

# Identification and Preliminary SAR Studies of (+)-Geodin as a Glucose Uptake Stimulator for Rat Adipocytes

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**Abstract** (+)-Geodin (**1**) was isolated from *Penicillium glabrum* AJ117540 with activity that stimulates glucose uptake by rat adipocytes. Unlike insulin it is active in the presence of wortmannin. Dihydrogeodin (**2**) and sulochrin (**3**) which are the precursors of (+)-geodin biosynthesis were also isolated from the same fungus. Preliminary SAR studies of **1** showed some analogues had enhanced activity. Especially, the activities of racemic geodin and dibromo analogue (**7a**) were comparable to that of the natural product.

**Keywords** (+)-geodin, glucose uptake stimulator, adipocytes

## Introduction

Insulin is a hormone, which regulates carbohydrate and lipid metabolisms, protein synthesis, and cell growth. The pathogenesis of type 2, non-insulin dependent diabetes mellitus (NIDDM) is complex, and not completely understood as yet, but appears to be the consequence of a combination of defective insulin production and secretion, and insulin resistance in peripheral target tissues [1]. Most of the drugs are insulin secretagogues or insulin sensitizers and their long term use causes resistance. Therefore, orally bioavailable small molecules which mimic insulin action are needed. In our search for novel antidiabetic agents with insulin-like effect, we screened over 6500 samples of natural product extracts for their ability to enhance glucose uptake activity on isolated rat adipocytes as an index. (+)-

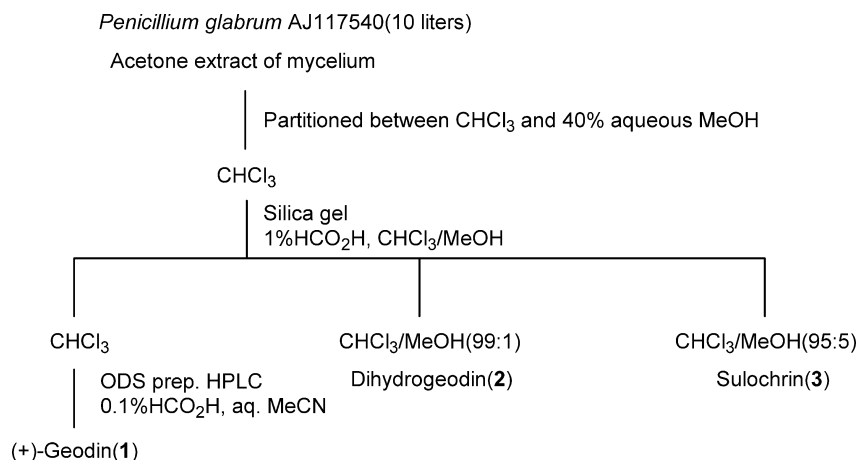
Geodin (**1**), a known fungal metabolite, was isolated from *Penicillium glabrum* AJ117540 as an active substance (Fig. 1). Dihydrogeodin (**2**) and sulochrin (**3**), the precursors of **1**, were also isolated from the same fungal extract. In this study, preliminary mechanistic insight and SAR are reported.

## Results and Discussion

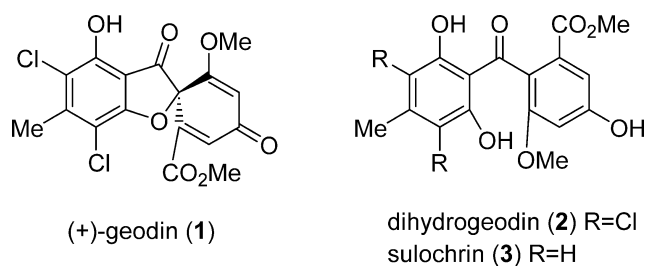
### Isolation and Identification of (+)-Geodin (**1**), Dihydrogeodin (**2**), and Sulochrin (**3**)

The fermentation broth was centrifuged to obtain mycelium, which was then extracted with acetone. The acetone extract was partitioned between  $\text{CHCl}_3$  and 40% aqueous MeOH. The  $\text{CHCl}_3$  extracts were combined and chromatographed on silica gel column with mixture of  $\text{CHCl}_3$  and MeOH containing 1% formic acid. Further purification was achieved by a preparative HPLC using an ODS column to give **1** as an active compound. **1** was previously isolated as a fungal metabolite with antibacterial activity [2] but its activity on glucose metabolism has been not reported so far. The related compound **2** and **3** were also isolated from same extract but they were devoid of glucose uptake stimulatory activity (Scheme 1).

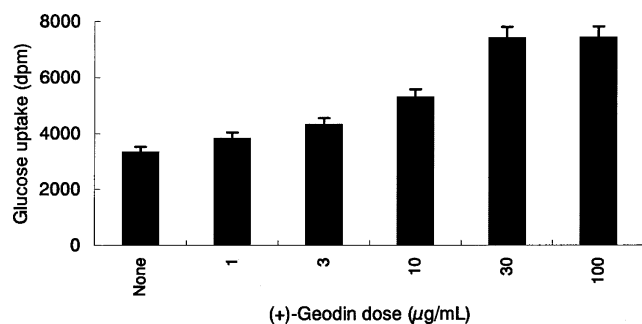
Compound **1** was obtained as a yellow solid. Negative ESI mass spectrum provided a molecular ion at  $m/z$  397 ( $\text{M}-\text{H}$ )<sup>-</sup>. Analyses of various NMR spectral data deduced that **1** was (+)-geodin. Finally, **1** was identified as (+)-geodin by comparison with an authentic specimen [3,4]. Compound **2** was obtained as a yellow solid, and **3** was as a



**Scheme 1** Isolation procedure for (+)-geodin (1), dihydrogeodin (2), and sulochrin (3)



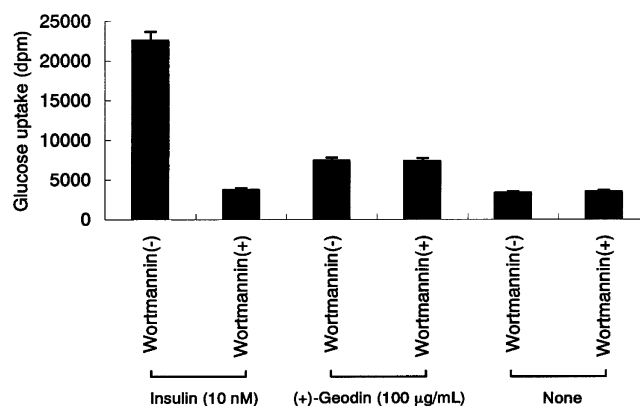
**Fig. 1** Structures of (+)-geodin (1), dihydrogeodin (2), and sulochrin (3).



**Fig. 2** (+)-Geodin-stimulated 2-deoxyglucose uptake by rat adipocytes.

Rat adipocytes were incubated for 30 minutes with various concentration of (+)-geodin. The cellular glucose uptake during further 5 minutes incubation with the addition of 2-deoxyglucose was determined.

white solid, respectively. The UV and <sup>1</sup>H NMR spectral data of **2** were good agreement with those of dihydrogeodin [4]. Therefore **2** was identified as dihydrogeodin. **3** was identified as sulochrin by comparison of the <sup>1</sup>H NMR and MS data with those of **2** (Fig. 1).

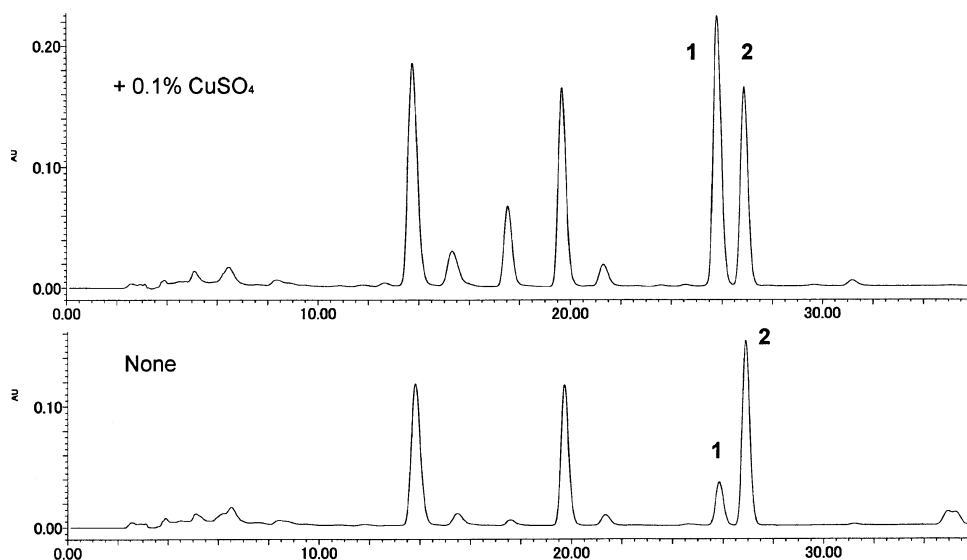


**Fig. 3** Effect of wortmannin on insulin and (+)-geodin-stimulated 2-deoxyglucose uptake by rat adipocytes.

Rat adipocytes were incubated for 30 minutes with 100 µg/ml of (+)-geodin with or without 0.1 µM of wortmannin. The cellular glucose uptake during further 5 minutes incubation with the addition of 2-deoxyglucose was determined.

### Glucose Uptake Activity

(+)-Geodin showed stimulation of glucose uptake by rat adipocytes in a dose dependent manner *in vitro*. Under the same conditions, 10 nM of insulin stimulated glucose uptake by 673% of that of basal uptake (Fig. 2). Zhang *et al.* reported that a small insulin mimetic, L-783,281 was isolated from a fungal extract of *Pseudomassaria* sp. It is a selective insulin receptor tyrosine kinase activator, and stimulates PI 3-kinase activation and phosphorylation of Akt kinase [5]. Insulin-stimulated glucose uptake was antagonized by 0.1 µM of wortmannin which is specific inhibitor of PI 3-kinase [6,7], however, the activity of **1** was not reduced (Fig. 3). Thus, it was deduced that (+)-geodin stimulates glucose uptake into cells by a different mechanism from those of insulin and L-783,281.



**Fig. 4** HPLC chromatograms of the acetone extract of *Penicillium glabrum* AJ117540.

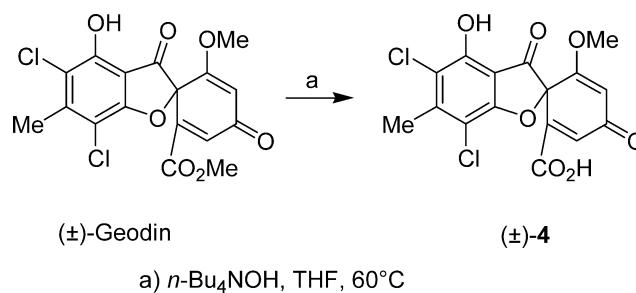
*Penicillium glabrum* AJ117540 was fermented in the producing medium with or without  $\text{CuSO}_4$ . HPLC was carried out on a column (Inertsil ODS-3, 0.6 i.d.  $\times$  15 cm) with linear gradient from 40% to 70% aqueous MeCN at a flow rate of 1.0 ml/minute.

#### Conversion of Dihydrogeodin to Racemic Geodin

The stimulating activity of glucose uptake of **1** prompted us to evaluate its activity *in vivo* and synthesize its analogues. However, the production of **1** (5~10 mg/liter) was not enough for derivatization and pharmacological tests *in vivo*. Further investigation revealed that mycelium extract of producing strain contained large quantities of dihydrogeodin (**2**), a biosynthetic precursor of (+)-geodin. Oxidation of **2** with DDQ gave 54% yield of racemic geodin [4], which was identical to **1** with respect to  $^1\text{H}$  NMR spectrum and chromatographic behavior on ODS HPLC. The activity of racemic **1** was comparable to that of the (+)-isomer.

#### Fermentation of AJ117540 Strain with $\text{CuSO}_4$

Sankawa *et al.* reported that *Aspergillus terreus* dihydrogeodin oxidase, enzyme which catalyzes the intramolecular stereospecific phenol oxidative coupling reaction, namely, converting dihydrogeodin to (+)-geodin, was a multicopper enzyme [3]. Fermentation of the producing strain in the medium containing  $\text{CuSO}_4$  resulted in improving productivity of (+)-geodin (up to 54 mg/liter) (Fig. 4). The amount of (+)-geodin reached the maximum after 3 days of growth without  $\text{Cu}^{2+}$ , however, addition of  $\text{CuSO}_4$  at this stage show little improvement in productivity (data not shown). These results indicated that the *P. glabrum* dihydrogeodin oxidase is also a copper enzyme.



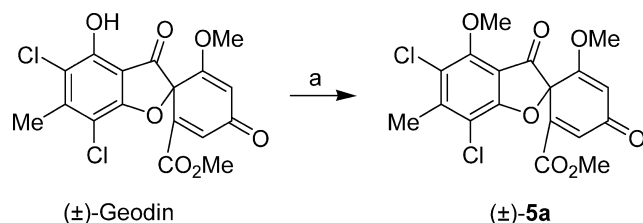
**Scheme 2**

#### Synthesis and Glucose Uptake Activity of Geodin Analogues

A series of geodin analogues were synthesized to evaluate stimulating activity of glucose uptake of related compounds. Since the stereochemistry of the spiro carbon is not important for the activity, SAR was studied with racemic derivatives. Treatment of ( $\pm$ )-geodin with tetra-*n*-butylammonium hydroxide [8] resulted in hydrolysis to give the carboxylic acid analogue erdin (**4**) (Scheme 2). The phenolic hydroxyl group of ( $\pm$ )-geodin was alkylated to afford various ether derivatives including methyl (**5a**), benzyl (**5b**), *tert*-butoxycarbonylmethyl (**5c**), and carboxymethyl ether (**5d**). The synthesis of methyl ether **5a** is outlined in Scheme 3. ( $\pm$ )-Geodin was reacted with methyl iodide in the presence of  $\text{K}_2\text{CO}_3$  in DMF to give methyl ether derivative **5a**. **5b~d** were also prepared in a similar method (Fig. 5). The derivatives **7** with bromine and

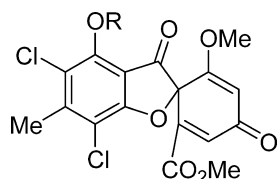
iodine atom in place of chlorine atom on benzene ring were also synthesized. Sulochrin (**3**) isolated from the same fungus was brominated with benzyltrimethylammonium tribromide to obtain **6**, which was converted to **7a** by oxidation with DDQ. Iodinated analogue **7b** was prepared from **3** by treatment with iodine/potassium iodide (Scheme 4).

Stimulation of glucose uptake in rat adipocytes by the



a) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt

**Scheme 3**



(±)-**5a** R=Me  
 (±)-**5b** R=Bn  
 (±)-**5c** R=CH<sub>2</sub>CO<sub>2</sub>t-Bu  
 (±)-**5d** R=CH<sub>2</sub>CO<sub>2</sub>H

**Fig. 5** Structures of racemic geodin analogues.

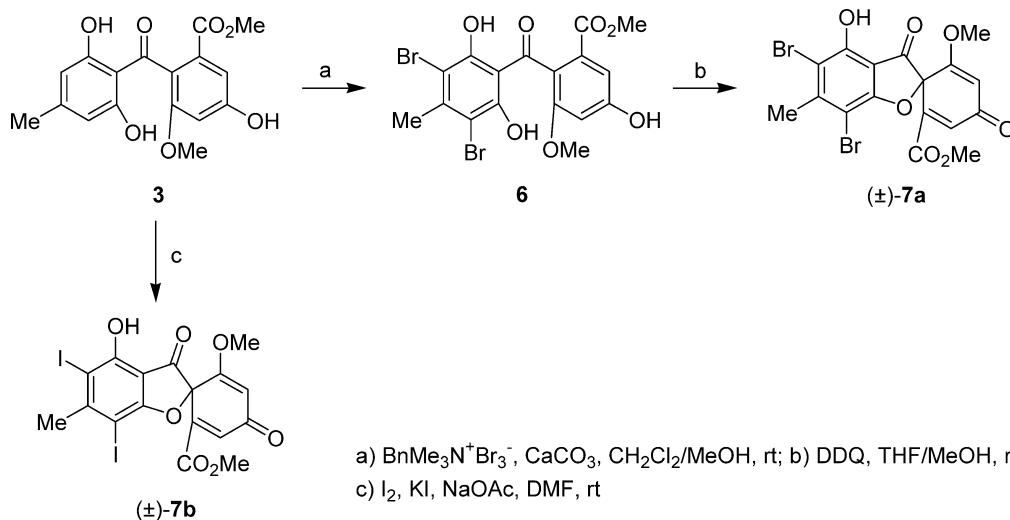
geodin analogues at a dose of 30 μg/ml are shown in Table 1.

The ether derivatives except for **5d**, and bromine or iodine substituted analogues retained activity, however, the activity of the ethers became weak dependent on the size of substituent. In contrast, more polar compounds **4** and **5d** were inactive. It is demonstrated that phenolic hydroxyl group is not necessary to exhibit desired activity. Inactive compounds may have low affinity for the target molecule or low permeability into cells. Under the assay conditions, geodin and its analogues did not show cytotoxic activity. Evaluation of the glucose uptake mechanism and pharmacological study *in vivo*, and further synthesis of analogues are in progress.

**Table 1** Various geodin analogues stimulated glucose uptake at a dose of 30 μg/ml

Compound	2-[ <sup>14</sup> C]-deoxyglucose uptake (T/C) <sup>a</sup>
(±)- <b>4</b>	1.09
(±)- <b>5a</b>	1.83
(±)- <b>5b</b>	1.62
(±)- <b>5c</b>	1.38
(±)- <b>5d</b>	1.08
(±)- <b>7a</b>	3.07
(±)- <b>7b</b>	2.07
(±)- <b>1</b>	2.44

<sup>a</sup> T/C=drug stimulated uptake/basal uptake.



**Scheme 4**

## Experimental

### General Methods

$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded with a Varian XL-300 spectrometer and JEOL JNM A-400 spectrometer. Mass spectra were recorded on JEOL MS700V spectrometer, JEOL JMS-HX110 spectrometer and Thermoelectron TSQ700 spectrometer. Optical rotation was recorded on a JASCO DIP-370. Silica gel column chromatography was carried out on Wakogel C-300. Silica gel TLC was carried out on Merck Silica gel 60F<sub>254</sub> 13792 and Silica gel 60F<sub>254</sub> 13794, respectively.

### Fermentation

Strain AJ117540 was isolated from a soil sample collected at Okinawa prefecture, Japan. The strain was identified to be *Penicillium glabrum* by morphological characteristics. A seed medium containing mashed potatoes 2.0%, glucose 0.5%, NZ-Case (Humco) 0.2%, yeast extract (Difco) 0.2%, NaCl 0.2%, and CaCO<sub>3</sub> 0.3% (pH 7.0) was inoculated with a slant culture of strain AJ117540, and incubated at 25°C on a rotary shaker (180 rpm) for 96 hours. The resultant seed culture was transferred to a hundred 500 ml Erlenmeyer flasks containing 100 ml of a producing medium composed of glucose 2.0%, mannitol 2.0%, glycerol 0.5%, corn germ meal 2.0%, EBIOS 1.0%, amino acid mixture for culture media (Ajinomoto) 3.0%, and CaCO<sub>3</sub> 0.4% (pH 6.8). The fermentation was carried out at 25°C on a rotary shaker (180 rpm) for 96 hours.

### Fermentation of Producing Strain with CuSO<sub>4</sub>

The fungus AJ117540 was fermented in the producing medium described above containing CuSO<sub>4</sub>·5H<sub>2</sub>O (0.1%) in the same condition.

### Isolation of (+)-Geodin (1), Dihydrogeodin (2), and Sulochrin (3)

The fermentation broth (10 liters) was centrifuged at 7000 rpm for 20 minutes to obtain mycelium, which was extracted with acetone (5 liters) at room temperature. The acetone extract was concentrated *in vacuo* to give a water suspension (600 ml). After addition of MeOH (400 ml), aqueous MeOH solution was extracted with CHCl<sub>3</sub> (500 ml×3). The CHCl<sub>3</sub> fractions were combined and concentrated to dryness. The brown oily residue (5.0 g) was applied to a silica gel column (80 g, 3.0 i.d.×27 cm) eluting with CHCl<sub>3</sub>, CHCl<sub>3</sub>/MeOH (99:1) and CHCl<sub>3</sub>/MeOH (99:5) containing 1% formic acid. The fraction eluted with CHCl<sub>3</sub> was purified on preparative reversed phase HPLC (column; Inertsil ODS-3, 1.0 i.d.×15 cm) with linear

gradient from 50% to 80% aqueous MeCN containing 0.1% formic acid at a rate of 3.0 ml/minute to yield 4 mg of (+)-geodin (1) as yellow solid. The fraction eluted with CHCl<sub>3</sub>/MeOH (99:1) gave 300 mg of dihydrogeodin (2) as yellow solid, and the fraction eluted with CHCl<sub>3</sub>/MeOH (95:5) gave 650 mg of crude sulochrin (3).

(+)-Geodin (1):  $[\alpha]_D^{22} +119^\circ$  (*c* 0.08, THF); ESI MS *m/z* 397 (M-H)<sup>-</sup>;  $^1\text{H}$  NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.13 (1H, d, *J*=1.2 Hz), 5.81 (1H, d, *J*=1.2 Hz), 3.89 (3H, s), 3.69 (3H, s), 2.56 (3H, s);  $^{13}\text{C}$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  193.3, 185.0, 167.9, 165.5, 163.4, 149.4, 146.6, 137.5, 137.0, 114.7, 109.4, 108.8, 104.4, 84.5, 57.0, 53.1, 18.7.

Dihydrogeodin (2): ESI MS *m/z* 399 (M-H)<sup>-</sup>;  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.71 (2H, br s), 10.06 (1H, br s), 6.92 (1H, d, *J*=2.1 Hz), 6.69 (1H, d, *J*=2.1 Hz), 3.65 (3H, s), 3.64 (3H, s), 2.42 (3H, s).

Sulochrin (3): ESI MS *m/z* 331 (M-H)<sup>-</sup>;  $^1\text{H}$  NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.41 (2H, br s), 9.95 (1H, br s), 6.88 (1H, d, *J*=1.8 Hz), 6.65 (1H, d, *J*=1.8 Hz), 6.06 (2H, s), 3.62 (3H, s), 3.61 (3H, s), 2.48 (3H, t, *J*=1.8 Hz).

### Conversion of Dihydrogeodin to Racemic Geodin

DDQ (105 mg, 0.46 mmol) was added to a solution of dihydrogeodin (107 mg, 0.27 mmol) in CHCl<sub>3</sub>/MeOH (1:1) (2 ml), and stirred for 3.0 hours at room temperature. After concentration, the resulting material was purified silica gel TLC developed with CHCl<sub>3</sub>/MeOH (97:3) containing 1% formic acid to give racemic geodin in 54% yield.

### Synthesis of Geodin Analogues

#### 5,7-Dichloro-4,6'-dimethoxy-6-methyl-3,4'-dioxospiro [benzofuran-2(3*H*),1'-[2,5]cyclohexadiene]-2'-carboxylic Acid (4)

40% aqueous tetra-*n*-butylammonium hydroxide (45  $\mu\text{l}$ ) was added to a solution of (+)-geodin (12 mg, 30  $\mu\text{mol}$ ) in THF (0.2 ml), and stirred at 60°C for 10 minutes. After addition of 5% HCl, the mixture was extracted with ethyl acetate. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The residue was purified by silica gel TLC developed with CHCl<sub>3</sub>/MeOH (95:5) containing 1% formic acid to yield 4.

43%; ESI MS *m/z* 383 (M-H)<sup>-</sup>;  $^1\text{H}$  NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  6.96 (1H, d, *J*=1.2 Hz), 5.88 (1H, d, *J*=1.2 Hz), 3.73 (3H, s), 2.54 (3H, s).

#### Methyl 5,7-Dichloro-4,6'-dimethoxy-6-methyl-3,4'-dioxospiro[benzofuran-2(3*H*),1'-[2,5]cyclohexadiene]-2'-carboxylate (5a)

K<sub>2</sub>CO<sub>3</sub> (2 mg, 14  $\mu\text{mol}$ ) and methyl iodide (3  $\mu\text{l}$ , 32  $\mu\text{mol}$ ) were added to a solution of ( $\pm$ )-geodin (4.5 mg, 11.2  $\mu\text{mol}$ ) in DMF (0.2 ml), and stirred for 5 hours at room



temperature. After addition of water, the mixture was extracted with ethyl acetate. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The residue was purified by silica gel TLC developed with  $\text{CHCl}_3$  to yield **5a**.

64%; FAB MS  $m/z$  413 ( $\text{M}+\text{H}$ )<sup>+</sup>; HR-MS  $m/z$  413.0202 ( $\text{M}+\text{H}$ )<sup>+</sup> calcd  $\text{C}_{18}\text{H}_{15}\text{O}_7^{35}\text{Cl}_2$  ( $\Delta +0.7$  mmu); <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.13 (1H, d,  $J=1.5$  Hz), 5.81 (1H, d,  $J=1.5$  Hz), 4.12 (3H, s), 3.74 (3H, s), 3.70 (3H, s), 2.59 (3H, s).

Methyl 4-Benzoyloxy-5,7-dichloro-6'-methoxy-6-methyl-3,4'-dioxospiro[benzofuran-2(3H),1'-[2,5]-cyclohexadiene]-2'-carboxylate (5b)

92%; FAB MS  $m/z$  489 ( $\text{M}+\text{H}$ )<sup>+</sup>; HR-MS  $m/z$  489.0506 ( $\text{M}+\text{H}$ )<sup>+</sup> calcd  $\text{C}_{24}\text{H}_{19}\text{O}_9^{35}\text{Cl}_2$  ( $\Delta -0.2$  mmu); <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.53 (2H, br d,  $J=7.2$  Hz), 7.34~7.40 (3H, m), 7.15 (1H, d,  $J=1.8$  Hz), 5.82 (1H, d,  $J=1.8$  Hz), 5.38 (1H, d,  $J=11.1$  Hz), 5.29 (1H, d,  $J=11.1$  Hz), 3.74 (3H, s), 3.68 (3H, s), 2.58 (3H, s).

Methyl 4-(tert-Butoxycarbonylmethoxy)-5,7-dichloro-6'-methoxy-6-methyl-3,4'-dioxospiro[benzofuran-2(3H),1'-[2,5]cyclohexadiene]-2'-carboxylate (5c)

91%; FAB MS  $m/z$  512 ( $\text{M}+\text{H}$ )<sup>+</sup>; HR-MS  $m/z$  512.0620 ( $\text{M}+\text{H}$ )<sup>+</sup> calcd  $\text{C}_{23}\text{H}_{22}\text{O}_9^{35}\text{Cl}_2$  ( $\Delta -2.1$  mmu); <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.12 (1H, d,  $J=2.1$  Hz), 5.79 (1H, d,  $J=2.1$  Hz), 5.02 (1H, d,  $J=15.9$  Hz), 4.95 (1H, d,  $J=15.9$  Hz), 3.72 (3H, s), 3.67 (3H, s), 2.59 (3H, s), 1.44 (9H, s).

Methyl 4-Carboxymethoxy-5,7-dichloro-6'-methoxy-6-methyl-3,4'-dioxospiro[benzofuran-2(3H),1'-[2,5]-cyclohexadiene]-2'-carboxylate (5d)

TFA (0.1 ml) was added to a solution of **5c** (5 mg, 9.7  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (0.5 ml), and stirred for 1 hour at room temperature. The reaction mixture was concentrated *in vacuo* to give carboxylic acid **5d**.

100%; ESI MS  $m/z$  457 ( $\text{M}+\text{H}$ )<sup>+</sup>; HR-MS  $m/z$  457.0088 ( $\text{M}+\text{H}$ )<sup>+</sup> calcd  $\text{C}_{19}\text{H}_{15}\text{O}_9^{35}\text{Cl}_2$  ( $\Delta -1.1$  mmu); <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.14 (1H, d,  $J=1.2$  Hz), 5.83 (1H, s), 4.89 (2H, br s), 3.75 (3H, s), 3.70 (3H, s), 2.62 (3H, s).

Methyl 3',5'-Dibromo-2',4,6'-trihydroxy-6-methoxy-4'-methylbenzophenone-2-carboxylate (6)

$\text{CaCO}_3$  (25 mg, 0.25 mmol) and benzyltrimethylammonium tribromide (95 mg, 0.24 mmol) were added to a solution of sulochrin (40 mg, 0.12 mmol) in  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (1 ml), and stirred for 30 minutes at room temperature. Then  $\text{CH}_2\text{Cl}_2$  was added into reaction mixture, and washed with 5% HCl. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , and concentrated *in vacuo*. The residue was purified by silica gel TLC developed with  $\text{CHCl}_3/\text{MeOH}$  (95 : 5) to give **6**.

63%; ESI MS  $m/z$  487 ( $\text{M}-\text{H}$ )<sup>-</sup>; <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3/\text{DMSO}-d_6$ )  $\delta$  6.93 (1H, d,  $J=1.5$  Hz), 6.57 (1H, d,  $J=1.5$  Hz), 3.63 (3H, s), 3.61 (3H, s), 2.52 (3H, s).

Methyl 5,7-Dibromo-4-hydroxy-6'-methoxy-6-methyl-3,4'-dioxospiro[benzofuran-2(3H),1'-[2,5]cyclohexadiene]-2'-carboxylate (7a)

Compound **6** was converted to **7a** by phenol oxidative coupling reaction with DDQ described above.

54%; FAB MS  $m/z$  487 ( $\text{M}+\text{H}$ )<sup>+</sup>; HR-MS  $m/z$  486.9023 ( $\text{M}+\text{H}$ )<sup>+</sup> calcd  $\text{C}_{17}\text{H}_{13}\text{O}_7^{79}\text{Br}_2$  ( $\Delta -0.5$  mmu); <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.15 (1H, d,  $J=1.5$  Hz), 5.83 (1H, d,  $J=1.5$  Hz), 3.74 (3H, s), 3.70 (3H, s), 2.69 (3H, s).

Methyl 4-Hydroxy-5,7-diiodo-6'-methoxy-6-methyl-3,4'-dioxospiro[benzofuran-2(3H),1'-[2,5]cyclohexadiene]-2'-carboxylate (7b)

Iodine (16 mg, 63  $\mu\text{mol}$ ), potassium iodide (11 mg, 66  $\mu\text{mol}$ ) and sodium acetate (5 mg, 61  $\mu\text{mol}$ ) were added to a solution of sulochrin (20 mg, 60  $\mu\text{mol}$ ) in DMF (1 ml). The reaction mixture was stirred for 14 hours at room temperature. Then ethyl acetate was added into reaction mixture, and washed with 5% sodium hydrogen sulfite. The organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was purified by silica gel TLC developed with  $\text{CHCl}_3/\text{MeOH}$  (96 : 4) to give **7b**.

11%; FAB MS  $m/z$  581 ( $\text{M}-\text{H}$ )<sup>-</sup>; HR-MS  $m/z$  580.8632 ( $\text{M}-\text{H}$ )<sup>-</sup> calcd  $\text{C}_{17}\text{H}_{11}\text{O}_7\text{I}_2$  ( $\Delta +3.7$  mmu); <sup>1</sup>H NMR (300 MHz,  $\text{DMSO}-d_6$ )  $\delta$  7.01 (1H, d,  $J=1.5$  Hz), 6.02 (1H, d,  $J=1.5$  Hz), 3.69 (3H, s), 3.64 (3H, s), 2.79 (3H, s).

### Preparation of Rat Adipocytes

Adipocytes were prepared from male Wistar rats (150~200 g). Epididymal fat pads (6.0 g) were cut into small pieces in the medium containing NaCl (130 mM), KCl (4.7 mM),  $\text{KH}_2\text{PO}_4$  (1.2 mM),  $\text{MgSO}_4$  (1.2 mM), CaCl<sub>2</sub> (1 mM), HEPES (25 mM) with 5% BSA (pH 7.6) (6 ml). After addition of type I collagenase (2.4%) into the medium, these tissues were treated with the enzyme for 40 minutes to obtain isolated adipocytes. After removal of collagenase by washing and flotation with fresh medium, the new buffer consisting with 2% BSA was added to give the suspension of adipocytes (45 ml).

### Glucose Uptake Activity

Adipocytes pretreated with each compound at 37°C for 30 minutes were incubated with further addition of 2-[<sup>14</sup>C]-deoxy-D-glucose (0.5  $\mu\text{Ci}$ ) for 5 minutes. The glucose uptake was terminated by the addition of 10  $\mu\text{M}$  of cytochalasin B followed by extraction with dinonylphthalate and centrifuging to obtain an oil layer for

determination of radioactivity of 2-[U-<sup>14</sup>C]-deoxy-D-glucose in the adipocytes.

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## References

1. Simpson IA, Cushman SW. Hormonal regulation of mammalian glucose transport. *Annu Rev Biochem* 55: 1059–1089 (1986), and references cited therein.
2. Marcus S. Antibacterial activities of geodin and erdin. *Biochem J* 41: 462–463 (1947)
3. Fujii I, Iijima H, Tsukita S, Ebizuka Y, Sankawa U. Purification and properties of dihydrogeodin oxidase from *Aspergillus terreus*. *J Biochem* 101: 11–18 (1987)
4. Kato T, Omori O, Iwasaki K, Inoue M. Synthetic studies on Sch 202596, an antagonist of the galanin receptor subtype GalR1: an efficient synthesis of (±)-geodin, the spirocoumaranone part of Sch 202596. *Tetrahedron* 58: 1289–1299 (2002)
5. Zhang B, Salituro G, Szalkowski D, Li Z, Zhang Y, Royo I, Viella D, Diez MT, Pelaez F, Ruby C, Kendall RL, Mao X, Griffin P, Galaycay J, Zierath JR, Heck JV, Smith RG, Mollar DE. Discovery of a small molecule insulin mimetic with antidiabetic activity in mice. *Science* 284: 974–977 (1999)
6. Okada T, Sakuma R, Fukui Y, Hazeki O, Ui M. Blockage of chemotactic peptide-induced stimulation of neutrophils by wortmannin as a result of selective inhibition of phosphatidylinositol 3-kinase. *J Biol Chem* 269: 3563–3567 (1994)
7. Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M. Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. *J Biol Chem* 269: 3568–3573 (1994)
8. Abdel-Magid AF, Cohen JH, Maryanoff CF, Shah RD, Villani FJ, Zhang F. Hydrolysis of polypeptide esters with tetrabutylammonium hydroxide. *Tetrahedron Lett* 39: 3391–3394 (1998)