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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

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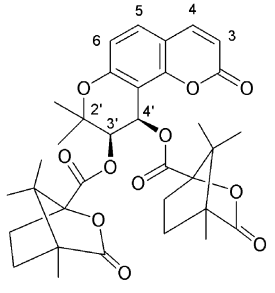
Abstract—Six 3-substituted 3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone derivatives (**3**–**8**) were synthesized from 3-methyl DCK (**2**). 3-Hydroxymethyl DCK (**6**) exhibited potent anti-HIV activity in H9 lymphocytes with EC_{50} and TI values of 1.87×10^{-4} μM and 1.89×10^{5}, respectively. These values are similar to those of DCK and better than those of AZT in the same assay.

In our previous research, 3′,4′-di-*O*-(*S*)-camphanoyl-(3′*R*,4′*R*)-(+)-*cis*-khellactone (DCK, **1**) (Fig. 1) was identified as a potent anti-HIV agent with an EC_{50} value of 2.56×10^{-4} μM and a remarkable therapeutic index (TI) of 1.37×10^{5} in H9 lymphocytes.[2,3] This lead compound was then modified to explore anti-HIV SARs. In particular, alkyl, alkoxy, aryl, and acyl groups were introduced at varying positions on the (+)-*cis*-khellactone skeleton. From this data, we identified several structural moieties essential for anti-HIV activity[4,5] (i.e., a 3′*R*,4′*R* configuration, resonance of the coumarin ring, and two bulky camphanoyl groups). Earlier results also indicated that substitution at the 3-, 4-, and 5-positions could enhance anti-HIV activity and reduce toxicity.[6,7] A methyl group was preferable to other alkyl groups, and 3-methyl, 4-methyl, and 5-methyl DCK analogues were more active than DCK and AZT in H9 lymphocytes with EC_{50} and TI values ranging from 5.25×10^{-5} to 2.39×10^{-7} μM and 2.15×10^{6} to 3.97×10^{8}, respectively.[5] However, the poor water-solubility of DCK and its active analogues could limit their further development as drug candidates.

In our continuing SAR study, our next synthetic efforts focused on introducing an amino or hydroxy group into the DCK structure both to improve water solubility and to explore the effects of polar functional groups on the anti-HIV activity. Herein, we report the synthesis of six 3-substituted DCK analogues (**3**–**8**) and their anti-HIV activity in H9 lymphocytes.

According to Scheme 1, 3-methyl DCK (**2**) was reacted with *N*-bromosuccinimide at a molar ratio of 1:1 in anhydrous benzene at reflux temperature for 6 h to produce 3-bromomethyl DCK (**3**) in a 77% yield.[8] If excess *N*-bromosuccinimide was used, 3-dibromomethyl DCK (**4**) was obtained also. Compound **3** was easily converted to other 3-substituted DCK analogues. The bromomethyl group in **3** was acetylated with acetic anhydride in the presence of sodium acetate at reflux for 3 h to afford 3-acetoxymethyl DCK (**5**) in a 79% yield. Hydrolysis of **5** in EtOH under acidic conditions[9] gave a



DCK (**1**)

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

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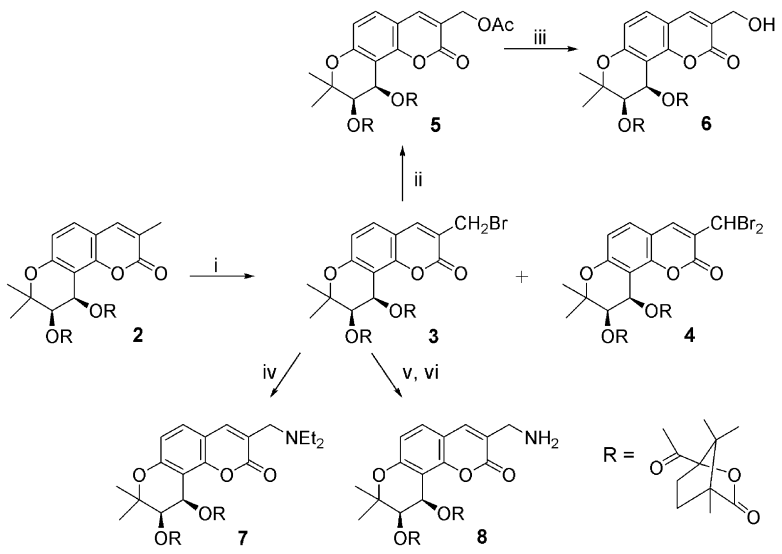

Scheme 1. Reagents, conditions, yields: (i) *N*-bromosuccinimide/benzene, reflux, 6 h, 77%; (ii) acetic anhydride/NaOAc, reflux, 3 h, 79%; (iii) EtOH/HCl (2 N), reflux, 4 h, 87%; (iv) diethylamine/toluene, reflux, 6 h, 71%; (v) hexamethylenetetramine/$CHCl_3$, reflux, 3 h: (vi) EtOH/HCl (2 N), 100 °C, 15 min, 76%.

Table 1. ^{1}H NMR data of 3-substituted-DCK analogues (**3**–**8**)

Proton	δ ppm (*J*, Hz)					
	3	**4**	**5**	**6**	**7**	**8**
4	7.76 (s)	8.24 (s)	7.69 (s)	7.67 (s)	7.81 (s)	7.67 (s)
5	7.42 (d, 8.7)	7.54 (d, 8.7)	7.42 (d, 8.7)	7.43 (d, 8.7)	7.43 (d, 8.7)	7.42 (d, 8.7)
6	6.84 (d, 8.7)	6.89 (d, 8.7)	6.83 (d, 8.7)	6.83 (d, 8.7)	6.81 (d, 8.7)	6.82 (d, 8.7)
3′	5.39 (d, 4.8)	5.39 (d, 4.8)	5.4 (d, 4.8)	5.39 (d, 4.8)	5.39 (d, 4.8)	5.39 (d, 4.8)
4′	6.64 (d, 4.8)	6.64 (d, 4.8)	6.64 (d, 4.8)	6.65 (d, 4.8)	6.65 (d, 4.8)	6.65 (d, 4.8)
Camphanoyl	2.51 (m)	2.48 (m)	2.49 (m)	2.49 (m)	2.50 (m)	2.50 (m)
CH_2 (×4)	2.24 (m)	2.23 (m)	2.24 (m)	2.22 (m)	2.22 (m)	2.23 (m)
	1.91 (m)	1.89 (m)	1.90 (m)	1.92 (m)	1.90 (m)	1.90 (m)
	1.68 (m)	1.63 (m)	1.62 (m)	1.66 (m)	1.69 (m)	1.69 (m)
Camphanoyl	0.98 (s)	0.98 (s)	0.95 (s)	0.98 (s)	0.98 (s)	0.98 (s)
CH_3 (×6)	1.02 (s)	1.03 (s)	1.03 (s)	1.00 (s)	1.01 (s)	1.01 (s)
	1.04 (s)	1.08 (s)	1.04 (s)	1.06 (s)	1.04 (s)	1.06 (s)
	1.09 (s)	1.09 (s)	1.08 (s)	1.08 (s)	1.09 (s)	1.08 (s)
	1.10 (s)	1.10 (s)	1.11 (s)	1.10 (s)	1.10 (s)	1.10 (s)
	1.11 (s)	1.11 (s)	1.12 (s)	1.11 (s)	1.11 (s)	1.11 (s)
2′-CH_3 (×2)	1.45 (s)	1.46 (s)	1.45 (s)	1.45 (s)	1.45 (s)	1.45 (s)
	1.49 (s)	1.50 (s)	1.49 (s)	1.49 (s)	1.49 (s)	1.49 (s)
Substituents at 3-position	4.38 (s, 2H, CH_2)	6.74 (s, 1H, CH)	5.00 (m, 2H, CH_2) 2.19 (s, 3H, $OCOCH_3$)	4.56 (m, 2H, CH_2)	3.47 (s, 2H, $CH_2NCH_2CH_3$) 2.60 (q, 4H, NCH_2CH_3) 1.07 (t, 6H, NCH_2CH_3)	3.79 (s, 2H, CH_2) 2.60 (br s, 2H, NH_2)

s = singlet, d = doublet, m = multiplet, t = triplet, q = quartet, br = broad

79% yield of 3-hydroxymethyl DCK (**6**). Alternatively, **3** was reacted with diethylamine in anhydrous toluene[8] at reflux temperature for 3 h to produce 3-diethylaminomethyl DCK (**7**) in a 71% yield. To synthesize 3-aminomethyl DCK (**8**), **3** was treated with hexamethylenetetramine[10] in refluxing $CHCl_3$ for 3 h, followed by hydrolysis in EtOH in the presence of a catalytic amount of HCl (2 N) to afford the target compound in a 76% yield. The ^{1}H NMR data of **3**–**8** are listed in Table 1.

The DCK analogues **3**–**8** were tested against HIV-1 replication in acutely infected H9 lymphocytes,[11] and the anti-HIV data are shown in Table 2. The 3-hydroxymethyl analogue, **6**, was the most promising compound and showed potent anti-HIV activity with an EC_{50} value of 1.88×10^{-4} μM and a remarkable TI of 188,032. These values are similar to those of DCK and much better than those of AZT in the same assay. The 3-dibromomethyl derivative, **4**, and the 3-acetoxymethyl analogue, **5**, also exhibited more potent anti-HIV

Table 2. Anti-HIV activities of 3-substituted DCK analogues (**3**–**8**) in acutely infected H9 lymphocytes

Compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	Therapeutic index[c]
3	3.38	8.54×10^{-2}	40
4	3.20	1.19×10^{-4}	26,890
5	3.34	1.03×10^{-3}	3243
6	35.35	1.88×10^{-4}	188,032
7	43.01	0.69	62
8	32.81	0.39	208
DCK (**2**)	35	2.56×10^{-4}	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]$TI = IC_{50}/EC_{50}$.

activity than AZT with EC_{50} values of 1.19×10^{-4} and 1.03×10^{-3} μM, respectively. However, both compounds were more cytotoxic than AZT, which resulted in lower TIs. In contrast, the 3-amino substituted compounds, **7** and **8**, were less active than DCK and AZT.

These results suggested that anti-HIV potency is maintained with a 3-hydroxymethyl substituent on the coumarin ring. The presence of a hydroxyl group may greatly change the molecular physicochemical properties in relation to biological action. A hydroxyl group can form H-bonds with water to improve molecular water solubility or with an active site on a biological target surface to increase affinity. In addition, the hydroxyl group could be readily linked with other functional groups to form a water soluble molecule or prodrug. Additional modification of DCK is in progress to improve pharmaceutical properties.

Acknowledgements

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11. HIV growth inhibition assay was conducted as described previously.[2–7]