Synthesis and Inhibitory Activities of Isochromophilone Analogues against gp120-CD4 Binding

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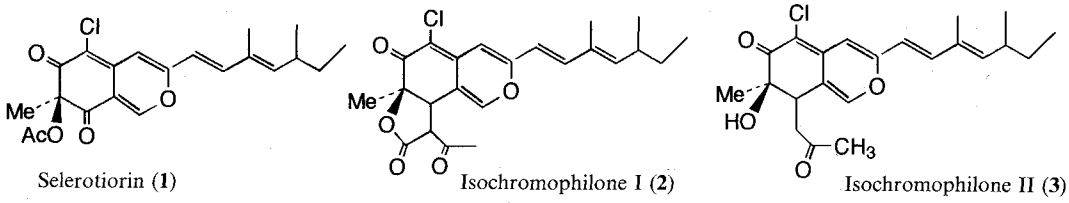
Several isochromophilone analogues were synthesized from sclerotiorin (**1**) by Wittig reactions and aldol condensation reaction. The structures of the products were elucidated from MS, elemental analysis, ^{1}H NMR and ^{13}C NMR spectra, and their inhibitory activities against gp120-CD4 binding were determined.

Sclerotiorin (**1**) occurs as a major metabolite in several species of monoverticillate Penicillia.[1] It was first isolated from a culture of *Penicillium* Van Beyma by CURTIN and REILLY.[2] Recently, in the screening program for new inhibitors against gp120-CD4 binding from microorganisms, isochromophilones Ia (**2**) and IIa (**3**), which have the same azaphilone skeleton and chlorine as **1**, were found as novel non-peptide inhibitors from a culture broth of *Penicillium* sp. FO-2338[3,4] (Chart 1). Although **1** was also found to be produced abundantly by the strain, its inhibitory activity against gp120-CD4 binding was much less than that of **2** and **3**. In order to understand the relationship between the structure and activity, we selected **1** as starting material to synthesize several derivatives, and determined their inhibitory activities against gp120-CD4 binding.

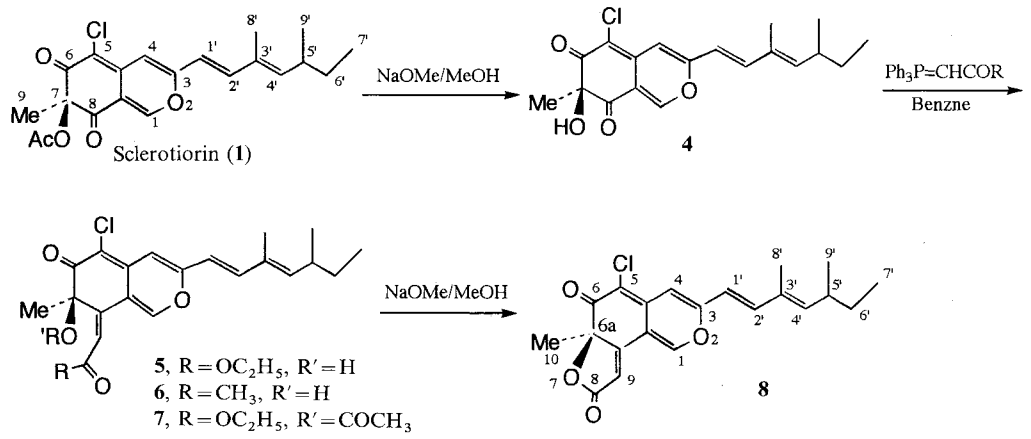
Results

The comparison of the structures of sclerotiorin (**1**) with isochromophilone Ia (**2**) and isochromophilone IIa (**3**) suggested that the 7-*O*-group and carbon-8 may be the groups principally responsible for the inhibitory activities. In order to prove this, at first, we tried the Wittig reaction at 8-carbonyl group of **1** with carbethoxymethylene-triphenylphosphorane. However, we didn't succeed by using **1** as starting material directly, so we performed the basic hydrolysis of the 7-*O*-acetyl group of **1** with sodium methoxide in refluxing methanol to obtain **4** in 89% yield. From **4** we obtained an olefinic ester **5** in 70% yield. The structure of **5** was elucidated from MS and ^{1}H NMR data. In ^{1}H NMR, the characteristic signal of the new product is the new olefinic proton (6.59 ppm, s, 10-H) and *O*-ethyl group (4.15 ppm, m, CH_2; 1.20 ppm, t, $J=7.8$ Hz, CH_3). The seqcis conformation of the new olefin of **5** was determined by NOE experiment between H-1 and H-10 (2%). Treatment of **4** with the Wittig agent 1-triphenylphosphoranylidene-2-propanone gave **6** in 30% yield. **5** was acetylated by acetic anhydride in pyridine at room temperature to yield **7** in 91%. Compound **8** was synthesized from **5** under basic condition by intramolecular esterification in 85% yield. The structure was elucidated by MS, ^{1}H NMR data, H-10 showed at 5.99 ppm (s), and NOE between H-1 and H-10 was approximately 5%.

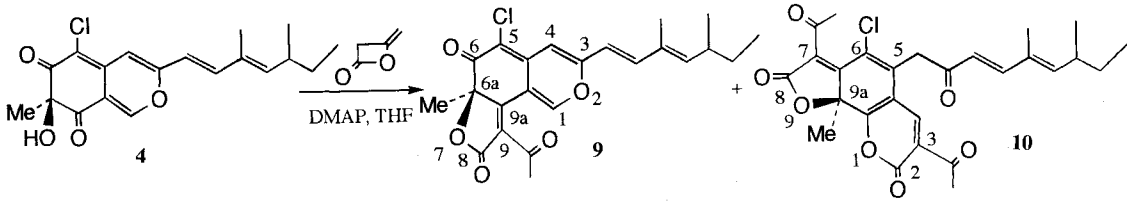
Chart 1.



Scheme 1.



Scheme 2.



To synthesize rubrorotiorin (**9**),[2] we conducted an intermolecular esterification reaction between **4** and diketene, and then an intramolecular aldol condensation reaction of the above product (Scheme 2). From this reaction **10** was also obtained as by-product in 3% yield. The structure of these products were elucidated by MS, ^{1}H NMR, ^{13}C NMR data, HMBC, HMQC and NOE. For the above products **5**, **6**, **7**, **8** and **9**, we conducted selective reduction of C-8, C-10 olefin as α,β-unsaturated carbonyl compounds with Birch reduction or sodium hydrogen telluride reduction reagent,[5,6] but didn't succeed. It may be due to several olefin bonds and lactone structure in these compounds are unstable to these reduction conditions.

The inhibitory activities against gp120-CD4 binding of these products were determined. Table 1 shows that compounds **5**, **8** and **9** inhibited gp120-CD4 binding with IC_{50} of 2.6, 8.1, and 13 μg/ml, respectively. These values rank with those of **2** and **3** (IC_{50}: 2.75 and 1.51 μg/ml, respectively). Compound **4** exhibited weaker inhibition (IC_{50}: 39 μg/ml), but compound **10** exhibited no inhibition at more than 100 μg/ml.

In conclusion, we have selected **1** as starting material to synthesize several isochromophilone analogues, and determined their inhibitory activities against gp120-CD4 binding. These results suggested that 7-hydroxyl group may be effective for the inhibition, but 8-carbonyl group may be not. Further studies will be reported later.

Table 1. Inhibitory activities against gp120-CD4 binding.

Compounds	IC_{50} (μg/ml)
1	>100
2	2.75
3	1.51
4	39
5	2.60
8	8.10
9	13
10	>100

Experimental

General Procedures

Melting points were measured on a Yamato melting point apparatus and not corrected. Fast atom bombardment mass spectra (FAB-MS) were taken on JEOL JMS-DX 300. IR spectra were obtained on a Perkin-Elmer 983G Infrared spectrometer. The ^{1}H NMR spectra were determined with a Varian VXR-300 and XL-400 spectrometers, in the solvent state, with tetramethyl-

silane (TMS) as an internal reference. Thin layer chromatography (TLC) was performed on kiesel gel $60F_{254}$ (Merck) plates, and spots were detected by ultraviolet (UV) and by spraying with 5% sulfuric acid solution. Column chromatography was conducted on silica gel 60 (70~230 mesh) (Merck).

7-Hydroxy-5-chloro-3-(3,5-dimethyl-1,3-heptadienyl)-7-methyl-6*H*-2-benzopyran-6,8(7*H*)-dione (**4**): **1** (500 mg, 1.30 mmol) was dissolved in anhydr. MeOH (100 ml), and NaOMe (70 mg, 1.26 mmol) was added. The solution was refluxed at 80°C for 5 hours, until no more starting material was found by TLC (*n*-hexane : acetone 3 : 1). Then the reaction solution was cooled to room temperature, and gradually acidified with Dowex 50w-1 (H^+) cation-exchange resin to pH 7~7.5. The resin was filtered and washed twice with MeOH. The filtrate was evaporated to dryness *in vacuo*, and purified by silica gel column chromatography (*n*-hexane : acetone 3 : 1) to yield **4** (400 mg, 89%).

4: Yellow powder, mp: 80~82°C, FAB-MS *m/z*: 349 $(M+1)^+$ (*m*-NBA as matrix), *Anal* Calcd for $C_{19}H_{21}O_4Cl$: C 65.51, H 6.03, Cl 10.06. Found: C 65.29, H 6.01, Cl 9.72. ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (3H, t, $J=7.8$ Hz, 7′-CH_3), 1.05 (3H, d, $J=7.2$ Hz, 9′-CH_3), 1.30 (1H, m, 6′-H), 1.44 (1H, m, 6′-H), 1.58 (3H, s, 9-CH_3), 1.84 (3H, s, 8′-CH_3), 2.48 (1H, m, 5′-H), 3.80 (1H, br s, 7-OH), 5.73 (1H, d, $J=10.2$ Hz, 4′-H), 6.08 (1H, d, $J=15.6$ Hz, 1′-H), 6.62 (1H, s, 4-H), 7.09 (1H, d, $J=15.6$ Hz, 2′-H), 7.93 (1H, s, 1-H).

5-Chloro-3-(3,5-dimethyl-1,3-heptadienyl)-7-hydroxy-7-methyl-8-(ethoxycarbonylmethyliden)-6*H*-2-benzopyran-6-one (**5**): **4** (400 mg, 1.15 mmol) was dissolved in anhydr. ether (30 ml), and $Ph_3P=CHCOOC_2H_5$ (446 mg, 1.28 mmol) was added. The solution was stirred at room temperature for 5 hours, until no more starting material was found by TLC (*n*-hexane : acetone 3 : 1). The solution was evaporated to dryness *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane : acetone 3 : 1) to yield **5** (340 mg, 70%).

5: Yellow powder, mp: 155~157°C, FAB-MS *m/z*: 419 $(M+1)^+$ (*m*-NBA as matrix), *Anal* Calcd for $C_{23}H_{27}O_4Cl$: C 66.03, H 6.46, Cl 8.37. Found: C 65.71, H 6.69, Cl 8.27. ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (3H, t, $J=7.8$ Hz, 7′-CH_3), 1.05 (3H, d, $J=7.2$ Hz, 9′-CH_3), 1.20 (3H, t, $J=7.8$ Hz, 10-$COOCH_2CH_3$), 1.28 (2H, m, 6′-H_2), 1.44 (3H, s, 9-CH_3), 1.83 (3H, s, 8′-CH_3), 2.47 (1H, m, 5′-H), 4.15 (2H, m, 10-$COOCH_2CH_3$), 4.27 (1H, br s, 7-OH), 5.66 (1H, d, $J=10.2$ Hz, 4′-H), 6.08 (1H, d, $J=15.6$ Hz, 1′-H), 6.59 (H, s, 10-H), 7.08 (1H, d, $J=15.6$ Hz, 2′-H), 8.07 (1H, s, 1-H).

5-Chloro-3-(3,5-dimethyl-1,3-heptadienyl)-7-hydroxy-7-methyl-8-(2-oxo-propyliden)-6*H*-2-benzopyran-6-one (**6**): **4** (300 mg, 0.862 mmol) was dissolved in anhydr. ether (30 ml), and $Ph_3P=CHCOCH_3$ (411 mg, 1.28 mmol) was added. The mixture was processed as described for **5** to yield **6** (87 mg, 30%).

6: Yellow powder, mp: 140~141°C, FAB-MS *m/z*: 389 $(M+1)^+$ (*m*-NBA as matrix), *Anal* Calcd for $C_{22}H_{25}O_4Cl$: C 68.04, H 6.44, Cl 9.02. Found: C 68.45, H 6.26, Cl 9.09. ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (3H, t, $J=7.8$ Hz, 7′-CH_3), 1.00 (3H, d, $J=7.2$ Hz, 9′-CH_3), 1.34 (2H, m, 6′-H_2), 1.44 (3H, s, 8-CH_3), 1.83 (3H, s, 8′-CH_3), 2.26 (3H, s, 10-$COCH_3$), 2.47 (1H, m, 5′-H), 4.32 (1H, br s, 7-OH), 5.68 (1H, d, $J=10.2$ Hz, 4′-H), 6.07 (1H, d, $J=15.6$ Hz, 1′-H), 6.57 (H, s, 10-H), 7.09 (1H, d, $J=15.6$ Hz, 2′-H), 8.27 (1H, s, 1-H).

5-Chloro-3-(3,5-dimethyl-1,3-heptadienyl)-7-acetyloxy-7-methyl-8-(ethoxycarbonylmethyliden)-6*H*-2-benzopyran-6-one (**7**): To a solution of **5** (100 mg, 0.24 mmol) in pyridine (20 ml), acetic anhydride (122 mg, 1.20 mmol, 5 eq) was added. The mixture was stirred at room temperature for 12 hours. The solution was poured into 0.5 N HCl aq. (20 ml), extracted with EtOAc (30 ml × 3), the extract was washed with saturated $NaHCO_3$ aq. and brine, dried with anhydrous Na_2SO_4, concentrated to dryness, and purified by silica gel column chromatography (*n*-hexane : acetone 5 : 1) to yield **7** (100 mg, 91%).

7: Yellow amorphous, FAB-MS *m/z*: 461 $(M+1)^+$ (*m*-NBA as matrix), *Anal* Calcd for $C_{25}H_{29}O_5Cl$: C 65.22, H 6.30, Cl 7.72. Found: C 65.10, H 6.42, Cl 7.90. ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (3H, t, $J=7.8$ Hz, 7′-CH_3), 0.99 (3H, d, $J=7.2$ Hz, 9′-CH_3), 1.24 (3H, t, $J=7.8$ Hz, 10-$COOCH_2CH_3$), 1.35 (2H, m, 6′-H_2), 1.54 (3H, s, 9-CH_3), 1.81 (3H, s, 8′-CH_3), 2.17 (3H, s, 7-OAc), 2.45 (1H, m, 5′-H), 4.14 (2H, m, 10-$COOCH_2CH_3$), 5.63 (1H, d, $J=10.2$ Hz, 4′-H), 5.85 (H, s, 10-H), 6.05 (1H, d, $J=15.0$ Hz, 1′-H), 6.51 (H, s, 4-H), 7.02 (1H, d, $J=15.0$ Hz, 2′-H), 8.12 (1H, s, 1-H).

5-Chloro-3-(3,5-dimethyl-1,3-heptadienyl)-6a-methyl-6*H*-furo[2,3-*h*]-2-benzopyran-6,8(6aH)-dione (**8**): **5** (330 mg, 0.79 mmol) was dissolved in anhydr. MeOH (50 ml), and NaOMe (158 mg, 1.54 mmol, 2 eq) was added. The solution was stirred at room temperature for 1 hour, until no more starting material was found by TLC (*n*-hexane : acetone 3 : 1). Then the solution was gradually acidified with Dowex50w-1(H^+) cationexchange resin to pH 7~7.5. The resin was filtered and washed twice with MeOH. The filtrate was evaporated to dryness *in vacuo*, and purified by silica gel column chromatography (*n*-hexane : acetone 4 : 1) to yield **8** (250 mg, 85%).

8: Yellow amorphous, FAB-MS *m/z*: 373 $(M+1)^+$ (*m*-NBA as matrix), *Anal* Calcd for $C_{21}H_{21}O_4Cl$: C 67.74, H 5.65, Cl 9.41. Found: C 67.82, H 5.57, Cl 9.70. ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, d, $J=7.8$ Hz, 7′-CH_3), 1.02 (3H, d, $J=7.2$ Hz, 9′-CH_3), 1.34 (1H, m, 6′-H), 1.44 (1H, m, 6′-H), 1.67 (3H, s, 9-CH_3), 1.85 (3H, s, 8′-CH_3), 2.48 (1H, m, 5′-H), 5.71 (1H, d, $J=10.2$ Hz, 4′-H), 5.99 (1H, s, 10-H), 6.09 (1H, d, $J=15.6$ Hz, 1′-H), 6.58 (1H, s, 4-H), 7.08 (1H, d, $J=15.6$ Hz, 2′-H), 7.63 (1H, s, 1-H).

9-Acetyl-5-chloro-3-(3,5-dimethyl-1,3-heptadienyl)-6a-methyl-6*H*-furo[2,3-*h*]-2-benzopyran-6,8(6aH)-dione (**9**): **4** (715 mg, 2.05 mmol) was dissolved in THF

(50 ml), Diketene (345 mg, 4.1 mmol, 2 eq) and DMAP (20 mg, 0.16 mmol, 2 eq) were added. The solution was stirred at room temperature for 5 hours, until no more starting material was found by TLC (*n*-hexane : acetone 3 : 1). The reaction solution was evaporated to dryness *in vacuo*, and purified by silica gel column chromatography (*n*-hexane : acetone 4 : 1) to yield **9** (460 mg, 54%) and 3,7-diacetyl-6-chloro-5-(2-oxo-5,7-dimethyl-3,5-nonadienyl)-9a-methyl-2*H*-furo[2,3-*h*]-1-benzopyran-2,8-dione (**10**) (28 mg, 3%).

9: Yellow amorphous, FAB-MS *m/z*: 415 $(M+1)^+$ (*m*-NBA as matrix), *Anal* Calcd for $C_{23}H_{24}O_5Cl$: C 66.67, H 5.80, Cl 8.45. Found: C 66.87, H 6.09, Cl 8.24. ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (3H, d, $J=7.6$ Hz, 7′-CH_3), 1.03 (3H, d, $J=7.2$ Hz, 9′-CH_3), 1.32 (1H, m, 6′-H), 1.45 (1H, m, 6′-H), 1.72 (3H, s, 9-CH_3), 1.87 (3H, s, 8′-CH_3), 2.48 (1H, m, 5′-H), 2.60 (3H, s, 10-$COCH_3$), 5.74 (1H, d, $J=9.2$ Hz, 4′-H), 6.09 (1H, d, $J=14.8$ Hz, 1′-H), 6.63 (1H, s, 4-H), 7.13 (1H, d, $J=14.8$ Hz, 2′-H), 8.84 (1H, s, 1-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.928 (7′-C), 12.353 (8′-C), 20.151 (9′-C), 26.333 (9-C), 29.694 (6′-C), 30.035 (10-CO*C*H_3), 30.081 (5′-C), 87.551 (7-C), 105.636 (4-C), 110.241 (1a-C), 115.627 (1′-C), 123.789 (10-C), 131.997 (3′-C), 139.530 (4a-C), 143.611 (2′-C), 149.384 (4′-C), 152.008 (1-C), 158.024 (3-C), 163.721 (8-C), 167.721 (5-C), 182.215 (10-*CO*CH_3), 183.072 (6-C), 194.277 (11-C).

10: Yellow amorphous, FAB-MS *m/z*: 455 $(M+1)^+$ (*m*-NBA as matrix). ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (3H, d, $J=7.6$ Hz, 7′-CH_3), 1.02 (3H, d, $J=7.2$ Hz, 9′-CH_3), 1.32 (1H, m, 6′-H), 1.44 (1H, m, 6′-H), 1.83 (3H, s, 8′-CH_3), 1.96 (3H, s, 9-CH_3), 2.50 (1H, m, 5′-H), 2.60 (3H, s, 14-CO*C*H_3), 2.78 (3H, s, 10-CO*C*H_3), 4.19 (1H, d, $J=16$ Hz, 4-H), 4.28 (1H, d, $J=16$ Hz, 4-H), 5.85 (1H, d, $J=9.2$ Hz, 4′-H), 6.24 (1H, d, $J=14.6$ Hz 1′-H), 7.38 (1H, d, $J=14.6$ Hz, 2′-H), 7.68 (1H, s, 1-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.903 (7′-C), 12.419 (9-*C*H_3), 14.937 (10-CO*C*H_3), 19.982 (9′-C), 25.916 (8′-C), 29.874 (6′-C), 30.428 (14-CO*C*H_3), 35.298 (5′-C), 41.958 (4-C), 84.705 (7-C), 122.649 (1′-C), 125.403 (4a-C), 128.938 (1-C), 128.991 (5-C), 130.379 (14-C), 131.737 (3′-C), 140.483 (6-C), 141.940 (1a-C), 142.668 (10-C), 150.724 (2′-C), 152.348 (4′-C), 152.909 (8-C), 166.300 (15-C), 189.116 (11-C), 193.190 (3-C), 200.686 (14-*CO*CH_3), 201.284 (10-*CO*CH_3).

Inhibition Experiments

The inhibitory activities against gp120-CD4 binding of these products were determined by enzyme-linked immunosorvent assay (ELISA) using recombinant soluble CD4 and recombinant gp120 as described by GILBERT, M., *et al.*[7] The reagents CD4 and gp120 and anti-CD4 used in the assay were generous gifts from Genentech Inc. (CA, U.S.A.).

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