HIV-1 Integrase Inhibition of Biscoumarin Analogues

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Nineteen biscoumarins bearing free and modified hydroxyl substituents at benzoyloxyphenyl linker have been synthesized by multiple step synthesis. Among these biscoumarins, thirteen were found to be active molecules against HIV-1 integrase (HIV-1 IN). The structure–activity relationship of the nineteen compounds on HIV IN may be useful for the design of potent therapeutic agents.

Key words biscoumarins; HIV-1 integrase; inhibitor

Current strategies for anti-HIV chemotherapy involve inhibition of virus adsorption, virus-cell fusion, reverse transcription, integration, translation, proteolytic cleavage, glycosylation, assembly, or release.[1,2] Because the integration is essential for the replication of HIV and the integrase, the enzyme responsive for integration, appears to be absent in the mammalian host, HIV-1 integrase (HIV-1 IN) represents a potential target for the development of non-toxic antiviral therapeutic agents.[3,4] Recently numerous coumarin compounds have been evaluated for inhibitory effects against HIV replication, and some of them have been found to inhibit different stages in the HIV replication cycle.[5] A natural tetrameric coumarin (I) shown potent integrase inhibitory activity (IC_{50}=0.8 μM for integration and 1.5 μM for 3′-processing) was first reported by Mazumder *et al.*[6] and the parent compound was then modified into simple components to determine the minimum active pharmacophore essential for potency. They disclosed a biscoumarin unit linked by a phenyl ring (II) was required for activity.[7]

Our previous studies focused mainly on the linker of phenyl ring. Replacement of the phenyl ring by aniline mustard (III) was achieved.[8] We reasoned the mustard moiety might react with nearby nucleophile of the binding site to form a covalent bonding and to improve the inhibitory activity. However, these compounds failed to enhance activity. We also prepared biscoumarins with a diversified modification on the linker.[9] Structure–activity relationship studies enabled us to know that the benzoyloxyphenyl linker (IV) seemed important for the inhibitory activity. These encouraging results prompted us to modify these lead compounds. Thus, we examined the effects of substitutions on the benzene ring of benzoyloxyphenyl linker. In the present paper, we report the synthesis and biological evaluation of a novel benzoyloxyphenyl linker (V) having either a monohydroxy or bishydroxy groups on the benzoyl ring.

Chemistry A general synthetic route for the preparation of target compounds (**30**—**47**) is depicted in Charts 2 and 4. Protection of the phenolic hydroxyl groups of commercially available polyphenolic acids (**1**—**8**) by acetylation with acetic anhydride in pyridine in the dark at room temperature gave (**9**—**16**). Key intermediate **17** was prepared from 4-hydroxybenzaldehyde with 4-hydroxycoumarin in ethanol under reflux. The first attempt to prepare target compounds by esterification of **17** with protected polyphenolic acids (**14**) in the presence of coupling reagents dicyclohexylcarbodiimide (DCC) and catalyst 4-dimethylaminopyridine (4-DMAP) in dichloromethane, followed by 3 N HCl hydrolysis to remove the protected phenols was unfruitful (Chart 3). The product was unexpected **18** other than **45**. Alternative synthesis of target compounds was successful by esterification of 4-hydroxybenzaldehyde with protected polyphenolic acids (**9**—**16**, **19**) and lipoic acid in the presence of coupling

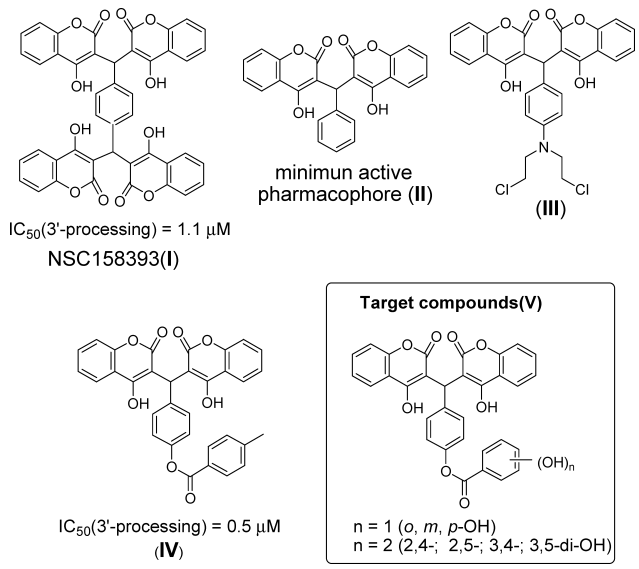

Chart 1. Molecular Structures of I—IV and Target Compounds V

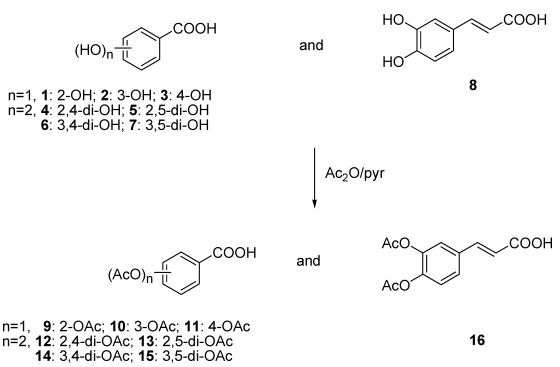

Chart 2

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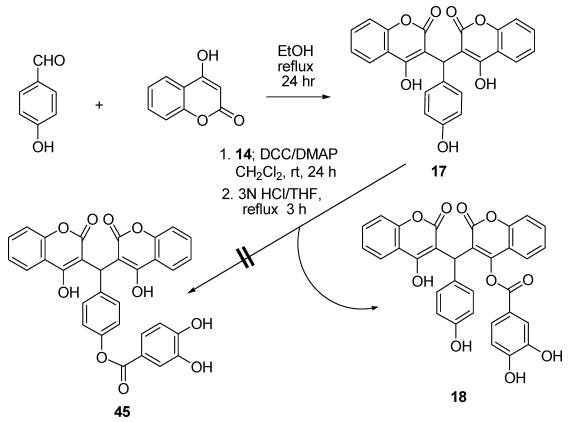

Chart 3

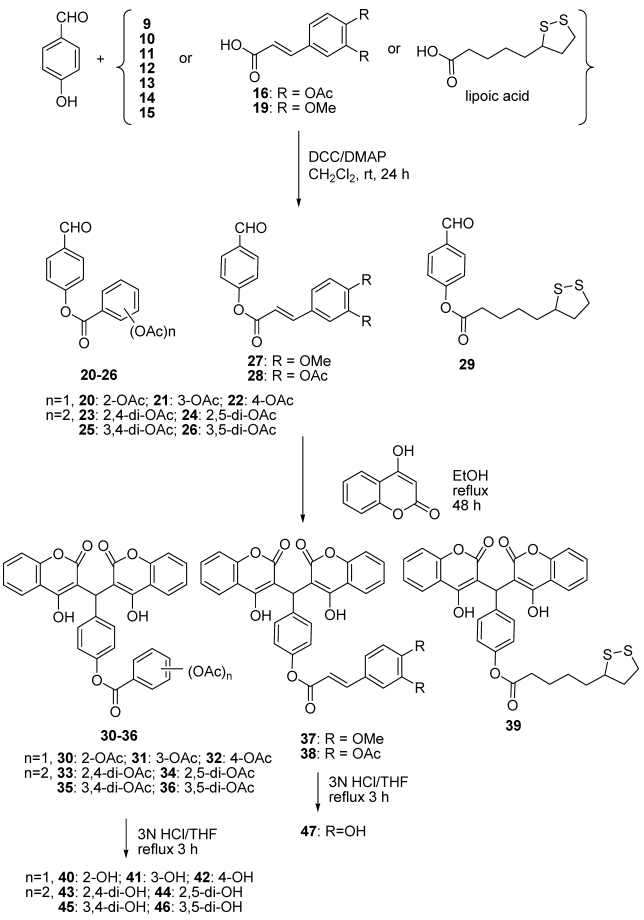

Chart 4

reagents DCC and DMAP in dichloromethane and then subsequently coupling reaction with 4-hydroxycoumarin in ethanol under reflux (Chart 4). All the structures of target compounds were established by NMR, IR, MS spectroscopy and elementary analysis. The proton on the benzylidene *tert*-carbon of **45** is found at δ 6.3 ppm, in agreement with previous studies on similar compounds.[8,9] However, an unexpected benzylidene proton upfield shift from δ 6.3 to 4.9 ppm is observed in **18**. The introduction of an ester instead of ketone group on the 4-hydroxycoumarin ring in **18** decreases electron withdrawing ability, which causes the benzylidene proton upfield shifting. Since it was found that there

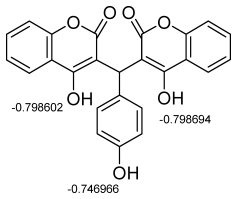

Fig. 1. The Values of Net-Charge at Three Oxygen Atoms of **17** by Hartree–Fock 3-21G(*) Calculation

Molecular orbital calculation was carried out by using the program SPARTAN '04 windows, version 1.0.3 of Wavefunction Inc.

were three reactive sites (two hydroxyls of 4-hydroxycoumarin and one hydroxyl of phenol) for the reaction of **17** with protected polyphenolic acids, molecular orbital calculation was carried out by using the program SPARTAN '04 windows, version 1.0.3 of Wavefunction Inc to verify the preferred reactive site for esterification. The net-charges by Hartree-Fock 3-21G(*) calculation for **17** are shown in Fig. 1. The values of net-charge at two oxygen atoms from hydroxyl of 4-hydroxycoumarin in **17** are nearly equal (-0.798602 and -0.798694, respectively), while the oxygen atom of phenol has a value of -0.746966. The net-charges at oxygen atoms from hydroxyls of 4-hydroxycoumarin are negatively larger than that at oxygen atom of phenol. Thus, the 4-hydroxycoumarin hydroxyl groups are more reactive than the phenol hydroxyl group in **17** for esterification, and these results agree with the experimental fact.

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 inhibitory activity in a 3′-processing assay which was performed as described previously.[3,10] 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. Table 1 lists the inhibitory activity of a series of mono- or bis-hydroxylated benzoyl biscoumarins against the recombinant wild-type HIV-1 IN enzyme. The inhibitory activity of NSC 158393 I and II were included for a comparison. Among nineteen compounds in this series, all were active, thirteen demonstrated activities less than 10 μM, four had activities in the range of 10 to 27 μM, and two showed activities at 37 and 53 μM, respectively. Compound **45** was the most potent in this series, with IC_{50} of 1.5 μM. The second-most-potent compound **35** was the acetate analog of **45**, with IC_{50} of 1.9 μM. Compounds were divided into two classes based on the free (class I) or modified (class II) hydroxyl substitute. All the compounds in class I, bearing free hydroxyl substitute(s) (**40**—**47**), except compound **44** and **46**, had a slight higher IN inhibitory activity in vitro than the corresponding compounds with modified hydroxyl substitute (**30**—**38**). The relative potency of the mono-hydroxyl compounds was in the order: 4-OH (**42**)>3-OH (**41**)>2-OH (**40**), whereas the bis-hydroxyl compounds was in the order: 3,4-di-OH (**45**)>2,4-di-OH (**43**)>2,5-di-OH (**44**)>3,5-di-OH

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

Entry	Compound	mp (°C)	3′-Processing (IC_{50} μM)
1	**18**	262—263	37.0
2	**30**	258—260	27.0
3	**31**	270—272	7.1
4	**32**	252—254	4.6
5	**33**	232—234	19.0
6	**34**	242—243	9.7
7	**35**	178—180	1.9
8	**36**	116—118	9.3
9	**37**	118—120	2.6
10	**38**	125—126	2.6
11	**39**	247—249	2.3
12	**40**	238—239	10.0
13	**41**	232—234	6.1
14	**42**	258—260	3.2
15	**43**	>300	4.9
16	**44**	262—264	25.0
17	**45**	210—212	1.5
18	**46**	177—181	53.0
19	**47**	266—268	2.6
	NSC 158393 (I)		1.1
	(II)		43.0

(**46**), suggesting the inhibitory activity is associated with the specific spatial disposition of the hydroxyl groups. Compound **47** (containing free hydroxyl caffeoyl) and **45** (having catechol) displayed considerable activity, with IC_{50} of 2.6 and 1.5 μM, respectively. It was of interesting that compound **37** (containing protected methoxy caffeoyl) and **38** (containing protected acetoxy caffeoyl) were equal potent to **47**, implying modification on bis-hydroxyl substitute tolerates activity. Class II compounds demonstrated similar trends to class I compounds on relative potency, except **33** and **36**. Additionally, compound **39**, replacing the caffeoyl with a potent antioxidative lipoyl moiety remained the potency, with IC_{50} of 2.3 μM.

In conclusion, we have designed and synthesized nineteen biscoumarins bearing free and modified hydroxyl substitute at the benzoyl ring. Among these easily available biscoumarins, thirteen were found to be active molecules against HIV-1 IN. These compounds may prove useful in structure–activity relationship studies on HIV IN and may ultimately lead to new classes of potent therapeutic agents. Further detailed studies of the biological activity of these compounds will reveal their full potential.

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.

Acetylation of Phenol(s) of Polyphenolic Acid, General Procedure to Obtain Compounds 9—16 To a solution of polyphenolic acid (20 mmol) was added acetic anhydride (6 eq) and pyridine (2 ml). The mixture was stirred at room temperature under dark for 4 h. Then poured onto 1 M H_3PO_4 (10 ml) cold solution. The mixture was extracted with ethyl acetate. The layers washed with brine and aq saturated sodium bicarbonate. The combined organic phase was dried under magnesium sulfate, filtered, and the solvent was removed under *vacuo*. The residue was crystallized from proper solvent to afford the corresponding acetoxy polyphenolic acids: 2-acetoxy benzoic acid (**9**), 3-acetoxy benzoic acid (**10**), 4-acetoxy benzoic acid (**11**), 2,4-diacetoxy benzoic acid (**12**), 2,5-diacetoxy benzoic acid (**13**), 3,4-diacetoxy benzoic acid (**14**), 3,5-diacetoxy benzoic acid (**15**), 3,4-diacetoxy cinnamic acid (**16**). These acetoxy polyphenolic acids were identified by melting point and ^{1}H-NMR. Data were in agreement with literature values.[11,12]

3,3′-(4-Hydroxybenzylidene)bis-4-hydroxycoumarin (17) A mixture of 4-hydroxybenzaldeyde (3.66 g, 30 mmol) and 4-hydroxycoumarin (9.73 g, 60 mmol) in absolute ethanol (100 ml) was heated under reflux for 24 h. The solvent was removed *in vacuo*. The residue was recrystallized from ethanol to give pale yellow powder **17** (11.4 g, 97%). *Rf*: 0.10 (*n*-hexane : EtOAc = 1 : 1). mp: 210—212 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 7.89—6.59 (m, 12H, ArH), 6.22 (s, 1H, CH). FAB-MS *m*/*z*: 428.09 (M+H$^+$). *Anal.* Cacld for $C_{25}H_{16}O_7$: C, 70.09; H, 3.76. Found: C, 69.87; H, 4.00.

4-(3,4-Dihydroxybenzoyloxy)coumarin-4′-hydroxycoumarin-4-hydroxyphenylmethane (18) To a solution of **14** (2.3 g, 10 mmol) and DCC (2.3 g, 11 mmol) in CH_2Cl_2 (30 ml) were added DMAP (122 mg, 1 mmol) and **17** (2.14 g, 5 mmol). The reaction mixture was stirred over night at room temperature. The mixture was filtered, and the solvent was removed *in vacuo*. The residue was recrystallized from methanol to give white intermediate (1.98 g, 61%). *Rf*: 0.30 (*n*-hexane : EtOAc = 1 : 1). mp: 230—233 °C (MeOH). ^{1}H-NMR ($CDCl_3$) δ: 8.09—7.12 (m, 15H, ArH), 5.20 (s, 1H, CH), 2.30 (s, 6H, CH_3). IR (KBr) cm^{-1}: 3512 (OH), 1725 (C=O). UV λ_{max} (EtOH) nm (log ε): 309 (4.13). FAB-MS *m*/*z*: 648.13 (M+H$^+$). *Anal.* Cacld for $C_{36}H_{24}O_{12}$: C, 66.67; H, 3.73. Found: C, 66.87; H, 4.00. A mixture of the intermediate (324 mg, 0.5 mmol) and 3 M HCl (10 ml) in THF (30 ml) was heated under reflux for 3 h and poured onto ice water (50 ml). The mixture was extracted with EtOAc. The layer was washed with water and brine. The organic extract was dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The residue was recrystallized from CH_2Cl_2 to give white powder **18** (210 mg, 75%). *Rf*: 0.15 (*n*-hexane : EtOAc = 1 : 1). mp: 262—263 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 9.95, 9.46 (s, 1H each, OH), 8.39—6.82 (m, 15H, ArH), 4.89 (s, 1H, CH). IR (KBr) cm^{-1}: 3624 (OH), 1808 (C=O). UV λ_{max} (EtOH) nm (log ε): 322 (4.23). FAB-MS *m*/*z*: 565.1 (M+H$^+$). *Anal.* Cacld for $C_{32}H_{20}O_{10}$: C, 68.09; H, 3.57. Found: C, 67.87; H, 3.87.

Esterification of Acetoxy (Poly)phenolic Acids or Lipoic Acid with 4-Hydroxybenzaldehyde, General Procedure to Obtain Compounds 20—29 To a solution of acid (10 mmol) and DCC (11 mmol) in CH_2Cl_2 (50 ml) were added DMAP (1 mmol) and 4-hydroxybenzaldehyde (11 mmol). 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 CH_2Cl_2/acetone (9/1) and recrystallized from methanol.

4-(2-Acetoxybenzoyloxy)benzaldehyde (**20**) as white powder in a yield of 79%: mp: 74—75 °C (*n*-hexane). ^{1}H-NMR ($CDCl_3$) δ: 9.87 (1H, s, CHO), 8.07—7.84 (8H, m, ArH), 2.17 (3H, s, $COCH_3$). FAB-MS *m*/*z*: 284.07 (M$^+$). *Anal.* Cacld for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. Found: C, 67.29; H, 4.51.

4-(3-Acetoxybenzoyloxy)benzaldehyde (**21**) as white powder in a yield of 84%: mp: 64—65 °C (*n*-hexane). ^{1}H-NMR ($CDCl_3$) δ: 10.01 (1H, s, CHO), 8.08—7.37 (8H, m, ArH), 2.34 (3H, s, $COCH_3$). FAB-MS *m*/*z*: 284.07 (M$^+$). *Anal.* Cacld for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. Found: C, 67.45; H, 4.11.

4-(4-Acetoxybenzoyloxy)benzaldehyde (**22**) as white powder in a yield of 53%: mp: 116—118 °C (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 9.88 (1H, s, CHO), 8.11—7.11 (8H, m, ArH), 2.21 (3H, s, $COCH_3$). FAB-MS *m*/*z*: 284.07 (M$^+$). *Anal.* Cacld for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. Found: C, 67.61; H, 4.33.

4-(2,4-Diacetoxybenzoyloxy)benzaldehyde (**23**) as white powder in a yield of 53%: mp: 152—154 °C (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 10.00 (1H, s, CHO), 8.25—7.02 (7H, m, ArH), 2.32, 2.29 (3H each, s, $COCH_3$). FAB-MS *m*/*z*: 342.07 (M$^+$). *Anal.* Cacld for $C_{18}H_{14}O_7$: C, 63.16; H, 4.12. Found: C, 63.29; H, 4.31.

4-(2,5-Diacetoxybenzoyloxy)benzaldehyde (**24**) as white powder in a yield of 70%: mp: 124—125 °C (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 10.00 (1H, s, CHO), 7.97—7.25 (7H, m, ArH), 2.32, 2.29 (3H each, s, $COCH_3$). FAB-MS *m*/*z*: 342.07 (M$^+$). *Anal.* Cacld for $C_{18}H_{14}O_7$: C, 63.16; H, 4.12. Found: C, 63.12; H, 4.30.

4-(3,4-Diacetoxybenzoyloxy)benzaldehyde (**25**) as white powder in a yield of 70%: mp: 100—101 °C (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 9.86 (1H, s, CHO), 7.94—7.09 (7H, m, ArH), 2.17 (6H, s, $COCH_3$). FAB-MS *m*/*z*: 342.07 (M$^+$). *Anal.* Cacld for $C_{18}H_{14}O_7$: C, 63.16; H, 4.12. Found: C, 63.08;

H, 4.45.

4-(3,5-Diacetoxybenzoyloxy)benzaldehyde (**26**) as white powder in a yield of 59%: mp: 110—111 °C (EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 9.87 (1H, s, CHO), 7.82—7.08 (7H, m, ArH), 2.17 (6H, s, $COCH_3$). FAB-MS *m/z*: 342.07 (M^+). *Anal.* Cacld for $C_{18}H_{14}O_7$: C, 63.16; H, 4.12. Found: C, 63.24; H, 4.03.

4-(3,4-Dimethoxycinnamoyloxy)benzaldehyde (**27**) as white powder in a yield of 59%: mp: 88—90 °C (MeOH). ^{1}H-NMR ($CDCl_3$) δ: 9.99 (s, 1H, CHO), 7.94—6.88 (m, 7H, ArH), 7.83, 6.48 (d, J=7.8 Hz, 1H each, CH), 3.92 (s, 6H, OCH_3). FAB-MS *m/z*: 284.07 (M^+). *Anal.* Cacld for $C_{18}H_{16}O_5$: C, 69.22; H, 5.16. Found: C, 69.35; H, 5.31.

4-(3,4-Diacetoxycinnamoyloxy)benzaldehyde (**28**) as white powder in a yield of 59%: mp: 91—92 °C (MeOH). ^{1}H-NMR ($CDCl_3$) δ: 10.00 (s, 1H, CHO), 7.95—7.25 (m, 7H, ArH), 7.81, 6.56 (d, J=7.8 Hz, 1H each, CH), 2.31, 2.30 (s, 3H each, CH_3). FAB-MS *m/z*: 368.09 (M^+). *Anal.* Cacld for $C_{20}H_{16}O_7$: C, 65.22; H, 4.38. Found: C, 65.29; H, 4.31.

4-(Liponyloxy)benzaldehyde (**29**) as an yellow oil in a yield of 83%: ^{1}H-NMR ($CDCl_3$) δ: 10.03 (s, 1H, CHO), 7.96, 7.31 (d, J=4.2 Hz, 2H each, ArH), 3.61—3.55 (m, 1H, H-6), 3.23—3.08 (m, 2H, H-8), 2.44—2.38 (m, 1H, H-7), 2.35 (t , J=7.2 Hz, 2H, H-2), 1.92—1.88 (m, 1H, H-7), 1.70—1.60 (m, 4H, H-3 and H-5), 1.53—1.40 (m, 2H, H-4). ^{13}C-NMR ($CDCl_3$) δ: 190.66, 171.07, 155.37, 133.99, 131.09, 122.26, 56.23, 40.20, 38.47, 34.53, 34.11, 28.61, 24.50. IR (KBr) cm^{-1}: 3425, 3120, 1676, 1076. FAB-MS *m/z*: 310.07 (M^+). *Anal.* Cacld for $C_{15}H_{18}O_3S_2$: C, 58.04; H, 5.84. Found: C, 58.35; H, 5.51.

Condensation of Substituted Aromatic Aldehydes with 4-Hydroxycoumarin (30—39),[6—8] General Procedure The reaction mixture of 4-hydroxycoumarin (2.2 eqivalent), aromatic aldehyde (1 equivalent) 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 certain solvents.

3,3′-[4-(2-Acetoxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**30**) as white powder in a yield of 63%: *Rf*: 0.1 (*n*-hexane : EtOAc=1 : 2). mp: 258—260 °C (hexane). ^{1}H-NMR (DMSO-d_6) δ: 8.18—6.98 (m, 16H, ArH), 6.28 (s, 1H, CH), 2.23 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3545, 1745. UV λ_{max} (EtOH) nm (log ε): 305 (4.18). FAB-MS *m/z*: 586.98 ($M+H^+$). *Anal.* Cacld for $C_{34}H_{22}O_{10}\cdot 2H_2O$: C, 65.60; H, 4.18. Found: C, 65.62; H, 3.99.

3,3′-[4-(3-Acetoxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**31**) as white powder in a yield of 60%: *Rf*: 0.15 (*n*-hexane : EtOAc=1 : 2). mp: 270—272 °C (hexane). ^{1}H-NMR (DMSO-d_6) δ: 8.00—7.06 (m, 16H, ArH), 6.28 (s, 1H, CH), 2.28 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3437, 1752. UV λ_{max} (EtOH) nm (log ε): 304 (4.19). FAB-MS *m/z*: 586.99 ($M+H^+$). *Anal.* Cacld for $C_{34}H_{22}O_{10}\cdot 2H_2O$: C, 65.60; H, 4.18. Found: C, 65.36; H, 4.19.

3,3′-[4-(4-Acetoxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**32**) as white powder in a yield of 58%: *Rf*: 0.20 (*n*-hexane : EtOAc=1 : 4). mp: 252—254 °C (AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 8.14—7.01 (m, 16H, ArH), 6.28 (s, 1H, CH), 2.30 (s, 3H, CH_3). IR (KBr) cm^{-1}: 3432, 1732. UV λ_{max} (EtOH) nm (log ε): 305 (4.18). FAB-MS *m/z*: 587.05 ($M+H^+$). *Anal.* Cacld for $C_{34}H_{22}O_{10}\cdot 2.5H_2O$: C, 64.26; H, 4.12. Found: C, 64.08; H, 4.08.

3,3′-[4-(2,4-Diacetoxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**33**) as white powder in a yield of 76%: *Rf*: 0.20 (*n*-hexane : EtOAc=1 : 4). mp: 232—234 °C (AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 8.18—6.98 (m, 15H, ArH), 6.27 (s, 1H, CH), 2.23, 2.29 (s, 3H each, CH_3). IR (KBr) cm^{-1}: 3436, 1648. UV λ_{max} (EtOH) nm (log ε): 307 (4.21). FAB-MS *m/z*: 644.98 ($M+H^+$). *Anal.* Cacld for $C_{36}H_{24}O_{12}\cdot 2H_2O$: C, 63.17; H, 4.12. Found: C, 63.15; H, 3.96.

3,3′-[4-(2,5-Diacetoxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**34**) as white powder in a yield of 60%: *Rf*: 0.20 (*n*-hexane : EtOAc=1 : 4). mp: 241—243 °C (AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 7.86—6.99 (m, 15H, ArH), 6.27 (s, 1H, CH), 2.24, 2.28 (s, 3H each, CH_3). IR (KBr) cm^{-1}: 3468, 1702. UV λ_{max} (EtOH) nm (log ε): 305 (4.18). FAB-MS *m/z*: 644.98 ($M+H^+$). *Anal.* Cacld for $C_{36}H_{24}O_{12}\cdot 1.5H_2O$: C, 64.01; H, 3.80. Found: C, 63.93; H, 3.98.

3,3′-[4-(3,4-Diacetoxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**35**) as white powder in a yield of 36%: *Rf*: 0.15 (CH_2Cl_2 : MeOH=19 : 1). mp: 178—180 °C (AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 7.99—7.07 (m, 15H, ArH), 6.30 (s, 1H, CH), 2.26, 2.29 (s, 3H each, CH_3). IR (KBr) cm^{-1}: 3511, 1706. UV λ_{max} (EtOH) nm (log ε): 301 (4.09). FAB-MS *m/z*: 645.0 ($M+H^+$). *Anal.* Cacld for $C_{36}H_{24}O_{12}\cdot H_2O$: C, 64.88; H, 3.78. Found: C, 65.11; H, 4.04.

3,3′-[4-(3,5-Diacetoxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**36**) as white powder in a yield of 33%: *Rf*: 0.15 (*n*-hexane : EtOAc=1 : 4). mp: 116—118 °C (AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 8.07—7.12 (m, 15H, ArH), 6.08 (s, 1H, CH), 2.31 (s, 6H, CH_3). IR (KBr) cm^{-1}: 3462, 1762. UV λ_{max} (EtOH) nm (log ε): 303 (4.07). FAB-MS *m/z*: 648.1 ($M+H^+$). *Anal.* Cacld for $C_{36}H_{24}O_{12}\cdot H_2O$: C, 64.88; H, 3.78. Found: C, 64.83; H, 3.88.

3,3′-[4-(3,4-Dimethoxycinnamoyloxy)-benzylidene]bis-4-hydroxycoumarin (**37**) as white powder in a yield of 50%: *Rf*: 0.15 (*n*-hexane : EtOAc=1 : 4). mp: 125—126 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 11.57, 11.30 (s, 1H each, OH), 8.18—6.86 (m, 15H, ArH), 7.78, 6.47 (d, J=7.8 Hz, 1H each, CH), 6.07 (s, 1H, CH), 3.91 (s, 6H, OCH_3). IR (KBr) cm^{-1}: 3471, 1821. UV λ_{max} (EtOH) nm (log ε): 313 (4.21). FAB-MS *m/z*: 619.16 ($M+H^+$). *Anal.* Cacld for $C_{36}H_{26}O_{10}$: C, 69.90; H, 4.24. Found: C, 69.91; H, 4.35.

3,3′-[4-(3,4-Diacetoxycinnamoyloxy)]bis-4-hydroxycoumarin (**38**) as white powder in a yield of 43%: *Rf*: 0.20 (*n*-hexane : EtOAc=1 : 4). mp: 118—120 °C (EtOHc). ^{1}H-NMR ($CDCl_3$) δ: 8.05—7.11 (m, 15H, ArH), 7.77, 6.54 (d, J=8.1 Hz, 1H each, =CH), 6.07 (s, 1H, CH), 2.30 (s, 6H, CH_3). IR (KBr) cm^{-1}: 3607, 1804. UV λ_{max} (EtOH) nm (log ε): 313 (4.28). FAB-MS *m/z*: 675.15 ($M+H^+$). *Anal.* Cacld for $C_{38}H_{26}O_{12}$: C, 67.66; H, 3.88. Found: C, 67.49; H, 4.06.

3,3′-[4-(Liponyloxy)benzylidene]bis-4-hydroxycoumarin (**39**) as yellow powder in a yield of 50%: *Rf*: 0.15 (*n*-hexane : EtOAc=1 : 4). mp: 247—249 °C (EtOAc). ^{1}H-NMR (DMSO-d_6) δ: 7.81—6.86 (m, 12H, ArH), 6.24 (s, 1H, CH), 3.61—3.55 (m, 1H, H-6), 3.23—3.08 (m, 2H, H-8), 2.44—2.38 (m, 1H, H-7), 2.35 (t, J=7.2 Hz, 2H, H-2), 1.88—1.92 (m, 1H, H-7), 1.70—1.60 (m, 4H, H-3 and H-5), 1.53—1.40 (m, 2H, H-4). IR (KBr) cm^{-1}: 3611, 1752. UV λ_{max} (EtOH) nm (log ε): 307 (4.15). FAB-MS *m/z*: 617.13 ($M+H^+$). *Anal.* Cacld for $C_{33}H_{28}O_8S_2\cdot H_2O$: C, 62.45; H, 4.76. Found: C, 6.27; H, 4.81.

Hydrolysis of the Acetate of 30—38, General Procedure to Obtain Compounds 40—47 The polyphenolic acids esters (5 mmol) was dissolved in a mixture of THF (30 ml) and aqeous 3 N HCl (30 ml). The reaction mixture was stirred for 2 d at room temperature and then extracted with ethyl acetate three times. The combined organic layers were washed with brine and water. The organic phase was dried under magnesium sulfate, filtered, and the solvent was removed *in vacuo* to give the residue. Pure products were obtained under recrystalliztion with certain solvents.

3,3′-[4-(2-Hydroxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**40**) as white powder in a yield of 96%: *Rf*: 0.2 (*n*-hexane : EtOAc=1 : 2). mp: 238—239 °C (MeOH). ^{1}H-NMR ($CDCl_3$) δ: 11.57, 11.30, 10.96 (s, 1H each, OH), 8.15—6.95 (m, 16H, ArH), 6.10 (s, 1H, CH). IR (KBr) cm^{-1}: 3479, 1808. UV λ_{max} (EtOH) nm (log ε): 316 (4.31). FAB-MS *m/z*: 549.0 ($M+H^+$). *Anal.* Cacld for $C_{32}H_{20}O_9$: C, 70.07; H, 3.68. Found: C, 70.45; H, 3.55.

3,3′-[4-(3-Hydroxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**41**) as white powder in a yield of 71%: *Rf*: 0.05 (CH_2Cl_2 : MeOH=19 : 1). mp: 232—234 °C (EtOAc). ^{1}H-NMR (DMSO-d_6) δ: 7.89—7.07 (m, 16H, ArH), 6.33 (s, 1H, CH). IR (KBr) cm^{-1}: 3611, 1782. UV λ_{max} (EtOH) nm (log ε): 322 (4.31). FAB-MS *m/z*: 549.0 ($M+H^+$). *Anal.* Cacld for $C_{32}H_{20}O_9$: C, 70.07; H, 3.68. Found: C, 70.05; H, 4.00.

3,3′-[4-(4-Hydroxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**42**) as white powder in a yield of 69%: *Rf*: 0.1 (CH_2Cl_2 : MeOH=19 : 1). mp: 258—260 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 7.95—6.88 (m, 16H, ArH), 6.29 (s, 1H, CH). IR (KBr) cm^{-1}: 3479, 1823. UV λ_{max} (EtOH) nm (log ε): 321 (4.36). FAB-MS *m/z*: 549.0 ($M+H^+$). *Anal.* Cacld for $C_{32}H_{20}O_9\cdot 2.5H_2O$: C, 64.76; H, 3.82. Found: C, 64.49; H, 4.01.

3,3′-[4-(2,4-Dihydroxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**43**) as white powder in a yield of 60%: *Rf*: 0.2 (CH_2Cl_2 : MeOH=9 : 1). mp: >300 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 10.59, 10.42 (s, 1H each, OH), 7.85—7.05 (m, 13H, ArH), 6.42 (d, J=4.2 Hz, 1H, ArH), 6.34 (d, J=1.2 Hz, 1H, ArH), 6.27 (s, 1H, CH). IR (KBr) cm^{-1}: 3612, 1682. UV λ_{max} (EtOH) nm (log ε): 313 (4.09). FAB-MS *m/z*: 565.2 ($M+H^+$). *Anal.* Cacld for $C_{32}H_{20}O_{10}\cdot 3H_2O$: C, 62.14; H, 3.74. Found: C, 62.45; H, 3.79.

3,3′-[4-(2,5-Dihydroxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**44**) as white powder in a yield of 44%: *Rf*: 0.12 (CH_2Cl_2 : MeOH=9 : 1). mp: 262—264 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 7.89—6.88 (m, 15H, ArH), 6.33 (s, 1H, CH). IR (KBr) cm^{-1}: 3619, 1829. UV λ_{max} (EtOH) nm (log ε): 307 (4.13). FAB-MS *m/z*: 565.0 ($M+H^+$). *Anal.* Cacld for $C_{32}H_{20}O_{10}\cdot 0.5H_2O$: C, 67.01; H, 3.60. Found: C, 66.87; H, 3.65.

3,3′-[4-(3,4-Dihydroxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**45**) as white powder in a yield of 43%: *Rf*: 0.10 (CH_2Cl_2 : MeOH=9 : 1). mp: 210—212 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 7.87—6.84 (m, 15H, ArH), 6.31 (s, 1H, CH). IR (KBr) cm^{-1}: 3571, 1722. UV λ_{max} (EtOH) nm (log ε): 322 (4.33). FAB-MS *m/z*: 565.2 ($M+H^+$). *Anal.* Cacld for $C_{32}H_{20}O_{10}\cdot 4.5H_2O$: C, 59.54; H, 3.95. Found: C, 59.78; H, 4.19.

3,3′-[4-(3,5-Dihydroxybenzoyloxy)benzylidene]bis-4-hydroxycoumarin (**46**) as white powder in a yield of 64%: *Rf*: 0.10 (CH_2Cl_2 : MeOH=19 : 1).

mp: 177—181 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 7.89—6.95 (m, 14H, ArH), 6.49 (s, 1H, ArH), 6.33 (s, 1H, CH). IR (KBr) cm^{-1}: 3546, 1722. UV λ_{max} (EtOH) nm (log ε): 313 (4.18). FAB-MS *m*/*z*: 565.1 (M+H$^+$). *Anal.* Cacld for $C_{32}H_{20}O_{10}\cdot 0.5H_2O$: C, 67.01; H, 3.60. Found: C, 66.87; H, 3.88.

3,3′-[4-(3,4-Dihydrox-cinnamoyloxy]bis-4-hydroxycoumarin (**47**) as yellow powder in a yield of 68%: *Rf*: 0.10 (CH_2Cl_2 : MeOH=9 : 1). mp: 266—268 °C (CH_2Cl_2). ^{1}H-NMR (DMSO-d_6) δ: 9.65, 9.18 (s, 1H each, OH), 7.81—6.77 (m, 15H, ArH), 7.62, 6.45 (d, *J*=8.1 Hz, 1H each, =CH), 6.25 (s, 1H, CH). IR (KBr) cm^{-1}: 3421, 1802. UV λ_{max} (EtOH) nm (log ε): 305 (4.18). FAB-MS *m*/*z*: 591.1 (M+H$^+$). *Anal.* Cacld for $C_{34}H_{22}O_{10}\cdot 4H_2O$: C, 61.64; H, 4.56. Found: C, 61.35; H, 4.32.

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′-TGACCAAGGGC-TAATTCACT-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 describsed previously.[8] 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 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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