*Recent Progress in the Development of Coumarin Derivatives as Potent Anti-HIV Agents**

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Abstract: Numerous plant-derived compounds have been evaluated for inhibitory effects against HIV replication, and some coumarins have been found to inhibit different stages in the HIV replication cycle. This review article describes recent progress in the discovery, structure modification, and structure–activity relationship studies of potent anti-HIV coumarin derivatives. A dicamphanoyl-khellactone (DCK) analog, which was discovered and developed in our laboratory, and calanolide A are currently in preclinical studies and clinical trials, respectively.
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Key words: anti-HIV coumarins; khellactone; DCK analogs; tetracyclic dipyranocoumarins; calanolides; inophyllums

1. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS), caused by the human immunodeficiency virus (HIV), results in life-threatening opportunistic infections and malignancies. HIV leads to the destruction and functional impairment of the immune system, subsequently destroying the body's ability to fight against infections.[1] Since first reported in 1981, it has spread rapidly through the human population and become one of the most devastating diseases faced by mankind.[2–4] As reported by UNAIDS and WHO, an estimated 40 million people globally were living with HIV or AIDS at the end of 2001. Five million people will be newly infected with HIV, including 1.8 million women and 0.8 million children under 15 years, and 3 million will die from AIDS in this year.[5]

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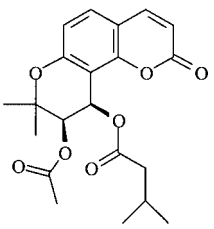
Current strategies for anti-HIV chemotherapy involve inhibition of virus adsorption, virus-cell fusion, reverse transcription, integration, translation, proteolytic cleavage, glycosylation, assembly, or release.[6,7] Each step could theoretically serve as a potential target for anti-HIV agents. Sixteen compounds (twenty-three formula) have been approved by the FDA to treat AIDS[8]: six nucleoside reverse transcriptase (RT) inhibitors (NRTIs)—AZT, ddC, ddI, d4T, 3TC, and abacavir; one new nucleotide RT inhibitor—tenofovir (Viread^{TM}), which was FDA approved in October 2001[9]; three non-nucleoside RT inhibitors (NNRTIs)—nevirapine, delavirding, and efavirenz; and six protease inhibitors (PIs)—saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, and lopinavir. However, rapid development of HIV resistance and toxicity of the drugs diminish their action.[10–13] Therefore, the development of new anti-HIV agents is focusing on novel structures and (or) new action mechanisms.

Numerous plant-derived compounds, including coumarins, have been evaluated for inhibitory effects on HIV replication *in vitro*.[14–16] Anti-HIV coumarins have been identified to inhibit viral adsorption, reverse transcription, protease inhibition and integration in the HIV replication cycle.[14] The coumarin inhibitors fall into four structural groups: (a) pyranocoumarins, such as khellactone derivates, for example, 3′R, 4′R-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK); (b) tetracyclic dipyranocoumarins, examples include calanolides A and B; (c) multimer coumarin derivates; and (d) other coumarins. This review will focus on recent discoveries and development of active coumarin derivatives, especially those discovered and developed in our laboratory.

2. KHELLACTONE ANALOGS AS ANTI-HIV AGENTS

Khellactone coumarins, which constitute a small branch of the coumarin family, are notable because of their extensive bioactivities, including anti-HIV, antitumor promoting,[17] and anti-platelet aggregation.[18] So far, more than 50 natural khellactone coumarins have been discovered. One member of this family, suksdorfin (**1**) (Fig. 1), a dihydroseselin type angular pyranocoumarin, was isolated from the methanolic extract of *Lomatium suksdorfii* through a bioactivity-directed fractionation. It suppressed viral replication in eleven separate acute HIV-1 (IIIB isolate) infections of H9 lymphocyte cells with an average EC_{50} value of 2.6 ± 2.1 μM. It also suppressed acute HIV-1 infections in fresh peripheral blood mononuclear cells, monocyte/macrophages, and U937 cells, a promonocytic cell line.[19]

Compound **1** represents a new class of potent anti-HIV agents that is structurally unique compared with other known anti-AIDS drugs. Its mechanism is also different from those of other known anti-HIV agents and remains unclear. In order to obtain more effective analogs and investigate the mechanism of action, **1** has been chosen as a lead compound for various structural modifications.

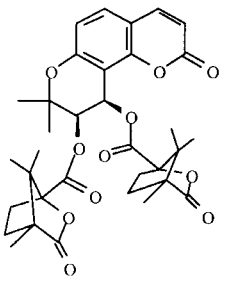


Suksdorfin (**1**)

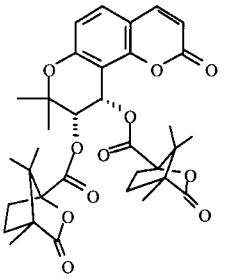
Figure 1. Structure of suksdorfin (**1**).

A. Pyrano-3′,4′ Stereoselectivity and Modification

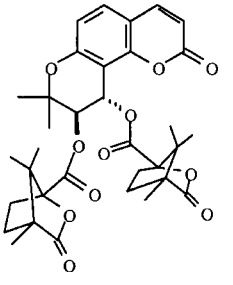
Compound **1** contains two chiral carbons, C-3′ and C-4′, with both possess R configurations. Huang[20,21] synthesized a series of dihydroseselin (khellactone) derivatives, including all four stereochemical isomers, 3′,4′-*cis* or *trans* (**3**–**38**) (Fig. 2). Among them, 3′R,4′R-di-*O*-(−)-



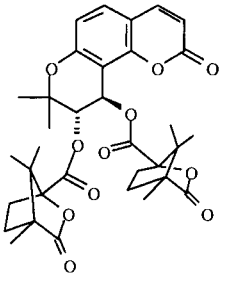
3'R, 4'R (**2**)



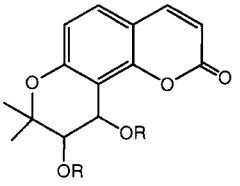
3'S, 4'S (**3**)

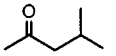
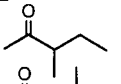
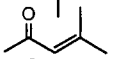
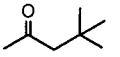


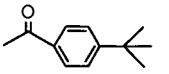
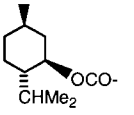
3'R, 4'S (**4**)



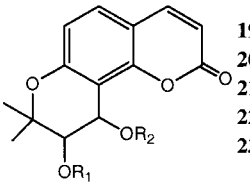
3'S, 4'R (**5**)

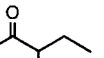


6. (±)-cis R=Ac
7. (±)-trans R=Ac
8. (±)-cis R= 
9. (±)-trans R=
10. (±)-cis R= 
11. (±)-cis R= 
12. (±)-cis R= 

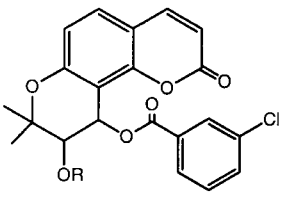
13. (±)-cis R= 
14. (+)-cis R= 
15. (-)-cis R=

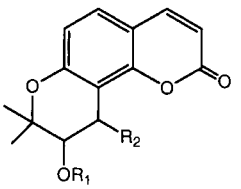
16. (±)-trans R=Me
17. (±)-trans R= COOPh
18. (±)-trans R=$COCH_2CMe_3$



19. (±)-cis R_1=H, R_2=
20. (±)-cis R_1=Ac, R_2= 
21. (±)-cis R_2=H, R_1=
22. (±)-trans R_1=H, R_2=
23. (±)-trans R_1=Ac, R_2=

24. (±)-trans R_1=CH_2Ph, R_2=Ac
25. (±)-trans R_1=Me, R_2=Ac
26. (±)-trans R_1=  R_2= 
27. (±)-trans R_1= R_2=Ac





28. (±)-trans R=Ac
29. (±)-trans R=Me
30. (±)-trans R=CH_2Ph
31. (±)-trans R= 
32. (±)-trans R= 

33. cis/trans R_1=H, R_2=N_3
34. cis/trans R_1=Ac, R_2=N_3
35. cis R_1=H, R_2=NHAc
36. trans R_1=H, R_2=NH_2
37. trans R_1=H, R_2=$NHCOCH_2CHMe_2$
38. trans R_1=Ac, R_2=$NHCOCH_2CHMe_2$

Figure 2. Modification of *cis*- and *trans*-khellactone derivatives.

camphanoyl-(+)-*cis*-khellactone (DCK) (**2**) had an EC_{50} of 4×10^{-4} μM and TI of 136,719, and was much more potent than the remaining stereo-isomers, **3** (3′S,4′S) (EC_{50} = 51 μM and TI 33.3), **4** (3′R,4′S) (EC_{50} = 6.4 μM and TI 1) and **5** (3′S,4′R) (EC_{50} = 32 μM and TI 1). Some *cis*- and *trans*-3′,4′-substituted compounds also showed weak activity; however, none exhibited better biological profiles than suksdorfin (**1**) (Table I).

The study data showed that a rigid stereochemistry of 3′ and 4′-configured khellactone derivatives is crucial for anti-HIV activity. Thus, most synthetic dihydroseselin analogs may show low anti-HIV activity because they are racemic mixtures (Fig. 2) Although it is difficult to isolate the optically pure 3′R, 4′R stereoisomer from a *cis* racemic mixture, asymmetric synthesis provides a solution. Xie[22] synthesized DCK (**2**) in up to 86% e.e. *via* a catalytic Sharpless asymmetric dihydroxylation (AD)[23,24] of seselin. Seven different chiral ligands were used for optimal enantioselectivity. Hydroquinidine (DHQD-R) type ligands led to 3′S,4′S configuration and hydroquinine (DHQ-R) type ligands gave 3′R, 4′R configuration as the main product. $(DHQ)_2PYR$ (hydroquinine 2,5-diphenyl-4,6-pyrimidinediyl diether) gave the highest R, R stereoselectivity. The reaction rate was faster at room temperature (rt) than at 0°C but resulted in lower % e.e. values. Methanesulfonamide improved the reaction rate at 0°C. By using OsO_4 as catalyst and $(DHQ)_2$-PHAL (hydroquinine 1,4-phthalazinediyl diether) as ligand at rt, a solid phase asymmetric synthesis successfully gave 91% e.e in the AD reaction.[25]

B. 3′R, 4′R Modification

The isovaleryl group in **1** can be overlapped with a similar structure in the rigid (−)-camphanoyl moiety in **2**. X-ray crystallographic analysis confirms that the isovaleryl group in **1** is more conformationally flexible and its terminal carbon atoms are disordered over two orientations, while in DCK (**2**), the 4′-camphanoyl group neighbors another bulky camphanoyl substituent making the isovaleryl moiety more rigid.[26]

When the two (−)-camphanoyl groups in DCK were replaced with (+)-camphanoyl groups (**43**), the anti-HIV activity ($EC_{50} = 6.60 \times 10^{-3}$ μM, TI $> 2.38 \times 10^4$) was still better than that of AZT but slightly lower than that of DCK. Compounds with aromatic acyl groups (**41** and **42**) or small acyl groups (**39** and **40**) on the 3′ and 4′-positions did not show anti-HIV activity. Mono-acyl-(+)-*cis*-khellactone derivatives did not suppress HIV-1 replication in H9 lymphocytes, even with one bulky acyl group (**44**, **46**, **48**, and **49**). Diester compounds with two different bulky acyl groups, 3′R-camphanoyl and 4′R-adamantanecarbonyl, exhibited anti-HIV activity but were less active than DCK (**50**, EC_{50} = 0.60 μM, TI 35.5) (Fig. 3). These results indicate that the volume, size, and shape of the camphanoyl group are as or even more important than its absolute configuration.[27]

C. Substituents on Coumarin Skeleton

Substitutions can be introduced at four positions on DCK's coumarin skeleton. Xie[28–30] synthesized a series of mono-substituted derivatives at the 3, 4, 5, and 6 positions.

The data in Table I show that mono-methyl substitution at the 3, 4, or 5 position (**51**, **52**, and **53**) greatly increased potency in comparison with DCK (**2**). Methoxylation at the 3, 4, or 5 position did not show the same effect as methylation; methoxylation at the 3 or 4 position (**55** and **56**) decreased the activity by 10-fold, while 5-methoxy DCK (**57**) retained activity. However, with both mono-methylation and methoxylation, substitution at the position 6 (**54** and **58**, respectively) dramatically decreased the activity. Analogs with other substituents, such as propyl (**59**), isopropyl (**60**), phenyl (**61**), and trifluoromethyl (**62**), at position 4 were less potent or inactive. The extremely high anti-HIV potency of 4-methyl-DCK (**52**) and 5-methyl-DCK (**53**) indicates that a methyl group on the coumarin ring probably fits well into a hydrophobic cleft on the target's active surface and greatly increases both the agent's target affinity and the desired pharmacological response. The larger volume of the propyl and isopropyl groups could affect compound planarity and interfere with target binding, while the

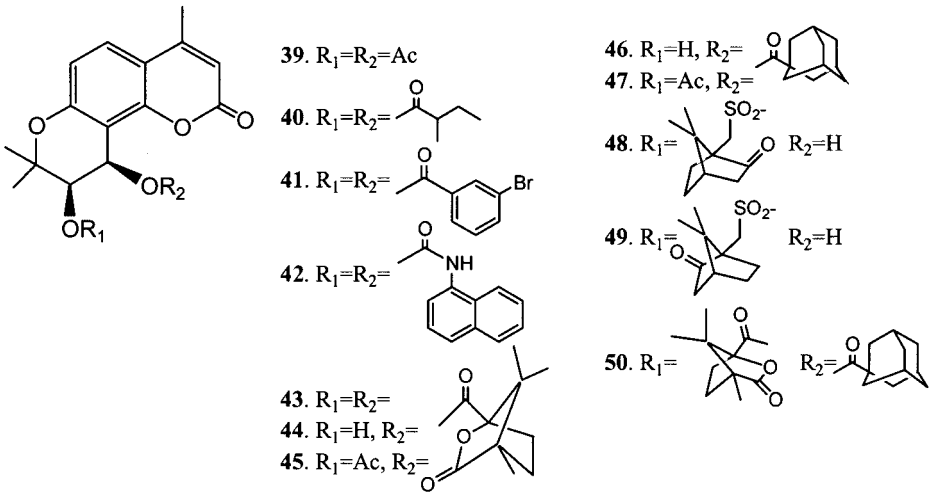


Figure 3. Modification of 3′R, 4′R-khellactone derivatives.

phenyl substituent (**61**) may not fit into the binding site. Although similar in size and shape to a methyl group, the trifluoromethyl group is likely incompatible with the hydrophobic region, based on the low activity of the trifluoromethyl analog (**62**). 4-Methyl-DCK (**52**) is synthesized in fewer steps than 5-methyl-DCK (**53**) *via* a four-step reaction sequence beginning with 4-methyl-7-hydroxyl-coumarin.[22,29,30] Thus, it has been chosen for additional preclinical research.

Disubstituted DCK analogs were also synthesized and screened for inhibition of HIV replication in H9 lymphocytes (Table I).[26] 4-Methyl-5-methoxy DCK (**70**) was the most promising compound and was as active as 4-methyl DCK (**52**) and much better than DCK and AZT in the same assay. Dimethyl DCK analogs **63**–**66** were more potent than AZT but less potent than DCK. 3-Chloro-4-methyl DCK (**67**) and 3,4-cyclohexano DCK (**69**) also showed potent anti-HIV activity. 3-Phenyl-4-methyl DCK (**68**) and 4-methyl-5-benzyloxy DCK (**72**) showed weak HIV-1 suppression, and 4-isopropyl-5-methyl DCK (**71**) was inactive in this assay. These results indicate that anti-HIV activity can be maintained when two methyl or other aliphatic substituent(s) are placed on the khellactone coumarin nucleus and that an aromatic substituent on the nucleus is not favorable. Comparing the molecular three-dimensional orientations and torsional angles of these molecules suggests that steric compression between two bulky substitutents at the 4- and 5-positions deforms the coumarin system. The [C(4)-C(4a)-C(5)-C(Me-5)] torsion angles are 42.9° and 29.5° in **71** and **64**, respectively, as computed by Sybyl software; thus, the 5-methyl groups are forced out of the resonance plane. However, the corresponding torsion angles in **70** and 4-methyl DCK (**52**) are 3.3° and 2.0°, respectively. An X-ray diffraction study of 4-methyl DCK confirmed the computational data.[26]

These data support the hypothesis that a planar system is an essential structural feature for potent anti-HIV activity in this compound class. Steric compression of the C(4) and C(5) coumarin substituents can reduce the overall planarity and, thus, resonance of the coumarin system, resulting in decreased or completely lost anti-HIV activity.

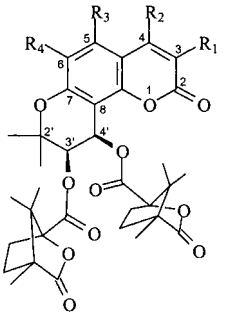
D. Coumarin Skeleton Modification

The thio-bioisosteres of DCK contain S rather than O in the 2-ketone moiety and retain anti-HIV activity in comparison to the substituted DCKs. Lawesson's reagent converted the pyranocoumarins to the corresponding pyranocoumarin thiones in 50–85% yields.[28] Among the khelthiolactone

analogs above, 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khelthiolactone **73**, 4-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khelthiolactone **74**, 3-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khelthiolactone **77**, and 5-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khelthiolactone **78** showed the highest potency in the H9-lymphocyte cell line, but were less active than correspondent DCK (**2**), 4-methyl-DCK (**52**), 3-methyl-DCK (**51**), and 5-methyl-DCK (**53**). However, in the CEM-SS cell line, **74** showed promising potency with $EC_{50} = 0.0635$ μM and TI > 3,149, and was more active than DCK and similar to 4-methyl-DCK[28] (Table II).

The DCK lactam analogs (**85**–**88**) were also synthesized asymmetrically and evaluated for anti-HIV activity against HIV-1 replication in H9 lymphocyte cells (Fig. 4). A novel 4-step synthesis procedure for the 8,8-dimethyl-2H,8H-pyrano[6,5-h]quinolin-2-ones began with 1,3-phenylenediamine. The subsequent procedures to make DCK lactam were similar to the synthesis of DCK except that the N was protected by BOC before Sharpless dihydroxylation and deprotected with TFA to release final products **87** and **88**.[31]

Table I. Structures and Anti-HIV Activities of Mono-Substituted (**53–59**) and Disubstituted (**60–69**) DCK Analogs in Acutely Infected H9 Lymphocytes



No.	Structure	EC_{50} (μM) [a]	TI [b]
Suksdorfin (**1**)		2.6 ± 2.1	30.6 ± 22.4
3'R,4'R DCK(**2**)	$R_1 = R_2 = R_3 = R_4 = H$	2.56×10^{-4}	1.37×10^{5}
3'S,4'S DCK (**3**)		51	33.3
3'R,4'S DCK (**4**)		6.4	1
3'S,4'R DCK (**5**)		32	1
51	$R_1 = CH_3, R_2 = R_3 = R_4 = H$	5.25×10^{-5}	$>2.15\times10^{6}$
52	$R_2 = CH_3, R_1 = R_3 = R_4 = H$	1.83×10^{-6}	$>6.89\times10^{7}$
53	$R_3 = CH_3, R_1 = R_2 = R_4 = H$	2.39×10^{-7}	$>3.97\times10^{8}$
54	$R_4 = CH_3, R_1 = R_2 = R_3 = H$	0.151	218
55	$R_1 = OCH_3, R_2 = R_3 = R_4 = H$	2.38×10^{-3}	$>6.43\times10^{4}$
56	$R_2 = OCH_3, R_1 = R_3 = R_4 = H$	2.99×10^{-3}	$>5.12\times10^{4}$
57	$R_3 = OCH_3, R_1 = R_2 = R_4 = H$	1.92×10^{-4}	$>7.97\times10^{5}$
58	$R_4 = OCH_3, R_1 = R_2 = R_3 = H$	15.8	>9.68

(*Continued*)

Table I. (*Continued*)

No.	Structure	EC_{50} (μM) [a]	TI [b]
59	$R_2 = CH_2CH_2CH_3$, $R_1 = R_3 = R_4 = H$	1.75×10^{-2}	$>8.63\times10^{3}$
60	$R_2 = CH(CH_3)_2$, $R_1 = R_3 = R_4 = H$	3.15×10^{-2}	$>4.79\times10^{3}$
61	$R_2 = C_6H_5$, $R_1 = R_3 = R_4 = H$	0.12	$>1.19\times10^{3}$
62	$R_2 = CF_3$, $R_1 = R_3 = R_4 = H$	1.81	>80.1
63	$R_1 = R_2 = CH_3$, $R_3 = R_4 = H$	1.92×10^{-3}	$>8.02\times10^{4}$
64	$R_2 = R_3 = CH_3$, $R_1 = R_4 = H$	4.19×10^{-3}	$>3.68\times10^{4}$
65	$R_1 = R_3 = CH_3$, $R_2 = R_4 = H$	9.10×10^{-3}	$>1.69\times10^{4}$
66	$R_2 = R_4 = CH_3$, $R_1 = R_3 = H$	4.69×10^{-3}	1.25×10^{3}
67	$R_1 = Cl$, $R_2 = CH_3$, $R_3 = R_4 = H$	2.01×10^{-3}	5.17×10^{4}
68	$R_1 = Ph$, $R_2 = CH_3$, $R_3 = R_4 = H$	43.7	>3.20
69	$R_1 = R_2 = (CH_2)_4$, $R_3 = R_4 = H$	2.12×10^{-3}	$>6.98\times10^{4}$
70	$R_2 = CH_3$, $R_3 = OCH_3$, $R_1 = R_4 = H$	7.21×10^{-6}	$>2.08\times10^{7}$
71	$R_2 = CH(CH_3)_2$, $R_3 = CH_3$, $R_1 = R_4 = H$	No suppression	
72	$R_2 = CH_3$, $R_3 = OCH_2C_6H_5$, $R_1 = R_4 = H$	1.54	87.7
AZT		0.045	41,667

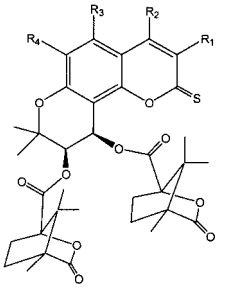
[a]Concentration which inhibits viral replication by 50%.
[b]Therapeutic index.

Compound **87**, the 4-methyl substituted lactam derivative, had potent anti-HIV activity in acutely infected H9 lymphocytes and was about 225-fold more active than AZT in the same assay. The interchange of O and its bioisostere NH retains the anti-HIV activity in DCK series derivatives because of the similar steric size of O and NH and their ability to act as hydrogen bond acceptors. However, when the nitrogen was protected by BOC and could not act as a hydrogen bond acceptor, the synthetic intermediates (**85** and **86**) did not inhibit HIV-1 replication. Thus, the hydrogen bond acceptor ability of NH or O in the lactam DCK or DCK skeleton is likely needed for activity, and may be involved in receptor binding.[31]

E. Approaches to Improve Water Solubility

The poor water-solubility of DCK and its active analogs limits their further development as drug candidates. Thus, introducing polar functional groups into the DCK structure could improve water solubility and provide the possibility of prodrugs. The first approach was preparation of (3′R, 4′R)-di-*O-cis*-acyl-3-carboxyl khellactones.[25] A parallel synthesis route was used to prepare this series of derivatives. After linking ethyl malonate with Wang resin, condensation with *O*-hydroxyaryl aldehyde provided the scaffold. The AD reaction on solid phase successfully gave 91% e.e. using $(DHQ)_2$-PHAL as ligand and OsO_4 as catalyst. After acylation, the resins were washed with DMF and DCM and then treated with 50% TFA in DCM to cleave the resultant 3-carboxy khellactones (**89**–**94**)

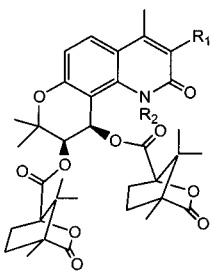
Table II. Structures and Anti-HIV Activities of Khelthiolactone Analogs (**73–84**) in Acutely Infected H9 Lymphocytes and CEM-SS Cell Line



Cmpd	Structure	H9 Lymphocytes		CEM-SS Cell Line	
		EC_{50} (μM)	TI	EC_{50} (μM)	TI
73	$R_1 = R_2 = R_3 = R_4 = H$	0.029	>5,390	0.307	13.7
74	$R_2 = CH_3$, $R_1 = R_3 = R_4 = H$	0.00718	>21,300	0.0635	>3,149
75	$R_2 = C_3H_7$, $R_1 = R_3 = R_4 = H$	0.128	>1,153	0.128	>1,153
76	R_2 = phenyl, $R_1 = R_3 = R_4 = H$	2.48	>56.6	2.48	>56.6
77	$R_1 = CH_3$, $R_2 = R_3 = R_4 = H$	0.0119	>12,900		
78	$R_3 = CH_3$, $R_1 = R_2 = R_4 = H$	0.0199	>7,690		
79	$R_3 = OCH_3$, $R_1 = R_2 = R_4 = H$	1.28	>117		
80	$R_4 = OCH_3$, $R_1 = R_2 = R_3 = H$	-	N/S		
81	$R_2 = R_3 = CH_3$, $R_1 = R_4 = H$	0.262	>571		
82	$R_2 = CH_3$, $R_3 = OCH_3$, $R_1 = R_4 = H$	1.46	>1000		
83	$R_1 = R_2 = CH_3$, $R_3 = R_4 = H$	0.0334	>4490		
84	$R_1 = C_6H_5$, $R_2 = CH_3$, $R_3 = R_4 = H$	4.95	6.24		
AZT		0.045	41,667	0.0038	>263
DCK (**2**)		2.56×10^{-4}	$>1.37\times10^{5}$	0.14-0.26	100
4-MeDCK(**52**)		1.83×10^{6}	$>6.89\times10^{7}$	0.0635	>3,150

(Fig. 5). However, none of these compounds showed promising activity, even the camphanoyl substituted **89**.

Next, a series of 3-substituted compounds was synthesized (**95**–**100**).[32] The data below show that anti-HIV potency is maintained with a polar substituent at position-3, for example, 3-hydroxymethyl (**98**) and 3-dibromomethyl (**95**). However, charged substituents, either negative [3-carboxyl (**89**–**94**)]



85, R_1 = H, R_2=BOC, no supression

86, $R_1 = CH_3$, R_2=BOC, no supression

87, R = H, EC_{50} = 0.00024 μM; TI = 119,333

88, R = CH_3, EC_{50} = 0.0046 μM; TI = 9,100

Figure 4. Structures and activities of the 4-methyl substituted DCK lactam analogs (**85 – 88**) in acutely infected H9 lymphocytes.

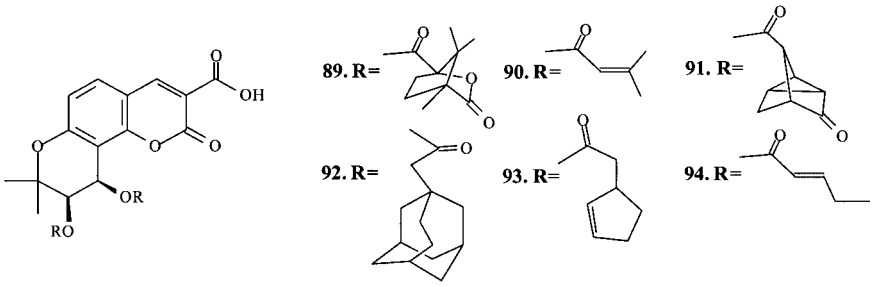


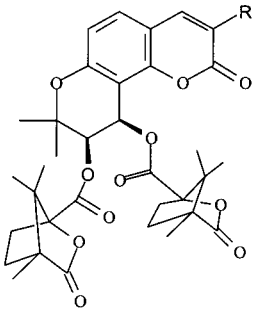
Figure 5. Structures of six (3′R, 4′R)-di-*O-cis*-actyl-3-carboxyl khellactones (**89 – 94**) with different 3′ and 4′ substituents by parallel synthesis.

or positive [3-amino (**99**) and (**100**)], are not favorable (Fig. 6). A hydroxyl group can form H-bonds with water to improve hydrophilicity or with an active site on a biological target to increase affinity. In addition, the hydroxyl group can be linked with other functional groups to form a water-soluble molecule or a prodrug.

F. Structure–Activity Relationship Conclusions

The following conclusions were drawn from the SAR studies of DCK analogs and derivatives:

1. Stereochemistry at the 3′ and 4′ positions should be R-configured.
2. The volume, size, and shape of the camphanoyl group are more important than the absolute configuration of its chiral carbon. However, (−)-camphanoyl analogs are most potent.
3. A planar coumarin system is probably an essential feature for potent anti-HIV activity. Steric compression between C(4) and C(5) substituents can reduce the overall planarity and, thus, resonance of the coumarin system, resulting in decreased or completely lost activity.
4. Methyl or other aliphatic substitutions on the coumarin nucleus are favorable for anti-HIV activity, whereas aromatic substituents are not.
5. Thio- and lactam 4-methyl-DCKs retain activity. In the CEM-SS cell line, the thio analog is more potent than 4-methyl-DCK.
6. 3-Hydroxymethyl and 3-dibromomethyl analogs retain potency, but 3-carboxyl and 3-amino analogs do not. Thus, polar but not negatively or positively charged substituents can be tolerated.



95. R = CH_2Br; EC_{50} = 0.0854 μM, TI = 40;

96. R = $CHBr_2$; EC_{50} = 1.19×10^{-4} μM, TI = 26,890;

97. R = CH_2OAc; EC_{50} = 1.03×10^{-3} μM, TI = 3243;

98. R = CH_2OH; EC_{50} = 1.88×10^{-4} μM, TI = 188,032;

99. R = CH_2NEt_2; EC_{50} = 0.69 μM, TI = 62;

100. R = CH_2NH_2; EC_{50} = 0.39 μM, TI = 208

Figure 6. Structures and activities of six 3-substituted dicamphanoyl-khellactone (DCK) analogs (**95 – 100**) in acutely infected H9 lymphocytes.

G. Mechanism of Action

Preliminary mechanism of action studies have been performed with DCK. It selectively inhibited virus replication in HIV-1 infected cells, but did not inhibit activity of three major HIV enzymes—HIV RT, protease, and integrase.[21] The studies did indicate that DCK inhibits HIV after viral entry but prior to viral DNA integration.

3. TETRACYCLIC DIPYRANOCOUMARINS AS ANTI-HIV AGENTS

Since their discovery in 1992, the calanolides, isolated from *Calophyllum lanigerum*, have been reported to be active against strains of HIV-1 in early stage reproduction.[33] Calanolides are tetracyclic dipyranocoumarins and their C-ring contains a gem-dimethyl group. Examples are (+)-calanolide A (**101**), (−)-calanolide B (costatolide) (**102**), and the cordatolides (**105** and **106**).[34] Another group of tetracyclic pyranocoumarins, the inophyllums, was isolated from the genus *Calophyllum*.[35–37] Both calanolides and inophyllums inhibit HIV-1 RT.[38] The most active inophyllums (**116**, **117**, and **118**) have slightly different stereochemistry than the potent calanolides (**101** and **102**) (Fig. 7).[39]

A. Modification of Calanolides

Many SAR studies[40,41] have focused on the three chiral centers in ring C of the prototype anti-HIV calanolide, (+)-calanolide A (**101**). Of all diastereomers, only **101** and **102**, which contain both 10,11-transmethylation and 12-(S)-OH chirality, display anti-HIV activity, with EC_{50} values of 0.18 ± 0.01 and 0.2 ± 0.1 μM, respectively. Neither their enantiomers (12-R-OH) nor epimeric alcohols, calanolide C (**107**), and epi-calanolide C (**108**), show noticeable anti-HIV activity. Because the two active calanolides (**101** and **102**) show similar potencies, the relative stereochemistry of C-10 to C-11 must underline an essential conformational feature. In addition, the inactivity of the enantiomeric calanolides indicates a critical stereoelectronic requirement at C-12.

Based on structural and functional modification of the C-12 hydroxyl group (**101**–**115**) (Fig. 7; Table III), a heteroatom is required at this position. Compounds **103** and **111**, without a hydroxyl group on C-12, did not show antiviral activity. $\Delta^{11,12}$ Olefination also diminished activity (**115**). Relative potencies of ketone (**104**), thiol (**109**), azide (**110**), amine (**113**), and acetylated derivatives (marginal activity, data not shown) also suggest stringent spatial and stereochemical requirements around C-12 (Table III).[40] However, both of the enantiomers of 12-oxocalanolide A (**104A** and **B**), synthetic intermediates containing one fewer chiral centers, were active against HIV in cytopathic assays. This result suggests that, for active analogs, the oxygen substituent can either be in the plane of the aromatic system or possess S configuration. Oxocalanolide A [**104** 12-(±), **104A** 12-(+)] exhibited similar antiviral activity, while (−)-12-oxocalanolide (**104B**) was less active. Interestingly, the racemic form, (±)-12-oxocalanolide (**104**), was consistently more active than the pure (+)-enantiomer (**104A**) implying a possible synergistic effect in the combination of the two enantiomers. In addition, unlike calanolides A and B, 12-oxocalanolides (**104A** and **B**) are also active against SIV (Table IV).[42]

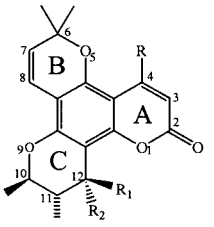
Currently, the only synthetic modification applied to the B ring of calanolides is 7,8-hydrogenation. The correspondent 7,8-dihydro derivatives, **114** and **112**, showed the same activity as the active calanolides A (**101**) and B (**102**) (Table III), respectively. However, activity was lost when the chiral character at C-12 was eliminated (**111** and **115**), which further confirms the importance of stereochemical requirements at C-12.

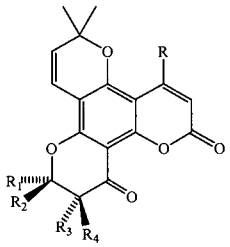
Activity of calanolides was affected not only by the types and relative stereochemistry of the C-10, -11, and -12 functional groups, but also by the C-4 substituent. Inophyllums, which have a C-4 phenyl, showed similar potency to the calanolides, which have a C-4 propyl, whereas cordatolide, the

C-4 methyl derivative (**105** and **106**), showed substantially lower activity.[37] These results suggest that a bulky group is necessary at C-4.

B. Modification of Inophyllums

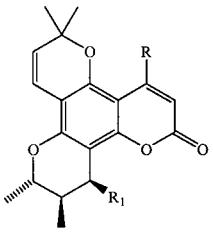
A series of inophyllums were isolated from the Malaysian tree *Calophyllum inophyllum* Linn and evaluated for inhibitory activity against HIV-1 RT. Among them, the most active compounds, inophyllum B (**116**) and inophyllum P (**117**) showed IC_{50} values against RT of 0.038 and 0.130 μM, respectively.[35] Pengsuparp [43] also reported the HIV-1 RT inhibition activity of soulattrolide [**118**, (−)

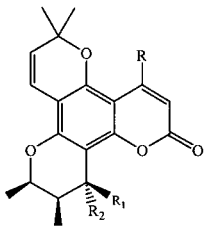


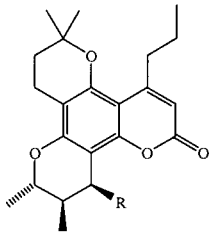


101. (+)-Calanolide A (R=n-pr, R_1=OH, R_2=H)
103. R=n-propyl, R_1=OMe, R_2=H
105. Cordatolide A (R=Me, R_1=OH, R_2=H)
106. Cordatolide B (R=Me, R_1=H, R_2=OH)
116. Inophyllum B (R=ph, R_1=OH, R_2=H)
117. Inophyllum P (R=ph, R_1=H, R_2=OH)

104A. (+)-12-Oxo-Calanolide (R=n-pr, R_1=R_4=Me, R_2=R_3=H)
104B.(-)-12-Oxo-Calanolide (R=n-pr, R_1=R_4=H, R_2=R_3=Me)
121. Inophyllum C (R=ph, R_1=R_4=H, R_2=R_3=Me)
122. Inophyllum E (R=ph, R_1=R_3=H, R_2=R_4=Me)



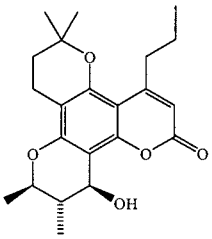


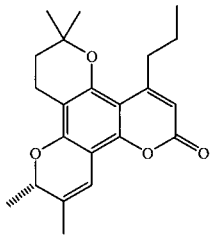


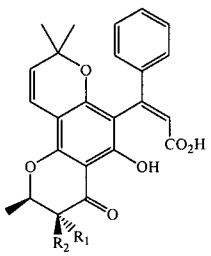
102. (-)-Calanolide B (R=n-pr, R_1=OH)
109. R=n-pr, R_1=SH
110. R=n-pr, R_1=N_3
118. Soulattrolide (R=ph, R_1=OH)

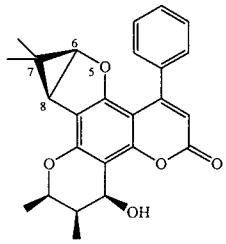
107. Calanolide C (R=n-pr, R_1=OH, R_2=H)
108. Epi-Calanolide C (R=n-pr, R_1=H, R_2=OH)
119. Inophyllum A (R=ph, R_1=OH, R_2=H)
120. Inophyllum D (R=ph, R_1=H, R_2=OH)

111. R=H
112. R=OH
113. R=NH_2









114

115

123. Calophyllic acid (R_1=Me, R_2=H)
124. Isocalophyllic acid (R_1=H, R_2=Me)

125. Inophyllum G-2

Figure 7. Structures of calanolides (**101–110**) and inophyllums (**111–125**).

Table III. HIV-1 Inhibitory Activities of Calanolide Analogs in NCI Primary Anti-HIV Screening[40] Assay*

Cmpd	EC_{50} (μM)	IC_{50} (μM) [c]	TI
101	0.18 ± 0.01	7.3 ± 1.8	40
102	0.2 ± 0.1	5.9 ± 1.9	31
103	--	--	Inactive
107 [33]	(maximum protection 30%) [d]	30	NM[d]
109	8.4 ± 1.1	>150	NM
110	1.6 ± 0.1	5.9 ± 2.2	4
111	--	--	Inactive
112	0.2 ± 0.02	8.2 ± 1.8	41
113	0.6 ± 0.1	5.8 ± 0.6	10
114	0.2 ± 0.1	5.2 ± 0.4	23
115	--	--	Inactive
116 [35]	1.4	55	39
117 [35]	1.6	55	16

*NCI primary anti-HIV screening was used to measure cytoprotective effect in human lymphoblastoid CEM-SS cell against HIV-1 (RF).
[c]Concentration which inhibits uninfected cell growth by 50%.
[d]Not measurable.

inophyllum P] in two different assays. The IC_{50} value was 0.73 μM, when RT was assessed for DNA-dependent DNA polymerase (DDDP), and 0.34 μM, when RT was assessed for RNA-dependent DP (RDDP). The inactivity of inophylums A (**119**) and D (**120**) suggests that the *trans* relationship between the 10 and 11 methyl groups is required in this compound class. The stereochemistry of the C-12 hydroxyl is not as critical as for the calanolides, since both **116** (12-S) and **117** (12-R) are active at submicromolar concentration. However, unlike 12-oxocalanolides (**104**), with a carbonyl group at this position, **121** and **122** showed only marginal activity. Several compounds with deformed (**123** and

Table IV. Anti-HIV Activities of 12-Oxo-Calanolide Enantiomers in Different HIV Virus Infected Cell Lines

Virus	Cells	Cmpd/EC_{50} (μM)			
		101	**104** (racemic)	**104A**	**104B**
HIV-1(IIIB)	CEM-SS	0.08	0.51	1	1.88
HIV-1(RF)	CEM-SS	0.1	0.4	0.9	3.41
HIV-1(SKI)	CEM-SS	0.09	0.17	0.17	0.27
HIV-2(ROD)	CEM-SS	>10	5.57	15.9	ND
SIV (DeltaB670)	174XCEM	>10	1.24	1.66	0.19

124), or rearranged tetracyclic ring (**125**) systems were inactive. These data indicate that the tetracyclic dipyranocoumarin system is also an essential key to retaining RT inhibitory activity.

C. Structure–Activity Relationship Conclusions

1. Bulky substituents are required at C-4 position.
2. Both calanolides and inophyllums require methyls at C-10 and C-11 of the chromanol ring to be trans diaxial.
3. Both calanolides and inophyllums require a hydrogen bond acceptor at C-12. In case of calanolides, the C-12 hydroxyl should be S configured, or carbonyl can be present. C-12 hydroxyl of inophyllums can be either S or R configured, but cannot be a carbonyl.

D. Mechanism of Action

Mechanistic *in vitro* assays indicated that calanolide A (**101**) inhibits RT in two different template-primer systems, primed ribosomal RNA template and homopolymeric poly rA- oligodT_{12-18} template/primer. Kinetic analysis with **101** revealed that it inhibits RT in a complex mechanism involving two binding sites. This action is likely due to the bi-bi ordered mechanism of RT, which requires template/primer binding prior to polymerization. At a certain concentration, **101** bound HIV-1 RT in a mutually exclusive fashion with the pyrophosphate analogs phosphoformic acid or 1-ethoxymethyl-5 ethyl-6-phenylthio-2-thiouracil, indicating that calanolide A (**101**) interacts with RT near the pyrophosphate binding site in addition to the active site.[44] Both Km for dTTP and Vm were affected with saturating rRNA concentration and unsaturating dTTP concentration. Unlike general NNRTIs, which noncompetively inhibit RT, compound **101** is at least partly competitive in respect to dNTP binding. Interestingly, the structurally related inophyllums inhibit RT in a simple noncompetitive manner with dNTP, which suggests that template-primer is required for inhibitor binding.[35,45]

E. In Vitro and In Vivo Study of Calanolides

Calanolide A (**101**), calanolide B (**102**), (+)-12-oxo-calanolide A (**104A**), and dihydrocalanolide B (**112**) were evaluated *in vitro* in combination with one or two current anti-HIV agents including NRTIs, NNRTIs, and PIs.[46,47] They exhibited different synergistic or additive antiviral interactions with those NRTIs, NNRTIs, and PIs. The *in vitro* anti-HIV assay suggests the best combination is the calanolides together with lamivudine and nelfinavir. No antagonistic anti-HIV drug interactions or synergistic toxicity was observed.[47] These properties make calanolides attractive candidates for therapeutic use.

In addition, calanolides are active against a wide range of viral strains in different cell lines.[41,48] They exhibit unique sensitivity profiles to drug resistant strains (Table V).[46] The calanolides retain activity against M184V and P236L (3TC and HEPT resistant, respectively) strains and showed strikingly enhanced potency against one of the most common mutations, Y181C (diphenylsulfone, nevirapine, E-BPTU, and UC38 resistant). All tested calanolides did not show activity against Y188H (**102** resistant), however, **102** was moderately active against T139I (**101** resistant).

Lipophilic **101** distributed readily into brain and lymph, which can be a part of viral reservoirs. Additionally, **101** suppressed HIV-1 (IIIB) replication in CEM-SS cells loaded on the hollow fibers in two separate physiological compartments, subcutaneous site and peritoneal cavity. Synergy with AZT was also confirmed *in vivo* along with its significant anti-HIV activity.[49] In a different study, compound **101** and its 7,8-dihydrogenated analog **114** were examined for bioavailability in CD2F1 mice at a dose of 50 mg/kg. Distribution and elimination of both compounds were similar as reflected by the pharmacokinetic parameters. However, apparent volume distribution (Vd) and oral clearance

Table V. Activities of Compounds **101**, **102**, and **112** Against Resistant Strains

Drugs to which isolates are resistant (mutation)	Cmpd/EC_{50} (μM)				
	101	**102**	**112**	Nevirapine	AZT
IIB (control)	0.1	0.2	0.2	0.01	0.05
Oxathinn carboxanilide (L100I)	>27	>270	>20	0.1	0.04
UC10-**102** (L103K)	>27	>270	>20	ND	0.003
Thiazolobenzimidazole (V108I)	24	4.4	3.5	0.3	0.04
TIBO-R82150 (A98G-V108I)	22	1.6	5.1	0.6	0.05
101 (T139I)	>27	4.5	>20	0.01	0.01
Diphenylsulfone (Y181C)	0.08	0.08	<0.01	5.9	0.01
Nevirapine(Y181C)	<0.01	<0.01	0.09	>38	0.03
Pyridinone (Y181C-L103N)	0.12	0.8	0.8	>38	0.01
E-BPTU (Y181C)	0.1	<0.08	<0.06	1.9	0.03
UC38 (Y181C)	0.2	<0.03	0.1	1.9	0.01
3TC (M184V)	0.3	1.3	1	0.01	0.02
102 (Y188H)	>27	>27	>27	ND	0.004
HEPT (P236L)	0.6	1.1	0.2	0.02	0.01

were significantly different for the two compounds after oral administration. **114** was more orally bioavailable than **101** without losing anti-HIV activity (F = 46.8 and 13.2%, respectively).[50]

Compound **101** is synthesized from phloroglucinol in five steps and is being investigated for clinical research.[51–54] A phase IA studies showed that **101** was generally well tolerated in doses up to 600 mg. In human subjects, plasma levels of **101** were higher than those predicted from animal studies. As expected from animal studies, gastrointestinal intolerance was not severe, but five subjects reported dyspepsia. The most common adverse event was an oily after taste in 19 of 32 patients and some reported transient dizziness. Calculated half-life in the 800 mg dosing cohort was approximately 20 hr.[55] In phase IB studies, **101** was administered orally to HIV infected subjects. Clinical and laboratory assessment on viral load and CD4 count indicated that antiviral effects of **101** appeared to be dose-dependent and maximized on day 14 or 16.[56]

Currently, Sarawak Medichem Pharmaceuticals has begun screening volunteers for combination therapy of calanolide A for treating AIDS, and hopes to progress to phase III clinical trial in late 2002.[57]

4. MISCELLANEOUS ANTI-HIV COUMARINS

A. Protease Inhibitor

HIV-1 protease has been identified as a significant target enzyme in AIDS research. Semi-synthetic anticoagulants such as warfarin (**126**) and phenprocoumon (**127**) were found to strongly inhibit cell free and cell mediated HIV infection (Fig. 8). These compounds are competitive micromolar

inhibitors of HIV-1 protease, and preliminary studies indicated that the pyran-2-one group, 4 hydroxyl group, and substitution at the 3-position are all necessary for activity.[58]

In a different study, a collaborative process involving mass screening, molecular modeling, protein crystallography, and chemical synthesis was used to design an optimized inhibitor of HIV-1 protease.[59] First, PD099560 (**128**) was identified as a nonpeptide competitive HIV-1 protease inhibitor from mass screening (Fig. 8). Then, inhibitor-enzyme binding was investigated computationally, using the program Autodock to assist the docking procedure and Monte-Carlo simulations along the energy evaluations. The crystal structure of **128** complexed with HIV-1 protease was solved at 3.0 A resolution. In both the modeled and X-ray complexes, the coumarin hydroxyl was

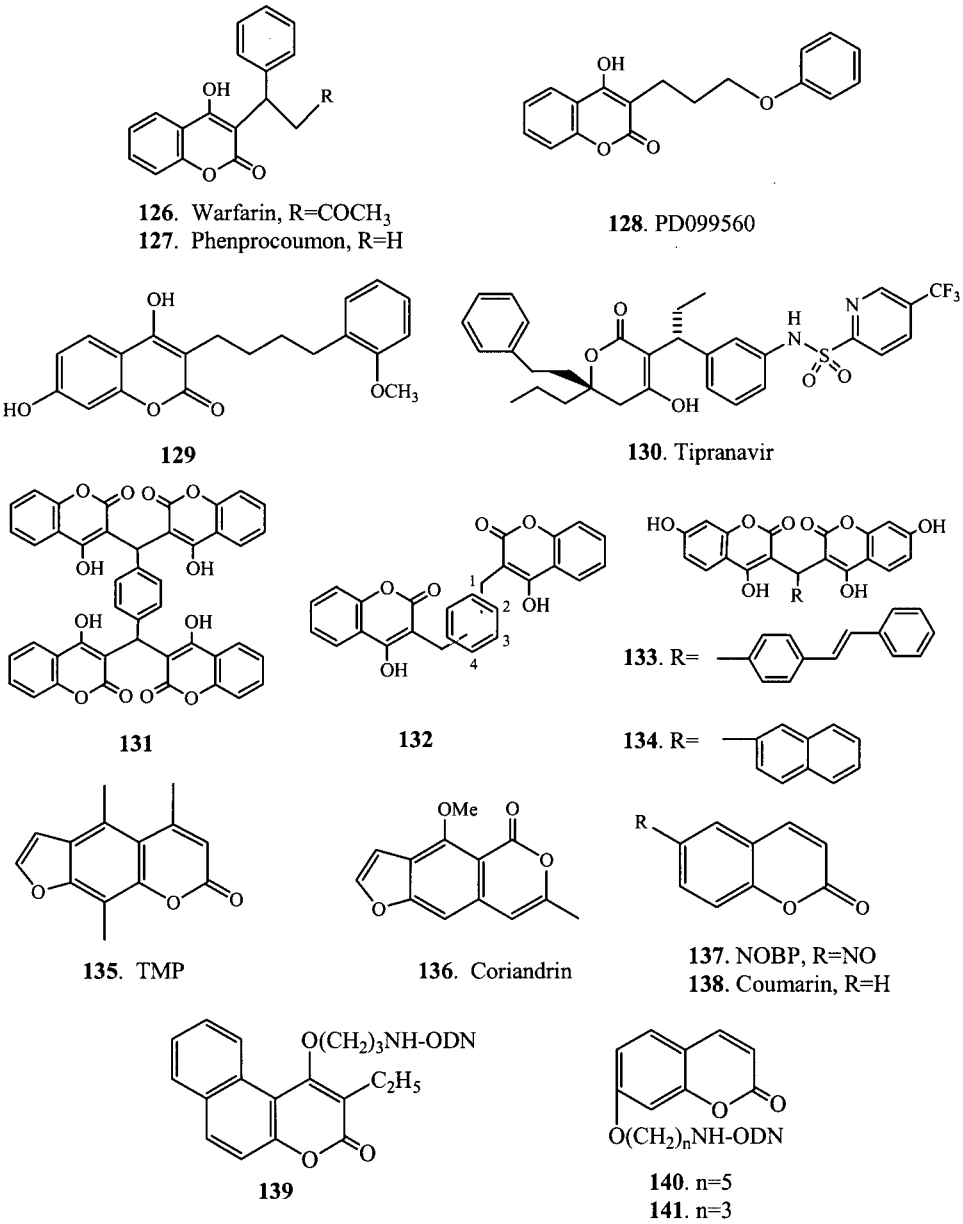


Figure 8. Miscellaneous anti-HIV coumarins.

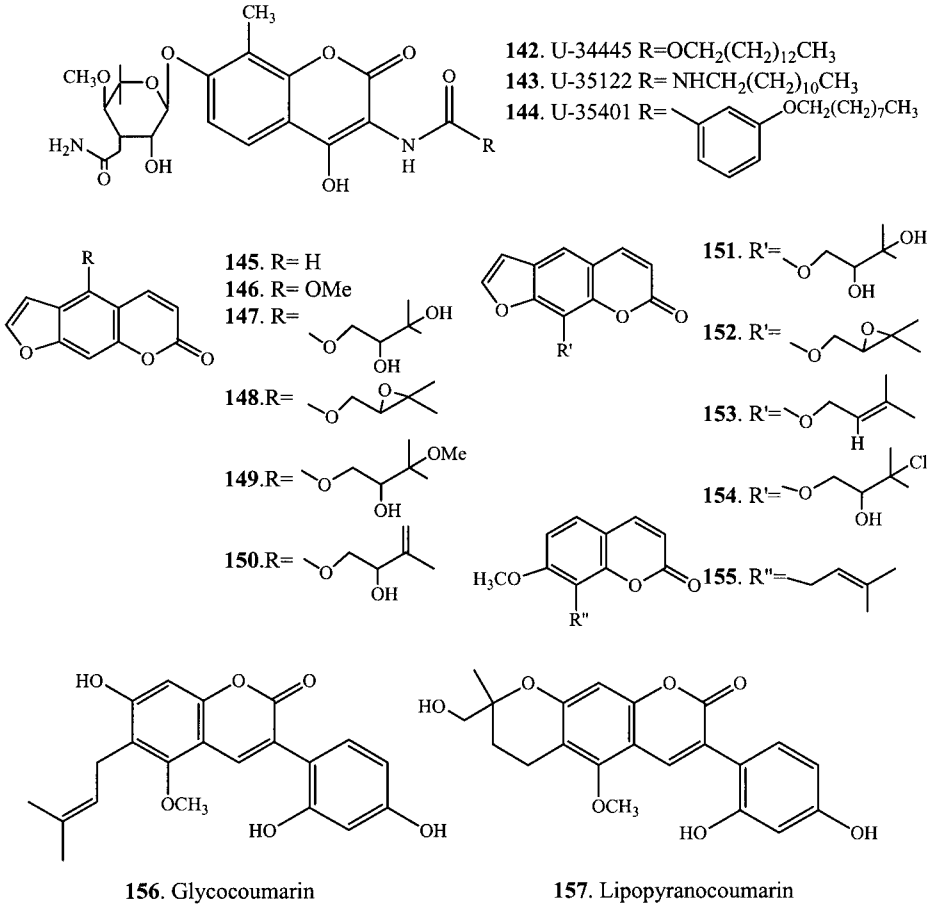


Figure 8. (*Countinued*)

positioned between two catalytic aspartates and the lactone ring oxygens interacted with the flap loop. The program GRID, which employs probes to determine favorable interaction sites on a molecular surface, was used to assist further inhibitor design. Modified **128**-analogs based on the computational study were more active than **128**; the most active compound was **129** (Fig. 8) with an IC_{50} of 0.52 μM in the HIV protease assay.[59,60] Further QSAR studies have lead to discovery of a sufonamide-containing 5,6-dihydro-4-hydroxy-2-pyrone, also called tipranavir (**130**), which is currently undergoing phase II clinical trial.[60–63]

B. Integrase Inhibitor

In addition to HIV RT and protease, HIV integrase is also a major chemotherapeutic target; however, no integrase inhibitors are currently marketed.[64] Among numerous HIV-1 integrase inhibitors under investigation, common structural features are two aryl units separated by a central linker. Such compounds include biscatechols and coumarins. Because catechols are cytotoxic, partly due to *in situ* oxidation to quinone species, coumarins have clinical advantages. A natural tetrameric coumarin (**131**) showed high anti-integrase activity (IC_{50} = 0.8 μM for integration and 1.5 μM for 3′-processing), and the parent compound was then dissected into simpler components to determine which features were essential for potency.[65] A biscoumarin unit linked by a phenyl ring was required

for activity within the test range. When the site of coumarin attachment was varied about the central phenyl linker, anti-integrase activity increased only slightly as the orientation varied from 1,1- (**132**) to 1,2- to 1,3-, but there was no significant change against 3′ processing. The IC_{50} values ranged between 11.1 ~ 38.8 μM for integration and 34.0 ~ 46.0 μM for 3′-processing. However, when 1,4-orientation was reached, potency dropped significantly for both activities, 69.2 μM for integration and 80.6 μM for 3′-processing. The structure of the central linker was then varied. Adding hydroxyl or amine functionality at the phenyl 4-position reduced inhibitory activity, while nitro or carboxyl groups had little effect. Replacing the phenyl ring with a heteroaryl ring, such as furan or thiophene, decreased inhibitory potency. Increasing the number of aryl rings on the central linker enhanced potency; the rigid stilbene analog (**133**) being the most potent (integration 3.7 μM, 3′-processing 5.5 μM) among the compounds synthesized. 7-Hydroxylated analogs were then synthesized based on the reported integrase inhibitory activities of several monomeric 7-hydroxy coumarins. 7-Hydroxylation was beneficial in a wide range of dimeric 4,7-hydroxycoumarins and led to a simplified coumarin integrase inhibitor without greatly sacrificing the potency of the tetrameric compound. In the studies reported, 3,3′-(2-naphthalenomethylene)-bis-[4,7-dihydroxycoumarin] (**134**) was the optimized structure with an IC_{50} of 3.6 μM for integration and 4.2 μM for 3′ processing.[66]

C. RT Inhibitor

HIV-1 RT is thought to have interaction with complementary oligodeoxynucleotide (ODN) primers at the 5′-end of the tRNA binding site as well as at the 3′-end of the primer. ODN derivatives containing specific intercalating groups, including acridine, phenazine, and ethidium, at the 5′ and/or 3′ ends are useful molecules in antisense and antigene strategies because they can form specific, more stable complexes with complementary nucleic acids. Some additional groups can stabilize nucleic acid duplexes complexed with enzymes, as reported with phenazine, ethidium, daunomycin, and deuteropurphyrin ODN complexes with HIV-1 RT. When several couromone and coumarin structures were conjugated to the 5′-end of ODNs (**139**–**141**) (Fig. 8), affinity toward HIV-1 RT increased, suggesting that these compounds may be functioning as primers. This action was confirmed when protection of RT by $tRNA^{lys3}$ decreased the complex formation between the enzyme and the conjugated ODN. Addition of $d(pT)_{16}$ did not alter this interaction. In general, addition of either homologous ODN (d(pT)n) or heterologous ODN (ODN-1: d(pDAGGTG))and ODN-2 d(pCCAAACA)) alone did not change the rate of polymerization catalyzed by HIV-1 RT in presence of poly(A)$d(pT)_{16}$ as template. The same ODNs conjugated to couromone or coumarin (**139**–**141**) did change the polymerization rate: either inhibition or slight activation followed by inhibition depending on the concentration. When ''chain terminator'' 3′ddT was added, the ligand-ODN complex was easily converted to a strong inhibitor.[67]

D. Other Mechanisms

Furanocoumarins, such as the psoralens and angelicin, are common constituents of many members of the Rutaceae and Apriaceae plant families. They are commonly UVA phototoxic toward cells, bacteria, fungi, and viruses. The toxicity is usually attributed to their ability to reversibly intercalate into nucleic acids, followed by light-activated production of monoadducts or biadducts with pyrimidine bases, especially thymines. Photoadducts are pyrone-side or furan-side monoadducts or crosslinked. Photomodified viral genomes cannot be transcribed into RNA/DNA, thus the furano-coumarins inhibit viral replication. Exposure of HIV-1 to 4,5′,8-trimethylpsoralen (TMP, **135**) drastically reduced syncytia formation in Molt-3 cells at 100 ng/ml and abolished syncytia formation at 1 μg/ml upon UVA radiation. Virus-infected cells treated with **135**, but not irradiated, were only slightly affected by the drug at 1 μg/ml.[68]

Coriandrin (**136**), a furanoisocoumarin from coriander, is much more photoreactive than psoralen but does not cross-link with DNA nor photosensitize human skin. Although HIV-1 was less sensitive

than SINV to coriandrin, psoralens may also have non-DNA targets, such as viral membranes, proteins or lipids bound covalently in a UVA-dependent manner.[69]

Retroviral zinc fingers with the sequence Cys-X2-Cys-X4-Hix-X4-Cys(CCHC) bind zinc stoichiometrically and with high affinity. Retroviral nucleocapsid and gag-precursor proteins from all known strains of retroviruses contain one of two copies of this invariant sequence. C-nitroso ligands, including the simple 3-nitrosobenzamide (NOBO) and the coumarin 6-nitroso-1,2-benzopyrone (NOBP, **137**) reportedly preferentially destabilize one of the two zinc fingers with concomitant loss of enzymatic activities confirmed by NMR studies. Retroviral CCHC zinc fingers participate in several nucleic acid interactions during the course of the viral life cycle, including the specific recognition of the viral genome during budding, RNA packaging into virions, and facilitation of reverse transcription. When HIV was pre-incubated with NOBO and **137**, viral infectivity was reduced in human lymphocytes, but when drugs and viruses were diluted separately before addition to the lymphocytes, essentially no effect was observed. Thus, activity depends on how long virus is exposed to the drug. Additionally, the intracellular lifetime of C-nitroso drugs is usually short and the drug is quickly converted to non-toxic products. Thus, a rapid one-hit cellular mechanism could explain the low toxicity.[70]

Poly (ADP-ribose) polymerase inhibitors suppress UV-induced HIV-1 gene expression. Such inhibitors include the R-NH_2 type ligands nicotineamide and 3-aminobenzamide, and they are known to inhibit poly-ADP ribosylation by competing with the substrate NAD. Because R-NH_2 type ligands are metabolic precursors of R-NO, the observed inhibition may be attributed partly to destabilization of retroviral CCHC zinc fingers of poly ADP ribose polymerase. Coumarin (**138**) also inhibits the activity of poly (ADP-ribose) polymerase, but through a different mechanism, by blocking the activation of the enzyme by its effector, double stranded DNA fragments.[71]

Novenamins contain a substructure of the antibiotic novobiocin, the sugar noviose attached to substituted coumarin residues. Novenamins such as U-34445 (**142**), U-35122 (**143**), and U-35401 (**144**) are specific inhibitors of the HIV-1 RNase H and do not interfere with polymerase function when tested at 100 μM. Dose response curves indicate that inhibitory action may be governed by strong amphophilicity, and activity is elicited only after a critical concentration threshold is reached. The IC_{50} values represent the sum of the inactive monomers and the active micelles and, consequently, are rather high. However, these compounds can serve as templates for the synthesis of optimized dimers, oligomers, or other simplified compounds.[72]

Initial reports have indicated that several other coumarins isolated from plant extracts exhibit anti-HIV IIIB activity *in vitro* in H9 lymphocytes; however, no additional investigations have been reported. Eight furanocoumarins from dried roots of *Ferula sumbul*, oxypeucedanin hydrate (**147**), oxypeucedanin (**148**), paburenol (**149**), oxypeucedanin methnolate (**150**), heraclenol (**151**), heraclenin (**152**), imperatorin (**153**), and osthol (**155**), were reported with EC_{50} values in the micromolar range.[73] Structurally similar coumarins, psoralen (**145**), heraclenin (**152**), pabulenol (**149**), saxalin (**154**), and bergapten (**146**) were isolated from Prangos *tschimganica* and inhibited HIV-1 replication in the same assay system.[74] Aesculetin (6,7,-dihydroxycoumarin) from *Artemisia capillaris* displayed an ED_{50} of 2.51 μg/ml and TI of 11.2 (Fig. 8; Table VI).[75]

Glycylcoumarin (**156**) and licopyranocoumarin (**157**) (Fig. 8) along with other phenolics isolated from licorice were reported to inhibit the cytopathic activity of HIV in the OKM-1 cell line at a concentration of 20 μg/ml without observable cytotoxicity.[76]

5. CONCLUSIONS

In a decade of extensive research, great progress has been achieved in the discovery of potential anti-HIV coumarins. Coumarins have been associated with several modes of action, including inhibition of viral adsorption (glycocoumarin, licopyranocoumarin), reverse transcription (calanolides and

Table VI. Compound **145–155** Inhibit HIV-1 (IIIB) Replication in H9 Lymphocytes

		IC_{50} (μM)	EC_{50} (μM)	TI
145	Psoralen [e]	19.1	0.1	19.1
146	Bergapten [e]	24.8	0.354	69.9
147	Oxypeucedanin hydrate [f]	21.1	10	2.11
148	Oxypeucedanin[f]	23.4	1.05	22.2
149	Oxypeucedanin metholate[f]	>100	33.3	3
150	Pabulenol [f]	16.7	6.38	2.61
151	Heraclenol [f]	>100	0.115	870
152	Heraclenin [e (f)]	19.8 (20.1)	7.29 (2.37)	2.71 (8.48)
153	Imperatorin [f]	>100	<0.1	>1000
154	Saxakin [e]	26.3	2.25	11.7
155	Osthol [f]	11.7	0.155	75.5

[e] AZT IC_{50} 500 μM, EC_{50} < 0.001 μM, TI > 500,000.[75]
[f] AZT IC_{50} 500 μM, EC_{50} 0.032 μM, TI 15,625.[74]

analogs), protease inhibition (3-substituted-4-hydroxycoumarins), and integration (tetrameric coumarins). Anti-HIV activity in subnanomolar potency has been achieved with synthetic analogs of suksdorfin, a khellactone pyranocoumarin. These potent dicamphanoyl-khellactones (DCKs) also are structurally and mechanistically unique from clinically used anti-HIV agents. The modes of action of DCK analogs have not been fully identified, but are the focus of ongoing studies. No plant-derived coumarin is currently in clinical use as an anti-HIV agent; however, excellent results have been shown in preclinical (DCK) studies and early clinical (calanolide) trials. Therefore, coumarin compounds showing significant activity and possessing unique HIV inhibitory mechanisms could have potential in future drug development and therapy.

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REFERENCES

1. Kanazawa S, Matija PB. Repression of MHC determinants in HIV infection. Microbes Infect 2001;3:467–473.
2. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, Saxon A. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men. N Engl J Med 1981;305: 1425–1431.

3. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vezinet-Brun F, Rouzioux C, Rozenbaum W, Montagnier L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983;220:868–871.
4. Gallo RC, Sarin PS, Gelmann EP, Robert-Guroff M, Richardson E, Kalyanaraman VS, Mann D, Sidhu GD, Stahl RE, Zolla-Pazner S, Leibowitch J, Popovic M. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 1983;220:865–867.
5. UNAIDS and WHO. AIDS epidemic update, Under Embargo. 2001.
6. Moore JP, Stevenson M. New targets for inhibitors of HIV-1 replication. Nat Rev Mol Cell Biol 2000;1: 40–49.
7. Miller MD, Hazuda DJ. New antiretroviral agents: Looking beyond protease and reverse transcriptase. Curr Opin Microbiol 2001;4:535–539.
8. FDA. Antiretroviral drugs approved by FDA for HIV. Information may be found at internet at: http:// www.fda.gov/oashi/aids/virals.html/
9. FDA. FDA talk paper: FDA approves Viread for HIV-1 infection. Information may be found at internet at: http://www.fda.gov/bbs/topics/ANSWERS/2001/ANS01111.html/
10. Swanstrom R, Erona J. Human immunodeficiency virus type-1 protease inhibitors: Therapeutic successes and failures, suppression and resistance. Pharmacol Ther 2000;86:145–170.
11. Loveday C. Nucleoside reverse transcriptase inhibitor resistance. JAIDS 2001;26:S10–S24.
12. Pellegrin I, Garrigue I, Caumont A, Pellegrin JL, Merel P, Schrive MH, Bonot P, Fleury H. Persistence of zidovudine-resistance mutations in HIV-1 isolates from patients removed from zidovudine therapy for at least 3 years and switched to a stavudine-containing regimen. AIDS (London, UK) 2001;15:1071–1073.
13. Fellay J, Boubaker K, Ledergerber B, Bernasconi E, Furrer H, Battegay M, Hirschel B, Vernazza P, Francioli P, Greub G, Flepp M, Telenti A. Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV Cohort Study. Lancet 2001;358:1322–1327.
14. Vlietinck AJ, De Bruyne T, Apers S, Pieters LA. Plant-derived leading compounds for chemotherapy of human immunodeficiency virus (HIV) infection. Planta Med 1998;64:97–109.
15. Hudson J, Towrs GHN. Phytomedicines as antivirals. Drugs Future 1999;24:295–320.
16. Yang SS, Cragg GM, Newman DJ, Bader JP. Natural product-based anti-HIV drug discovery and development facilitated by the NCI developmental therapeutics Program. J Nat Prod 2001;64:265–277.
17. Nishino H, Okuyama T, Takata M, Shibata S, Tokuda H, Takayasu J, Hasegawa T, Nishino A, Ueyama H, Iwashima A. Studies on the anti-tumor-promoting activity of naturally occurring substances. IV. Pd-II [(+)anomalin, (+)praeruptorin B], a seselin-type coumarin, inhibits the promotion of skin tumor formation by 12-O-tetradecanoylphorbol-13-acetate in 7,12-dimethylbenz[a]anthracene-initiated mice. Carcinogenesis (London) 1990;11:1557–1561.
18. Tsai IL, Wun MF, Teng CM, Ishikawa T, Chen IS. Anti-platelet aggregation constituents from Formosan Toddalia asiatica. Phytochemistry 1998;48:1377–1382.
19. Lee TT, Kashiwada Y, Huang L, Snider J, Cosentino M, Lee KH. Suksdorfin: An anti-HIV principle from Lomatium suksdorfii, its structure-activity correlation with related coumarins, and synergistic effects with anti-AIDS nucleosides. Bioorg Med Chem Lett 1994;2:1051–1056.
20. Huang L, Kashiwada Y, Cosentino LM, Fan S, Lee KH. 3′,4′-di-O-(−)-camphanoyl-(+)-*cis*-khellactone and related compounds: A new class of potent anti-HIV agents. Bioorg Med Chem Lett 1994;4:593–598.
21. Huang L, Kashiwada Y, Cosentino LM, Fan S, Chen CH, McPhail AT, Fujioka T, Mihashi K, Lee KH. Anti-AIDS Agents. 15. Synthesis and anti-HIV activity of dihydroseselins and related analogs. J Med Chem 1994;37:3947–3955.
22. Xie L, Crimmins MT, Lee KH. Anti-AIDS agents. 22. Asymmetric synthesis of 3′,4′-di-O-(−)-camphanoyl-(+)-*cis*-khellactone (DCK), a potent anti-HIV agent. Tetrahedron Lett 1995;36:4529–4532.
23. Mehltretter GM, Dobler C, Sundermeier U, Beller M. An improved version of the sharpless asymmetric dihydroxylation. Tetrahedron Lett 2000;41:8083–8087.
24. Kolb HC, VanNieuwenhze MS, Sharpless KB. Catalytic asymmetric dihydroxylation. Chem Rev (Washington DC, US) 1994;94:2483–2547.
25. Xia Y, Yang ZY, Brossi A, Lee KH. Asymmetric solid-phase synthesis of (3′R,4′R)-di-O-*cis*-acyl 3-carboxyl khellactones. Org Lett 1999;1:2113–2115.
26. Xie L, Takeuchi Y, Cosentino LM, McPhail AT, Lee KH. Anti-AIDS agents. 42. Synthesis and anti-HIV activity of disubstituted (3′R,4′R)-3′,4′-di-O-(S)-camphanoyl-(+)-*cis*-khellactone analogues. J Med Chem 2001;44:664–671.

27. Xie L, Takeuchi Y, Cosentino LM, Lee KH. Anti-AIDS agents. 37.synthesis and structure-activity relationships of (3′R,4′R)-(+)-*cis*-khellactone derivatives as novel potent anti-HIV agents. J Med Chem 1999;42:2662–2672.
28. Yang ZY, Xia Y, Xia P, Cosentino LM, Lee KH. Anti-AIDS agents. 31. Synthesis and anti-HIV activity of 4-substituted 3′,4′-di-O-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) thiolactone analogs. Bioorg Med Chem Lett 1998;8:1483–1486.
29. Xie L, Takeuchi Y, Cosentino LM, Lee KH. Anti-AIDS agents 33 synthesis and anti-HIV activity of monomethyl substituted 3′,4′-di-O-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) analogs. Bioorg Med Chem Lett 1998;8:2151–2156.
30. Takeuchi Y, Xie L, Cosentino LM, Lee KH. Anti-AIDS agents—XXVIII. Synthesis and anti-HIV activity of methoxy substituted 3′,4′-di-O-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) analogs. Bioorg Med Chem Lett 1997;7:2573–2578.
31. Yang ZY, Xia Y, Xia P, Brossi A, Cosentino LM, Lee KH. Anti-AIDS agents part 41: Synthesis and anti-HIV activity of 3′,4′-di-O-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) lactam analogues. Bioorg Med Chem Lett 2000;10:1003–1005.
32. Xie L, Allaway G, Wild C, Kilgore N, Lee KH. Anti-AIDS agents part 47. Synthesis and anti-HIV activity of 3-substituted 3′,4′-di-O-(S)-camphanoyl-(3′R,4′R)-(+)-*cis*-khellactone derivatives. Bioorg Med Chem Lett 2001;11:2291–2293.
33. Kashman Y, Gustafson KR, Fuller RW, Cardellina JH II, McMahon JB, Currens MJ, Buckheit RW, Jr., Hughes SH, Cragg GM, Boyd MR. HIV inhibitory natural products. Part 7. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. J Med Chem 1992;35:2735–2743.
34. Dharmaratne HRW, Wanigasekera WMAP, Mata-Greenwood E, Pezzuto JM. Inhibition of human immunodeficiency virus type 1 reverse transcriptase activity by cordatolides isolated from *Calophyllum cordato-oblongum*. Planta Med 1998;64:460–461.
35. Patil AD, Freyer AJ, Eggleston DS, Haltiwanger RC, Bean MF, Taylor PB, Caranfa MJ, Breen AL, Bartus HR. The inophyllums, novel inhibitors of HIV-1 reverse transcriptase isolated from the Malaysian tree, *Calophyllum inophyllum* Linn. J Med Chem 1993;36:4131–4138.
36. Spino C, Dodier M, Sotheeswaran S. Anti-HIV coumarins from *Calophyllum* seed oil. Bioorg Med Chem Lett 1998;8:3475–3478.
37. McKee TC, Covington CD, Fuller RW, Bokesch HR, Young S, Cardellina JH II, Kadushin MR, Soejarto DD, Stevens PF, Cragg GM, Boyd MR. Pyranocoumarins from tropical species of the genus *Calophyllum*: A chemotaxonomic study of extracts in the National Cancer Institute collection. J Nat Prod 1998;61:1252–1256.
38. De Clercq E. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med Res Rev 2000;20:323–349.
39. Patil AD, Hertzberg RP, Dreyer GB, Freyer AJ, Westley JW, Chenera B, West ML. Coumarin derivatives as retroviral inhibitors. 1994; PCT:WO9414789.
40. Galinis DL, Fuller RW, McKee TC, Cardellina JH II, Gulakowski RJ, McMahon JB, Boyd MR. Structure-activity modifications of the HIV-1 inhibitors (+)-calanolide A and (−)-calanolide B. J Med Chem 1996;39:4507–4510.
41. Zembower DE, Liao S, Flavin MT, Xu ZQ, Stup TL, Buckheit RWJ, Khilevich A, Mar AA, Sheinkman AK. Structural analogs of the calanolide anti-HIV agents. Modification of the *trans*-10,11-dimethyldihydropyran-12-ol ring (ring C). J Med Chem 1997;40:1005–1017.
42. Xu ZQ, Buckheit RW, Jr., Stup TL, Flavin MT, Khilevich A, Rizzo JD, Lin L, Zembower DE. In vitro anti-human immunodeficiency virus (HIV) activity of the chromanone derivative, 12-oxocalanolide A, a novel NNRTI. Bioorg Med Chem Lett 1998;8:2179–2184.
43. Pengsuparp T, Serit M, Hughes SH, Soejarto DD, Pezzuto JM. Specific inhibition of human immunodeficiency virus type 1 reverse transcriptase mediated by soulattrolide, a coumarin isolated from the latex of *Calophyllum teysmannii*. J Nat Prod 1996;59:839–842.
44. Currens MJ, Mariner JM, McMahon JB, Boyd MR. Kinetic analysis of inhibition of human immunodeficiency virus type-1 reverse transcriptase by calanolide A. J Pharmacol Exp Ther 1996;279:652–661.
45. Taylor PB, Culps JS, Debouk C, Johnson RK. Kinetic and mutational analysis of human immunodeficiency virus type 1 reverse transcriptase inhibition by inophyllums, a novel class of non-nucleotide inhibitors. J Biol Chem 1994;269:6325–6331.
46. Buckheit RW, Jr., White EL, Eliakas-Boltz V, Russell J, Stup TL, Kinjerski TL, Osterling MC, Weigand A, Bader JP. Unique anti-human immunodeficiency virus activities of the nonnucleoside reverse transcriptase

inhibitors calanolide A, costatolide, and dihydrocostatolide. Antimicrob Agents Chemother 1999;43: 1827–1834.
47. Buckheit RW, Jr., Russell JD, Xu ZQ, Flavin M. Anti-HIV-1 activity of calanolides used in combination with other mechanistically diverse inhibitors of HIV-1 replication. Antiviral Chem Chemother 2000;11: 321–327.
48. Currens MJ, Gulakowski RJ, Mariner JM, Moran RA, Buckheit RW, Jr., Gustafson KR, McMahon JB, Boyd MR. Antiviral activity and mechanism of action of calanolide A against the human immunodeficiency virus type-1. J Pharmacol Exp Ther 1996;279:645–651.
49. Xu ZQ, Hollingshead MG, Borgel S, Elder C, Khilevich A, Flavin MT. In vivo anti-HIV activity of (+)-calanolide A in the hollow fiber mouse model. Bioorg Med Chem Lett 1999;9:133–138.
50. Newman RA, Chen W, Madden TL. Pharmaceutical properties of related calanolide compounds with activity against human immunodeficiency virus. J Pharm Sci 1998;87:1077–1080.
51. Ishikawa T, Oku Y, Tanaka T, Kumamoto T. An approach to anti-HIV-1 active calophyllum coumarin synthesis: An enantioselective construction of 2,3-dimethyl-4-chromanone ring by quinine-assisted intramolecular Michael-type addition. Tetrahedron Lett 1999;40:3777–3780.
52. Rehder KS, Kepler JA. Total synthesis of (±)-calanolide A. Synth Commun 1996;26:4005–4021.
53. Flavin MT, Rizzo JD, Khilevich A, Kucherenko A, Sheinkman AK, Vilaychack V, Lin L, Chen W, Mata E. Synthesis, chromatographic resolution, and anti-human immunodeficiency virus activity of (±)-calanolide A and its enantiomers. J Med Chem 1996;39:1303–1313.
54. Tanaka T, Kumamoto T, Ishikawa T. Enantioselective total synthesis of anti HIV-1 active (+)-calanolide A through a quinine-catalyzed asymmetric intramolecular oxo-Michael addition. Tetrahedron Lett 2000;41: 10229–10232.
55. Creagh T, Ruckle JL, Tolbert DT, Giltner J, Eiznhamer DA, Dutta B, Flavin MT, Xu ZQ. Safety and pharmacokinetics of single doses of (+)-calanolide A, a novel, naturally occurring nonnucleoside reverse transcriptase inhibitor, in healthy, human immunodeficiency virus-negative human subjects. Antimicrob Agents Chemother 2001;45:1379–1386.
56. Sherer R, Dutta B, Anderson R, Laudetteaboulhab J. A phase IB study of (+)-calanolide A in HIV-1-infected, antiretroviral therapy naive patients. 7th Conference on Retroviruses and Opportunistic Infections. 2000.
57. Sarawak Medichem Pharmaceuticals, Inc. Calanolide A development plan. Information may be found at: www.sarawak-medichem.com/cala/dev.html/
58. Tummino PJ, Ferguson D, Hupe D. Competitive inhibition of HIV-1 protease by warfarin derivatives. Biochem Biophys Res Commun 1994;201:290–294.
59. Lunney EA, Hagen SE, Domagala JM, Humblet C, Kosinski J, Tait BD, Warmus JS, Wilson M, Ferguson D, Hupe D, Tummino PJ, Baldwin ET, Bhat TN, Liu B, Erickson JW. A novel nonpeptide HIV-1 protease inhibitor: Elucidation of the binding mode and its application in the design of related analogs. J Med Chem 1994;37:2664–2677.
60. Janakiraman MN, Mullen CR, Strohback JW. Crystallography and the design of non-peptidic HIV protease inhibitors; 4-hydroxycoumarins, 4-hydroxy-2-pyrones, and tetronic acid. American Crystallographic Association Annual Meeting. 1995.
61. Chong KT, Pagano PJ. In vitro combination of PNU-140690, a human immunodeficiency virus type 1 protease inhibitor, with ritonavir against ritonavir-sensitive and -resistant clinical isolates. Antimicrob Agents Chemother 1997;41:2367–2373.
62. Thaisrivongs S, Strohbach JW. Structure-based discovery of Tipranavir disodium (PNU-140690E): A potent, orally bioavailable, nonpeptidic HIV protease inhibitor. Biopolymers 1999;51:51–58.
63. Skulnick HI, Johnson PD, Howe WJ, Tomich PK, Chong KT, Watenpaugh KD, Janakiraman MN, Dolak LA, McGrath JP. Structure-based design of sulfonamide-substituted non-peptidic HIV protease inhibitors. J Med Chem 1995;38:4968–4971.
64. d'Angelo J, Mouscadet JF, Desmaele D, Zouhiri F, Leh H. HIV-1 integrase: The next target for AIDS therapy? Pathol Biol 2001;49:237–246.
65. Mazumder A, Wang S, Neamati N, Nicklaus M, Sunder S, Chen J, Milne GWA, Rice WG, Burke TR, Jr., Pommier Y. Antiretroviral agents as inhibitors of both human immunodeficiency virus type 1 integrase and protease. J Med Chem 1996;39:2472–2481.
66. Zhao H, Neamati N, Hong H, Mazumder A, Wang S, Sunder S, Milne GWA, Pommier Y, Burke TR, Jr. Coumarin-based inhibitors of HIV integrase. J Med Chem 1997;40:242–249.
67. Martyanov IV, Zakharova OD, Sottofattori E, Pyshnyi DV, Yurchenko EY, Babbi P, Mazzei M, Balbi A, Andreola ML, Litvak S, Tarrago-Litvak L, Nevinsky GA. Interaction of oligonucleotides conjugated to substituted chromones and coumarins with HIV-1 reverse transcriptase. Antisense Nucleic Acid Drug Dev 1999;9:473–480.

68. Miolo G, Tomanin R, De Rossi A, Dall'Acqua F, Zacchello F, Scarpa M. Antiretroviral activity of furocoumarins plus UVA light detected by a replication-defective retrovirus. J Photochem Photobiol B Biol 1994;26:241–247.
69. Hudson JB, Graham EA, Harris L, Ashwood-Smith MJ. The unusual UVA-dependent antiviral properties of the furoisocoumarin, coriandrin. Photochem Photobiol 1993;57:491–496.
70. Rice WG, Schaeffer CA, Harten B, Villinger F, South TL, Summers MF, Henderson LE, Bess JW, Jr., Arthur LO. Inhibition of HIV-1 infectivity by zinc-ejecting aromatic C-nitroso compounds. Nature (London) 1993;361:473–475.
71. Yamagoe S, Kohda T, Oishi M. Poly(ADP-ribose) polymerase inhibitors suppress UV-induced human immunodeficiency virus type 1 gene expression at the posttranscriptional level. Mol Cell Biol 1991; 11:3522–3527.
72. Althaus IW, Franks KM, Langley KB, Kezdy FJ, Peterson T, Baxser SE, Decker DE, Dolak LA, Ulrich RG, Reusser F. The amphiphilic properties of novenamines determine their activity as inhibitors of HIV-1 RNase H. Experientia 1996;52:329–335.
73. Wu TS, Tsang ZJ, Wu PL, Lin FW, Li CY, Teng CM, Lee KH. New constituents and antiplatelet aggregation and anti-HIV principles of *Artemisia capillaris*. Bioorg Med Chem 2001;9:77–83.
74. Zhou P, Takaishi Y, Duan H, Chen B, Honda G, Itoh M, Takeda Y, Kodzhimatov OK, Lee KH. Coumarins and bicoumarin from *Ferula sumbul*: Anti-HIV ctivity and inhibition of cytokine release. Phytochemistry 2000;53:689–697.
75. Shikishima Y, Takaishi Y, Honda G, Ito M, Takeda Y, Kodzhimatov OK, Ashurmetov O, Lee KH. Chemical constituents of *Prangos tschimganica*; structure elucidation and absolute configuration of coumarin and furanocoumarin derivatives with anti-HIV activity. Chem Pharm Bull 2001;49:877–880.
76. Hatano T, Yasuhara T, Miyamoto K, Okuda T. Anti-human immunodeficiency virus phenolics from licorice. Chem Pharm Bull 1988;36:2286–2288.

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