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GS-164, a small synthetic compound, stimulates tubulin polymerization by a similar mechanism to that of Taxol

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Abstract *Purpose*: During our search for new microtubule effectors as anticancer agents, we have found that a small synthetic molecule designated GS-164 interferes with the assembly of porcine microtubule proteins and has cytotoxic activity against a wide range of human tumor cell lines. In this study, we investigated mode of action of the compound in comparison with Taxol and colcemid. *Methods*: To gain an insight into the mode of action of GS-164, we used an in vitro microtubule polymerization assay and a flow-cytometric measurement technique. Microtubule organization and the level of tubulin polymerization in HeLa cells were also examined by immunofluorescence microscopy and cytoskeletal protein analyses, respectively. *Results*: GS-164 stimulated assembly of microtubule proteins in vitro in a concentration-dependent and a GTP-independent manner. Furthermore, as with Taxol, the microtubule polymerization induced by GS-164 was antagonized by podophyllotoxin, a tubulin polymerization inhibitor, and microtubules formed by GS-164 were resistant to disassembly by calcium or low temperatures. GS-164 in the micromolar range arrested the cell cycle of HeLa cells in the mitotic phase leading to cell death. GS-164 also increased the amounts of cellular microtubules in HeLa cells, resulting in the formation of microtubule bundles. *Conclusion*: These results indicate that GS-164 stimulates microtubule assembly by a similar mechanism to that of Taxol. A comparative conformational analysis of GS-164 and Taxol suggested that the structure of the former mimics the minimum essential sites of Taxol required to exert the Taxol-like activities described above. Although the cytotoxicity of GS-164 against human tumor cells was 1000-fold lower than that of Taxol and GS-164 was one-tenth as active as Taxol in vitro, these findings pave the way for synthesizing clinically useful anticancer agents using GS-164 as a lead compound.

Key words GS-164 · Taxol · Mitotic agent · Microtubule · Anticancer

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Introduction

Taxol (paclitaxel) [17, 21, 26, 39, 46] is an exciting new therapeutic agent with antitumor activity against ovarian, breast, and lung carcinomas. This compound is extracted from the bark of the Pacific yew tree *Taxus brevifolia*, as well as from needles and stems of this and other *Taxus* species, and its complex chemical structure has been determined [57]. Its activity as an antitumor agent was recognized in an early preclinical screening program at the NCI using P388 murine leukemia-bearing mice [11]. Interest in this compound arises from not only its clinical activity against poorly responsive solid tumors but also from its unique mechanism of action. It promotes tubulin polymerization and stabilizes microtubules that result in the inhibition of cell migration and chromosome segregation by blocking the transit of cycling cells in the G_2/M phase [48].

A principal obstacle to the wider therapeutic use of Taxol is limited supply. Since Taxol can be obtained only in low yields (40–165 mg/kg bark) from the very slow growing Pacific yew trees which are sparsely distributed in the Northern hemisphere [17, 21, 26, 39, 46], alternative approaches for obtaining Taxol have been explored, such as isolation from renewable foliage and other tissues of *Taxus* species [1, 13, 25, 27, 29], production in tissue culture of *Taxus* plant cells [12, 32], production by culture of *Taxomyces andreanae*, a Taxol-producing endophytic fungus isolated from a Pacific yew tree [50], and semisynthesis of the drug or its analogues such as Taxotere from Baccatin III or related taxoid

metabolites that are more readily available from renewable sources [2, 15, 19, 43, 56]. Although the total synthesis of Taxol has also been achieved by Nicolaou et al. [40] and Holton et al. [20], it is not yet commercially viable owing to the multiple synthetic processes involved.

In this report, we describe a novel small synthetic compound designated GS-164, which stimulates tubulin polymerization and stabilizes microtubules. GS-164 has activities similar to those of Taxol in vitro and in vivo. The conformations of GS-164 and Taxol were compared, and the structure-activity relationships of these drugs are discussed.

Materials and methods

Materials and human cell lines

GS-164 (Fig. 1) was synthesized by Cyclan Co. (Moscow, Russia). Taxol, colchicine, colcemid, podophyllotoxin, bovine serum albumin and a monoclonal antibody against β-tubulin (TUB2.1) were obtained from Sigma Co. (St. Louis, Mo.). All other chemicals were reagent grade. The following human cell lines were purchased from the American Type Culture Collection (Rockville, Md.): colon adenocarcinomas SW48, SW620 and SW948, breast adenocarcinomas MCF-7, MDA-MB231, MDA-MB435S, MDA-MB453 and MDA-MB468, hepatocellular carcinoma HepG2, lung small-cell carcinoma H69, pancreatic carcinoma MIA PaCa-2, fibrosarcoma HT-1080, prostate adenocarcinoma PC-3, embryonal rhabdomyosarcoma RD, and osteogenic sarcoma Saos-2. The following human cell lines were purchased from Flow Laboratories (Irvine, UK): metastatic pancreas adenocarcinoma AsPC-1, colon adenocarcinoma SW480, and epidermoid carcinoma A431. The human epitheloid carcinoma HeLa S3, colon adenocarcinoma WiDr, lung carcinoma A549, and melanoma G361 were obtained from the Institute for Fermentation (Osaka, Japan).

Cytotoxicity assay

Cytotoxicity was assayed colorimetrically by the tetrazolium salt (MTT) method [37]. The cytotoxic activity of the drugs was determined in terms of the IC_{50} (μM), the concentration required to inhibit 50% of the cell growth. Cell viability was determined by trypan blue exclusion.

Preparation of microtubule proteins and polymerization assay

Microtubule protein was prepared from porcine brains according to the polymerization–depolymerization procedure described by Li et al. [30]. Polymerization of microtubule proteins was followed by turbidity measurement (400 nm, Beckman DU250 spectrophotometer) at 37 °C using 0.7 mg of microtubule protein per ml of a reaction mixture [100 mM 4-morpholinoethanesulfonic acid (MES), 1 mM ethyleneglycol bis(2-aminoethylether) tetraacetic acid (EGTA), 0.5 mM 2-mercaptoethanol (2ME) and 0.5 mM $MgSO_4$ (pH 6.5)]. Drugs dissolved in ethanol were added at the start of the reaction.

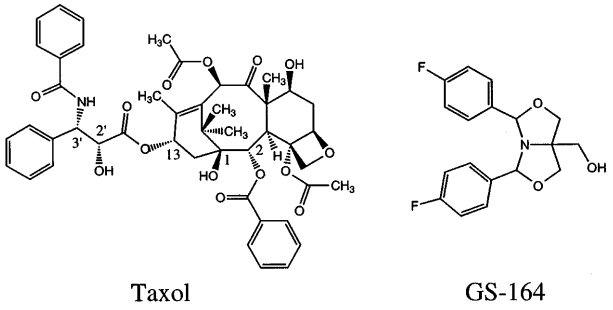

Fig. 1 Structures of GS-164 and Taxol

Analysis for cellular tubulin and actin polymerization level

The cytoskeletal fraction containing microtubule, actin and other cellular network proteins was prepared from mammalian cells as described by Thrower et al. [54] with slight modification. Briefly, 2 ml cell suspension (1×10^6 cells) of HeLa S3 cells cultured in the presence or absence of a drug was centrifuged at 2000 g for 5 min at 4 °C, and the pellet was resuspended in 2 ml lysis buffer [0.1 M piperazine-N,N'-bis(2-ethanesulfonic acid) (Pipes), 1 mM EGTA, 1 mM $MgSO_4$, 30% glycerol, 5% DMSO, 5 mM GTP, 1 mM DTT, 1 mM $N\alpha$-p-tosyl-L-arginine methyl ester (TAME), 0.05 mg/ml aprotinin, 0.02% sodium azide and 0.125% NP-40 (pH 6.9)] and incubated at 37 °C for 20 min. Lysis of cells was verified by light microscopy. Buffer (1 ml) lacking NP-40 was added to the lysed cell suspension, and then the mixture was centrifuged at 200 000 g for 90 min at 37 °C. After the supernatant was aspirated, 0.2 ml of a depolymerization buffer (0.1 M MES, 1 mM $MgSO_4$, 10 mM $CaCl_2$, 5 mM GTP, 1 mM TAME, 0.05 mg/ml aprotinin and 0.02% sodium azide, pH 6.9) was added to the pellet. The solution was homogenized with a glass pestle and allowed to stand for 1 h on ice to ensure depolymerization of cytoskeletal proteins. Fractions thus obtained were electrophoresed on 10% polyacrylamide gels and transferred to nitrocellulose filters (Hybond-ECL; Amersham) using a semidry electroblotter (Bio-rad). Beta-tubulin protein conjugated with TUB2.1 [36] was detected with the enhanced chemiluminescence system (ECL; Amersham). Proteins on the electrophoresed gel were stained with Coomassie brilliant blue. The identity of actin was confirmed by immunoblotting with actin-specific antibody and sensitivity to cytochalasin B.

Immunofluorescence microscopy

The cellular microtubule organization was observed by fluorescence microscopy using the procedure of Ohta et al. [41]. Briefly, HeLa cells (10^5 cells/ml) were cultured in chamber slides for 20 h in the presence or absence of GS-164 and fixed with 4% formaldehyde in phosphate-buffered saline (PBS) for 30 min. The fixed cells were washed with PBS twice and incubated with 0.3% Nonidet P-40 for 10 min. After washing with PBS twice, the cells were incubated with TUB2.1 (200 µl/ml, 1:200 dilution) for 1 h at 37 °C, washed twice, then incubated with fluorescein-conjugated goat antimouse immunogloblin antibody (Cappel, Malvern, Pa.) for 30 min. The immunostained slides were then rinsed before mounting in Immunon (Lipshaw Pittsburgh, Pa.) and viewed with a Nikon Diaphot photomicroscope (objective, ×40).

Flow-cytometric measurement

DNA contents were measured by flow cytometry as described by Garcia et al. [14]. Briefly, HeLa cells (70–80% confluence) were trypsinized and fixed in cold methanol. The cells were then washed, centrifuged, resuspended in PBS containing 60 µg/ml RNase and incubated for 30 min at 37 °C. Before analysis, the cells were stained with 20 µg/ml of propidium iodide. DNA levels were measured using a Becton Dickinson Immunocytometry System 2350 (San Jose, Calif.).

Comparative conformational analysis

Stable conformations of GS-164 and Taxol were generated by molecular dynamics and molecular mechanics calculations using a Discover CVFF force field (ver. 2.95, Biosym Technologies).

Conformers of Taxol were constructed by systematically bond-rotating the substituents at positions 2 and 13, fixing the taxane skeleton to the most stable conformation which was determined using molecular dynamics at 2000 K. The resulting structures were minimized. For GS-164, each isomer was energy-minimized. The pharmacophore was predicted using Apex-3D (ver. 1.4.3, Biosym Technologies and DCL System International) based on the stable conformations of GS-164 and Taxol.

Results

Cytotoxicity against human tumor cell lines

The cytotoxic activities of GS-164, Taxol and colchicine are shown in Table 1. GS-164 suppressed the growth of almost all human cell lines tested, whereas the potency of the cytotoxicity of GS-164 was about one-thousandth that of the control mitotic poisons. However, the gross cytotoxicity profiles of the three agents for these cell lines were similar.

Stimulation of microtubule polymerization in vitro

The incubation of porcine microtubule protein with various concentrations of GS-164 induced the polymerization of microtubules as monitored spectrophotometrically by measuring the increase in turbidity. Although there was no substantial microtubule polymerization in the absence of GS-164, a concentration-dependent increase in the rate and extent of microtubule assembly was observed with GS-164 and stimulation reached a maximum at over 400 μ*M* (Fig. 2). Electron microscopic observation of the product induced by GS-164 revealed a typical cylindrical microtubule structure, not merely microtubule aggregation, and there was no increase in the turbidity of the reaction mixture when bovine serum albumin or human immunoglobulin was used instead of porcine microtubule protein (Fig. 2, and data not shown). Neither the rate nor the extent of GS-164-induced microtubule polymerization were influenced by adding GTP to the reaction mixture as is found with Taxol [47] (data not shown). Furthermore, microtubule polymers formed in the presence of GS-164 were stable against the depolymerizing effect of 1 m*M* $CaCl_2$ and exposure to cold (4 °C) as are the polymers formed in the presence of Taxol [47] (Fig. 3). Antimitotic drugs such as colchicine and podophyllotoxin antagonize the

Table 1 Growth-inhibitory activity of GS-164, Taxol and colchicine against various human cell lines. Each cell line was cultured in the presence or absence of a drug in a 96-well plate for 72 h. Proliferation was evaluated by the MTT assay [37]. Initial density was 2×10^4 cells/ml (*NT* not tested)

Cell line	IC_{50} (μ*M*)		
	GS-164	Taxol	Colchicine
HeLa	9	0.023	0.005
MIA PaCa-2	14.1	0.011	0.011
A549	18.8	0.009	0.043
G361	17.5	0.017	0.018
WiDr	20.7	0.016	0.012
SW48	12	0.007	0.01
SW480	>100	0.117	0.063
SW620	11	NT	NT
SW948	20	NT	NT
MCF-7	5	0.0009	0.0063
MDA-MB231	13.6	0.011	0.013
MDA-MB435S	5.6	NT	NT
MDA-MB453	11	NT	NT
MDA-MB468	6.1	NT	NT
H69	24.6	0.009	0.012
AsPC-1	38.3	0.01	0.028
Saos-2	10.2	0.0042	0.0058
RD	17.7	0.0021	0.0058
Hep G2	22.7	0.005	0.03
HT1080	10.8	0.02	0.012
A431	>100	0.048	0.043
PC-3	18	NT	NT

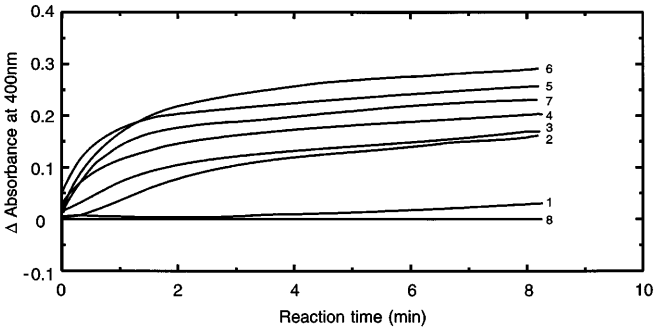

Fig. 2 Effect of GS-164 on the polymerization of microtubule protein in vitro. Polymerization of microtubule protein was measured as described in Materials and methods. After 5 μ*M* Taxol (*2*), 40 μ*M* (*3*), 100 μ*M* (*4*), 180 μ*M* (*5*) or 440 μ*M* (*6*) GS-164 or 1 m*M* GTP (*7*) was added to a reaction mixture containing microtubule protein (0.7 mg/ml) at 0 °C or 440 μ*M* GS-164 (*8*) was added to a reaction mixture containing bovine serum albumin (1.0 mg/ml) at 0 °C, the temperature was shifted to 37 °C and the turbidity of the solution was measured at an absorbance of 400 nm. (*lane 1* untreated control)

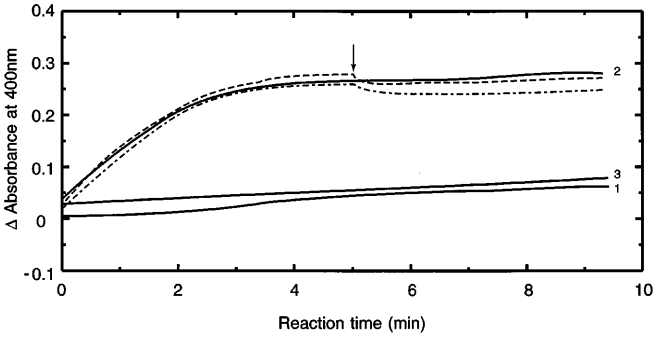

Fig. 3 Effect of podophyllotoxin on the polymerization of microtubule protein induced by GS-164 in vitro. After 40 μ*M* GS-164 (*2*) or 40 μ*M* GS-164 and 50 μ*M* podophyllotoxin (*3*) was added to a reaction mixture containing microtubule protein (0.7 mg/ml) at 0 °C, the temperature was shifted to 37 °C and the turbidity of the solution was measured at an absorbance of 400 nm. At the time indicated by the *arrow*, $CaCl_2$ was added at a final concentration of 1 m*M* (----) or the temperature of the reaction mixture was shifted at 4 °C (---) (*lane 1* untreated control)

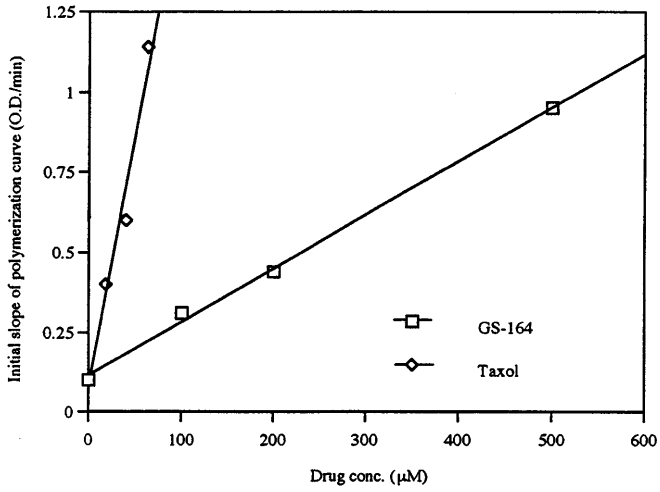

Fig. 4 Relative ability of GS-164 and Taxol to stimulate polymerization of microtubule protein in vitro. Polymerization of microtubule protein was assayed at various concentrations of GS-164 and Taxol as described in Materials and methods. The maximal assembly rate at each concentration of drug was determined by the initial slope of each polymerization curve as the absorbance increase at 400 nm per minute

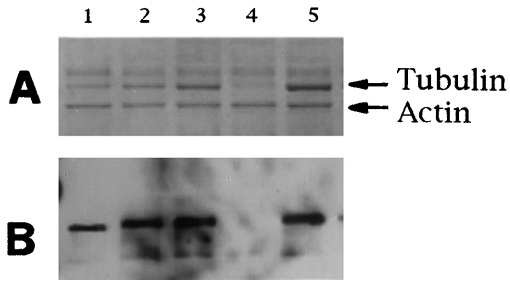

Fig. 5A,B Effects of GS-164, Taxol and colcemid on the levels of tubulin and actin polymers in HeLa S3 cells. HeLa S3 cells were cultured in the absence (*lane 1*) or the presence of 15 μ*M* (*lane 2*) and 30 μ*M* (*lane 3*) GS-164, 0.3 μ*M* colcemid (*lane 4*) and 1 μ*M* Taxol (*lane 5*) for 4 h. Cytoskeletal proteins in each lane were detected by staining with Coomassie brilliant blue after electrophoresis on a 10% polyacrylamide gel (**A**), and tubulin proteins were also detected by blotting with a monoclonal antibody against human β-tubulin (TUB2.1) after transfer of the electrophoresed gel to a nitrocellulose filter as described in Materials and methods (**B**). The identity of actin proteins was confirmed by the molecular weight and by immunoblotting with an actin-specific monoclonal antibody (data not shown). The locations of tubulin and actin proteins on the gel are indicated by the *arrows*

microtubule polymerization induced by Taxol at concentrations substoichiometric to that of microtubule [28]. Under our assay conditions, podophyllotoxin interfered with GS-164-induced microtubule polymerization, and the inhibition was almost complete at equimolar concentrations of GS-164 and podophyllotoxin (Fig. 3).

Though stimulation of microtubule polymerization is maximal with equivalent amounts of Taxol and tubulin on a molar basis [28], excess GS-164 was required to induce the maximum turbidity of polymers (Fig. 2). Figure 4 shows a comparison of the concentration-dependent assembly rate stimulated by Taxol and GS-164. The stimulatory activity of microtubule polymerization by GS-164 was one-tenth that of Taxol.

Stimulation of cellular tubulin polymerization

As described above, GS-164 had microtubule polymerization-stimulatory activity in vitro and cytotoxicity against a wide range of human tumor cells. To gain insight into whether the activity of GS-164 to induce microtubule assembly directly contributed to its cytotoxic activity, we examined the cellular tubulin polymerization level in HeLa cells exposed to GS-164. Figure 5 shows that GS-164 apparently increased the tubulin polymerization level dose-dependently. However, like Taxol and colchicine, GS-164 hardly affected the actin polymerization level, indicating that it specifically affected microtubule formation in the treated cells. Although we did not characterize the slowest migrating protein on the gel, its amount was not affected by treatment with any compound tested (Fig. 5A).

Effect on cell cycle progression and cellular microtubule organization

Mammalian cells incubated with Taxol arrest in the G_2/M phase of the cell cycle and cellular microtubules are abnormally assembled either in abundant arrays of disorganized microtubules in parallel "bundles" or in mitotic asters that do not require centrioles for formation [14]. The formation of these structures is dose- and time-dependent. Asters have also been observed in mitotic cells and bundles in interphase cells [14]. As shown in Fig. 6, the proportion of cells in the G_2/M phase increased in HeLa cells after exposure to GS-164 as well as Taxol and colcemid; this is commonly observed with mitotic toxins. Furthermore, the microtubule organization in HeLa cells incubated with GS-164 differed markedly from that of the nontreated cells as shown by immunofluorescence microscopy using an anti-β-tubulin monoclonal antibody (Fig. 7). Microtubule bundles were predominant and abnormal mitotic asters were virtually absent in HeLa cells (Fig. 7). In contrast, asters were prevalent in CHO cells at high concentrations of GS-164 (data not shown). Although this different response to GS-164 between these two cell lines might be a consequence of a lower sensitivity of CHO cells to the cytotoxic effects of GS-164, farther experiments are needed to clarify this issue.

Comparative conformational analysis

To understand the structural elements of GS-164 responsible for its tubulin-polymerizing activity, we analyzed the stable conformations of both GS-164 and Taxol, and estimated the structural similarity between these compounds. By conformational search for the

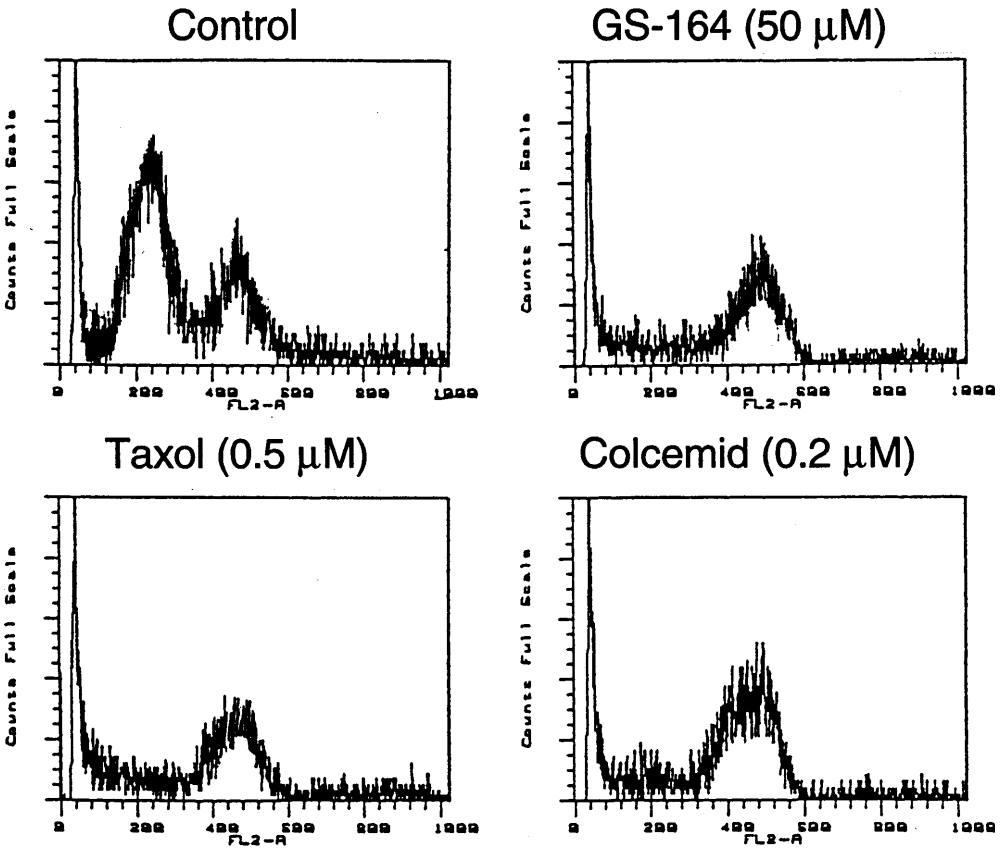

Fig. 6 Effect of GS-164, Taxol and colcemid on HeLa S3 cell cycle progression. Exponentially proliferating HeLa cells were cultured in the absence or presence of 50 μ*M* GS-164, 0.5 μ*M* Taxol or 0.2 μ*M* colcemid. The cells were harvested 24 h later for analytical DNA flow cytometry as described in Materials and methods

substituents at positions 2 and 13 with fixing the taxane skeleton of Taxol to the most stable, 24 probable conformations of Taxol were obtained within 20 kcal/mol. GS-164 generated four isomers because of the presence of two asymmetric carbons at comparable energy levels. Using these stable conformations of GS-164 and Taxol for the pharmacophore analysis, we determined that one of two phenyl groups of GS-164 was homologous to the phenyl group in the benzoyloxy side-chain at position 2 of Taxol and the other group of GS-164 was homologous to the phenyl ring at position 3′. Furthermore, the hydroxyl group and the one ring oxygen of GS-164 may correspond to the hydroxyl group at position 1 and the ester oxygen at position 2 of Taxol, respectively (Fig. 8). We propose that the structure of GS-164 (*R*/*R* isomer) mimics the part of the Taxol structure that is critical for Taxol to exert the activity reported [4, 7, 8, 16, 18, 19, 22, 42, 51, 53, 55].

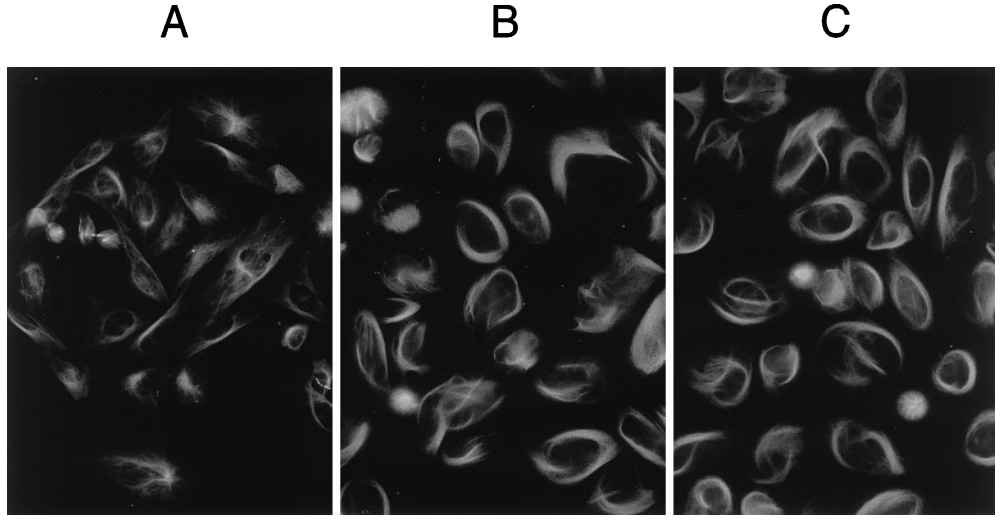

Fig. 7A–C Indirect immunofluorescence staining of GS-164-induced microtubules in HeLa S3 cells. HeLa S3 cells were cultured in the absence (**A**) or presence of 0.1 μ*M* Taxol (**B**) and 50 μ*M* GS-164 (**C**) for 20 h at 37 °C. The cells were then fixed and stained with an anti-β-tubulin monoclonal antibody as described in Materials and methods

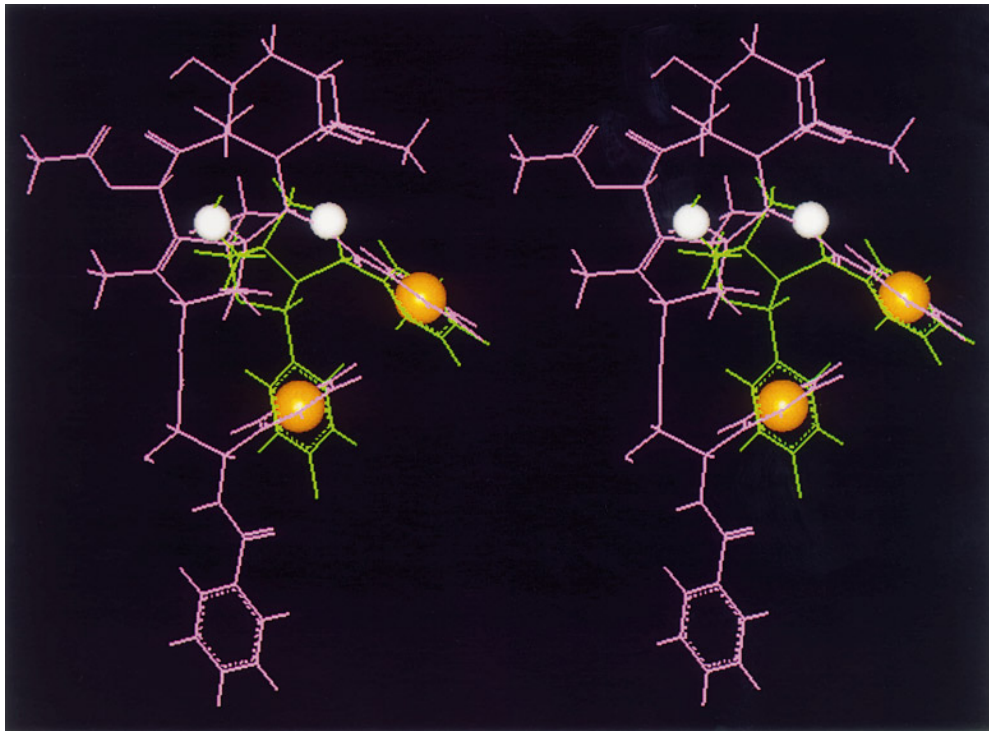

Fig. 8 Structural similarity between GS-164 and Taxol. A stereoscopic view was obtained by overlapping GS-164 (*green*) and Taxol (*magenta*) with two benzene rings (*orange spheres*) and two oxygen atoms (*white spheres*) in similar positions

Discussion

We demonstrated that a novel low-molecular-weight compound, GS-164, can stimulate microtubule assembly by a similar mechanism to that of Taxol by the following in vitro and in vivo experimental evidence. In the in vitro microtubule polymerization assay, (1) GS-164 induced microtubule assembly in the absence of GTP in a concentration-dependent manner (Fig. 2), (2) the microtubules formed by the drug were resistant to disassembly by calcium or low temperatures and (3) podophyllotoxin interfered with the microtubule polymerization induced by GS-164 (Fig. 3). In addition, in the cell-based assay, GS-164 increased the amounts of cellular microtubules in HeLa cells without affecting those of actin filaments (Fig. 5). Furthermore, GS-164 induced microtubule bundles (Fig. 7), and arrested the cell cycle progression of HeLa cells in the G_2/M phase leading to cell death (Fig. 6). All of these activities exerted by GS-164 have also been attributed to Taxol.

In addition to the activities described above, Taxol inhibits the transition from G_0 to S phase when serum-starved fibroblasts are stimulated by growth factors, suggesting that Taxol also affects the interphase cytoskeleton by disrupting the normal function of the cell membrane, transmembrane signalling, intracellular transport and/or locomotion [24, 45]. Jordan et al. [23] and Long and Fairchild [31] have also shown that low concentrations of Taxol inhibit the progression of mitotic cells to G_1 phase by interfering with spindle formation without affecting other microtubule functions, suggesting that this is the primary cytotoxic mechanism of Taxol. Although we have shown G_2/M phase arrest by GS-164 in this study, it remains to be determined whether GS-164 interferes with other cell-cycle transitions. Thus, to elucidate the mode of action of GS-164 in more detail, further experiments to compare the activities of GS-164 and Taxol at equipotent doses are required.

Taxol may modulate either the interaction of growth factors with their receptors or the resulting intracellular signalling [3, 10, 33, 34, 44, 49, 58]. For example, Bogdan and Ding [3] and other groups [10, 33, 34, 58] have shown that Taxol downregulates tumor necrosis factor-α (TNF-α) receptors on macrophages and induces TNF-α and interleukin-1 (IL-1) expression in these cells as observed with lipopolysaccharide. However, GS-164 did not stimulate TNF-α production by macrophages under experimental conditions whereas Taxol did (data not shown). Burkhart et al. [6] have reported that the effect of Taxol on TNF-α expression is distinct from its microtubule-stabilizing activity. Thus these biological responses to Taxol might be due to its side-chain effect, which is not related to tubulin polymerization-stimulatory activity.

Recently, Bollag et al. [5] have reported that epothilones A and B, 16-membered macrolides isolated from an extract of the myxobacterium *Sorangium cellulosum*, mimic the biological effects of Taxol and bind to the same microtubule-binding site as Taxol in a displacement competition assay. Although all the biological activities of these compounds are almost equipotent to those of Taxol, they differ from Taxol in that they are also effective against multidrug-resistant tumor cells against which Taxol is ineffective. This might be due to

the failure of P-glycoprotein to recognize epothilones as substrates because the structures of epothilones are totally different from that of Taxol.

Our comparative conformational analysis of GS-164 and Taxol suggested that GS-164 mimics the minimum essential structural elements of taxol responsible for its tubulin polymerization-stimulating activity (Fig. 8). Guéard et al. [19] have postulated that during the binding of Taxol to tubulin, two structural features of Taxol which constitute part of "the southern hemisphere" of the molecule [51] can be assumed to be critical for the recognition: the hydrophobic area including the benzoate group at position 2 could locate in the core region of the protein, and the phenyl group at position 3′ and the hydroxyl group at the position 2′ could stabilize the drug–protein complex through direct interaction. Considerable experimental evidence supports the importance of the benzoyloxy group at position 2 and the phenyl group at position 3′ of Taxol for microtubule assembly and cytotoxic properties [4, 7, 8, 16, 18, 22, 42, 53, 55]. In particular, Mastropaolo et al. [35] have recently reported crystal structure data on Taxol indicating that the benzoate group at position 2 and the phenyl group at position 3′ are close to each other and possibly influence each other's orientation in a polar environment. Thus, these data are in good agreement with our assumption based on the conformation analysis that the two phenyl groups, the hydroxy group and the one oxygen of GS-164 correspond to the two aromatic rings, the hydroxy group at position 1 and the ester oxygen at position 2 of Taxol, respectively. However, other elements of Taxol quite certainly contribute to its remarkably potent activities, since GS-164 is far weaker than Taxol in the various biological activities shown in this study. For example, Neidigh et al. [38] and Chordia et al. [9] have reported that 4-deacetoxy-Taxol is significantly less active than Taxol in tubulin polymerization activity, suggesting the importance of the 4-acetyl group of Taxol. Furthermore, the hydroxyl group at position 2′ of Taxol has been shown to increase the affinity of Taxol for tubulin [52]. The cytotoxicity of GS-164 against human tumor cells was 1000-fold lower than that of Taxol (Table 1) whereas, when assayed in vitro, microtubule polymerization-stimulatory activity of the drug was one-tenth that of Taxol (Fig. 4). This discrepancy between the in vitro and the in vivo activities of GS-164 and Taxol might be a consequence of insufficient uptake of GS-164 through the cellular membrane of animal cells. This suggests that by introducing hydrophobic side-chains into GS-164, its in vivo activity could be improved up to the in vitro level. Although detailed structure-activity relationships of GS-164 derivatives in comparison with those of Taxol are needed to elucidate the structural elements of Taxol contributing to its potent tubulin polymerization-stimulatory activity, the finding that a structurally simple compound such as GS-164 shows this interesting activity substantiates the importance of the two phenyl groups at positions 2 and position 3′ of Taxol. More importantly, GS-164 could be the mother compound from which to develop clinically useful anticancer agents by synthetic approaches.

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