15-Deoxyspergualin InhibitsAkt Kinase Activation and
Phosphatidylcholine Synthesis*

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15-Deoxyspergualin (DSG) strongly inhibited growth of mouse EL-4 lymphoma cells in vitro and in vivo. It significantly prolonged the survival days of EL-4-transplanted mice. In vitro study revealed that its antiproliferative effect appeared only after 2 days of treatment. At that time, protein synthesis was significantly inhibited rather than DNA and RNA synthesis. Furthermore, DSG induced apoptosis without arresting the cell cycle. p70 S6 kinase (p70S6K), a key molecule in protein synthesis, was inhibited by 2 days of treatment of DSG. Akt, an upstream kinase of p70S6K, was also deactivated by 2 days of treatment of DSG. Hsp90 is reported to bind to and stabilize Akt kinase and also to bind to DSG. Yet DSG did not inhibit the binding of Hsp90 to Akt kinase. PI3-kinase, an activator of Akt, was not affected by DSG treatment. However, when we looked into phospholipid synthesis, we found that DSG inhibited phosphatidylcholine (PC) synthesis strongly rather than phosphatidylinositol even by 1 day of treatment. Moreover, DSG failed to inhibit Akt kinase activation and PC synthesis in DSG-less sensitive human K562 leukemia cells. These results demonstrate that DSG inhibits tumor cell growth through the inhibition of protein synthesis and induction of apoptosis, which is caused by the down-regulation of Akt kinase and p70S6K. It is also indicated that the down-regulation of Akt kinase by DSG should not depend on PI3-kinase and Hsp90. There might be possible involvement of PC in Akt kinase activity.

Protein synthesis is regulated by many molecules. Among them, p70 S6 kinase (p70S6K),1 a serine/threonine kinase, is one of the key molecules. p70S6K phosphorylates the 40-S ribosomal S6 protein, resulting in the translational up-regulation of mRNAs (1, 2). p70S6K is activated by the phosphatidylinositol 3-kinase (PI3K)-Akt pathway or FRAP (a mammalian target of rapamycin) (3) upon stimulation of growth signals. Rapamycin is an inhibitor of p70S6K activation (4, 5) and inhibits FRAP by forming a complex with the immunophilin FK506-binding protein (6), which binds to and inhibits FRAP (7). PI3K consists of p110 catalytic and p85 regulatory subunits (8) and generates the intracellular amounts of phosphatidylinositol-3,4-biphosphate (PIP₂) and phosphatidylinositol-3, 4,5-triphosphate (PIP₃) (9). Wortmannin is an inhibitor of PI3K and inhibits various downstream events of PI3K including Akt and p70S6K activations (10–13). Akt (also known as protein kinase B) is also a serine/threonine kinase, which mediates PI3K-regulated biological events. By stimulation, Akt is recruited from the cytosol to the plasma membrane and is phosphorylated at two key regulatory sites, Thr-308 in the catalytic domain and Ser-473 at the C terminus (14). This translocation requires PI3K activation and the pleckstrin homology domain, through which Akt directly interacts with PIP₂ or PIP₃ (13, 15, 16). The membrane-bound Akt is then phosphorylated by 3-phosphoinositide-dependent kinase-1 (PDK1) (17). One of targets of Akt is p70S6K, and the PI3K-Akt pathway activates p70S6K (12). Akt also plays an important role in a cell survival signaling pathway and inactivates BAD, a Bcl-2 family protein, rendering it incapable of blocking Bcl-2 activity (18, 19).

15-Deoxyspergualin (DSG) is the most potent synthetic analogue of spergualin, which was isolated as an antitumor compound from microbial cultured broth (20–22). DSG inhibits growth of various tumor cell lines in vitro and in vivo (23, 24). Furthermore, DSG has a potent immunosuppressive effect and has been used clinically as an immunosuppressant (25, 26). It is reported that DSG binds to heat shock proteins (HSPs) such as Hsc70 and Hsp90 and inhibits their functions (27, 28). In respect to the antitumor effect, DSG is reported to inhibit the cell cycle progression at G1 phase (29, 30) and tumorigenic angiogenesis (31, 32). However, the precise mechanism of DSG action remains to be elucidated. We have been studying the mechanism of DSG action concerning its antitumor effect. DSG has a spermidine and a guanidine moiety in its structure. Focusing on its structural characteristic, we have recently reported that the spermidine moiety has cell binding activity and that the guanidine moiety has cytotoxic activity (33). Moreover, it is suggested that its action should not be related to polyamine synthesis. In this study, we have further investigated the precise mechanism of DSG action on tumor cell growth. Finally, we have found that DSG inhibits Akt kinase activation and phosphatidylcholine synthesis.

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—DSG was provided from Takara Shuzou Co. Ltd. (Otsu, Japan). [3H]Thymidine, [3H]uridine, [3H]leucine, [γ-32P]ATP, [32P]phosphoric acid, and [14C]choline chloride were purchased from PerkinElmer Life Sciences. Antibodies used for Western blotting and immunoprecipitation were the following: anti-p70S6K (sc-230), anti-Akt (sc-1618), anti-Hsc70 (sc-1059), anti-Hsp90 (sc-7947) were from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phospho-Akt was from New England Biolabs (Beverly, MA); anti-PI3K p85 was from Upstate Biotechnology (Lake Placid, NY).

Cells—Mouse EL-4 lymphoma cells and human K562 leukemia cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine

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1 The abbreviations used are: p70S6K, p70 S6 kinase; DSG, 15-deoxyspergualin; PI, phosphatidylinositol; PI3K, phosphatidylinositol 3-kinase; PIP₂, phosphatidylinositol-3,4-biphosphate; PIP₃, phosphatidylinositol-3,4,5-triphosphate; PC, phosphatidylcholine; FRAP, FKBP12-rapamycin-associated protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HSP, heat shock proteins.
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serum JRH Biosciences, Lenexa, KS), 100 units/ml penicillin G, and 100 μg/ml streptomycin at 37 °C with 5% CO2.

**Experimental Chemotherapy**—Female C57BL/6 mice, 6 weeks old, were purchased from Charles River Breeding Laboratories (Yokohama, Japan) and maintained in a specific pathogen-free barrier facility. Mice (five per group) were transplanted with EL-4 cells (1 × 105) intraperitoneally or subcutaneously, and DSG was administered intraperitoneally the day after the tumor inoculation. The antitumor effect was evaluated by T/C (%) calculated from the median survival time of the control group to that of the treated group as described (34).

**Cell Growth**—Cells were inoculated into 96-well plates at 3000 cells/well and incubated with or without DSG for the indicated times. Tumor volume was estimated using the formula: Tumor volume (mm3) = (length × width)2/2. The growth was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described (34).

**Cell Cycle Analysis**—(Cells × 105) were cultured in 10-cm dishes with or without DSG for 3 days and then fixed with ice-cold 70% ethanol. The fixed cells were treated with 0.1% RNase (Sigma) at 37 °C for 15 min and then resuspended in phosphate-buffered saline containing 50 μg/ml propidium iodide (Sigma). DNA fluorescence was measured with a FACSCalibur (BD PharMingen).

**DNA Fragmentation**—Cells were cultured in 10-cm dishes with or without DSG for 3 days, and then 1 × 106 cells were washed with phosphate-buffered saline and lysed in 100 μl of lysis buffer containing 0.5% Triton X-100, 10 mM Tris-HCl (pH 7.4), and 10 mM EDTA at room temperature for 10 min. The supernatant fractions were collected by centrifugation at 15,000 rpm for 10 min and treated with RNase A at 37 °C for 1 h. The DNA in these fractions was precipitated overnight with 20 μl of 5 mM NaCl and 120 μl of 2-isopropanol at −20 °C. DNA was dissolved in 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA buffer and separated on 1% agarose gels.

**Macromolecular Synthesis**—Cells were inoculated into 12-well plates at 3 × 104 cells/ml and cultured with or without DSG for the indicated times. For the last 1 h of the culture, [3H]thymidine, [3H]uridine, or [3H]leucine was added to the culture at 1 μCi/ml. The radioactivities in 10% trichloroacetic acid-insoluble materials were counted using a liquid scintillation counter.

**Preparation of Cell Lysates and Western Blotting**—(Cells × 105) were cultured in 35-mm dishes with or without test chemicals for the indicated times. The cells were washed twice with ice-cold phosphate-buffered saline containing 100 μM Na2VO4 and then lysed in a lysis buffer (20 mM Hepes, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 50 mM NaF, 50 mM β-glycerophosphate, 1 mM Na3VO4, and 25 μg/ml each of antipain, leupeptin, and pepstatin). Equal protein extracts were separated by SDS-PAGE and transferred onto Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA). Enhanced chemiluminescence (Amersham Biosciences) was used to visualize the immunoblot signals.

**Immunoprecipitation**—Cell lysates were prepared as described above, and equal amounts of protein were incubated with the indicated antipeptide antisera and immune complexes were collected on protein A-Sepharose beads (Amersham Biosciences) or protein G-agarose beads (Santa Cruz Biotechnology) and washed four times in immunoprecipitation buffer (20 mM Hepes, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 50 mM NaF, 50 mM β-glycerophosphate, 0.1 mM phenylmethylsulfonyl fluoride, and 0.2 mM Na2VO4). S6 Kinase Assay—Cells (3 × 105) were cultured in 35-mm dishes with or without test chemicals for the indicated times. The cell lysates were prepared and immunoprecipitated with anti-p70S6K antibody as described above. The S6 kinase activity of the immune complexes was determined by using S6 peptide as a substrate as instructed by the manufacturer (Upstate Biotechnology). Kinase activity was quantitated as the counts per minute of 32P label incorporated into the S6 peptide.

**PI3K Assay**—PI3K activity was measured with a modification of the method of Coffer et al. (35). Cells were resuspended for 30 min at 4 °C in a lysis buffer (1% Triton X-100, 20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 10 mM Na2PO4, 2 mM EDTA, 50 mM NaF, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na2VO4, and 25 μg/ml each of antipain, leupeptin, and pepstatin). The lysates were incubated for 1 h at 4 °C in an anti-PI3K phosphotyrosine antibody, and then further incubated with protein A-Sepharose beads for 1 h at 4 °C. The immune complexes were washed three times with a lysis buffer and twice with 10 mM Tris-HCl, pH 7.4, containing 1 mM Na2VO4. Reactions were performed by adding 100 μg of sonicated phosphatidylinositol, 20 μCi of [γ-32P]ATP, 200 μM adenosine, 30 mM MgCl2, and 35 μM ATP in a total volume of 60 μl. After 20 min at 37 °C, 100 μl of 1 M HCl and 200 μl of CHCl3-MeOH (1:1) were added to stop reaction. After centrifugation and removal of the upper layer, 80 μl of MeOH-HCl (1:1) was added. After further centrifugation, lipids were separated on a silica gel TLC plate (Merck) with a solvent system of CHCl3-MeOH-NH4OH (45:35:10). Radiolabeled spots on a TLC plate were analyzed by autoradiography.

**Phospholipid Synthesis**—Phospholipid synthesis was assessed by the method of Horwitz et al. (36) with a modification. Cells (3 × 105) were incubated with or without DSG for the indicated times. The cells were washed twice with phosphate-buffered saline and further incubated in phosphate-free minimum essential medium containing 10% dialyzed fetal bovine serum and 10 μCi/ml [32P]phosphoric acid for 1 h at 37 °C. The cells were pelleted by a brief centrifugation and resuspended in 0.5 ml of ice-cold 10% trichloroacetic acid. The acid-insoluble materials were washed once with water and resuspended in 0.5 ml of water. Lipids were extracted by vigorous vortex for 2 min with 1.25 ml of MeOH and 0.625 ml of CHCl3. After 10 min left at room temperature, lipids were further extracted by vigorous vortex for 30 s with 0.625 ml of CHCl3 and then with 0.625 ml of water. After centrifugation at 2,500 rpm for 5 min, 1 ml of CHCl3 layer was removed and dried up. The extracted lipids were dissolved in CHCl3-MeOH (1:1) and separated on a potassium oxalate-pretreated silica gel TLC with a solvent system of CHCl3-acetone-MeOH-acetic acid-water (40:15:13:12:8). Radiolabeled spots on a TLC plate were analyzed by autoradiography. To measure phosphatidylcholine synthesis, cells were labeled in choline-free minimum essential medium containing 10% dialyzed fetal bovine serum and 0.5 μCi/ml [14C]choline chloride for 1 h at 37 °C.

**RESULTS**

**Antitumor Effect of DSG**—As reported previously, DSG inhibited growth of mouse EL-4 cells strongly with an IC50 of 0.02 μg/ml (33). We first examined whether DSG inhibits the growth of EL-4 cells in vivo like other leukemias such as P388 and L1210 cells (23, 24). As a result, DSG significantly prolonged the survival days of EL-4 intraperitoneal-transplanted mice as well as subcutaneous-transplanted mice without any

**TABLE I**

| Dose | Mean survival days | T/C | % | Mean survival days | T/C |
|------|-------------------|-----|---|-------------------|-----|
| mg/kg | days ± SD | % | days ± SD | % |
| 0    | 12.3 ± 2.0       | 100 | 14.7 ± 2.0 | 100 |
| 0.78 | 18.4 ± 2.1*      | 149.8 | 20.4 ± 2.1* | 138.6 |
| 3.1  | 20.2 ± 1.1*      | 164.4 | 22.0 ± 1.1* | 149.5 |
| 12.5 | 22.0 ± 1.4*      | 179.1 | 25.2 ± 1.4* | 171.3 |

* Significant difference (Student’s t test, P < 0.001) as compared with untreated group.

**FIG. 1**

Antitumor effect of DSG on subcutaneous-transplanted EL-4 cells. C57BL/6 mice were inoculated subcutaneously with 105 EL-4 cells on day 0. DSG was administered intraperitoneally on days 1–9. The antitumor effect of DSG was measured by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described (34).
toxicity (Table I). At 12.5 mg/kg, DSG completely inhibited the growth of subcutaneous-transplanted EL-4 cells for 8 days even after the last administration (Fig. 1). Because the antitumor effect of DSG was well pronounced against EL-4 cells in vitro and in vivo, we used EL-4 cells throughout this study.

**DSG Inhibits Protein Synthesis and Induces Apoptosis—**In vitro study of time course evaluation of growth revealed that the antiproliferative effect of DSG appeared only after 2 days of treatment. As shown in Fig. 2A, DSG did not affect the cell number after 1 day of treatment; however, it reduced the growth after 2 days of treatment. After 3 days of treatment, the cell number did not change, and it seemed to be arresting the cell cycle at 0.1 μg/ml DSG. However, flow cytometric analysis showed that the apparent cell cycle arrest by DSG treatment was negligible, but the population of cells in sub-G1 was increased about two times by 0.1 μg/ml DSG, suggesting apoptosis induction (Fig. 2B). In fact, more degradation of the cellular DNA was detected by DSG at 0.1 μg/ml (Fig. 2C). Thus, DSG induced apoptosis of tumor cells.

To examine the mechanism of DSG action on tumor cell growth, macromolecular synthesis including DNA, RNA, and protein was measured. Although DSG did not affect all three syntheses by 1 day of treatment, it inhibited them after 2 days of treatment. Among them, protein synthesis was the most strongly inhibited, and even at 0.01 μg/ml DSG inhibited it about 70% by 2 days of treatment (Fig. 3). On the other hand, DNA synthesis was moderately inhibited by DSG, and RNA synthesis was transiently inhibited only by the 2 days of treatment (Fig. 3). Thus, these results indicated that DSG affected tumor growth mainly through the inhibition of protein synthesis and induction of apoptosis.

**DSG Inhibits p70S6K Activation—**Because DSG inhibited protein synthesis strongly rather than DNA synthesis, we then examined the effect of DSG on p70S6K, a key molecule of...
protein synthesis. Although rapamycin, an inhibitor of \( p70S6K \) activation (4, 5), completely inhibited \( p70S6K \) activity only by 4 h of treatment, DSG reduced it by 48 h of treatment at 0.1 \( \mu g/ml \) (Fig. 4). Inhibitory effect of DSG on \( p70S6K \) was moderate as compared with rapamycin, but DSG inhibited \( p70S6K \) activity dose dependently by 2 days of treatment (Fig. 4).

\[ \text{p70S6K is hyperphosphorylated upon its activated state and migrates slowly on SDS-PAGE (4, 5). Anti-p70S6K antibody also revealed that the inactive form of p70S6K, a high mobility band, appeared by 4 h of treatment of rapamycin (Fig. 4B). In contrast, the inactive form of p70S6K slightly appeared only by 2 days of treatment of DSG (Fig. 4B). p70S6K autophosphorylates itself upon activation (4). However, DSG did not inhibit p70S6K directly in vitro (data not shown).} \]

DSG Deregulates Akt Kinase—We next determined whether DSG inhibits Akt kinase, an upstream effector of \( p70S6K \).

Although DSG did not affect the amount of Akt kinase even by 2 days of treatment, DSG significantly reduced the phosphorylated form of Akt kinase (an active form) at 0.1 \( \mu g/ml \) (Fig. 5). It is reported that DSG binds to Hsc70 and Hsp90 (27, 28). Furthermore, Hsp90 is reported to bind to and stabilize Akt kinase (37). To determine whether DSG deregulates Akt through dissociation of Akt with HSPs, we examined the effect of DSG on Hsc70 and Hsp90. As a result, DSG did not affect the amount of Hsc70 and Hsp90 proteins (Fig. 5). When the amount of Akt bound to HSPs was evaluated by immunoprecipitation with anti-Akt antibody, Akt-bound Hsc70 was not detected, but Akt-bound Hsp90 was apparently detected in EL-4 cells. However, DSG did not decrease the amount of Hsp90 bound to Akt kinase (Fig. 5).

Effect of DSG on PI3K—Since PI3K is an activator of Akt kinase, the effect of DSG on PI3K was evaluated. After 2 days...
of treatment, immunoprecipitates with anti-PI3K were assessed for the activity using PI as a substrate. As a result, wortmannin, an inhibitor of PI3K, inhibited PI3K activity in EL-4 cells, but DSG weakly inhibited it only at 10 μg/ml (Fig. 6A). Furthermore, DSG did not inhibit PI3K activity directly in vitro (data not shown). Because PI3K activity is dependent on the amount of intracellular phosphoinositides, we examined whether DSG inhibits PI3K indirectly through reducing cellular PI. Phospholipid synthesis revealed that DSG partially inhibited PI synthesis, but it inhibited PC synthesis strongly (Fig. 6, B and C). To confirm the effect of DSG on PC synthesis, EL-4 cells were labeled with [14C]choline instead of [32P]phosphoric acid. The labeled PC showed that DSG inhibited PC synthesis dose-dependently only by 1 day of treatment (Fig. 6D).

Inhibition of Akt Kinase and PC Synthesis by DSG Correlates with Growth Inhibition—We next examined whether the inhibition of Akt kinase and PC synthesis is critical for the effect of DSG. When compared with EL-4 cells (IC50 of 0.02 μg/ml), the growth of K562 cells was less sensitive to DSG (IC50 of 0.46 μg/ml) (Fig. 7A). DSG did not reduce an active form of Akt kinase in K562 cells (Fig. 7B). Furthermore, it did not affect PC synthesis at 1 μg/ml even by 2 days of treatment (Fig. 7C). These results suggest that strong growth inhibition by DSG is achieved through the down-regulation of Akt kinase and PC synthesis.

DISCUSSION

Because DSG has a spermidine moiety, mechanistic studies of DSG had been first done to study polyamine synthesis. Then, DSG was reported to inhibit spermidine synthase, spermine synthase, and polyamine oxidase (38). Moreover, DSG was reported to be unstable in serum because of degradation by amine oxidase and found to be a good inhibitor of amine oxidase (39). Using other amine oxidase inhibitors, two modes of cytotoxic action of DSG were suggested, one dependent on and one independent of amine oxidase in serum (40, 41). However, using EL-4 cells, our recent results have suggested other possibilities. As reported previously, modification of polyamine metabolism did not affect DSG action on EL-4 cells (33). Erwin and Pegg (42) reported that DSG activates spermidine/spermine acetyl transferase activity. However, DSG did not increase spermidine/spermine acetyl transferase activity in EL-4 cells even by 2 days of treatment (data not shown). Therefore, we focused on the growth inhibitory mechanism instead of the
Akt, but DSG did not affect it (Fig. 5), suggesting that DSG postulated that DSG should modulate Akt through interfering nase (37), and DSG associates with Hsp90 (27, 28). It is easily Akt activation (Fig. 5). Hsp90 binds to and stabilizes Akt ki-
molecule of p70S6K. As a result, we found that DSG inhibited p70S6K directly, we next examined Akt kinase, an upstream 2 days of treatment (Fig. 4). Because DSG did not inhibit found that DSG inhibited p70S6K activation (Fig. 4). However, 2 days of treatment even at high doses (Fig. 2). Because the cell 27770 

Hibasami et al. (43) reported that DSG arrests the cell cycle 

Akt pathway, we are now studying the precise mechanism of DSG action on the PI3K pathway.

In conclusion, the results obtained in this study demonstrate that DSG inhibits tumor cell growth through the inhibition of protein synthesis and induction of apoptosis by the down-
regulation of Akt kinase in a PI3K- and Hsp90-independent manner. Although EL-4 cells rapidly grew (<13h doubling time), the antiproliferative effect of DSG clearly appeared only by 2 days of treatment. Therefore, DSG will modulate some other intracellular events first, and the accumulated intracel-
luar changes must result in the down-regulation of Akt kinase. The PC synthesis pathway is one of candidates for a real target of DSG. To evaluate the inhibition of PC synthesis by DSG on Akt pathway, we are now studying the precise mechanism of DSG action on the PC synthesis pathway.

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REFERENCES
1. Terada, N., Patel, H. R., Takase, K., Kohno, K., Nairn, A. C., and Gelfand, E. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11477–11481
2. Jefferies, H. B. J., Reinhard, C., Kozma, S. C., and Thomas, G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4441–4445
3. Heitmian, J., Muroya, N. R., and Hall, M. N. (1991) Science 253, 905–909
4. Price, D. J., Grove, J. R., Calvo, V., Auvrich, J., and Bierer, B. E. (1992) Science 257, 973–977
5. Kuo, C. J., Chung, J., Fiorentino, D. F., Flanagan, W. M., Blenis, J., and Crabtree, G. R. (1992) Nature 358, 70–73
6. Schreiber, S. L. (1991) Science 251, 283–287
7. Brown, E. J., Beal, P. A., Keith, C. T., Chen, J., Shin, T. B., and Schreiber, S. L. (1995) Nature 377, 441–446
8. Vlahos, C. J., and Matter, W. F. (1992) FEBS Lett. 309, 242–248
9. Dekker, L. V., and Segal, A. W. (2000) Science 287, 982–985
10. Klippel, A., Kavanaugh, W. M., Pot, D., and Williams, L. T. (1997) Mol. Cell. Biol. 17, 338–344
11. Franke, T. F., Yang, S. I., Chan, T. O., Datta, K., Kozlaukas, A., Morrison, D. K., Kaplan, D. R., and Tisseria, P. N. (1996) Cell 81, 725–736
12. Burgering, B. M. T., and Coffer, P. J. (1995) Nature 376, 599–602
13. Franke, T. P., Kaplan, D. R., Cantley, L. C., and Toker, A. (1997) Science 275, 665–668
14. Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., and Hemmings, B. A. (1996) EMBO J. 15, 6541–6551
15. James, S. R., Downes, C. P., Gigg, G., Groth, S. J., Holmes, A. B., and Alessi, D. R. (1996) Biochem. J. 315, 709–713
16. Andjelkovic, M., Alessi, D. R., Meier, B., Fernandez, A., Lamb, N. J. C., Frels, M., Cron, P., Cohen, P., Luoqong, J. M., and Hemmings, B. A. (1997) J. Biol. Chem. 272, 31515–31524
17. Stephens, L., Anderson, K., Stokey, D., Edjromand-Bromage, H., Painter, G. F., Holmes, A. B., Gaffney, P. R. J., Reese, C. B., McCormick, F., Temp, P., Coadwell, J., and Hawkins, P. T. (1998) Science 276, 710–714
18. Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotob, Y., and Greenberg, M. E. (1997) Cell 91, 251–261
19. Peso, L., Gonzalez-Garcia, M., Page, C., Herrera, R., and Nunez, G. (1997) Science 278, 687–689
20. Takeuchi, T., Inuma, H., Kunimoto, S., Masuda, T., Ishizuka, M., Takeuchi,
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M., Hamada, M., Naganawa, H., Kondo, S., and Umezawa, H. (1981) J. Antibiot. (Tokyo) 34, 1619–1621
21. Umeda, Y., Moriguchi, M., Kuroda, H., Nkamura, T., Iinuma, H., Takeuchi, T., and Umezawa, H. (1985) J. Antibiot. (Tokyo) 38, 886–898
22. Iwasawa, H., Kondo, S., Ikeda, D., Takeuchi, T., and Umezawa, H. (1982) J. Antibiot. (Tokyo) 35, 1665–1669
23. Plowman, J., Steadman D., Harrison, J., Trader, M. W., Daniel P., Griswold, J., Chadwick, M., McComish, M. F., Silveira, D. M., and Zaharko, D. (1987) Cancer Res. 47, 685–689
24. Nishikawa, K., Shibasaki, C., Hiratsuka, M., Arakawa, M., Takahasi, K., and Takeuchi, T. (1991) J. Antibiot. (Tokyo) 44, 1101–1109
25. Nemoto, K., Hayashi, M., Abe, F., Nkamura, T., Ishizuka, M., and Umezawa, H. (1987) J. Antibiot. (Tokyo) 40, 561–562
26. Masuda, T., Mizutani, S., Iijima, M., Odai, H., Suda, H., Ishizuka, M., Takeuchi, T., and Umezawa, H. (1987) J. Antibiot. (Tokyo) 40, 1612–1618
27. Nadeau, K., Nadler, S. G., Saudnier, M., Tepper, M. A., and Walsh, C. T. (1994) Biochemistry 33, 2561–2567
28. Nadler, S. G., Tepper, M. A., Schacter, B., and Mazzucco, C. E. (1992) Science 258, 484–486
29. Nishikawa, K., Shibasaki, C., Uchida, T., Takahashi, K., and Takeuchi, T. (1991) J. Antibiot. (Tokyo) 44, 1237–1246
30. Hiratsuka, M., Kuramochi, H., Takahasi, K., Takeuchi, T., and Oshima, M. (1991) Jpn. J. Cancer Res. 82, 1065–1068
31. Okawa, T., Shimamura, M., Ashino-Puse, H., Iwaguchi, T., Ishizuka, M., and Takeuchi, T. (1991) J. Antibiot. (Tokyo) 44, 1033–1035
32. Okawa, T., Hasegawa, M., Morita, I., Murata, S.-I., Ahino, H., Shimamura, M., Kiue, A., Hamaokah, K., Kawano, M., Ishizuka, M., and Takeuchi, T. (1992) Anti-Cancer Drugs 3, 293–299
33. Kawada, M., Somono, T., Iinuma, H., Masuda, T., Ishizuka, M., and Takeuchi, T. (2000) J. Antibiot. (Tokyo) 53, 705–710
34. Fukazawa, H., Mizuno, S., and Uehara, Y. (1995) Anal. Biochem. 228, 83–90
35. Coffer, P. J., Geijser, N., Mrabet, L., Schweizer, R. C., Maikoe, T., Raaijmakers, J. A. M., Lammers, J.-W. J., and Koenderman, L. (1998) Biochem. J. 329, 121–130
36. Horwitz, J., and Perlman, R. L. (1987) in Methods in Enzymology (Conn, P. M., and Means, A. R., eds) Vol. 141, p. 169, Academic Press, New York
37. Sato, S., Fujita, N., and Tsuuro, T. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10832–10837
38. Hibasami, H., Tsukada, T., Suzuki, R., Takano, K., Takaji, S., Takeuchi, T., Shirakawa, S., Murata, T., and Nakashima, K. (1991) Anticancer Res. 11, 325–330
39. Kunimoto, S., and Takeuchi, T. (1994) J. Antibiot. (Tokyo) 47, 1130–1135
40. Kunimoto, S., Nosaka, C., Xu, C. Z., and Takeuchi, T. (1989) J. Antibiot. (Tokyo) 42, 116–122
41. Kuramochi, H., Hiratsuka, M., Nagamine, S., Takehashi, K., Nakamura, T., Takeuchi, T., and Umezawa, H. (1988) J. Antibiot. (Tokyo) 41, 234–238
42. Erwin, B. G., and Pegg, A. E. (1986) Biochem. J. 238, 581–587
43. Oda, C., Toyoda, K., and Nemat, K. (1998) Immunology 95, 370–376