Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5855–5857
Bioorganic & Medicinal Chemistry Letters

Anti-AIDS agents. Part 62: Anti-HIV activity of 2′-substituted 4-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (4-methyl DCK) analogs☆

Qian Zhang,[a] Ying Chen,[a] Peng Xia,[a,*] Yi Xia,[b] Zheng-Yu Yang,[b] Donglei Yu,[b] Susan L. Morris-Natschke[b] and Kuo-Hsiung Lee[b,*]

[a]*Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai 200032, China*
[b]*Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, USA*

Received 13 August 2004; accepted 15 September 2004
Available online 12 October 2004

Abstract—Four 4-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (4-methyl DCK) analogs (**7a**–**d**) with different alkyl substituents at the 2′-position were synthesized and evaluated for inhibition of HIV-1 replication in H9 lymphocytes. 2′-Methyl-2′-ethyl-4-methyl DCK (**7b**) was more potent (EC_{50} = 0.22 μM, TI > 175) than the other three compounds (**7a**, **7c**, and **7d**), but significantly less potent than 4-methyl DCK (**2**, EC_{50} = 0.0059 μM, TI > 6600). The bioassay results indicated that the 2′-substituents had a strong effect on the anti-HIV activity, and *gem*-dimethyl substitution at the 2′-position was greatly preferable to larger alkyl substituents or hydrogen atoms.

1. Introduction

3′,4′-Di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK, **1**) was discovered as a potent anti-HIV agent with an EC_{50} value of 2.56×10^{-4} μM and a therapeutic index (TI) of 1.37×10^{5} in H9 lymphocytic cells in our previous research.[2] Further research studies indicated that 3-methyl DCK, 4-methyl DCK, and 5-methyl DCK were much more potent than DCK and AZT in the same assay 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.[3,4] A preliminary mechanistic study showed that the DCK analog 3-hydroxymethyl-4-methyl DCK inhibits HIV reverse transcriptase (RT), but has a novel mechanism of action compared to current anti-HIV/AIDS drugs. More recent studies indicated that DCK analogs act at a point in the virus life cycle immediately following the target for AZT and nevirapine, which are RT inhibitors.[5] Additional mechanism of action studies are ongoing; therefore, further structure modification will be helpful in exploring interactions with the viral target and the mechanism of DCK analogs.

In recent structure modifications,[6,7] two new series of 4-methyl DCK (**2**) analogs were synthesized based on the concept of bioisosterism, namely, by replacing the ring oxygen atom of DCK with a sulfur atom. The bioassay results suggested that the 2′-substituents might have a significant effect on the anti-HIV activity. This finding prompted us to synthesize additional DCK analogs with different substituents at the 2′-position and examine the relationship with anti-HIV activity (Fig. 1).

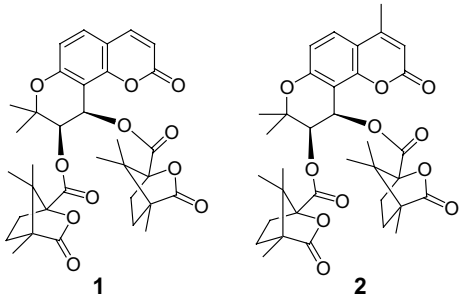


Figure 1. Structures of **1** and **2**.

☆See Ref. 1.

*Corresponding authors. Tel.: +86 21 54237563 (P.X.); tel.: +1 919 962 0066; fax: +1 919 966 3893 (K.-H.L.); e-mail addresses: pxia@shmu.edu.cn; khlee@unc.edu

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.09.032

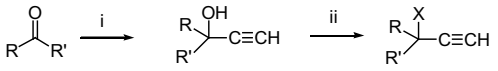
2. Chemistry

The propargyl ethers **4a–d** were synthesized via the reaction of 4-methyl-7-hydroxycoumarin with 3-bromoprop-1-yne, 3-chloro-3-methylpent-1-yne, 3-chloro-3-ethylpent-1-yne, or 3-chloro-3-methylhex-1-yne, respectively, in the presence of K_2CO_3 and KI by refluxing in acetone or heating in DMF at 80–90 °C (Scheme 1). The propargyl bromides were obtained from the halogenation of corresponding propargyl alcohols, which were prepared by ethynylation of different carbonyl compounds (Scheme 2). Thermo ring closure of **4a–d** by refluxing in *N*,*N*-dimethylaniline or heating in DMF at 80–90 °C afforded **5a–d**. Compounds **5b** and **5c** are racemic mixtures.

Asymmetric dihydroxylation[8] of **5a–d** followed by acylation with (*S*)-camphanic chloride gave the desired 4-methyl DCK analogs with different alkyl substituents at the 2′-position (**7a–d**) (Scheme 1).[9]

3. Results and discussion

Table 1 shows the inhibitory activities against HIV-1 replication of compounds **7a–d** together with DCK, 4-methyl DCK, and AZT as reference compounds. 4-Methyl DCK (**2**), which has *gem*-dimethyl substitution at the 2′-position, was the most potent compound with an EC_{50} value of 0.0059 μM and a therapeutic index (TI) of >6600 in H9 lymphocytic cells. Replacing just one 2′-methyl group with an ethyl group resulted in a dramatic decrease of anti-HIV activity. 2′-Methyl-2′-ethyl-4-methyl DCK (**7b**) was ca. 10^2-fold less active than 4-methyl DCK (**2**). When the 2′-substituents became bulkier, the activity decreased further. 2′-Methyl-



Scheme 2. Reagents and conditions: (i) *t*-BuOK, acetylene gas, THF, 0 °C, >99%; (ii) PBr_3 or $SOCl_2$, rt, 40–50%.

Table 1. Anti-HIV activity of DCK analogs in acutely infected H9 lymphocytes*

Compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
7a	>41.1	6.9	>6.0
7b	>38.4	0.22	>175
7c	>37.6	6.4	>5.9
7d	>37.6	2.84	>13.2
DCK (**1**)[d]	>16.1	0.049	>328
4-Me DCK (**2**)[d]	>38.9	0.0059	>6600
AZT	1872	0.048	39,000

* This assay was performed by Panacos, Inc. The general procedure was described previously.[5]

[a] Concentration that inhibits uninfected H9 cell growth by 50%.

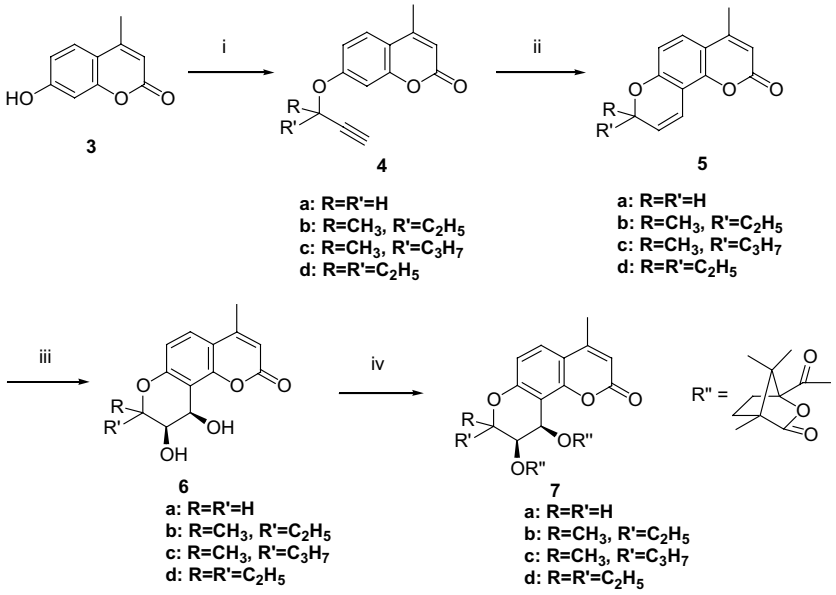
[b] Concentration that inhibits viral replication by 50%.

[c] TI = therapeutic index IC_{50}/EC_{50}.

[d] EC_{50} and TI for DCK and 4-methyl DCK were 2.56×10^{-4} μM, 1.83×10^{-6} μM, and 1.37×10^5, 6.89×10^7, respectively, in previous screenings and publication.[2]

2′-propyl-4-methyl DCK (**7c**) and 2′,2′-diethyl-4-methyl DCK (**7d**) were ca. 30- and 10-fold less potent, respectively, than **7b**. The 2′-unsubstituted compound, 2′,2′-dihydro-4-methyl DCK (**7a**), showed weak potency, similar to **7c**.

Based on these findings, we speculate that the space around the 2′-position is very tight when a DCK analog



Scheme 1. Reagents and conditions: (i) 3-bromoprop-1-yne (R = R′ = H), 3-chloro-3-methylpent-1-yne (R = CH_3, R′ = C_2H_5), 3-chloro-3-methylhex-1-yne (R = CH_3, R′ = C_3H_7), and 3-chloro-3-ethylpent-1-yne (R = R′ = C_2H_5), respectively, K_2CO_3, KI in acetone reflux or in DMF at 80–90 °C, 32–95%; (ii) diethylaniline, reflux, or DMF 80–90 °C, 11–85%; (iii) $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, $(DHQ)_2$-PHAL, K_2CO_3, 75–98%; (iv) (*S*)-camphanic chloride, DMAP/CH_2Cl_2, 80–88%.

binds with its target enzyme. Thus, there is a space requirement at this position for optimal anti-HIV activity. To date, two methyl groups at the 2′-position are preferred to other alkyl substituents for enhanced anti-HIV activity in the DCK series. Synthesis of analogs with other 2′-functional groups and mechanism studies of this compound series are under investigation.

Acknowledgements

This research was supported by grants from the National Natural Science Foundation of China (No. 20272010) and Research Foundation for University PhD Project of China Education Ministry (No. 20020246069) awarded to P. Xia, and Grant AI-33066 from the National Institute of Allergies and Infectious Diseases awarded to K. H. Lee.

References and notes

1. For Part 61, see: Zhu, X. K.; Guan, J.; Xiao, Z.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem.* **2004**, *12*, 4267.
2. Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Chen, C. H.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, K. H. *J. Med. Chem.* **1994**, *37*, 3947.
3. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. *J. Med. Chem.* **1999**, *42*, 2662.
4. Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **2003**, *23*, 322.
5. Xie, L.; Yu, D.; Wild, C.; Allaway, G.; Turpin, J.; Smith, P. C.; Lee, K. H. *J. Med. Chem.* **2004**, *47*, 756.
6. Chen, Y.; Zhang, Q.; Zhang, B.; Xia, P.; Xia, Y.; Yang, Z. Y.; Kilgore, N.; Wild, C.; Morris-Natschke, S. L.; Lee, K. H. *Bioorg. Med. Chem.*, in press.
7. Xia, P.; Yin, Z. J.; Chen, Y.; Zhang, Q.; Zhang, B. N.; Xia, Y.; Yang, Z. Y.; Kilgore, N.; Wild, C.; Morris-Natschke, S. L.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3341.
8. Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M.; Xu, D.; Zhang, X. L. *J. Org. Chem.* **1992**, *57*, 2768.
9. Physical and spectral data for **7a–d**:
2′,2′-Dihydro-4-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**7a**) mp: 170 °C (dec); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85–1.12 (18H, m, camphanoyl CH_3), 1.64–2.60 (2H × 4, m, camphanoyl CH_2), 2.48 (3H, s, CH_3-4), 4.31 (2H, m, CH_2-2′), 6.13 (1H, d, J = 6.0 Hz, H-3), 5.51 (1H, m, H-3′), 6.80 (1H, m, H-4′), 6.88 (1H, d, J = 8.9 Hz, H-6), 7.55 (1H, d, J = 8.9 Hz, H-5); MS *m/z* (%): 608 (M^+, 3.65), 427 ([M−camphanoyl]$^+$, 3.35), 411 ([M−*O*−camphanoyl]$^+$, 2.17); HR-MS: calcd for $C_{33}H_{36}O_{11}$ 608.22577, found: 608.22521.
2′-Ethyl-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**7b**) mp: 166 °C (dec); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85–1.12 (m, 21H, camphanoyl CH_3 and CH_2CH_3-2′), 1.38–2.52 (m, 13H, camphanoyl CH_2, CH_3-2′ and CH_2CH_3-2′), 2.39 (3H, s, CH_3-4), 6.10 (1H, d, J = 5.3 Hz, H-3), 5.46–5.55 (1H, m, H-3′), 6.64–6.83 (1H, m, H-4′), 6.85 (1H, d, J = 8.8 Hz, H-6), 7.53 (1H, d, J = 8.8 Hz, H-5); MS *m/z* (%): 650 (M^+, 5.41), 453 ([M−*O*−camphanoyl]$^+$, 5.40); HR-MS: calcd for $C_{36}H_{42}O_{11}$ 650.27271, found 650.27216.
2′-Propyl-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**7c**) mp: 140 °C (dec); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88–1.13 (21H, m, camphanoyl CH_3 and $CH_2CH_2CH_3$-2′), 1.40–2.52 (15H, m, camphanoyl CH_2, CH_3-2′ and $CH_2CH_2CH_3$-2′), 2.39 (3H, s, CH_3-4), 6.11 (1H, d, J = 5.5 Hz, H-3), 5.43–5.53 (1H, m, H-3′), 6.65–6.82 (1H, m, H-4′), 6.84 (1H, d, J = 8.9 Hz, H-6), 7.53 (1H, d, J = 8.9 Hz, H-5); MS *m/z* (%): 664 (M^+, 3.65), 467 ([M−*O*−camphanoyl]$^+$, 2.33); HR-MS: calcd for $C_{37}H_{44}O_{11}$ 664.28837, found 664.28781.
2′,2′-Diethyl-4-methyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**7d**) mp: 150 °C (dec); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.93–1.13 (24H, m, camphanoyl CH_3 and 2 × CH_2CH_3-2′), 1.56–2.51 (12H, m, camphanoyl CH_2 and 2′-CH_2CH_3 × 2), 2.39 (3H, s, CH_3-4), 6.10 (1H, d, J = 6.3 Hz, H-3), 5.60–5.62 (1H, m, H-3′), 6.68–6.77 (1H, m, H-4′), 6.86 (1H, d, J = 9.0 Hz, H-6), 7.53 (1H, d, J = 9.0 Hz, H-5); MS *m/z* (%): 664 (M^+, 3.65), 467 ([M−*O*−camphanoyl]$^+$, 5.20); HR-MS: calcd for $C_{37}H_{44}O_{11}$ 664.28837, found 664.28960.