Bioorganic & Medicinal Chemistry 18 (2010) 7203–7211

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Anti-AIDS agents 84. Synthesis and anti-human immunodeficiency virus (HIV) activity of 2′-monomethyl-4-methyl- and 1′-thia-4-methyl-(3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) analogs ☆

Shi-Qing Xu [a], Xin Yan [a], Ying Chen [a,*], Peng Xia [a,*], Keduo Qian [b], Donglei Yu [b], Yi Xia [b], Zheng-Yu Yang [b], Susan L. Morris-Natschke [b], Kuo-Hsiung Lee [b,c,*]

[a] *Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai 201203, China*
[b] *Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360, USA*
[c] *Chinese Medicine Research and Development Center and Department of Pharmacy, China Medical University and Hospital, Taichung, Taiwan*

ARTICLE INFO

Article history:
Received 25 June 2010
Revised 9 August 2010
Accepted 16 August 2010
Available online 19 August 2010

Keywords:
2′-Monomethyl-4-methyl-(3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) analogs
1′-Thia-4-methyl-(3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) analogs
Anti-HIV activity
Structure–activity relationship (SAR)

ABSTRACT

In a continuing investigation into the pharmacophores and structure–activity relationship (SAR) of (3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone (DCK) as a potent anti-HIV agent, 2′-monomethyl substituted 1′-oxa, 1′-thia, 1′-sulfoxide, and 1′-sulfone analogs were synthesized and evaluated for inhibition of HIV-1 replication in H9 lymphocytes. Among them, 2′*S*-monomethyl-4-methyl DCK (**5a**)‡ and 2′*S*-monomethyl-1′-thia-4-methyl DCK (**7a**) exhibited potent anti-HIV activity with EC_{50} values of 40.2 and 39.1 nM and remarkable therapeutic indexes of 705 and 1000, respectively, which were better than those of the lead compound DCK in the same assay. In contrast, the corresponding isomeric 2′*R*-monomethyl-4-methyl DCK (**6**) and 2′*R*-monomethyl-1′-thia-4-methyl DCK (**8**) showed much weaker inhibitory activity against HIV-1 replication. Therefore, the bioassay results suggest that the spatial orientation of the 2′-methyl group in DCK analogs can have important effects on anti-HIV activity of this compound class.

1. Introduction

Since first reported in the 1980s, acquired immunodeficiency syndrome (AIDS) has spread rapidly through the human population and become one of the most devastating diseases facing mankind.[1,2] This disease is caused by infection with the human immunodeficiency virus (HIV) and results in life-threatening opportunistic infections and malignancies. The increasing incidence of HIV drug resistance together with many serious side effects and long-term complications in patients diminish the action of current anti-HIV drugs. Consequently, the development of new anti-HIV agents continues to focus on novel structures or new action mechanisms.

In our previous research, 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK, **1**, Fig. 1) demonstrated extremely potent inhibitory activity against HIV-1 replication in H9 lymphocytic cells.[3] In subsequent structural modification studies, numerous DCK derivatives were synthesized and more than 20 DCK analogs have shown promising inhibitory activity against HIV-1 replication in H9 lymphocytes, MT-2 cell lines, and MT-4 cell lines, respectively.[4] Among them, 3-methyl, 4-methyl, and 5-methyl substituted DCKs were much more potent than DCK and zidovudine (AZT) in the same assay.[5] Initial structure–activity relationships (SAR) have been established for this compound type.[4] In addition, a preliminary mechanistic study showed that DCK is a unique inhibitor of HIV-1 reverse transcriptase (RT). It did not significantly affect RNA-dependent DNA polymerase activity when poly-rA or poly-rC was used as template, while it did affect DNA-dependent DNA polymerase activity of HIV-1 RT when poly-dA or poly-dC was used

Abbreviations: DCK, (3′*R*,4′*R*)-3′,4′-di-*O*-(*S*)-camphanoyl-(+)-*cis*-khellactone; SAR, structure–activity relationships; AIDS, acquired immunodeficiency syndrome; HIV-1, human immunodeficiency virus type 1; AZT, zidovudine; RT, reverse transcriptase; DMF, dimethyl formamide; AD, asymmetric dihydroxylation; DMAP, 4-(dimethylamino)pyridine; $(DHQ)_2PHAL$, hydroquinine 1,4-phthalazinediyl diether; MCPBA, 3-chloroperoxybenzoic acid.

☆ Anti-AIDS Agents 84. For part 83, see Zhou, T.; Shi, Q.; Lee. K. H. Anti-AIDS Agents 83. Efficient microwave-assisted one-pot preparation of angular 2,2-dimethyl-2*H*-chromone containing compounds. *Tetrahedron Lett.* **2010**, 4382.

* Corresponding authors. Tel.: +86 21 54237563 (P.X.); tel.: +1 919 962 0066; fax: +1 919 966 3893 (K.H.L.).

E-mail addresses: yingchen71@fudan.edu.cn (Y. Chen), pxia@fudan.edu.cn (P. Xia), khlee@unc.edu (K.-H. Lee).

‡ X-ray data of **5a**: DEPOSIT NUMBER CCDC 678968.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.08.031

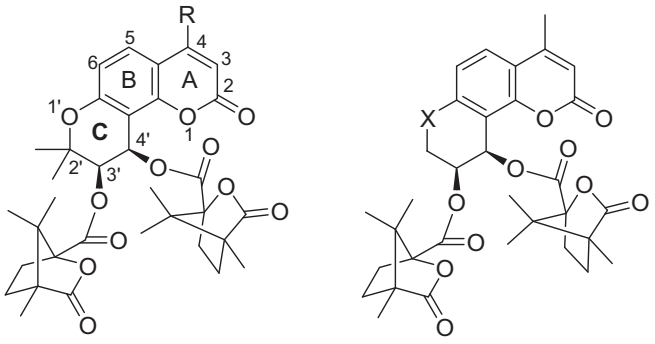


Figure 1. Structures of previously synthesized DCK analogs (**1**–**4**).

as template.[6] Moreover, a time-of-addition study suggested that DCK analogs are strongly synergistic with approved drugs, such as AZT, and act at a point in the virus life cycle immediately following the target for AZT and nevirapine.[7]

1.1. Design

In our prior SAR study of the DCK C-ring, we found that the size of the 2′-alkyl substituent(s) had a significant effect on the antiviral activity. *gem*-Dimethyl substitution (**2**) was preferable, and in contrast, either no substitution (**3**) or larger alkyl substituents dramatically decreased activity. The rank order of activity was two hydrogen atoms (unsubstituted) ≈ methyl + propyl < *gem*-diethyl < *gem*-dimethyl.[8] In a continuing effort to identify the pharmacophores of the 2′-position, 2′-monomethyl substituted 4-methyl DCK analogs were designed to further explore SAR at this position. We also extended our new design to 1′-thia derivatives, because bioisosteric replacement of sulfur for oxygen has also resulted in potent inhibitory effects on HIV-1 replication.[9] The structures of the newly designed compounds (**5**–**12**) are shown in Figure 2. This paper reports their syntheses and bioassay data, as well as the X-ray crystallographic structure of **5a**.

2. Results and discussion

2.1. Chemistry

Analogs **5a**, **5b**, and **6** were synthesized as illustrated in Scheme 1. 3-Chloro-1-butyne (**14**) was prepared by reacting 3-butyne-2-ol (**13**) with thionyl chloride in pyridine.[10] 4-Methyl-7-hydroxycoumarin (**15**) was treated with **14** in dimethyl formamide (DMF) or acetone in the presence of anhydrous potassium carbonate and potassium iodide at 75–80 °C to produce the corresponding racemic mono-methyl propargyl ether **16**, followed by thermal rearrangement in refluxing *N*,*N*-diethylaniline to form intermediate **17**. Sharpless asymmetric dihydroxylation (AD) of **17** afforded two racemic mixtures **18** and **19**, with yields of 64% and 24%, respectively.[11] After acylation of **18** with optically pure (*S*)-(−)-camphanic chloride in anhydrous CH_2Cl_2 at room temperature with 4-(dimethylamino)pyridine (DMAP) as acid scavenger, diastereoisomers **5a** and **5b** were separated by column chromatography on silica gel [petroleum ether–EtOAc, 3:1 (v/v)] in 68% and 18% yields, respectively. Compound **6** was obtained by acylation of **19** with (*S*)-(−)-camphanic chloride in anhydrous CH_2Cl_2 at room temperature in 84% yield. In Sharpless AD, osmylation preferentially takes place at the less hindered carbon of the double bond neighboring a methyl group, and the chiral auxiliary agent plays a decisive role on the stereoselectivity.[12–16] In our synthetic scheme, hydroquinine 1,4-phthalazinediyl diether ($(DHQ)_2PHAL$) was used as the chiral auxiliary; therefore, diol **18a** was obtained as the main product. The absolute configuration of **5a** (2′*S*,3′*S*,4′*R*) was confirmed by X-ray diffraction spectroscopy (Fig. 3). Because of the directing effect of $(DHQ)_2PHAL$ and the steric effect of the 2′α-methyl (2′*S*), formation of diol **19b** was unfavorable; therefore, the amount of corresponding camphanoyl ester was too small to quantify. The three chiral centers of the C ring in **6** were defined as 2′*R*, 3′*S*, and 4′*R*.

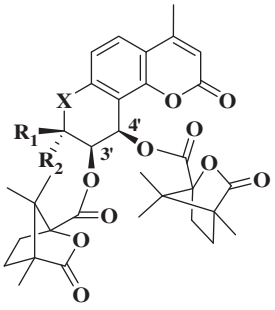
The preparation of **7a**, **7b**, and **8** is illustrated in Scheme 2. In a modified procedure,[17,18] intermediate 4-methyl-7-mercaptocoumarin (**22**) was obtained by reacting 4-methyl-7-hydroxy coumarin (**15**) with dimethylthiocarbamoyl chloride in DMF in the presence of anhydrous potassium carbonate, followed by a rearrangement at 220–230 °C, then hydrolysis with methanolic KOH and acidification with HCl. The remaining synthetic steps were similar to those in Scheme 1 detailed above. However, diastereoisomers **7a** and **7b** could not be separated by flash chromatography on silica gel or crystallization. ^{1}H NMR spectroscopy showed that the ratio of diastereoisomers **7a** and **7b** was about 1:1. Finally, after exploring many different HPLC columns and conditions, diastereoisomers **7a** and **7b** were successfully separated on an Alltima HP column (Grace, 22 mm × 150 mm, 10 μm) with acetonitrile–water 70:30. The chemical shifts of protons at 2′-, 3′-, and 4′-positions in ^{1}H NMR spectra of **5a**, **5b**, and **6** showed the same patterns as those of the corresponding **7a**, **7b**, and **8**.

Scheme 3 illustrates the synthesis of **9a**, **9b**, **10**, **11a**, **11b**, and **12**. The diasteromeric mixture of **7a** and **7b** was reacted with one equivalent of 3-chloroperoxybenzoic acid (MCPBA) in CH_2Cl_2 to afford a diastereomeric mixture of sulfoxides **9a** and **9b**, which was again treated with one equivalent of MCPBA in CH_2Cl_2 to give a diasteromeric mixture of sulfones **11a** and **11b**.[19] Compounds **10** and **12** were prepared by similar oxidation of **8**. The pure diastereomers of mixtures **9** and **11** were not isolated, because the mixtures did not show antiviral activity (Table 1).

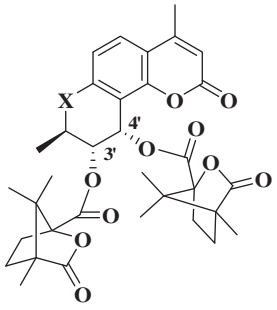
2.2. Biological activity

Compounds **5**–**12** were evaluated in HIV-1 infected H9 lymphocytes in parallel with AZT and prior DCK analogs **2–4**. The bioassay data are summarized in Table 1.

2′*S*-Methyl-4-methyl DCK (**5a**) showed significant anti-HIV activity with an EC_{50} value of 0.0402 μM and remarkable TI value of 705, which were slightly better than those of the 2′-dimethyl lead compound (**2**, 4-Me-DCK, EC_{50}: 0.126 μM, TI 301) in the same assay system. The 2′*R*-methyl isomer (**6**) was inactive at the test concentrations, and compound **3**, with no substitution at the 2′-position, exhibited dramatically reduced activity (EC_{50}: 16.2 μM). These results demonstrated that the spatial location of the 2′-methyl has a strong impact on the anti-HIV activity of DCK analogs. The same trend was also observed in the 1′-thia DCK series, where the EC_{50} values of **7a** (2′*S*-Me), **8** (2′*R*-Me), and **4** (2′-H_2) were 0.0391, 1.26, and 0.99 μM, respectively. Because the same rank order of potency was found in both DCK and 1′-thia DCK series [2′*S*-methyl (**5a** and **7a**) > 2′-H_2 (**3** and **4**) > 2′*R*-methyl (**6** and **8**)], we concluded that 2′*S*-methyl (α-methyl) substitution was essential for enhanced antiviral potency among Type I compounds (see Fig. 2). In comparison, 2′*R*-methyl (β-methyl) was unfavorable and led to decreased antiviral activity. Moreover, we observed that 1′-thia DCK analogs generally had a slightly better antiviral profile than the DCK series analogs. With oxygen at the 1′-position, **6** and **3** showed significantly reduced activity compared with **5a**, whereas the corresponding 1′-thia analogs, **8** and **4**, retained more activity (lower fold reduction) compared with **7a**. This result may possibly be due to a better interaction of S rather than O with the binding pocket, considering the extra size



Type I (3',4' β)



Type II (3',4' α)

Compound	Structure	X	R_1	R_2
5a	**I**	O	H	CH_3
5b	**II**	O		
6	**I**	O	CH_3	H
7a	**I**	S	H	CH_3
7b	**II**	S		
8	**I**	S	CH_3	H
9a	**I**	S=O	H	CH_3
9b	**II**	S=O		
10	**I**	S=O	CH_3	H
11a	**I**		H	CH_3
11b	**II**			
12	**I**		CH_3	H

Figure 2. Structures of 2′-monomethyl DCK and 1′-thia DCK analogs.

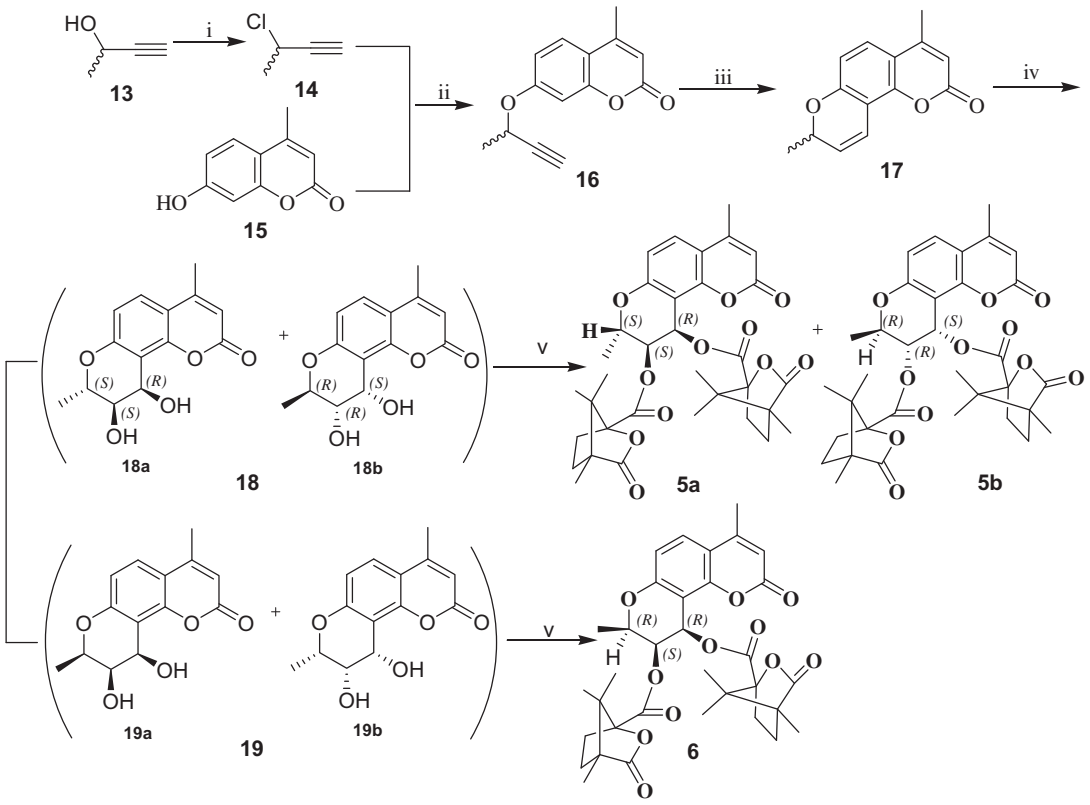
and electrons carried by the S atom, which may fit into the binding pocket better. Based on these results, we postulated that the 1′ and 2′ positions are important pharmacophores for the anti-HIV activity of DCK analogs.

The low potency and inactivity of analogs **5b** (1′-oxa; 2′*R*,3′*R*,4′*S*) and **7b** (1′-thia; 2′*R*,3′*S*,4′*S*), respectively, in which the 2′-methyl is β and 3′,4′-dicamphanoyl groups are α (Type II in Fig. 2), were in accord with the preferred configuration (β) and spatial demand for the two camphanoyls found previously with DCK compounds.[3,4] In addition, all 1′-sulfoxide and 1′-sulfone analogs (**9**–**12**) showed much weaker activity than the corresponding 1′-thia-4-methyl DCK analogs (**7a** + **7b** or **8**) or were inactive. The reason for the poor activities of these analogs might be that sulfone and sulfoxide have extra oxygen atoms that may cause some steric repulsion or loss of hydrogen bonding accepter function.

To confirm RT is the biological target of the newly synthesized 2′-monomethyl DCK analogs, compound **5a** was evaluated against viral enzyme RT and compared with **2**. From the results, we can see **5a** potently inhibited RT activity with an IC_{50} value of 6.7 μM compared to the previous best hit **2** (IC_{50}: 10.4 μM), suggesting that the monomethyl analogs may function similarly to DCKs as RT inhibitors.

3. Conclusion

In conclusion, 2′-monomethyl substituted DCKs, as well as 1′-thia, 1′-sulfoxide, and 1′-sulfone DCK analogs, were designed and synthesized in this study. We discovered the following SAR conclusions. (1) In both the DCK and 1′-thia DCK Type I series, 2′*S* configuration (α-methyl substitution) was preferable to 2′*R* (β-methyl substitution) for anti-HIV activity. 2′*S*-Methyl analogs also showed more potent activity than corresponding 2′-H analogs, suggesting that the stereochemistry at the 2′-position has an important influence on the DCK series. (2) 1′-Thia DCK analogs generally showed a better antiviral profile than the DCK analogs. (3) Converting 1′-thia-4-methyl DCK analogs to the corresponding 1′-sulfoxide- and 1′-sulfone-4-methyl DCK analogs resulted in decreased or completely abolished activity. (4) Consistent with prior SAR, β orientations of the two camphanoyls (Type I) at the 3′ and 4′ positions were favorable for anti-HIV activity.



Scheme 1. Reagents and conditions: (i) $SOCl_2$, pyridine; (ii) K_2CO_3, KI in DMF or acetone, 75–80 °C; (iii) *N*,*N*-diethylaniline, reflux; (iv) $K_2OsO_2(OH)_4$, $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3 in *t*-butanol/H_2O (v/v = 1:1), ice bath; (v) (*S*)-camphanic chloride, DMAP in CH_2Cl_2, rt.

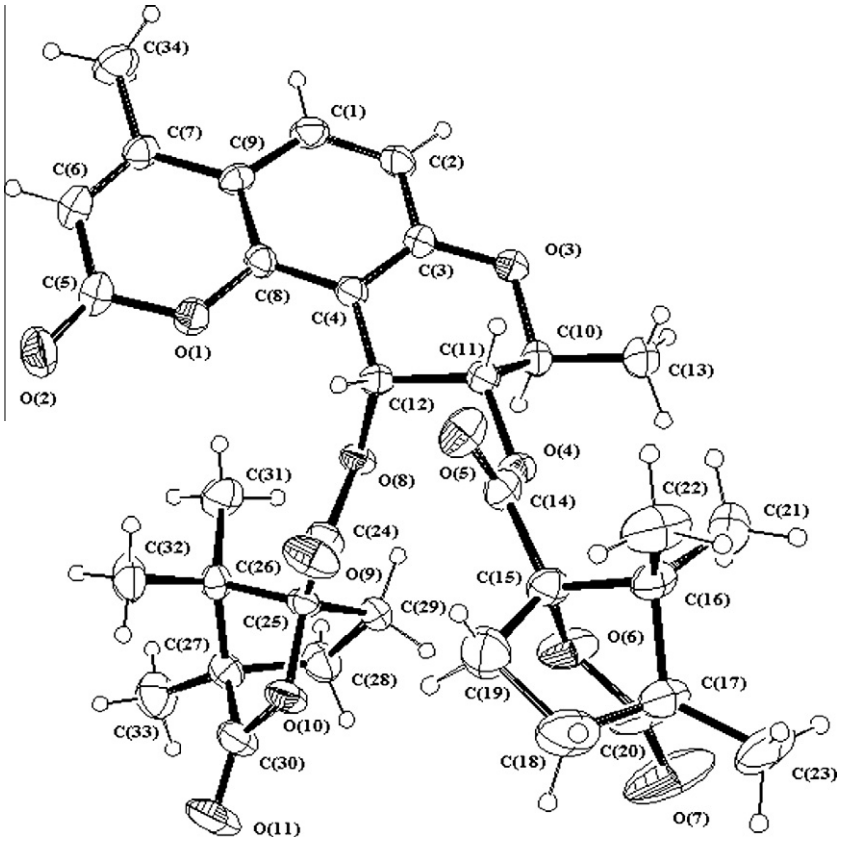
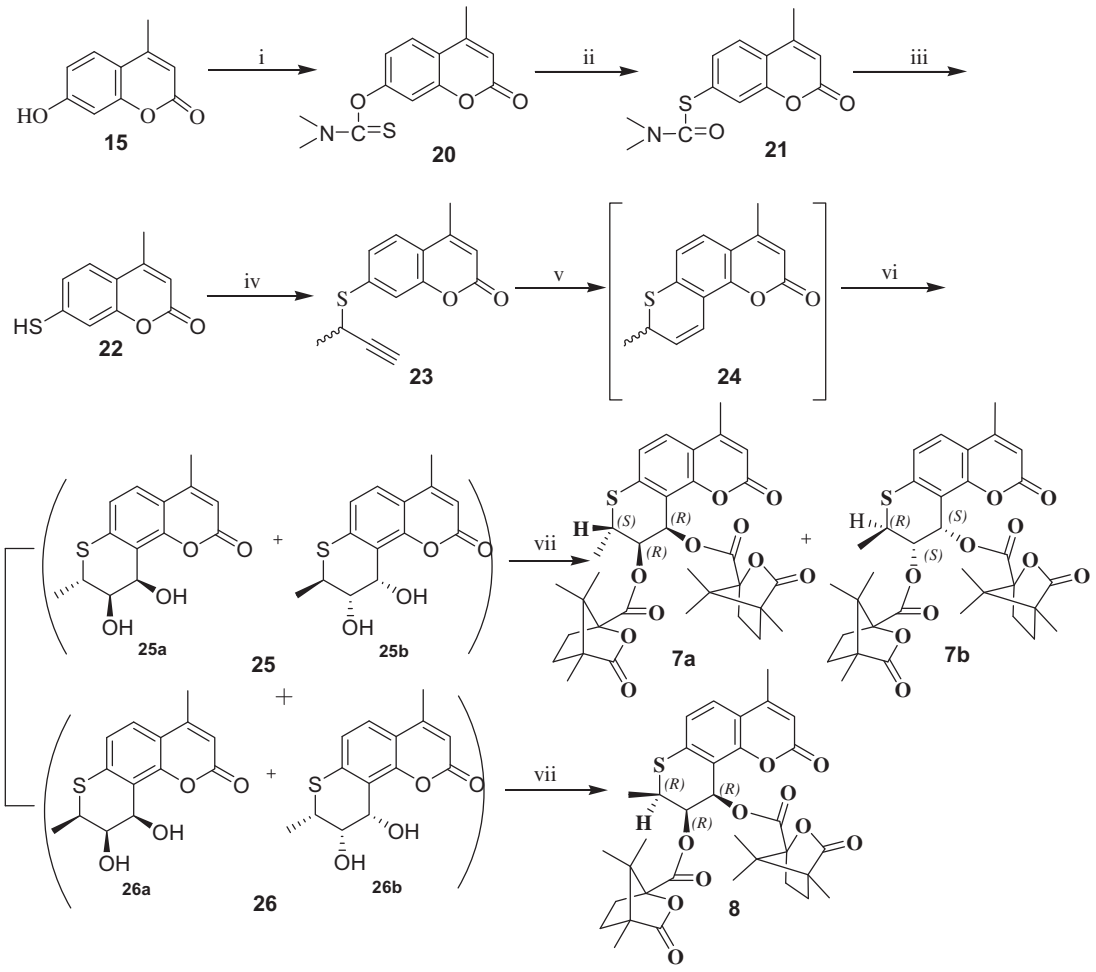


Figure 3. X-ray structure of 2′*S*-monomethyl-4-methyl DCK (**5a**).



Scheme 2. Reagents and conditions: (i) dimethylthiocarbamoyl chloride, K_2CO_3 in DMF, 55–60 °C; (ii) heating at 220–230 °C; (iii) KOH in MeOH/N_2, reflux, concentrated HCl; (iv) 3-chloro-1-butyne, K_2CO_3, KI in DMF or acetone/N_2, 75–80 °C; (v) *N*,*N*-diethylaniline, reflux; (vi) $K_2OsO_2(OH)_4$, $(DHQ)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3 in *t*-butanol/H_2O (v/v = 1:1), ice bath; (vii) (*S*)-camphanic chloride, DMAP in CH_2Cl_2, rt.

4. Experimental section

4.1. Chemistry

Melting points were measured with the capillary tube method without correction. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker-DPX 400 MHz spectrometer. The solvent used was $CDCl_3$ unless indicated. Elemental analyzes were measured with Elementar Vario EL. Mass spectra were measured with HP5973N analytical mass spectrometers. High resolution mass spectra (HRMS) were measured on a Shimadzu LCMS-IT-TOF with ESI interface. All target compounds were analyzed for C, H and gave values within 0.4% of the theoretical values. Optical rotations were measured with a Jasco P-1020 digital polarimeter at 17.5 °C at the sodium D line. The diastereoisomeric excess percentages were determined from intensity of protons at the 3′-position in the ^{1}H NMR spectra. Thin-layer chromatography (TLC) was performed on PLC silica gel 60 F_{254} plates.

4.2. 3-Chloro-1-butyne (14)

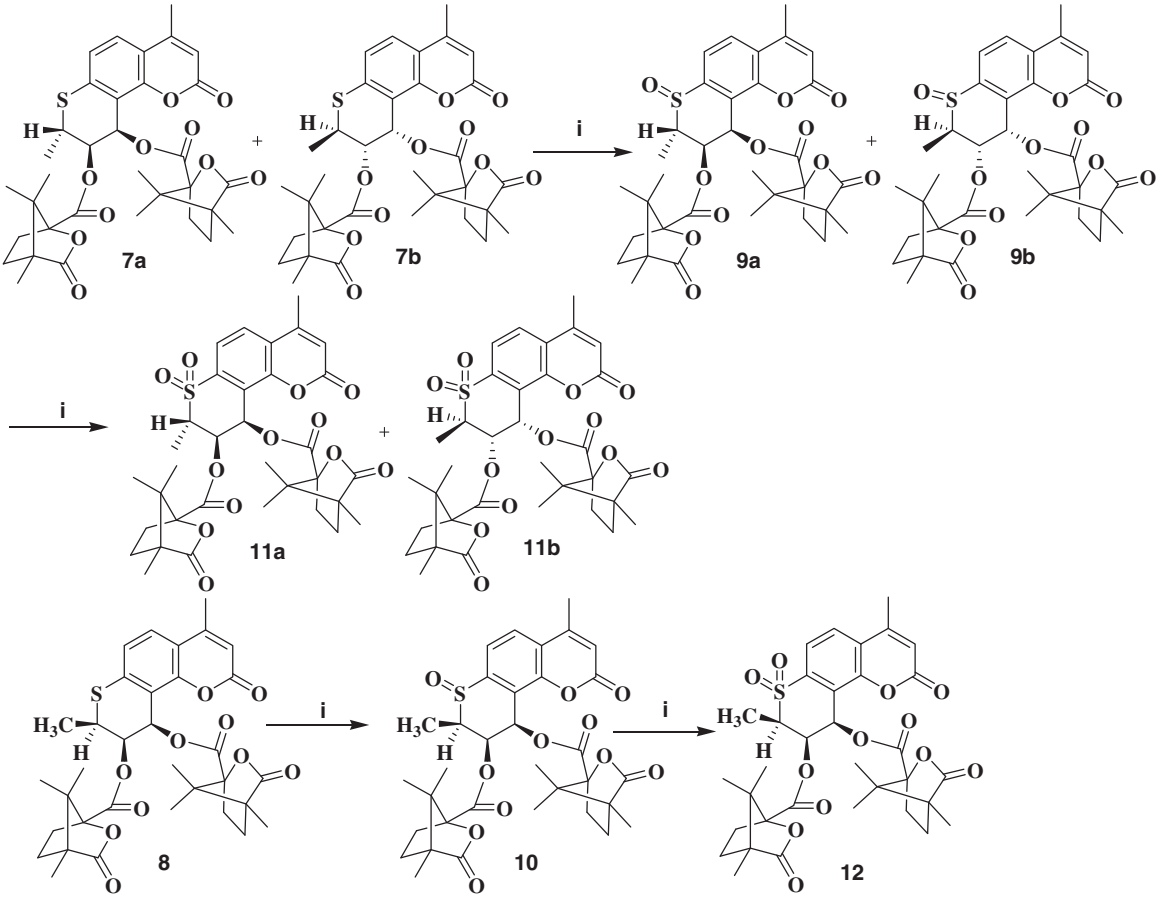
Under stirring, 3-butyne-2-ol (**13**) (7.8 mL, 0.1 mol) was added dropwise to a mixture of thionyl chloride (7.3 mL, 0.1 mol) and anhydrous pyridine (0.8 g) at 0 °C over 0.5 h. The mixture was stirred for additional 2 h at 0 °C, allowed to warm to rt within 0.5 h, heated at 50 °C for 1 h. Then pyridine was added to adjust the pH of the mixture to 7–8. After filtration, the filtrate was directly used in the next reaction.

4.3. 7-(But-3-yn-2-yloxy)-4-methyl-2*H*-chromen-2-one (16)

A mixture of **15** (1.41 g, 8 mmol), K_2CO_3 (4.41 g, 32 mmol), KI (266 mg, 1.6 mmol), and 3-chloro-1-butyne in DMF (15 mL) was stirred for 2 h at 75–80 °C. Ice water (120 mL) was added. The precipitate was filtered and purified by column chromatography on silica gel (petroleum ether–EtOAc, 5:1) to provide **16** as a white solid (1.42 g, 78%), mp 134–135 °C. ^{1}H NMR δ 1.71 (3H, d, J = 6.65 Hz, 8-CH_3), 2.40 (3H, d, J = 1.17 Hz, 4-CH_3), 2.52 (1H, d, J = 1.96 Hz, –C*CH*), 4.92 (1H, m, 8-C*H*), 6.16 (1H, d, J = 1.17 Hz, 3-H), 6.93 (1H, d, J = 2.74 Hz, 8-H), 6.97 (1H, dd, J = 8.00, 2.35 Hz, 6-H), 7.52 (1H, d, J = 8.60 Hz, 5-H). EI-MS m/z (%): 228 (M^+, 45).

4.4. 2′,4-Dimethylseselin (17)

A mixture of **16** (1.3 g, 5.7 mmol) and *N*,*N*-diethylaniline (12 mL) was refluxed for 4 h. The solvent was removed in vacuo and the residue was purified by column chromatography on silica gel (petroleum ether–EtOAc, 7:1) to provide **17** as a white solid (1.0 g, 77%), mp 115–116 °C. ^{1}H NMR δ 1.48 (3H, d, J = 6.65 Hz, 2′-CH_3), 2.37 (3H, d, J = 1.17 Hz, 4-CH_3), 5.10 (1H, m, 2′-C*H*), 5.76



Scheme 3. Reagent: (i) one equivalent 3-chloroperoxybenzoic acid in CH_2Cl_2.

(1H, dd, J_1 = 9.98 Hz, J_2 = 3.33 Hz, 3′-H), 6.12 (1H, d, J = 1.17 Hz, 3-H), 6.74 (1H, d, J = 8.61 Hz, 6-H), 6.95 (1H, dd, J_1 = 9.78 Hz, J_2 = 1.18 Hz, 4′-H), 7.34 (1H, d, J = 8.61 Hz, 5-H). EI-MS m/z (%): 228 (M^+, 21).

4.5. 2′,4-Dimethyl-(+)-*cis*-khellactone (18) and (19)

Compound **17** (684 mg, 3.0 mmol), $(DHQ)_2$-PHAL (82 mg, 0.105 mmol), $K_3Fe(CN)_6$ (4.94 g, 15 mmol), K_2CO_3 (2.08 g, 15 mmol) were dissolved in *t*-BuOH/H_2O (120 mL, v/v, 1:1) at rt. Then the solution was cooled to 0 °C and $K_2OsO_2(OH)_4$ (38 mg, 0.105 mmol) was added under stirring. The mixture was stirred at 0 °C for 70 h. Then sodium sulfite (4 g) was added and stirred at rt for 1 h. The mixture was extracted with EtOAc three times. The combined organic layer was dried over Na_2SO_4, and then solvent was removed. The residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH, 140:1) to provide **19** (188 mg, 24%, $CHCl_3$–MeOH, 60:1, R_f 0.07) and **18** (503 mg, 64%, $CHCl_3$–MeOH, 60:1, R_f 0.05) as white solids. Compound **18**, mp 231–234 °C. ^{1}H NMR (DMSO) δ 1.38 (3H, d, J = 6.26 Hz, 2′-CH_3), 2.36 (3H, s, 4-CH_3), 3.42 (1H, m, 3′-*CH*), 4.25 (1H, m, 2′-*CH*), 4.85 (1H, m, 4′-*CH*), 5.12 (1H, d, J = 6.65 Hz, 3′-OH), 5.36 (1H, d, J = 4.69 Hz, 4′-OH), 6.19 (1H, s, 3-H), 6.79 (1H, d, J = 8.60 Hz, 6-H), 7.58 (1H, d, J = 9.0 Hz, 5-H). EI-MS m/z (%): 262 (M^+, 28). Compound **19**, mp 252–255 °C. ^{1}H NMR (DMSO) δ 1.36 (3H, d, J = 6.65 Hz, 2′-CH_3), 2.36 (3H, s, 4-CH_3), 3.80 (1H, m, 3′-*CH*), 4.27 (1H, m, 2′-*CH*), 4.96 (1H, m, 4′-*CH*), 5.09 (1H, d, J = 5.09 Hz, 3′-OH), 5.13 (1H, d, J = 6.26 Hz, 4′-OH), 6.18 (1H, s, 3-H), 6.78 (1H, d, J = 9.0 Hz, 6-H), 7.56 (1H, d, J = 8.6 Hz, 5-H). EI-MS m/z (%): 262 (M^+, 37).

Table 1
Anti-HIV activity of analogs **5**–**12**[a]

Compound	IC_{50} (μM)	EC_{50} (μM)	TI
5a	28.4	<0.040	>705.6
5b	40.2	2.260	17.78
6	40.2	No suppression	No suppression
7a	39.1	<0.039	>1000
7b	39.1	No suppression	No suppression
8	39.1	1.260	31.06
9a + **9b** (2.4:1)	38.2	No suppression	No suppression
10	38.2	24.400	1.57
11a + **11b** (3.2:1)	37.3	3.370	11.07
12	37.3	No suppression	No suppression
2 (4-Me-DCK)	>39.2	0.126	301.2
3	41.1	16.200	2.54
4	40.1	0.990	40.58
AZT	500	0.010	43,848

[a] All data presented are averages of at least three separate experiments performed by Panacos Pharmaceuticals Inc., Gaithersburg, MD. EC_{50}: concentration that inhibits HIV-1_{IIIB} replication by 50%. IC_{50}: concentration that inhibits uninfected H9 cell growth by 50%. TI = IC_{50}/EC_{50}. No suppression: there was no inhibition at concentrations below the IC_{50}.

4.6. (2′*S*,3′*S*,4′*R*)-2′,4-Dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (5a) and (2′*R*,3′*R*,4′*S*)-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (5b)

Compound **18** (152 mg, 0.58 mmol) was acylated with (*S*)-(−)-camphanic chloride (504 mg, 2.32 mmol) in anhydrous CH_2Cl_2 (15 mL) in the presence of DMAP (426 mg, 3.48 mmol) for 0.5 h at rt. After the removal of the solvent under reduced pressure,

the residue was purified by using silica gel chromatography (petroleum ether–EtOAc, 3:1) to give product **5b** (65 mg, 18%, petroleum ether–EtOAc, 5:2, R_f 0.76) and product **5a** (245 mg, 68%, petroleum ether–EtOAc, 5:2, R_f 0.75) as white solids. Compound **5a**, mp 263–266 °C. Crystals suitable for single crystal X-ray diffraction were grown from EtOH. ^{1}H NMR δ 0.96–1.11 (18H, m, 6 × –CH_3 in camphanoyl), 1.61–2.54 (8H, m, 4 × –CH_2 in camphanoyl), 1.46 (3H, d, J = 6.26 Hz, 2′-CH_3), 2.39 (3H, d, J = 1.18 Hz, 4-CH_3), 4.51 (1H, m, 2′-*CH*), 5.15 (1H, m, 3′-H), 6.13 (1H, d, J = 1.57 Hz, 3-H), 6.71 (1H, d, J = 3.52 Hz, 4′-H), 6.86 (1H, d, J = 8.99 Hz, 6-H), 7.54 (1H, d, J = 8.99 Hz, 5-H). $[\alpha]_D$ +28.9 (c 0.9, CH_2Cl_2). EI-MS m/z (%): 622 (M^+, 8). Anal. Calcd for $C_{34}H_{38}O_{11}$: C, 65.58; H, 6.15. Found: C, 65.44; H, 6.24.

Compound **5b**, mp 237–239 °C. ^{1}H NMR δ 0.82 (3H, s, –CH_3 in camphanoyl), 1.04–1.12 (15H, m, –CH_3 × 5 in camphanoyl), 1.64–2.57 (8H, m, 4 × –CH_2 in camphanoyl), 1.45 (3H, d, J = 6.26 Hz, 2′-CH_3), 2.39 (3H, d, J = 1.17 Hz, 4-CH_3), 4.62 (1H, m, 2′-*CH*), 5.27 (1H, m, 3′-H), 6.11 (1H, d, J = 1.17 Hz, 3-H), 6.81 (1H, d, J = 3.52 Hz, 4′-H), 6.86 (1H, d, J = 8.61 Hz, 6-H), 7.54 (1H, d, J = 8.61 Hz, 5-H). $[\alpha]_D$ −24.0 (*c* 1.0, $CHCl_3$). EI-MS m/z (%): 622 (M^+, 6). Anal. Calcd for $C_{34}H_{38}O_{11}$: C, 65.58; H, 6.15. Found: C, 65.37; H, 6.28.

4.7. (2′*R*,3′*S*,4′*R*)-2′,4-Dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (6)

Compound **19** (63 mg, 0.245 mmol) was acylated with (*S*)-(−)camphanic chloride (171 mg, 0.78 mmol) in anhydrous CH_2Cl_2 (10 mL) in the presence of DMAP (150 mg, 1.23 mmol) for 0.5 h at rt. After the removal of the solvent under reduced pressure, the residue was submitted to silica gel chromatography separation ($CHCl_3$–CH_3OH, 120:1) to afford product **6** as a white solid (128 mg, 84%), mp >280 °C. ^{1}H NMR δ 0.98–1.11 (18H, m, CH_3 × 6 in camphanoyl), 1.64–2.57 (8H, m, 4 × –CH_2 in camphanoyl), 1.46 (3H, d, J = 6.60 Hz, 2′-CH_3), 2.39 (3H, d, J = 1.22 Hz, 4-CH_3), 4.48 (1H, m, 2′-*CH*), 5.63 (1H, m, 3′-H), 6.12 (1H, d, J = 0.98 Hz, 3-H), 6.73 (1H, d, J = 4.88 Hz, 4′-H), 6.88 (1H, d, J = 8.79 Hz, 6-H), 7.51 (1H, d, J = 9.03 Hz, 5-H). $[\alpha]_D$ +111 (*c* 1.0, CH_2Cl_2). ESI-MS m/z (%): 645 (M+Na^+). Anal. Calcd for $C_{34}H_{38}O_{11}$: C, 65.58; H, 6.15. Found: C, 65.56; H, 6.36.

4.8. *O*-4-Methyl-2-oxo-2*H*-chromen-7-yl dimethylcarbamothioate (20)

Compound **15** (4.4 g, 25 mmol), K_2CO_3 (7.6 g, 55 mmol), and dimethylthiocarbamoyl chloride (6.18 g, 50 mmol) were dissolved in DMF (45 mL). After stirring at 65–70 °C for 1 h, ice water (300 mL) was added, and the precipitate was filtered and recrystallized from EtOAc to afford **20** as a yellow solid (5.8 g, 88%), mp 214–216 °C (lit.[17] mp 208–209 °C). ^{1}H NMR 2.44 (3H, s, 4-CH_3), 3.38 (3H, s, N-CH_3), 3.47 (3H, s, N-CH_3), 6.28 (1H, s, 3-H), 7.07 (1H, s, 8-H), 7.26 (1H, 6-H), 7.60 (1H, d, J = 8.18 Hz, 5-H).

4.9. *S*-4-Methyl-2oxo-2*H*-chromen-7-yl dimethylcarbamothioate (21)

Compound **20** (5.0 g, 19.0 mmol) was heated to 220–230 °C for 1 h. The crude product was recrystallized from EtOH to give **21** as a yellow solid (4.2 g, 84%), mp 160–162 °C (lit.[17] mp 159–160 °C).

4.10. 4-Methyl-7-mercapto-2*H*-chromen-2-one (22)

Under nitrogen, compound **21** (3.6 g, 13.7 mmol) and potassium hydroxide (2.34 g, 41.6 mmol) were dissolved in MeOH (800 mL). The mixture was refluxed for 1.5 h with stirring. After cooling to rt, the mixture was acidified with concentrated HCl, and MeOH was removed under vacuum. Then ice water (400 mL) was added, and the reaction mixture was filtered. The precipitate was recrystallized from MeOH to afford **22** as a yellow solid (2.1 g, 81%), mp 137–138 °C (lit.[17] mp 138–139 °C).

4.11. 7-(But-3-yn-2-ylthio)-4-methyl-2-methylene-2*H*-chromene (23)

Following the same procedure for the preparation of **16**, except under nitrogen, **23** was obtained from **22** as a white solid (yield, 75%): mp 124–126 °C. ^{1}H NMR δ 1.62 (3H, d, J = 6.65 Hz, 8-CH_3), 2.42 (3H, d, J = 1.56 Hz, 4-CH_3), 2.37 (1H, d, J = 1.35 Hz, –*CCH*), 4.04 (1H, m, 8-*CH*), 6.26 (1H, d, J = 1.17 Hz, 3-H), 7.43 (1H, d, J = 2.35 Hz, 8-H), 7.32 (1H, dd, J_1 = 8.60 Hz, J_2 = 1.95 Hz, 6-H), 7.52 (1H, d, J = 8.60 Hz, 5-H). EI-MS m/z (%): 244 (M^+, 50), 229 (base).

4.12. 1′-Thia-2′,4-dimethylseselin (24)

Following the same procedure for the preparation of **17, 24** was obtained from **23** as a yellow solid (yield, 56%, estimated by ^{1}H NMR). The crude **24** was used directly in the preparation of **25** and **26**.

4.13. 1′-Thia-2′,4-dimethyl-(+)-*cis*-khellactone (25) and (26)

Following the same procedure for the preparation of **18** and **19, 25** (yield 52%, mp 226–227 °C, $CHCl_3$–MeOH, 80:1, R_f 0.14) and **26** (yield 16%, mp 242–243 °C, $CHCl_3$–MeOH, 80:1, R_f 0.15) were obtained from **24** as white solids. Compound **25**. ^{1}H NMR (DMSO) δ 1.31 (3H, d, J = 6.25 Hz, 2′-CH_3), 2.38 (3H, s, 4-CH_3), 3.47 (1H, m, 3′-*CH*), 3.63 (1H, m, 2′-*CH*), 5.07 (1H, m, 4′-*CH*), 5.19 (1H, d, J = 6.65 Hz, 3′-OH), 5.44 (1H, d, J = 4.7 Hz, 4′-OH), 6.30 (1H, d, J = 1.18 Hz, 3-H), 7.06 (1H, d, J = 8.60 Hz, 6-H), 7.55 (1H, d, J = 8.22 Hz, 5-H). EI-MS m/z (%): 278 (M^+, 26). Compound **26**. ^{1}H NMR (DMSO) δ 1.50 (3H, d, J = 7.04 Hz, 2′-CH_3), 2.38 (3H, d, J = 1.17 Hz, 4-CH_3), 3.31 (1H, m, 3′-*CH*), 3.97 (1H, m, 2′-*CH*), 5.07 (1H, m, 4′-*CH*), 5.14 (1H, d, J = 4.30 Hz, 3′-OH), 5.32 (1H, d, J = 5.48 Hz, 4′-OH), 6.30 (1H, d, J = 1.18 Hz, 3-H), 7.08 (1H, d, J = 8.22 Hz, 6-H), 7.55 (1H, d, J = 8.21 Hz, 5-H). EI-MS m/z (%): 278 (M^+, 22).

4.14. (2′*S*,3′*R*,4′*R*)-1′-Thia-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (7a) and (2′*R*,3′*S*,4′*S*)-1′-thia-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (7b)

Following the same procedure for the preparation of **5a** and **5b, 7a** (yield 42%, mp 175–177 °C) and **7b** (yield 42%, mp 153–155 °C) were obtained from **25** as yellow solids. The diastereoisomers (**7a**) and (**7b**) were separated by semi-preparative isolation on Alltima HP column with CH_3CN–water 70:30. Compound **7a** (HPLC, CH_3CN–water 70:30, R_f 0.49). 1H NMR δ 0.95 (3H, s, –CH_3 in camphanoyl), 1.05–1.12 (15H, m, –CH_3 × 5 in camphanoyl), 1.58–2.60 (8H, m, 4 × –CH_2 in camphanoyl), 1.33 (3H, d, J = 6.6 Hz, 2′-CH_3), 2.38 (3H, 4-CH_3), 3.85 (1H, m, 2′-*CH*), 5.33 (1H, dd, J_1 = 11.7 Hz, J_2 = 2.7 Hz, 3′-H), 6.17 (1H, 3-H), 6.85 (1H, d, J = 3.0 Hz, 4′-H), 7.04 (1H, d, J = 8.4 Hz, 6-H), 7.46 (1H, d, J = 8.4 Hz, 5-H). $[\alpha]_D$ −171.9 (*c* 0.70, CH_2Cl_2); HRMS (MALDI-DHB) calcd mass for $C_{34}H_{38}O_{10}S$ [M^++Na] 661.2083, found 661.2085. Compound **7b** (HPLC, CH_3CN–water 70:30, R_f 0.39). ^{1}H NMR δ 0.75 (3H, s, –CH_3 in camphanoyl), 1.05–1.12 (15H, m, –CH_3 × 5 in camphanoyl), 1.58–2.60 (8H, m, 4 × –CH_2 in camphanoyl), 1.34 (3H, d, J = 6.65 Hz, 2′-CH_3), 2.38 (3H, d, J = 1.0 Hz, 4-CH_3), 3.90 (1H, m, 2′-*CH*), 5.42 (1H, dd, J_1 = 11.4 Hz, J_2 = 3.0 Hz, 3′-H), 6.17 (1H, d, J = 1.0 Hz, 3-H), 6.95 (1H, d, J = 3.0 Hz, 4′-H), 7.04 (1H, d, J = 8.7 Hz, 6-H), 7.46 (1H, d, J = 8.7 Hz, 5-H). $[\alpha]_D$ +135.6 (*c* 0.83, CH_2Cl_2); HRMS (MALDI-DHB) calcd mass for $C_{34}H_{38}O_{10}S$ [M^++Na] 661.2083, found 661.2086.

4.15. (2′*R*,3′*R*,4′*R*)-1′-Thia-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (8)

Following the same procedure for the preparation of **6, 8** was obtained from **26** as a yellow solid (yield, 78%), 260–263 °C. ^{1}H NMR δ 0.82–1.11 (18H, m, $-CH_3 \times 6$ in camphanoyl), 1.64–2.58 (8H, m, $4 \times -CH_2$ in camphanoyl), 1.10 (3H, d, J = 5.86 Hz, 2′-CH_3), 2.39 (3H, d, J = 1.10 Hz, 4-CH_3), 3.32 (1H, m, 2′-*CH*), 5.80 (1H, m, 3′-H), 6.18 (1H, d, J = 1.10 Hz, 3-H), 6.76 (1H, d, J = 4.40 Hz, 4′-H), 7.08 (1H, d, J = 8.44 Hz, 6-H), 7.49 (1H, d, J = 8.43 Hz, 5-H). $[\alpha]_D$ +102.5 (*c* 0.8, CH_2Cl_2). ESI-MS *m*/*z* (%): 661 ($M+Na^+$). Anal. Calcd for $C_{34}H_{38}O_{10}S$: C, 63.93; H, 6.00; S, 5.02. Found: C, 63.72; H, 6.12; S, 5.15.

4.16. 1′-Sulfoxide-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (9a) and (9b)

A mixture of **7a** and **7b** (150 mg, 0.235 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Then the solution was cooled to 0 °C and 90% MCPBA (44 mg, 0.235 mmol) was added portionwise and stirred for 15 min. The solution was stirred for an additional 10 min at rt. After dilution with water (60 mL), the mixture was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, and then solvent was removed in vacuo. The residue was recrystallized from MeOH to afford a mixture of **9a** and **9b** as a white solid (138 mg, 90%), mp 273–276 °C. ^{1}H NMR spectra showed the ratio of diastereoisomers (**9a**) and (**9b**) was about 2.4:1. ^{1}H NMR δ 0.76–1.25 (18H, s, $-CH_3 \times 6$ in camphanoyl), 1.55–2.59 (8H, m, $4 \times -CH_2$ in camphanoyl), 1.55 (3H, d, J = 7.15 Hz, 2′-CH_3), 2.49 (3H, s, 4-CH_3), 3.59 (0.69H, m, 2′-*CH*), 3.72 (0.31H, m, 2′-*CH*), 6.04 (1H, m, 3′-H), 6.40 (1H, 3-H), 6.95 (0.71H, d, J = 3.30 Hz, 4′-H), 7.08 (0.29H, d, J = 3.12 Hz, 4′-H), 7.75 (1H, d, J = 8.24 Hz, 6-H), 7.86 (1H, d, J = 8.24 Hz, 5-H). $[\alpha]_D$ +22.2 (*c* 0.9, CH_2Cl_2). ESI-MS *m*/*z* (%): 677.2 ($M+Na^+$). Anal. Calcd for $C_{34}H_{38}O_{11}S$: C, 62.37; H, 5.85; S, 4.90. Found: C, 62.54; H, 5.79; S, 4.93.

4.17. 1′-Sulfone-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (11a) and (11b)

A mixture of compound **9a** and **9b** (105 mg, 0.16 mmol), 90% MCPBA (44 mg, 0.21 mmol) in anhydrous CH_2Cl_2 (8 mL) was stirred for 30 min at rt. Then diluted with water (50 mL), the mixture was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, and then solvent was removed. The residue was recrystallized from MeOH to afford a mixture of **11a** and **11b** as a white solid (92 mg, 86%), mp >270 °C. ^{1}H NMR spectra showed the ratio of diastereoisomers (**11a**) and (**11b**) was about 3.2:1. ^{1}H NMR δ 0.77–1.24 (18H, s, $-CH_3 \times 6$ in camphanoyl), 1.55–2.58 (8H, m, $4 \times -CH_2$ in camphanoyl), 1.55 (3H, d, J = 7.04 Hz, 2′-CH_3), 2.49 (3H, d, J = 1.17 Hz, 4-CH_3), 3.96 (0.77H, m, 2′-*CH*), 4.10 (0.23H, m, 2′-*CH*), 5.75 (1H, m, 3′-H), 6.40 (1H, d, J = 1.18 Hz, 3-H), 6.97 (0.76H, d, J = 3.25 Hz, 4′-H), 7.11 (0.24H, d, J = 3.13 Hz, 4′-H), 7.91 (2H, 6-H, and 5-H). $[\alpha]_D$ −5.0 (*c* 1.0, CH_2Cl_2). ESI-MS *m*/*z* (%): 669 (M^+-1). Anal. Calcd for $C_{34}H_{38}O_{12}S$: C, 60.88; H, 5.71; S, 4.78. Found: C, 60.96; H, 5.84; S, 4.80.

4.18. (8*R*,9*R*,10*R*)-1′-Sulfoxide-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (10)

Following the same procedure for the preparation of **9a** and **9b, 10** was obtained from **8** as a white solid (yield, 82%), mp 246–248 °C. ^{1}H NMR δ 0.85–1.25 (18H, m, $-CH_3 \times 6$ in camphanoyl), 1.60–2.58 (8H, m, $4 \times -CH_2$ in camphanoyl), 1.46 (3H, d, J = 7.43 Hz, 2′-CH_3), 2.49 (3H, s, 4-CH_3), 3.48 (1H, m, 2′-*CH*), 6.38 (1H, m, 3′-H), 6.39 (1H, s, 3-H), 6.89 (1H, d, J = 4.30 Hz, 4′-H), 7.78 (1H, d, J = 8.21 Hz, 6-H), 7.89 (1H, d, J = 8.21 Hz, 5-H). $[\alpha]_D$ +21.8 (*c* 1.1, CH_2Cl_2). ESI-MS *m*/*z* (%): 677 ($M+Na^+$).

4.19. (8*R*,9*R*,10*R*)-1′-Sulfone-2′,4-dimethyl-3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (12)

A mixture of compound **8** (64 mg, 0.10 mmol), 90% MCPBA (60 mg, 0.30 mmol) in anhydrous CH_2Cl_2 (6 mL) was stirred for 45 min at rt. The reaction mixture was diluted with CH_2Cl_2 (60 mL), and washed with 10% Na_2CO_3 and brine. The organic layer was dried over Na_2SO_4. After removal of the solvent, the residue was separated by column chromatography on silica gel (petroleum ether–acetone, 3:1) to provide product **12** as a white solid (57 mg, 82%), mp 203–205 °C. ^{1}H NMR δ 0.79–1.12 (18H, m, $-CH_3 \times 6$ in camphanoyl), 1.68–2.58 (8H, m, $4 \times -CH_2$ in camphanoyl), 1.65 (3H, d, J = 6.65 Hz, 2′-CH_3), 2.49 (3H, s, 4-CH_3), 3.59 (1H, m, 2′-*CH*), 6.29 (1H, m, 3′-H), 6.41 (1H, s, 3-H), 6.91 (1H, d, J = 4.30 Hz, 4′-H), 7.92 (2H, 6-H, and 5-H). $[\alpha]_D$ −40.0 (*c* 1.0, CH_2Cl_2). ESI-MS *m*/*z* (%): 693 ($M+Na^+$).

4.20. Biological assays

4.20.1. HIV-1 infectivity assay against non-drug-resistant strain in H9 lymphocytes

This assay was performed by Panacos Pharmaceuticals, Inc. as follows. The human T-cell line, H9, was maintained in continuous culture with L-glutamine at 5% CO_2 and 37 °C .Test samples were first dissolved in dimethyl sulfoxide. The following were the final drug concentrations routinely used for screening 100, 20, 4 and 0.8 μg/mL. For agents found to be active, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value could be determined. Test samples were prepared, and to each sample well was added 90 μL of media containing H9 cells at 3×10^5 cells/mL and 45 μL of virus inoculum (HIV-1 IIIB isolate) containing 125 $TCID_{50}$. Control wells containing virus and cells only (no drug) and cells only (no virus or drug) were also prepared. A second set of samples was prepared identical to the first and added to cells under identical conditions without virus (mock infection) for toxicity determinations (IC_{50} defined below). In addition, AZT and 4-methyl DCK was also assayed during each experiment as positive drug controls. On days 1 and 4 post-infection (PI), spent media was removed from each well and replaced with fresh media. On day 6 PI, the assay was terminated and cultured supernatants were harvested for analysis for virus replication by p24 antigen capture. The compound toxicity was determined by XTT using the mock-infected sample wells. If a test sample inhibited virus replication and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; EC_{50}, the concentration of test sample that was able to suppress HIV replication by 50%; and the therapeutic index (TI), the ratio of the IC_{50} to EC_{50}.

4.20.2. Assay for RT enzymatic inhibition

The RT activity of HIV-1 DH012 was determined in the presence of various concentrations of the tested compounds using a Roche colorimetric HIV-1 RT assay kit following the protocol provided by the manufacturer.

Acknowledgments

This research was supported by the Grants from the National Natural Science Foundation of China awarded to P.X. (No. 20272010) and Y.C. (No. 30200348 and 30873164, respectively, and the Research Grant (2002046069) for Ph.D. program from the National Education Administration of China awarded to P.X., and Grant AI-33066 from the National Institute of Allergies and

Infectious Diseases awarded to K.H.L. Thanks are also due to the National Drug Innovative Program (Grant No. 2009ZX09301-011) for partial support.

Supplementary data

Additional information on compound purity including elemental analysis, high-resolution mass spectral data, and HPLC analysis results of the target compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.031.

References and notes

1. Gottlieb, M. S.; Schroff, R.; Schanker, H. M.; Weisman, J. D.; Fan, P. T.; Wolf, R. A.; Saxon, A. N. *Engl. J. Med.* **1981**, *305*, 1425.
2. Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868.
3. 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.
4. (a) Yu, D.; Suzuki, M.; Xie, L.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **2003**, *23*, 322; (b) Yu, D.; Morris-Natschke, S. L.; Lee, K. H. *Med. Res. Rev.* **2007**, *27*, 108; (c) Xie, L.; Guo, H. F.; Lu, H.; Zhuang, X. M.; Zhang, A. M.; Wu, G.; Ruan, J. X.; Zhou, T.; Yu, D.; Qian, X.; Lee, K. H.; Jiang, S. *J. Med. Chem.* **2008**, *25*, 7689; (d) Tang, J.; Qian, K.; Zhang, B. N.; Chen, Y.; Xia, P.; Yu, D.; Xia, Y.; Yang, Z. Y.; Chen, C. H.; Morris-Natschke, S. L.; Lee, K. H. *Bioorg. Med. Chem.* **2010**, *18*, 4363.
5. Xie, L.; Takeuchi, Y.; Cosentino, L. M.; Lee, K. H. *J. Med. Chem.* **1999**, *42*, 2662.
6. Huang, L.; Yuan, X.; Yu, D.; Lee, K. H.; Chen, C. H. *Virology* **2005**, *332*, 623.
7. Xie, L.; Yu, D.; Wild, C.; Allaway, G.; Turpin, J.; Smith, P. C.; Lee, K. H. *J. Med. Chem.* **2004**, *47*, 756.
8. Zhang, Q.; Chen, Y.; Xia, P.; Xia, Y.; Yang, Z. Y.; Yu, D. L.; Morris-Natschke, S. L.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5855.
9. Chen, Y.; Zhang, Q.; Zhang, B. N.; Xia, P.; Xia, Y.; Yang, Z. Y.; Kilgore, N.; Wild, C.; Morris-Natschke, S. L.; Lee, K. H. *Bioorg. Med. Chem.* **2004**, *12*, 6383.
10. Jacobs, T. L.; Petty, W. L.; Teach, E. G. *J. Am. Chem. Soc.* **1960**, *82*, 4094.
11. Sharpless, K. B.; Amberg, W.; Bennani, Y. L. *J. Org. Chem.* **1992**, *57*, 2768.
12. Zhou, W. S.; Huang, L. F.; Sun, L. Q.; Pan, X. F. *Tetrahedron Lett.* **1991**, *32*, 6745.
13. Takatsuto, S.; Ikekawa, N. *Chem. Pharm. Bull.* **1984**, *32*, 2001.
14. McMorris, T. C.; Patil, P. A. *J. Org. Chem.* **1993**, *58*, 2338.
15. Crispino, G. A.; Sharpless, K. B. *Synthesis* **1993**, *8*, 777.
16. Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
17. Sebok, P.; Timar, T.; Eszenyi, T.; Patonay, T. *J. Org. Chem.* **1995**, *59*, 6318.
18. Tanaka, M.; Kai, T.; Sun, X. L.; Takayanagi, H.; Uda, T.; Furuhata, K. *Chem. Pharm. Bull.* **1995**, *43*, 1844.
19. Gabbutt, C. D.; Hepworth, J. D.; Heron, B. M. *Tetrahedron* **1995**, *51*, 13277.