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Anti-AIDS agents 66: Syntheses and anti-HIV activity of phenolic and aza 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) derivatives

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Abstract—New phenolic and aza 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) analogs were synthesized and assayed for inhibition of HIV-1 IIIB replication in H9 lymphocytes. Compound **16**, 4-methyl-1′-aza-DCK (4-methyl-aza-DCK), was less lipophilic than 4-methyl-DCK, and retained sub-micromolar anti-HIV activity with EC_{50} and TI values of 0.77 μM and >42, respectively. Moreover, it showed moderately improved metabolic stability. Introduction of phenolic hydroxyl groups to 4-methyl-DCK decreased lipophilicity significantly, but did not improve metabolic stability and also decreased activity.

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1. Introduction

3′,4′-Di-*O*-*S*-(−)-camphanoyl-3′*R*,4′*R*-(+)-*cis*-khellactone (DCK, Fig. 1) is a potent anti-HIV agent that suppresses HIV replication with sub-micromolar EC_{50} values and acts by inhibiting reverse transcriptase DNA-dependent DNA polymerization activity.[1] Initial structure–activity relationships (SAR) have been established for this compound type.[2,3] The previously synthesized DCK derivatives were substituted with methyl [for instance, 4-methyl-DCK (Fig. 1)], methoxy, halogen, and hydroxymethyl groups on the (+)-*cis*-khellactone nucleus. These non-polar groups were shown to be beneficial for activity at positions 3, 4, and 5, but detrimental at position-6. Slightly more polar hydroxymethyl derivatives were moderately active, even when the substituent was introduced at position-6. The previous studies also indicated that, despite their high potencies, most DCK derivatives show low oral bioavailability in male Sprague–Dawley rats and are rapidly metabolized both in vitro and in vivo.[4] The most recent metabolic study of DCKs suggested camphanoyl moieties as the target of metabolism;[5] however, they are also reported to be the important determinants for the activity.[6] Thus, we were also concerned with improving these pharmaceutical parameters, which are significant to efficient drug design.[7,8]

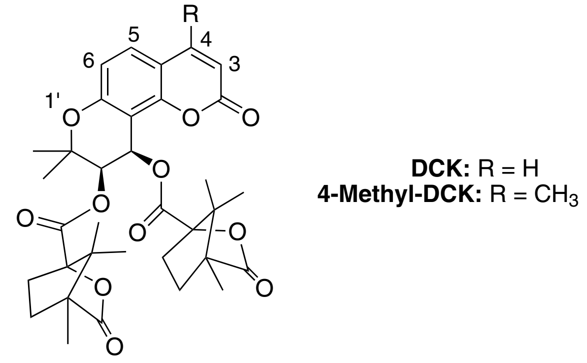

Figure 1. Structures of DCK and 4-methyl-DCK.

In this study, we introduced a phenolic hydroxyl (a moderately polar isostere of a methyl group) directly on the khellactone nucleus or nitrogen (an isostere of oxygen) in the dimethylpyran-ring in order to extend SAR beyond the prior lipophilic substituents. In addition, introduction of polar substituents can also enhance

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Keywords: DCK; Khellactone; HIV; aza-DCK.

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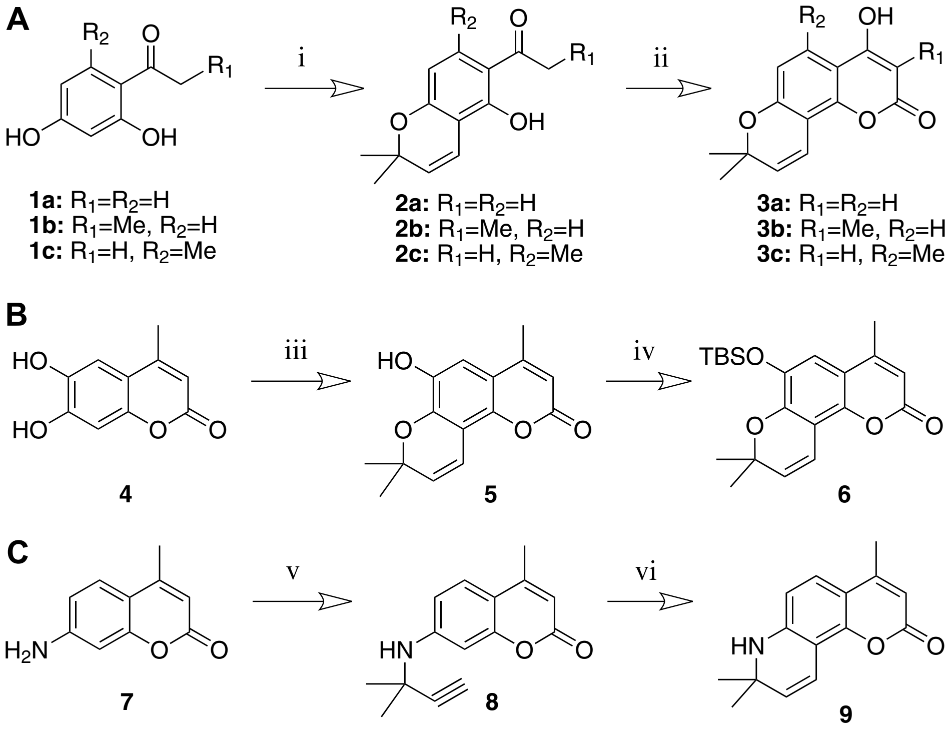
aqueous solubility, an important parameter for oral bioavailability. The new more polar analogs were postulated to have lower lipophilicity (in terms of log *D*) compared to the non-polar DCK equivalents. The log *D* values for a subset of these compounds were calculated by using ACD/Log *D* Sol Suite software. The calculated log *D* values (pH 7.4) of **13a**, **15**, and **16** are 0.52, 1.91, and 3.97, respectively, while our lead compound 4-methyl-DCK has a calculated log *D* value of 4.82. Accordingly, compounds **13a**, **15**, and **16**, and related compounds were synthesized and evaluated in vitro against HIV-1 replication in H9 lymphocytes. The in vitro metabolic stabilities of these compounds were also tested with human liver microsomes under oxidative conditions.

2. Synthesis

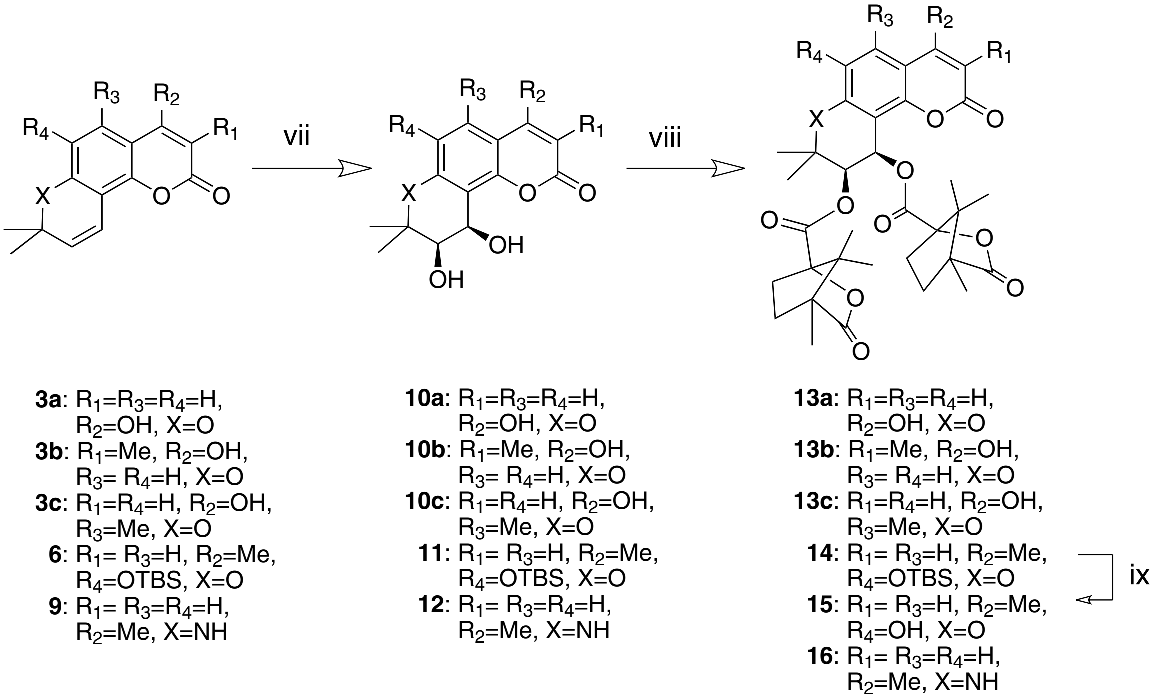
Seselins (**3a–c**) were synthesized as shown (Scheme 1). In order to introduce a hydroxyl at the 4-position of DCK, the 2′,2′-dimethylpyran-ring was added prior to coumarin formation (Scheme 1A), in contrast to prior synthetic methodology for DCK analogs. Chromenes **2a–c** were synthesized by refluxing **1a–c**, which were commercially available (**1a–b**) or prepared by Friedel Crafts acetylation of resorcinol[9] (**1c**), with 4,4-dimethoxy-2-methyl-2-butanol in pyridine at 150 °C. Cyclization using diethyl carbonate gave the desired 4-hydroxyseselins (**3a–c**).[10,11] Introduction of hydroxyl at the seselin 6-position was achieved by condensing a 6,7-dihydroxycoumarin with 4,4-dimethoxy-2-methyl-2-butanol in refluxing pyridine (Scheme 1B). Commercially available 4-methylesculetin (**4**) was used as the substrate for the initial SAR investigation. The structural arrangement of the pyran ring of the product seselin **5** was confirmed to be 6-hydroxyseselin based on NOESY data, and was distinguished from its isomer. 4-Methyl-1′-azaseselin (**9**) was synthesized from the corresponding 7-aminocoumarin (**7**) by Aza-Claisen rearrangement (Scheme 1 C). Addition of alkyne to **7** was catalyzed by copper and cuprous chloride in water and triethylamine.[12] The product **8** was then heated in the presence of copper chloride to obtain 4-methyl-1′-azaseselin (**9**).[13] According to Scheme 2, the asymmetric dihydroxylation of **3a–c**, **6**, and **9** was accomplished using a catalytic sharpless asymmetric dihydroxylation (SAD) with AD-mix-α [$K_2OsO_2(OH)_4$ (cat.), $(DHQ)_2Pyr$, K_2CO_3, and K_3FeCN_6]. Esterification of the khellactones with camphanoyl chloride gave the final products 4-hydroxy-DCKs **13a–c** and 4-methyl-1′-aza-DCK **16.** 6-Hydroxy-4-methyl-DCK **15** was obtained by deprotecting intermediate **14**.

3. Results and discussion

The log *D* values of target compounds at pH 7.4 were calculated by using ACD/Log *D* Sol Suite software and are listed in Table 1. These DCK analogs were incubated with human liver microsomes under oxidative conditions, and substrate depletion rates from the microsomal incubation were analyzed by high-performance liquid



Scheme 1. Synthesis of seselins. Reagents and conditions: (A) Synthesis of 4-hydroxyseselins. (i) 4,4-Dimethoxy-2-methyl-2-butanol [$HO(CH_3)_2CCH_2CH(OMe)_2$], pyridine 150 °C; (ii) diethyl carbonate, NaH, 220 °C, 30 min. (B) Synthesis of 6-hydroxyseselin. (iii) 4,4-Dimethoxy-2-methyl-2-butanol, pyridine 150 °C; (iv) imidazole, TBSCl. (C) Synthesis of 1′-azaseselin. (v) 3-Chloro-3-methyl-1-butyne, CuCl, Cu, Et3N, H2O, reflux; (vi) CuCl, THF, reflux.



3a: R_1=R_3=R_4=H, R_2=OH, X=O
3b: R_1=Me, R_2=OH, R_3= R_4=H, X=O
3c: R_1=R_4=H, R_2=OH, R_3=Me, X=O
6: R_1= R_3=H, R_2=Me, R_4=OTBS, X=O
9: R_1= R_3=R_4=H, R_2=Me, X=NH

10a: R_1=R_3=R_4=H, R_2=OH, X=O
10b: R_1=Me, R_2=OH, R_3= R_4=H, X=O
10c: R_1=R_4=H, R_2=OH, R_3=Me, X=O
11: R_1= R_3=H, R_2=Me, R_4=OTBS, X=O
12: R_1= R_3=R_4=H, R_2=Me, X=NH

13a: R_1=R_3=R_4=H, R_2=OH, X=O
13b: R_1=Me, R_2=OH, R_3= R_4=H, X=O
13c: R_1=R_4=H, R_2=OH, R_3=Me, X=O
14: R_1= R_3=H, R_2=Me, R_4=OTBS, X=O
15: R_1= R_3=H, R_2=Me, R_4=OH, X=O
16: R_1= R_3=R_4=H, R_2=Me, X=NH

Scheme 2. Syntheses of DCK analogs. Reagents and condition: (vii) SAD: $K_2OsO_2(OH)_4$, K_2CO_3, $K_3Fe(CN)_6$, $(DHQ)_2Pyr$, *t*-BuOH/H_2O (v/v, 1:1), 0 °C; (viii) (*S*)-(−)-camphanoyl chloride, pyridine, CH_2Cl_2; (ix) CeF, THF.

Table 1. Anti-HIV activity data and calculated lipophilicity values of DCK analogs

Compound	log*D*[a]	EC_{50}[b] (μM)	IC_{50}[c] (μM)	TI[d]
13a	0.52	15	>40	>3.0
13b	1.11	2.1	31	15
13c	1.50	1.2	14	12
15	1.91	1.5	>38	>25
16	3.97	0.77	>32	>42
4-Methyl-DCK	4.82	0.02	>10	>500
AZT		0.05	1900	38,000

[a] From ACD/Log*D* Sol Suite software.
[b] EC_{50}, concentration required to inhibit the replication of HIV in cell culture by 50%.
[c] IC_{50}, concentration required to reduce host cell viability by 50%.
[d] TI, in vitro therapeutic index, ratio of IC_{50}:EC_{50}.

Table 2. Metabolic stability values of selected DCK analogs

Compound	$t_{1/2}$ in vitro[a] (min)	CL_{int}[a] (L/min/kg)
13a	ND[b]	ND
13b	3.5 ± 0.5	2.0 ± 0.3
13c	ND	ND
15	3.1 ± 0.2	2.3 ± 0.3
16	9.6 ± 0.7	0.7 ± 0.1
4-Methyl-DCK	3.4 ± 0.3	2.0 ± 0.2

[a] See calculations in Section 5.
[b] ND, not determined.

chromatography (HPLC) linked with a UV detector. In vitro half life and intrinsic clearance values (Cl_{int}), which are frequently used in both academia and the pharmaceutical industry to estimate in vivo metabolic stabilities of drug candidates,[14–16] were also calculated (Table 2). In vitro half-lives of the synthesized phenolic DCKs (**13b** and **15**) were 3.1–3.5 min, similar to half-lives reported previously for methyl and methoxy DCKs,[5] under the same conditions. The results suggest that modifications of the coumarin ring do not influence in vitro oxidative metabolism, regardless of the type of modification, although impact on the anti-HIV activity is significant. The findings also would predict similarly rapid in vivo metabolism and low bioavailability. In contrast, the 1′-aza-DCK analog (**16**) differed from the other DCK analogs and had a longer in vitro half life, close to 10 min. The estimate of in vivo intrinsic clearance utilized scaling factors applied to the observed in vitro data, and allowed comparison with data obtained using different protein concentrations. The Cl_{int} value of **16** (0.7 L/min/kg) is still larger than reported values of high clearance drugs;[17] however, in consideration of the high protein binding (unpublished data), **16** is likely to show more moderate clearance in vivo. Additionally, although hydroxymethyl DCK is reported to show moderate bioavailability and in vitro half life in rats,[4] compound **16** is the only known analog so far that has comparable in vitro half life in human liver microsomes.

The synthesized DCK analogs were assayed for anti-HIV activity using the HIV-1 IIIB strain in H9 lymphocytes (Table 1). Introduction of a phenolic hydroxyl at position-4 (**13a**) lowered the log*D* value significantly, but was also highly detrimental to the activity as **13a** showed an EC_{50} value of only 15 μM. Addition of a 3- or 5-methyl (**13 b**, **13c**) to 4-hydroxy-DCK (**13a**) increased lipophilicity on the coumarin nucleus and improved activity by almost 10-fold, which suggested that lipophilic modification on a polar DCK was beneficial, similar to phenomena observed with other DCK analogs.

Among all compounds synthesized, the 1′-aza-DCK analog (**16**) showed the most promising anti-HIV activity, even though the introduction of the secondary amine in the dimethylpyran-ring only slightly lowered the lipophilicity (compare log *D* values of the bioisosteric isomer **16** and 4-methyl-DCK, Table 1). In addition, while 4-methyl-DCK and the phenolic DCKs **13b** and **15** showed similar metabolic stabilities, 4-methyl-1′-aza-DCK (**16**) was moderately more stable (Table 2). These results indicate that, although many publications suggest that lipophilicity contributes to metabolic lability, the position of polar modification is more important than the overall lipophilicity for the metabolic stability of this compound type. Combined with the previous reports, this study indicates that the pyran ring may be a good target for altering metabolic stability of DCKs.

4. Conclusion

Phenolic and aza DCK derivatives were synthesized and evaluated for anti-HIV activity. The most promising analog in this study, 4-methyl-1′-aza-DCK (**16**), had lower lipophilicity than the parent compound (4-methyl-DCK), retained anti-HIV activity in the sub-micromolar range, and exhibited improved metabolic stability. From the activity profiles of our synthesized DCK analogs, lipophilicity around the coumarin ring appeared to be required for potent anti-HIV activity. Our extended SAR of the DCK derivatives will be valuable in the design and development of DCK derivatives. Further studies to develop derivatives with improved potency and pharmaceutical profiles are in progress in our laboratory.

5. Experimental

5.1. Chemistry

5.1.1. General procedures. Melting points were measured with a Fisher Johns melting point apparatus without correction. The proton nuclear magnetic resonance (^1H NMR) spectra were measured on a Varian 300 MHz spectrometer using TMS as internal standard. Mass spectra were obtained on an Agilent 1100 MSD ion trap mass spectrometer or PE-SCIEX API-3000 with turbo ion spray source. Thin-layer chromatography (TLC) was performed on a precoated silica gel GF plate purchased from Analtech, Inc. Silica gel (200–400 mesh) from Natland (Durham, NC) was used for column chromatography. All other chemicals were obtained from Aldrich, Inc., unless otherwise noted.

5.1.2. General procedure for synthesis of dimethylchromenes (2a–c) and 6-hydroxy-4-methylseselin (5). Substrate (2′,4′-dihydroxyacetophenone **1a**, 2′,4′-dihydroxypropiophenone **1b**, 2′,4′-dihydroxy-6′-methylacetophenone **1c**, or 4-methylesculetin **4**), dry pyridine, and 4,4-dimethoxy-2-methyl-2-butanol were heated together at 150 °C for 5 h. The same quantity of the 4,4-dimethoxy-2-methyl-2-butanol was added and the heating was continued for additional 5 h. The solvent was evaporated and the brown residue was isolated on silica gel using hexane–EtOAc to obtain the product.

5.1.2.1. 6-Acetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene (2a). Yield: 46% (starting with 1.52 g **1a**); mp 90–92 °C; MS-ESI+ (*m*/*z*, %): 241 (M^++Na, 100), 219 (M^++1, 78); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.44 (6H, s, 2-$(CH_3)_2$), 2.53 (3H, s, $COCH_3$), 5.58 (1H, d, $J = 10.1$ Hz, H-4), 6.33 (1H, d, $J = 9.0$ Hz, H-8), 6.70 (1H, d, $J = 10.1$ Hz, H-3), 7.51 (1H, d, $J = 9.0$ Hz, H-7), 12.86 (1H, s, OH).

5.1.2.2. 6-Propionyl-5-hydroxy-2,2-dimethyl-2*H*-chromenene (2b). Yield: 48% (starting with 415 mg of **1b**); mp 70–72 °C; MS-ESI+ (*m*/*z*, %): 233 (M^++1,100); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.20 (3H, t, $J = 7.3$ Hz, CH_3), 1.43 (6H, s, 2-$(CH_3)_2$), 2.91 (2H, q, $J = 7.3$ Hz, CH_2), 5.56 (1H, d, $J = 9.9$ Hz, H-4), 6.31 (1H, d, $J = 9.0$ Hz, H-8), 6.70 (1H, d, $J = 9.9$ Hz, H-3), 7.53 (1H, d, $J = 9.0$ Hz, H-7), 13.05 (1H, s, OH).

5.1.2.3. 6-Acetyl-5-hydroxy-2,2,7-trimethyl-2*H*-chromene (2c). Yield: 38% (starting with 238 mg of **1c**); mp 53–55 °C; MS-ESI− (*m*/*z*, %): 232 (M^-,100); ^1H NMR ($CDCl_3$, 300 MHz) δ 2.60 (3H, s, CH_3), 1.43 (6H, s, 2-$(CH_3)_2$), 2.53 (3H, s, $COCH_3$), 5.52 (1H, d, $J = 9.9$ Hz, H-4), 6.19 (1H, s, H-8), 6.69 (1H, d, $J = 9.9$ Hz, H-3), 13.65 (1H, s, OH).

5.1.2.4. 6-Hydroxy-4-methylseselin (5). Yield: 58% (starting with 481 mg of **4**); mp 190–192 °C; MS-ESI− (*m*/*z*, %): 258 (M^-, 30), 257 (M^-−1, 100); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.50 (6H, s, 2′-$(CH_3)_2$), 2.34 (3H, s, CH_3), 5.56 (1H, s, OH), 5.73 (1H, d, $J = 10.3$ Hz, H-3′), 6.14 (1H, H-3), 6.89 (1H, d, $J = 10.3$ Hz, H-4′), 6.99 (1H, s, H-5).

5.1.3. General procedure for synthesis of 4-hydroxyseselins (3a–c). Sodium hydride (60% dispersion in oil) was added slowly to a solution of the chromene derivative in dry diethyl carbonate. The mixture was slowly heated to reflux and stirred for 20 min. After cooling, MeOH was added carefully. Diethyl ether was added and the solution was extracted with water. The aqueous extracts were acidified with HCl (10%) and then extracted with EtOAc. The combined organic extracts were dried and recrystallized using EtOAc to obtain the product.

5.1.3.1. 4-Hydroxyseselin (3a). Yield: 38% (starting with 436 mg of **2a**); mp >210 °C sublimed; MS-ESI− (*m*/*z*, %): 244 (M^-, 40), 243 (M^-−1, 100); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.43 (6H, s, 2′-$(CH_3)_2$), 5.43 (1H, s, H-3), 5.67 (1H, d, $J = 9.9$ Hz, H-3′), 5.71 (1H, d, $J = 9.0$ Hz, H-6), 6.83 (1H, d, $J = 9.9$ Hz, H-4′), 7.60 (1H, d, $J = 9.0$ Hz, H-5).

5.1.3.2. 4-Hydroxy-3-methylseselin (3b). Yield: 91% (starting with 324 mg of **2b**); mp >190 °C sublimed; MS-ESI− (*m*/*z*, %): 258 (M^-, 30), 257 (M^-−1, 100);

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.46 (6H, s, 2′-$(CH_3)_2$), 2.12 (3H, s, 3-CH_3), 5.68 (1H, d, $J = 9.9$ Hz, H-3′), 6.69 (1H, br s, OH), 6.74 (1H, d, $J = 8.6$ Hz, H-6), 6.87 (1H, d, $J = 9.9$ Hz, H-4′), 7.59 (1H, d, $J = 8.6$ Hz, H-5).

5.1.3.3. 4-Hydroxy-5-methylseselin (3c). Yield: 80% (starting with 232 mg of **2c**); mp 185–187 °C; MS-ESI− (*m*/*z*, %): 257 ($M^-{-}1$, 100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.41 (6H, s, 2′-$(CH_3)_2$), 2.60 (3H, s, 5-CH_3), 5.61 (1H, s, H-3), 6.49 (1H, d, $J = 6.8$ Hz, H-3′), 6.80 (1H, d, $J = 6.8$ Hz, H-4′).

5.1.4. Synthesis of 6-(*tert*-butyldimethylsilanyloxy)-4-methylseselin (6). Compound **5** (125 mg) was protected with *tert*-butyldimethylsilyl (TBS) by reacting with TBS-Cl and imidazole in THF at rt overnight. Yield: 90%; mp 135–136 °C; MS-ESI+ (*m*/*z*, %): 373 ($M^+{+}1$, 100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.20 (6H, s, $Si(CH_3)_2$), 1.03 (9H, s, $SiC(CH_3)_3$)1.53 (6H, s, 2′-$(CH_3)_2$), 2.33 (3H, s, CH_3), 5.70 (1H, d, $J = 10.1$ Hz, H-3′), 6.12 (1H, H-3), 6.92 (1H, d, $J = 10.1$ Hz, H-4′), 6.87 (1H, s, H-5).

5.1.5. Synthesis of 7-amino-4-methylcoumarin (7). Aminophenol and ethyl acetoacetate were mixed on graphite/K10 (2:1, w/w) and heated at 130 °C for 30 min. After cooling, the reaction mixture was filtered and the crude residue was chromatographed on silica gel using CH_2Cl_2–MeOH to obtain the product. Yield: 25% (starting from 3 g aminophenol); mp 222–223 °C, MS-ESI− (*m*/*z*, %): 174 ($M^-{-}1$, 100); ^{1}H NMR (acetone-d_6, 300 MHz) δ 2.38 (3H, d, $J = 1.1$ Hz, 4-CH_3), 5.94 (1H, q, $J = 1.1$ Hz, H-3), 6.50 (1H, d, $J = 2.2$ Hz, H-8), 6.65 (1H, dd, $J = 8.6$, 2.2 Hz, H-6), 7.45 (1H, $J = 8.6$ Hz, H-5).

5.1.6. Synthesis of 7-(1,1-dimethyl-prop-2-ynylamino)-4-methylcoumarin (8). Compound **7** (700 mg), 3-chloro-3-methylbut-1-yne, copper powder, and copper (I) chloride in 50% aqueous acetone were heated to reflux overnight. After cooling, the reaction mixture was filtered and the crude residue was chromatographed on silica gel using CH_2Cl_2–MeOH to obtain the product. Yield: 84%; mp 181–183 °C; MS-ESI− (*m*/*z*, %): 240 ($M^-{-}1$, 100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.66 (6H, s, 1-$(CH_3)_2$), 2.35 (3H, s, 4-CH_3), 2.43 (1H, s, CCH), 4.24 (1H, s, NH), 6.02 (1H, s, H-2), 6.71 (1H, d, $J = 8.8$ Hz, H-6), 6.94 (1H, s, H-8), 7.37 (1H, s, $J = 8.8$ Hz, H-5).

5.1.7. Synthesis of 4,8,8-trimethyl-7,8-dihydro-2*H*-pyrano[2,3-*f*]quinolin-2-one (9). Amine **8** (120 mg) and copper (I) chloride were heated in refluxing THF to obtain the product azaseselin in 98% yield. Mp 125–127 °C, MS-ESI− (*m*/*z*, %): 240 ($M^-{-}1$, 100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.35 (6H, s, 2-$(CH_3)_2$), 2.31 (3H, s, CH_3), 4.11 (1H, s, NH), 5.54 (1H, d, $J = 9.9$ Hz, H-3), 5.95 (1H, H-6), 6.33 (1H, d, $J = 8.2$ Hz, H-10), 6.87 (1H, d, $J = 9.9$ Hz, H-4), 7.18 (1H, d, $J = 8.2$ Hz, H-9).

5.1.8. General procedure for asymmetric dihydroxylation. A mixture of $K_3Fe(CN)_6$ (150 mg, 0.75 mmol), K_2CO_3 (105 mg, 0.75 mmol), and 2,5-diphenyl-4,6-bis(9-*O*-dihydroquinyl)-pyrimidine [$(DHQ)_2$Pyr] (4.4 mg, 0.005 mmol), $K_2OsO_2(OH)_4$ (0.005 mmol), was dissolved in 5 mL of *t*-BuOH/H_2O (v/v, 1:1) at rt. The solution was cooled to 0 °C and methanesulfonamide (0.25 mmol) added with stirring. When the solution turned from light yellow to orange, the substituted seselin compound (0.25 mmol) was added. The mixture was stirred at 0 °C for 2–4 days. Reaction was monitored using TLC, and at completion, $Na_2S_2O_5$ (excess), water, and $CHCl_3$ were added. After being stirred for 0.5 h at rt, the mixture was extracted with $CHCl_3$ three times. The residue was separated by column chromatography to obtain the pure substituted (+)-*cis*-khellactones.

5.1.8.1. 4-Hydroxy-(+)-*cis*-khellactone (10a). Yield: 58% (starting with 133 mg of **3a**); mp 186–187 °C; MS-ESI− (*m*/*z*, %): 277 ($M^-{-}1$, 100); ^{1}H NMR δ 1.42, 1.45 (each 3H, s, 2′-$(CH_3)_2$), 3.86 (1H, d, $J = 4.7$ Hz, H-3′), 5.17 (1H, d, $J = 4.7$ Hz, H-4′), 6.82 (1H, d, $J = 9.1$ Hz, H-6), 7.85 (1H, d, $J = 9.1$ Hz, H-5).

5.1.8.2. 4-Hydroxy-3-methyl-(+)-*cis*-khellactone (10b). Without isolation, compound **10b** was treated with camphonyl chloride in the next step.

5.1.8.3. 4-Hydroxy-5-methyl-(+)-*cis*-khellactone (10c). Yield: 63% (starting with 129 mg of **3c**); mp 197–200 °C; MS-ESI+ (*m*/*z*, %): 293 ($M^+{+}1$, 100); MS-ESI− (*m*/*z*, %): 291 ($M^-{-}1$, 100); ^{1}H NMR (CD_3OD) δ 1.39, 1.42 (each 3H, s, 2′-$(CH_3)_2$), 2.63 (3H, s, 5-CH_3), 3.73 (1H, d, $J = 4.7$ Hz, H-3′), 5.01 (1H, d, $J = 4.7$ Hz, H-4′), 6.56 (1 H, s, H-6).

5.1.8.4. 6-(*tert*-Butyldimethylsilanyloxy)-4-methyl-(+)-*cis*-khellactone (11). Yield: 82% (starting with 38 mg of **6**); mp 148–150 °C; MS-ESI− (*m*/*z*, %): 407 ($M^+{+}1$, 100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.18, 0.20 (each 3H, s, $Si(CH_3)_2$), 1.03 (9H, s, $SiC(CH_3)_3$), 1.44, 1.47 (each 3H, s, 2′-$(CH_3)_2$), 2.36 (3H, s, CH_3), 3.16 (1H, d, $J = 6.2$ Hz, 3′-OH), 3.85 (1H, t, $J = 5.1$ Hz, H-3′), 3.91 (1H, d, $J = 5.1$ Hz, 4′-OH), 5.20 (1H, t, $J = 6.2$ Hz, 4′-H), 6.14 (1H, s, H-3), 6.96 (1H, s, H-5).

5.1.8.5. (9*S*,10*R*)-9,10-Dihydroxy-4,8,8-trimethyl-7,8,9,10-tetrahydro-2*H*-pyrano[2,3-*f*]quinolin-2-one (12). Yield: 78% (starting with 82 mg of **9**); mp 255–257 °C; MS-ESI− (*m*/*z*, %): 274 ($M^-{-}1$, 100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29, 1.34 (each 3H, s, 2′-$(CH_3)_2$), 2.35 (3H, s, CH_3), 3.75 (1H, d, $J = 5.1$ Hz, H-3′), 5.99 (1H, s, H-3), 5.19 (1H, d, $J = 5.1$ Hz, H-4′), 6.43 (1H, d, $J = 9.0$ Hz, H-6), 7.31 (1H, d, $J = 9.0$ Hz, H-5).

5.1.9. General procedure for esterification of khellactones. Excess (*S*)-(−)-camphanoyl chloride (greater than 0.5 mmol) and (+)-*cis*-khellactone were reacted in pyridine/CH_2Cl_2 for 1–2 days at rt. The mixture was extracted with EtOAc and separated by column chromatography to obtain the substituted DCK derivatives.

5.1.9.1. 4-Hydroxy-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (13a). Yield: 38% (starting with 100 mg of **10a**); mp 197–199 °C; MS-ESI− (*m*/*z*, %): 637.5 (M^-−1, 100); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.94, 0.98, 1.06, 1.07, 1.11, 1.11 (each 3H, s, camphanoyl CH_3), 1.46, 1.50 (each 3H, s, 2′-$(CH_3)_2$), 1.69, 1.88, 2.21, 2.46 (each 2H, m, camphanoyl CH_2), 5.38 (1H, d, *J* = 9.1 Hz, H-3′), 5.68 (1H, s, H-3), 6.64 (1H, d, *J* = 9.1 Hz, 4′-H), 6.81 (1H, d, *J* = 4.7 Hz, H-6), 7.79 (1H, d, *J* = 4.7 Hz, H-5).

5.1.9.2. 4-Hydroxy-3-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (13b). Yield: 23% (starting with 50 mg of **3b**); mp 183–185 °C; MS-ESI− (*m*/*z*, %): 651.3 (M^-−1, 100); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.01, 1.04, 1.07, 1.11, 1.13, 1.14 (each 3H, s, camphanoyl CH_3), 1.48, 1.52 (each 3H, s, 2′-$(CH_3)_2$), 1.73, 1.89, 2.21, 2.52 (each 2H, m, camphanoyl CH_2), 2.20 (3H, s, 3-CH_3), 5.41 (1H, d, *J* = 4.7 Hz, H-3′), 6.69 (1H, d, *J* = 4.7 Hz, H-4′), 6.87 (1H, d, *J* = 8.9 Hz, H-6), 7.36 (1H, d, *J* = 8.9 Hz, H-5).

5.1.9.3. 4-Hydroxy-5-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (13c). Yield: 20% (starting with 50 mg of **10c**); mp 174–176 °C, MS-ESI− (*m*/*z*, %): 651.3 (M^-−1, 100); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.96, 1.01, 1.04, 1.05, 1.07, 1.08, 1.11 (each 3H, s, camphanoyl CH_3), 1.35, 1.48, (each 3H, s, 2′-$(CH_3)_2$), 1.73, 1.89, 2.21, 2.52 (each 2H, m, camphanoyl CH_2), 2.55 (3H, s, 5-CH_3), 4.04 (1H, d, *J* = 4.7 Hz, H-3′), 5.64 (1H, s, H-3), 6.46 (1H, d, *J* = 4.7 Hz, H-4′), 6.54 (1H, s, H-6).

5.1.9.4. 6-(*tert*-Butyldimethylsilanyloxy)-4-methyl-3′, 4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (14). Yield: 59% (starting with 50 mg of **11**); mp 138–139 °C; MS-ESI+ (*m*/*z*, %): 789.4 (M^++Na, 100); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.20 (6H, s, $Si(CH_3)_2$), 0.98, 1.01, 1.03, 1.07, 1.10, 1.11 (each 3H, s, camphanoyl CH_3), 1.03 (9H, s, $SiC(CH_3)_3$), 1.48, 1.50 (each 3H, s, 2′-$(CH_3)_2$), 1.66, 1.91, 2.21, 2.49 (each 2H, m, camphanoyl CH_2), 2.34 (3H, s, CH_3), 5.37 (1H, d, *J* = 4.8 Hz, H-3′), 6.11 (1H, s, H-3), 6.65 (1H, d, *J* = 4.8 Hz, 4′-H), 7.02 (1H, s, H-5).

5.1.9.5. 9,10-Di-*O*-camphanoyl-4,8,8-trimethyl-7,8,9,10-tetrahydro-2*H*-pyrano[2,3-*f*]quinolin-2-one (16). Yield: 5% (starting with 100 mg of **12**); mp 227–229 °C; MS-ESI− (*m*/*z*, %): 634.7 (M^-−1, 30); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.97, 0.98, 1.06, 1.09, 1.09, 1.10 (each 3H, s, camphanoyl CH_3), 1.25, 1.27 (each 3H, s, 2′-$(CH_3)_2$), 1.67, 1.91, 2.21, 2.49 (each 2H, m, camphanoyl CH_2), 2.32 (3H, s, CH_3), 5.26 (1H, d, *J* = 4.6 Hz, H-3′), 5.95 (1H, s, H-3), 6.46 (1H, d, *J* = 8.6 Hz, H-6), 6.67 (1H, d, *J* = 4.6 Hz, 4′-H), 7.36 (1H, d, *J* = 8.6 Hz, H-5).

5.1.10. Synthesis of 6-hydroxy-4-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (15). Compound **14** (114 mg) was stirred with CeF in THF at rt for 5 min to remove the silyl protecting group. Yield: 36%, mp 255–257 °C; MS-ESI− (*m*/*z*, %): 651.6 (M^-−1, 100); ^1H NMR ($CDCl_3$, 300 MHz) δ 0.98, 1.00, 1.07, 1.09, 1.10, 1.11 (each 3H, s, camphanoyl CH_3), 1.51, 1.53 (each 3H, s, 2′-$(CH_3)_2$), 1.67, 1.91, 2.21, 2.49 (each 2H, m, camphanoyl CH_2), 2.36 (3H, s, CH_3), 5.42 (1H, d, *J* = 4.8 Hz, H-3′), 5.49 (1H, s, 6-OH), 6.14 (1H, s, H-3), 6.68 (1H, d, *J* = 4.8 Hz, 4′-H), 7.16 (1H, s, H-5).

5.2. Biological studies

5.2.1. Anti-HIV assay. HIV growth inhibition assay was performed as described previously.[4]

5.2.2. Metabolic stability studies

5.2.2.1. Sample preparations. DCKs were brought to a final concentration of 1 μM with 100 mM sodium phosphate buffer at pH 7.4, which contained 0.1 mg/mL human liver microsome and 5 mM $MgCl_2$. Incubation volumes were 1.5 mL. Reactions were started by adding 150 μL of NADPH (15 mM) after 10-min incubation at 37 °C, and stopped by addition of 3 volumes of ice-cold acetonitrile. Incubations of all samples were run in duplicate, and for control incubations, NADPH was omitted. For each sample, 200 μL aliquots were taken out at 0, 5, 10, 15, 30, and 60 min time points. After addition of acetonitrile, 15 μL of 0.01 mg/mL acetonitrile solution of DCK was added to supernatants as an internal standard prior to centrifugation. The mixture was centrifuged at 15,000 rpm for 5 min. Supernatants were then evaporated to dryness under nitrogen using an N-Evap analytic evaporator (Organomation Associates Inc., Berlin, MA), and the residue was reconstituted with 150 μL of acetonitrile and water with 0.1% HOAc (3:7, v/v). An aliquot (75 μL) was injected onto the HPLC with UV detector.

5.2.2.2. HPLC-UV conditions. Analysis was carried out on an HP Series 1100 (Hewlett-Packard, Palo Alto, CA), consisting of an autosampler, a degasser, a binary pump, and a UV detector. An Axxiom ODS column 150 × 4.6 mm ID, 5 μm (Thomson Instrument Co., Springfield, VA) connected to a RP-18 guard column (15 × 3.2 mm ID, 7 μm) (Brownlee, San Jose, CA) was isocratically eluted with acetonitrile and 25 mM HOAc in water (55:45, v/v) at a flow rate of 1.5 mL/min. Detection wavelength was set at 320 nm. Chromatograms were recorded on a Hewlett-Packard Chemstation A.05.01. (Hewlett-Packard, Palo Alto, CA).

5.2.2.3. Calculations. In the determination of the in vitro half life ($t_{1/2}$), the analyte/internal-standard peak height ratios were converted to percentage drug remaining, using the initial time (0 min) peak height ratio values as 100%. The slope of the linear regression from log percentage remaining versus incubation time relationships ($-k_{el}$) was used in the conversion to in vitro $t_{1/2}$; $t_{1/2} = 0.693/k_{el}$.

Conversion to Cl_{int} (in units of L/min/kg) was done using the following formulas for the enzyme kinetic and half life methods, respectively:[14]

$$\mathrm{Cl}_{\mathrm{int}} = \frac{0.693}{\text{invitro } t_{1/2}} \cdot \frac{\text{ml incubation}}{\text{mg microsomes}} \cdot \frac{44.8 \text{ mg microsomes}}{\text{g liver}} \cdot \frac{20.7 \text{ g liver}}{\text{kg per body weight}}$$

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