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ANTI-AIDS AGENTS 31.[1] SYNTHESIS AND ANTI-HIV ACTIVITY OF 4-SUBSTITUTED 3',4'-DI-*O*-(-)-CAMPHANOYL-(+)-*Cis*-KHELLACTONE (DCK) THIOLACTONE ANALOGS

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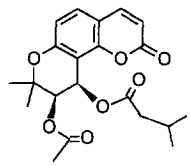
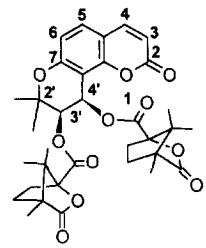
Abstract: Four DCK-thiolactone analogs (**3–6**) were synthesized asymmetrically and evaluated for anti-HIV activity against HIV-1 replication in H9 lymphocyte cells. Based on the functionality on the thiolactone-coumarin nucleus, activity was in the order: methyl > H > propyl > benzyl. 4-Methyl-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khelthiolactone (**4**) exhibited extremely potent anti-HIV activity with EC_{50} and therapeutic index values of 0.00718 μM and > 21,300, respectively.

Suksdorfin (**1**), isolated from the fruit of *Lomatium suksdorfii*, is a khellactone with interesting biological properties, especially its anti-HIV activity.[2] Modification of **1** yielded 3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khellactone (**DCK**) (**2**), which demonstrated extremely potent inhibitory activity against HIV-1 replication in H9 lymphocytic cells with an EC_{50} value of 0.000256 μM and therapeutic index of 136,719, and was more potent than AZT as an anti-HIV agent in this assay.[3,4] In an attempt to determine the mechanism of anti-HIV activity, suksdorfin and DCK were tested for anti-HIV-RT activity. Both compounds showed no activity in an *in vitro* RT assay using the poly(A) as the template.[3] Their mechanisms of action are currently under investigation.



1 (Suksdorfin)



2 (DCK)

These results strongly suggest the need for more in-depth evaluation of both mechanism of action and essential pharmacophoric elements in this compound class. Therefore, selected modifications of the three constitutive building blocks of DCK (i.e., khellactone moiety, substitutions at the 3' and 4' positions, and substitutions R_3 ~ R_6 on the coumarin nucleus) were warranted. Among 42 khellactone derivatives with different *O*-acyl- and /or *O*-alkyl- groups at the 3', and 4' positions, an *R*-configuration and di-*O*-(-)-camphanoyl moities at the 3'- and 4'-positions are very important to and required for anti-HIV activity in

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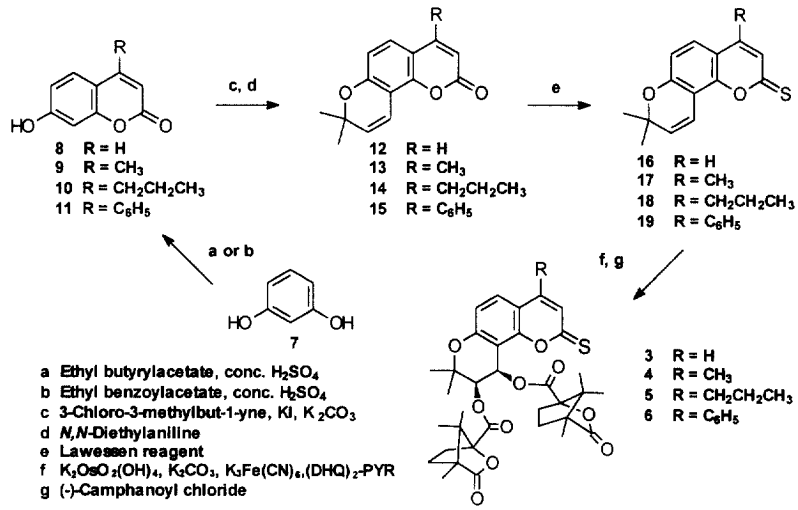
this type of compound.[3,4] Introducing additional substitutions at the 3-, 4-, 5-, or 6-position led to four isomeric methoxy substituted DCK analogs, respectively. Among them, anti-HIV activity of the 4- and 5-methoxy DCK analogs was better than AZT in the same assay.[5]

As an extension to these studies, we explored modification of the khellactone nucleus, including synthesis of bioisosteres of **2**, replacing O with S in the 2-ketone moiety. These modifications will give us more information about structure-activity relationships, which will eventually help to explore the mechanisms of action. In this paper, we report the synthesis and anti-HIV activity of 4-unsubstituted (**3**), 4-methyl- (**4**), 4-propyl- (**5**) and 4-benzyl- (**6**) 3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khelthiolactone.

The synthesis of DCK thiolactone analogs (**3–6**) was accomplished by a 5- or 6-step sequence illustrated in Scheme 1.

Scheme 1 Synthesis of DCK Thiolactone and 4-Substituted 3',4'-di-*O*-(-)Camphanoyl-(+)-*cis*-khelthiolactones



4-Propyl-7-hydroxycoumarin (**10**) and 4-benzyl-7-hydroxycoumarin (**11**) were prepared from the commercially available resorcinol (**7**) and ethyl butyrylacetate or ethyl benzoylacetate, respectively, in the presence of sulfuric acid. Compounds **10** and **11**, along with commercially available 7-hydroxycoumarin (**8**) and 4-methyl-7-hydroxycoumarin (**9**) were converted in two steps to form seselin (**12**), 4-methyl- (**13**), 4-propyl- (**14**), and 4-benzyl-seselin (**15**), respectively. The key intermediates, thiolactone seselins (**16–19**), were prepared by refluxing substituted seselins (**12–15**) with Lawessen reagent in toluene, respectively. As in the asymmetric synthesis of DCK,[6] the four thiolactone seselin analogs (**16–19**) were asymmetrically dihydroxylated using $(DHQ)_2$-PYR as a chiral catalyst, and then were esterified with (-)-(*S*)-camphanoyl chloride at room temperature for 48 h to obtain **3–6**, respectively.[7] In this compound type, asymmetric dihydroxylation is highly

stereoselective with percent enantiomeric excess (% e.e.) ranging from 72–92%.[8] The *cis*-khelthiolactone derivatives with 3'*R*, 4'*R* configuration are the predominant diasteroisomers.

Table1. Anti-HIV Activity of DCK and Its Thiolactone Analogs in Acutely Infected H9 Lymphocytes[9]

Compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
3	> 156[d]	0.0290	> 5,390
4	> 153[d]	0.00718	> 21,300
5	> 148[d]	0.128	>1,153
6	> 140[d]	2.48	> 56.6
DCK	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 = therapeutic index 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.

Table 1 shows the anti-HIV activities of DCK thiolactone analogs **3–6**, with AZT and DCK included in the same experiment for comparison. The results indicated that **4** had very potent anti-HIV activity in acutely infected H9 lymphocytes with an EC_{50} value of 0.00718 μM and a remarkable therapeutic index of >21,300. Compound **3** also was more active than AZT with an EC_{50} value of 0.0290 μM. However, in comparing the activity between thiolactone and ketone compounds, the EC_{50} and TI values of thiolactone analogs (**3**,**4**) were not comparable to those of DCK or 4-methyl DCK (unpublished data).[10] Therefore, the ketone moiety appears important in maintaining the anti-HIV-1 activity. In addition, the size of the substitutions on the coumarin nucleus (at the 4-position) plays an important role in the anti-HIV activity. Introduction of a methyl group at the 4-position (**4**) led to enhanced activity compared with the unsubstituted thiolactone DCK (**3**). Larger or bulky group at the 4- position reduced the anti-HIV inhibition dramatically. Further modification of DCK analogs for better pharmacological properties and SAR studies is in progress.

Acknowledgement. This investigation was supported by grant AI-33066 from the National Institute of Allergies and Infectious Diseases awarded to K. H. Lee.

References and notes:

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7. **3',4'-Di-*O*-(-)-camphanoyl-(+)-*cis*-khelthiolactone (3)** (% d.e. 92): mp 182–184°C; $[\alpha]_D$ + 93.3° (c 0.4, $CHCl_3$); ^1H NMR δ 7.44 (d, *J* = 8.8 Hz, 1H, H-5), 7.40 (d, *J* = 9.4 Hz, 1H, H-4), 7.10 (d, *J* = 9.4 Hz, 1H, H-3), 6.86 (d, *J* = 8.8 Hz, 1H, H-6), 6.75 (d, *J* = 4.7 Hz, 1H, H-4'), 5.43 (d, *J* = 4.7 Hz, 1H, H-3'), 2.50, 2.25, 1.90, 1.58 (each m, 2H, camphanoyl CH_2), 1.53, 1.47 (each s, 3H, 2'-CH_3), 0.94—1.13 (m, 18H in total, camphanoyl CH_3); HRMS calcd for $C_{34}H_{38}O_{10}S$ 638.2186, Found 638.2185.

   4-Methyl-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khelthiolactone (4) (% d.e. 91): mp 180–182°C; $[\alpha]_D$ + 101.0° (c 0.4, $CHCl_3$); ^1H NMR δ 7.58 (d, *J* = 8.9 Hz, 1H, H-5), 7.04 (s, 1H, H-3), 6.89 (d, *J* = 8.9 Hz, 1H, H-6), 6.75 (d, *J* = 4.9 Hz, 1H, H-4'), 5.42 (d, *J* = 4.9 Hz, 1H, H-3'), 2.34 (s, 3H, 4-CH_3), 2.55, 2.25, 1.94, 1.70 (each m, 2H, camphanoyl CH_2), 1.50, 1.47 (each s, 3H, 2'-CH_3), 0.93—1.15 (m, 18H in total, camphanoyl CH_3); HRMS calcd for $C_{35}H_{40}O_{10}S$ 652.2342, Found 652.2357.

   4-Propyl-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khelthiolactone (5) (% d.e. 72): mp 145–147°C; $[\alpha]_D$ + 20.0° (c 0.2, $CHCl_3$); ^1H NMR δ 7.61 (d, *J* = 8.9 Hz, 1H, H-5), 7.03 (s, 1H, H-3), 6.88 (d, *J* = 8.9 Hz, 1H, H-6), 6.74 (d, *J* = 4.6 Hz, 1H, H-4'), 5.43 (d, *J* = 4.6 Hz, 1H, H-3'), 2.65 (t, 2H, 4-αCH_2), 2.02 (m, 2H, 4-βCH_2), 1.25 (m, 3H, 4-γCH_3), 2.52, 2.24, 1.92, 1.71 (each m, 2H, camphanoyl CH_2), 1.52, 1.47 (each s, 3H, 2'-CH_3), 0.93—1.15 (m, 18H in total, camphanoyl CH_3); HRMS calcd for $C_{37}H_{44}O_{10}S$ 680.2655, Found 680.2633 .

   4-Benzyl-3',4'-di-*O*-(-)-camphanoyl-(+)-*cis*-khelthiolactone (6) (% d.e. 84): mp 106–108°C; $[\alpha]_D$ + 21.7° (c 0.12, $CHCl_3$); ^1H NMR δ 7.44–7.55 (m, 5H, 4-C_6H_5), 7.40 (d, *J* = 9.0 Hz, 1H, H-5), 7.10 (s, 1H, H-3), 6.84 (d, *J* = 9.0 Hz, 1H, H-6), 6.77 (d, *J* = 4.7 Hz, 1H, H-4'), 5.46 (d, *J* = 4.7 Hz, 1H, H-3'), 2.50, 2.27, 1.91, 1.70 (each m, 2H, camphanoyl CH_2), 1.52, 1.47 (each s, 3H, 2'-CH_3), 0.99—1.17 (m, 18H in total, camphanoyl CH_3); HRMS calcd for $C_{40}H_{42}O_{10}S$ 714.2498, Found 714.2489.
8. The percent enantiomeric excess was determined by ^1H NMR analysis of the bis-(-)-camphanic esters.
9. **HIV Growth Inhibition Assay**. HIV growth inhibition assay was performed as described previously.[5]
10. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem. Lett.* submitted