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Anti-AIDS Agents Part 41:† Synthesis and Anti-HIV Activity of 3′,4′-di-*o*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) Lactam Analogues

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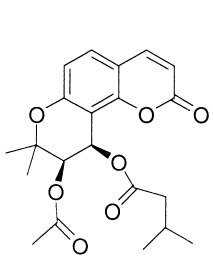
Abstract—DCK lactam analogues were synthesized and evaluated for anti-HIV activity against HIV-1 replication in H9 lymphocyte cells. 4-Methyl-DCK lactam (**11a**) exhibited potent anti-HIV activity with EC_{50} and therapeutic index values of 0.00024 μM and 119,333, respectively.

Suksdorfin (**1**), isolated from the fruit of *Lomatium suksdorfii*, is a khellactone with interesting biological properties, especially its anti-HIV activity.[1] 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. Previous SAR studies indicated that an angular pranocoumarin skeleton and di-*O*-(−)-camphanoyl moieties of *R*-configuration at the 3′- and 4′-positions are very important to and required for anti-HIV activity in this compound type. 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.[2,3] Their mechanisms of action are currently under investigation.

Bioisosterism represents one approach used by the medicinal chemists for the rational modification of a lead compound into safer and more clinically effective agents. Based on the principle of isosteric replacement, a series of bioisosteres of DCK, replacing O with S in the 2-ketone moiety, were synthesized and evaluated for anti-HIV activity against HIV replication in both H9 and CEM-SS cell lines. We recently discovered that



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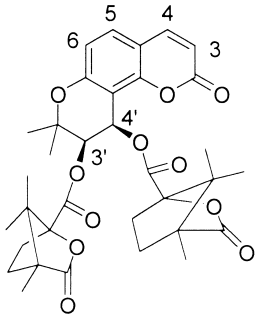
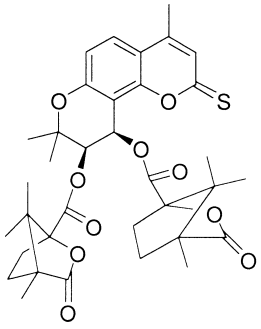


2 (DCK)



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†For Part 40, see Xia, Y.; Yang, Z. Y.; Brossi, A.; Lee, K. H. *Org. Lett.* **1999**, *1*, 2113.

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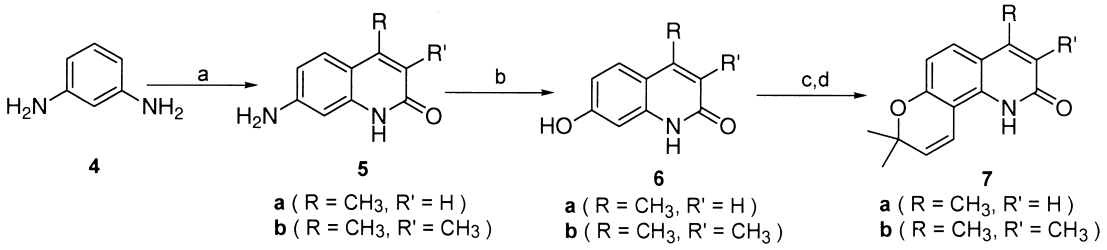
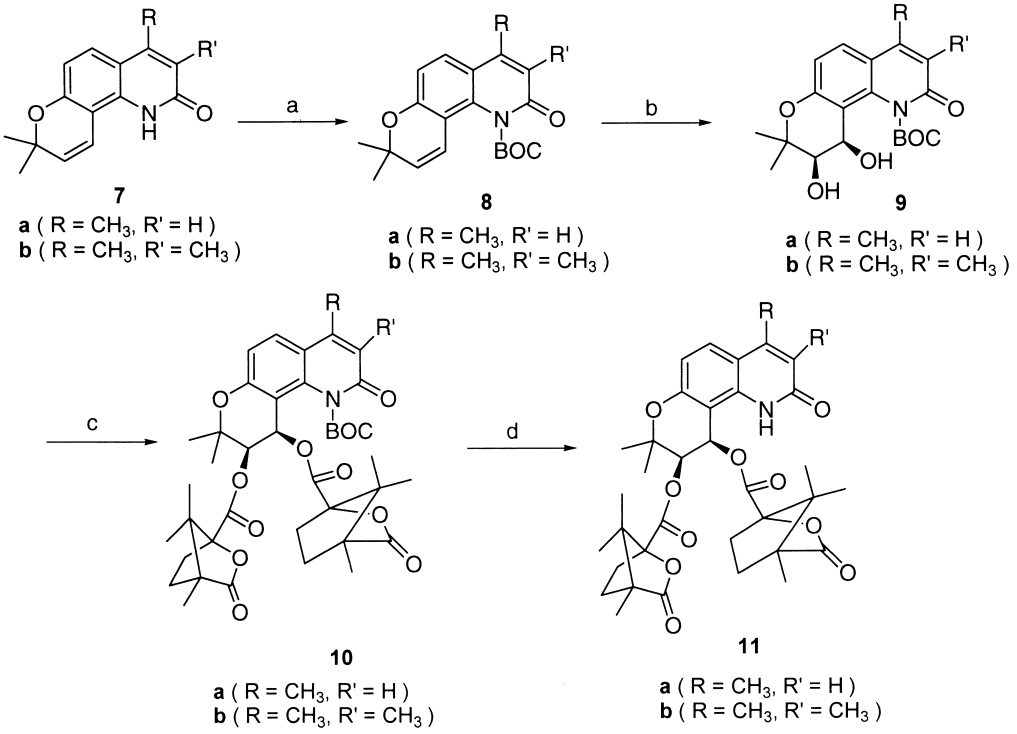
4-methyl-DCK thiolactone (**3**) not only has potent anti-HIV activity,[4] but also shows different sensitivity in different cell lines.[5]

In addition, interchanging groups in bioactive molecules is a valuable concept to derive new molecules that often show different solubility and bioavailibity, and therefore, provide interesting activity profiles. As an extension to these studies, we explored modification of the pyranocoumarin nucleus, including synthesis of DCK lactam analogues. The interchange of O and NH is well known and has been successfully employed in the development of various pharmacological agents. The similar steric size and the ability of these functional groups to act as hydrogen bond acceptors likely are responsible for their successful use as bioisosteres. These modifications will give us more information about structure–activity relationships, and eventually help to explore the mechanism of action. In this paper, we report the synthesis and anti-HIV activity of some DCK lactam analogues. We have developed a novel 4-step procedure for the general preparation of 8,8-dimethyl-2*H*,8*H*-pyrano[6,5-*h*]quinolin-2-ones (Scheme 1).[6] First, a Knorr cyclization was used to give 7-amino-quinolin-2-ones (**5**), followed by diazotization and hydrolysis to form 7-hydroxyquinolin-2-ones(**6**). Subsequently, a nucleophilic substitution reaction and a regiospecific Claisen rearrangement at high temperature provided the designed compounds (**7**). However, the unprotected lactam compounds (**7**) were not successful on the Sharpless's asymmetric dihydroxylation (AD) reaction. As shown in Scheme 2, we chose to protect the amine as a carbamate using the BOC group. Compounds **9a** and **9b** were asymmetrically dihydroxylated using $(DHQ)_2$-PYR as a chiral catalyst, and then were esterified with (−)-(*S*)-camphanoyl chloride and deprotected with TFA to obtain the desired compounds **11a** and **11b**, respectively.[7] In this compound type, asymmetric dihydroxylation is highly stereoselective with percent enantiomeric excess (% ee) ranging from 89 to 93%.[8]

Table 1 shows the anti-HIV activities of DCK lactam analogues and their two and three ring precursors **5a**–**11**, with AZT and DCK included in the same experiment



Scheme 1. Synthesis of 8,8-dimethyl-2*H*,8*H*-pyrano[6,5-*h*]quinolin-2-ones. (a) $RCOCHR'COOC_2H_5$; (b) $NaNO_2/H_2SO_4$, H_2O; (c) 3-chloro-3-methybut-1yne; (d) *N*,*N*-diethylaniline.



Scheme 2. Synthesis of DCK lactam analogues. (a) Di-*tert*-butyl dicarbonate, Et_3N; (b) $K_2OsO_2(OH)_4$, K_2CO_3, $K_3Fe(CN)_6$, $(DHQ)_2$-PYR; (c) (−)-(*S*)-camphanoyl chloride; (d) TFA.

Table 1. Anti-HIV activity of DCK and its lactam analogues in acutely infected H9 lymphocytes[9]

Compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
5a	104		No suppression
6a	125		No suppression
7a			Did not dissolve
8a			Precipitated in the media
9a			Precipitated in the media
10a	>157[d]		No suppression
11a	28	0.00024	119,333
11b	42	0.0046	9100
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.

for comparison. The results indicated that **11a** had very potent anti-HIV activity in acutely infected H9 lymphocytes with an EC_{50} value of 0.00024 μM and a therapeutic index of 119,333. Compound **11a** was about 225-fold more active than AZT as comparing their EC_{50} values in this assay. Compound **11b** also was active with an EC_{50} value of 0.0046 μM. However, increasing the number of methyl groups from one (**11a**) to two (**11b**) reduced the anti-HIV inhibition about 23-fold as comparing their EC_{50} values. Compounds **5a**–**10a** showed no anti-HIV activity or precipitated when added in the assay media, which confirmed that the unique camphonyl groups at the 3′ and 4′ positions are structural requirements for the inhibition of HIV-1 replication. The EC_{50} value of lactam analogue **11b** was not comparable to that of DCK. However, **11b** was less toxic (higher IC_{50}) than DCK. Interestingly, the hydrogen atom in the lactam ring skeleton seemed to be important for anti-HIV activity; the *N*-protected **10a** was completely inactive. Mechanism studies of DCK and DCK lactam analogues are ongoing. Further modification of DCK analogues for better pharmacological properties and SAR studies are in progress.

Acknowledgements

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References and Notes

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6. Yang, Z. Y.; Xia, Y.; Xia, P.; Brossi, A.; Lee, K. H. *Tetrahedron Lett.* **1999**, *40*, 4505.
7. **(9R,10R)-9,10-di-*O*-(−)-camphanoyl-4,8,8-trimethyl-1,8,9,10-tetrahydro-2*H*-pyrano[2,3-*h*]quinolin-2-one (11a)** (% de 93): mp 156–158 °C; $[\alpha]_D + 98.0°$ (*c* 0.4, $CHCl_3$); ^1H NMR δ 7.64 (d, $J = 8.9$ Hz, 1H, H-5), 6.79 (d, $J = 8.9$ Hz, 1H, H-6), 6.59 (d, $J = 4.5$ Hz, 1H, H-10), 6.34 (s, 1H, H-3), 5.43 (d, $J = 4.5$ Hz, 1H, H-9), 2.42 (s, 3H, 4-CH_3), 2.47, 2.17, 1.91, 1.68 (each m, 2H, camphanoyl CH_2), 1.52, 1.47 (each s, 3H, 8-CH_3), 0.92–1.25 (m, 18H in total, camphanoyl CH_3); HRMS calcd for $C_{35}H_{41}NO_{10}$ 635.2730, found 635.2733.
8. The percent enantiomeric excess was determined by ^1H NMR analysis of the bis-(−)-camphanic esters.
9. HIV growth inhibition assay was performed as described previously.[4]