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Synthesis and Anti-HIV Activity of Trisubstituted (3′R, 4′R)-3′,4′-Di-*O*-(S)-camphanoyl-(+)-*cis*-khellactone (DCK) Analogs

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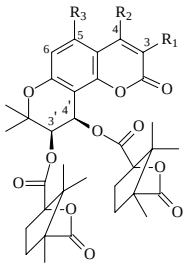
Abstract: To further explore the potential of DCK analogs as anti-HIV drug candidates, ten new tri-substituted (3′R,4′R)-3′,4′-di-*O*-(S)-camphanoyl-(+)-*cis*-khellactone (DCK) derivatives (**4-13**) were designed, synthesized, and evaluated against HIV replication in MT4 cells and H9 lymphocytes.

Keywords: anti-HIV agent, DCK analogs, synthesis.

In our prior studies, 3′, 4′-di-*O*-(S)-camphanoyl-(+)-*cis*-khellactone (DCK, **1**, **Figure 1**) and its derivatives including mono- and di-substituted DCK analogs were identified as a novel class of potent anti-HIV agents[1-4]. Because of its high potency and efficient synthesis, 4-methyl-DCK **2**[5] was chosen as a drug candidate for preclinical studies. However, the low solubility and poor oral bioavailability of 4-methyl-DCK limited its further development. Because high molecular hydrophobicity might be one reason for the failure of 4-methyl-DCK as a drug candidate, our subsequent modification focused on improving water solubility. Mono- and disubstituted DCK analogs with polar functional groups were first explored[6], and the results indicated that hydrophilic groups could be introduced at the 3-position on the coumarin nucleus without adversely affecting potency. Therefore, in our continuing studies, 5-methoxy-4-methyl-DCK **3**[4] served as a new lead, because it exhibited similar anti-HIV potency to **2**, but had a lower predicted

Figure 1 Lead compounds



1 DCK, $R_1 = R_2 = R_3 = H$
2 $R_1 = R_3 = H$, $R_2 = CH_3$
3 $R_1 = H$, $R_2 = CH_3$, $R_3 = OCH_3$

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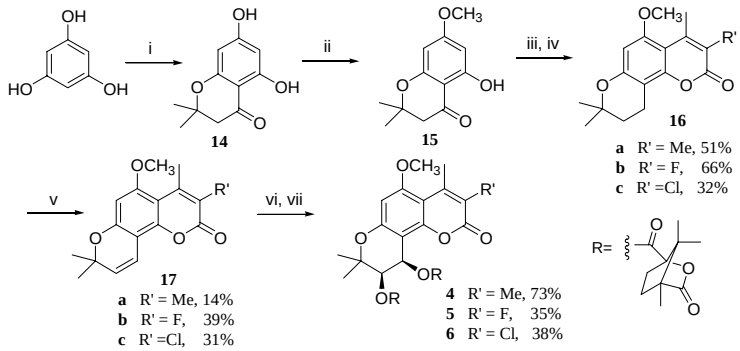
log P value (4.84) than **2** (5.09)[7]. Therefore, we have currently produced trisubstituted DCK analogs by modifying the 3-position of compound **3**. Herein, we report the synthesis of ten new trisubstituted DCK analogs **4-13** and their anti-HIV data in MT4 and H9 cell lines.

Target compounds **4-6** were synthesized as shown in **Scheme 1**. Compound **15** was prepared according to literature[4] and its carbonyl group was reduced with $NaBH_4$. Then the 3,4-substituted lactone rings were formed by Pechmann reactions with ethyl 2-methylacetoacetate, ethyl 2-fluoroacetoacetate, or ethyl 2-chloroacetoacetate, respectively, to give corresponding trisubstituted coumarin compounds **16a, 16b,** and **16c**. Next, compounds **16a-c** were converted into the corresponding trisubstituted seselin derivatives **17a-c** by dehydrogenation with DDQ in anhydrous 1,4-dioxane, respectively. Finally, Sharpless asymmetric dihydroxylation followed by acylation with (S)-camphanic chloride gave trisubstituted DCK analogs **4-6**, respectively.

As shown in **Scheme 2**, trisubstituted DCK analogs **7-13** were synthesized from **4**. Compound **4** was first treated with N-bromosuccinimide at a molar ratio of 1:1 in refluxing anhydrous benzene to afford 3-bromomethyl product **7**. The 3-bromomethyl moiety in **7** can be readily converted to different functional groups by S_N reactions. Compound **7** was treated with piperidine or diethylamine in refluxing anhydrous toluene for 6 h, followed by salt formation with HCl to provide the corresponding aminomethyl hydrochlorides **8** and **9**, respectively. When treated with sodium cyanide or sodium nitrite, compound **7** was con- verted to products **10** or **11**, respectively. Using the same methods in literature[5], compounds **12** and **13** were obtained from **7**. Structures of **4-13** were confirmed from ^{1}H NMR and MS spectral data. Their percent diasteromeric excess (% *d.e.*) was also calculated using ^{1}H NMR.

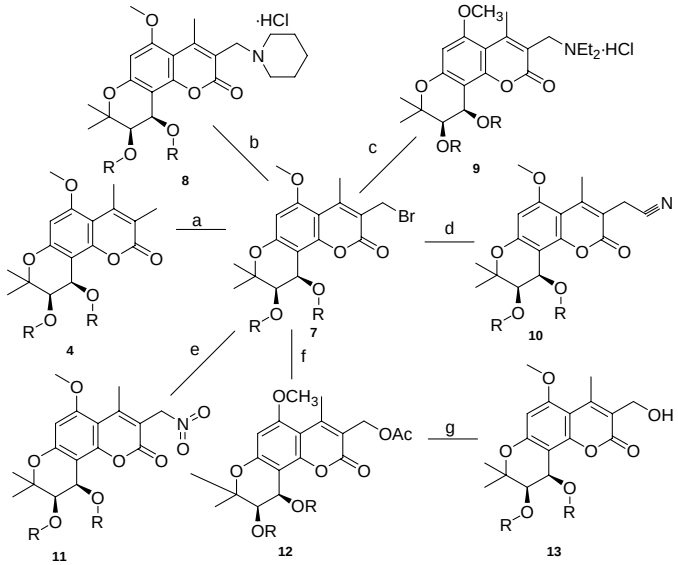
DCK analogs **4-13** were tested against HIV-1 replication in acutely infected MT4 and H9 lymphocytes, and the data are shown in **Table 1**. In the MT4 cell line assay, **13** was the most promising compound with an EC_{50} value of 0.00129 μmol/L and a TI value of > 55,690. This compound also showed high potency in H9 lymphocytes (EC_{50} =0.052μmol/L, TI > 688). This result was consistent with prior data found in this cell line with 3-hydroxymethyl DCK and 3-hydroxymethyl-4-methyl DCK[5]. In the current

Scheme 1 Synthesis of trisubstituted DCK analogs **4-6**



Reagents and conditions: i, $(CH_3)_2C{=}CCOOH$, $BF_3{\bullet}Et_2O$, 70 °C; ii, CH_3I/K_2CO_3, acetone, reflux; iii, $NaBH_4$ in aq. KOH/ toluene, reflux; iv, ethyl 2-substituted acetoactate, $CH_2Cl_2/BF_3{\bullet}Et_2O$, N_2 protection, reflux; v, DDQ, 1,4-dioxane, reflux; vi, $K_3Fe(CN)_6$, K_2CO_3, $(DHQ)_2$-PYR, and $K_2OsO_2{\bullet}2H_2O$ in *t*-BuOH/H_2O (v/v = 1:1), ice-bath; vii, (S)-camphanic chloride, anhydrous CH_2Cl_2/Py, r.t..

Scheme 2 Synthesis of trisubstituted DCK analogs **7-13**



a, NBS, benzene, reflux; b, (i) piperidine, anhydrous toluene, reflux; (ii) Et_2O•HCl; c, (i) $HNEt_2$, anhydrous toluene, reflux; (ii) Et_2O•HCl; d, NaCN/DMSO, 60℃; e, $NaNO_2$/DMF, r.t.; f, NaOAc/Ac_2O, reflux; g, H^+/EtOH, reflux.

Table 1 Anti-HIV activity of trisubstituted DCK analogs **4-13** in MT4 and H9 cell lines

Compd.	Log P	MT4 cell line [e]			H9 cell line		
		IC_{50} (μmol/L) [a]	EC_{50} (μmol/L) [b]	TI[c]	IC_{50} (μmol/L)	EC_{50} (μmol/L)	TI
4	5.12	>73.53	2.94	>25	>36.76	1.95	>19
5	4.36	15.06	0.45	33	>37.65	0.018	>2,092
6	4.74	>71.28	2.85	>25	>35.64	1.80	>20
7	5.32	>65.88	0.24	>275	>32.93	1.62	>20
8	—	>76.64	2.50	>31	21.70	2.19	10
9	—	>63.45	>63.45	1	22.75	2.27	10
10	4.94	>70.92	0.11	>645	>35.46	0.11	>322
11	0.80	>68.96	1.90	>36	>34.48	0.27	>128
12	4.24	>67.75	39.60	>2	>33.88	1.50	>23
13	4.11	>71.84	0.00129	>55,690	>35.92	0.052	>688
4-Me-DCK **2** [d]	5.09	—	—	—	23.60	0.00594	3973
AZT	—	>187.27	0.0055	>34,049	1873	0.0168	111,488

[a] Concentration that inhibits uninfected cell growth by 50%. [b] Concentration that inhibits viral replication by 50%. [c] TI (therapeutic index) = IC_{50}/EC_{50}. [d] $EC_{50} = 1.83\times10^{-6}$ μmol/L and TI = 6.89×10^{7} in our previous study[3]. [e] Assays in the MT-4 cell line were performed by Beijing Institute of Microbiology & Epidemiology, Beijing, China.

studies, **5** was the most promising compound with an EC_{50} value in H9 lymphocytes, similar to AZT (EC_{50} = 0.0168 μmol/L) and better than DCK in the same assay. Compound **10** showed HIV inhibitory activity (EC_{50} = 0.11 μmol/L) in both MT4 and H9 cell lines. The remaining compounds, except **9** and **12**, all showed anti-HIV activity in both assays with EC_{50} values ranging from 0.24-2.94 μmol/L. The two water-soluble amine salts **8** and **9** were generally less active than the other analogs. The calculated log P

values of **4-13** indicated that the presence of a polar group at the 3-position of the coumarin ring could be potential to improve molecular water-solubility.

Acknowledgments

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References and Notes

1. L. Huang, Y. Kashiwada, L. M. Cosentino, *et al*., *Bioorg. Med. Chem. Lett.*,**1994**, *4*, 593.
2. L. Huang, Y. Kashiwada, L. M. Cosentino, *et al*., *J. Med. Chem*., **1994**, *37*, 3947.
3. L. Xie, Y. Takeuchi, L. M. Cosentino, K. H. Lee, *J. Med. Chem*., **1999**, *42*, 2662.
4. L. Xie, Y. Takeuchi, L. M. Cosentino, A. T. McPhail, K. H. Lee, *J. Med. Chem*., **2001**, *44*, 664.
5. L. Xie, Y. Takeuchi, L. M. Cosentino, K. H. Lee, *Bioorg. Med. Chem. Lett*., **1998**, *8*, 2151.
6. L. Xie, D. L. Yu, C. Wild, *et al., J. Med. Chem.*, **2004**, *47*, 756.
7. Log P values were calculated with HYPERCHEM 7.0.
8. **4**: 87% *d.e.*; m.p. 171-172 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.95-1.09 (m.s, 18H, CH_3×6), 1.43 and 1.49 (s, each 3H, CH_3-2′), 1.65, 1.90, 2.20 and 2.38 (m, each 2H, CH_2), 2.49 (s, 3H, CH_3-4), 2.08 (s, 3H, CH_3-3), 3.86 (s, 3H, OCH_3), 5.33 (d, 1H, *J* = 4.8 Hz, H-3′), 6.25 (s, 1H, ArH), 6.53 (d, 1H, *J* = 4.8 Hz, H-4′); MS *m*/*z* (%): 680 (M, 5). **5**: 84% *d.e.*; m.p. 170-171°C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.97-1.11 (ms, 18H, CH_3×6), 1.44 and 1.48 (s, each 3H, CH_3-2′), 1.70, 1.94, 2.22 and 2.48 (m, each 2H, CH_2), 2.53 (s, 3H, CH_3-4), 3.89 (s, 3H, OCH_3), 5.36 (d, 1H, *J* = 4.8 Hz, H-3′), 6.31 (s, 1H, ArH), 6.55 (d, 1H, *J* = 4.8 Hz, H-4′); MS (ESI+) *m*/*z* (%): 702 (M+NH_4, 100). **6**: 84% *d.e.*; m.p. 243-245 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.97-1.12 (m.s, 18H, CH_3×6), 1.44 and 1.49 (s, each 3H, CH_3-2′), 1.66, 1.87, 2.20 and 2.50 (m, each 2H, CH_2), 2.74 (s, 3H, CH_3-4), 3.90 (s, 3H, OCH_3), 5.36 (d, 1H, *J* = 4.8 Hz,, H-3′), 6.31 (s, 1H, ArH), 6.55 (d, 1H, *J* = 4.8 Hz, H-4′); MS (ESI+) m/z (%): 718 (M+NH_4, 100), 720 (M+2+NH_4, 30). **7**: 87% *d.e.*; m.p. 159-162 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.97-1.11 (m.s, 18H, CH_3×6), 1.43 and 1.49 (s, each 3H, CH_3-2′), 1.66, 1.87, 2.20 and 2.50 (m, each 2H, CH_2), 2.63 (s, 3H, CH_3-4), 3.90 (s, 3H, OCH_3), 4.52 (s, 2H, CH_2-3), 5.35 (d, 1H, *J* = 4.8 Hz, H-3′), 6.29 (s, 1H, ArH), 6.57 (d, 1H, *J* = 4.8 Hz, H-4′); MS (FAB) *m*/*z* (%): 761 (M+3, 2), 759 (M+1, 2). **8**: 88% *d.e.*; free base with m.p. 165-167 °C; ^{1}H NMR (D_2O, δ ppm): 0.85-1.08 (m.s, 18H, CH_3×6), 1.50 (s, 6H, CH_3-2′), 1.40-2.25 (m, 10H, CH_2), 2.52 (m, 2H, CH_2), 2.75 (s, 3H, CH_3-4), 3.05 (m, 2H, CH_2), 3.50 (m, 4H, CH_2), 3.97 (s, 3H, OCH_3), 4.34 (s, 2H, CH_2-3), 5.55 (d, 1H, *J* = 4.8 Hz, H-3′), 6.58 (s, 1H, ArH), 6.62 (d, 1H, *J* = 4.8 Hz, H-4′); MS (FAB) *m*/*z* (%): 765 (M+2, 100). **9**: 81% *d.e.*; free base with m.p. 186-188 °C; ^{1}H NMR (D_2O, δ ppm): 0.85-1.09 (m.s, 18H, CH_3×6), 1.32 (s, 6H, NCH_2CH_3), 1.46 (s, 6H, CH_3-2′), 1.68 (m, 2H, CH_2), 2.07 (m , 4H, CH_2), 2.50 (m 2H, CH_2), 2.73 (s, 3H, CH_3-4), 3.25 (m, 4H, NCH_2), 3.91 (s, 3H, OCH_3), 4.30 (s, 2H, CH_2-3), 5.55 (d, 1H, *J* = 4.8 Hz, H-3′), 6.58 (s, 1H, ArH) , 6.65 (d, 1H, *J* = 4.8 Hz, H-4′); MS (FAB) *m*/*z* (%) 753 (M+2, 70). **10**: 86% *d.e.*; m.p. 165-167 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.97-1.12 (m.s, 18H, CH_3×6), 1.43 and 1.49 (s, each 3H, CH_3-2′), 1.70, 1.92, 2.20 and 2.49 (m, each 2H, CH_2), 2.68 (s, 3H, CH_3-4), 3.71 (s, 2H, CH_2-3), 3.91 (s, 3H, OCH_3), 5.36 (d, 1H, *J* = 4.8 Hz, H-3′), 6.31 (s, 1H, ArH), 6.56 (d, 1H, *J* = 4.8 Hz, H-4′); MS (ESI+) *m*/*z* (%): 706 (M+1, 40). **11**: 85% *d.e.*; m.p. 148-150 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.97-1.11 (m.s, 18H, CH_3×6), 1.44 and 1.50 (s, each 3H, CH_3-2′), 1.63, 1.91, 2.22 and 2.50 (m, each 2H, CH_2), 2.63 (s, 3H, CH_3-4), 3.89 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2-3), 5.36 (d, 1H, *J* = 4.8 Hz, H-3′), 6.29 (s, 1H, ArH), 6.58 (d, 1H, *J* = 4.8 Hz, H-4′); MS (ESI+) *m*/*z* (%): 714 (M-NO+NH_4, 100). **12**: 82% *d.e.*; m.p. 165-166 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.97-1.11 (m.s, 18H, CH_3×6), 1.43 and 1.49 (s, 3H, CH_3-2′), 1.68, 1.92, 2.23 and 2.50 (m, each 2H, CH_2), 2.06 (s, 3H, CH_3CO), 2.60 (s, 3H, CH_3-4), 3.92 (s, 3H, OCH_3), 5.12 (s, 2H, CH_2-3), 5.36 (d, 1H, *J* = 4.8 Hz, H-3′), 6.28 (s, 1H, ArH), 6.56 (d, 1H, *J* = 4.8 Hz, H-4′); MS (FAB) *m*/*z* (%): 738 (M, 12). **13**: 91% *d.e.*; m.p. 158-160 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 0.97-1.13 (m.s, 18H, CH_3×6), 1.43 and 1.49 (s, each 3H, CH_3-2′), 1.70, 1.92, 2.20 and 2.49 (m, each 2H, CH_2), 2.63 (s, 3H, CH_3-4), 3.89 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2-3), 5.36 (d, 1H, *J* = 4.8 Hz, H-3′), 6.31 (s, 1H, ArH), 6.58 (d, 1H, *J* = 4.8 Hz H-4′); MS (ESI+) *m*/*z* (%):719 (M+Na, 100), 714 (M+NH_4, 53).

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