

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 dimer; 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 non-toxic 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 ($IC_{50}=1.5\ \mu M$) and strand transfer ($IC_{50}=0.8\ \mu M$). 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 dimer 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 dimer appending with hydrophobic moiety (**IV**). In this report, we describe the synthesis of chemically modified coumarin dimer 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-sulfonyloxy-

benzaldehyde (**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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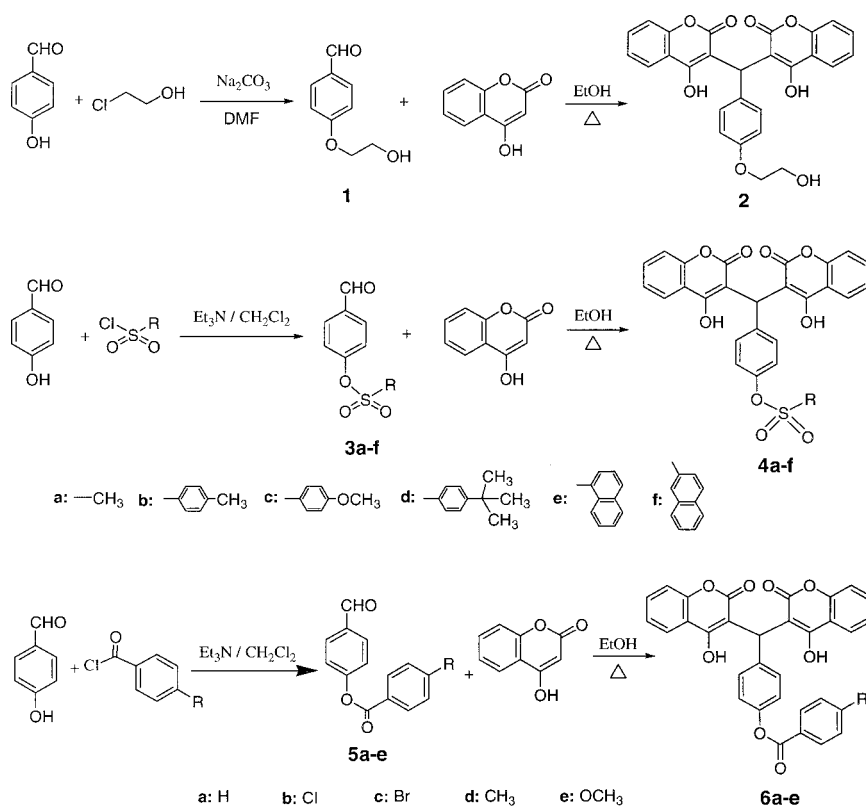


Chart 1

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

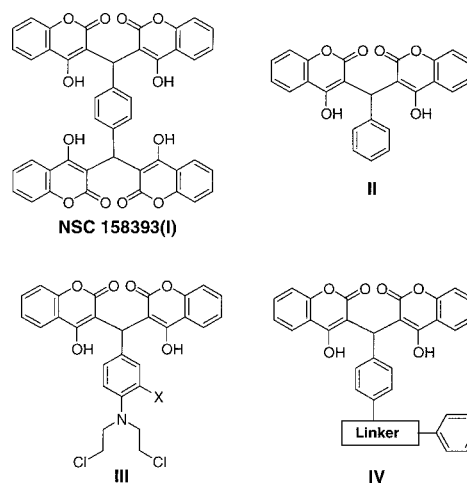


Fig. 1

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). ¹H- and ¹³C-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 P₂O₅ for at least 12 h unless otherwise specified. Elemental analyses were obtained from Perkin-Elmer 2400 Elemental Analyzer.

ume of 20 μ l 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 MgCl₂, 25 mM NaCl, 1 mM DTT]. Products were separated on a 18% acrylamide/urea denaturing gel and quantified on a phosphorimager 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 μ l. 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 μ g/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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