Available online at www.sciencedirect.com

ScienceDirect

Bioorganic & Medicinal Chemistry 15 (2007) 1127–1137

Bioorganic & Medicinal Chemistry

Ring substituent effects on biological activity of vinyl sulfones as inhibitors of HIV-1

D. Christopher Meadows,[a,c] Tino Sanchez,[b] Nouri Neamati,[b]
Thomas W. North[c] and Jacquelyn Gervay-Hague[a,*]

[a]*Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA*
[b]*Department of Pharmaceutical Sciences, School of Pharmacy, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90089, USA*
[c]*Center for Comparative Medicine and Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616, USA*

Received 30 June 2006; revised 4 October 2006; accepted 11 October 2006
Available online 13 October 2006

Abstract—In a previous study, we prepared a small library of chicoric acid analogs that possessed both potent anti-integrase and antiviral activity. It was also shown that active compounds fell into one of two groups: those that inhibited an early stage in viral replication and those that inhibited at a later stage. In this study, a series of vinyl geminal disulfone-containing compounds possessing a range of ring substituents has been synthesized to probe the impact of structure on inhibitory mechanisms. Four active compounds were identified using HIV drug susceptibility assays. Three of the inhibitors possessing either no substituents or electron-withdrawing substituents on the aromatic rings led to high levels of cytotoxicity and antiviral activity. Intrigued by the potential implications of electronic effects on activity, we probed whether the active compounds could be nonspecifically reacting via 1,4-addition. To investigate this hypothesis, the compounds were incubated with glutathione and upon LC/MS analysis, molecular ion peaks corresponding to both mono and double addition adducts were identified. Second, we synthesized analogs lacking the ability to participate in 1,4-addition and tested them for antiviral activity and cytotoxicity, and found the compounds inactive for both activities. Taken together, the studies reported herein suggest that compounds lacking electron-donating substituents on the aromatic ring are promiscuous acceptors of biological nucleophiles, whereas compounds possessing electron-donating substituents seem to resist addition or at least be more selective and significantly less toxic.

1. Introduction

According to a recent report by the United Nations (June 2, 2005), HIV-1 infection continues to expand worldwide and treatment is available for only 12% of those who need it.[1] Current antiretroviral treatment for HIV-1 infection has been seriously complicated by drug resistance. Both vertical and horizontal transmission of drug-resistant variants has been demonstrated and as more people have begun antiviral therapy, the transmission of drug-resistant and multiple drug-resistant strains has increased.[2,3] Resistance has been observed for all drugs currently used against the virus and is expected to occur with any newly developed ones. Additional candidates against existing targets, and drugs with novel modes of action are needed to help combat resistant virus responsible for treatment failure and ultimately death.

HIV-1 integrase (IN) is one target for which there is no FDA approved drug currently available. IN is responsible for inserting the viral DNA into the host chromosome where it can then be transcribed into viral mRNA and translated into viral proteins that then assemble into virions which bud from the cell membrane and subsequently infect other cells.[4] In this process, IN performs two catalytic functions. In the cytoplasm, IN associates with newly reverse transcribed viral dsDNA and cleaves a dinucleotide from each 3′-end of the viral genome in a step termed 3′-processing. Once 3′-processing is complete, the viral genome, IN, and other associated cellular and viral proteins (the preintegration complex) are translocated into the nucleus where IN

Keywords: HIV-1; HIV integrase; Vinyl sulfones.

[*] Corresponding author. Tel.: +1 530 754 9577; fax: +1 530 752 8995; e-mail: gervay@chem.ucdavis.edu

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.10.017

inserts the recessed 3′-ends directly into the phosphodiester backbone of the cellular DNA in a step termed strand transfer or integration. Following repair of the integration intermediate using cellular enzymes, the viral genome is integrated into the host chromosome.

While many compounds have been reported to inhibit IN in purified enzyme assays, the majority of these do not also possess requisite antiviral activity in the cellular environment, and if they do, it cannot be concluded that anti-IN activity is necessarily the source of viral suppression.[5] For example, chicoric acid analogs were one of the first classes of compounds reported to inhibit IN in the low micromolar range both in enzymatic and cellular assays (Fig. 1).[6] It was later found, however, that IN was not the true cellular target of the chicoric acids, rather interference of viral entry to the cell probably through interaction with envelope glycoprotein gp120.[7] One possible reason for the lack of anti-IN activity of the chicoric acids in cells could be due to the charged nature of the molecules preventing passage through the cell membrane. Through the synthesis of neutral analogs of chicoric acid (Fig. 1), we hypothesized that the molecules could better traverse the cellular membrane and could then exert their anti-IN activity.

Previously, we reported the design and synthesis of disulfone-containing analogs of chicoric acid and demonstrated that they did indeed inhibit both IN and viral replication with nanomolar to micromolar activity (Fig. 1).[8] In addition, we discovered through cellular time-of-addition assays that one of the disulfone-containing compounds had an activity profile that correlated with dextran sulfate and T20, two compounds known to interfere with early events in viral replication. It is intriguing that the minor structural differences between **1** and **2** correspond to such mechanistic diversity (early stage vs later stage inhibition). In an effort to probe what structural features are important for the mechanistic variation of the disulfone compounds, we designed and synthesized a wide range of ring-functionalized compounds. During the course of this study we observed a trend: compounds possessing only electron-withdrawing ring substituents or lacking ring substituents led to greatly increased cytotoxicity. Herein, we report the effect of electronic structure on antiviral activity and cytotoxicity.

2. Results and discussion

Compounds **3**–**20** were prepared as previously described using a unique bis-phosphonate reagent and an appropriate aromatic aldehyde in a Horner–Emmons–Wadsworth reaction.[8–10] Reduction of the resulting olefins using either catalytic hydrogenation or superhydride® provided the fully saturated analogs. Table 1 lists the compounds synthesized and the aldehydes reacted according to Scheme 1.

The compounds in Table 1 were initially screened against purified IN to determine if any possessed activity against either 3′-processing or integration. In the assay, wild-type IN is incubated with the test compound and a ^{32}P-labeled oligonucleotide. An aliquot of this mixture is then analyzed for 3′-processed and strand transfer products by gel electrophoresis. As can be seen from Table 2, most of the compounds were found to be inactive against IN ($IC_{50} \geqslant 100\ \mu M$) with respect to both catalytic activities. However, **4** demonstrated moderate activity against both functions, **6** displayed relatively potent inhibition of integration and moderate activity against 3′-processing.

Having established that most of the newly synthesized compounds were inactive against IN, we next screened the compounds for antiviral activity aimed at alternate steps in replication, namely viral entry. The assay we currently use for HIV-1 has recently been described.[11,12] As indicated in Table 3, four of the 19 new compounds (**6**, **9**, **11**, and **17**) were found to possess antiviral activity in the low micromolar range. Although **4** showed IN

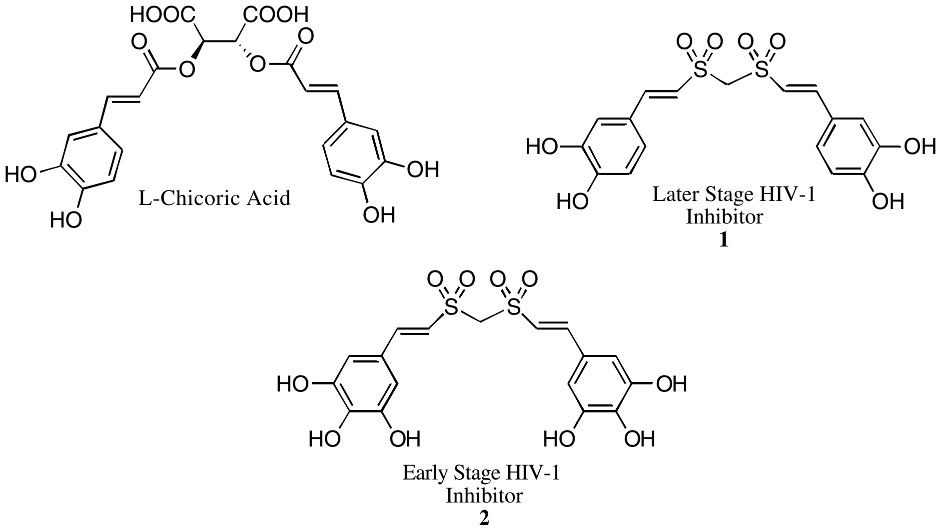
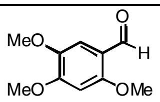
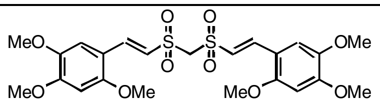
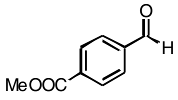
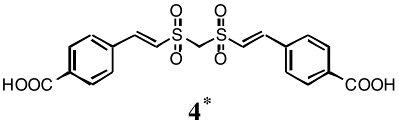
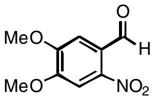
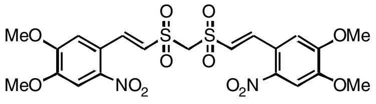
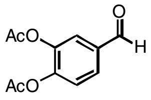
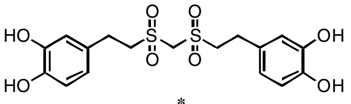
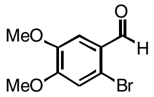
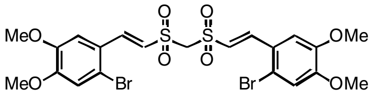
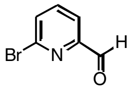
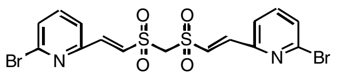
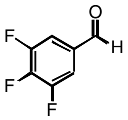
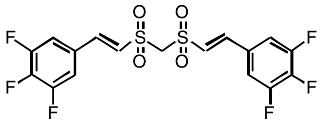
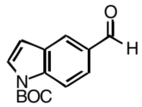
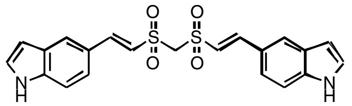
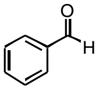
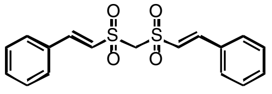
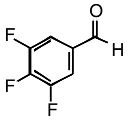
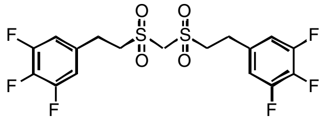
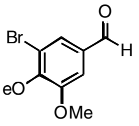
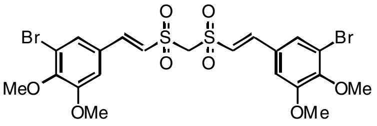
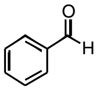
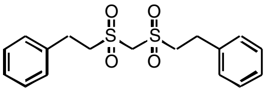
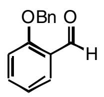
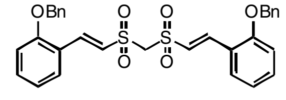
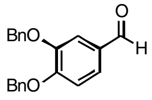
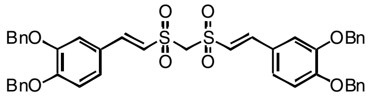
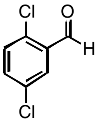
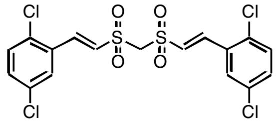
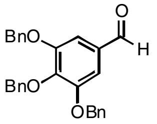
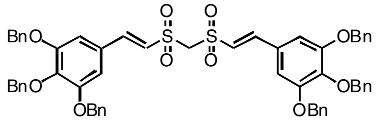
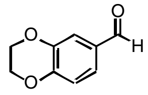
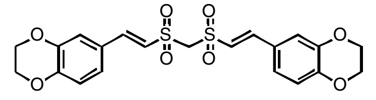
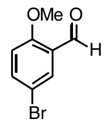
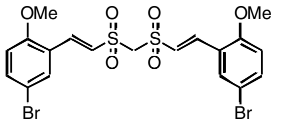
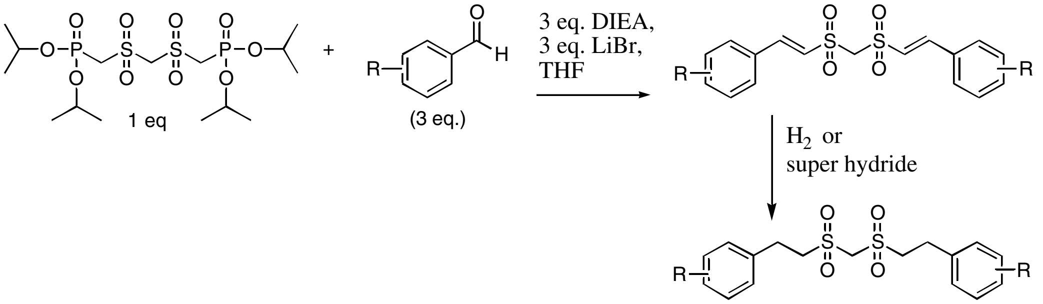


Figure 1. L-Chicoric acid and neutral disulfone-containing analog.

Table 1. Geminal disulfones prepared

Aldehyde	Coupled Product	Aldehyde	Coupled Product (*After Deprotection)
	 **3**		 **4***
	 **5**		 **6***
	 **7**		 **8**
	 **9**		 **10**
	 **11**		 **12**
	 **13**		 **14**
	 **15**		 **16**
	 **17**		 **18**
	 **19**		 **20**

inhibition, it did not exhibit antiviral activity suggesting that membrane permeability may be a problem with this analog. Active compounds were then tested for cytotoxicity in a standard cell proliferation assay (MTS assay) in HeLa H1-JC.37 cells. Compounds **9**, **11**, and **17** were found to have highly toxic effects. With **9** and **17** containing electron-withdrawing substituents and **11** possessing no substituents, we reasoned that these



Scheme 1. Synthesis of biaryl/disulfone compounds via Horner–Emmons–Wadsworth chemistry.

Table 2. HIV-1 IN inhibition data

Compound	IC_{50} (μM)	
	3′-processing	Integration
3	>1000	>1000
4	33 ± 3	33 ± 4
5	500 ± 50	500 ± 50
6	40 ± 10	17 ± 6
7	850 ± 60	500 ± 100
8	>1000	900 ± 50
9	>100	>100
10	>100	95 ± 5
11	>100	>100
12	900 ± 50	333 ± 90
13	>100	>100
14	>100	>100
15	>100	>100
16	>100	>100
17	>100	>100
18	>100	>100
19	>100	>100
20	>100	>100

Table 3. Antiviral activity, cytotoxicity, and antiviral selectivity data for the IN inhibitors

Compound	Antiviral activity[a] EC_{50} ± SE (μM)	Cytotoxicity[b] IC_{50} (μM)	Antiviral selectivity IC_{50}/EC_{50}
1[8]	2.4 ± 0.3[8]	190 ± 7.0[8]	78 ± 6.7[8]
2[8]	0.3 ± 0.03[8]	170 ± 6.0[8]	560 ± 35[8]
3	>10	NT	N/A
4	>10	NT	N/A
5	>10	NT	N/A
6	6.2 ± 1.7	170.2 ± 5.3	27 ± 7.2
7	>10	NT	N/A
8	>10	NT	N/A
9	2.0 ± 0.04	7.8 ± 0.2	3.9 ± 0.02
10	>10	NT	N/A
11	1.2 ± 0.2	12.0 ± 0.2	9.9 ± 1.5
12	>10	110.0 ± 0.4	N/A
13	>10	NT	N/A
14	>10	>150	N/A
15	>10	NT	N/A
16	>10	NT	N/A
17	3.4 ± 0.01	2.6 ± 0.07	0.77
18	>10	NT	N/A
19	>10	NT	N/A
20	>10	NT	N/A

N/A, cannot be calculated without cytotoxicity data.
NT, not tested.

[a] EC_{50} values are means ± SE from three separate determinations.

[b] IC_{50} values are the average of two experiments.

compounds may react with biological molecules via conjugate addition and that the resulting toxicity was the source of antiviral activity.

The possibility of conjugate addition was probed in three ways. First, we synthesized fully saturated analogs of both **9** and **11** (**12** and **14**, respectively) and tested anti-HIV activity; both of the compounds were found to be inactive. Second, **12** and **14** were tested in the MTS assay to see if there was a difference in cytotoxicity between the saturated and unsaturated pairs (Table 2). There was more than a 10-fold difference in toxicity between the saturated and unsaturated compounds implicating the double bond as the source of the observed toxicity. Nevertheless, we did not feel confident in identifying conjugate addition as the source of activity without further evidence. Orientation effects imposed by unsaturation could also be responsible for cell death as has been observed for the cytotoxic combretastatins where *cis* oriented analogs possess toxicity and *trans* orientations are inactive.[13–15] To distinguish this possibility, **9** and **11** were incubated with glutathione in phosphate-buffered saline (PBS) at 37 °C for four days in an analogous manner as for the drug susceptibility assay. After four days, the mixtures were analyzed by LC/FABMS for peaks corresponding to mono and/or double addition adducts. The mass spectra of **9** and **11** indeed showed peaks corresponding to both mono and double addition products suggesting that the compounds act as promiscuous acceptors of nucleophilic biological molecules and taken together with the previous data, we conclude that this is the most likely source of toxicity (Figs. 2–5).

The glutathione addition experiment was also conducted on the most potent disulfone compound identified thus far: the 3,4,5-trihydroxyl compound (**2**). In this case, neither ion peaks corresponding to the mono nor the double addition adducts were observed in the mass spectrum (data not shown) suggesting that resonance-donating effects of the three phenolic groups make the double bond less susceptible to 1,4-addition. Although these studies do not discount the possibility of 1,4-addition as the mode of action for less toxic compounds, it demonstrates that these are at least more selective in their Michael accepting

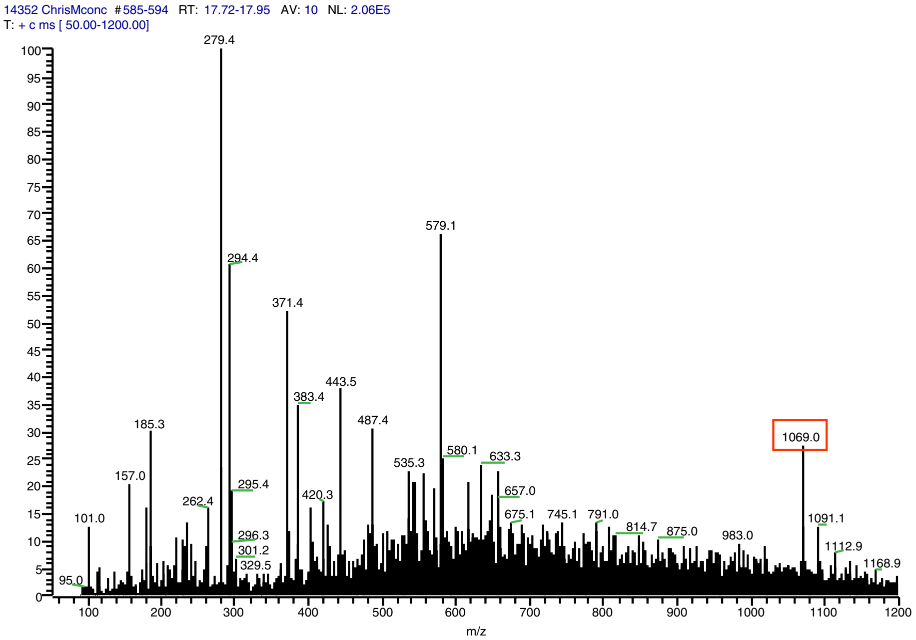


Figure 2. Mass spectrum of **9** incubated with glutathione; molecular ion of double addition product is highlighted (M^+ = 1069.0).

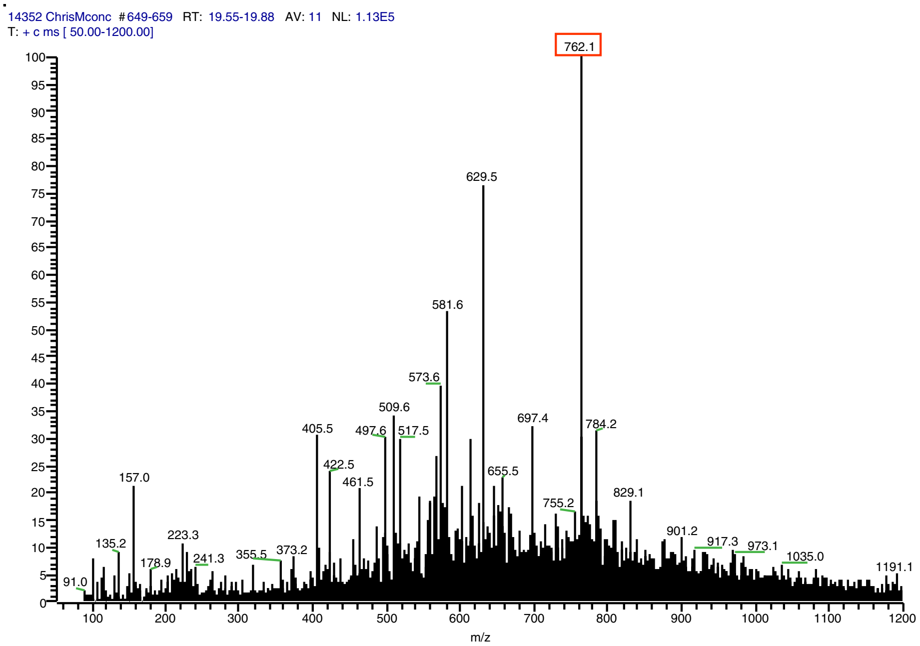


Figure 3. Mass spectrum of **9** incubated with glutathione; molecular ion of mono addition product is highlighted (M^+ = 762.1).

capabilities, that is, perhaps the aromatic groups serve to advantageously place the vinyl sulfone near a pertinent biological nucleophile.

3. Conclusion

Vinyl geminal disulfones are potentially an important class of antivirals, possessing nanomolar to micromolar activity. Vinyl sulfones are already known for their ability to inhibit a variety of cysteine proteases.[16,17] The cysteine protease inhibitors are typically peptidomimetic structures providing specificity and strategic placement of the vinyl sulfone enabling 1,4-addition with the active site cysteine residue. It is possible that vinyl geminal disulfone anti-HIV compounds act in a similar fashion, with the aromatic rings engendering specificity and serving to advantageously place the vinyl sulfone for reaction with a relevant nucleophile. This study suggests that a small subset of vinyl geminal disulfones with no ring substituents or only electron-withdrawing ring substituents possess electron-poor

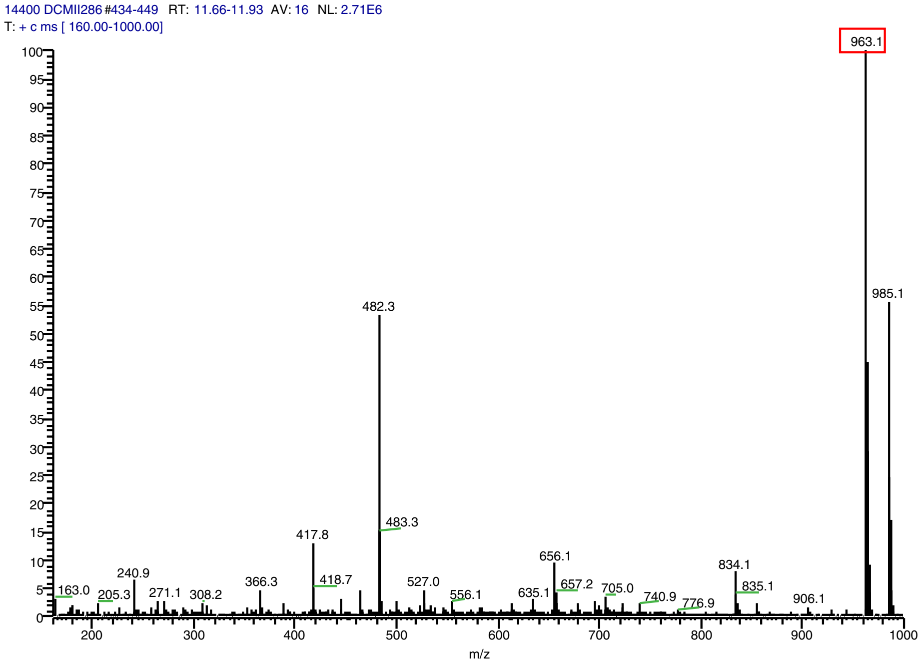


Figure 4. Mass spectrum of **11** incubated with glutathione; molecular ion of double addition product is highlighted ($M^+ = 963.1$).

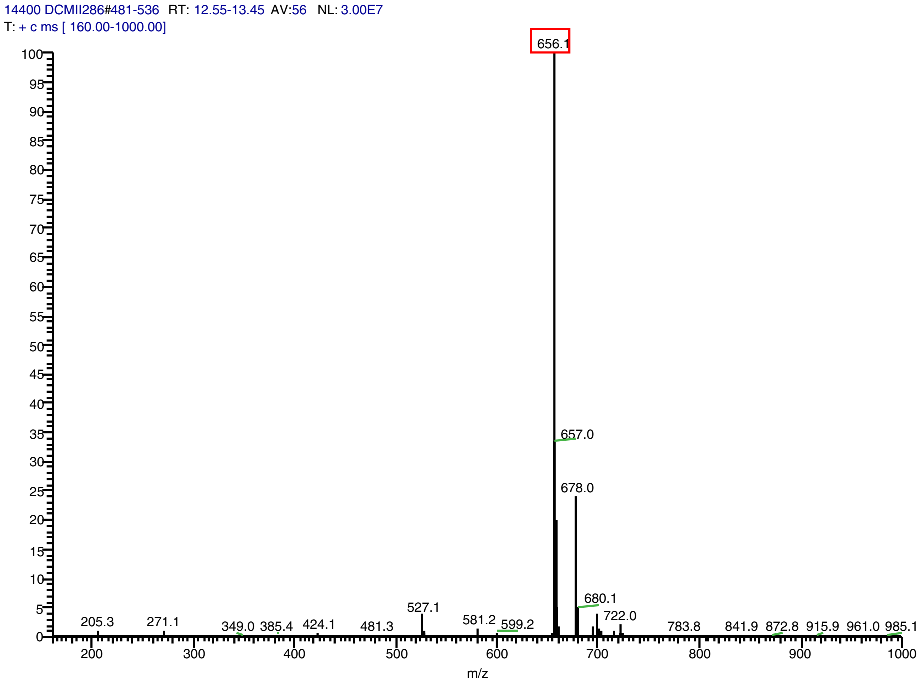


Figure 5. Mass spectrum of **11** incubated with glutathione; molecular ion of mono addition product is highlighted ($M^+ = 656.1$).

conjugated systems, acting as promiscuous acceptors of biological nucleophiles. As lead optimization continues, because of the associated toxicity, molecules lacking any electron-donating ring substituents should be avoided. However, it may be possible to exploit this reactivity by using electron-withdrawing substituents in combination with electron-donating functionalities or other targeting agents to confer a greater degree of selectivity.

4. Experimental

4.1. General procedures

Elemental analyses were obtained from Desert Analytics Laboratory, Tucson, AZ, USA. All materials were obtained from commercial sources and used without additional purification. The aromatic aldehydes used were bought from Aldrich Chemical Company, Inc.

The THF used for reaction was 99.9% anhydrous, inhibitor free in sureseal bottles also obtained from Aldrich Chemical Company, Inc. All glassware for reactions under anhydrous conditions was flame-dried prior to use. Flash chromatography was performed on Silica Gel Geduran (40–63 μm) 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.

4.2. General procedure A

In a flame-dried flask, 1.0 equiv of the disulfone reagent,[10] 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 reaction mixture was stirred overnight before being 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.

4.2.1. Bis(*trans*-β-2,4,5-trimethoxy-styrenesulfonyl)methane (3). To a solution of disulfone reagent[10] (203 mg, 0.41 mmol) in THF (1 mL) was added 1.21 mL of 1 M potassium *tert*-butoxide in THF followed by 2,4,5-trimethoxybenzaldehyde (239 mg, 1.2 mmol) and allowed to mix overnight. Then approximately 2 mL of 0.1 M HCl was added followed by ethyl acetate. The whole solution was filtered and washed with water and ether to give 110 mg (51% yield) of the title compound. ^{1}H NMR (DMSO-d_6, 600 MHz) 3.69 (s, 6H), 3.82 (s, 6H), 3.84 (s, 6H), 5.35 (s, 2H), 6.65 (s, 2H), 7.11 (s, 2H), 7.18 (d, 2H, $J = 15.5$ Hz), 7.59 (d, 2H, $J = 15.5$ Hz). ^{13}C NMR (DMSO-d_6, 150 MHz) 55.82, 55.95, 56.20, 97.15, 111.26, 112.06, 122.67, 139.47, 142.79, 153.20, 154.25, 219.92. HRFABMS (+) calcd $C_{23}H_{29}O_{10}S_2$ (M+H): 529.1124, found: 529.1190 (M+H). Anal. ($C_{23}H_{28}O_{10}S_2$) C, H.

4.2.2. Bis(*trans*-β-4-carboxy-styrenesulfonyl)methane (4). To a solution of disulfone reagent[10] (300 mg, 0.6 mmol) in THF (2.5 mL) was added LiBr (156 mg, 1.8 mmol) followed by DIEA (313 μL, 1.8 mmol). Methyl 4-formylbenzoate (246 mg, 1.5 mmol) was then added and mixed for 2.5 h. Then approximately 200 μL of acetic acid was added followed by water and ethyl acetate. Hexanes were added and then whole solution was filtered and washed with water and ether to give 241 mg (86% yield) of the methyl ester. ^{1}H NMR (DMSO-d_6, 600 MHz) 3.86 (s, 6H), 5.63 (s, 2H), 7.51 (d, 2H, $J = 15.6$ Hz), 7.62 (d, 2H, $J = 15.6$ Hz), 7.77 (d, 4H, $J = 8.2$ Hz), 7.92 (d, 4H, $J = 8.2$ Hz). ^{13}C NMR (DMSO-d_6, 150 MHz) 52.33, 71.28, 128.64, 129.13, 129.57, 131.55, 136.33, 143.31, 165.48. HRFABMS (−) calcd $C_{21}H_{19}O_8S_2$ (M−H): 463.0521, found: 463.0527 (M−H). Anal. Calcd $C_{21}H_{20}O_8S_2$: C, 54.30; H, 4.34. Found: C, 53.52; H, 4.79.

To a solution of the methyl ester in DMSO (1 mL) was added 1 mL of 1 M NaOH and heated with a heat gun for about 1 min. Then concentrated HCl was added until the solution was acidic and then filtered and washed with water to give 54 mg (73%) of the title compound after drying. ^{1}H NMR (DMSO-d_6, 600 MHz) 5.62 (s, 2H), 7.51 (d, 2H, $J = 15.6$ Hz) 7.64 (d, 2H, $J = 15.6$ Hz), 7.79 (d, 2H, $J = 8.3$ Hz) 7.96 (d, 2H, $J = 8.3$ Hz). ^{13}C NMR (DMSO-d_6, 150 MHz) 74.41, 128.40, 129.01, 129.53, 129.72, 129.77, 129.94, 132.91, 135.97, 143.39, 166.51. FABMS (+) calcd $C_{19}H_{16}O_8S_2$ (M+Na): 459.03, found: 460.2 (M+Na). Anal. ($C_{19}H_{16}O_8S_2$) C, H.

4.2.3. Bis(*trans*-β-3,4-methoxy-6-nitro-styrenesulfonyl)-methane (5). To a solution of disulfone reagent[10] (200 mg, 0.4 mmol) in THF (4 mL) was added 1.2 mL of 1 M lithium *tert*-butoxide in THF followed by 3,4-dimethoxy-6-nitrobenzaldehyde (80%) (300 mg, 1.2 mmol) and mixed overnight. The solution was filtered and washed with 0.1 N HCl, THF, and ether to give 93 mg of the title compound. ^{1}H NMR (DMSO-d_6, 600 MHz) 3.83 (s, 6H), 3.87 (s, 6H), 5.53 (s, 2H), 7.14 (s, 4H), 7.46 (d, 2H, $J = 14.8$ Hz) 7.88 (d, 2H, $J = 14.8$ Hz). ^{13}C NMR (DMSO-d_6, 150 MHz) 56.24, 56.38, 107.77, 110.06, 128.60, 140.65, 141.59, 150.29, 152.41. HRFABMS (−) calcd $C_{21}H_{21}O_{12}S_2N_2$ (M−H): 557.0536, found: 557.0539 (M−H). Anal. ($C_{21}H_{22}O_{12}S_2N_2$) C, H, N.

4.2.4. Bis(2-(3,4-dihydroxyphenyl)-ethylsulfonyl)methane (6). A solution of 56 mg of compound **1**[8] and 7 mg of palladium on carbon (10%) was dissolved in 5 mL of ethyl acetate and put on the parr shaker at a pressure of 57 psi. The mixture stayed on the parr shaker for 4 days, monitoring by TLC with fresh catalyst added daily. At the end of the 4 days, the mixture was filtered through a pad of Celite®. The crude oil recovered was subjected to column chromatography (30% acetone/69% toluene/1% acetic acid; $R_f = 0.16$) to give 20 mg (39%) of the title compound. ^{1}H NMR (acetone-d_6, 300 MHz) δ 3.01 (t, 4H, $J = 8.4$ Hz), 3.66 (t, 4H, $J = 8.4$ Hz), 4.89 (s, 2H), 6.64 (dod, 2H, $J = 8.1$ Hz, $J = 1.8$ Hz), 6.78 (d, 2H, $J = 8.1$ Hz), 6.78 (s, 2H), 7.89 (brs, 4H). ^{13}C NMR (acetone-d_6, 75 MHz) δ 27.68, 56.16, 68.77, 116.33, 116.41, 120.71, 130.03, 144.85, 146.10. FT-IR (film): ν 3433 (O–H), 2916 (C–H), 1657 (arom C=C), 1321, 1124 (SO_2). Anal. Calcd for $C_{17}H_{20}O_8S_2$: C, 49.03; H, 4.84. Found: C, 50.62; H, 4.84.

4.2.5. Bis(*trans*-β-6-bromo-3,4-dimethoxy-styrenesulfonyl)methane (7). To a solution of disulfone reagent[10] (300 mg, 0.6 mmol) in THF (3 mL) was added LiBr (156 mg, 1.8 mmol) followed by DIEA (420 μL, 2.4 mmol). Bromoveratraldehyde (441 mg, 1.8 mmol) was then added and mixed overnight. Then approximately 100 μL of acetic acid was added followed by water and ethyl acetate. The ethyl acetate layer was dried and ether was added, and the solution was kept in the freezer overnight to produce a solid which was filtered to give 278 mg (75%) of the title compound. ^{1}H NMR (DMSO-d_6, 600 MHz) 3.75 (s, 6H), 3.81 (s, 6H), 5.49 (s, 2H), 7.16 (s, 2H), 7.25 (s, 2H), 7.40 (d, 2H, $J = 15.3$ Hz), 7.65 (d, 2H, $J = 15.3$ Hz). ^{13}C NMR (acetone-d_6, 150 MHz) 55.75, 56.08, 71.64, 110.32, 115.52, 117.56, 122.98, 126.19, 142.69, 148.45, 152.14. HRFABMS (+) calcd $C_{21}H_{22}O_8S_2Br_2$: 625.9103, found: 625.9131. Anal. Calcd for $C_{21}H_{22}O_8S_2Br_2$ (1.1 H_2O) : C, 39.03; H, 3.78. Found: C, 38.72; H, 3.84.

4.2.6. Bis(*trans*-2-bromo-6-(2-methanesulfonyl-vinyl)-pyridine) (8). The disulfone reagent[10] (182 mg, 0.36 mmol) and 6-bromo-2-pyridine-carboxaldehyde (223 mg, 1.20 mmol) were reacted according to general procedure A. After removal of the solvent, the crude product was subjected to column chromatography (hexanes/ethyl acetate 1:1, $R_f = 0.32$) to yield 112 mg (61%) of the title compound. ^{1}H NMR (acetone-d_6, 400 MHz) δ 5.43 (s, 2H), 7.60 (d, 2H, $J = 15.2$ Hz), 7.63–7.78 (m, 6H), 7.87 (d, 2H, $J = 15.2$ Hz). ^{13}C NMR (acetone-d_6, 100 MHz) δ 72.47, 126.21, 130.94, 131.78, 141.18, 143.03, 143.15, 152.63. FT-IR (KBr): v 3070, 1572, 1432 (C=C str), 1331, 1119 (SO_2 str), 985 (*trans* C=C str). Anal ($C_{15}H_{12}Br_2N_2O_4S_2$) C, H, N.

4.2.7. Bis(*trans*-β-3,4,5-trifluoro-styrenesulfonyl)methane (9). The disulfone reagent[10] (219 mg, 0.44 mmol) was combined with 3,4,5-trifluorobenzaldehyde (0.16 mL, 1.44 mmol) according to general method A. After removal of solvent, the crude material was subjected to column chromatography (8% acetone/92% toluene, $R_f = 0.32$) to yield 149 mg (74%) of the title compound. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 5.56 (s, 2H), 7.48 (d, 2H, $J = 15.5$ Hz), 7.51 (d, 2H, $J = 15.5$ Hz), 7.69 (m, 4H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 71.98, 114.48 (d, $J = 16.5$ Hz), 129.43, 129.71, 141.04 (d, $J = 253.5$ Hz), 142.83, 151.03 (dd, $J = 7.75$ Hz, $J = 247.5$ Hz). FT-IR (KBr): v 3070, 1616, 1535 (C=C str), 1442 (–CH_2– str), 1336, 1149 (SO_2 str), 1247 (Ph–F str), 974 (*trans* C=C str). Anal ($C_{17}H_{10}F_6O_4S_2$): C, H.

4.2.8. Bis(*trans*-β-1-indole-5-styrenesulfonyl)methane (10). 1-(*tert*-Butyloxycarbonyl)-indole-5-carboxaldehyde (202 mg, 0.82 mmol) and the disulfone reagent[10] (138 mg, 0.27 mmol) were reacted according to general procedure A. The resulting crude solid was subjected to column chromatography (30% ethyl acetate/70% hexanes, $R_f = 0.22$) to yield 169 mg (57%) of the desired purified product. ^{1}H NMR ($CDCl_3$, 400 MHz) 1.67 (s, 18H), 4.73 (s, 2H), 6.56 (d, 2H, $J = 3.6$ Hz), 7.20 (d, 2H, $J = 15.6$ Hz), 7.48 (dd, 2H, $J = 8.8$ Hz, $J = 2.0$ Hz), 7.62 (d, 2H, $J = 3.6$ Hz), 7.66 (d, 2H, $J = 1.6$ Hz), 7.76 (d, 2H, $J = 15.6$ Hz), 8.14 (d, 2H, $J = 8.8$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) 28.22, 74.36, 84.56, 107.50, 115.90, 122.55, 122.95, 124.77, 126.35, 127.49, 131.07, 137.21, 147.74, 149.31. The protected diindole (94 mg, 0.15 mmol) was then dissolved in 3 mL of dichloromethane and 0.25 mL of trifluoroacetic acid was added. The mixture was stirred for 3 h at which time the solvent was evaporated and then coevaporated with toluene. Two milliliters of water was then added and the solid was filtered off. The filtered solid was resuspended in 3 mL of water and this was lyophilized to give 56 mg (88%) of the desired deprotected product. ^{1}H NMR (DMSO-d_6, 400 MHz) 5.45 (s, 2H), 6.50 (d, 2H, $J = 3.2$ Hz), 7.22 (d, 2H, $J = 15.6$ Hz), 7.43 (m, 6H), 7.63 (d, 2H, $J = 15.6$ Hz), 7.89, (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz) 72.37, 102.31, 112.22, 121.18, 121.97, 123.15, 123.46, 127.03, 127.86, 137.67, 146.79. FT-IR (KBr): v 2921 (C–H), 1326, 1144 (SO_2), 1199 (C–N), 974 (*trans* C=C). Anal. Calcd for $C_{21}H_{18}N_2O_4S_2$: C, 59.14; H, 4.25. Found: C, 58.30; H, 4.28.

4.2.9. Bis(*trans*-(2-methanesulfonyl-vinyl)-benzene) (11). The disulfone reagent[10] (103 mg, 0.21 mmol) and benzaldehyde (0.07 mL, 0.68 mmol) were combined according to general procedure A. After removal of solvent, the crude material was subjected to column chromatography (40% ethyl acetate/60% hexanes, $R_f = 0.43$) to yield 65 mg (88%) of the title product. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.68 (s, 2H), 7.21 (d, 2H, $J = 15.6$ Hz), 7.38–7.54 (m, 10H), 7.67 (d, 2H, $J = 15.6$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 74.11, 124.38, 129.18, 129.31, 131.77, 132.09, 147.02. FT-IR (film): v 3060, 1612, 1448 (C=C), 1323, 1124 (SO_2 str), 976 (*trans* C=C str). Anal. Calcd for $C_{17}H_{16}O_4S_2$: C, 58.60; H, 4.63. Found: C, 58.21; H, 4.63.

4.2.10. Bis(2-(3,4,5-trifluorophenyl)-ethylsulfonyl)methane (12). The vinyl trifluoro derivative, **9** (106 mg, 0.23 mmol) was dissolved in dry THF and cooled to −78 °C. To this was added 0.58 mL (0.58 mmol) of 1 M $LiEt_3BH$ (Super-Hydride) in THF. The mixture was stirred overnight and was allowed to come to room temperature. The reaction was quenched by pouring into 50 mL of 1 M HCl and then extracted into EtOAc (3× 50 mL). The combined organic fractions were then washed with water and brine. The solvent was evaporated and chromatographed in 30% EtOAc/hexanes ($R_f = 0.16$) to give 78 mg (73%) of the title compound. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.11 (4H, dt, $J = 8.0$ Hz, $J = 2.4$ Hz), 3.71 (4H, dt, $J = 8.0$ Hz, $J = 2.4$ Hz), 5.55 (2H, s), 7.31 (4H, dd, $J = 8.0$ Hz, $J = 2.4$ Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 26.12, 53.99, 67.67, 113.35 (dd, $J_{C\text{–}F2} = 21.2$ Hz, $J_{C\text{–}F3} = 5.3$ Hz), 135.42 (m), 137.60 (dt, $J_{C\text{–}F} = 245.9$ Hz, $J_{C\text{–}F2} = 16.0$ Hz), 150.08 (ddd, $J_{C\text{–}F} = 245.6$ Hz, $J_{C\text{–}F2} = 9.9$ Hz, $J_{C\text{–}F3} = 3.8$ Hz). HRMS calcd $C_{17}H_{14}$- $F_6O_4S_2$ (M+NH_4): 478.05814, found: 478.058410. Anal. Calcd for $C_{17}H_{14}F_6O_4S_2$: C, 44.35; H, 3.06. Found: C, 45.18; H, 2.96.

4.2.11. Bis(*trans*-1-bromo-5-(2-methanesulfonyl-vinyl)-2,3-dimethoxy-benzene) (13). The disulfone reagent[10] (138 mg, 0.28 mmol) of the disulfone reagent (**1**) and 5-bromoveratraldehyde (223 mg, 0.91 mmol) were used according to general method A. Following removal of solvent, 5 mL of ethyl acetate was added from which a white solid precipitated, this was filtered off and rinsed with cold ethyl acetate to give 93 mg (53%) of the title compound (mp = 238 °C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.78 (s, 6H), 3.81 (s, 6H), 5.47 (s, 2H), 7.33 (s, 2H), 7.40 (d, 2H, $J = 16.0$ Hz), 7.46 (d, 2H, $J = 16.0$ Hz), 7.49 (s, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 56.20, 60.23, 71.36, 112.46, 116.96, 125.36, 126.47, 129.40, 143.65, 147.90, 153.33. FT-IR (KBr): *v* 3064, 1616, 1491 (C=C str), 1468 (–CH$_2$– str), 1315, 1124 (SO$_2$ str), 1282, 1047 (Ph–O–C str), 1228 (C–O str), 970 (*trans* C=C str). Anal. ($C_{21}H_{22}Br_2O_8S_2$) C, H.

4.2.12. Bis(*trans*-(2-methanesulfonyl-ethyl)-benzene) (14). Compound **11** (64 mg, 0.18 mmol) was dissolved in 3 mL of ethyl acetate. To this was added 6 mg of palladium (10 wt %) on carbon powder (wet) and allowed to react on the Parr shaker overnight at 60 psi. This mixture was filtered over a pad of Celite® 503 and subjected to column chromatography (ethyl acetate/hexanes 1:2, $R_f = 0.40$) to yield 6 mg (10%) after spilling of some title product. ^{1}H NMR (CDCl$_3$, 400 MHz) δ 3.14 (t, 4H, $J = 8$ Hz), 3.66 (t, 4H, $J = 8$ Hz), 3.98 (s, 2H), 7.22–7.35 (m, 10H). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 28.43, 55.24, 68.33, 127.62, 128.84, 129.24, 136.47. FT-IR (film): *v* 3012 (C=C str), 1456 (–CH$_2$– str), 1311, 1128 (SO$_2$ str). Anal. ($C_{17}H_{20}O_4S_2$) C, H.

4.2.13. Bis(1-benzyloxy-*trans*-2-(2-methanesulfonyl-vinyl)-benzene) (15). The disulfone reagent[10] (201 mg, 0.40 mmol) and 2-benzyloxybenzaldehyde (282 mg, 1.33 mmol) were used according to general method A. Following removal of solvent, the crude solid was purified by column chromatography (30% ethyl acetate/70% hexanes, $R_f = 0.36$) to yield 108 mg (48%) of the title compound as a foamy solid. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 4.56 (s, 2H), 5.17 (s, 4H), 6.98 (d, 2H, $J = 8.1$ Hz), 7.32–7.49 (m, 18H), 7.93 (d, 2H, $J = 15.6$ Hz). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 70.66, 74.18, 112.98, 121.13, 121.28, 125.21, 127.41, 128.32, 128.87, 131.34, 133.28, 136.12, 142.61, 158.25. FT-IR (film): *v* 3064, 1599, 1486 (C=C str), 1452 (–CH$_2$– str), 1317, 1124 (SO$_2$ str), 1250, 1051 (Ph–O–C str), 1223 (C–O str), 984 (*trans* C=C str). Anal. ($C_{31}H_{28}O_6S_2$) C, H.

4.2.14. Bis(1,2-benzyloxy-*trans*-5-(2-ethanesulfonyl-vinyl)-benzene) (16). The disulfone reagent[10] (219 mg, 0.44 mmol) and 3,4-benzyloxybenzaldehyde (419 mg, 1.44 mmol) were reacted according to general method A. After removal of solvent, 4 mL of acetone was added from which a white solid precipitated. This solid was filtered and washed with cold acetone to give 134 mg (40%) of the title compound (mp = 194–195 °C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.07 (s, 4H), 5.14 (s, 4H), 5.42 (s, 2H), 7.05 (d, 2H, $J = 6.3$ Hz), 7.18 (d, 2H, $J = 11.4$ Hz), 7.22 (d, 2H, $J = 11.7$ Hz), 7.32–7.43 (m, 24H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 70.49, 70.65, 72.45, 113.86, 114.23, 124.06, 124.82, 125.65, 127.68–128.60 m, 136.83, 145.30, 148.41, 151.23. FT-IR (KBr): *v* 3064, 1597, (C=C str), 1456 (–CH$_2$– str), 1323, 1140 (SO$_2$ str), 1271, 1022 (Ph–O–C str), 1211 (C–O str), 979 (*trans* C=C str). Anal. Calcd for $C_{45}H_{40}O_8S_2$: C, 69.93; H, 5.22. Found: C, 69.44; H, 5.19.

4.2.15. Bis(*trans*-1,4-dihloro-2-(2-methanesulfonyl-vinyl)-benzene) (17). The disulfone reagent[10] (224 mg, 0.45 mmol) and 2,5-dichlorobenzaldehyde (258 mg, 1.47 mmol) were reacted according to general procedure A. After removal of solvent, 142 mg (65%) of the title compound was precipitated out of 4 mL of acetone and filtered (mp = 246–247 °C). ^{1}H NMR (DMSO-d_6, 400 MHz) 5.68 (s, 2H), 7.50–7.56 (m, 4H), 7.64 (d, 2H, $J = 15.6$ Hz), 7.69 (d, 2H, $J = 15.6$ Hz) δ 7.93 (d, 2H, $J = 2$ Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 71.05, 128.41, 130.47, 131.31, 131.71, 132.50, 132.64, 132.85, 138.74. FT-IR (KBr): *v* 3068, 1608, 1462 (C=C str), 1340, 1149 (SO$_2$ str), 1099 (Ph–Cl str), 970 (*trans* C=C str). Anal. ($C_{17}H_{12}Cl_4O_4S_2$) C, H.

4.2.16. Bis(1,2,3-benzyloxy-*trans*-(2-ethanesulfonyl-vinyl)-benzene) (18). The disulfone reagent[10] (130 mg, 0.26 mmol) and 3,4,5-tribenzyloxybenzaldehyde were used according to general method A. After removal of the solvent, 4 mL of acetone was added to the pale yellow oil and a white solid precipitated. This solid was filtered and washed with cold acetone to give 159 mg (62%) of the title compound (mp = 216 °C). ^{1}H NMR (acetone-d_6, 300 MHz) δ 4.94 (s, 4H), 5.09 (s, 8H), 5.50 (s, 2H), 7.18–7.47 (m, 38H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 70.48, 70.78, 74.54, 108.22, 127.72–128.76 (m), 136.82, 136.98, 137.61, 140.01, 145.93, 152.07, 152.53. FT-IR (KBr): *v* 3064, 1614, 1504 (C=C str), 1454 (–CH$_2$– str), 1329, 1122 (SO$_2$ str), 1248 (Ph–O–C str), 972 (*trans* C=C str). Anal. ($C_{59}H_{52}O_{10}S_2$) C, H.

4.2.17. Bis(6-(2-methanesulfonyl-vinyl)-2,3-dihydro-benzo[1,4]dioxine) (19). The disulfone reagent[10] (221 mg, 0.44 mmol) and 1,4-benzodioxan-6-carboxaldehyde (240 mg, 1.45 mmol) were reacted according to general method A. Following solvent removal, the crude mixture was subjected to column chromatography (8% acetone/92% toluene, $R_f = 0.26$) to yield 86 mg (42%) of the title compound. ^{1}H NMR (CDCl$_3$, 500 MHz) δ 4.29 (d$_{app}$, 8H, $J = 13$ Hz), 4.64 (s, 2H), 6.88 (d, 2H, $J = 8.5$ Hz), 7.00 (d, 2H, $J = 15.5$ Hz), 7.05 (m, 4H), 7.53 (d, 2H, $J = 15.5$ Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 63.88, 64.40, 71.74, 117.39, 117.43, 122.81, 123.69, 125.25, 143.37, 144.40, 146.21. FT-IR (film): *v* 3060, 1600, 1510 (C=C str), 1325, 1122 (SO$_2$ str), 1292, 1066 (Ph–O–C str), 976 (*trans* C=C str). Anal. ($C_{21}H_{20}O_8S_2$) C, H.

4.2.18. Bis(4-bromo-2-(2-methanesulfonyl-vinyl)-1-methoxy-benzene) (20). The disulfone reagent[10] (175 mg, 0.35 mmol) and 5-bromo-2-methoxybenzaldehyde (248 mg, 1.15 mmol) were reacted according to general procedure A. After removal of the solvent, 188 mg (95%) of the title compound was precipitated from 5 mL of dichloromethane (mp = 282 °C). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.82 (s, 6H), 5.50 (s, 2H), 7.02 (d, 2H, $J = 8.7$ Hz), 7.43 (d, 2H, $J = 15.6$ Hz), 7.54 (d, 2H, $J = 15.6$ Hz), 7.57 (dd, 4H, $J = 8.7$ Hz, $J = 2.4$ Hz), 7.79 (d, 2H, $J = 2.4$ Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 56.15, 71.23, 112.27, 114.13, 122.24, 127.71, 132.03, 135.31, 138.20, 157.44. FT-IR (KBr): ν 3091, 1614, 1487 (C=C str), 1462 (–CH_2– str), 1317, 1124 (SO_2 str), 1257 (Ph–O–C str), 1217 (C–O str), 1012 (Ph–Br str), 976 (*trans* C=C str). Anal. ($C_{19}H_{18}Br_2O_6S_2$) C, H.

Elemental analysis:

Compound	Calculated	Found
2	$C_{21}H_{20}O_8S_2$: C, 54.30; H, 4.34	C, 53.52; H, 4.79
3	$C_{19}H_{16}O_8S_2$ ($0.5H_2O$): C, 51.23; H, 3.85	C, 51.27; H, 3.65
4	$C_{23}H_{28}O_{10}S_2$ ($0.5H_2O$): C, 51.39; H, 5.44	C, 51.49; H, 5.06
5	$C_{17}H_{20}O_8S_2$: C, 49.03; H, 4.84	C, 50.62; H, 4.84
6	$C_{21}H_{22}O_{12}S_2N_2$ ($1H_2O$): C, 43.75; H, 4.20; N, 4.86	C, 43.88; H, 3.89, N, 4.65
7	$C_{15}H_{12}Br_2N_2O_4S_2$(0.6 acetone): C, 37.60; H, 2.76; N, 5.16	C, 37.42; H, 2.59; N, 5.24
8	$C_{21}H_{22}O_8S_2Br_2$ ($1.1H_2O$): C, 39.03; H, 3.78	C, 38.72; H, 3.84
9	$C_{21}H_{18}N_2O_4S_2$: C, 59.14; H, 4.25	C, 58.30; H, 4.28
10	$C_{17}H_{10}F_6O_4S_2$: C, 44.74; H, 2.21	C, 44.63; H, 2.23
11	$C_{17}H_{14}F_6O_4S_2$: C, 44.35; H, 3.06	C, 45.18; H, 2.96
12	$C_{17}H_{16}O_4S_2$: C, 58.60; H, 4.63	C, 58.21; H, 4.63
13	$C_{17}H_{20}O_4S_2$(1.5 acetone): C, 60.12; H, 6.59	C, 60.26; H, 6.48
14	$C_{21}H_{22}Br_2O_8S_2$: C, 40.27; H, 3.54	C, 40.23; H, 3.51
15	$C_{45}H_{40}O_8S_2$: C, 69.93; H, 5.22	C, 69.44;, H, 5.19
16	$C_{31}H_{28}O_6S_2$: C, 66.41; H, 5.03	C, 66.81; H, 5.05
17	$C_{59}H_{52}O_{10}S_2$: C, 71.93; H, 5.32	C, 71.61; H, 5.34
18	$C_{17}H_{12}Cl_4O_4S_2$(0.5 DMSO): C, 41.16; H, 2.48	C, 41.29; H, 2.38
19	$C_{21}H_{20}O_{10}S_2$: C, 50.80; H, 4.06	C, 50.63; H, 4.39
20	$C_{21}H_{20}O_8S_2$: C, 54.30; H, 4.34	C, 54.24, H, 4.32
21	$C_{19}H_{20}O_6S_2$: C, 51.81; H, 4.58	C, 52.04; H, 4.60
22	$C_{19}H_{18}Br_2O_6S_2$($0.5H_2O$): C, 39.67; H, 3.30	C, 39.75; H, 3.34

4.3. Purified enzyme assays

4.3.1. Biological materials, chemicals, and enzymes. All compounds were dissolved in DMSO and the stock solutions were stored at −20 °C. The $\gamma[^{32}P]$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.

4.3.2. Preparation of oligonucleotide substrates. The oligonucleotides 21top, 5′-GTGTGGAAAATCTCTAG CAGT-3′ and 21bot, 5′-ACTGCTAGAGATTT TCCACAC-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 $\gamma\ [^{32}P]$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 mini-column (USA Scientific) to separate annealed double-stranded oligonucleotide from unincorporated material.

4.3.3. 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, and 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:

$$\%\ I = 100 \times [1 - (D - C)/(N - C)]$$

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 IC_{50} values were determined by plotting the logarithm of drug concentration versus percent inhibition to obtain concentration that produced 50% inhibition.

4.4. Cell-based assays

4.4.1. Focal infectivity assay. Drug-susceptibility of HIV-1 to inhibitors was determined with a focal infectivity assay (FIA) as previously described.[11,12] Immunostaining was performed using the monoclonal antibody 22-6[12] at a 1/800 dilution followed by horseradish peroxidase-labeled goat anti-mouse (Kirkegaard and Perry Labs) at 1/1000 dilution. Foci were counted under a dissecting microscope at 30–100× 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 with six determinations per experiment.

4.4.2. Cytotoxicity. Toxicity 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 (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.

Acknowledgment

This work was supported by the National Institutes of Health (GM60917), University of California AIDS Research Program (D02D400).

References and notes

1. www.unaids.org.
2. Duwe, S.; Brunn, M.; Altmann, D.; Hamouda, O.; Schmidt, B.; Walter, H.; Pauli, G.; Kucherer, C. *J. Acquir. Immune Defic. Syndr.* **2001**, *26*, 266.
3. Ammaranond, P.; Cunningham, P.; Oelrichs, R.; Suzuki, K.; Harris, C.; Leas, L.; Grulich, A.; Cooper, D. A.; Kelleher, A. D. *J. Clin. Virol.* **2003**, *26*, 153.
4. Craigie, R. *J. Biol. Chem.* **2001**, *276*, 23213.
5. Pommier, Y.; Marchand, C.; Neamati, N. *Antiviral Res.* **2000**, *47*, 139.
6. Robinson, W. E., Jr.; Reinecke, M. G.; Abdel-Malek, S.; Jia, Q.; Chow, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6326.
7. 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. *Mol. Pharmacol.* **2000**, *58*, 641.
8. Meadows, D. C.; Matthews, T. B.; North, T. W.; Hadd, M. J.; Kuo, C. L.; Neamati, N.; Gervay-Hague, J. *J. Med. Chem.* **2005**, *48*, 4526.
9. Hadd, M. J.; Smith, M. A.; Gervay-Hague, J. *Tetrahedron Lett.* **2001**, *42*, 5137.
10. Gervay-Hague, J.; Hadd, M. J. US Patent No. 60/138,986.
11. Murry, J. P.; Higgins, J.; Matthews, T. B.; Huang, V. Y.; Van Rompay, K. K. A.; Pedersen, N. C.; North, T. W. *J. Virol.* **2003**, *77*, 1120.
12. Giuffre, A. C.; Higgins, J.; Buckheit, R. W., Jr.; North, T. W. *Antimicrob. Agents Chemother.* **2003**, *47*, 1756.
13. Gaukroger, K.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; McGown, A. T. *J. Org. Chem.* **2001**, *66*, 8135.
14. Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200.
15. Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 257.
16. Roush, W. R.; Gwaltney, S. L., II; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.* **1998**, *120*, 10994.
17. Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Bromme, D. *J. Med. Chem.* **1995**, *38*, 3193.