DESIGN AND SYNTHESIS OF PHOTOACTIVATABLE COUMARIN-CONTAINING HIV-1 INTEGRASE INHIBITORS

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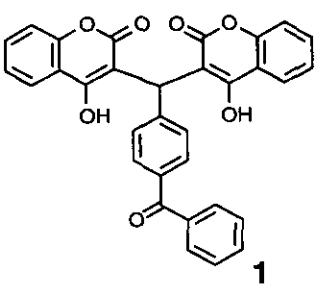
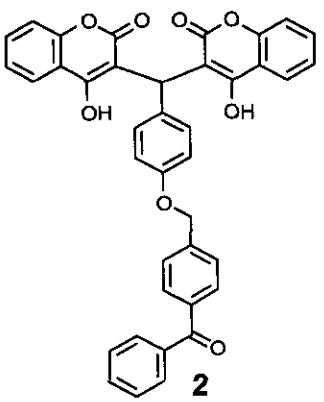
Abstract- Three dimeric coumarin analogues (**1** - **3**) were prepared, each containing the photoactivatable benzophenone moiety. These compounds exhibited low micromolar IC_{50} values against HIV-1 integrase mediated 3'-processing and strand transfer, and may represent useful probes for elucidating enzyme-inhibitor interactions.

To aid the design of HIV-1 integrase inhibitors as potential anti-AIDS drugs,[1,2] it is important to gain information concerning enzyme-inhibitor interactions. Unfortunately, the three dimensional structure of the HIV-1 integrase catalytic core has not yet been determined with an inhibitor bound.[3] The expanding use of benzophenone photophores in photoaffinity labeling provides one approach toward understanding the binding of ligands to target proteins.[4] As part of our efforts to develop HIV-1 integrase inhibitors,[5-9] we have reported a class of highly potent coumarin-containing compounds.[10] Among these latter agents were germinal coumarin dimers prepared by reaction of a appropriate aldehyde linkers with 4-hydroxycoumarin. This lead us to design photoactivatable coumarin-based HIV-1 integrase inhibitors, which could be synthesized from suitable benzophenone-containing aldehydes. Herein we reported the design, synthesis and HIV-1 integrase inhibitory potency of three benzophenone-containing coumarin dimers **1** - **3**.



1



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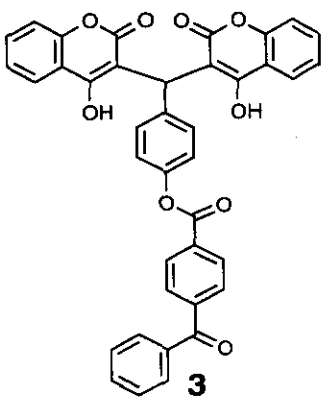
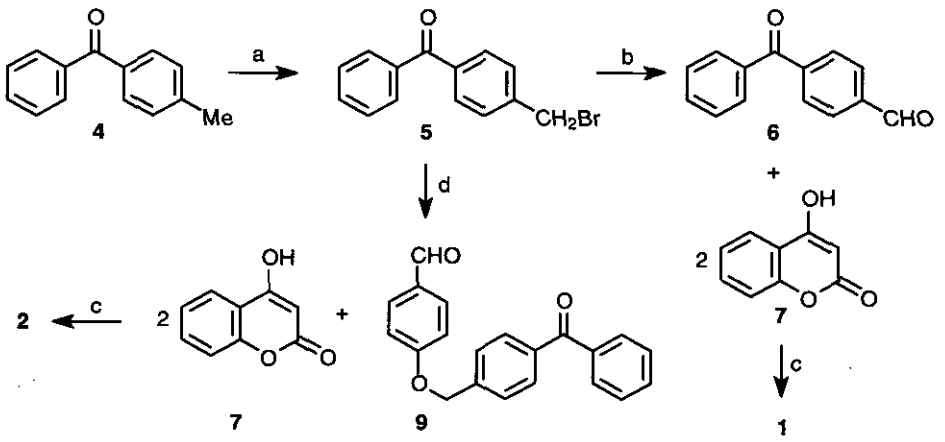


3

Synthesis

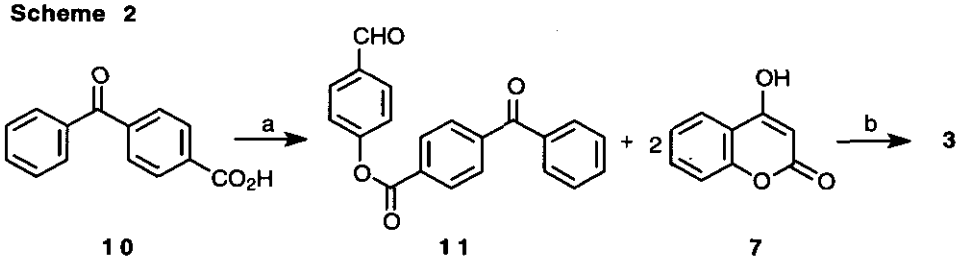
4-Methylbenzophenone (**4**) was treated with NBS using *tert*-butyl peroxide as inducer to provide 4-bromomethylbenzophenone (**5**) in 47% yield,[11] which was then oxidized using tetrabutylammonium periodate[12] to give 4-phenylcarbonylbenzaldehyde (**6**) (59%).[13] Condensation of **6** with 4-hydroxycoumarin (**7**) using our previously reported method[10] gave 3,3'-(4-phenylcarbonylbenzylidene)-bis-(4-hydroxycoumarin) (**1**) in high yield (78%, Scheme 1). Alternatively, treatment of 4-bromomethylbenzophenone (**5**) with 4-hydroxybenzaldehyde (**8**) and anhydrous potassium carbonate provided 4-(4'-phenylcarbonylbenzyloxy)benzaldehyde (**9**) (66%), which was then condensed with 4-hydroxycoumarin (**7**) to give 3,3'-[(4-phenylcarbonylbenzyloxy)benzylidene]-bis-(4-hydroxycoumarin) (**2**) (92%, Scheme 1). Commercially available 4-phenylcarbonylbenzoic acid (**10**) and 4-hydroxybenzaldehyde (**8**) were esterified by treatment with DCC to provide 4-formylphenyl 4-phenylcarbonylbenzoate (**11**) in 65% yield, which was then condensed with 4-hydroxycoumarin as above to yield 3,3'-[4-(phenylcarbonylbenzoyloxy)benzylidene]-bis-(4-hydroxycoumarin) (**3**) (92%, Scheme 2).

Scheme 1



Reagents: a) NBS, CCl_4, cat. *tert*-BuOOH; b) Bu_4NIO_4, dioxane; c) EtOH; d) 4-Hydroxybenzaldehyde (**8**), K_2CO_3, DMF.

Scheme 2



Reagents: a) 4-Hydroxybenzaldehyde (**8**), DCC, Et_2O-dioxane (1:1); b) EtOH.

RESULTS AND DISCUSSION

Benzophenone photophores have proven to be very useful in a variety of different systems.[4] In this paper we report the synthesis of benzophenone aldehydes (**6**, **9** and **11**), which upon further condensation with 4-hydroxycoumarin, provided three dimeric coumarin analogues (**1** - **3**) that exhibited high HIV-1 integrase inhibitory potency (Table 1). By undergoing photo-induced covalent tagging of the ligand binding domain of the integrase enzyme, these inhibitors may potentially represent important probes for studying the interaction of inhibitors with the catalytic core.

Table 1

Compound	HIV-1 Integrase IC_{50} (μM)	
	3'-Processing	Strand Transfer
1	18.6	10
2	9.9	10
3	11.5	4.2

EXPERIMENTAL SECTION

Chemistry. Melting points were taken on a Mel Temp II melting point apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlab Inc., Norcross, GA. IR (KBr) spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer and ^{1}H NMR data were obtained on a Bruker AC250 (250 MHz) instrument. Fast atom bombardment mass spectra (FABMS) were acquired with a VG Analytical 7070E mass spectrometer under the control of a VG 2035 data system. Flash column chromatography was performed using E. Merck silica gel 60 (particle size, 0.04-0.063 mm).

4-Bromomethylbenzophenone (5). A solution of 4-methylbenzophenone (**4**) (1.96 g, 10 mmol) *N*-bromosuccinimide (1.95 g, 11 mmol), and *tert*-butyl peroxide (30 mg) in carbon tetrachloride (100 mL) was refluxed overnight under irradiation with a sun lamp. After cooling to rt, the succinimide was filtered off, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (EtOAc : hexane, 1:5) and crystallized from acetone-hexane to provide **5** as a white solid (1.3 g, 47%), mp 110-112 °C (lit.,[11] 103-105 °C). ^{1}H NMR ($CDCl_3$) δ 7.8-7.76 (m, 4H), 7.62-7.55 (m, 1H), 7.51-7.44 (m, 4H), 4.52 (s, 2H). Anal. Calcd for $C_{14}H_{11}OBr$: C, 61.11; H. 4.03. Found: C, 61.08; H, 4.08.

4-Phenylcarbonylbenzaldehyde (6). 4-Bromomethylbenzophenone (**5**) (550 mg, 2 mmol) in dioxane (2.5 mL) was treated with tetrabutylammonium periodate (550 mg, 2 mmol) at reflux overnight. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (EtOAc : hexane, 1:3) and crystallized from acetone-hexane to provide **6** as a white solid (246 mg, 59%), mp 57-59 °C (acetone-hexane)(lit.,[13] 62-65 °C).^{1}H NMR ($CDCl_3$) δ 10.12 (s, 1H), 8.01-7.9 (m, 3H), 7.81-7.78 (m, 2H), 7.62-7.47 (m, 4H). Anal. Calcd for $C_{14}H_{10}O_2$: C, 79.98; H, 4.79. Found: C, 80.00; H, 4.84.

3,3'-(4-Phenylcarbonylbenzylidene)-bis-(4-hydroxycoumarin) (1). A mixture of 4-hydroxycoumarin (**7**) (324 mg, 2 mmol) and 4-phenylcarbonylbenzaldehyde (**6**) (210 mg, 1 mmol) in EtOH (5 mL) was refluxed overnight. After cooling to rt, the white solid was filtered and dried in vacuo to provide **1** as a white solid (403 mg, 78%), mp 269-271 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 7.89-7.86 (m, 2H), 7.72-7.69 (m, 2H), 7.63-7.5 (m, 7H), 7.34-7.25 (m, 6H), 6.93 (br s, 2H), 6.40 (s, 1H); IR (KBr) 3448, 1664, 1654, 1648, 1560, 766; FABMS m/z 517 (MH^+). Anal. Calcd for $C_{32}H_{20}O_7$: C, 74.41; H, 3.90. Found: C, 74.32; H, 3.96.

4-(4'-Phenylcarbonylbenzyloxy)benzaldehyde (9). A solution of 4-hydroxybenzaldehyde **8**) (244 mg, 2 mmol) and 4-bromomethylbenzophenone (**5**) (550 mg, 2 mmol) in DMF (6 mL) was treated with anhydrous potassium carbonate (553 mg, 4 mmol) and the resulting mixture was heated at 60-70 °C overnight. The solid was filtered off through silica gel and DMF was removed under reduced pressure. The residue was purified by crystallization to provide **9** as a white solid (420 mg, 66%); mp 94-96 °C (acetone-hexane). ^{1}H NMR ($CDCl_3$) δ 9.89 (s, 1H), 7.87-7.78 (m, 6H), 7.59-7.45 (m, 5H), 7.08 (m, 2H); 5.23 (s, 2H). Anal. Calcd for $C_{21}H_{16}O_3$: C, 79.73; H, 5.10. Found C, 79.45; H, 5.22.

3,3'-(4-Phenylcarbonylbenzyloxybenzylidene)-bis-(4-hydroxycoumarin) (2). Reaction of 4-hydroxycoumarin (**7**) (324 mg, 2 mmol) and 4-[(4'-phenylcarbonyl)benzyloxy]benzaldehyde (**9**) (316 mg, 1 mmol) in EtOH (5 mL) as described above for the preparation of **1**, provided **2** as a white solid (575 mg, 92%), mp 121-126 °C (EtOH). ^{1}H NMR (DMSO-d_6) δ 7.86 (dd, J = 7.9, 1.4 Hz, 2H), 7.76-7.72 (m, 4H), 7.64-7.53 (m, 7H), 7.34-7.26 (m, 4H), 7.05 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H),

6.26 (s, 1H), 5.60 (br s, 2H), 5.16 (s, 2H); IR (KBr) 3448, 1664, 1618, 1560, 1508, 1279, 763; FABMS *m/z* 623 (MH^+); Anal. Calcd for $C_{39}H_{26}O_8 \cdot {}^1/_4H_2O$: C, 74.69; H, 4.26. Found: C, 74.63; H, 4.36.

4-Formylphenyl 4-phenylcarbonylbenzoate (11). To a solution of 4-hydroxybenzaldehyde (**8**) (610 mg, 5 mmol) and 4-benzoylbenzoic acid (**10**) (1.13 g, 5 mmol) in ether-dioxane (1:2, 30 mL) was added DCC (1.05 g, 5.1 mmol) and the mixture was stirred overnight at rt. The dicyclohexylurea was removed by filtration, and the filtrate taken to dryness. Residue was purified by silica gel chromatography (Et_2O : hexane, 1:3) and crystallized from acetone-hexane to provide **11** as a white solid (550 mg, 65%), mp 132.5-134 °C (acetone-hexane). ^1H NMR ($CDCl_3$) δ 10.03 (s, 1H), 8.31 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.91 (d, *J* = 8.3 Hz, 2H), 7.83-7.74 (m, 3H), 7.64-7.42 (m, 4H). Anal. Calcd for $C_{21}H_{14}O_4$) C, 76.36; H, 4.27. Found: C, 76.24; H, 4.40.

3,3'-[(4-Phenylcarbonylbenzoyloxy)benzylidene]-bis-(4-hydroxycoumarin) (3). Reaction of 4-hydroxycoumarin (**7**) (540 mg, 3.32 mmol) and 4-formylphenyl 4-phenylcarbonylbenzoate (**11**) (550 mg, 1.66 mmol) in EtOH (8 mL) as described above for the preparation of **1,** provided **3** as a white solid (575 mg, 92%), mp 191-193 °C (EtOH). ^1H NMR (DMSO-d_6) δ 8.27 (d, *J* = 8.4 Hz, 2H), 7.91-7.88 (m, 4H), 7.79-7.68 (m, 3H), 7.61-7.54 (m, 4H), 7.35-7.13 (m, 8H), 6.35 (s, 1H), 6.20 (br s, 2H); IR (KBr) 3448, 1670, 1654, 1618, 1560, 1508, 1263, 760; FABMS *m/z* 637 (MH^+). Anal. Calcd for $C_{39}H_{24}O_9$: C, 73.58; H, 3.80. Found: C, 73.43; H, 3.93.

Integrase Assay. Determination of IC_{50} values against isolated HIV-1 integrase perparations was performed as previously reported.[14]

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