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Anti-AIDS agents. Part 56: Synthesis and anti-HIV activity of 7-thia-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (7-thia-DCK) analogs☆

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Abstract—Two thia-DCK analogs (**3a**,**b**) were synthesized and evaluated for inhibition of HIV-1 replication in H9 lymphocytes. Compound **3a** showed potent anti-HIV activity with an EC_{50} value of 0.14 μM and a therapeutic index of 1110. However, the corresponding 6-*tert*-butyl-substituted compound (**3b**) showed no suppression. The bioassay results indicated that thia-DCK analogs merit attention as potential HIV-1 inhibitors.
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1. Introduction

Acquired immunodeficiency syndrome (AIDS), which is caused by human immunodeficiency virus (HIV) infection, has become a serious global threat to human health and life. The US FDA has now approved 19 anti-HIV drugs for clinical use. However, these drugs cannot completely eliminate HIV in the human body and also show side effects and toxicity and are subject to drug resistance by mutated virus. Thus, it is necessary to further explore novel anti-HIV agents with new mechanisms of action.

Suksdorfin (**1**, Fig. 1), isolated from the fruit of *Lomatium suksdorfii*, is a khellactone with interesting biological properties, especially anti-HIV activity.[2] Modification of **1** has already provided 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (DCK) (**2**, Fig. 1), which showed extremely potent inhibitory activity against HIV-1 replication in H9 lymphocytic cells with an EC_{50} value of 2.56×10^{-4} μM and a therapeutic index (TI) of 1.37×10^{5}.[3] In further structural research,

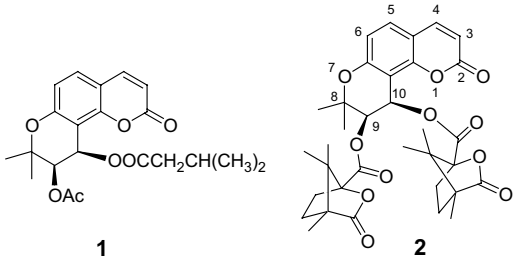

Figure 1. Structures of **1** and **2**.

numerous DCK derivatives were synthesized and evaluated for activity in an in vitro anti-HIV assay. Among them, 3-methyl, 4-methyl, 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. A preliminary mechanistic study showed that DCK and its analogs did not inhibit HIV-RT, integrase, or protease and might inhibit HIV-1 replication by a novel mechanism. Currently, their mechanism of action is still under investigation.

Based on the concept of bioisosterism, we designed a new series of analogs by replacing the oxygen atom in ring C (pyran ring) of DCK with a sulfur atom. Such

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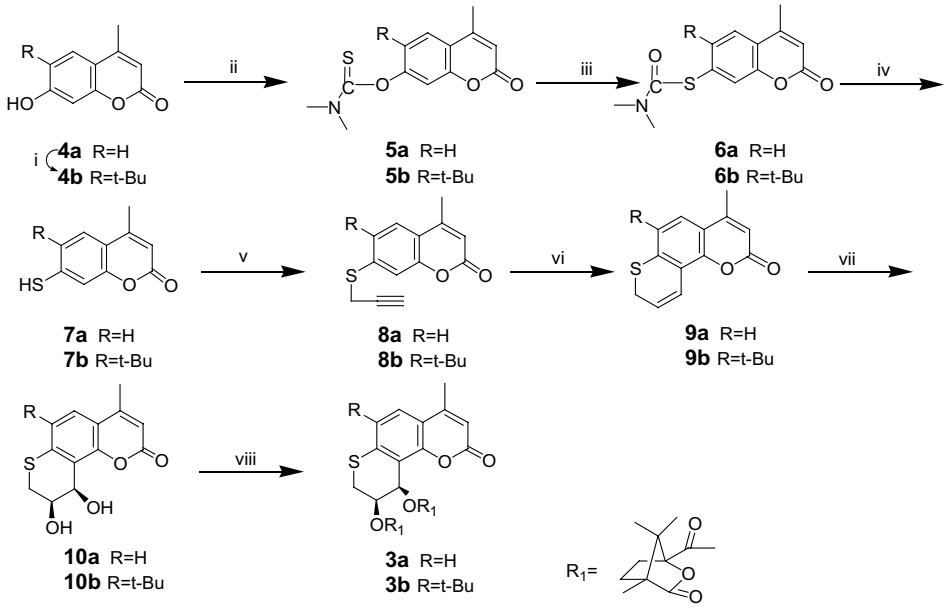

Scheme 1. Reagents and conditions: (i) *t*-BuOH, H_3PO_4/P_2O_5; (ii) dimethylthiocarbamoyl chloride, DMAP/CH_2Cl_2; (iii) heating; (iv) (1) $NaOCH_3$/MeOH, (2) HCl; (v) 3-bromo-1-propyne, K_2CO_3, KI, acetone/N_2; (vi) *N*,*N*-diethylaniline, reflux; (vii) (1) $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$, $(DHQ)_2$–PHAL, K_2CO_3, (2) Na_2SO_3; (viii) camphanic chloride, DMAP/CH_2Cl_2.

novel 7-thia-DCK analogs would provide more insight into the SAR of this molecular scaffold. In this paper, we report the synthesis and anti-HIV bioassay results of two initial 7-thia-4-methyl-8-nonsubstituted DCK analogs. Further structural modification and biological screening are ongoing.

2. Chemistry

As shown in Scheme 1, the reaction of 7-hydroxy-4-methyl-coumarin (**4a**) with *tert*-butanol in the presence of H_3PO_4 and P_2O_5 at 80 °C gave 7-hydroxy-4-methyl-6-*tert*-butyl-coumarin (**4b**). Compounds **5a** and **5b**, which were obtained by reacting intermediates **4a** and **4b** with dimethylthiocarbamoyl chloride, underwent rearrangement upon heating followed by hydrolysis in the presence of $NaOCH_3/CH_3OH$ and acidification with HCl to afford the key intermediate 7-mercapto-coumarins (**7a**,**b**).[4,5] The 6-*tert*-butyl- and 6-nonsubstituted-7-mercapto-coumarins were then converted to the corresponding seselin analogs (**9a**,**b**) by alkylation with 3-bromo-1-propyne in the presence of potassium iodide and potassium carbonate in acetone at room temperature, followed by cyclization in *N*,*N*-dimethylaniline at reflux temperature. Finally, asymmetric dihydroxylation[6] of (**9a**,**b**) followed by acylation with camphanic chloride gave the desired 7-thia-DCK analogs (**3a**,**b**).

3. Results and discussion

Compounds **3a** and**3b** were screened for anti-HIV activity in H9 lymphocytes, and their bioassay data are shown in Table 1. The 8-nonsubstituted analog **3a** exhibited potent inhibitory activity against HIV-1 replication with an EC_{50} value of 0.14 μM and TI value of 1110. In the same assay, the EC_{50}/TI values of AZT and DCK were 0.036 μM/51,972 and 0.000256 μM/136,719, respectively. These bioassay results indicated that thia-DCK analogs could be potential anti-HIV agents. Interestingly, compound **3b** with a *tert*-butyl group at the 6-position showed no anti-HIV-1 activity. This result suggested that the 6-position might be crucial for potency against HIV replication and sensitive to modification. A bulky group at this position might not be accommodated at the target receptor. The bioactivity comparison between **3a** and DCK implies that two methyl groups at the 8-position might be favorable for anti-HIV activity. In order to gain more information on this thia-DCK analog class, further modification is under investigation.

Table 1. Anti-HIV activity of compounds **3a** and **3b** in acutely infected H9 lymphocytes

Compound	IC_{50} (μM)[a]	EC_{50} (μM)[b]	TI[c]
3a	155[d]	0.141	1110
3b	126[d]	No suppression	No suppression
DCK[e]	35	0.000256	136,719
AZT	1871	0.036	51,972

[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 DMSO, which is used to solubilize the agents tested.
[e] The data for DCK were cited in Ref. 7.

4. Experimental

Melting points were measured with the capillary tube method without correction. ^{1}H NMR spectra were determined on Bruker-AM-300, DPX300 and

DRX-400 MHz spectrometers using TMS as internal standard. The solvent used was $CDCl_3$ unless otherwise indicated. Optical rotations were measured with a Jasco P-1020 digital polarimeter at 17.4 °C at the sodium D-line. MS and HRMS were measured on HP5989A and Concept 1H mass spectrometers.

4.1. 4-Methyl-6-(*tert*-butyl)-7-hydroxy-2*H*-chromen-2-one (4b)

tert-Butanol (10 g, 0.134 mol) was added to a mixture of 7-hydroxy-4-methyl-2*H*-chromen-2-one (3.0 g, 17.0 mmol), H_3PO_4 (200 mL), and P_2O_5 (15 g) at 60–70 °C. After stirring for 1 h, additional P_2O_5 (15 g) and *tert*-butanol (15 g) were added. This addition was repeated every 2 h, and the reaction was continued for an additional 11 h at the same temperature. The reaction mixture was then cooled to rt, poured into 1500 mL ice water, and filtered to afford crude product, which was purified by column chromatography on silica H with an eluent of petroleum–EtOAc = 3:1 to provide pure **4b** (1.45 g, yield 36.7%), mp 243–244 °C; MS (*m*/*z*, %): 232 (M^+, 22.08), 217 (M^+-15, 100); ^1H NMR: δ 1.45 (9H, s, *t*-Bu), 2.45 (3H, s, 4-CH_3), 6.14 (1H, s, 3-H), 7.32 (1H, s, 8-H), 7.46 (1H, s, 5-H), 7.55–7.59 (1H, br, OH).

4.2. 4-Methyl-6-(*tert*-butyl)-7-[(*N*,*N*-dimethyl)thiocarbamoyloxy]-2*H*-chromen-2-one (5b)

Under nitrogen, dimethylthiocarbamoyl chloride (3.2 g, 26 mmol) and DMAP (3.16 g, 26 mmol) were added to a solution of compound **4b** (1.0 g, 4.3 mmol) in CH_2Cl_2 (60 mL). After 96 h, the solvent was removed in vacuo, and the residue was poured into ice water and filtered. Compound **5b** was obtained as white solid (1.35 g, yield 98.2%, mp 234–238 °C), which was used in next reaction without purification. An analytical sample was purified by column chromatography on silica H (eluent: petroleum–EtOAc = 6:1), mp 236–237 °C. MS (*m*/*z*, %): 319 (M^+-1, 17.27), 88 (100); ^1H NMR: δ 1.41 (9H, s, *t*-Bu), 2.44 (3H, d, J = 0.92 Hz, 4-CH_3), 3.43 (3H, s, N–CH_3), 3.50 (3H, s, N–CH_3), 6.25 (1H, d, J = 0.97 Hz, 3-H), 7.04 (1H, s, 8-H), 7.58 (1H, s, 5-H).

4.3. 4-Methyl-6-(*tert*-butyl)-7-[(*N*,*N*-dimethyl)carbamoylmercapto]-2*H*-chromen-2-one (6b)

Compound **5b** (1.40 g) was heated to 230–250 °C with stirring for 3 h. The crude product (**6b**) (1.4 g) was obtained in quantitative yield. An analytical sample was obtained by recrystallization from EtOAc, mp 186–187 °C. MS (*m*/*z*, %): 318 (M^+-1, 6.71), 88 (100); ^1H NMR: δ 1.52 (9H, s, *t*-Bu), 2.44 (3H, d, J = 1.27 Hz, 4-CH_3), 3.06 (6H, br, N–$(CH_3)_2$), 6.29 (1H, d, J = 1.23 Hz, 3-H), 7.47 (1H, s, 8-H), 7.64 (1H, s, 5-H).

4.4. 4-Methyl-6-(*tert*-butyl)-7-mercapto-2*H*-chromen-2-one (7b)

Compound **6b** (1.89 g, 5.92 mmol) was hydrolyzed in the presence of $NaOCH_3/CH_3OH$ (5 mL, 25% w/v) in 80 mL of MeOH under nitrogen for 36 h. The reaction mixture was then acidified with dry HCl. After being diluted with 100 mL CH_2Cl_2 and washed with 40 mL H_2O, the aqueous layer was extracted with CH_2Cl_2 three times. The organic phase was dried over Na_2SO_4. Finally, removal of the solvent furnished a light yellow solid (870 mg, yield 59.2%), mp 176–180 °C. MS (*m*/*z*, %): 248 (M^+, 52.27), 233 (M^+-15, 100); ^1H NMR: δ 1.51 (9H, s, *t*-Bu), 2.41 (3H, s, –CH_3), 3.87 (1H, s, –SH), 6.20 (1H, s, 3-H), 7.18 (1H, s, 8-H), 7.53 (1H, s, 5-H).

4.5. 4-Methyl-6-(*tert*-butyl)-7-(prop-2-ynylthio)-2*H*-chromen-2-one (8b)

Under nitrogen, a mixture of compound **7b** (500 mg, 2.0 mmol), K_2CO_3 (5.57 g, 40.3 mmol), KI (50 mg, 0.30 mmol), and 3-bromo-1-propyne (956 mg, 8.37 mmol) in 100 mL acetone was stirred for 15 min at rt. Water (50 mL) was added to the reaction mixture, which was then extracted with EtOAc four times. The organic phase was dried over Na_2SO_4 and filtered. After removing the solvent in vacuo, the residue was purified by column chromatography on silica H (eluent: petroleum–EtOAc = 8:1) to give **8b** (330 mg, yield 57.2%), mp 158–160 °C. MS (*m*/*z*, %): 286 (M^+, 3.45), 271 (M^+-15, 77.14); ^1H NMR: δ 1.46 (9H, s, *t*-Bu), 2.20 (1H, t, J = 1.62 Hz, 3′-H), 2.35 (3H, s, 4-CH_3), 3.66 (2H, d, J = 1.62 Hz, 1′-H), 6.17 (1H, s, 3-H), 7.37 (1H, s, 8-H), 7.49 (1H, s, 5-H).

4.6. 4-Methyl-6-(*tert*-butyl)-8*H*-thiopyrano[6,5-*h*]-2*H*-chromen-2-one (9b)

Under nitrogen, compound **8b** (200 mg, 0.7 mmol) was heated to reflux in *N*,*N*-diethylaniline for 75 min. The solvent was removed in vacuo. The residue was purified by column chromatography on silica H (eluent: petroleum–EtOAc = 30:1) to afford **9b** as a yellow solid (158 mg, yield 79%), mp 142–144 °C. MS (*m*/*z*, %): 286 (M^+, 57.26), 271 (M^+-15, 100); ^1H NMR: δ 1.48 (9H, s, *t*-Bu), 2.35 (3H, s, –CH_3), 3.27 (2H, dd, J_1 = 1.2 Hz, J_2 = 5.3 Hz, 8-H), 6.10 (1H, m, J_1 = 5.3 Hz, J_2 = 10.4 Hz, 9-H), 6.15 (1H, s, 3-H), 7.15 (1H, d, J = 10.1 Hz, 10-H), 7.39 (1H, s, 5-H).

4.7. (9*R*,10*R*)-9,10-Dihydroxy-4-methyl-6-(*tert*-butyl)-8*H*,9*H*,10*H*-thiopyrano[6,5-*h*]-2*H*-chromen-2-one (10b)

A mixture of $K_3Fe(CN)_6$ (0.92 g, 2.8 mmol), K_2CO_3 (0.38 g, 2.8 mmol), $K_2OsO_2(OH)_4$ (8 mg, 0.02 mmol), and [(DHQ)$_2$–PHAL] (20 mg, 0.03 mmol) was dissolved in 48 mL of *t*-BuOH/H_2O (v/v, 1:1) at rt. The solution was cooled to 0 °C, compound **9b** was added, and the reaction continued for 6 h at 0–5 °C. Then, Na_2SO_3 (684 mg, 0.54 mmol) was added. After stirring for 2 h at rt, the mixture was extracted with $CHCl_3$ five times and EtOAc four times. The combined organic layers were dried over Na_2SO_4, and then solvent was removed. The residue was purified by column chromatography on silica H (eluent: petroleum–EtOAc = 2:1) to afford **10b** as a white solid (126 mg, yield 71.3%), mp 227–228 °C. MS (*m*/*z*, %): 320 (M^+, 57.12), 276 (M^+-45, 100); ^1H NMR: δ 1.53 (9H, s, *t*-Bu), 2.42 (3H, s, 4-CH_3), 2.85 (1H, dd, J_1 = 3.6 Hz, J_2 = 11.9 Hz, 8-H), 3.18 (1H, d,

J = 9.7 Hz, 9-OH), 3.33 (1H, t, J = 11.9 Hz, 8-H), 3.77 (1H, s, 10-OH), 4.13 (1H, m, J_1 = 3.7 Hz, J_2 = 9.7 Hz, 9-H), 5.40 (1H, t, J = 3.5 Hz, 10-H), 6.21 (1H, s, 3-H), 7.53 (1H, s, 5-H); ^{1}H NMR ($CDCl_3$+D_2O): δ 1.53 (9H, s, *t*-Bu), 2.42 (3H, s, 4-CH_3), 2.84 (1H, dd, J_1 = 3.6 Hz, J_2 = 11.9 Hz, 8-H), 3.31 (1H, t, J = 11.9 Hz, 8-H), 4.11 (1H, m, J = 11.9 Hz, 9-H), 5.36 (1H, d, J = 3.7 Hz, 10-H), 6.21 (1H, s, 3-H), 7.53 (1H, s, 5-H); HRMS: $C_{17}H_{20}O_4S$ calcd 320.10823. Anal. 320.10750.

4.8. (9*R*,10*R*)-9,10-Dicamphanoyloxy-4-methyl-6-(*tert*-butyl)-8*H*,9*H*,10*H*-thiopyrano[6,5-*h*]-2*H*-chromen-2-one (3b)

Compound **10b** (140 mg, 0.44 mmol) was acylated with (*S*)-(−)-camphanic chloride (379 mg, 1.75 mmol) in 40 mL CH_2Cl_2 in the presence of DMAP (320 mg, 2.62 mmol) for 1 h at rt. After removing the solvent, the target product **3b** (200 mg) was obtained by column chromatography on silica H (eluent: petroleum–EtOAc = 2:1), yield 94.1%, mp 256–258 °C. MS (*m*/*z*, %): 680 (M^+, 23.53), 482 (M^+−camphanoyl group, 20.13), 285 (M^+−2 × camphanoyl group, 100); ^{1}H NMR: δ 0.77–2.58 (24H, m, H in camphanoyl group), 1.55 (9H, s, *t*-Bu), 2.40(3H, s, 4-CH_3), 2.84 (1H, m, 8-H), 3.52 (1H, m, 8-H), 5.89 (1H, m, 9-H), 6.18 (1H, s, 3-H), 6.87 (1H, m, 10-H), 7.59 (1H, s, 5-H); $[\alpha]_D$ −37.39 (*c* 0.0023, $CDCl_3$), HRMS: $C_{33}H_{26}O_{10}S$ calcd 680.26552. Anal. 680.26686.

4.9. 7-*N*,*N*-Dimethylthiocarbamoyloxy-4-methyl-2*H*-chromen-2-one (5a)

7-Hydroxy-4-methyl-2*H*-chromen-2-one (1.76 g, 10 mmol) and DMAP (2.44 g, 20 mmol) were solubilized in 15 mL DMF, and reacted with dimethylthiocarbamoyl chloride (2.47 g, 20 mmol) for 3 h at rt. The mixture was poured into 400 mL ice water and filtered to afford crude product **5a** (2.27 g, yield 86.3%), mp 213–215 °C (lit.[4] mp 208–209 °C). Recrystallization from acetone afforded a pure sample, mp 215–217 °C.

4.10. 7-*N*,*N*-Dimethylcarbamoylmercapto-4-methyl-2*H*-chromen-2-one (6a)

Compound **5a** (1.50 g, 5.70 mmol) was heated to 225–230 °C with stirring for 1 h. The crude product (**6a**) was recrystallized from acetone to give light yellow crystals (1.31 g, yield 87.3%), mp 160–162 °C [lit.[4] mp 159–160 °C].

4.11. 4-Methyl-7-mercapto-2*H*-chromen-2-one (7a)

Under nitrogen, compound **6a** (5.0 g, 19 mmol) was hydrolyzed in the presence of $NaOCH_3$/MeOH (30 mL, 25% w/v) in 400 mL MeOH for 19 h at rt, then acidified with dry HCl. After being diluted with 100 mL H_2O, the reaction mixture was extracted with CH_2Cl_2 three times. The organic phase was dried over Na_2SO_4. Finally, removal of the solvent furnished **7a** as a light yellow solid (2.7 g, yield 74.0%), mp 137–138 °C [lit.[4] mp 138–139 °C].

4.12. 4-Methyl-7-prop-2-ynylthio-2*H*-chromen-2-one (8a)

Under nitrogen, a mixture of compound **7a** (2.60 g, 13.3 mmol), K_2CO_3 (28.0 g, 203 mmol), KI (337 mg, 2.03 mmol), and 3-bromo-1-propyne (6 mg, 47 mmol) in 100 mL acetone was stirred for 45 min at rt. Water (100 mL) was added, and the mixture extracted four times with $CHCl_3$. The organic phase was dried over Na_2SO_4 and filtered. After removing the solvent, the residue was purified by column chromatography on silica H (eluent: petroleum–EtOAc = 15:1) to obtain pure compound **8a** (2.77 g), yield 89.13%, mp 127–128 °C. MS (*m*/*z*, %): 229 (M^+−1, 100), 230 (M^+, 69.62); ^{1}H NMR: δ 2.29 (1H, s, 3′-H), 2.37 (3H, s, 4-CH_3), 3.72 (2H, s, 1′-H), 6.72 (1H, s, 3-H), 7.26–7.36 (2H, m, 6-H, 8-H), 7.54 (1H, d, J = 8.33 Hz, 5-H); IR (cm^{-1}): 2119 (alkyne).

4.13. 4-Methyl-8*H*-thiopyrano[6,5-*h*]-2*H*-chromen-2-one (9a)

Under nitrogen, compound **8a** (512 mg, 2.22 mmol) was heated to reflux in *N*,*N*-diethylaniline for 3 h. The solvent was removed in vacuo and the residue was purified by column chromatography on silica H (eluent: petroleum–EtOAc = 8:1) to afford compound **9a** (397 mg, yield 77.6%), mp 164–167 °C. MS (*m*/*z*, %): 230 (M^+, 100), 299 (M^+−1, 99); ^{1}H NMR: δ 2.33 (3H, s, 4-CH_3), 3.42 (2H, dd, J_1 = 1.5 Hz, J_2 = 5.2 Hz, 8-H), 5.997 (1H, m, J_1 = 5.2 Hz, J_2 = 10.4 Hz, 9-H), 6.15 (1H, s, 3-H), 7.07 (1H, d, J = 10.4 Hz, 10-H), 7.07 (1H, d, J = 8.3 Hz, 6-H), 7.25 (1H, d, J = 8.3 Hz, 5-H).

4.14. (9*R*,10*R*)-9,10-Dihydroxy-4-methyl-8*H*,9*H*,10*H*-thiopyrano[6,5-*h*]-2*H*-chromen-2-one (10a)

A mixture of $K_3Fe(CN)_6$ (4.29 g, 13.1 mmol), K_2CO_3 (1.79 g, 13.1 mol), $K_2OsO_2(OH)_4$ (38 mg, 0.10 mmol), and [(DHQ)$_2$–PHAL] (98 mg, 0.12 mmol) was solubilized in 174 mL of *t*-BuOH/H_2O (v/v, 1:1) at rt. The solution was cooled to 0 °C, compound **9a** (600 mg, 2.6 mmol) was added, and reaction was continued for 32 h at 0–5 °C. Then, Na_2SO_3 (3.20 g, 25.6 mmol) was added. After stirring for 2 h at rt, the mixture was extracted with $CHCl_3$ five times and EtOAc nine times. The combined organic layers were dried over Na_2SO_4, and then solvent was removed. The residue was purified by column chromatography on silica H (eluent: petroleum–EtOAc = 2:1) to afford dihydroxy compound **10a** (353 mg, yield 51.3%), mp 182–185 °C. MS (*m*/*z*, %): 264 (M^+, 63.63), 220 (M^+−44, 100), 192 (7-SH-heterocycle core, 69.47); ^{1}H NMR (DMSO-d_6): δ 2.35 (3H, s, 4-CH_3), 2.69 (1H, dd, J_1 = 2.8 Hz, J_2 = 11.5 Hz, 8-H), 3.27 (1H, t, J = 11.6 Hz, 8-H), 3.82 (9-H), 5.03 (1H, br, 10-H), 5.40 (2H, m, 2 × OH), 6.26 (1H, s, 3-H), 7.07 (1H, d, J = 8.5 Hz, 6-H), 7.53 (1H, d, J = 8.6 Hz, 5-H); ^{1}H NMR (DMSO-d_6 + D_2O): δ 2.31 (3H, s, 4-CH_3), 2.68 (1H, dd, J_1 = 2.9 Hz, J_2 = 11.6 Hz, 8-H), 3.24 (1H, t, J = 11.8 Hz, 8-H), 3.83 (1H, m, J_1 = 2.8 Hz, J_2 = 8.8 Hz, 9-H), 5.01 (1H, s, 10-H), 6.20 (1H, s, 3-H), 7.05 (1H, d, J = 8.6 Hz, 6-H), 7.50 (1H, d, J = 8.6 Hz, 5-H); HRMS $C_{13}H_{12}O_4S$ calcd 264.04563. Anal. 264.04125.

4.15. (9*R*,10*R*)-9,10-Dicamphanoyloxy-4-methyl-8*H*,9*H*,10*H*-thiopyrano[6,5-*h*]-2*H*-chromen-2-one (3a)

Compound **10a** (200 mg, 0.76 mmol) was acylated with (*S*)-(−)-camphanic chloride (656 mg, 3.02 mmol) in 60 mL CH_2Cl_2 in presence of DMAP (5.54 g, 4.54 mmol) for 1 h at rt. After removing the solvent, the target product **3a** (450 mg) was obtained by column chromatography on silica H (eluent: petroleum–EtOAc = 2:1), yield 95.2%, mp 177–179 °C. MS (*m*/*z*, %): 624 (M^+, 11.68), 229 ($M^+-2\times$ camphanoyl group, 100); ^1H NMR: δ 0.77–2.61 (24H, m, H in camphanoyl group), 2.40 (3H, s, 4-CH_3), 2.81 (1H, m, 8-H), 3.55 (1H, m, 8-H), 5.56 (1H, m, 9-H), 6.19 (1H, s, 3-H), 6.87 (1H, m, 10-H), 7.09 (1H, d, J = 8.48 Hz, 6-H), 7.48 (1H, d, J = 8.33 Hz, 5-H); $[\alpha]_D$ −53.40 (*c* 0.002, $CDCl_3$), HRMS: $C_{33}H_{26}O_{10}S$ calcd 624.20292. Anal. 624.20460.

5. HIV growth inhibition assay in H9 lymphocytes

The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal serum (FCS) supplemented with L-glutamine) at 5% CO_2 and 37 °C. Aliquots of this cell line were used in experiments only when in log phase of growth. Test samples were first dissolved in dimethyl sulfoxide (DMSO). The following were the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The mock-infected aliquot was used for toxicity determinations (IC_{50}). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 infectious units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 infectious units/cell was added to the first aliquot of H9 cells. The other aliquot of H9 cells received only culture medium and then was incubated under identical conditions as the HIV-infected H9 cells. After a 4 h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive infected control/negative drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on day 4 for use in-house p24 antigen ELLSA assay. P24 antigen is protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts on a Coulter counter on the mock-infected H9 lymphocytes, which had either received culture medium (no toxicity) or test sample or AZT.

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References and notes

1. Yu, D.; Brossi, A.; Kilgore, N.; Wild, C.; Allaway, G.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1575.
2. Lee, T. T.; Kashiwada, Y.; Huang, L.; Snider, J.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem.* **1994**, *2*, 1051.
3. Huang, L.; Kashiwada, Y.; Cosentino, L. M.; Fan, S.; Chen, C. H.; McPhail, A. T.; Fujioka, T.; Mihashi, K.; Lee, H. K. *J. Med. Chem.* **1994**, *37*, 3947.
4. Sebök, P.; Tímár, T.; Eszenyi, T. *J. Org. Chem.* **1995**, *59*, 6318.
5. Tanaka, M.; Kai, T.; Sun, X. L.; Takayanagi, H.; Uda, T.; Furuhata, K. *Chem. Pharm. Bull.* **1994**, *43*, 1844.
6. 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.
7. Yang, Z. Y.; Xia, Y.; Xia, P.; Brossi, A.; Cosentino, L. M.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1003.