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Antitumor Agents. 134.† New Shiraiachrome-A- and Calphostin-C-Related Perylene Derivatives as Cytotoxic and Antiviral Agents and Inhibitors of Protein Kinase C

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Shiraiachrome-A and -B have been isolated from the mycelium of the Chinese bamboo fungus *Shiraia bambusicola* as the cytotoxic principles. A series of new perylene derivatives (7–27) related to Shiraiachrome-A and -B as well as Calphostin-C have been synthesized and evaluated for their cytotoxicity, antiviral activity, and inhibitory activity against protein kinase C. The results indicated that 11 and 12 are potent cytotoxic agents against HCT-8, RPMI-7951, and TE-671 solid tumor cells, whereas 24 and 26 demonstrated strong antiviral activity against HSV-1 and HSV-2. Compound 10 is an inhibitor of protein kinase C.

As part of our continuing search among Chinese medicinal plant materials for new potent anti-solid tumor agents,[1] the methanolic extract of the mycelium of the fungus *Shiraia bambusicola* P. Henn (Hypocreaceae) was found to show significant (ED_{50} <20 μg/mL) cytotoxicity in the A-549 and HCT-8 solid tumor cells. Subsequent bioassay-directed fractionation in HCT-8 in vitro led to the isolation and characterization of Shiraiachrome-A (1) and -B (2) as two major cytotoxic principles (Figure 1). Compounds 1 and 2 and their related substances were previously reported as fungal metabolites which exert photodynamic activity towards bacteria and fungi from *S. bambusicola*.[2] However, compound 1 has been shown to have potent cytotoxicity against HCT-8 colon carcinoma for the first time.

Previously, compounds possessing a basic skeleton similar to 1, such as calphostin-C (3) and related substances have been reported as novel and specific inhibitors of protein kinase C and cytotoxic agents against HeLa S3 and MCF-7 breast carcinoma cells.[3,4] On the other hand, the other related aromatic polycyclic diones isolated from plants of *Hypericum* species, hypericin (4) and pseudohypericin (5), showed potent antiretroviral activity including anti-human inmmunodeficiency virus.[4,5] The antiretroviral activity of 4 and 5 was suggested to be attributable to the inhibition of some phosphorylation involved by protein kinase C during viral infection of cells.[4] These facts prompted our synthesis and evaluation of various 3,10-perylenequinones (7–27) related to 1 and 2. Compounds 1 and 2 are both 3,10-perylenequinone derivatives. Compound 3 was previously isolated from the culture broth of a fungi *Cladosporium cladosporioides*.

Chemistry

Scheme I showed the preparation of all perylene derivatives from their corresponding naphthoquinones or naphthalene. We found that heating 5-hydroxy-1,4-

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Table I. Cytotoxicity, Antivirial Activity, and PKC Activity of Perylene Derivatives (ED_{50}, mg/mL)[a]

entry	A-549	HCT-8	RPMI-7951	TE-671	KB	P388	HSV-1	HSV-2	PKC (μM)
1	2.1	0.55	–	–	5.5	0.54	5	5	44[c]
2	4.64	3.18	–	–	0.55	0.39	5	5	NT[c]
7	*b*	–	8.90	5.21	–	4.35	–	–	>218
8	–	–	–	–	–	–	–	–	>218
10	–	–	–	–	–	–	–	–	14[c]
11	3.84	0.56	0.05	0.68	0.54	0.36	–	–	82
12	5.41	4.16	0.05	0.61	0.80	0.64	–	–	>218
13	–	–	–	–	–	–	–	–	89
14	–	–	5.63	–	5.57	–	–	–	>40[d]
15	–	–	5.50	–	5.50	–	–	–	>40[d]
16	–	–	–	–	–	–	–	–	>40[d]
17	–	–	–	–	–	–	–	–	>40[d]
19	–	–	–	–	–	–	–	–	>218
20	–	–	–	–	–	–	–	–	>218
21	–	–	–	–	–	–	–	–	95
23	–	–	–	–	–	–	–	–	200
24	–	–	–	–	–	–	2	10	>218
25	–	–	–	–	–	–	–	–	>218
26	–	–	–	–	–	–	3	15	>218
27	–	–	–	–	–	–	–	–	>218

[a] ED_{50} was the concentration of drug which affords 50% reduction in cell number after a 3-day incubation. Each compound was examined with three concentrations at 0.1, 1, and 10 μg/mL. The ED_{50} value was established on the basis of the degree of inhibition at these three concentrations. [b] "–": Inactive at 10 μg/mL. [c] For entry 1 and 2 should read, phorbol binding was inhibited 80–100% at 38 μM. NT = not tested in the mixed micelle assay. [d] For entry 14, 15, 16, and 17 should read, highest concentration tested = 40 μM.

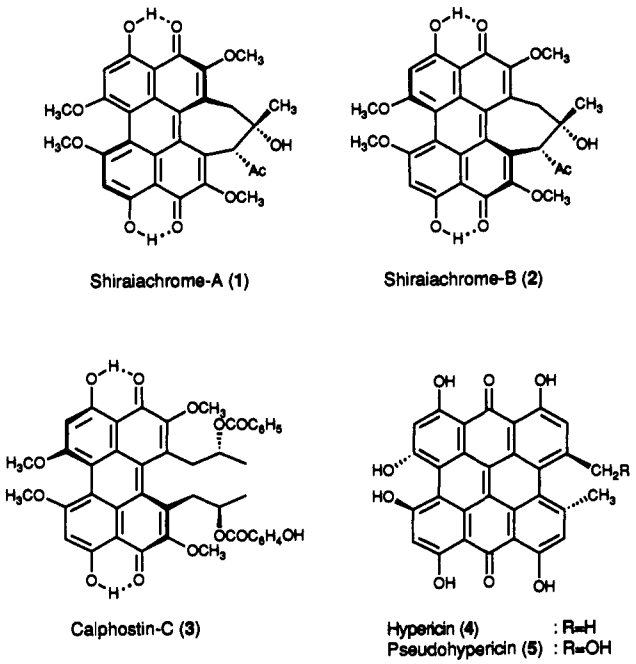

Figure 1.

naphthoquinone (18) with anhydrous aluminum chloride is an easier way to obtain 19 than Fatiadi's method.[6] Compound 19 was previously synthesized by Kraus et al. and found to have no antiretroviral activity.[5] Compounds 7 and 10 were synthesized according to Fatiadi's procedures.[6] All amino derivatives (8, 13, and 21) were prepared by nitric acid nitrolization, followed by titanium(III) chloride reduction,[7] or subsequent acetylation. Compound 23 was synthesized by heating 4,5-dihydroxynaphthalene-2,7-disulfonic acid disodium salt (22) with anhydrous aluminum chloride.

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Results and Discussion

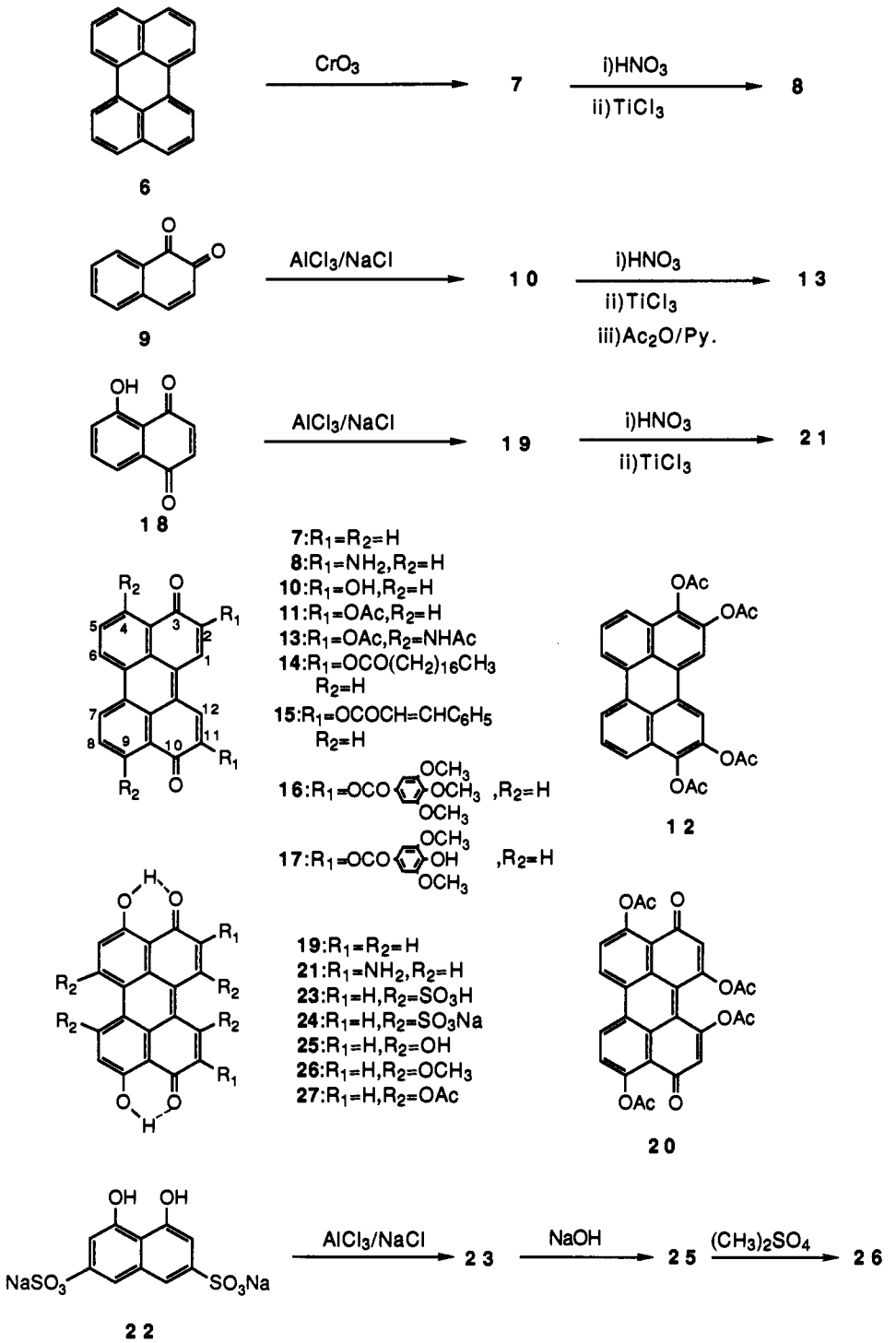
As shown in Table I, a comparison of the cytotoxicity of the three basic skeletons (7, 10, and 19) of 1–3 indicated that only the more lipophilic hydroxyl-group-lacking 7 demonstrated marginal cytotoxicity against TE-671 and P-388 tumor cells. The introduction of the hydrophilic amino group into 8 and 21 also led to the loss of cytotoxicity. The cytotoxicity was regained when the lipophilic acetyl group was introduced into 11 and 12. Both 11 and 12 exhibited comparable cytotoxicity against A-549, HCT-8, KB, and P-388 tumor cells compared to 1 and 2. In addition, they are also quite cytotoxic against RPMI-7951 and TE-671, in which 1 and 2 were not cytotoxic. It is also noteworthy, however, that 11 and 12 are quite comparable in cytotoxicity to the natural products (1 and 2) versus all cultures as mentioned above except for HSV-1 and -2 against which they are inactive. Replacement of the two acetyl groups of 11 with other acyl groups, such as those shown in 14–17, all gave rise to less active (14,15) or inactive compounds (16,17), indicating the two acetyl groups at the R_1 position (C-2 and C-11) are required for the enhanced cytotoxicity. The introduction of the acetyl groups or other moieties, such as OH, OCH_3, SO_3H, and SO_3Na at R_2 (C-1 and C-12) all resulted in the loss of cytotoxicity as seen in 23–27 and 20. On the other hand, compound 10 showed strong inhibitory activity against protein kinase C. However, this activity decreased upon introduction of stearoyl, cinnamoyl, 3,4,5-trimethoxybenzoyl, and 3,5-dimethoxy-4-hydroxybenzoyl groups. A screening of compounds 1–27 indicated that 1 and 2 as well as 24 and 26 showed potent anti-HSV-1 and anti-HSV-2 activities.

Experimental Section

General Experimental Procedures. All melting points were taken in a Fischer-John melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrometer. NMR spectra were obtained from a Bruker AC-300 300-MHz instrument. All chemical shifts were reported in ppm from TMS. Analytical and preparative thin-layer chromatographies (TLC) were carried out on Merck precoated silica gel 60 F-254 and Analtech GF, respectively. EM Kieselgel 60 (230–400 mesh) was used for column chromatography.

2,11-Diamino-3,10-perylenequinone (8). Compound 7 (prepared from 6 by Fatiadi's methods, 500 mg, 1.8 mmol) was

Scheme I



heated with concentrated nitric acid (25 mL) in an oil bath (100 °C) for 3 h with stirring. After cooling, the reaction mixture was poured into 200 mL of ice–water. The resulting precipitate was dissolved in acetic acid (500 mL) by heating and then filtered. After adding the 12% titanium(III) chloride in 21% HCl solution (50 mL), the reaction mixture was allowed to stand for 15 min, then neutralized with 20% sodium hydroxide solution, and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and purified by column chromatography [silica gel (25 g), using chloroform–methanol (20:1) as eluant] to give **8** (48 mg, 8%): amorphous; mp 201–203 °C; IR (KBr) 3323 (NH), 1630 (C=O of quinone), 1590 (C=C), 1298, 1245 cm^{-1}; ^1H NMR (d_6-DMSO) δ 3.69 (s, 4 H, NH), 7.27 (s, 2 H, 1-H, 12-H), 7.89 (br s, 2 H, 6-H, 7-H), 8.41 (t, 2 H, J = 7.0 Hz, 5-H, 8-H), 8.64 (d, 2 H, J = 7.0 Hz, 4-H, 9-H). Anal. ($C_{20}H_{12}N_2O_2$) C, H, N.

2,11-Diacetoxy-3,10-perylenequinone (11) and 2,3,10,11-tetraacetoxyperylene (12). A mixture of 2,11-dihydroxy-3,10-perylenequinone (**10**) (200 mg, 0.64 mmol) (prepared from **9** by Fatiadi's methods), acetic anhydride (5 mL), and pyridine (5 mL) was stirred for 36 h at room temperature and then poured into ice–water (50 mL). The solution was extracted with ethyl acetate. After evaporation of solvent, the residue was column chromatographed on silica gel (30 g) using chloroform–methanol (100:1) as an eluant to afford **11** (46 mg, 14%) and **12** (159 mg, 48%). Compound **11**: pale yellow crystals; mp 225–226 °C; IR (KBr) 3130 (aromatic C—H), 1752 (C=O of ester), 1637 (C=O of quinone), 1579 (C=C), 1395, 1190, 1172 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.41 (s, 6 H, OAc × 2), 7.68 (s, 2 H, 1-H, 12-H), 7.69 (t, 2 H, J = 7.7 Hz, 5-H, 8-H), 8.38 (d, 2 H, J = 7.7 Hz, 4-H, 9-H), 8.50 (d, 2 H, J = 7.7 Hz, 6-H, 7-H). Anal. ($C_{24}H_{14}O_6$) C, H.

Compound **12**: deep yellow crystals; mp 286–287 °C; IR (KBr) 3130 (aromatic C—H), 1760 (C=O of ester), 1593 (C=C), 1393, 1369, 1200 cm^{-1}; ^1H NMR (d_6-DMSO) δ 2.38, 2.50 (both s, each

6 H, OAc × 4), 7.67 (t, 2 H, J = 7.9 Hz, 5-H, 8-H), 7.83 (d, 2 H, J = 7.9 Hz, 6-H, 7-H), 8.30 (s, 2 H, 1-H, 12-H), 8.49 (d, 2 H, J = 7.9 Hz, 4-H, 9-H); ^{13}C NMR (d_6-DMSO) δ 20.31 (OCOCH_3 × 2), 20.55 (OCOCH_3 × 2), 117.55, 121.78, 121.83, 126.42, 128.37, 128.74 (2 C), 130.32, 134.14, 140.39, 168.46 (O$COCH_3$ × 4). Anal. ($C_{28}H_{20}O_8$) C, H.

2,11-Diacetoxy-4,9-diacetaamido-3,10-perylenequinone (13). Compound **10** (210 mg, 0.67 mmol) was heated with concentrated nitric acid (50 mL) in an oil bath (130–135 °C) for 3 h with stirring. After cooling, the reaction mixture was poured into ice–water (300 mL) and then extracted with ethyl acetate. The solvent was removed under reduced pressure, and the residue was dissolved into acetic acid (50 mL). To the acetic acid solution was added the 12% titanium(III) chloride in 21% HCl solution (10 mL). After the mixture was allowed to stand for 15 min, it was neutralized with 20% NaOH solution and then extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated. The residue was acetylated with acetic anhydride (5 mL) and pyridine (5 mL) for 24 h at room temperature and worked up in the usual way to yield **13** (25 mg, 7%): amorphous; mp 234–235 °C; IR (KBr) 3420 (NH), 3150 (aromatic C-H), 1712 (C=O of quinone), 1575, 1395 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 6 H, OCOCH_3 × 2), 2.48 (s, 6 H, NHCOCH_3 × 2), 7.92 (s, 2 H, 1-H, 12-H), 8.72 (d, 2 H, J = 7.5 Hz, 5-H, 8-H), 8.96 (d, 2 H, J = 7.5 Hz, 6-H, 7-H), 9.32 (s, 2 H, N$HCOCH_3$ × 2). Anal. ($C_{28}H_{22}N_2O_8$) C, H, N.

2,11-Bis(stearoyloxy)-3,10-perylenequinone (14). The mixture of **10** (200 mg, 0.64 mmol), stearoyl chloride (1.0 mL), and pyridine (10 mL) was heated at 100 °C for 2 h. After cooling, the undissolved **10** (153 mg) was recovered by filtration. The filtrate was stirred for 24 h at room temperature and then poured into ice–water. The solution was extracted with chloroform. After evaporation of solvent, the residue was column chromatographed on silica gel (50 g). Elution of the column with chloroform furnished **14** (98 mg, 77%) plus the recovering of 150 mg of **10**. Compound **14**: reddish amorphous; mp 156–157 °C; IR (KBr) 2910, 2845, 1745 (C=O of ester), 1630 (C=O of quinone), 1580, 1250, 1330 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6 H, J = 6.8 Hz, CH_3CH_2 × 2), 1.0–2.03 (m, 60 H, CH_2 × 30), 2.75 (t, 4 H, J = 7.5 Hz, OCOCH_2 × 2), 7.57 (s, 2 H, 1-H, 12-H), 7.93 (d, 2 H, J = 6.5 Hz, 4-H, 9-H), 8.67 (d, 2 H, J = 6.5 Hz, 6-H, 7-H), 8.94 (t, 2 H, J = 6.5 Hz, 5-H, 8-H). Anal. ($C_{56}H_{78}O_6$) C, H.

2,11-Bis(cinnamoyloxy)-3,10-perylenequinone (15). A mixture of **10** (200 mg, 0.57 mmol), cinnamoyl chloride (200 mg), and pyridine (10 mL) was treated by an analogous procedure used for the preparation of **14** described above to afford **15** (88 mg, 87%) plus recovering 145 mg of **10**. Compound **15**: mp 291–292 °C; IR (KBr) 3050 (CH=CH), 1715 (C=O of ester), 1630 (C=O of quinone), 1612 (CH=CH), 1575, 1325, 1115 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.73 (d, 2 H, J = 16.0 Hz, COCH=CHC_6H_5), 7.43 (m, 6 H, 6 H of C_6H_5 × 2), 7.62 (m, 4 H, 4 H of C_6H_5 × 2), 7.96 (t, 2 H, J = 7.5 Hz, 5-H, 8-H), 7.99 (d, 2 H, J = 16.0 Hz, COCH=CHC_6H_5), 8.15 (s, 2 H, 1-H, 12-H), 8.69 (d, 2 H, J = 7.5 Hz, 4-H, 9-H), 8.89 (d, 2 H, J = 7.5 Hz, 6-H, 7-H). Anal. ($C_{38}H_{22}O_6$) C, H.

2,11-Bis[(3′,4′,5′-trimethoxybenzoyl)oxy]-3,10-perylenequinone (16). To a mixture of **10** (200 mg, 0.57 mmol) and pyridine (10 mL) was added a chloroform (5 mL) solution containing 3,4,5-trimethoxybenzoyl chloride (500 mg). The mixture was further treated in the same way as the procedure used above for the preparation of **14** to yield **16** (116 mg, 75%) plus the recovering of 130 mg of **10**. Compound **16**: mp 296–297 °C; IR (KBr) 1730 (C=O of ester), 1640 (C=O of quinone), 1580, 1330, 1165 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 12 H, OCH_3 × 4), 3.93 (s, 6 H, OCH_3 × 2), 7.43 (s, 4 H, OCOC_6H_2 × 2), 7.73 (t, 2 H, J = 7.0 Hz, 5-H, 8-H), 7.98 (s, 2 H, 1-H, 12-H), 8.15 (s, 2 H, 1-H, 12-H), 8.41 (d, 2 H, J = 7.0 Hz, 4-H, 9-H), 8.46 (d, 2 H, J = 7.0 Hz, 6-H, 7-H). Anal. ($C_{40}H_{30}O_{12}$) C, H.

2,11-Bis(3′,5′-dimethoxy-4′-hydroxybenzoyl)oxy]-3,10-perylenequinone (17). A solution of **16** (36 mg) in methylene chloride (15 mL) was added to iodotrimethylsilane (0.20 mL). The mixture was stirred for 8 h at room temperature and then quenched with methanol (1 mL). After the solvent was evaporated, the residue was column chromatographed on silica gel (35 g) to furnish **17** (20 mg, 55%): yellow needles; mp 296–297 °C; IR (KBr) 3400 (OH), 1725 (C=O of ester), 1635 (C=O of quinone), 1580, 1330, 1120 cm^{-1}; ^{1}H NMR (d_6-DMSO) δ 3.85 (s, 12 H, OCH_3 × 4), 7.42 (s, 4 H, 4 H of benzoyl), 8.07 (t, 2 H, J = 7.5 Hz, 5-H, 8-H), 8.53 (d, 2 H, J = 7.5 Hz, 4-H, 9-H), 8.70 (s, 2 H, 1-H, 12-H), 9.22 (d, 2 H, J = 7.5 Hz, 6-H, 7-H), 9.65 (s, 2 H, OH × 2). Anal. ($C_{38}H_{26}O_{12}$) C, H.

4,9-Dihydroxy-3,10-perylenequinone (19). A mixture of 5-hydroxy-1,4-naphthoquinone (**18**, 1.0 g, 5.75 mmol), anhydrous aluminum chloride (7.5 g), and sodium chloride (1.0 g) was heated for 3 h in an oil bath (190–200 °C) with stirring. After cooling, the water (50 mL) was added very carefully. The precipitate was collected and washed with water and acetone to afford crude **19** (838 mg, 93%). Recrystallization from nitrobenzene to give **19**: dark red crystals; mp >365 °C; IR (KBr) 3150 (aromatic C—H), 1625 (quinone C=O), 1395 cm^{-1}. Anal. ($C_{20}H_{10}O_4$) C, H.

1,4,9,12-Tetraacetoxy-3,10-perylenequinone (20). Compound **19** (500 mg, 1.59 mmol) was suspended in pyridine (100 mL) and acetic anhydride (100 mL). The mixture was treated with a supersonic water bath (Branson 5200) for 4 days. After the insoluble substance was filtered out, the solution was poured into ice–water and extracted with chloroform. The solvent was removed under reduced pressure, and the residue was further purified by preparative TLC to afford **20** (68 mg, 11%): yellow, amorphous; mp 178–179 °C; IR (KBr) 3120, 3060 (aromatic C—H), 1745 (ester), 1658 (C=O of quinone), 1608, 1585 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.18, 2.27 (each s, both 6 H, OAc × 4), 6.18 (s, 2 H, 2-H, 11-H), 7.16 (d, 2 H, J = 5.0 Hz, 5-H, 8-H), 8.62 (d, 2 H, J = 5.0 Hz, 6-H, 7-H); ^{13}C NMR ($CDCl_3$) δ 18.55, 19.69 (both OCOCH_3), 113.79, 124.16, 124.48, 125.20, 141.03, 149.79, 150.09, 150.66, 162.82, 165.04, 177.35 ($COCH_3$). Anal. ($C_{28}H_{18}O_{10}$) C, H.

2,11-Diamino-4,9-dihydroxy-3,10-perylenequinone (21). Compound **19** (1.0 g, 3.18 mmol) was heated with concentrated nitric acid (50 mL) in an oil bath (130–135 °C) for 3 h. The reaction mixture was worked up by the same procedure used for preparation of **8** to give **21**: dark red amorphous; mp 256–257 °C, IR (KBr) 3360 (NH), 3130 (aromatic C—H), 1630 (C=O of quinone), 1600 (C=C), 1550, 1390 cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.24 (s, 2 H, NH × 2), 7.40 (d, J = 6 Hz, 2 H, 5-H, 8-H), 7.41 (d, J = 6 Hz, 2 H, 6-H, 7-H), 7.90 (br s, 2 H, 1-H, 12-H). Anal. ($C_{20}H_{12}N_2O_4$) C, H, N.

1,6,7,12-Tetrasulfo-4,9-dihydroxy-3,10-perylenequinone (23) and Its Sodium Salt (24). 4,5-Dihydroxynaphthalene-2,7-disulfonic acid disodium salt dihydrate (**22**, 2.0 g, 5.0 mmol) was heated with anhydrous aluminum chloride (15 g) and sodium chloride (2.0 g) for 80 h in an oil bath (205–210 °C). After cooling, water (100 mL) was added carefully. After the solution was allowed to stand for 1 night, the precipitate (1.18 g, 74%) was collected. The precipitate was further purified by Sephadex LH-20 column chromatography (3.5 × 25 cm) using H_2O as an eluant to afford **23**: black amorphous; mp >365 °C; IR (KBr) 3600–2200 (br, SO_3^-), 1625 (C=O of quinone), 1600, 1577 (C=C), 1370, 1210, 1160, 1050, 1030 cm^{-1}; ^{1}H NMR (D_2O) δ 6.78 (s, 2 H, 2-H, 11-H), 7.46 (s, 2-H, 5-H, 8-H); ^{13}C NMR (D_2O) δ 109.19, 118.73, 120.90, 137.21, 143.61, 143.91, 143.97, 156.04, 156.19, 156.24. Anal. ($C_{20}H_{10}O_{16}S_4$) C, H, S.

Compound **23** (200 mg, 0.32 mmol) was dissolved into a solution of sodium hydroxide (50.5 mg) in water (5 mL). The solution was evaporated under reduced pressure to provide **24**: black amorphous; mp >365 °C; IR (KBr) 3500–2500 (br, SO_3^-), 1630 (C=O of quinone), 1560 (C=C), 1445, 1390 cm^{-1}. Anal. ($C_{20}H_{10}O_{16}S_4Na_4$) C, H, S.

1,4,6,7,9,12-Hexahydroxy-3,10-perylenequinone (25). A mixture of **23** (1.5 g, 2.37 mmol), sodium hydroxide (3.0 g), and water (10 mL) was heated in an oil bath. After the temperature was elevated gradually to 200 °C to remove water, it was further heated for 2 h at 250 °C. After cooling, water (50 mL) was added and the solution was neutralized with 10% HCl to pH 2–3. The precipitate was collected and washed with water and acetone to afford crude **25** (134 mg, 15%). Crude **25** was purified by Sephadex LH-20 column chromatography (3.5 × 20 cm) to yield **25**: black amorphous; mp >365 °C; IR (KBr) 3420 (OH), 3160 (aromatic C—H), 1620 (br, quinone C=O), 1375, 1200, 1042 cm^{-1}. Anal. ($C_{20}H_{10}O_8$) C, H.

1,6,7,12-Tetramethoxy-4,9-dihydroxy-3,10-perylenequinone (26). A mixture of **23** (3.0 g, 4.73 mmol), sodium hydroxide (6.0 g), and water (2 mL) was heated in an oil bath. After the temperature was elevated gradually to 200 °C to remove water, it was further heated for 2 h at 250 °C. After cooling, water (50 mL) was added to dissolve the residue. Dimethyl sulfate (7.5 mL) was added dropwise over 30 min to the resulting solution. It was adjusted to pH 8–9 and stirred for 2 h at 50 °C. After cooling, the precipitate was collected and washed with water and acetone to afford **26** (450 mg, 21%): black amorphous; mp >300 °C dec; IR (KBr) 3450 (OH), 3140 (aromatic C—H), 1630 (quinone C=O), 1395 cm^{-1}. Anal. ($C_{24}H_{18}O_8$) C, H.

1,6,7,12-Tetraacetyl-4,9-dihydroxy-3,10-perylenequinone (27). Compound **25** (600 mg, 1.59 mmol) was suspended in acetic anhydride (30 mL) and pyridine (30 mL). The mixture was heated in an oil bath (100 °C) for 48 h. The solvent was removed in vacuo, and the residue was purified by column chromatography (SiO_2, 20 g) using chloroform–methanol (100:1) as eluant to furnish **27** (115 mg, 13%): yellow amorphous; mp 200–201 °C; IR (KBr) 3150 (aromatic C—H), 1680 (C=O of ester), 1625 (C=O of quinone), 1590 (C=C), 1482, 1397, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.17, 2.18, 2.42 (all s, 6 H each, OAc × 6), 6.53 (s, 2 H, 2-H, 11-H), 7.14 (s, 2 H, 5-H, 8-H). Anal. ($C_{28}H_{18}O_{12}$) C, H.

Cytotoxic Assay. Assay for cytotoxicity in various tumor cells were carried out according to the procedures described previously.[8,9,10] The tumor cells included in this study were A-549 lung carcinoma, HCT-8 colon carcinoma, RPMI-7951 melanoma, TE-671 meduloblastoma, KB epidermoid carcinoma of nasopharynx, and P-388 lymphocytic leukemia cells.

Protein Kinase C Assay. The assay was performed by a modification of the mixed micelle assay as described elsewhere.[11] The reaction mixture in a total volume of 250 μL contained 0.3% Triton X-100 with 6 mol% phosphatidylserine (Avanti) and 0.5 mol% dioleoylglycerol (Avanti) in 29 mM Hepes buffer, pH = 7.5, 10 mM magnesium chloride, 100 μM calcium chloride, and 20 μM [^{32}P]ATP. Compound was dissolved in DMSO, and 10 μL was added to the assay. DMSO alone was added in the control assay reactions. The reaction was started with 20 μL of protein kinase C (PKC) (1 μg/mL). The reaction was terminated with 0.5 mL ice-cold trichloroacetic acid followed by 100 μL of bovine serum albumin (1 mg/mL). The precipitate was collected by vacuum filtration using GF/C filters and quantified by counting in a β scintillation counter.

Anti HSV-1/HSV-2 Assay. Confluent Vero cells were infected with 3PFU/Cell of HSV-1 (Kos) or HSV-2 (333) in RPMI-1640 medium containing 2% FCS. Two-fold serial dilutions of the drugs (final concentration: 100, 50, 25, 12.5 μg/mL) were added during the innoculation phase. After 24 h, the CPE was observed under the microscope.

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3′-Spiro Nucleosides, a New Class of Specific Human Immunodeficiency Virus Type 1 Inhibitors: Synthesis and Antiviral Activity of [2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xylo- and -ribofuranose]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole 2″,2″-Dioxide] (TSAO) Pyrimidine Nucleosides

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A series of 3′-spiro nucleosides have been synthesized and evaluated as anti-HIV-1 agents. Reaction of *O*-mesylcyanohydrins of furanos-3′-ulosyl nucleosides with base afforded [1-[2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xylo- and -ribofuranosyl]]-3′-spiro-5″-[4″-amino-1″,2″-oxathiole 2″,2″-dioxide] derivatives of thymine, uracil and 4-*N*-acetylcytosine **11** and **12**. Desilylation of **11** and **12** gave the full deprotected 3′-spiro xylo- and ribofuranosyl nucleosides **13** and **14** or the partially 5′-O-deprotected-3′-spiro β-D-*xylo*- and -*ribo*-nucleosides **15** and **16**, or 2′-O-deprotected-3′-spiro β-D-*ribo*-nucleoside **17**. 2′-Deoxygenation of **17** afforded 2′-deoxy-3′-spiro β-D-*erythro*-pentofuranosyl derivative **18**. These 3′-spiro derivatives were evaluated for their anti-HIV-1 activity. All 3′-spiro nucleosides having a *xylo* configuration did not show any anti-HIV-1 activity. 3′-Spiro *ribo*-nucleosides with none or only one silyl group at C-2′ or C-5′ or the 2′-deoxy derivative were also inactive at subtoxic concentrations. However, 3′-spiro *ribo*-nucleosides having two silyl groups at C-2′ and C-5′ were potent and selective inhibitors of HIV-1. None of the nucleoside analogues that showed anti-HIV-1 activity proved inhibitory to the replication of HIV-2 or SIV.

Introduction

Azidothymidine (AZT, Retrovir) and DDI (dideoxyinosine) are, so far, the only drugs approved for the clinical treatment of acquired immunodeficiency syndrome (AIDS).[1-4] However, they also induce bone marrow suppression (AZT), peripheral neuropathy (DDI), pancreatitis (DDI), and other side effects.[3-6] AZT and DDI have

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