Chemical Library and Structure–Activity Relationships of 11-Demethyl-12-oxo Calanolide A Analogues as Anti-HIV-1 Agents

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(+)-Calanolide A (**1**) as a natural product was previously found as an inhibitor of HIV-1 reverse transcriptase. In our further investigation of its template, racemic 11-demethyl-12-oxo calanolide A (**15**), which had two fewer chiral carbon centers at the C-11 and C-12 positions than (+)-calanolide A, had a comparably inhibitory activity and better therapeutic index (EC_{50} = 0.11 μM, TI = 818) against HIV-1 in vitro. A library based on its structural core was then designed and synthesized with introduction of nine diversity points in this article. The evaluations of anti-HIV-1 activity in vitro concluded their structure–activity relationships (SARs). A novel compound (10-bromomethyl-11-demethyl-12-oxo calanolide A, **123**) was identified to have much higher inhibitory potency and therapeutic index (EC_{50} = 2.85 nM, TI > 10,526) than those of the class compound against HIV-1. This finding provided a very important clue that modifications of the C ring at the C-10 position may be conducted to obtain drug candidates with better activity against HIV-1.

Introduction

Human immunodeficiency virus (HIV[a]) is the cause of acquired immunodeficiency syndrome (AIDS). Since its first report in 1981,[1] AIDS has spread rapidly through the human population worldwide. The development of chemotherapeutic strategies for AIDS has recently been considered one of the most challenging scientific projects.[2] The United States Food and Drug Administration (FDA) has approved several anti-HIV drugs, including seven nucleoside reverse transcriptase inhibitors (NRTIs), one nucleotide reverse transcriptase inhibitor (NtRTI), three nonnucleoside reverse transcriptase inhibitors (NNRTIs), nine protease inhibitors (PIs), and one fusion inhibitor.[3] Although highly active antiretroviral therapy (HAART) using a combination of anti-HIV drugs has been highly effective in suppressing the HIV load and decreasing mortality in AIDS patients, the emergence of drug resistance among HIV carriers, the unavoidable viral recurrency after drug treatments, and the toxicity of the therapies have made the continued search for novel anti-HIV drugs necessary.[4,5]

In 1992, Kashman[6] reported (+)-calanolide A (**1**, Chart 1), isolated from a tropical rainforest plant of the species *Calophyllum lanigerum*, to be active against HIV-1, with a half-maximal effective concentration (EC_{50}) of 0.1 μM and therapeutic index in the range of 16–279 in various cell lines.[7] (+)-Calanolide A inhibits not only wild-type HIV-1 but also clinically isolated resistant strains such as A17 (Y181C mutant). Studies (phase I) of single doses and multiple escalating doses of (+)-calanolide A have demonstrated its safety and a pharmacokinetic profile, which could significantly reduce viral load in HIV patients with a dose of 600 mg (bid) in a monotherapeutic trial, worthy of further clinical study.[8,9] Interestingly, it was demonstrated that (+)-calanolide A also inhibited *Mycobacterium* tuberculosis (TB) at a maximum inhibitory concentration (MIC) of 3.13 μg/mL.[10] The data generated in these studies have shed light on the application of natural products as therapeutic agents for AIDS and/or TB.

(+)-Calanolide A is the first natural product identified as active against HIV-1 and has recently been investigated continuously in phase II/III clinical trials. However, no final report or other communication has been evaluated by the US FDA since the beginning of clinical trials in 1997. Its lower inhibitory potency may be the reason accounting for such long-term clinical studies.

Since then, some other coumarin analogues, such as (+)-inophyllum B (**2**)[11] and (+)-cordatolide A (**3**, Chart 1),[12] have also been isolated from plants of the genus *Calophyllum* and identified to be specific HIV-1 reverse transcriptase inhibitors. They have the common scaffold, tetracyclic dipyranocoumarin, but different groups substituted at the C-4 position. Apparently, there are three chiral carbon centers in the scaffold at the C-10, -11, and -12 positions. Because of their limited availability from natural resources, the total synthesis of these compounds and their derivatives has already been undertaken.[7,13–23]

In our previous studies[15,16] (Scheme 1) using phloroglucinol (**6**) as the starting material, racemic calanolide A was obtained through the consecutive construction of the three skeletal rings, coumarin (rings A and B, **7**), 2,3-dimethylchromanone (ring C, **8**), and 2,2-dimethylchromene (ring D, **10**). The acylation of 5,7-dihydroxy-4-propyl-2*H*-chromen-2-one (**7**) and ring closure were achieved simultaneously to produce **8** with the Friedel–Crafts reaction by using tigloyl chloride in the presence of $AlCl_3$. Compound **10** was then obtained by condensation of **8** with 1,1-diethoxy-3-methyl-2-butene (**11**) under pyridine catalysis. The 10,11-*trans* and -*cis* isomers were separated from the

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[a] Abbreviations: HIV-1, human immunodeficiency virus (type I); SARs, structure–activity relationships; AIDS, acquired immunodeficiency syndrome; FDA, Food and Drug Administration (USA); NRTIs, nucleoside reverse transcriptase inhibitors; NtRTI, nucleotide reverse transcriptase inhibitor; NNRTIs, nonnucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; HAART, highly active antiretroviral therapy; EC_{50}, half-maximal effective concentration; TB, *Mycobacterium* tuberculosis; MIC, maximum inhibitory concentration; PPA, polyphosphoric acid; TBAF, tetrabutyl ammonium fluoride; THF, tetrahydrofuran; TLC, thin-layer chromatography; tosyl, 4-toluenesulfonyl; EDC·HCl, 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride.

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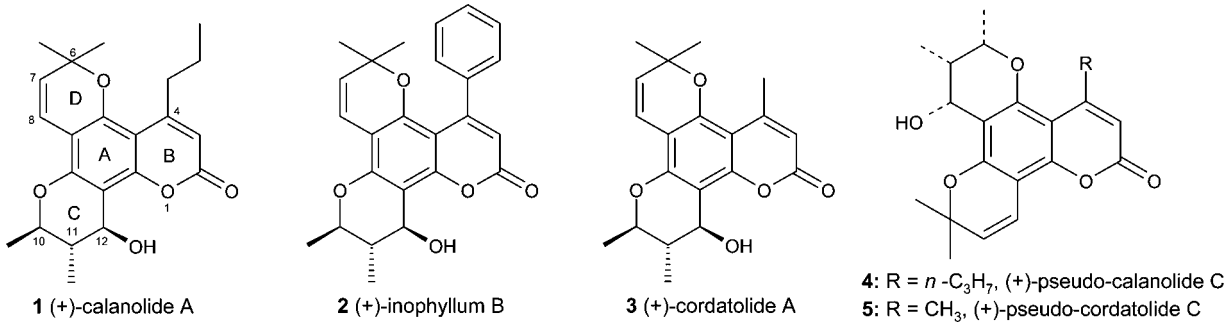
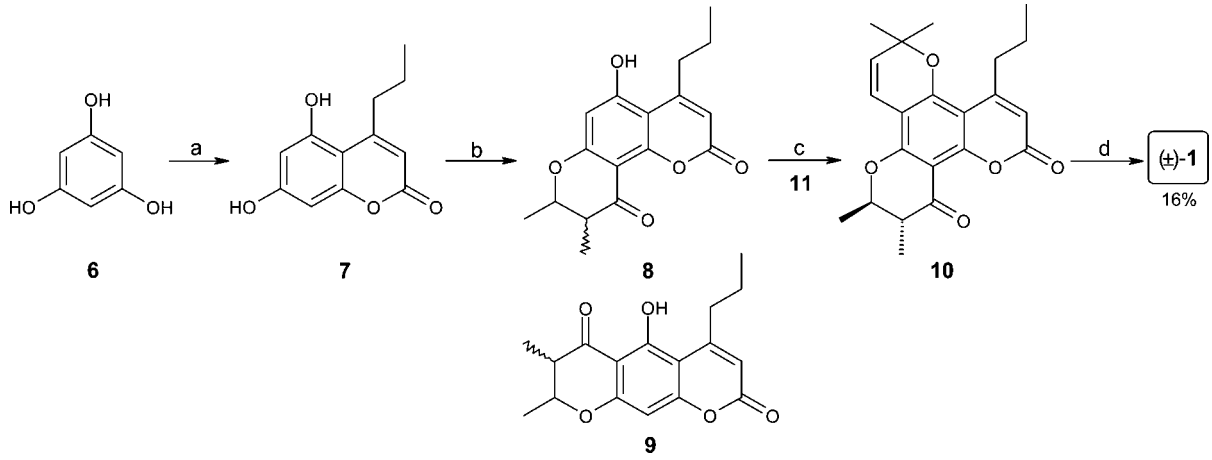
Chart 1. Chemical Structures of the Biologically Active Natural Dipyranocoumarins

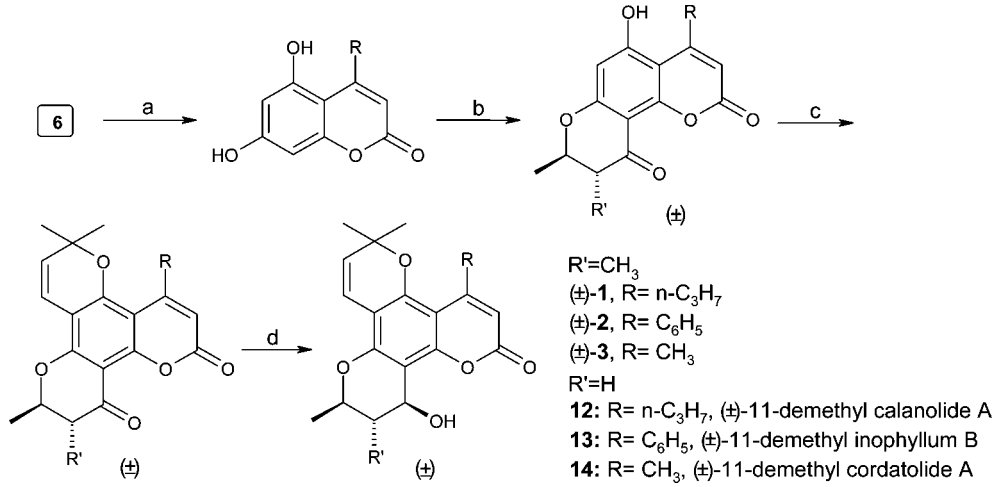


Scheme 1. Synthesis of Racemic Calanolide A by Zhou[a]



[a] Reagents and conditions: (a) $C_3H_7COCH_2COOC_2H_5$, H_2SO_4; (b) (i) tiglic acid, $SOCl_2$, reflux, (ii) $AlCl_3$, $PhNO_2/CS_2$, reflux, 16 h, (iii) column chromatography; (c) pyridine, toluene, reflux, 6 h; (d) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, 0 °C.

Scheme 2. Optimized Synthetic Route of the Racemic *Calophyllum* Coumarins[a]



[a] Reagents and conditions: (a) $RCOCH_2COOC_2H_5$, HCl/CH_3OH; (b) tiglic acid (or crotonic acid), PPA; (c) 1,1-diethoxy-3-methyl-2-butene (**11**), pyridine/toluene, microwave irradiation, 140 °C, 150 W, 120 psi, 20 min; (d) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, 0 °C.

mixture by silica gel column chromatography. Racemic inophyllum B and cordatolide A were also prepared by using this synthetic strategy.[24,25] The chemical resolution of racemic calanolide A and cordatolide A was developed.[26]

Further improvements of the reaction conditions were made. For instance, polyphosphoric acid (PPA) was first used as both catalyst and solvent to obtain the angular chromanone **8**,[27] and microwave irradiation was recently used to significantly accelerate the construction of ring D (**10**). The PPA method gave the highest ratio of the anticipated angular compound **8** (70%) to the unavoidable linear compound **9** (9%). Obviously, such improvements markedly facilitated the total synthesis of calanolide A, inophyllum B, cordatolide A, and their 11-demethyl analogues with fewer reaction steps and higher overall yields (Scheme 2).[28]

Previous studies have demonstrated that (±)-11-demethyl calanolide A (**12**) also has inhibitory activity against HIV-1, with an EC_{50} value of 0.31 μM and a range of therapeutic index from 21 to 169.[29] It inhibited different HIV-1 strains in cell culture, especially the NNRTI (L697661)-resistant strain, con-

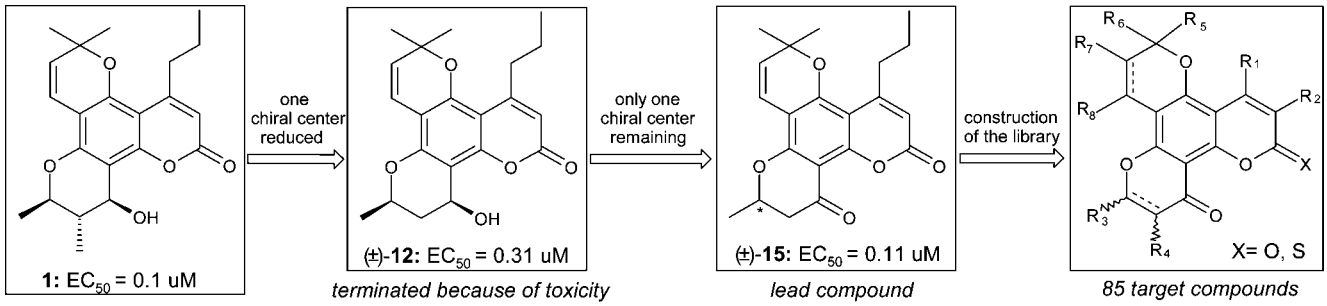
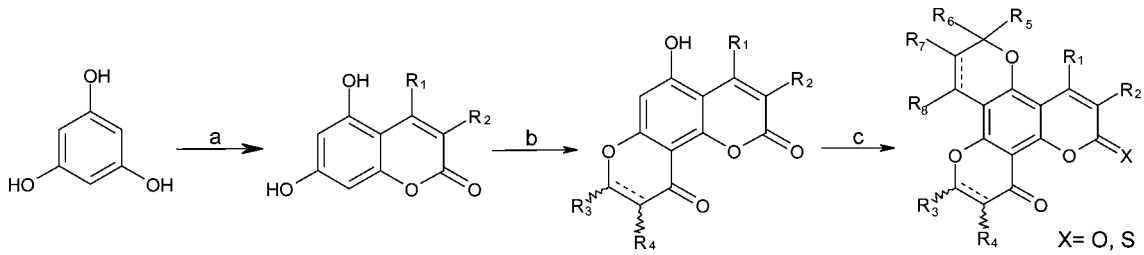


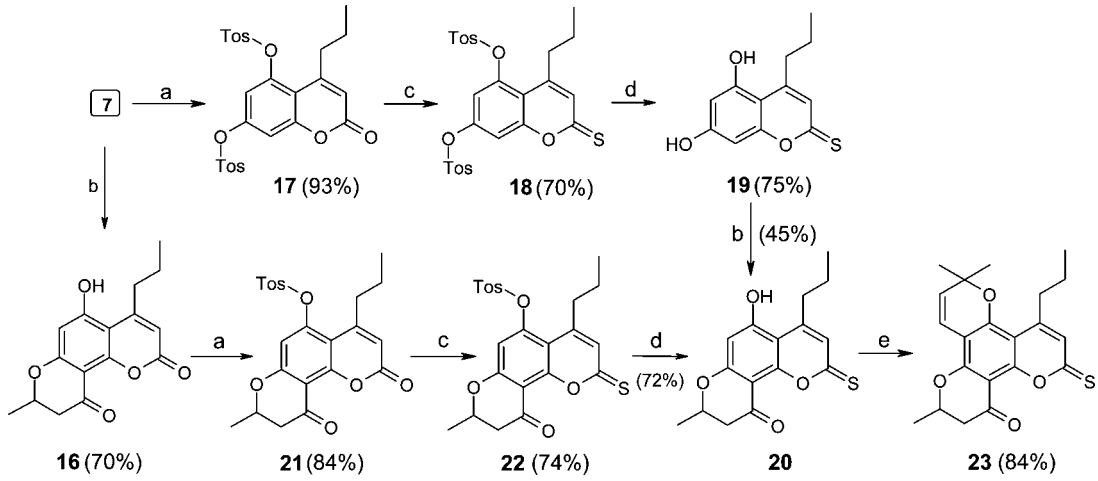
Figure 1. Identification of the lead compound and construction of the library.

Scheme 3. Synthetic Route for the Tetracyclicdipyranocourmines Library Construction[a]



[a] Reagents and conditions: (a) $R_1COCH(R_2)COOC_2H_5$, HCl/CH_3OH; (b) α,β-unsaturated acids, PPA; (c) α,β-unsaturated acetals, microwave irradiation.

Scheme 4. Synthesis of the Thio-Substituted Target Compound[a]



[a] Reagents and conditions: (a) *p*-methylbenzenesulfonyl chloride, pyridine; (b) crotonic acid, PPA; (c) P_2S_5, xylene, reflux, 5 h; (d) TBAF, THF, reflux; (e) compound **11**, pyridine/toluene, microwave irradiation, 140 °C, 150 W, 120 psi, 20 min.

taining the clinic Y181C mutant. In particular, compound **12** also exerted synergistic effects in combination with indinavir, AZT, and T-20.[29]Obviously, the removal of the methyl group at the C-11 position made it an attractive candidate for drug development because it could be synthesized much more conveniently without consideration of *trans*/*cis* configurations in ring C. Unfortunately, this research was terminated because of the toxicity of the compound for cells tested both in vitro and in mice because of its relatively lower inhibitory potency.[29]

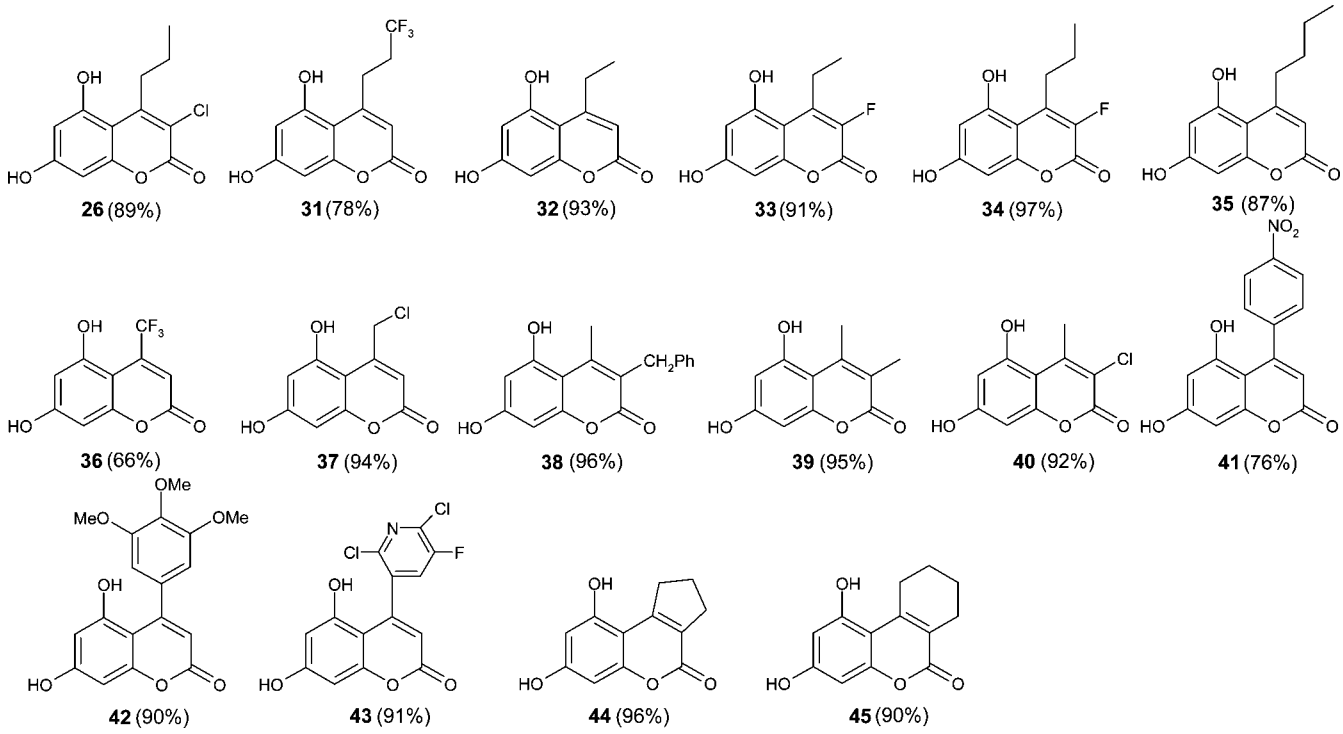
Fortunately, 11-demethyl-12-oxo calanolide A (**15**), which is the precursor of (±)-11-demethyl calanolide A synthesis, also has similar inhibitory activity against HIV-1, with an EC_{50} value of 0.11 μM and a better therapeutic index (818). Because there is only one chiral carbon center remaining in the structure, this result encouraged us to pursue further studies of its structure–activity relationships (SARs) by using our combinatorial chemistry technology. Therefore, a chemical library based on tetracyclic dipyranocoumarin (**15**) was constructed (Figure 1, Scheme 3).[28] In all, nine diversity points were finally introduced through structural modifications, focusing on rings B, C, and D. A total of 85 aimed compounds were synthesized in parallel and then biologically evaluated against HIV-1 in this study.

Chemistry

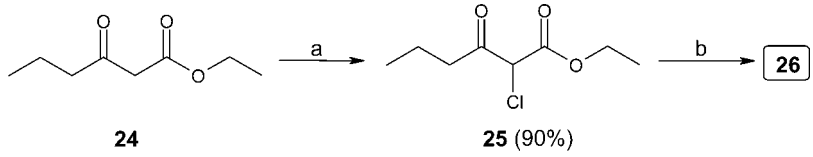
Diversity of Ring B. The replacement of the C═O of the ester with C═S in ring B was first explored. We successfully replaced the C═O with a C═S in the presence of thionation reagent.[30] Scheme 4 illustrates the practical strategies used to achieve this replacement. The hydroxyl group(s) was protected by a tosyl[31] group when the oxygen atom was substituted under P_2S_5 or Lawesson's reagent in either method.

The selective removal of the 5,7-ditosyl protective groups of compound **18** in the presence of tetrabutyl ammonium fluoride (TBAF) was also observed in our experiments. The tosyl group at the 5-*O* position was selectively removed in the presence of TBAF at room temperature, whereas the other tosyl protecting

Chart 2. Diversities of R_1 and R_2 in Coumarins

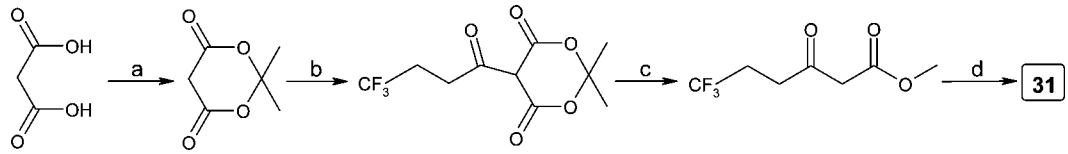


Scheme 5. Synthetic Route of Chloro-Substituted Coumarin at R_2[a]



[a] Reagents and conditions: (a) SO_2Cl_2, ether; (b) compound **6**, HCl/CH_3OH.

Scheme 6. Introduction of the $CH_2CH_2CF_3$ Group of R_1[a]



[a] Reagents and conditions: (a) acetone, concentrated H_2SO_4; (b) $CF_3CH_2CH_2COOH$, EDC·HCl; (c) CH_3OH, reflux; (d) compound **6**, HCl/CH_3OH.

group at 7-*O* had to be removed at a higher reaction temperature, such as under solvent refluxing conditions or microwave irradiation. This selective deprotection of the two protective groups was used as an alternative strategy during our following structural modifications focusing on ring C.

To explore the diversities of R_1 and R_2 on ring B, besides commercially available β-oxo esters, two methods of varying β-oxo esters were used to construct 3,4-substituted coumarins (Chart 2) through Pechmann condensation, as outlined in Schemes 5[32] and 6.[33]

The corresponding coumarins diversified at the R_1 and R_2 positions (**26**, **31**–**45**) were illustrated in Chart 2.

The coumarins were reacted with crotonic acid in the presence of PPA to offer their corresponding angular chromanones, respectively (**46**–**61**, Chart 3), and also to produce some linear byproducts (**62**–**70**, Chart 4).

When compound **42** was reacted with crotonic acid in the presence of PPA to produce its chromanone, the 7-*O*-acylated (**71**) and 5,7-di-*O*-acylated (**72**, Chart 5) intermediates were also observed.

Compound **52** was further modified through the nucleophilic substitutions to attain compounds **73** and **74**. Compound **52** also could be dimerized to offer **75** through piperazine as a linker (Chart 6).

The purified angular chromanones (Chart 3) were condensed with compound **11** under parallel microwave irradiation to produce their target tetracyclic dipyranocoumarin compounds (**76**–**91**). Compound **82** was further modified through the nucleophilic substitutions to attain compounds **92** and **93**. Compound **86** was reduced by $SnCl_2 \cdot 2H_2O/HCl$ that converted the electron-withdrawing nitro group into an electron-donating amino group (**94**, Table 1).

Chart 3. Diversities of R_1 and R_2 in Angular Chromanones

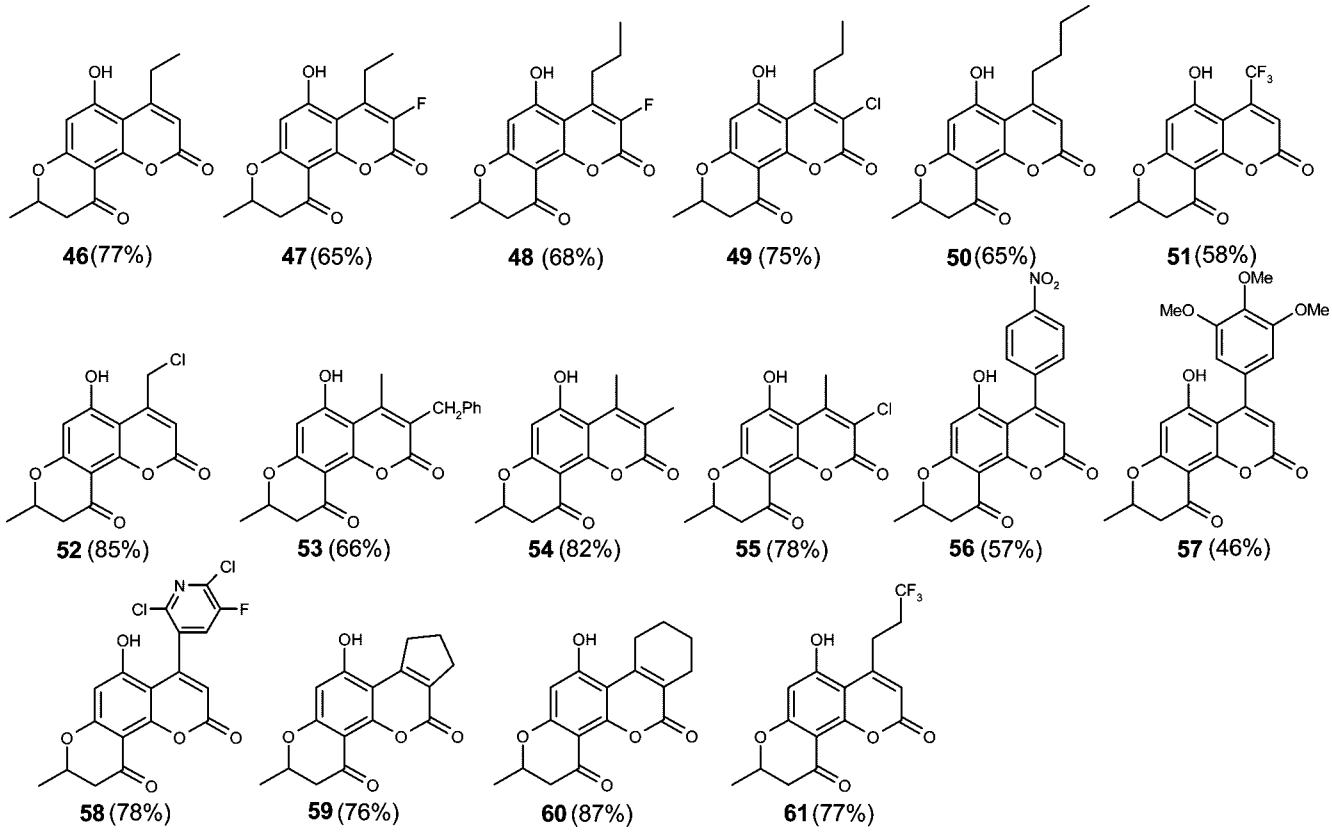
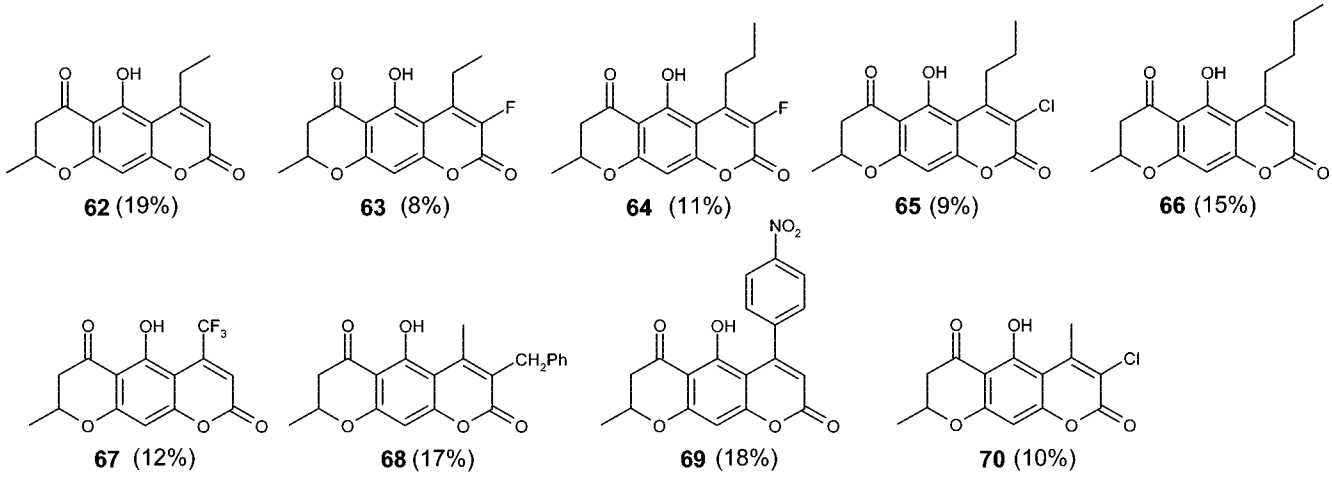


Chart 4. Diversities of R_1 and R_2 in Linear Chromanones



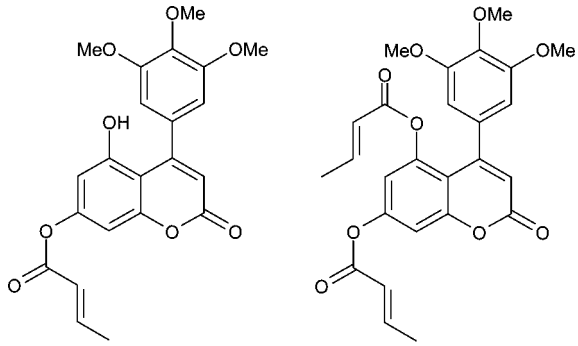
Diversity of Ring C. The following commercially available or premade α,β-unsaturated acids (Chart 7) were used as the building blocks for the introduction of diversities at the R_3 and R_4 positions on ring C.

Compound **7** was reacted with the α,β-unsaturated acids mentioned above in the presence of PPA to produce their corresponding angular chromanones, respectively (**95**–**103**), and also some linear byproducts (**104**–**106**, Chart 8).

Further eliminating the last chiral center of **15** was also investigated in this article. The anticipated compound should be 10,11-didemethyl-12-oxo-calanolide A. According to the method described above, acrylic acid was used to construct the target angular chromanone in PPA. However, only three byproducts were identified, 7-*O*-acylated (**107**), 5-*O*-acylated (**108**), and 5,7-di-*O*-acylated (**109**) intermediates from the reactant mixture, instead of the anticipated closed-ring compound. The replacement of PPA with CF_3SO_3H was also tested but only produced a novel byproduct (**110**) that was 7-*O*-acylated with a closed ring at the C-8 position (Scheme 7).

Interestingly, when compound **7** was reacted with crotonic acid in the presence of CF_3SO_3H, another closed-ring product (**111**) with 6-acylation was produced, together with a linear byproduct (**112**, Scheme 8). Unfortunately, we could not produce the corresponding tetracyclic product from compound **111** by direct condensation with compound **11**, which would have been an analogue of pseudocalanolide C[34] (**4**, Chart 1). The chemical shift of the hydroxyl group in compound **111** was 12.483 ppm, which was downfield when compared with that of compound **16** (11.760 ppm). This indicates that a hydrogen bond formed between the adjacent carbonyl and hydroxyl groups. Perhaps,

Chart 5. 7-*O*-Acylated and 5,7-Di-*O*-acylated Coumarins as Side Products



71 (6%) **72** (8%)

this hydrogen bond reduced the reactant activity of the hydroxyl group, rendering the construction of the chromene ring (D ring) unsuccessful.

Surprisingly, when the following α,β-unsaturated acids were reacted with compound **7** in the presence of PPA, halogen atoms and substituted phenyl groups were all removed. Only the common 8,9-unsaturated chromanone (**113**, Chart 9) was obtained in each reaction. The detailed mechanisms are currently under further investigation.

The angular chromanone compounds produced (**95**–**103**, **110**, and **113**) were successfully condensed with compound **11** under parallel microwave irradiation to generate their corresponding tetracyclic dipyranocoumarin compounds (**114**–**124**, Table 2).

Diversity of Ring D. To introduce diversity at points R_5, R_6, and R_7, the selected building blocks, α,β-unsaturated acetals (**125**–**132**, Chart 10), were freshly synthesized with the corresponding commercially available α,β-unsaturated aldehydes and EtOH in the presence of $HC(OEt)_3$.[15]

Under parallel microwave irradiation, the angular chromanone compounds were reacted with acetal **125** to generate 6-methyl-substituted tetracyclic dipyranocoumarin compounds at the R_5 (or R_6) positions (**133**–**139**, Table 3).

With **126** as the building block, 6-ethyl-substituted tetracyclic dipyranocoumarin compounds at the R_5 (or R_6) positions were obtained (**140**–**144**, Table 4).

With **127** as the building block, 6-*n*-propyl-substituted tetracyclic dipyranocoumarin compounds at the R_5 (or R_6) positions were produced (**145**–**151**, Table 5).

With **128** as the building block, 6-propene-substituted tetracyclic dipyranocoumarin compounds at the R_5 (or R_6) positions were obtained (**152**–**157**, Table 6).

With **129** as the building block, 6-*n*-butyl-substituted tetracyclic dipyranocoumarin compounds at the R_5 (or R_6) positions were produced (**158**–**165**, Table 7).

With **130** as the building block, 6-phenyl-substituted tetracyclic dipyranocoumarin compounds at the R_5 (or R_6) positions were obtained (**166**–**173**, Table 8).

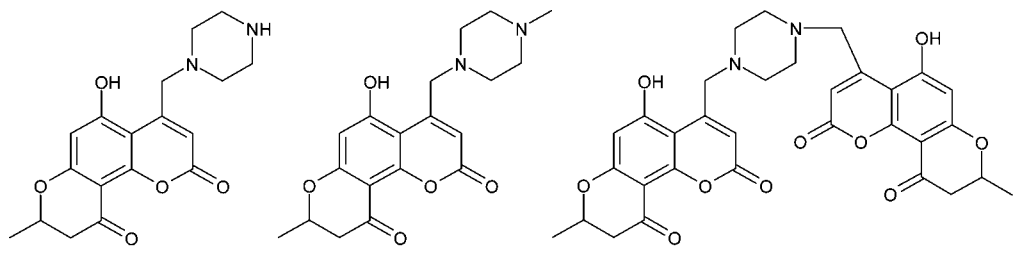
Chart 6. Nucleophilic Substitutions in the Angular Chromanones

Under parallel microwave irradiation, the angular chromanone compounds were reacted with acetal **131** to offer the 7-methyl-substituted tetracyclic dipyranocoumarin compounds at the R_7 positions (**174**–**178**, Table 9), along with their side products with $\Delta^{7,8}$-saturation by EtOH (**179**–**181**, Table 10). When compound **16** was reacted with acetal **132** under microwave irradiation, the anticipated *o*-NO_2-phenyl-substituted tetracyclic dipyranocoumarin compound (**182**, Table 9) along with a byproduct (**183**, Table 10) was yielded, which was also further reduced by $SnCl_2 \cdot 2H_2O$ to offer a *o*-NH_2-phenyl-substituted product (**184**, Table 9).

Novel Synthetic Strategy for the Construction of Ring D and Ring C. As mentioned above, the selective removal of the two protective tosyl groups at O-5 and O-7 of compound **17** was able to be adopted (Scheme 9). When the tosyl group at the O-5 position was primarily deprotected in the presence of TBAF at room temperature (**185**), the chromene ring (ring D) was able to be constructed through subsequent condensation with compound **11** (**186**). Afterward, the remaining tosyl group at the O-7 position could be removed by TBAF under solvent refluxing or microwave irradiation conditions to attain **187**. Finally, the chromanone ring (ring C) was constructed through the reaction with acyl chloride in the presence of a strong Lewis acid, such as BF_3/Et_2O.[35] This strategy allowed us to introduce the molecular diversities into ring C at the last reaction step in a parallel manner, which made the synthesis and purification of the final products much easier.

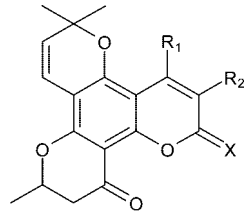
Structure–Activity Relationships (SARs) of 11-Demethyl-12-oxo Calanolide A Analogues against HIV-1. The inhibitory activities against HIV-1 of all compounds were tested in vitro by using a pseudotyped viral assay, as described previously.[36] Zembower et al.[37] previously reported that 10,11-*trans* or 10,11-*cis* 12-ketones of calanolide A analogues exerted an inhibitory activity against HIV-1 but with a lower potency than that of (+)-calanolide A. Here, we report for the first time that 11-demethyl-12-oxo calanolide A (**15**) is active against HIV-1, with an EC_{50} value of 0.11 μM and a higher therapeutic index (818) than that of (+)-calanolide A. This result indicates that the chiral carbon centers at the 11 and 12 positions are unnecessary for its anti-HIV-1 activity. This result encouraged us to prepare a chemical library of compound **15** to further study the structure–activity relationships of compound **15**. Thus, a chemical library with a maximum of nine diversity points on the scaffold was designed and synthesized in this study.

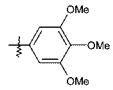
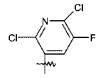
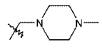
The C═O group was first replaced with a C═S group on ring B, according to the isostere principle. This modification resulted in an inactive compound (**23**). Position 4 (R_1) on ring B was linked with a short alkyl chain. A two- or three-carbon chain was required to maintain a similar level of activity, such as an ethyl (**76**, EC_{50} = 0.27 μM, TI > 1000) or an *n*-propyl (**15**, EC_{50} = 0.11 μM, TI = 818) substitution. However, a longer (*n*-butyl group) or shorter (methyl group) chain at R_1 was



73 (68%) **74** (81%) **75** (19%)

Table 1. Diversities of R_1 and R_2 in Target Compounds



compd	R_1	R_2	X	Yield (%)	Inhibition (%, 1.0 μM)	Toxicity (10 μM)[a]	EC_{50} (TI)[b]
15	*n*-C_3H_7	H	O	87	73.1	*c*	0.11 μM (818)
23	*n*-C_3H_7	H	S	84	*c*	*c*	*c*
76	C_2H_5	H	O	84	73.2	*c*	0.27 μM (> 1,000)
77	C_2H_5	F	O	85	*c*	++	*c*
78	*n*-C_3H_7	F	O	86	45.3	++	*c*
79	*n*-C_3H_7	Cl	O	85	68.1	*c*	0.37 μM (243–730)
80	*n*-C_4H_9	H	O	89	*c*	*c*	*c*
81	CF_3	H	O	87	*c*	*c*	*c*
82	CH_2Cl	H	O	80	*c*	*c*	*c*
83	CH_3	$CH_2C_6H_5$	O	82	*c*	*c*	*c*
84	CH_3	CH_3	O	79	*c*	*c*	*c*
85	CH_3	Cl	O	80	*c*	*c*	*c*
86	*p*-$NO_2C_6H_4$	H	O	75	51.9	*c*	*c*
87		H	O	77	58	*c*	*c*
88		H	O	81	*c*	*c*	*c*
89	cyclopentyl		O	71	*c*	*c*	*c*
90	cyclohexyl		O	79	67	*c*	*c*
91	$CH_2CH_2CF_3$	H	O	72	*c*	*c*	*c*
92		H	O	64	*c*	*c*	*c*
93		H	O	75	48.3	*c*	*c*
94	*p*-$NH_2C_6H_4$	H	O	63	*c*	*c*	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index (TI) was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

Chart 7. Diversities of R_3 and R_4

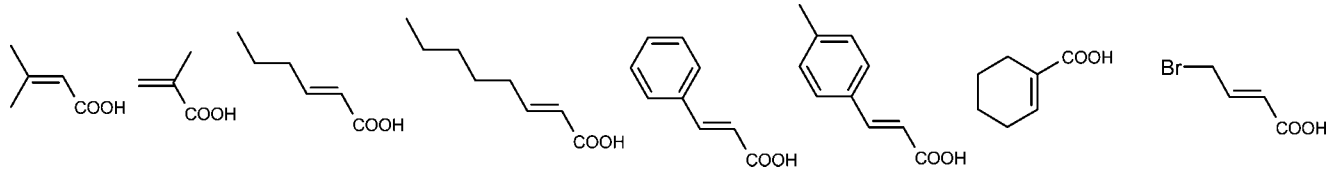
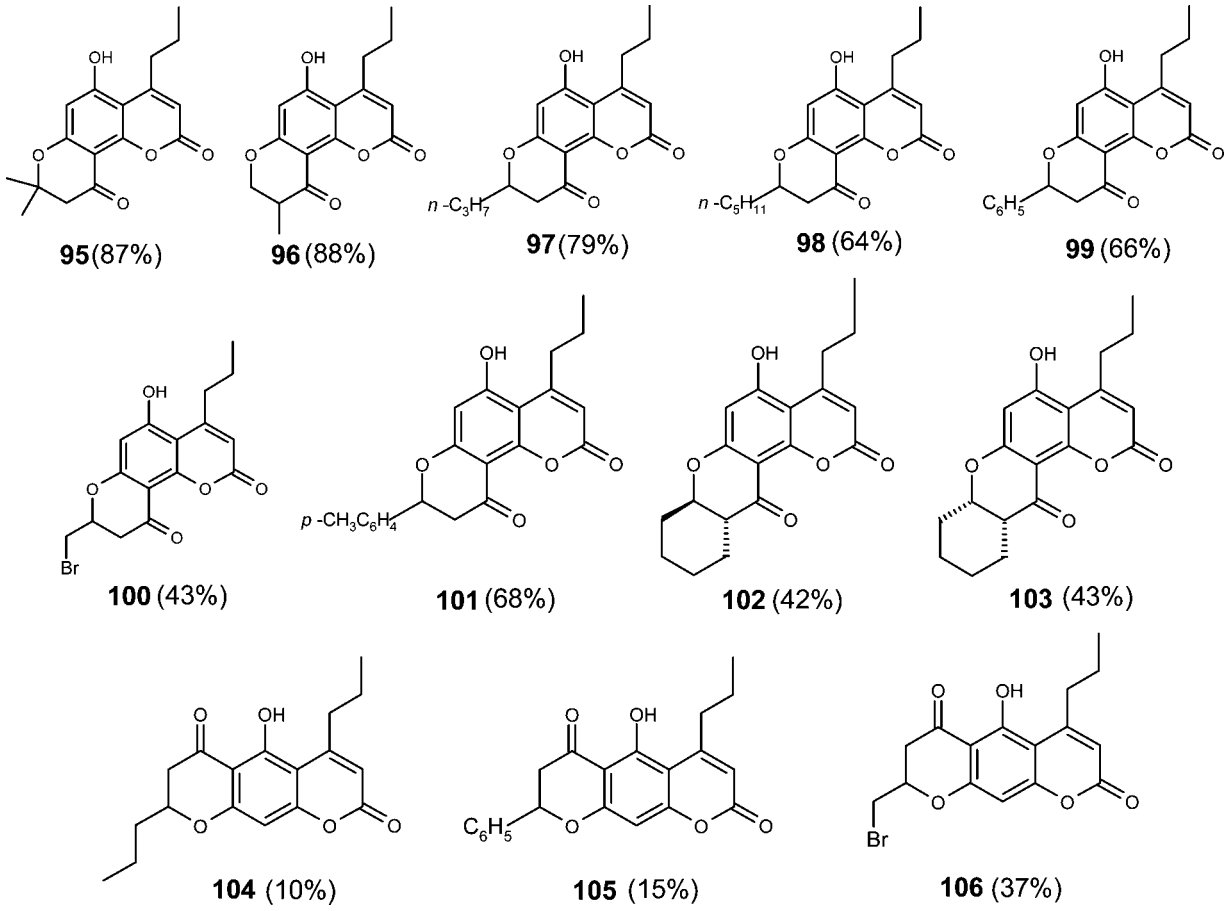
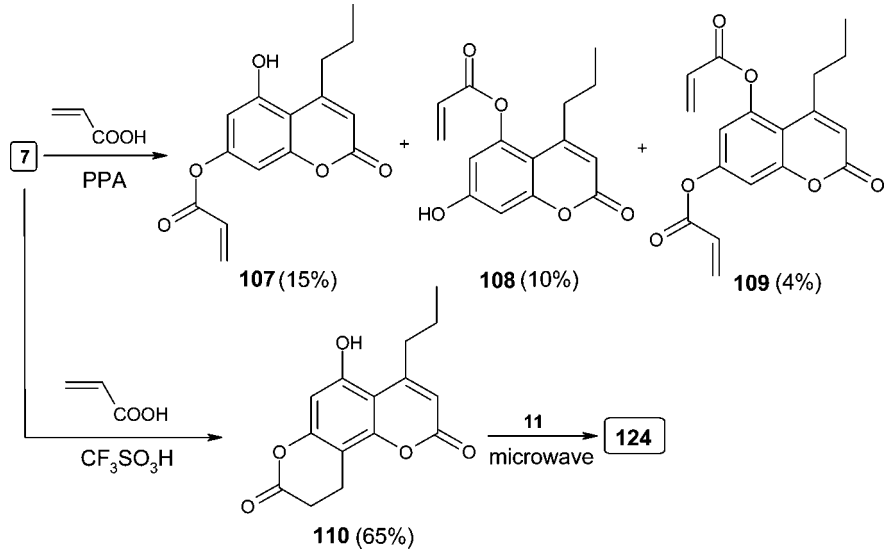


Chart 8. Diversities of R_3 and R_4 in Chromanones



Scheme 7. Side Products with Acrylic Acid as the Building Block



unfavorable. The replacement of R_1 with the aromatic groups (phenyl, substituted phenyl, or aromatic heterocycle) or piperazine (or *N*-methyl piperazine) made the compounds inactive. Furthermore, halogenations of **15** at R_1, such as with CF_3, CH_2Cl, or $(CH_2)_2CF_3$, also caused a loss of activity. Cyclization at R_1 and R_2 with a five- or six-membered ring to constrain the conformation did not improve its biological activity. From such multiple modifications and their results, we concluded that a hydrophobic R_1 of a proper length is necessary and is thought to engage in a compact hydrophobic interaction with the reverse transcriptase of HIV-1.

R_2 of ring B was modified with halogenation (F or Cl atom). Chlorine substitution (**79**) produced a similar level of activity ($EC_{50} = 0.37\ \mu M$, TI = 243–730) to that of (+)-calanolide A, whereas fluorine substitution (**77**, **78**) was both useless and cytotoxic at 10 μM. An alternative strategy to modify R_2 with hydrophobic methyl or benzyl groups also did not result in the anticipated anti-HIV-1 activity. Thus, modifications at the R_2

Scheme 8. Crotonic Acid in the Presence of CF_3SO_3H Offered a Novel Side Product

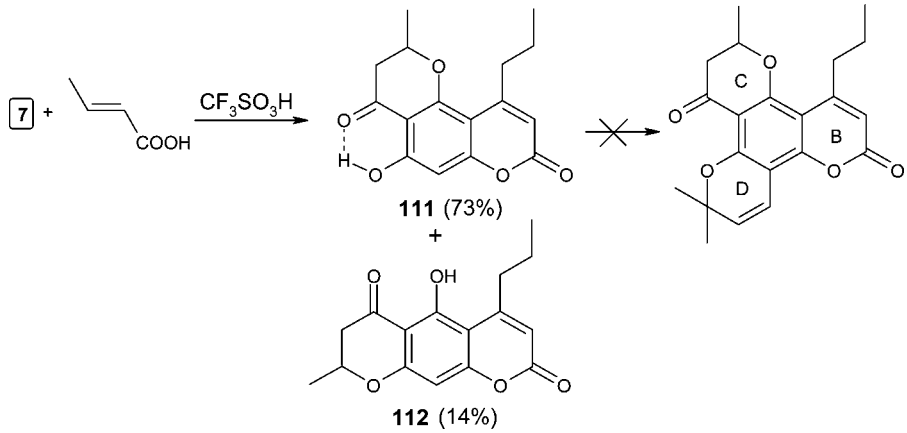


Chart 9. Some Special α,β-Unsaturated Acids and Their Common Chromanone

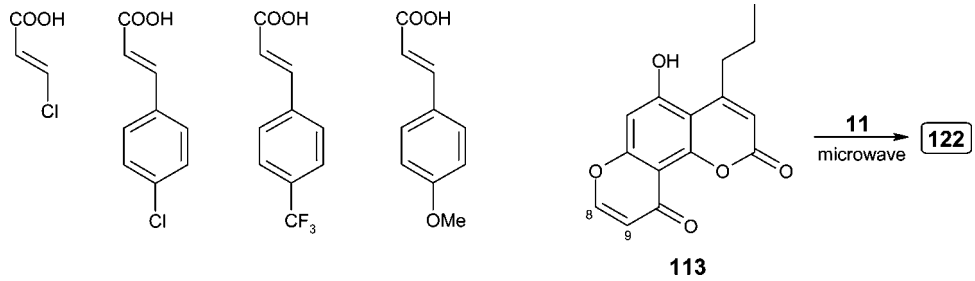
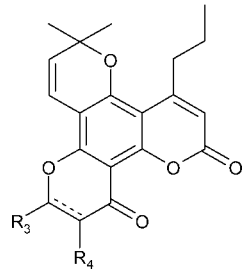


Table 2. Diversities of R_3 and R_4 in Target Compounds



compd	R_3	R_4	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
114	di-CH_3	H	80	68.3	*c*	0.64 μM (47–140)
115	H	CH_3	74	38.9	++	*c*
116	*n*-C_3H_7	H	81	61.9	+	32.5 nM (102–308)
117	*n*-C_5H_{11}	H	80	46.4	+	*c*
118	C_6H_5	H	82	62.7	+	*c*
119	*p*-$CH_3C_6H_4$	H	85	57	*c*	*c*
120	*trans*-cyclohexyl		80	40	+	*c*
121	*cis*-cyclohexyl		84	59.3	+	*c*
122	H $\Delta^{10,11}$	H	71	64.9	*c*	*c*
123	$BrCH_2$	H	88	93.5	*c*	2.85 nM (>10,526)
124	carbonyl,12-CH_2	H	72	*c*	*c*	*c*

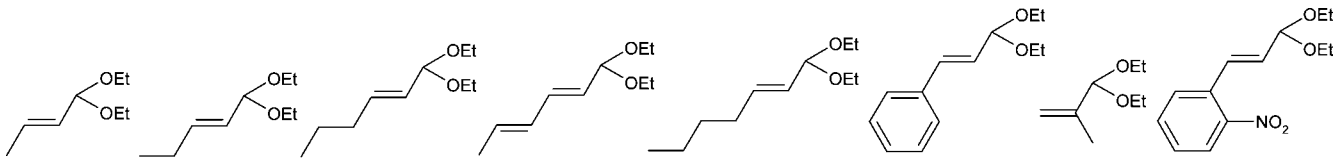
[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

position of ring B were not useful in the search for novel highly active agents against HIV-1.

R_3 and R_4 of ring C were modified by various substitutions. 10,10-Dimethyl-11-demethyl-12-oxo calanolide A (**114**) was still active against HIV-1, with an EC_{50} value of 0.64 μM. However, its therapeutic index was in a lower range of 47–140. Although an extended hydrophobic chain increased the activity to the nanomolar level (EC_{50} = 32.5 nM), the novel compound (**116**) still had a lower therapeutic index (102–308). Other modifications of ring C at R_3 and R_4, such as the movement of the methyl group from C-10 to C-11 (**115**), aromatization of C-10 (**118** and **119**), cyclization (**120** and **121**), alkenes at the C-10 and C-11 positions (**122**), and reversal of the direction of ring C (**124**), did not cause significant biological improvement.

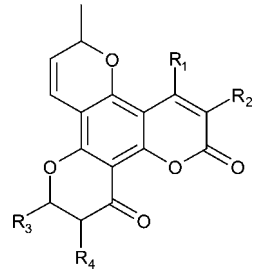
C-10 bromomethylated **15** (**123**) was originally designed as an intermediate for further modification at the C-10 position through nucleophilic substitutions. Interestingly, **123** showed greater anti-HIV-1 activity (2.85 nM) and therapeutic index (>10 526). Although it may serve the toxic potentialities to the molecule during long-term treatment because the bromine easily generates an alkylating reactive intermediate, this finding provided a very important clue that modifications of the C ring at the C-10 position may be conducted to obtain drug candidates with activity against HIV-1. Thus, mimicking bromine at the

Chart 10. Diversities of R_5, R_6, and R_7



125 (86%) **126** (82%) **127** (88%) **128** (76%) **129** (69%) **130** (59%) **131** (81%) **132** (64%)

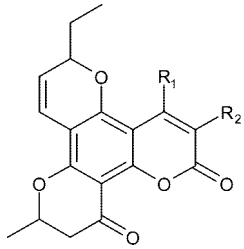
Table 3. Target Compounds With R_5 (or R_6) = CH_3



compd	R_1	R_2	R_3	R_4	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
133	*n*-C_3H_7	H	H	CH_3	79	62.4	++	*c*
134	C_2H_5	H	CH_3	H	59	57.4	*c*	*c*
135	*n*-C_3H_7	H	CH_3	H	62	*c*	*c*	*c*
136	*n*-C_3H_7	Cl	CH_3	H	64	55.7	*c*	*c*
137	*n*-C_4H_9	H	CH_3	H	61	75.4	*c*	*c*
138	C_2H_5	F	CH_3	H	65	39.5	*c*	*c*
139	*n*-C_3H_7	F	CH_3	H	66	*c*	*c*	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

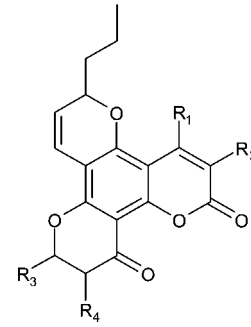
Table 4. Target Compounds with R_5 (or R_6) = C_2H_5



compd	R_1	R_2	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
140	C_2H_5	H	64	*c*	*c*	*c*
141	*n*-C_3H_7	H	68	*c*	*c*	*c*
142	*n*-C_3H_7	Cl	71	*c*	*c*	*c*
143	C_2H_5	F	70	*c*	*c*	*c*
144	*n*-C_3H_7	F	65	*c*	*c*	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

Table 5. Target Compounds with R_5 (or R_6) = *n*-C_3H_7



compd	R_1	R_2	R_3	R_4	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
145	*n*-C_3H_7	H	H	CH_3	69	*c*	*c*	*c*
146	C_2H_5	H	CH_3	H	72	97	++	*c*
147	*n*-C_3H_7	H	CH_3	H	55	54.1	*c*	*c*
148	*n*-C_3H_7	Cl	CH_3	H	73	*c*	*c*	*c*
149	*n*-C_4H_9	H	CH_3	H	76	*c*	*c*	*c*
150	C_2H_5	F	CH_3	H	68	*c*	*c*	*c*
151	*n*-C_3H_7	F	CH_3	H	62	*c*	*c*	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

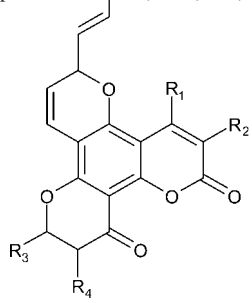
C-10 position is being undertaken in our laboratory with the aims of producing potential druggable properties and tolerated toxicity.

R_5 or R_6 of ring D was diversified with lipophilic or alkene chains or phenyl groups in this study. Many combinations of R_5 or R_6 modifications together with diversity at R_1, R_2, R_3, and R_4 were synthesized. In a short summary, modifications only at R_5 or R_6 of ring D did not improve the compounds' activities against HIV-1. A longer lipophilic chain and aromatization at R_5 or R_6 of ring D significantly increased the cytotoxicity of the compounds in vitro, for example compounds **159**, **167**, **169**, and **170**.

The movement of the 6,6-dimethyl group to the C-7 position (compounds **174**–**178**) and the saturation of the bond between the C-7 and C-8 positions of ring D (compounds **179**–**181** and **183**) caused a loss of activity against HIV-1.

In summary, a chemical library of 11-demethyl-12-oxo calanolide A was designed and synthesized with nine diversity points. Evaluation of the anti-HIV-1 activities of the compounds in vitro indicated that (1), in ring B, a hydrophobic R_1 of the proper length is necessary and modifications at the R_2 position of ring B are not useful in the search for novel highly active agents against HIV-1; (2) at R_3 and R_4 of ring C, an extended hydrophobic chain at the C-10 position results in a compound (**116**) active against HIV-1 at nanomolar concentrations (EC_{50}

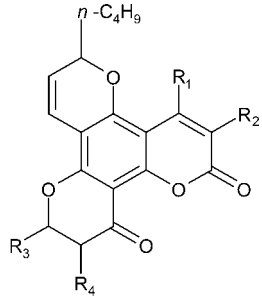
Table 6. Target Compounds with R_5 (or R_6) = ($CH{=}CHCH_3$)



compd	R_1	R_2	R_3	R_4	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
152	n-C_3H_7	H	H	CH_3	65	37.8	*c*	*c*
153	n-C_3H_7	H	CH_3	H	63	*c*	*c*	*c*
154	n-C_3H_7	Cl	CH_3	H	58	57.3	*c*	*c*
155	C_2H_5	H	CH_3	H	67	56.4	*c*	*c*
156	C_2H_5	F	CH_3	H	66	46.2	*c*	*c*
157	$CH_2CH_2CF_3$	H	CH_3	H	56	*c*	*c*	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

Table 7. Target Compounds with R_5 (or R_6) = n-C_4H_9



compd	R_1	R_2	R_3	R_4	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
158	n-C_3H_7	H	H	CH_3	71	35.2	+	*c*
159	C_2H_5	H	CH_3	H	78	96.7	++	*c*
160	n-C_3H_7	H	CH_3	H	56	*c*	*c*	*c*
161	n-C_3H_7	Cl	CH_3	H	71	*c*	*c*	*c*
162	n-C_4H_9	H	CH_3	H	79	*c*	*c*	*c*
163	C_2H_5	F	CH_3	H	76	*c*	*c*	*c*
164	n-C_3H_7	F	CH_3	H	58	*c*	*c*	*c*
165	$CH_2CH_2CF_3$	H	CH_3	H	74	53.6	*c*	*c*

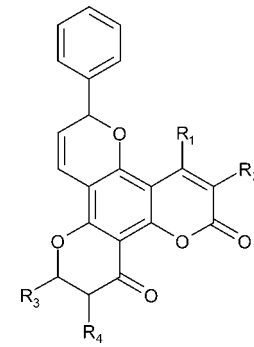
[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

= 32.5 nM), although the therapeutic index is still low (102–308); (3) a longer lipophilic chain and aromatization at R_5 or R_6 of ring D significantly increase its cytotoxicity in vitro; and (4) however, 10-bromomethyl-11-demethyl-12-oxo calanolide A (**123**) showed the greater anti-HIV-1 activity (EC_{50} = 2.85 nM) and therapeutic index (>10 526). This finding provided a very important clue that modifications of the C ring at the C-10 position may be conducted to obtain drug candidates with activity against HIV-1.

Experimental Section

Cell-Based Assays. Serial diluted drugs (10 μM, 1 μM, 100 nM, 10 nM, 1 nM, and 0.1 nM) were tested against 100 TCID50 HIVADA infection in a pseudovirus-based assay, as we previously described.[36] The assay quantifies the activity of a drug to inhibit HIV-induced reporter luciferase activity. The viral infection was determined on the third day by measuring the reporter luciferase activity in TZM. CD4+. CCR5+ cells postinfection by using commercially available kits. Antiviral data were reported as the quantity of drug required to inhibit production by percentage. Controls included (1) infected cells without adding any drugs as a baseline; (2) pseudovirus with a nonspecific envelope (e.g., HCV) that cannot infect TZM. CD4+. CCR5+ cells; and (3) an HIV-specific RT inhibitor as a positive control (e.g., AZT).

Table 8. Target Compounds with R_5 (or R_6) = C_6H_5



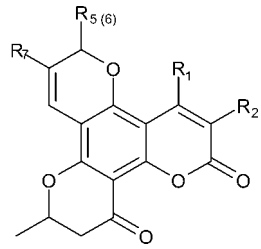
compd	R_1	R_2	R_3	R_4	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
166	n-C_3H_7	H	H	CH_3	70	*c*	+	*c*
167	C_2H_5	H	CH_3	H	64	96.4	++	*c*
168	n-C_3H_7	H	CH_3	H	66	*c*	*c*	*c*
169	n-C_3H_7	Cl	CH_3	H	69	96.3	+++	*c*
170	n-C_4H_9	H	CH_3	H	62	96.4	++	*c*
171	C_2H_5	F	CH_3	H	75	*c*	*c*	*c*
172	n-C_3H_7	F	CH_3	H	68	*c*	*c*	*c*
173	$CH_2CH_2CF_3$	H	CH_3	H	58	47.6	*c*	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

The cell toxicity of the compounds against HIV was evaluated by similar microtiter assays with TZM. CD4+. CCR5+ and Vero cells. Serial diluted drugs (10 mM, 1 mM, 100 μM, 10 μM, 1 μM, and 0.1 μM) were tested in cells by using the cell viability assay, which is commercially available (Promega). The toxicity data are reported as the quantity of a drug required to reduce cell viability by percentage.

General Synthetic Strategy. All the reagents and chemicals were obtained from commercial suppliers and used as received. Melting points are uncorrected and were determined without correction with a Yanaco micromelting point apparatus. Reaction progress was monitored by thin-layer chromatography (TLC) on Qing Dao silica gel 60 F-254 with UV detection. Flash column chromatography was performed with silica gel 60 (160–200 mesh) from Qing Dao Haiyang Chemical Factory with petroleum and ethyl acetate as the eluent solvents. ^{1}H NMR spectra were recorded with Mercury-300, -400, and INOVA-500 spectrometers in DMSO-d_6 solution unless otherwise indicated. Chemical shifts are reported in parts per million (δ) relative to the solvent signal, and coupling constant (J) values are reported in hertz. The spin multiplicities were indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet), and b (broad). Auto HPLC-MS analysis was performed on a ThermoFinnigan LCQ-Advantage mass spectrometer equipped with an Agilent pump, an Agilent detector, an Agilent liquid handler, and a fluent splitter. The employed column was a Kromasil C18 column (4.6 μm, 4.6 mm × 50 mm) from DIKMA. The eluent was a mixture of acetonitrile and water containing 0.05% HCOOH with a linear gradient from 5:95 (v:v) acetonitrile−H_2O to 95:5 (v:v) acetonitrile−H_2O over 5 min at a 1 mL/min flow rate. A portion of the eluent (5%) was split into the MS system. The UV detection was carried out at a UV wavelength of 254 nm. HR-MS data were recorded with an Agilent LC-MSD/TOF mass spectrometer. Infrared spectra were recorded with an Impaco 400 FT-IR spectrophotometer. Elemental analyses were performed with a Carlo Erba 1106 elemental analyzer, where analyses are indicated by the symbols of the elements; the analytical results obtained were within

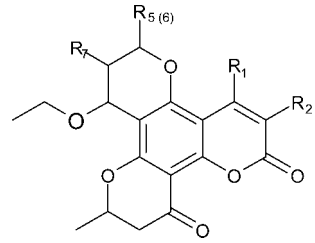
Table 9. Diversities of $R_{5\ (6)}$ and R_7 in the Target Compounds



compd	R_1	R_2	$R_{5\ (6)}$	R_7	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
174	C_2H_5	H	H	CH_3	49	*c*	*c*	*c*
175	*n*-C_3H_7	H	H	CH_3	53	*c*	*c*	*c*
176	*n*-C_3H_7	Cl	H	CH_3	51	49.1	*c*	*c*
177	*n*-C_3H_7	F	H	CH_3	47	47.1	*c*	*c*
178	C_2H_5	F	H	CH_3	49	*c*	*c*	*c*
182	*n*-C_3H_7	H	*o*-NO_2-C_6H_4	H	44	52.6	*c*	*c*
184	*n*-C_3H_7	H	*o*-NH_2-C_6H_4	H	81	*c*	*c*	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

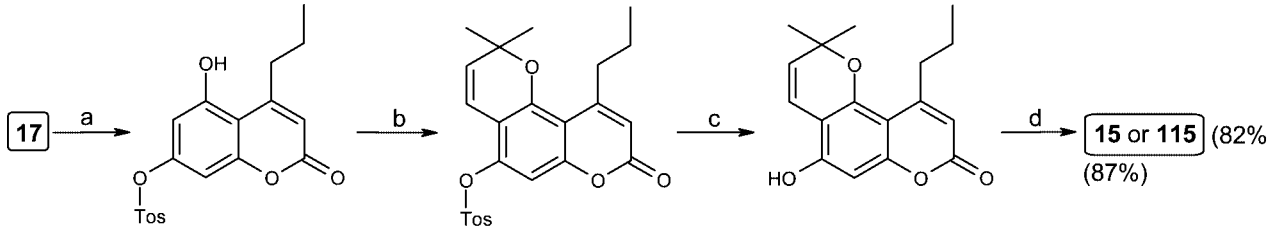
Table 10. Side Products with $\Delta^{7,8}$-Saturation by EtOH



compd	R_1	R_2	$R_{5\ (6)}$	R_7	yield (%)	inhibition (%, 1.0 μM)	toxicity (10 μM)[a]	EC_{50} (TI)[b]
179	*n*-C_3H_7	H	H	CH_3	26	*c*	*c*	*c*
180	C_2H_5	H	H	CH_3	19	*c*	*c*	*c*
181	*n*-C_3H_7	Cl	H	CH_3	21	51.7	*c*	*c*
183	*n*-C_3H_7	H	*o*-NO_2-C_6H_4	H	25	61	+	*c*

[a] "+", "++", and "+++" mean that <10%, 10–80%, and >80% of the host cells of the virus died, respectively. [b] Therapeutic index was calculated by a formula of IC_{50}/EC_{50}. [c] Not detectable.

Scheme 9. Novel Synthetic Strategy of the Target Compounds[a]



[a] Reagents and conditions: (a) 1.5 equiv of TBAF, THF, rt, 3 h; (b) compound **11**, microwave irradiation in toluene/pyridine, 140 °C, 150 W, 120 psi, 20 min; (c) 10 equiv of TBAF, THF, reflux (or microwave irradiation); (d) (i) crotonic acid (or 2-methyl acrylic acid), $SOCl_2$, reflux, (ii) BF_3/Et_2O, reflux.

±0.4% of the theoretical values. Microwave heating was carried out with a Discover microwave synthesizer (CEM Corporation, NC).

Typical procedures and compounds are only described in the article. All characterizations of synthesized compounds are detailed in the Supporting Information. The original spectra of various compounds can be obtained from the authors.

General Method for the Synthesis of Coumarins. To an equimolar mixture of pholoroglucinol (**6**) and β-keto-ethyl acetate was added 50 mL of methanol saturated with dry hydrochloride gas under stirring until it was completely dissolved at room temperature. The precitipate was then collected and dried. With this method, compounds **26** and **31**–**45** were prepared, respectively.

5-Hydroxy-8-methyl-4-propyl-8,9-dihydro-2*H*,10*H*-pyrano[2,3-*f*]chromene-2,10-dione (16) and 5-Hydroxy-8-methyl-4-propyl-7,8-dihydro-2*H*,6*H*-pyrano[3,2-*g*]chromene-2,6-dione (112). Compound **7** (2.20 g, 10 mmol) and crotonic acid (1.29 g, 15 mmol) were dissolved in 100 mL of PPA and were mixed with mechanical stirring at 90 °C for 20 min. The reactant mixture was poured into 200 mL of ice–water and stirred; a yellow solid was precipitated, subsequently. The solid was filtered and washed with water. The obtained mixture was purified by column chromatography to give angular compound **16** (2.01 g, 70%) as target and linear compound **112** (260 mg, 9%) as byproduct. **16**: ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 11.760 (s, 1H, OH), 6.282 (s, 1H, 6-H), 6.025 (s, 1H, 3-H), 4.635 (m, 1H, 8-H), 2.863 (t, 2H, J = 7.5 Hz, 4-C$\underline{H}_2$CH$_2$CH$_3$),

2.648 (dd, 1H, J = 11.1 Hz, 16.5 Hz, 9-He), 2.562 (dd, 1H, J = 4.2 Hz, 16.5 Hz, 9-Ha), 1.552 (sex, 2H, J = 7.5 Hz, 7.2 Hz, 4-$CH_2C\underline{H}_2CH_3$), 1.385 (d, 3H, J = 6.0 Hz, 8-CH_3), 0.924 (t, 3H, J = 7.2 Hz, 4-$CH_2CH_2C\underline{H}_3$). ESI-MS (m/z): 289.1 $[M + H]^+$ (calcd MW = 288.30). **112**: ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 13.900 (s, 1H, OH), 6.450 (s, 1H, 10-H), 6.042 (s, 1H, 3-H), 4.753 (m, 1H, 8-H), 2.971 (dd, 1H, J = 12.3 Hz, 17.4 Hz, 7-He), 2.875 (t, 2H, J = 7.5 Hz, 4-$C\underline{H}_2CH_2CH_3$), 2.793 (dd, 1H, J = 3.0 Hz, 17.4 Hz, 7-Ha), 1.584 (sex, 2H, J = 7.5 Hz, 7.2 Hz, 4-$CH_2C\underline{H}_2CH_3$), 1.437 (d, 3H, J = 6.3 Hz, 8-CH_3), 0.948 (t, 3H, J = 7.2 Hz, 4-$CH_2CH_2C\underline{H}_3$). ESI-MS (m/z): 289.1 $[M + H]^+$ (calcd MW = 288.30).

With this method, compounds **20**, **46**–**72**, **95**–**109**, and **113** were prepared, respectively.

1,1-Diethoxy-3-methyl-2-butene (**11**) was prepared as previously described.[15,16] Compounds **125**–**132** were prepared by the same method.

6,6,10-Trimethyl-4-propyl-10,11-dihydro-2*H*,6*H*,12*H*-dipyrano[2,3-*f*:2′,3′-*h*]chromene-2,12-dione (15). To the compound **16** (14 mg, 0.05 mmol) and **11** (0.2 mmol) in a microwave reaction tube was added 2 mL of toluene and 2 drops of pyridine. The reaction was continued for 20 min under the conditions of 150 W and 140 °C. The reactants were purified by silica gel chromography to gain compound **15** as an off-white powder with mp 114–116 °C. The yield was 86%. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 6.566 (d, 1H, J = 9.9 Hz, 8-H), 6.086 (s, 1H, 3-H), 5.774 (d, 1H, J = 9.9 Hz, 7-H), 4.707 (m, 1H, J = 6.3 Hz, 12.0 Hz, 3.9 Hz, 10-H), 2.837 (t, 2H, J = 7.5 Hz, 4-$C\underline{H}_2CH_2CH_3$), 2.708 (dd, 1H, J = 12.0 Hz, 16.5 Hz, 11-He), 2.606 (dd, 1H, J = 3.9 Hz, 16.5 Hz, 11-Ha), 1.537 (sex, 2H, J = 7.5 Hz, 7.2 Hz, 4-$CH_2CH_2CH_3$), 1.488, 1.450 (2s, 6H, CH_3), 1.439 (d, 3H, J = 6.3 Hz, 10-CH_3), 0.963 (t, 3H, J = 7.2 Hz, 4-$CH_2CH_2C\underline{H}_3$). ESI-MS (m/z): 355.1 $[M + H]^+$ (calcd MW = 354.41).

With this method, compounds **23**, **76**–**91**, **114**–**124**, **133**–**183**, and **186** were prepared, respectively.

2-Oxo-4-propyl-2*H*-chromene-5,7-diyl Bis(4-methylbenzenesulfonate) (17). Compound **7** (5 mmol) was dissolved in pyridine and cooled down in an ice bath. *p*-Methylbenzenesulfonyl chloride (6 equiv) was then added with stirring. The reaction was continued at room temperature overnight. The reactants were poured into ice–water under strong stirring. The precipitate was filtered and washed with water. After completely drying, a white solid (**17**) was obtained as 2.45 g, yield 93%, mp 239–241 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 7.762 (m, 4H, Ar-H), 7.499 (m, 4H, Ar-H), 7.150 (d, 1H, J = 2.4 Hz, 8-H), 6.781 (d, 1H, J = 2.4 Hz, 6-H), 6.322 (s, 1H, 3-H), 2.691 (t, 2H, J = 7.5 Hz, 9-CH_2), 2.429, 2.408 (2s, 6H, 14, 17-CH_3), 1.413 (sex, 2H, J = 7.5 Hz, 7.2 Hz, 10-CH_2), 0.792 (t, 3H, J = 7.2 Hz, 11-CH_3). ESI-MS (m/z): 529.1 $[M + H]^+$ (calcd MW = 528.60). IR (KBr) cm^{-1}: 3087, 2962, 2877, 1743, 1612, 1421, 1379, 1194.

With this method, compound **21** was also prepared.

4-Propyl-2-thioxo-2*H*-chromene-5,7-diyl Bis(4-methylbenzenesulfonate) (18). Compound **17** (3 mmol) was dissolved in xylene, and 5 equiv of P_2S_5 was added. The reaction was continued for 4 h at room temperature. The excess P_2S_5 was then filtered off. The filtrate was concentrated under reduced pressure. The residue was finally purified by silica gel chromatography to attain 1.14 g of compound **18** with mp 198–201 °C. The yield is 70%. ^{1}H NMR (300 MHz, DMSO-d_6): 7.768 (dd, 4H, J = 8.4 Hz, 6.9 Hz, Ar-H), 7.496 (dd, 4H, J = 8.1 Hz, 6.6 Hz, Ar-H), 7.345 (d, 1H, J = 2.7 Hz, 8-H), 7.082 (s, 1H, 3-H), 6.859 (d, 1H, J = 2.4 Hz, 6-H), 2.637 (t, 2H, J = 7.5 Hz, 9-CH_2), 2.426, 2.403 (2s, 6H, 14, 17-CH_3), 1.403 (sex, 2H, J = 7.5 Hz, 7.2 Hz, 10-CH_2), 0.785 (t, 3H, J = 7.2 Hz, 11-CH_3). ESI-MS (m/z): 545.1 $[M + H]^+$ (calcd MW = 544.67). IR (KBr) cm^{-1}: 3087, 2974, 2881, 1614, 1597, 1385, 1144. Anal. Calcd for $C_{26}H_{24}O_7S_3 \cdot 0.5H_2O$: C, 56.40; H, 4.55. Found: C, 56.52; H, 4.40.

With this method, compound **22** was also prepared.

5,7-Dihydroxy-4-propyl-2*H*-chromene-2-thione (19). Compound **18** (1.5 mmol) was dissolved in THF, and 3 equiv of TBAF was added. The reaction was refluxed for 10 h and then concentrated under reduced pressure. The residue was redissolved again in ethyl acetate. The excess TBAF was then washed off with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified to yield 266 mg of compound **19** in 75% (yellow powder) with mp 204–206 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 10.901 (s, 1H, 7-OH), 10.634 (s, 1H, 5-OH), 6.766 (s, 1H, 3-H), 6.358 (d, 1H, J = 2.4 Hz, 8-H), 6.344 (d, 1H, J = 2.4 Hz, 6-H), 2.826 (t, 2H, J = 7.5 Hz, 9-CH_2), 1.578 (sex, 2H, J = 7.5 Hz, 7.2 Hz, 10-CH_2), 0.932 (t, 3H, J = 7.2 Hz, 11-CH_3). ESI-MS (m/z): 237.1 $[M + H]^+$ (calcd MW = 236.29). Anal. Calcd for $C_{12}H_{12}O_3S$: C, 61.00; H, 5.12. Found: C, 60.84; H, 5.14.

With this method, compound **187** was also prepared.

Ethyl 2-chloro-3-oxohexanoate (**25**) was prepared according to ref 31 as a light yellow liquid with a yield of 90%.

2,2-Dimethyl-1,3-dioxane-4,6-dione (**28**) was prepared according to ref 32 as a white needle crystal with a yield of 62%.

2,2-Dimethyl-5-(4,4,4-trifluorobutanoyl)-1,3-dioxane-4,6-dione (29). Compound **28** (288 mg, 2 mmol) and 4,4,4-trifluorobutanoic acid (284 mg, 2 mmol) were dissolved in 5 mL of THF. EDC·HCl (340 mg, 2.2 mmol) was then added to the mixture. The reactants were reacted at room temperature for 5 h. The solvent was removed through evaporation. The residue was dissolved with 20 mL of ethyl acetate and then washed with 1 M HCl solution (3 × 15 mL). The organic layer was dried with anhydrous Na_2SO_4. The solvent was removed, and the residue was then ready for the next synthesis.

Methyl 6,6,6-Trifluoro-3-oxohexanoate (30). The residue of **29** was dissolved in 10 mL of methanol, and 4-methylbenzenesulfonic acid in catalytical amount was added. The mixture was reflux for 4 h and then concentrated through evaporation. The residue was ready for the next synthesis.

5-Hydroxy-8-methyl-4-(piperazin-1-ylmethyl)-8,9-dihydro-2*H*,10*H*-pyrano[2,3-*f*]chromene-2,10-dione (73). Compound **52** (0.05 mmol) was dissolved in 10 mL of THF, and 2 mL of piperazine was added to the solution. The reaction mixture was refluxed for 4 h and then purified by column chromatography. Compound **73** was obtained as a light yellow powder with mp 256–258 °C. The yield was 68%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 12.820 (s, 1H, 5-OH), 9.520 (s, 1H, NH), 7.652 (s, 1H, 6-H), 6.643 (s, 1H, 3-H), 4.605 (m, 1H, J = 6.0 Hz, 12.4 Hz, 2.4 Hz, 8-H), 3.810 (m, 4H, CH_2), 3.121 (s, 2H, 4-CH_2), 3.029 (m, 4H, CH_2), 2.869 (dd, 1H, J = 12.4 Hz, 17.2 Hz, 9-He), 2.709 (dd, 1H, J = 2.4 Hz, 17.2 Hz, 9-Ha), 1.409 (d, 3H, J = 6.0 Hz, 8-CH_3). ESI-MS (m/z): 345.2 $[M + H]^+$ (calcd MW = 344.37). HRESIMS obsd m/z 345.1454, calcd for $C_{18}H_{21}N_2O_5^+$ 345.1450.

With this method, compounds **74**, **75**, **92**, and **93** were prepared, respectively.

4-(4-Aminophenyl)-6,6,10-trimethyl-10,11-dihydro-2*H*,6*H*,12*H*-dipyrano[2,3-*f*:2′,3′-*h*]chromene-2,12-dione (94). To a solution of compound **86** (0.02 mmol) in a sufficient volume of ethanol was added 5 equiv of 38% HCl and 15 equiv of $SnCl_2 \cdot 2H_2O$. After refluxing for 2 h, the reactants were poured into a 40% NaOH solution under stirring. The product was extracted with DCM (3 × 20 mL) and purificed by silica gel chromography to gain 5.1 mg of the final product (white powder, mp 149–151 °C). The yield was 63%. ^{1}H NMR (400 MHz, DMSO-d_6): 7.089 (m, 2H, 4-Ar-H), 6.492 (m, 2H, 4-Ar-H), 6.477 (d, 1H, J = 10.0 Hz, 8-H), 6.275 (d, 1H, J = 8.4 Hz, 3-H), 5.577 (d, 1H, J = 10.0 Hz, 7-H), 5.495 (d, 2H, J = 6.0 Hz, NH_2), 4.647 (m, 1H, J = 6.0 Hz, 3.2 Hz, 12.4 Hz, 10-H), 2.840 (dd, 1H, J = 12.4 Hz, 15.6 Hz, 11-He), 2.677 (dd, 1H, J = 3.2 Hz, 15.6 Hz, 11-Ha), 1.453 (d, 3H, J = 6.0 Hz, 10-CH_3), 1.273, 1.224 (2s, 6H, 6-CH_3). ESI-MS (m/z): 404.1 $[M + H]^+$ (MW = 403.44). HRESIMS obsd m/z 404.1495, calcd for $C_{24}H_{22}NO_5^+$ 404.1498.

With this method, compound **184** was also prepared.

5-Hydroxy-4-propyl-9,10-dihydro-2*H*,8*H*-pyrano[2,3-*f*]chromene-2,8-dione (110). To the mixture of compound **7** (0.2 mmol) and acrylic acid (0.2 mmol) was added 5 mL of CF_3SO_3H. After the reaction was continued for 4 h at 60 °C, the reactants were poured into ice–water under stirring. The crude was then

collected after extraction with ethyl acetate (3 × 15 mL) and drying with $MgSO_4$, and then it was concentrated under reduced pressure. A white powder (**110**, 35.6 mg) was finally attained by column chromography with mp 166–167 °C in a yield of 65%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 11.034 (s, 1H, 5-OH), 6.600 (s, 1H, 6-H), 6.053 (s, 1H, 3-H), 2.852 (m, 6H, 4, 9, 10-CH_2), 1.571 (m, 2H, 4-$CH_2C\underline{H}_2CH_3$), 0.969 (t, 3H, J = 7.2 Hz, 4-$CH_2CH_2C\underline{H}_3$). ESI-MS (m/z): 275.1 $[M + H]^+$ (calcd MW = 274.27). Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.45; H, 5.20. HRESIMS obsd m/z 275.0918, calcd for $C_{15}H_{15}O_5^+$ 275.0919.

With this method, compound **111** was also prepared.

5-Hydroxy-2-oxo-4-propyl-2*H*-chromen-7-yl 4-Methylbenzenesulfonate (185). Compound **17** (1 mmol) was dissolved in 10 mL of THF, and TBAF (1.5 mmol) was added. The mixture was stirred at room temperature and monitored by TLC. The solvent was evaporated in vacuo, and the residue was dissolved in ethyl acetate (3 × 15 mL) and then washed with water (3 × 50 mL). The organic layer was dried with anhydrous Na_2SO_4 and then concentrated. The residue was purified by column chromatography. The final product was obtained with a yield of 88% and mp 155–157 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 11.261 (s, 1H, OH), 7.790 (m, 2H, Ar-H), 7.499 (m, 2H, Ar-H), 6.521 (d, 1H, J = 2.4 Hz, 8-H), 6.472 (d, 1H, J = 2.4 Hz, 6-H), 6.104 (s, 1H, 3-H), 2.859 (t, 2H, J = 7.5 Hz, CH_2), 2.422 (s, 3H, CH_3), 1.547 (sex, 2H, J = 7.5 Hz, 7.2 Hz, CH_2), 0.921 (t, 3H, J = 7.2 Hz, CH_3). ESI-MS (m/z): 375.1 $[M + H]^+$ (MW = 374.42).

6,6,11-Trimethyl-4-propyl-10,11-dihydro-2*H*,6*H*,12*H*-dipyrano[2,3-*f*:2′,3′-*h*]chromene-2,12-dione (115) under the Novel Strategy. Compound **187** (0.2 mmol) was dissolved in 5 mL of BF_3/Et_2O with an argon bubble, and crotonic acyl chloride (0.3 mmol) was added dropwise. The reaction was stirred and heated at 50 °C and monitored by TLC. The mixture was poured into ice–water and extracted with ethyl acetate (3 × 20 mL). The organic layer was dried by anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography. The target compound was obtained with a yield of 82% and mp 108–109 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 6.565 (d, 1H, J = 9.9 Hz, 8-H), 6.105 (s, 1H, 3-H), 5.789 (d, 1H, J = 9.9 Hz, 7-H), 4.630 (dd, 1H, J = 5.1 Hz, 11.1 Hz, 10-He), 4.256 (t, 1H, J = 11.1 Hz, 10-Ha), 2.846 (m, 3H, 11-CH, 4-$C\underline{H}_2CH_2CH_3$), 1.562 (sex, 2H, J = 7.5 Hz, 7.2 Hz, 4-$CH_2C\underline{H}_2CH_3$), 1.492, 1.469 (2s, 6H, CH_3), 1.052 (d, 3H, J = 6.9 Hz, 11-$C\underline{H}_3$), 0.968 (t, 3H, J = 7.2 Hz, 4-$CH_2CH_2C\underline{H}_3$). ESI-MS (m/z): 355.1 $[M + H]^+$ (calcd MW = 354.41).

With this method, compound **15** was also resynthesized.

Conclusion

A library was designed and synthesized in this article. A novel compound (10-bromo-methyl-11-demethyl-12-oxo calanolide A, **123**) was identified to have much higher inhibitory potency and therapeutic index (EC_{50} = 2.85 nM, TI > 10,526) than that of (+)-calanolide A against HIV-1 through the assays in vitro.

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Supporting Information Available: Complete spectroscopic and analytical data for the preparation of all intermediates and target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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