4,4′-(NHCOCH$_2$Cl)$_2$	46.5	46.5			
37	NSC 53674	4,4′-(NHCHO)$_2$	>100	>100		>100	>100
38	NSC 36807	4,4′-(NHCO-24-cholestanol-12-OH)$_2$	>100	>100		>100	>100
39	NSC 39859	4,4′-[NHCH$_2$S(CH$_2$)$_{11}$CHMe$_2$]$_2$[d]	90	90		>100	>100
40	NSC 40337	4,4′-[NHCOCH$_2$NHCH(CO$_2$H)CH$_2$CH$_2$SMe]$_2$	>100	>100			
41	NSC 40502	4,4′-[NHCH$_2$NHCH(CH$_2$CH$_2$SMe)C(O)O(CH$_2$)$_{15}$Me]$_2$	>100	>100			
42	NSC 95632	4,4′-[NHCO(CH$_2$)$_3$N(C$_6$H$_{10}$)$_2$]$_2$	>100	>100			
43	NSC 176305	4,4′-[(NHN═C(CN)$_2$]$_2$	25.9 ± 6.7 (38.3)	21.9 ± 1.9 (38.0)	63.5		
44	NSC 176307	4,4′-[NHN═C(COMe)$_2$]$_2$	>100	90			
45	NSC 20636	3,3′-[NHC(═NH)NHC(NH$_2$)═NH]$_2$ · HCl	>100	>100		>100	>100
46	NSC 85650	3,3′-(SO$_2$SCCl$_3$)$_2$	20.7 ± 6.9 (38.0)	21.3 ± 7.9 (40.0)	28.0	>100	>100
47	NSC 38049	3,3′-(CHO)$_2$, 4,4′-(OH)$_2$	0.6 ± 0.2 (1.5)	1.3 ± 0.2 (0.8)	5.7		
48	NSC 689434	4,4′-(OAc)$_2$	>100	>100		3.0	NR[e]
49		3,3′-(COMe)$_2$, 4,4′-(OH)$_2$	>100	>100			
50	NSC 689435	3,3′-(NO$_2$)$_2$, 4,4′-(OH)$_2$	>100	>100		30.3	NR
51	NSC 689416	3,4-(CO)$_2$O, 3′,4′-(CO)$_2$O	20.9 ± 1.7	18.6 ± 2.0		>100	>100
52	NSC 689437	3,4-(COOH)$_2$, 3′,4′-(COOH)$_2$	29.8 ± 8.0	29.5 ± 5.0		50.2	NR

[a] A diagram of the basic structure of the compounds listed is shown above the table.
[b] Values in parentheses refer to HIV-2 IN inhibition.
[c] Values with standard deviations are means from three independent experiments.
[d] Me, methyl.
[e] NR, not reached due to cytotoxicity.

Synthesis (general methods). Melting points were taken on a Mel Temp II melting point apparatus, and uncorrected values are reported. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, Ga. Infrared (IR) (KBr) spectra were recorded on a Perkin-Elmer 1600 Fourier-transformed–infrared spectrometer, and ^{1}H nuclear magnetic resonance (^{1}H NMR) data were obtained on a Bruker AC250 (250 MHz) instrument. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system.

4,4′-Diacetoxydiphenylsulfone (compound 48). To a solution of 4-hydroxyphenyl sulfone (1 g, 4 mmol in anhydrous dichloromethane [20 ml]), was added triethylamine (1.2 g, 12 mmol), and the reaction mixture was stirred for 5 min at room temperature, at which time the mixture became clear. Acetyl chloride (0.78 g, 10 mmol) was then added dropwise at 0°C, and the mixture was stirred for an additional 30 min at room temperature. A white solid formed, which was removed by filtration, and the filtrate was diluted with chloroform (50 ml), washed with brine, and then dried over anhydrous sodium sulfate. Solvent was removed under reduced pressure, and the resulting residue was crystallized from dichloromethane-hexane to yield product as a white solid (1.24 g, 93% yield, 163 to 166°C mp). ^{1}H NMR ($CDCl_3$) δ 7.94 (d, J = 8.8 Hz, 4-H), 7.23 (d, J = 8.8 Hz, 4-H), 2.29 (s, 6-H). Analysis calculated for $C_{16}H_{14}O_6S$: C, 57.48; H, 4.22. Found: C, 57.41; H, 4.23.

3,3′-Diacetyl-4,4′-dihydroxydiphenylsulfone (compound 49). A mixture of 4-acetoxyphenyl sulfone (1 g, 3 mmol) and finely powdered anhydrous aluminum chloride (2.71 g, 20.3 mmol) was heated under argon slowly from room temperature to 160 to 165°C and maintained at this temperature for 3 h. After cooling to room temperature, the reaction mixture was treated with crushed ice (20 g) followed by concentrated hydrochloric acid (to pH ~2), extracted with ethanol acetate (EtOAc), and dried over anhydrous sodium sulfate. Purification by silica gel flash chromatography (EtOAc-hexane, 1:1) provided product as a white solid (0.72 g, 72% yield, 195 to 196°C (EtOAc-hexane) mp (in the literature [25], 189 to 190°C mp). ^{1}H NMR (DMSO-D_6) δ 12.19 (s, 2-H), 8.25 (d, J = 2.4 Hz, 2-H), 7.99 (dd, J = 8.8, 2.4 Hz, 2-H), 7.14 (d, J = 8.8 Hz, 2-H), 2.65 (s, 6-H). FABMS m/z 335 (MH^+). Analysis Calculated for $C_{16}H_{14}O_6S$: C, 57.48; H, 4.22. Found: C, 57.54; H, 4.27.

3,3′-Dinitro-4,4′-dihydroxydiphenylsulfone (compound 50). Fuming nitric acid (3.2 ml, 77.0 mmol) was added to a stirred suspension of 4-hydroxyphenyl sulfone (750 mg, 3.0 mmol) in chloroform (50 ml) at room temperature over 30 min, and the reaction mixture was stirred (1.5 h). The solvent was removed under reduced pressure at room temperature, and the residue was treated with cold 6 N hydrochloric acid. The product was collected by filtration, washed (H_2O), and then air dried to provide a light yellow solid (760 mg, 74% yield, 235°C mp) (in the literature [15], 230 to 233°C mp). ^{1}H NMR (DMSO-D_6) δ 12.38 (br, 2-H), 8.43 (d, J = 2.4 Hz, 2-H), 8.05 (dd, J = 8.9, 2.4 Hz, 2-H), 7.27 (d, J = 8.9 Hz, 2-H); FABMS m/z 339 (M-H). Analysis calculated for $C_{12}H_8N_2O_8S$: C, 42.36; H, 2.37. Found: C, 42.51; H, 2.43.

3,3′,4,4′-Diphenylsulfonetetracarboxylic dianhydride (compound 51). This compound is commercially available (TCI America, Portland, Oreg.).

3,3′,4,4′-Diphenylsulfonetetracarboxylic acid (compound 52). A suspension of 3,3′,4,4′-diphenylsulfonetetracarboxylic dianhydride (1.8 g, 5.0 mmol) in H_2O (20 ml) was refluxed (overnight). Upon cooling (5 to 10°C), a solid formed, and this solid was collected by filtration and dried at 80 to 100°C overnight to yield product as a white solid (1.28 g, 65% yield, 288 to 290°C mp) (in the literature [21], 281 to 290°C mp). ^{1}H NMR (DMSO-D_6) δ 8.26 (m, 2-H), 8.22 (m, 2-H), 7.87 (d, J = 7.9 Hz, 2-H). IR (KBr) 3,004 (br), 1,708, 1,424, 1,290 cm^{-1}. FABMS m/z 393 (M-H). Analysis Calculated for $C_{16}H_{10}O_{10}S$: C, 48.74; H, 2.56. Found: C, 48.57; H, 2.66.

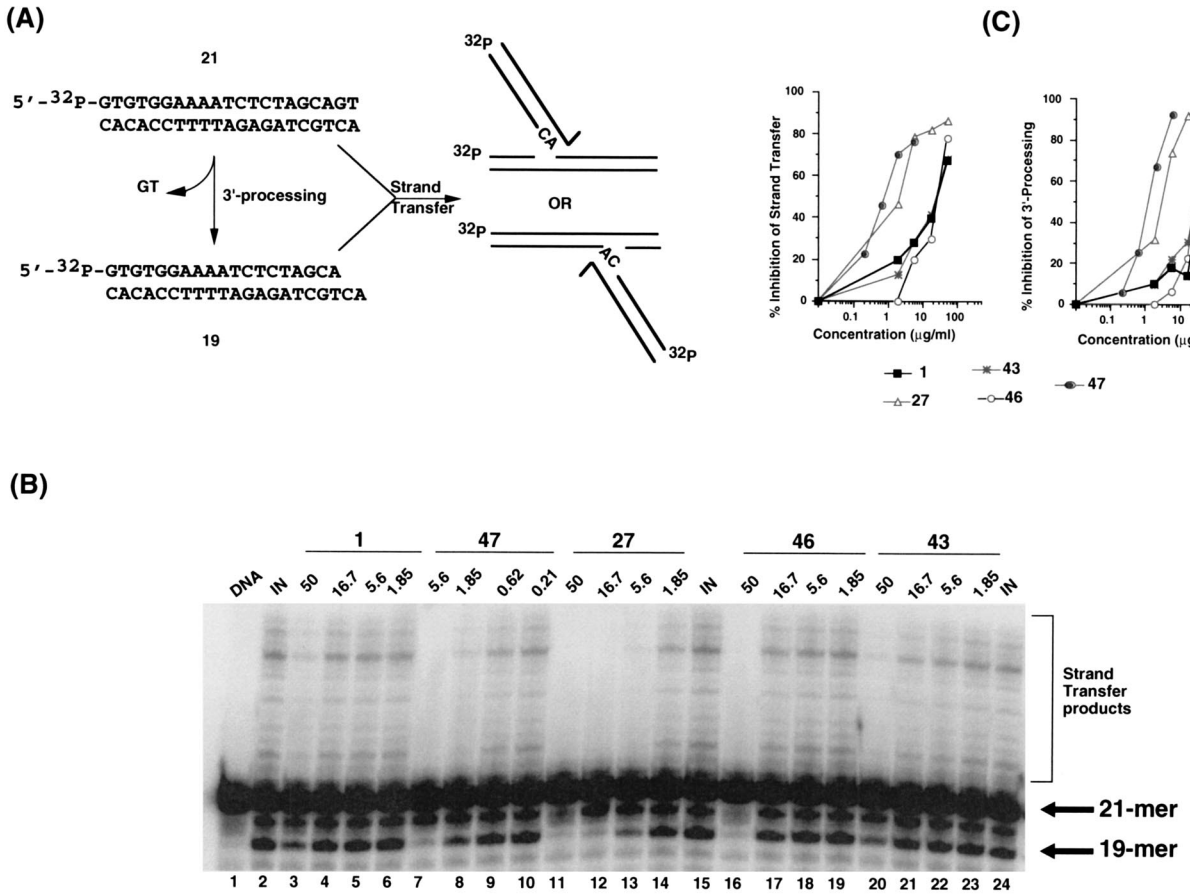


FIG. 1. HIV-1 IN catalytic assay (3′ processing and strand transfer). (A) A 21-mer blunt-end oligonucleotide corresponding to the U5 end of the HIV-1 proviral DNA, 5′ end labeled with ^{32}P, is reacted with purified HIV-1 IN. The initial step involves nucleolytic cleavage of two bases from the 3′ end, resulting in a 19-mer oligonucleotide. The second, or strand transfer, step involves joining this recessed 3′ end to the 5′ end of an IN-induced break in another identical oligonucleotide, which serves as the target DNA. (B) Concentration-dependent inhibition of HIV-1 IN by a series of sulfones. The results of electrophoresis in a 20% denaturing acrylamide gel show 19-mer 3′ processing products, the substrate 21-mer oligonucleotide, and higher-molecular-weight strand transfer products. Drug concentrations (in micrograms per milliliter) are indicated above each lane. See Tables 2 and 3 for an explanation of the compound numbers. (C) Graph showing the quantitation of results presented in panel B. Inhibition was calculated after phosphorimager quantification.

RESULTS

From hundreds of diarylsulfones available in the NCI drug repository, we selected 47 representative compounds containing various substituents, while ignoring polyhydroxylated derivatives. Five additional compounds were either synthesized or obtained from commercial sources. All of these compounds were initially tested at 100 μg/ml and were categorized as active only if they exhibited noticeable inhibitory potency below this concentration. Compounds were loosely divided into two classes based on the electronic nature of the substituents. For example, Table 2 lists anti-IN activities of a series of diarylsulfones containing electron-withdrawing substituents. Among 30 compounds in this series, 20 were inactive (>100 μg/ml), 9 had activities in the range of 50 to 100 μg/ml, and 2 demonstrated activities less than 20 μg/ml. Compound 27 was the most potent in this series, with IC_{50}s of 2.9 ± 0.7 and 2.5 ± 0.5 μg/ml (values are means ± standard deviations) for 3′ processing and strand transfer, respectively, against HIV-1 IN. The second-most-potent compound was the difluoro analog compound 1, with IC_{50}s of 51.0 ± 8.8 and 59.0 ± 8.9 μg/ml. These results show that at least one of the hydroxyl groups of the catechols could be replaced by a nitro group and the other could be replaced with a very strong electron-withdrawing group. Additionally, replacing a hydroxyl with a stronger nucleophile, such as a thiol group, increased potency. The difference in potency shown by compounds 1 and 13 compared to compounds 5, 7, and 10 may be due to charge distribution on the aromatic ring. The abilities of selected compounds to inhibit HIV-2 IN were evaluated (results are summarized in Tables 2 and 3). All compounds tested exhibited comparable potencies against both HIV-1 and HIV-2 IN, supporting a notion that a conserved region of IN is targeted.

Table 3 lists the anti-HIV-1 IN activities of a series of diarylsulfones containing *meta* and/or *para* substituents. In this series of compounds, a majority of which have electron-donating groups, 10 analogs were inactive, 2 had activities in the range of 40 to 90 μg/ml, and 5 had IC_{50}s of less than 33 μg/ml. The most potent compound in this series was the dialdehyde compound 47, with IC_{50}s of 0.6 ± 0.2 and 1.3 ± 0.2 μg/ml. The diamino analog compound 33 showed high potency with IC_{50}s of 4.5 ± 3.0 and 4.9 ± 3.0 μg/ml for 3′ processing and strand transfer, respectively. The remaining compounds which contained reactive functional groups, had potencies of less than 33 μg/ml. Among these groups of compounds, it seems that an aldehyde or an amino group can substitute for a hydroxyl group. The compounds bearing single reactive functional

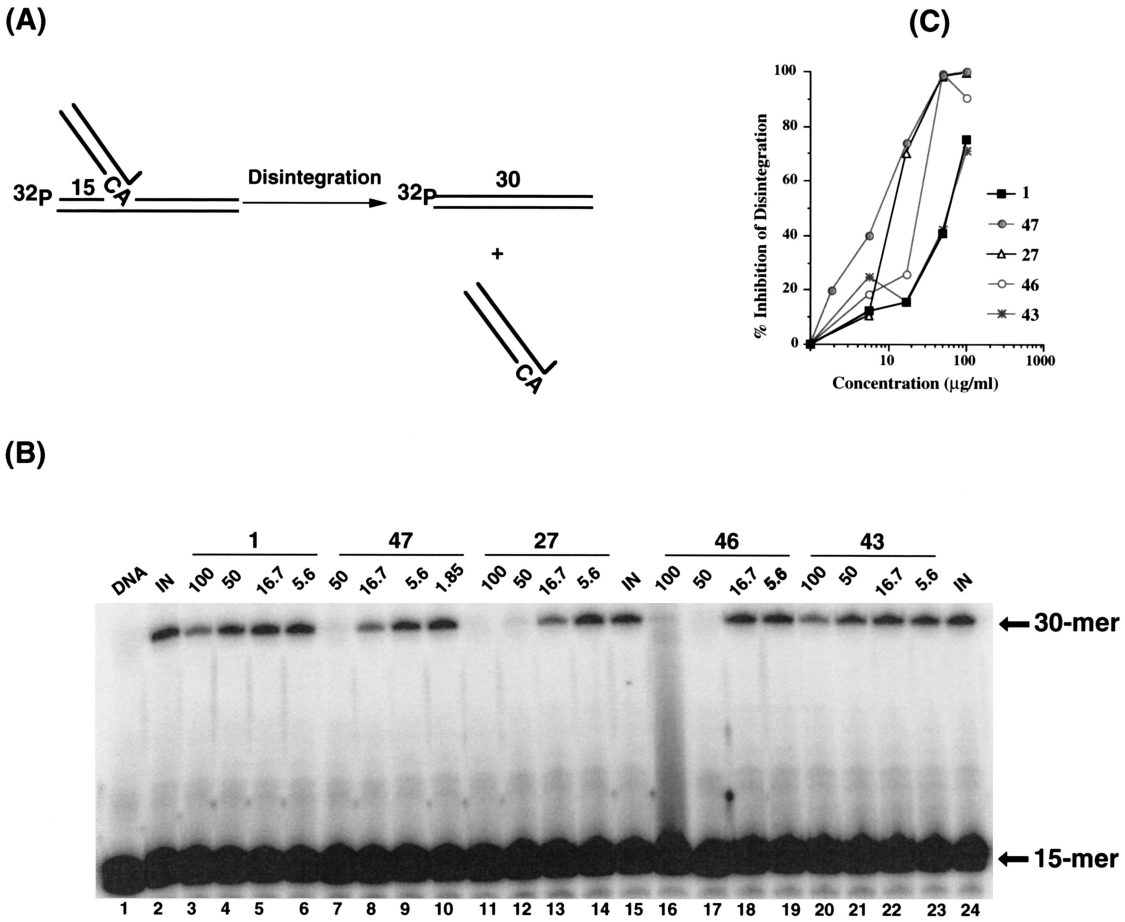


FIG. 2. HIV-1 IN disintegration assay using the core truncated mutant IN^{50-212}. (A) The substrate oligonucleotide mimics a strand transfer step product, i.e., a Y oligonucleotide containing 15-mer oligonucleotide 5′ end labeled with ^{32}P. HIV-1 IN mediates the disintegration generating a 30-mer oligonucleotide. (B) Concentration-dependent inhibition of HIV-1 IN by a series of sulfones. The results of electrophoresis in a 20% denaturing acrylamide gel show the original 15-mer and the 30-mer disintegration products. Drug concentrations (in micrograms per milliliter) are indicated above each lane. See Tables 2 and 3 for an explanation of the compound numbers. (C) Graph showing the quantitation of results presented in panel B. Inhibition was calculated after phosphorimager quantification.

groups, such as compounds 31, 36, 43, and 46, did not require an extra *ortho*-hydroxyl for activity. It is interesting that the 4,4′-diamino analog compound 32 and 4,4′-dihydroxy sulfone were totally inactive even at concentrations higher than 5 mM (data not shown), whereas the 3,3′-diamino,4,4′-dihydroxy was highly active, thus showing that compounds with hydrogen bond donor capabilities and *ortho* substitution have high IN activity in vitro. The tetracarboxylic dianhydride compound 51 and its free acid derivative compound 52 exhibited similar potencies; however, when one of the acidic groups is replaced with a nitro group, as in compounds 13 and 28, the potency is decreased by a factor of 3 to 4. Interestingly, when the nitro group is preserved and the acidic group is replaced by a basic amino group, as in compound 25, the potency is decreased even further, thus implying the importance of a carboxyl moiety. The tetranitro derivative compound 2 and other derivatives containing nitro groups such as compounds 11 to 14, all exhibited moderate potencies, suggesting the importance of a nitro group versus other electron-withdrawing groups such as tetrachloro derivative compound 16. Furthermore, it seems that derivatives with acetyl and acyl groups had reduced potency whereas those containing an aldehyde or a carboxylic acid exhibited high potency.

Five of the potent compounds were selected for further studies utilizing HIV-1 IN mutants and another DNA-binding enzyme, topoisomerase I. Representative gels illustrating a comparison among these compounds are shown in Fig. 1 to 3. Initially, a dual assay which measures the extents of both 3′ processing and strand transfer by using a blunt-ended 21-mer duplex oligonucleotide was used (Fig. 1). The 3′ processing reaction or endonuclease cleavage liberates the 3′-terminal dinucleotide GT to yield a 19-mer oligonucleotide from a 21-mer duplex substrate (Fig. 1A). Subsequent transesterification results in the insertion of one 3′ processed oligonucleotide into another oligonucleotide, generating higher-molecular-weight products with slower migration than the 21-mer substrate. In addition to catalyzing the 3′ processing and strand transfer reactions described above, IN can also catalyze an apparent reversal of a later step known as disintegration (Fig. 2A). This reaction is also inhibited by several sulfones (Fig. 2). To probe the site of binding on IN, the IN deletion mutant IN^{50-212}, lacking both the amino-terminal zinc finger region and the carboxyl-terminal DNA-binding domain, was used. All of the active sulfones inhibited this mutant, which implies that the binding of sulfones to the IN core region is responsible for IN inhibition. The dialdehyde and dithiol compounds 47 and 27 exhibited the highest potencies of the compounds tested. Generally, higher IC_{50}s for the disintegration step are obtained

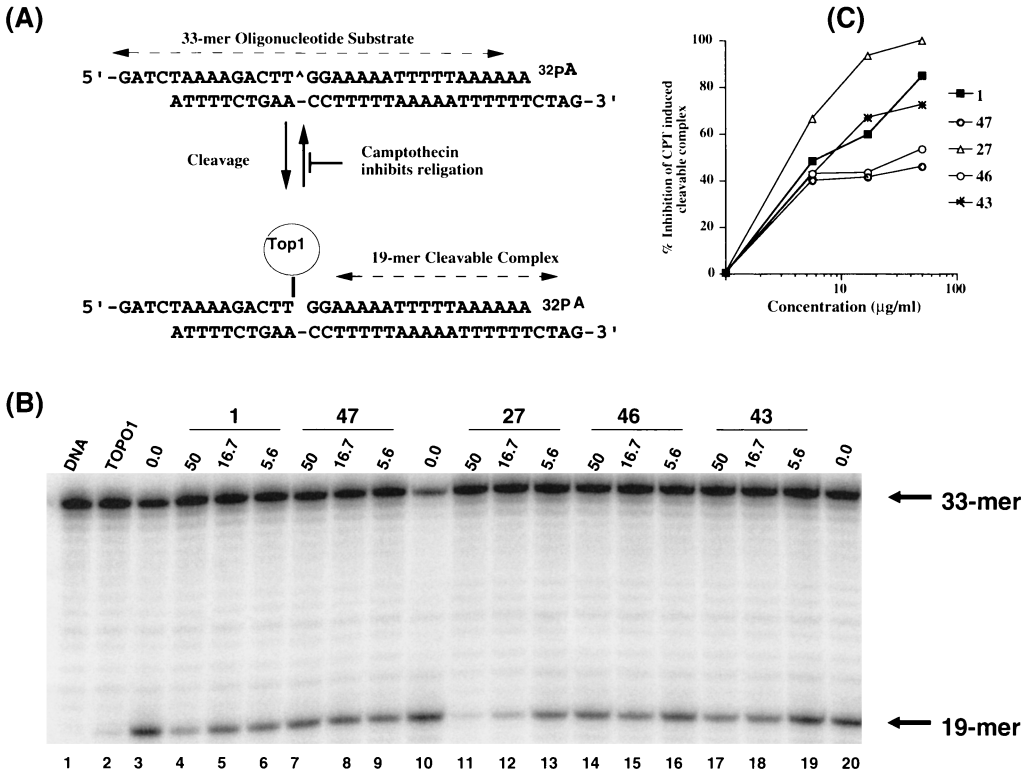


FIG. 3. Topoisomerase I induced DNA cleavage assay. (A) A 33-mer oligonucleotide bearing a strong topoisomerase cleavage site in its center (Top1), 3′ end ^{32}P labeled, was reacted with eukaryotic topoisomerase I in the absence or presence of camptothecin as described in Materials and Methods. The topoisomerase I-induced cleavage resulted in a 19-mer oligonucleotide. (B) Inhibition of camptothecin-induced cleavable complex formation by diarylsulfones. Lane 1, DNA alone; lane 2, DNA plus topoisomerase I; lanes 3 to 20, topoisomerase I plus camptothecin (10 μM) and the indicated concentrations (in micrograms per milliliter) of drugs, as indicated above the lanes. See Tables 2 and 3 for an explanation of the compound numbers. (C) Graph showing the quantitation of results presented in panel B. Inhibition was calculated after phosphoimager quantification. CPT, camptothecin.

when the deletion mutant IN^{50-212} is used, suggesting that the full native enzyme is required for optimal HIV-1 IN catalysis.

Selectivity of inhibition by diarylsulfones against mammalian topoisomerase I was examined (Fig. 3). In an assay specific for inhibition of camptothecin-induced cleavable complex (24), the potencies of diarylsulfones were different. For example, compounds 46 and 47 were not potent inhibitors (IC_{50}s, >50 μg/ml) whereas compounds 1, 27, and 43 were effective inhibitors (IC_{50}s, <10 μg/ml) of camptothecin-induced cleavable complex. The induction of a cleavable complex in the presence of these sulfones was also assayed, and none showed any detectable formation of cleavable complex (data not shown). Therefore, there is variation among sulfones both in their ability to inhibit different DNA-binding enzymes and also to bind to DNA with high affinity. For example, compounds 46 and 47 inhibited IN with a selectivity index of >2 and >50, respectively, over that of topoisomerase I. However, such selectivity was not observed with compounds 1, 27, and 43.

The important roles played by sulfones as antibacterial, antimalarial, and antileprotic agents are well documented (6, 7, 14). Sulfones and sulfanilamides are known to directly inhibit dihydropteroate synthase (DHPS) or are converted by this enzyme to a toxic derivative that inhibits subsequent enzymes in the folate biosynthetic pathway. Although there seems to be no obvious homology between HIV-1 IN and DHPS, several sulfones and sulfanilamides have been shown to be inhibitors of both of these enzymes. We thus extended our studies to include the following sulfa drugs (data not shown): sulfamethoxazole, 5-methylsulfadiazine, *N*-(2-amino-4-pyrimidinyl)sulfanilamide, sulfathiazole, sulfadimethoxine, sulfamethazine, sulfisomidine, sulfaquinoxaline, sulfadiazine, sulfisoxazole, *N′*-(4,5-dimethyloxazole-2-yl)sulfanilamide, sulfasalazine, 3-nitro-1-(8-quinolysulfonyl)-1,2,4-triazole, *N*4-(2-amino-4-pyrimidinyl)sulfanilamide, phenylsulfathiazole, 2-amino-5-(4-nitrophenylsulfonyl)thiazole, *N*-(6-aminohexyl)-5-chloronaphthalene-1-sulfonamide, succinylsulfathiazole, and 4-carboxybenzenesulfonamide. Sulfisoxazole and sulfasalazine were the most active derivatives, and the IC_{50}s for these drugs were around 50 and 100 μg/ml for both 3′ processing and integration, respectively. Most of the sulfa drugs containing the $NH_2C_6H_6SO_2$ moiety were inactive. This is in agreement with diarylsulfone drugs containing NH_2 or OH groups on the phenyl rings. In contrast to DHPS inhibitors (6, 7), it seems that neither the H bond-donating groups nor basicity of the ring is required for IN activity.

Since a majority of compounds examined in this study did not fully protect HIV-1-infected T4 lymphocyte CEM cells, as shown by measuring the protective effect against HIV-1, there is a need for further improvement of sulfone-based inhibitors. Compounds with high or moderate potency against HIV-1 IN exhibited considerable cytotoxicity. For example, compounds 2, 11, 14, 19, 27, and 52 all exhibited moderate to high potency in an isolated system against HIV-1 IN. However, they were cytotoxic at low micromolar concentrations. Thus, no obvious protection against HIV-1-infected cells was observed (Tables 2 and 3). However, compound 3 [50% effective concentration [EC_{50}], 0.26 ± 0.05 μg/ml; IC_{50}, 44.7 μg/ml) exhibited remarkable potency in cell-based assays, and compound 12 (EC_{50},

3.4 ± 0.8 μg/ml; IC_{50}: 15.6 ± 1.4 μg/ml) was also confirmed to be moderately potent in this assay. Although these two compounds were inactive in our IN assay, they were effective inhibitors of HIV-1 reverse transcriptase (20). Interestingly, not all compounds that exhibited high potency against reverse transcriptase were confirmed active in cellular assays (1, 20). We also did not observe a direct correlation between anti-IN activities and in vitro activities in cellular assays.

DISCUSSION

The mechanisms of cytotoxicity and anti-HIV-1, and anti-IN activity remain a critical issue for this class of compounds and all other inhibitors reported thus far. It seems possible to identify critical factors necessary for IN activity in isolated enzyme systems. Unfortunately, information gained in such systems does not always translate to activities observed in cellular assays. The question of whether anti-IN activity in isolated enzyme systems correlates with general anti-HIV activity remains. Studies from several laboratories have concluded that in some instances, there is a good correlation between activities in isolated enzyme models and the in vitro cellular data (see, for example, reference 27). However, there are many reasons why isolated enzyme data do not necessarily translate into cellular activity. In light of this, a more robust model system needs to be evaluated, and toward this end we have been engaging in several different studies to address this issue. For example, we are exploring the site of enzyme-drug interaction, using a photoaffinity probe (23). Several of our water-soluble inhibitors will be evaluated in cocrystallization experiments with the native enzyme for X-ray structure determinations. We are also exploring inhibitory activities of the drugs against several IN mutants.

Comparative analysis of a panel of structurally similar sulfones has revealed several factors contributing to the mechanisms of anti-HIV-1 IN activity of this class of compounds. For example, replacement of one hydroxyl group with an amino, thiol, aldehyde, or carboxyl group resulted in no loss of potency. However, electron-withdrawing groups such as nitro and fluoro groups influence IN inhibitory potency. Interestingly, precedents in the literature suggest that the electronic nature of substituents on the phenyl ring greatly influences the antibacterial and antimalarial activities of sulfones (6, 7, 14).

The findings of the present study could have important implications for the designing of more potent sulfone-based inhibitors. For example, compounds containing a mercapto group adjacent to an amino, hydroxyl, or carboxyl group ought to have anti-IN activity. Presently, we are engaged in synthesizing novel compounds based on sulfones and other inhibitors to obtain an optimal IN inhibitor.

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