Chemical Modification of Coumarin Dimer and HIV-1 Integrase Inhibitory Activity

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A systematic series of chemically modified coumarin dimmers has been synthesized and tested for their inhibitory activity against HIV-1 integrase. We observed that modified coumarin dimmers containing hydrophobic moiety on the linker display potent inhibitory activities.

Key words chemical modification; coumarin dimmer; HIV-1 integrase; inhibitor

Integration of reversely transcribed viral DNA of the human immunodeficiency virus type 1 into chromosomal DNA of newly infected cells is an essential step of the viral replicative cycle. HIV-1 integrase (HIV-1 IN) is responsible for the integration. This enzyme catalyzes two reactions referred to as 3′-processing (cleavage of a dinucleotide from each end of viral DNA) and strand transfer (insertion of the viral DNA into the host cellular DNA).[1—3] Because the integration process is essential for the replication of HIV and the enzyme appears to be absent in the mammalian host, HIV-1 IN represents a potential target for the development of nontoxic antiviral therapeutic agents.[4,5] Systematic screening of potential inhibitors has been undertaken using mostly purified integrase-based assays. From such screens several integrase inhibitor classes have now been identified.[6—10] The tetrameric 4-hydroxycoumarin (NSC 158393, **I**) was first reported against HIV-1 IN by Mazumder *et al.*[11] with potent inhibition of both 3′-processing (IC_{50}=1.5 μM) and strand transfer (IC_{50}=0.8 μM). In order to delineate structural features of NSC 158393 necessary for high inhibitory activity, Zhao *et al.*[12] disclosed the minimum active pharmacophore (**II**), *i.e.* a coumarin dimmer containing an aryl substituent on the central linker methylene. We also recently reported 4-hydroxycoumarin dimmers bearing aniline mustard moiety (**III**)[13] and reasoned the mustard moiety might react with nearby nucleophile of the coumarin–IN complex binding site to form a covalent bonding and to improve the inhibitory activity. However, these compounds seem to fail forming covalent binding at coumarin–IN complex binding site because the compounds with the appending of aniline mustard did not markedly increase the inhibitory activity. This finding indicates a probably non-nucleophile or hydrophobic region at the coumarin–IN complex binding site. Therefore we pay our efforts on the synthesis of coumarin dimmer appending with hydrophobic moiety (**IV**). In this report, we describe the synthesis of chemically modified coumarin dimmer analogues and their inhibitory activity on HIV-1 IN.

Chemistry

The synthetic route to the target compounds (**2**), (**4a**—**f**) and (**6a**—**e**) are outlined in Chart 1. Starting 4-hydroxybenzaldehyde was transformed to their corresponding 4-(2-hydroxyethoxy)benzaldehyde (**1**), alkyl- and aryl-sulfonyloxybenzaldehyde (**3a**—**f**), and arylcarbonyloxybenzaldehyde (**3a**—**e**) by using 2-chloroethanol, alkyl- and aryl-sulfonyl chloride, and 4-substituted benzoyl chloride, respectively. Subsequent coupling with 4-hydroxycoumarin provided target compounds (**2**), (**4a**—**f**) and (**6a**—**e**) in moderate to high yield.

Results and Discussion

HIV-1 IN inhibition assays were carried out in the presence of low amounts of purified recombinant integrase (50 nM) in the presence of 7.5 mM Mg^{2+} as the cationic cofactor, using 21-mer double-strand oligonucleotide substrate. The compounds were screened for their inhibitory activity in a 3′-processing assay which was performed as described previously.[14] Effects against the strand transfer activity were evaluated during the same assay from the homologous integration events and found to be no significant differences from 3′-processing inhibition, thus confirming that coumarins affect equally both steps of the integration reaction. The inhibitory effects of 3′-processing are summarized in Table 1. All compounds tested were found to have good to potent HIV-1 IN inhibitory activity against the recombinant wild-type enzyme. The inhibitory activity of NSC 158393 (**I**) and **II** were included for a comparison. On the basis of their structure, the compounds tested can be roughly classified as (A), compounds with aryl-substituted attached on the benzoyloxy- or sulfonyloxy-linker (**4b**—**f**, **6a**—**e**) and (B), compounds without aryl-substituted attached on the linker (**2**, **4a**). Class A compounds exhibited potent inhibitory activity with IC_{50} value in the range of 0.5—3.9 μM. Their inhibitory activities were superior to **II** and comparable to NSC 158393 (I). However, Class B compounds showed moderate to non inhibitory activity with IC_{50} value 23.7 and >100 μM, respectively. It is of interest to note: First that compounds with an aryl-substituted linker (**4b**—**f**, **6a**—**e**) possessed higher inhibitory activity than compounds without this substitution (**2**, **4a**); And second, that a benzoyloxy- or sulfonyloxy-linker seemed necessary for the inhibitory activity since compounds **2** devoid of benzoyloxy or sulfonyloxy linkage did not show any activity. Based on this observation, we postulate that an interaction formed between a hydrophobic surface located in the close vicinity of the coumarin–IN complex binding site and the aromatic moiety linked to coumarin

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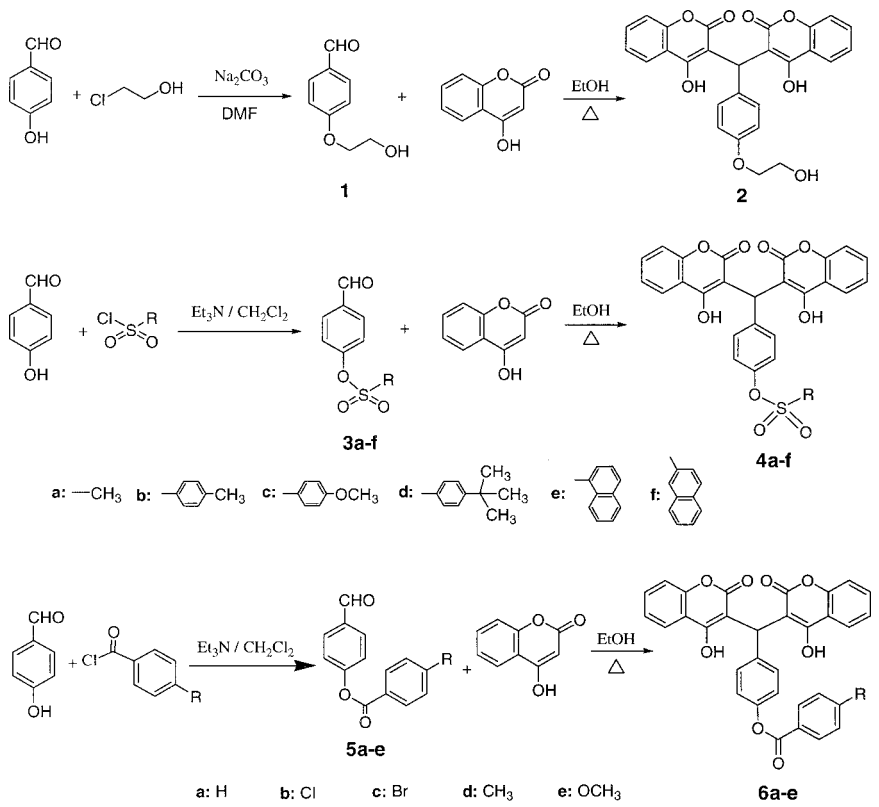

Chart 1

Table 1. HIV-1 Integrase Inhibitory Activities of Modified Coumarin Dimmers

Entry	Compound	Yield (%)	3′-Processing (IC_{50} μM)
1	**2**	66	>100.0
2	**4a**	81	23.7
3	**4b**	83	3.9
4	**4c**	66	1.9
5	**4d**	57	3.1
6	**4e**	56	1.5
7	**4f**	56	2.1
8	**6a**	67	2.6
9	**6b**	70	2.9
10	**6c**	68	1.1
11	**6d**	64	0.5
12	**6e**	60	3.1
13	NSC 158393 (**I**)		1.1
14	(**II**)		43.0

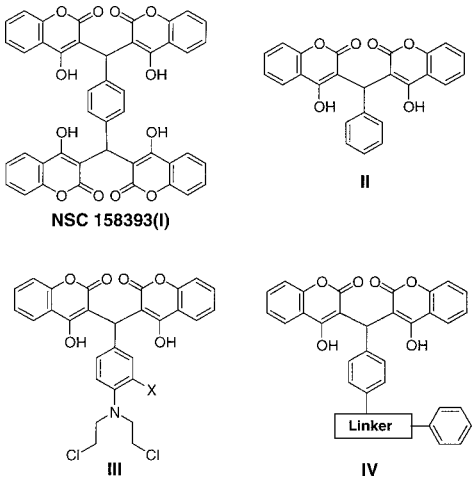

Fig. 1

molecule is important for strengthening the anchorage of the active compound. However, this assumption needs further verification.

In conclusion, twelve new chemically modified coumarin dimmer analogues have been synthesized. Among these easily available coumarin dimmers, ten were found to be active molecules against HIV-1 IN. These active molecules all contain hydrophobic moiety on the linker, which might have some contribution on the inhibitory activity. This finding may be useful in future studies concerned with the development of potent new HIV-1 IN inhibitors.

Experimental

General Melting points (mp) were taken on a BUCHI 530 apparatus and are uncorrected. Merck Art No105554 plates precoated with Silica gel 60 containing fluorescent indicator were used for thin-layer chromatography, and Silica gel 60 (Merck Art No 109385, 230—400 mesh) was employed for column chromatography. Evaporations were carried out at <50 °C using a rotary evaporator at reduced pressure (water aspirator). ^{1}H- and ^{13}C-NMR spectra were obtained at Varian 300 NMR spectrometer at 300 and 75 MHz, respectively. Where necessary, deuterium exchange experiments were used to obtained proton shift assignments. Mass spectra were recorded on a JEOL J.M.S-300 spectrophotometer. Analytical samples were dried under reduced pressure at 78 °C in the presence of P_2O_5 for at least 12 h unless otherwise specified. Elemental analyses were obtained from Perkin-Elmer 2400 Elemental Analyzer.

4-(2-Hydroxyethoxy)-benzaldehyde[15] (1) The reaction mixture of 2-chloroethanol (3.3 g, 41 mmol), 4-hydroxybenzaldehyde (5.0 g, 41 mmol), sodium carbonate (5.9 g, 56 mmol) and DMF (15 ml) was heated under reflux for 24 h. After cooling, the mixture was poured into water. Extraction with ethyl acetate, drying with magnesium sulfate the mixture was concentrated under reduced pressure. Column chromatography of the residue on silica gel with 1 : 1 *n*-hexane/EtOAc as eluent gave **1** (4.4 g, 65%) as a light brown oil. *Rf* 0.45 (*n*-hexane/EtOAc=1/1). ^{1}H-NMR ($CDCl_3$) δ: 3.79—4.25 (m, 4H, CH_2), 6.96 (d, 2H, J=8.5 Hz), 7.77 (d, 2H, J=8.5 Hz), 9.81 (s, 1H, CHO). MS *m/z*: 166 (M^+). *Anal.* Calcd for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 64.96; H, 6.20.

Sulfonic Acid 4-Formylphenyl Ester (3a—f). General Procedure Freshly distilled methanesulfonyl chloride (2.33 g, 20 mmol) was added to a solution of 4-hydroxybenzaldehyde (1.24 g, 10 mmol) in dry pyridine (10 ml) under ice bath. The reaction mixture was stirred at room temperature for 4 h and then poured into 10% HCl solution. Extraction with ethyl acetate, washing with saturated sodium bicarbonate and brine, drying with magnesium sulfate the mixture was concentrated under reduced pressure. Column chromatography of the residue on silica gel with 1 : 1 *n*-hexane/EtOAc as eluent gave **3a** (1.3 g, 64%) as a light brown oil.

3b—**f** were obtained under the same condition.

Methanelsulfonic Acid 4-Formylphenyl Ester (**3a**): *Rf* 0.55 (*n*-hexane/EtOAc=1/1). mp: 62—63 °C (lit.[16] 60—61 °C). ^{1}H-NMR ($CDCl_3$) δ: 3.22 (s, 3H, CH_3), 7.46, 7.76 (d, 2H each, J=8.5 Hz, Ar), 10.0 (s, 1H, CHO). MS *m/z*: 200 (M^+). *Anal.* Calcd for $C_8H_8O_4S$: C, 47.99; H, 4.03. Found: C, 48.02; H, 4.20.

Toluenesulfonic Acid 4-Formylphenyl Ester (**3b**): *Rf* 0.67 (*n*-hexane/EtOAc=1/1). mp: 76—77 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H, CH_3), 7.15—7.84 (m, 8H, Ar-H), 9.96 (s, 1H, CHO). MS *m/z*: 276 (M^+). *Anal.* Calcd for $C_{14}H_{12}O_4S$: C, 60.86; H, 4.38. Found: C, 60.62; H, 4.20.

4-Methoxybenzenesulfonic Acid 4-Formylphenyl Ester (**3c**): *Rf* 0.70 (*n*-hexane/EtOAc=1/1). mp: 86—87 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.89 (s, 3H, OCH_3), 6.97—7.85 (m, 8H, Ar-H), 9.97 (s, 1H, CHO). MS *m/z*: 292 (M^+). *Anal.* Calcd for $C_{14}H_{12}O_5S$: C, 57.52; H, 4.14. Found: C, 57.62; H, 4.27.

4-*tert*-Butylbenzenesulfonic Acid 4-Formylphenyl Ester (**3d**): *Rf* 0.63 (*n*-hexane/EtOAc=1/1). mp: 115—116 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (s, 9H, CH_3), 7.19—7.87 (m, 8H, Ar-H), 9.98 (s, 1H, CHO). MS *m/z*: 318 (M^+). *Anal.* Calcd for $C_{17}H_{18}O_4S$: C, 64.13; H, 5.70. Found: C, 63.98; H, 5.57.

1-Naphthalenesulfonic Acid 4-formylphenyl Ester (**3e**): *Rf* 0.54 (*n*-hexane/EtOAc=1/1). mp: 123—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.69—8.20 (m, 11H, Ar-H), 9.92 (s, 1H, CHO). MS *m/z*: 312 (M^+). *Anal.* Calcd for $C_{17}H_{12}O_4S$: C, 65.37; H, 3.87. Found: C, 65.68; H, 3.57.

2-Naphthalenesulfonic Acid 4-Formylphenyl Ester (**3f**): *Rf* 0.46 (*n*-hexane/EtOAc=1/1). mp: 83—84 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.16—8.01 (m, 9H, Ar-H), 8.37 (s, 2H, Ar-H), 9.92 (s, 1H, CHO). MS *m/z*: 312 (M^+). *Anal.* Calcd for $C_{17}H_{12}O_4S$: C, 65.37; H, 3.87. Found: C, 65.44; H, 3.66.

Benzoic Acid 4-Formylphenyl Ester (5a—e). General Procedure Benzoyl chloride (2.8 g; 20 mmol) was added to a solution of 4-hydroxybenzaldehyde (1.24 g, 10 mmol) in dry pyridine (20 ml) under ice bath. The reaction mixture was stirred at room temperature for 8 h. Extraction with ethyl acetate, drying with magnesium sulfate the mixture was concentrated under reduced pressure. Column chromatography of the residue on silica gel with 1 : 1 *n*-hexane/EtOAc as eluent gave **5a** (1.45 g, 64%) as a yellow crystal.

5b—**e** were obtained under the same condition.

Benzoic Acid 4-Formylphenyl Ester (**5a**): *Rf* 0.46 (*n*-hexane/EtOAc=1/1). mp: 119—120 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.23—8.49 (m, 9H, Ar-H), 9.98 (s, 1H, CHO). MS *m/z*: 226 (M^+). *Anal.* Calcd for $C_{14}H_{10}O_3$: C, 74.33; H, 4.46. Found: C, 74.64; H, 4.46.

4-Chlorobenzoic Acid 4-Formylphenyl Ester (**5b**): *Rf* 0.38 (*n*-hexane/EtOAc=4/1). mp: 104—105 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.39—8.14 (m, 8H, Ar-H), 10.02 (s, 1H, CHO). MS *m/z*: 260 (M^+). *Anal.* Calcd for $C_{14}H_9ClO_3$: C, 64.51; H, 3.48. Found: C, 64.64; H, 3.46.

4-Bromobenzoic Acid 4-Formylphenyl Ester (**5c**): *Rf* 0.56 (*n*-hexane/EtOAc=1/1). mp: 176—177 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.33—7.99 (m, 8H, Ar-H), 9.96 (s, 1H, CHO). MS *m/z*: 305 (M^+). *Anal.* Calcd for $C_{14}H_9BrO_3$: C, 55.11; H, 2.97. Found: C, 55.44; H, 3.05.

4-Methylbenzoic Acid 4-Formylphenyl Ester (**5d**): *Rf* 0.35 (*n*-hexane/EtOAc=4/1). mp: 74—75 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.45 (s, 3H, CH_3), 7.26—8.09 (m, 8H, Ar-H), 10.01 (s, 1H, CHO). MS *m/z*: 240 (M^+). *Anal.* Calcd for $C_{15}H_{12}O_3$: C, 74.99; H, 5.03. Found: C, 74.64; H, 5.24.

4-Methoxybenzoic Acid 4-Formylphenyl Ester (**5e**): *Rf* 0.25 (*n*-hexane/EtOAc=4/1). mp: 106—107 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.90 (s, 3H, OCH_3), 6.98—8.13 (m, 8H, Ar-H), 10.01 (s, 1H, CHO). MS *m/z*: 256 (M^+). *Anal.* Calcd for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72. Found: C, 70.14; H, 5.00.

Condensation of Benzaldehydes with 4-Hydroxycoumarin (2, 4a—f, 6a—e).[17,18] General Procedure The reaction mixture of 4-hydroxycoumarin (2 eq), benzaldehyde (1 eq) in ethanol was heated under reflux for 18 h. The mixture was concentrated under reduced pressure to furnished product. Pure products were obtained under recrystalliztion with certain solvents.

3,3′-[4-(2-Hydroxyethoxy)benzylidene]bis(4-hydroxy-coumarin) (**2**): *Rf* 0.16 (*n*-hexane/EtOAc=1/4). mp: 176—177 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 3.69, 3.91 (m, 2H each, OCH_2CH_2OH), 6.28 (s, 1H, CH), 6.78—7.91 (m, 12H, Ar-H). MS *m/z*: 472 (M^+). *Anal.* Calcd for $C_{27}H_{20}O_8$: C, 68.64; H, 4.27. Found: C, 68.49; H, 4.51.

3,3′-[4-[(Methylsulfonyl)oxy]benzylidene]bis(4-hydroxy-coumarin) (**4a**): *Rf* 0.32 (*n*-hexane/EtOAc=1/4). mp: 235—236 °C (Actone). ^{1}H-NMR (DMSO-d_6) δ: 3.34 (s, 3H, CH_3), 6.30 (s, 1H, CH), 7.20—7.90 (m, H, Ar-H). MS *m/z*: 506 (M^+). *Anal.* Calcd for $C_{26}H_{18}O_9S$: C, 61.66; H, 3.58. Found: C, 61.33; H, 3.65.

3,3′-[4-[[(4-Methylphenyl)sulfonyl]oxy]benzylidene]bis(4-hydroxy-coumarin) (**4b**): *Rf* 0.34 (*n*-hexane/EtOAc=1/4). mp: 240—241 °C (Actone). ^{1}H-NMR (DMSO-d_6) δ: 2.39 (s, 3H, CH_3), 6.27 (s, 1H, CH), 6.83—7.87 (m, 16H, Ar-H). MS *m/z*: 582 (M^+). *Anal.* Calcd for $C_{32}H_{22}O_9S$: C, 65.97; H, 3.81. Found: C, 66.01; H, 3.82.

3,3′-[4-[[(4-Methoxyphenyl)sulfonyl]oxy]benzylidene]bis(4-hydroxy-coumarin) (**4c**): *Rf* 0.41 (*n*-hexane/EtOAc=1/4). mp: 246—247 °C (Actone). ^{1}H-NMR (DMSO-d_6) δ: 3.89 (s, 3H, OCH_3), 6.05 (s, 1H, CH), 6.92—8.09 (m, 16H, Ar-H). MS *m/z*: 598 (M^+). *Anal.* Calcd for $C_{32}H_{22}O_{10}S$: C, 64.21; H, 3.70. Found: C, 64.26; H, 3.60.

3,3′-[4-[[(4-*tert*-Butylphenyl)sulfonyl]oxy]benzylidene]bis(4-hydroxy-coumarin) (**4d**): *Rf* 0.27 (*n*-hexane/EtOAc=1/4). mp: 149—150 °C (Actone). ^{1}H-NMR (DMSO-d_6) δ: 1.26 (s, 9H, CH_3), 6.34 (s, 1H, CH), 6.89—7.92 (m, 16H, Ar-H). MS *m/z*: 624 (M^+). *Anal.* Calcd for $C_{35}H_{28}O_9S$: C, 67.30; H, 4.52. Found: C, 67.03; H, 4.76.

3,3′-[4-[[(1-Naphthalyl)sulfonyl]oxy]benzylidene]bis(4-hydroxy-coumarin) (**4e**): *Rf* 0.21 (*n*-hexane/EtOAc=1/4). mp: >300 °C (Actone). ^{1}H-NMR (DMSO-d_6) δ: 6.20 (s, 1H, CH), 6.69—8.65 (m, 19H, Ar-H). MS *m/z*: 618 (M^+). *Anal.* Calcd for $C_{35}H_{22}O_9S$: C, 67.95; H, 3.58. Found: C, 68.02; H, 3.58.

3,3′-[4-[[(2-Naphthalyl)sulfonyl]oxy]benzylidene]bis(4-hydroxy-coumarin) (**4f**): *Rf* 0.30 (*n*-hexane/EtOAc=1/1). mp: 230—232 °C (Actone). ^{1}H-NMR (DMSO-d_6) δ: 6.29 (s, 1H, CH), 6.88—8.54 (m, 19H, Ar-H). MS *m/z*: 618 (M^+). *Anal.* Calcd for $C_{35}H_{22}O_9S$: C, 67.95; H, 3.58. Found: C, 67.75; H, 3.57.

3,3′-[4-(Benzoyloxy)benzylidene]bis(4-hydroxy-coumarin) (**6a**): *Rf* 0.43 (*n*-hexane/EtOAc=1/4). mp: 200—201 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 6.37 (s, 1H, CH), 7.12—8.14 (m, 17H, Ar-H). MS *m/z*: 532 (M^+). *Anal.* Calcd for $C_{32}H_{20}O_8$: C, 72.18; H, 3.79. Found: C, 72.16; H, 3.65.

3,3′-[4-(4-Chlorobenzoyloxy)benzylidene]bis(4-hydroxy-coumarin) (**6b**): *Rf* 0.21 (*n*-hexane/EtOAc=1/4). mp: 147—148 °C (Acetone). ^{1}H-NMR (DMSO-d_6) δ: 6.41 (s, 1H, CH), 7.14—8.12 (m, 16H, Ar-H). MS *m/z*: 567 (M^+). *Anal.* Calcd for $C_{32}H_{19}ClO_8$: C, 67.79; H, 3.38. Found: C, 67.49; H, 3.61.

3,3′-[4-(4-Bromobenzoyloxy)benzylidene]bis(4-hydroxy-coumarin) (**6c**): *Rf* 0.18 (*n*-hexane/EtOAc=1/4). mp: 159—160 °C (Acetone). ^{1}H-NMR (DMSO-d_6) δ: 6.38 (s, 1H, CH), 7.14—8.06 (m, H, Ar-H). MS *m/z*: 611 (M^+). *Anal.* Calcd for $C_{32}H_{19}BrO_8$: C, 62.86; H, 3.13. Found: C, 63.02; H, 3.44.

3,3′-[4-(4-Methylbenzoyloxy)benzylidene]bis(4-hydroxy-coumarin) (**6d**): *Rf* 0.26 (*n*-Hexane/EtOAc=1/4). mp: 164—165 °C (Acetone). ^{1}H-NMR (DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 6.32 (s, 1H, CH), 7.09—8.02 (m, 16H, Ar-H). MS *m/z*: 546 (M^+). *Anal.* Calcd for $C_{33}H_{22}O_8$: C, 72.52; H, 4.06. Found: C, 72.59; H, 4.40.

3,3′-[4-(4-Methoxbenzoyloxy)benzylidene]bis(4-hydroxy-coumarin) (**6e**): *Rf* 0.48 (*n*-hexane/EtOAc=1/4). mp: 159—160 °C (Acetone). ^{1}H-NMR (DMSO-d_6) δ: 3.85 (s, 3H, CH_3), 6.37 (s, 1H, CH), 7.08—8.05 (m, 16H, Ar-H). MS *m/z*: 562 (M^+). *Anal.* Calcd for $C_{33}H_{22}O_9$: C, 70.46; H, 3.94. Found: C, 70.28; H, 4.16.

HIV-1 Integrase Inhibitory Assay. Oligonucleotides Oligonucleotides were purchased from Eurogentec and further purified on 18% acrylamide/urea denaturing gel. U5B: GTGTGGAAAATCTCTAGCA; U5B-2: GTGTGGAAAATCTCTAG; U5A: 5′-ACTGCTAGAGATTTTCCACAC; ST1: AGTGAATTAGCCCTTGGTCA-biotine; ST2: 5′-TGACCAAGGGCTAATTCACT-biotine; U5B and U5B-2 were radiolabeled using T4 polynucleotide kinase for respectively 3′-processing and strand transfer reactions.

HIV-1 Integrase Assays Wild-type HIV-1 integrase was purified as described previously.[19] 3′-Processing assay was performed in a reaction vol-

ume of 20 μl containing 0.025 pmol of labeled U5A/U5B double-stranded DNA substrate and 1 pmol of integrase in buffer A [20 mM Hepes (pH 7.2), 10 mM $MgCl_2$, 25 mM NaCl, 1 mM DTT]. Products were separated on a 18% acrylamide/urea denaturing gel and quantified on a phosphoimager using ImageQuant software (AmershamPharmaciaBiotech). Strand transfer reactions were performed in triplicate in 96-well plates using 0.25 pmol of labeled U5A/U5B-2 double-stranded DNA substrate, 12 pmol of ST1/ST2 3′-biotinylated target DNA and 2 pmol of integrase in buffer A in a final volume of 40 μl. Radiolabeled reaction products were bound to Streptavidin-coated magnetic beads (DynaL), washed twice in buffer B (PBS buffer supplemented with 0.025% tween 20 and 10 μg/ml BSA) and quantified on a beta radiation counter. Inhibition in the presence of drugs is expressed as the fractional product in percent of the control without drug.

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