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Anti-AIDS Agents —XXVIII.[1] Synthesis and Anti-HIV Activity of Methoxy Substituted 3',4'-Di-*O*-(-)-Camphanoyl-(+)-*Cis*-Khellactone (DCK) Analogues

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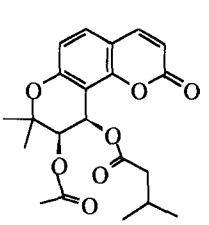
Abstract: Four isomeric methoxy substituted DCK analogues (**3–6**) were asymmetrically synthesized from different starting materials. 5-Methoxy-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (**5**) exhibited extremely potent anti-HIV activity against HIV-1 replication in H9 lymphocyte cells with EC_{50} and therapeutic index values of 0.00038 μM and >402,632, respectively, which are better than those of DCK and AZT in this assay.

In order to develop more potent anti-HIV agents, we are continuing our efforts to isolate novel anti-HIV compounds from natural products and to modify the identified active principles. Accordingly, the isolation of suksdorfin (**1**) as an anti-HIV agent[2] from *Lomatium suksdorfii* led to our synthesis of 42 khellactone derivatives.[3,4] Among them, 3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (DCK) (**2**) exhibited very potent inhibitory activity against HIV-1 replication in H9 lymphocyte cells with EC_{50} and therapeutic index values of 0.000256 μM and 136,719, and was more potent than AZT as an anti-HIV agent in this assay. However, three stereoisomers of DCK showed much lower anti-HIV activity than **2**. The other khellactone derivatives with different *O*-acyl- and/or *O*-alkyl groups at the 3'and 4' positions were inactive or toxic in the assay. The results indicated that the *R*- configuration and di-*O*-(-)-camphanoyl substitution at the 3'and 4' positions are very important for anti-HIV activity in this type of compound.



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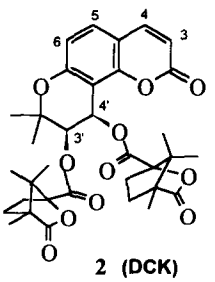

2 (DCK)

As an extension to these studies, we plan to introduce additional substituents at the 3-, 4-, 5-, and 6-positions of the coumarin nucleus. In this paper, we report the synthesis and anti-HIV activity of 3-methoxy (**3**), 4-methoxy (**4**), 5-methoxy (**5**) and 6-methoxy (**6**)-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone.

Our synthetic strategy was first to obtain four isomeric methoxy substituted 7-hydroxycoumarin (**8**, **12**, **17**, and **19**) and methoxy substituted seselin (**9**, **13**, **18**, and **20**) intermediates, and then to stereoselectively synthesize DCK analogues **3–6.** The synthetic routes are shown in Scheme 1. 3-Methoxy-7-hydroxycoumarin (**8**) was prepared in a 44% yield from the commercially available 2,4-dihydroxybenzaldehyde (**7**) and a mixture of sodium methoxyacetate and methoxyacetyl chloride in DMF. 4,7-Dihydroxycoumarin (**11**) was obtained in a 28% yield by the reaction of 1,3-dihydroxybenzene (**10**) and malonic acid in the presence of $BF_3{\cdot}Et_2O$. Compound **11** was then selectively methylated at the 4-hydroxy group[5] to give 4-methoxy-7-hydroxycoumarin (**12**) in a 60% yield. 1,3,5-Trihydroxybenzaldehyde (**14**) was first reacted with Ac_2O/Py in CH_2Cl_2 to give 2,4-diacetoxy-6-hydroxybenzaldehyde (**15**) (50% yield) together with 2,4,6-triacetoxybenzaldehyde (**16**). However, **16** could be easily converted to **15** by heating in MeOH/Py. Compound **15** then successively underwent a Wittig reaction with Ph_3P=CHCOOMe, methylation of the 6-hydroxy group with CH_3I, deprotection of the 2,4-dihydroxy groups, and cyclization of the coumarin ring to obtain 5-methoxy-7-hydroxycoumarin (**17**) with an overall yield of 45%. 6-Methoxy-7-hydroxycoumarin (**19**) is commercially available. 3-Methoxy (**9**), 4-methoxy (**13**), 5-methoxy (**18**), and 6-methoxy (**20**) seselin were separately prepared from **8**, **12**, **17**, and **19**, respectively, according to a procedure reported in the literature.[6] The yields ranged from 35–60%. As in the asymmetric synthesis of DCK,[7] the four isomeric methoxy substituted seselin analogues (**9**, **13**, **18**, and **20**) were asymmetrically dihdroxylated using $(DHQ)_2$-PYR[8] as a chiral catalyst, and then were esterified with (-)-(*S*)-camphanoyl chloride at room temperature for 48 h to obtain **3–6**, respectively.[9] The asymmetric dihydroxylation for this kind of compound is highly stereoselective with percent enantiomeric excess (% e.e.) ranging from 75 ~ >90%.[10] The *cis*-khellactone derivatives with *3'R, 4'R* configuration[4,7] are the predominant diastereoisomers. The ^{1}H NMR data of **3–6** are shown in Table 1.

The anti-HIV activities of **3–6** are shown in Table 2. The results indicated that **5** has very potent anti-HIV activity in acutely infected H9 lymphocytes with an EC_{50} value of 0.00038 μM and a remarkable therapeutic index of >402,632, which are better than those of DCK and AZT in this assay. Compound **4** also exhibited more potent anti-HIV activity than AZT with an EC_{50} value of 0.00276 and therapeutic index of >51,000. However, these values were not comparable to those of DCK. Compound **3** also was more active than AZT with an EC_{50} value of 0.006, but its therapeutic index value was lower than that of AZT. In contrast, compound **6** was much less active than the lead compound DCK. These results suggested that introducing methoxy group at the 4- or 5-position of DCK could lead to enhanced anti-HIV activity, with the 5-methoxy group being the most effective. Further modification of DCK for better pharmacological properties is in progress.

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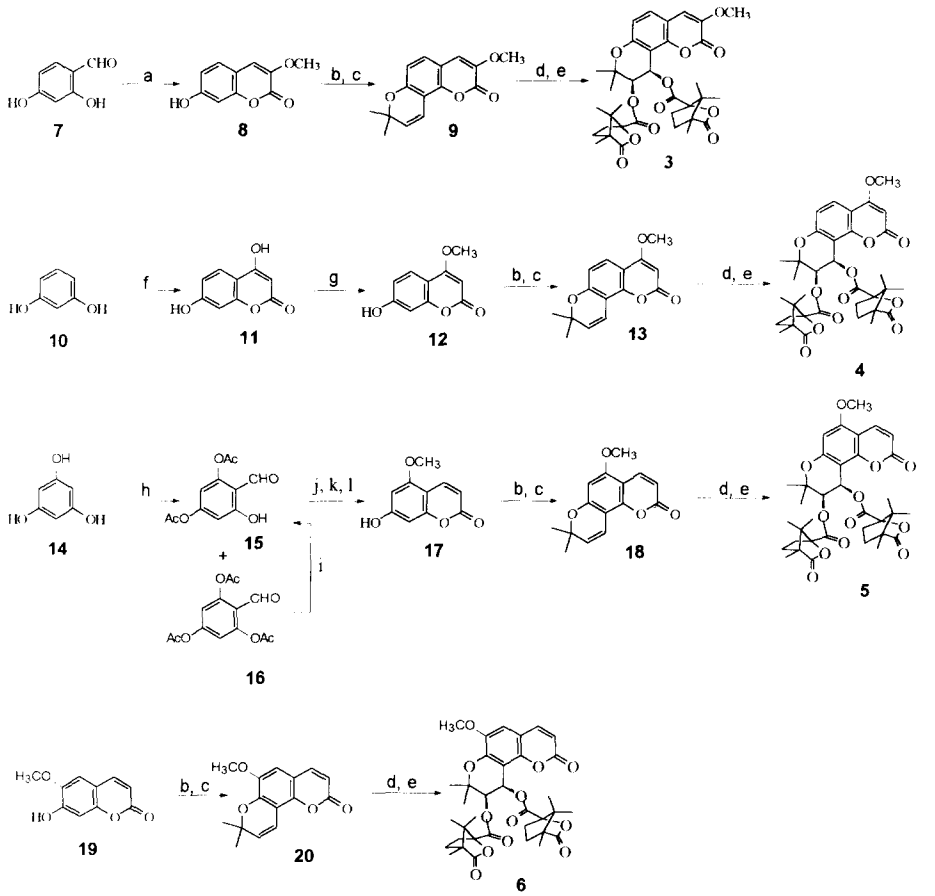

Scheme 1. Synthesis of mono-methoxy substituted 3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactones (**3**–**6**)

a. $MeOCH_2COONa$, $MeOCH_2COCl$ in DMF
b. 3-Chloro-3-methylbut-1-yne, KI, K_2CO_3 in DMF
c. N,N-Diethylaniline, reflux
d. $K_2OsO_2(OH)_4$, K_2CO_3, $K_3Fe(CN)_6$, $(DHQ)_2$-PYR in t-BuOH/H_2O, 0°C
e. (-)-Camphanoyl chloride, pyridine in CH_2Cl_2
f. $CH_2(COOH)_2$, $BF_3{\cdot}Et_2O$; g. MeOH/H_2SO_4
h. Ac_2O, pyridine in CH_2Cl_2; i. MeOH, Py
j. Ph_3P=CHCOOMe in DMF; k. MeI, K_2CO_3 in DMF
l. $MeCH_2CH_2OH$, DMAP, reflux 3 hs

Table 1. ^{1}H NMR Data of Methoxy Substituted-DCK Analogues (3–6)

Proton δppm (*J*)	3	4	5	6
H-3	3.88 (s, OCH_3)	5.53 (s)	6.14 (d, 9.8)	6.27 (d, 9.8)
H-4	6.78 (s)	3.97 (s, OCH_3)	7.97 (d, 9.8)	7.60 (d, 9.8)
H-5	7.34 (d, 8.8)	7.74 (d, 8.8)	3.90 (s, OCH_3)	6.90 (s)
H-6	6.83 (d, 8.8)	6.81 (d, 8.8)	6.25 (s)	3.92 (s, OCH_3)
H-3'	5.39 (d, 4.8)	5.38 (d, 4.8)	5.34 (d, 4.8)	5.40 (d, 4.8)
H-4'	6.64 (d, 4.8)	6.64 (d, 4.8)	6.60 (d, 4.8)	6.65 (d, 4.8)
CH_2 (x 4)	2.48 (m)	2.48 (m)	2.49 (m)	2.48 (m)
	2.22 (m)	2.24 (m)	2.22 (m)	2.10 (m)
	1.92 (m)	1.92 (m)	1.90 (m)	1.97 (m)
	1.69 (m)	1.68 (m)	1.71 (m)	1.72 (m)
CH_3 (x8)	1.55 (s, 3H)	1.49 (s, 3H)	1.55 (s, 3H)	1.52 (s, 3H)
	1.47 (s, 3H)	1.45 (s, 3H)	1.50 (s, 3H)	0.98-1.14 (m.s.)
	1.43 (s, 3H)	0.93-1.14 (m.s.)	1.44 (s, 3H)	
	0.93-1.12 (m.s.)		0.98-1.14 (m.s.)	

Table 2. Anti-HIV Activity of DCK and Its Analogues (3–6) in Acutely Infected H9 Lymphocytes[11]

Compound	IC_{50} (μM) [a]	EC_{50} (μM) [b]	Therapeutic index [c]
3	>153[d]	0.006	>25,500
4	>153[d]	0.00276	>51,000
5	>153[d]	0.000138	>402,632
6	>153[d]	24.5	>9.68
DCK (2)	35	0.000256	136,719
AZT	1875	0.045	41,667

[a]concentration that inhibits uninfected H9 cell growth by 50%.
[b]concentration that inhibits viral replication by 50%.
[c]TI = IC_{50}/EC_{50}.
[d]Maximum IC_{50} value possible for this assay due to the presence of DMSO which is used to solubilize the agents tested.

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8. $(DHQ)_2$-PYR: Hydroquinine 2,5-diphenyl-4,6-pyrimidinediyl diether.
9. **3-Methoxy-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (3)** (% d.e. 80): mp 147–50 °C; $[\alpha]_D$ +12.9° (*c* 0.715, $CHCl_3$); MS (CI–NH_3) *m/z* (%): 670 ($M+NH_4^+$, 100); EA for $C_{35}H_{40}O_{12} \cdot \frac{1}{2} H_2O$: Theory: C, 63.53; H, 6.25. Found: C, 63.57; H, 6.41.

   4-Methoxy-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (4) (% d.e. 73): mp 174–6 °C; $[\alpha]_D$ +2.34° (*c* 0.685, $CHCl_3$); MS (CI–NH_3) *m/z* (%): 670 ($M+NH_4^+$, 75); EA for $C_{35}H_{40}O_{12} \cdot \frac{1}{2} H_2O$: Theory: C, 63.53; H, 6.25. Found: C, 63.33; H, 6.39.

   5-Methoxy-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (5) (% d.e. 86): mp 168–70 °C; $[\alpha]_D$ –4.44° (*c* 0.45, $CHCl_3$); MS (CI–NH_3) *m/z* (%): 670 ($M+NH_4^+$, 60); EA for $C_{35}H_{40}O_{12} \cdot \frac{1}{2} H_2O$: Theory: C, 63.53; H, 6.25. Found: C, 63.52; H, 6.26

   6-Methoxy-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (6) (% d.e. >95): mp 262-4 °C; $[\alpha]_D$ –18.26° (*c* 0.5, $CHCl_3$); MS (EI) *m/z* (%): 652 (M^+, 20); EA for $C_{35}H_{40}O_{12} \cdot 2\frac{1}{2} H_2O$: Theory: C, 60.25; H, 6.50. Found: C, 60.22; H, 6.92.
10. The percent enantiomeric excess was determined by ^1H NMR analysis of the bis-(-)-camphanic esters.
11. **HIV Growth Inhibition Assay**. The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum [FCS] supplemented with L-glutamine at 5% CO_2 and 37 °C. Aliquots of this cell line were only used in experiments when in log-phase of growth. Test samples were first dissolved in dimethyl sulfoxide (DMSO). The following were the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL, but for active agents additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value could be achieved. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The mock-infected was used for toxicity determinations (IC_{50}). The stock virus used for

these studies typically had a $TCID_{50}$ value of 10^4 Infectious Units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 Infectious Units/cell was added to the first aliquot of H9 cells. The other aliquot of H9 cells only received culture medium and then was incubated under identical conditions as the HIV-infected H9 cells. After a 4 hour incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive infected control/negative drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on Day 4 for use in our in-house p24 antigen ELISA assay. P24 antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts by a Coulter Counter on the mock-infected H9 cells which had either received culture medium (no toxicity) or test sample or AZT.

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