Synthesis and HIV-1 Integrase Inhibition of Novel Bis- or Tetra-Coumarin Analogues

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Present studies were undertaken on the preparation of synthetic analogues of bis- or tetra-coumarins and their activity against HIV-1 integrase (HIV-1 IN). Among these coumarin analogues, compounds 14, 16 and 18 were found to be potent molecules against HIV-1 IN at IC_{50} values of 0.96, 0.58, and 0.49 μM, respectively. The results provided a tool for guiding the further design of more potent antiviral agents and for predicting the affinity of related compounds.

Key words bis-coumarin; tetra-coumarin; human immuno-deficiency virus-1 integrase; inhibitor

Acquired immuno-deficiency syndrome (AIDS) is a major pandemic caused by human immuno-deficiency virus (HIV) and considerable efforts are being completed for years to build up drugs against the outrageous disease. The HIV genome encodes for the protease, reverse transcriptase and integrase enzymes. Combination antiviral therapy with protease and reverse transcriptase inhibitors has established the potential therapeutic usefulness of antiviral therapy for treatment for AIDS.[1)] However, the capability of HIV to rapidly develop drug resistance, together with toxicity problems, requires the expansion of additional classes of antiviral drugs. Integrase is an attractive target for antivirals[2—4)] because it is vital for HIV replication and, unlike protease and reverse transcriptase, there are no known counterparts in the host cell. Integrase catalyzes two reactions; 3′-end processing, in which two deoxynucleotides are removed from the 3′ ends of the viral DNA and the strand transfer reaction, in which the processed 3′ ends of the viral DNA are covalently ligated to the host chromosomal DNA.[5—7)] Integration of the proviral DNA is a key step in allowing viral DNA to become permanently inserted into the host genome. This step is essential for the subsequent transcription of the viral genome which leads to production of new viral genomic RNA and viral proteins needed for the production of the next round of infectious virus. Essentially, furthermore, integrase uses a single active site to hold two different configurations of DNA substrates, which may restrict the ability of HIV to develop drug resistance to integrase inhibitors. 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.[8—12)] One of them, Raltegravir (MK-0518) was recently shown to be active in patients thereby demonstrating the potential of anti-integrase agents.[13,14)] The tetrameric 4-hydroxycoumarin (NSC 158393, I) was first descripted against HIV-1 integrase (HIV-1 IN) by Mazumder *et al.*[15)] with potent inhibition of both 3′-processing (IC_{50}=1.5 μM) and strand transfer (IC_{50}=0.8 μM). They also delineated the minimum active pharmacophore of NSC 158393 necessary for inhibitory activity,[16)] *i.e.*, a coumarin dimmer containing an aryl substituent on the central linker methylene (II). In previous papers, we have reported the synthesis of 4-hydroxycoumarin dimmers bearing aniline mustard moiety[17)] 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 did not markedly increase the inhibitory activity and seemed to fail forming covalent binding at coumarin–IN complex binding site. We also reported biscoumarins with a diversified modification on the linker[18)] and enabled us to know that the benzoyloxyphenyl linker (III) seemed important for the inhibitory activity. These encouraging results prompted us to modify these lead compounds. Further structural theoretical studies by Long *et al.*[19)] on the binding site of HIV-1 IN allow establishing that the spatial distance between two adjacent active sites (one from each dimer) are very important for inhibitory activity. Thus, we postulate that compounds (**7**, **8**, **12**—**14**, **18**) with the chain extension on the linker may be more beneficial to enhance the activity. Afterwards, according to the instability of ester bond on the benzoyloxyphenyl linker, we have replaced an amide bond instead, such as compound **4**. With the aim to examine our assumption and find more potent new anti-HIV agents, in this paper we describe the synthesis and inhibitory activity of a series of novel bis- or tetra-coumarins against HIV-1 IN.

Chemistry

The target compounds in this study were synthesized as shown in Charts 2—4. Starting 4-carboxybenzaldehyde was transformed to its corresponding 4-methoxyphenyl 4-formylbenzoate (**1**) and 4-formyl-*N*-(4-methoxyphenyl)benzamide (**2**) by the reaction with 4-methoxyphenol and 4-methoxyani-

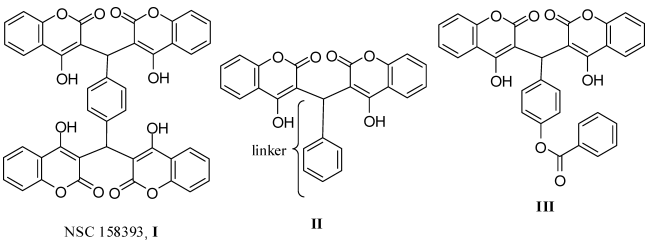

Chart 1. Molecular Structures of I—III

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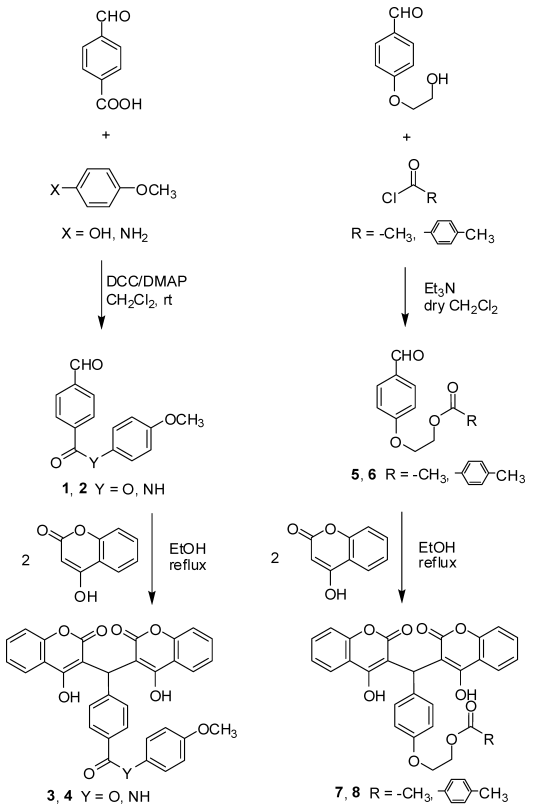

Chart 2

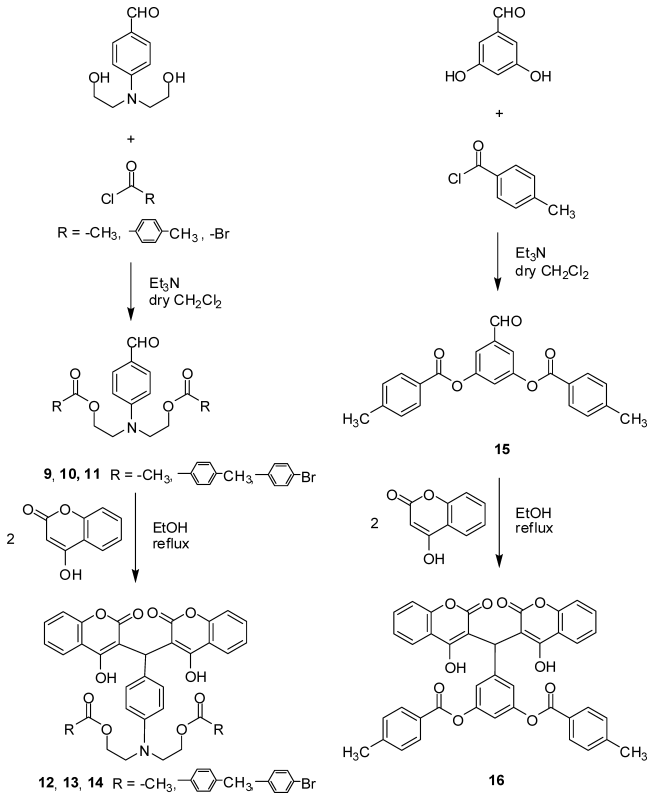

Chart 3

line in a mixture of dicyclocarbodiimide (DCC) and dimethylamonopyridine (DMAP) in dichloromethane at room temperature for over night, respectively. 4-(2-Hydroxy-ethoxy)-benzaldehyde, a reaction product of 4-hydroxybenzaldehyde and 2-chloroethanol was converted to 2-(4-formylphenoxy)-ethyl acetate (**5**) and 2-(4-formylphenoxy)ethyl 4-methylbenzoate (**6**) by the triethylamine catalyzed coupling reaction with acetyl chloride and *p*-toluoyl chloride in dry methylene chloride, at room temperature for 6 h, respectively. Subsequent condensation of **1**, **2**, **5**, and **6** with 2 equivalents 4-hydroxycoumarin in ethanol under reflux for 2 d provided target compounds **3**, **4**, **7**, and **8**, respectively in moderate to high yield. 4-[Bis-(2-hydroxy-ethyl)-amino]-benzaldehyde was treated with acetyl chloride, *p*-toluoyl chloride or 4-bromobenzoyl chloride in a mixture of triethylamine in dry methylene chloride at room temperature for 6 h furnished compounds **9**—**11**, respectively. At the same reaction condition, compound **15** was obtained by the reaction of 3,5-dihydroxybenzaldehyde with *p*-toluoyl chloride. Subsequent condensation of **9**—**11** and **15** with 2 equivalents 4-hydroxycoumarin provided target compounds **12**—**14** and **16**, respectively. Compound **18**, the tetra-coumarin with the longest extended linker was synthesized in two steps. Esterification of 4-carboxybenzaldehyde and 4-hydroxybenzaldehyde in a mixture of dicyclocarbodiimide (DCC) and dimethylamonopyridine (DMAP) in dichloromethane at room temperature for over night gave 4-formylphenyl 4-formylbenzoate (**17**). And condensation of **17** with 4 equivalents 4-hydroxycoumarin in ethanol under reflux for 2 d provided target compounds **18**. All the target compounds were characterized by NMR, MS, IR and elemental analysis. Both analytical and spectral data of all the compounds are in full agreement with

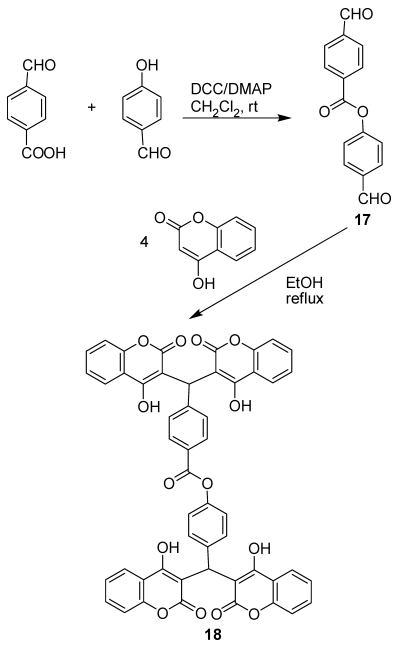

Chart 4

the proposed structures.

Results and Discussion

HIV-1 IN Inhibition 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

Table 1. HIV-1 Integrase Inhibitory Activity of Modified Coumarin Derivatives

Entry	Compound	IC_{50} (μM)
1	**3**	3.1
2	**4**	23.1
3	**7**	96.0
4	**8**	23.1
5	**12**	102
6	**13**	1.8
7	**14**	0.96
8	**16**	0.58
9	**18**	0.49
	NSC 158393 (I)	1.1
	(II)	43.0

inhibitory activity in a 3′-processing assay which was performed as described previously.[20,21] Effects against the strand transfer activity were evaluated during the same assay from the homologous integration events and found to be no significantly different from 3′-processing inhibition, thus confirming that coumarins affect equally both steps of the integration reaction. Table 1 lists the inhibitory activity of a series of bis- or tetra-coumarin analogues against the recombinant wild-type HIV-1 IN enzyme. The inhibitory activity of NSC 158393 (I) and (II) were included for a comparison. Among nine compounds in this series, three demonstrated potent activities with IC_{50} less than 1.0 μM, two displayed activities less than 4.0 μM, two showed activities at 23.1 μM, and two showed weak activities with IC_{50} larger than 90 μM, respectively. Compound **18**, the tetra-coumarin with the longest extended linker was the most potent one in this series, with IC_{50} of 0.49 μM. Compounds **14** and **16**, with the bis-coumarin which linked with bis-benzoyl moiety instead of bis-coumarin like NSC 158393 (I), possessed IC_{50} of 0.96 and 0.58 μM, respectively. It was of interesting that the existing of bis-benzoyl moiety in **14** and **16** seemed to serve important role on potency, because **12**, only with bis-acetoxy moiety, and **7** or **8**, with only mono-benzoyl moiety, showed a remarkable decreased activity. Further, compound **18** was more active than tetra-coumarin NSC 158393 (I). This is the first finding that the extended linker gained increased activity.

Conclusion

In summary, we have described the synthesis and HIV-IN inhibitory activity of novel bis- or tetra-coumarin analogues. The results showed that most of these analogues exhibited moderate or high activity against HIV-IN. The most active compound **18** exhibited about 2-fold more potent activity than lead NSC 158393 (I). This result confirmed our assumption that spatial distance between each coumarin unit and linker are very important for inhibitory activity. The introduction of an extended linker between two bis-coumarin might improve the inhibitory activity. As a result our studies provide useful indicators for guiding the further rational design of more potent new HIV-IN inhibitor.

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 obtain 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.

General Procedure for Compounds 1, 2 and 17 To a solution of 4-carboxybenzaldehyde (6.9 mmol) and DCC (10.1 mmol) in CH_2Cl_2 (50 ml) were added DMAP (1 mmol) and 6.9 mmol of 4-methoxyphenol, 4-methoxyaniline or 4-hydroxybenzaldehyde, respectively. The reaction mixture was stirred over night at room temperature. The mixture was filtered, and the solvent was removed *in vacuo*. The residue was purified by flash chromatography on silica gel with *n*-hexane/EtOAc (9/1) and recrystallized from methanol.

4-Methoxyphenyl 4-Formylbenzoate (**1**): Yield: 1.17 g (66%), a white powder, mp 105—106 °C. ^{1}H-NMR ($CDCl_3$) δ: 10.01 (s, 1H, CHO), 8.13—6.98 (m, 8H, ArH), 3.90 (s, 3H, CH_3). MS *m/z*: 257 (MH^+). *Anal.* Cacld for $C_{15}H_{12}O_4$: C, 70.31, H, 4.72. Found: C, 70.04, H, 4.54.

4-Formyl-*N*-(4-methoxyphenyl)benzamide (**2**): Yield: 0.95 g (54%), a white powder, mp 183—184 °C. ^{1}H-NMR (DMSO-d_6) δ: 10.39 (s, 1H, NH), 10.10 (s, 1H, CHO), 8.12—7.16 (m, 8H, ArH), 3.83 (s, 3H, CH_3). MS *m/z*: 256. (MH^+). *Anal.* Cacld for $C_{15}H_{13}NO_3$: C, 70.58, H, 5.13, N, 5.49. Found: C, 70.47, H, 5.41, N, 5.66.

4-Formylphenyl 4-Formylbenzoate (**17**): Yield: 1.2 g (68%), a white powder, mp 123—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 10.16, 10.04 (s, 1H each, CHO), 8.38—7.42 (m, 8H, ArH). MS *m/z*: 255 (MH^+). *Anal.* Cacld for $C_{15}H_{10}O_4$: C, 70.86, H, 3.96. Found: C, 71.04, H, 3.92.

General Procedure for Compounds 5, 6, 9—11 and 15 To an ice cold solution of certain alcohols (6.5 mmol) in triethylamine (5 ml) and dry dichloromethane (20 ml) were added acetyl or benzoyl chloride (6.5 mmol), respectively. The mixture was stirred at room temperature for 6 h and then poured onto ice. The mixture was extracted with ethyl acetate three times. The organic layers washed with brine, dried under anhydrous magnesium sulfate. The filtrate was concentrated under *vacuo*. The residue was purified by flash chromatography on silica gel with *n*-hexane/EtOAc (5/1) and crystallized from *n*-hexane to afford the corresponding products **5**, **6**, **9**—**11** and **15**.

2-(4-Formylphenoxy)ethyl Acetate (**5**): Yield: 0.87 g (64%), a white powder, mp 93—95 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.88 (s, 1H, CHO), 7.83—7.20 (m, 4H, ArH), 4.68 (t, J=5.0 Hz, 2H, CH_2CH_2), 4.39 (t, J=4.9 Hz, 2H, CH_2CH_2), 2.30 (s, 3H, CH_3). MS *m/z*: 209 (MH^+). *Anal.* Cacld for $C_{11}H_{12}O_4$: C, 63.45, H, 5.81. Found: C, 63.64, H, 5.84.

2-(4-Formylphenoxy)ethyl 4-Methylbenzoate (**6**): Yield: 0.8 g (43%), a white powder, mp 91—92 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.89 (s, 1H, CHO), 7.95—7.03 (m, 8H, ArH), 4.68 (t, J=5.0 Hz, 2H, CH_2CH_2), 4.39 (t, J=4.9 Hz, 2H, CH_2CH_2), 2.40 (s, 3H, CH_3). MS *m/z*: 285 (MH^+). *Anal.* Cacld for $C_{17}H_{16}O_4$: C, 71.82, H, 5.67. Found: C, 71.94, H, 5.84.

4-(Bis-(2-acetoxy-ethyl)-amino)-benzaldehyde (**9**): Yield: 1.39 g (73%), a white powder, mp 39—40 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.89 (s, 1H, CHO), 7.21—6.95 (m, 4H, ArH), 4.55 (t, J=6.0 Hz, 4H, CH_2O), 3.93 (t, J=6.0 Hz, 4H, NCH_2), 2.41 (s, 6H, CH_3). MS *m/z*: 294 (MH^+). *Anal.* Cacld for $C_{15}H_{19}NO_5$: C, 61.42, H, 6.53, N, 4.78. Found: C, 61.31, H, 6.44, N, 4.53.

4-(Bis(2-(4-methyl-benzoyloxy)-ethyl)-amino)-benzaldehyde (**10**): Yield: 2.0 g (98%), a yellow powder, mp 140—141 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.76 (s, 1H, CHO), 8.02—6.92 (m, 12H, ArH), 4.54 (t, J=6.0 Hz, 4H, CH_2O), 3.91 (t, J=6.0 Hz, 4H, NCH_2), 2.40 (s, 6H, CH_3). MS *m/z*: 446 (MH^+). *Anal.* Cacld for $C_{27}H_{27}NO_5$: C, 72.79, H, 6.11, N, 3.14. Found: C, 72.75, H, 5.74, 3.14.

4-(Bis(2-(4-bromobenzoyloxy)-ethyl)-amino)-benzaldehyde (**11**): Yield: 1.9 g (51%), a yellow powder, mp 125—126 °C. ^{1}H-NMR ($CDCl_3$) δ: 9.76 (s, 1H, CHO), 7.83—6.90 (m, 12H, ArH), 4.54 (t, J=6.0 Hz, 4H, CH_2O), 3.90 (t, J=6.0 Hz, 4H, NCH_2). MS *m/z*: 576 (MH^+). *Anal.* Cacld for $C_{25}H_{21}Br_2NO_5$: C, 52.20, H, 3.68, N, 2.43. Found: C, 52.30, H, 3.61, N, 2.51.

3,5-Di-((4-methylbenzoyl)oxy)benzaldehyde (**15**): Yield: 2.07 g (85%), a white powder, mp 95—96 °C. ^{1}H-NMR ($CDCl_3$) δ: 10.03 (s, 1H, CHO), 8.11—7.32 (m, 11H, ArH), 2.47 (s, 3H, CH_3). MS *m/z*: 375 (MH^+). *Anal.* Cacld for $C_{23}H_{18}O_5$: C, 73.79, H, 4.85. Found: C, 73.84, H, 5.02.

General Procedure for Compounds 3, 4, 7, 8, 12—14, 16 and 18 The reaction mixture of 4-hydroxycoumarin (2.2 eq for entry 1—8; 4.2 eq for entry 9), aromatic aldehyde (1 eq) in ethanol was heated under reflux for

48 h. The mixture was concentrated under reduced pressure to furnished product. Pure products were obtained under recrystalliztion with methanol.

3,3′-(4-(4-Methoxybenzoyl)oxy)-benzylidene)bis-4-hydroxycoumarin (**3**): Yield: 60%, a white powder, mp 192—193 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.05—7.08 (m, 16H, ArH), 6.37 (s, 1H, CH), 3.85 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3622, 1736, 1663. MS *m/z*: 563 (MH^+). *Anal.* Cacld for $C_{33}H_{22}O_9$: C, 70.46, H, 3.94. Found: C, 70.16, H, 3.93.

4-(Bis(4-hydroxy-2-oxo-2*H*-chromen-3-yl)methyl)-*N*-*p*-tolylbenzamide (**4**): Yield: 52%, a white powder, mp >270 °C. ^{1}H-NMR (DMSO-d_6) δ: 10.05 (s, 1H, NH), 7.81—7.09 (m, 16H, ArH), 6.31 (s, 1H, CH), 3.93 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3400, 3332, 1653. MS *m/z*: 562 (MH^+). *Anal.* Cacld for $C_{33}H_{23}NO_8$: C, 70.58, H, 4.13, N, 2.49. Found: C, 70.70, H, 4.38, N, 2.51.

3,3′-(4-(2-(4-Acetoxy)-ethoxy)-benzylidene)bis-4-hydroxycoumarin (**7**): Yield: 65%, a white powder, mp 228—229 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.84—6.79 (m, 12H, ArH), 6.24 (s, 1H, CH), 4.53 (s, 2H, CH_2OCO), 4.25 (s, 2H, $ArOCH_2$), 2.25 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3437, 1752, 1712. MS *m/z*: 515 (MH^+). *Anal.* Cacld for $C_{29}H_{22}O_9$: C, 67.70, H, 4.31. Found: C, 67.34, H, 3.99.

3,3′-(4-(2-(4-Methylbenzoyloxy)-ethoxy)-benzylidene)bis-4-hydroxycoumarin (**8**): Yield: 78%, a white powder, mp 225—226 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.83—6.78 (m, 16H, ArH), 6.23 (s, 1H, CH), 4.52 (t, J=5.1 Hz, 2H, CH_2CH_2), 4.24 (t, J=5.1 Hz, 2H, CH_2CH_2), 2.34 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3412, 1712, 1653. MS *m/z*: 591 (MH^+). *Anal.* Cacld for $C_{35}H_{26}O_9$: C, 71.18, H, 4.44. Found: C, 71.34, H, 4.12.

3,3′-(4-(Bis[2-(acetoxy)ethyl]amino)-benzylidene)bis-4-hydroxycoumarin (**12**): Yield: 47%, a yellow powder, mp 211—212 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.81—6.55 (m, 12H, ArH), 6.13 (s, 1H, CH), 4.08 (t, J=5.2 Hz, 4H, CH_2O), 3.50 (t, J=5.2 Hz, 4H, NCH_2), 1.96 (s, 6H, CH_3). IR (KBr) cm^{-1}: 3433, 1736, 1232. MS *m/z*: 600 (MH^+). *Anal.* Cacld for $C_{33}H_{29}NO_{10}$: C, 66.10, H, 4.88 , N, 2.34. Found: C, 66.49, H, 4.73, N, 2.27.

3,3′-(4-(Bis-(2-(4-methylbenzoyloxy)-ethyl)-amino)-benzylidene)-bis-4-hydroxycoumarin (**13**): Yield: 67%, a dark red powder, mp 175—176 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.84—6.71 (m, 20H, ArH), 6.19 (s, 1H, CH), 4.36 (t, J=5.6 Hz, 4H, CH_2O), 3.71 (t, J=5.6 Hz, 4H, NCH_2), 2.33 (s, 6H, CH_3). IR (KBr) cm^{-1}: 3633, 1707, 1271. MS *m/z*: 752 (MH^+). *Anal.* Cacld for $C_{45}H_{37}NO_{10}$: C, 71.89, H, 4.96, N, 1.86. Found: C, 71.60, H, 4.62, N, 1.89.

3,3′-(4-(Bis-(2-(4-bromobenzoyloxy)-ethyl)-amino)-benzylidene)-bis-4-hydroxycoumarin (**14**): Yield: 78%, a pink powder, mp 222—223 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.85—6.72 (m, 20H, ArH), 6.19 (s, 1H, CH), 4.37 (t, J=5.5 Hz, 4H, CH_2O), 3.72 (t, J=5.2 Hz, 4H, NCH_2). IR (KBr) cm^{-1}: 3477, 1713, 1271. MS *m/z*: 882 (MH^+). *Anal.* Cacld for $C_{43}H_{31}NO_{10}Br_2$: C, 58.59, H, 3.54, N, 1.59. Found: C, 58.51, H, 3.32, N, 1.56.

3,3′-(3,5-Di-((4-methylbenzoyl)oxy)-benzylidene)bis-4-hydroxycoumarin (**16**): Yield: 46%, a white powder, mp >270 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.95—6.93 (m, 19H, ArH), 6.38 (s, 1H, CH), 2.35 (s, 6H, CH_3). IR (KBr) cm^{-1}: 3477, 1730, 1658. MS *m/z*: 681 (MH^+). *Anal.* Cacld for $C_{41}H_{28}O_{10}$: C, 72.35 , H, 4.15. Found: C, 72.57, H, 4.42.

4-(Bis(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl-4′-(bis(4-hydroxy-2-oxo-2*H*-chromen-3-yl)methyl)benzoate (**18**): Yield: 75%, a white powder, mp >270 °C. ^{1}H-NMR (DMSO-d_6) δ: 7.93—7.02 (m, 24H, ArH), 6.34, 6.28 (s, 1H each, CH). IR (KBr) cm^{-1}: 3445, 1674, 1271. MS *m/z*: 867 (MH^+). *Anal.* Cacld for $C_{51}H_{30}O_{14}$: C, 70.67, H, 3.49. Found: C, 70.37, H, 3.71.

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.[15,16] 3′-Processing assay was performed in a reaction volume 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 Image Quant software (Amersham Pharmacia Biotech). 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 Streptavidincoated 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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