The Geranylgeranyltransferase-I Inhibitor GGTL-298 Arrests Human Tumor Cells in G0/G1 and Induces p21WAF1/CIP1/SDI1 in a p53-independent Manner*

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Recently we have shown that in fibroblasts (NIH 3T3 and Rat-1 cells) inhibition of protein geranylgeranylation leads to a G0/G1 arrest, whereas inhibition of protein farnesylation does not affect cell cycle distribution. Here we demonstrate that in human tumor cells the geranylgeranyltransferase-I (GGTase-I) inhibitor GGTL-298 blocked cells in G0/G1, whereas the farnesyltransferase (FTase) inhibitor FTI-277 showed a differential effect depending on the cell line. FTI-277 accumulated Calu-1 and A-549 lung carcinoma and Colo 357 pancreatic carcinoma cells in G0/M, T-24 bladder carcinoma, and HT-1080 fibrosarcoma cells in G0/G1, but had no effect on cell cycle distribution of pancreatic (Panc-1), breast (SKBr 3 and MDAMB-231), and head and neck (A-253) carcinoma cells. Furthermore, treatment of Calu-1, Panc-1, Colo 357, T-24, A-253, SKBr 3, and MDAMB-231 cells with GGTL-298, but not FTI-277, induced the protein expression levels of the cyclin-dependent kinase inhibitor p21WAF, HT-1080 and A-549 cells had a high basal level of p21WAF, and GGTL-298 did not further increase these levels. Furthermore, GGTL-298 also induces the accumulation of large amounts of p21WAF mRNA in Calu-1 cells, a cell line that lacks the tumor suppressor gene p53. There was little effect of GGTL-298 on the cellular levels of another cyclin-dependent kinase inhibitor p27KIP as well as cyclin E and cyclin D1. These results demonstrate that GGTase-I inhibitors arrest cells in G0/G1 and induce accumulation of p21WAF in a p53-independent manner and that FTase inhibitors can interfere with cell cycle events by a mechanism that involves neither p21WAF nor p27KIP. The results also point to the potential of GGTase-I inhibitors as agents capable of restoring growth arrest in cells lacking functional p53.

Mutated forms of the small G-protein Ras are found in about 30% of all human cancers, including 95% of pancreatic and 50% of colon cancers (1). Prenylation at the Ras C terminus is required for Ras-mediated malignant transformation, and farnesyltransferase (FTase) inhibitors are presently being evaluated as potential antitumor agents by a number of research groups (for reviews, see Refs. 2 and 3). Because there is now some evidence that KB-Ras, which is the most commonly mutated form of Ras, may be geranylgeranylated as well as farnesylated (4–8), geranylgeranyltransferase-I (GGTase-I) inhibitors have been included in the search for potential anticancer agents (9–12). While several reports have established the ability of FTase inhibitors to block oncogenic Ras signaling and tumor growth in mouse models (13, 14), GG- Tase-I inhibitors have received less attention. It is generally assumed that GGTase-I inhibitors would exert a gross toxic effect because the number of geranylgeranylated proteins in the cell exceeds that of farnesyalted proteins by about a factor of 5 (15). Recent reports from our group have suggested, however, that GGTL-298, a CAAX peptidomimetic GGTase-I inhibitor, may not be simply toxic (9–11, 16). For example, we showed that GGTL-298 arrested cells in the G0/G1 phase of the cell cycle, induced apoptosis, and enhanced nitric-oxide synthase-2 induction by IL-1β. In this report we extended our studies to investigate the effects of GGTL-298 and the FTase specific inhibitor FTI-277 on cell cycle events that may contribute to their growth arrest activity.

Very early, it was suggested by Pardee (17) that, at various stages of the cell cycle, so-called “checkpoints” existed whose function was to ensure that cell growth occurred only if a number of minimum requirements were met. Today, it is widely accepted that the cell cycle has several such “checkpoints” which receive signals from a variety of processes to ensure that a cell replicates only if its genetic code is intact and functional and only if the cell is metabolically prepared for the next phase of the cell cycle. The restriction point in G1 has become the subject of intense research efforts, and some of the processes regulating the G1 to S transition are now beginning to be understood (for a review, see Ref. 18). In a G1 arrested cell, transcriptional activity is low due to a major transcriptional activator, E2F, being bound to and inactivated by the hypophosphorylated form of the retinoblastoma (Rb) protein. Upon phosphorylation through cyclin-dependent kinases (CDKs), Rb dissociates from E2F, resulting in an increase in transcriptional activity mediated by free E2F (reviewed in Ref. 19). CDKs have been found to be the catalytic subunits in complexes containing a CDK and a cyclin as the regulatory subunit. The activity of cyclin/CDK complexes is regulated by varying expression levels of the cyclins, whereas CDK levels

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1 The abbreviations used are: FTase, farnesyltransferase; GGTase-I, geranylgeranyltransferase-I; IL, interleukin; CDK, cyclin-dependent kinase; TGF-β, transforming growth factor-β; PAGE, polyacrylamide gel electrophoresis.
remain fairly constant throughout the cell cycle (reviewed in Ref. 20).

More recently, it was shown that cyclin/CDK activity is also regulated through a number of cyclin-dependent kinase inhibitors. The first such inhibitor was p21WAF, which was described by four independent research groups to be a universal inhibitor of cyclin-dependent kinases (21–24). Research on the role of the tumor suppressor p53 as a mediator of G1 arrest in response to DNA damage led to the discovery that p21WAF is a downstream effector of p53 (25). It was also shown that the p21WAF promoter contained a binding site for p53 and that p53 acted as a transcriptional activator for p21WAF (22). Another CDK inhibitor, p27KIP, was first discovered in HeLa cells as a heat-stable inhibitory activity of cyclin E and D1 kinase activities (26), and in the yeast two-hybrid system it was shown to interact with cyclin D1/CDK2 (27). It was subsequently found that p27KIP was involved in the growth arrest caused by transforming growth factor-β (TGF-β) and contact inhibition, and since then it has been suggested that p27KIP mediates general antiproliferative effects in response to certain growth inhibitory signals (28).

Both p21WAF and p27KIP bind to and inactivate a variety of cyclin/CDK complexes, and existing data indicate that both cyclin-dependent kinase inhibitors inhibit cyclin/CDK activity by stoichiometric binding to the cyclin regulatory subunit (reviewed in Ref. 28). While it appeared that p27KIP mediated a more general antiproliferative response, p21WAF seemed to be associated with DNA damage. However, recent results suggest that p21WAF not only mediates growth arrest and apoptosis in response to DNA damage but may also be responsible for the antiproliferative effects of certain growth inhibitory stimuli, such as TGF-β (29), mimosine (30), okadaic acid, and 12-O-tetradecanoylphorbol-13-acetate (31), and differentiating agents (32). In all of these cases, p21WAF induction seemed to be independent of p53 function. Additional evidence for the existence of p53-dependent and -independent p21WAF-induction pathways arises from a recent study showing that TGF-β and p53 act through distinct elements on the p21WAF promoter (33).

Although there is now a growing body of evidence that p21WAF can mediate a p53-independent cell cycle arrest, the function and regulation of p21WAF in response to cytoinhibitory agents remains unclear. In this report, we present evidence that inhibition of protein geranylgeranylation leads to a G0/G1 block and a p53-independent induction of p21WAF. Furthermore, the work also demonstrates that inhibition of protein farnesylation results in either no effect on cell cycle distribution or accumulation of cells in G0/G1, or G1/M, depending on the type of human tumor cells. In all cases, however, these effects of FTI-277 are not due to changes in the levels of p21WAF or p27KIP.

**EXPERIMENTAL PROCEDURES**

**Synthesis of CAAX Peptidomimetics**—The FTase-specific peptidomimetic FTI-277 and the GGTase-I-specific peptidomimetic GGTTI-298 were synthesized as described previously (9, 10, 34). The purity and the chemical structures were proved by analytical high performance liquid chromatography, 1H NMR, elemental analyses, and high resolution mass spectrometry.

**Cells and Culture**—All human tumor cell lines except Colo 357 were purchased from ATCC (Rockville, MD). Colo 357 was a kind gift from Channing Der (University of North Carolina) (35). Panc-1 and Colo 357 were grown in Dulbecco's modified Eagle's medium, SKBr3 and MDAMB-231 were grown in RPMI 1640 medium, A-549 was grown in F12K medium, and HT-1080 was grown in minimal essential medium. All cell lines were propagated in a humidified 10% CO2 incubator at 37°C supplemented with 1% penicillin-streptomycin (Life Technologies, Inc.) and 10% fetal bovine serum (Atlanta Biologicals). All media were purchased from Life Technologies, Inc. (Baltimore, MD) except F12K was from Sigma.

**Western Blotting**—Cells were plated at low density (1.5 × 10^5) in 100-mm Petri dishes, treated with vehicle or inhibitors every 24 h for 48 h, harvested, and lysed in HEPES lysis buffer as described (13). 20-μg lysates were then electrophoresed on a 12.5% SDS-PAGE mini-gel, transferred to nitrocellulose, and immunoblotted with antibodies against p21WAF (C-19, Santa Cruz Biotechnology, Santa Cruz, CA), p27KIP (G173-524, PharMingen, San Diego, CA), cyclin E (C19, Santa Cruz Biotechnology), cyclin D1 (PRAD1, Santa Cruz Biotechnology), or Rap1A/Krev-1 (121, Santa Cruz Biotechnology) antibodies. Positive antibody reactions were visualized using peroxidase-conjugated secondary antibodies and an enhanced chemiluminescence detection system as described (36).

**Northern Blotting**—Calu-1 cells were grown to 30% confluency and treated with vehicle or inhibitors for 48 h. Total RNA was isolated as described in Chomczynski and Sacchi (37). Briefly, cells were solubilized in 4 M guanidinium isothiocyanate, proteins and DNA were extracted with phenol-chloroform at pH 4, and RNA was precipitated from the aqueous phase with isopro pyl alcohol. Pellets were washed with ethanol and redissolved in 0.5% SDS, and the amount of RNA was determined by UV spectroscopy. RNA aliquots (10–15 μg) were electrophoresed on a 1% agarose gel, transferred to nylon hybridization membranes (GeneScreen, NEN Life Science Products) and blotted with radiolabeled p21WAF and cyclophilin cDNA probes (32). Hybridized membranes were exposed to x-ray film for 1–3 days depending on signal intensity.

**Flow Cytometry**—Cells were plated in 100-mm Petri dishes and treated with inhibitors or vehicle as described for the Western blot procedure, and nuclei were prepared as described (38). Briefly, cells were harvested by gentle scraping, pelleted by low speed centrifugation, resuspended in 200 μl of citrate buffer (250 mM sucrose, 40 mM trisodium citrate, 5% Me2SO, pH 7.6), and frozen at −80°C. Before staining, cells were thawed quickly and treated with 450 μl of trypsin (30 μg/ml) followed by treatment with 375 μl of trypsin inhibitor (0.5 mg/ml) and RNase A (0.1 mg/ml). Nuclei were stained with 375 μl of ice-cold staining solution (0.4 mg/ml propidium iodide, 1.16 mg/ml spermine × 4 HCl, 0.5 mM Tris-base, pH 7.6). DNA content was determined in a FACStar flow cytometer (Becton-Dickinson, San Jose, CA). Unless otherwise stated, the proportions of cell in G0/G1, S, and G2/M phase were calculated from their DNA histogram using the BDIS Consort software package (Ver. 3.0, Becton-Dickinson).

**RESULTS**

**GGTI-298 Arrests Human Tumor Cells in the G1/G0 Phase of the Cell Cycle**—We have recently shown that the GGTase-I inhibitor GGTI-298 arrested NIH 3T3 and Rat-1 cells in the G1/G0 phase of the cell cycle, whereas the FTase inhibitor FTI-277 had no effect on the cell cycle distribution of these cells. This study, however, was carried out in fibroblasts, and the effects of these inhibitors on epithelial cells where most human tumors originate from is not known. Furthermore, the effects of these inhibitors on cell cycle events that are involved in their ability to inhibit growth of tumors have not been investigated. We therefore used eight human carcinoma cell lines of epithelial origin (two pancreatic (Panc-1 and Colo 357), two breast (SKBr3 and MDAMB-231), two lung (Calu-1 and MDAMB-231), two bladder (A-253), and one fibrosarcoma (HT-1080) to evaluate the effects of FTI-277 and GGTI-298 on cell cycle events. We first concentrated on three cell lines, Calu-1, A-253, and T-24. Cells were treated with vehicle or inhibitors as described under “Experimental Procedures,” and their DNA content was analyzed by flow cytometry after staining with propidium iodide. Fig. 1 shows that Calu-1, A-253, and T-24 cells treated with vehicle contained about 40% of the cells in G0/G1, 40% in S, and 20% in G2/M. GGTI-298 accumulated 70 to 90% of the cells in G0/G1. A similar effect on cell cycle distribution was observed with lovastatin, an inhibitor of protein geranylgeranylation and farnesylation. Thus, GGTI-298 and lovastatin led to a G1/G0 arrest in Calu-1, T-24, and A-253 cells. We next determined whether or not GGTI-298 can induce a G0/G1 block in other human tumor cell lines. Table
A-549, and Colo 357 cells showed a G2/M enrichment after T-24 and HT-1080 cells accumulated in G0/G1; and Calu-1, affected by treatment with FTI-277 (Fig. 1 and Table I). However, A-253, Panc-1, SKBr3, and MDAMB-231 cells was little af-

Effect of FTI-277 on Cell Cycle Distribution in Human Tumor Cells Is Cell-type-dependent—In contrast to FTI-277, which induced a G0/G1 block in all cells evaluated, the effects of FTI-277 were cell-type-dependent. The cell cycle distribution of Calu-1, A-253, and T-24 were little or no p21WAF. In contrast to cells treated with vehicle, those treated with GGTI-298 or lovastatin accumulated large quantities of p21WAF. However, treatment of cells with FTI-277 did not result in induction of the protein levels of p21WAF (Fig. 2, top panel). Higher concentrations of FTI-277 (up to 20 μM) did not affect p21WAF expression (data not shown). To determine whether this effect is specific to p21WAF, we carried out similar studies investigating the effects of prenyltranferase inhibitors on the protein levels of another cyclin-dependent kinase inhibitor, p27KIP. In contrast to the results with p21WAF, exponentially growing human tumor cells, treated with vehicle only, expressed detectable levels of p27KIP (Fig. 2, bottom panel). Treatment of Calu-1, A-253, and T-24 with lovastatin, GGTI-298, or FTI-277 did not affect protein levels of p27KIP (Fig. 2, bottom panel).

The effect of GGTI-298, FTI-277, and lovastatin on the levels of p21WAF mRNA were next investigated by Northern blot analysis. Fig. 3 shows that exponentially growing Calu-1 cells expressed low levels of p21WAF mRNA. Treatment with GGTI-298 and lovastatin, but not FTI-277, induced accumulation of large amounts of p21WAF mRNA. To determine whether the induction of p21WAF correlates with inhibition of protein geranylgeranylation and cell cycle arrest, we treated Calu-1 cells with various concentrations of GGTI-298 and evaluated p21WAF protein levels, Rap1A processing (a small G-protein that is exclusively geranylgeranylated by GGTagase-I), and cell cycle distribution as described under “Experimental Proce-

![Fig. 1. GGTI-298 arrests cells in the G0/G1 phase of the cell cycle.](image)

![Fig. 2. Treatment with GGTI-298 results in elevated protein levels of p21WAF but not p27KIP.](image)

### Table I

**Effects of GGTI-298 and FTI-277 on cell cycle phase distribution in human tumors**

| Cell line  | Percent G0/G1 | Percent G2/M |
|-----------|---------------|--------------|
| Control   | FTI-277       | GGTI-298     |
| Calu-1    | 53            | 34           | 83           | 16 | 42 | 10 |
| Panc-1    | 55            | 46           | 84           | 21 | 24 | 18 |
| Colo-357  | 51            | 43           | 87           | 15 | 28 | 7  |
| T-24      | 37            | 63           | 98           | 26 | 20 | 2  |
| A-253     | 38            | 34           | 86           | 19 | 24 | 8  |
| SKBr3     | 50            | 51           | 85           | 13 | 22 | 5  |
| MDAMB-231 | 55            | 60           | 93           | 17 | 16 | 3  |
| HFT1080   | 32            | 78           | 83           | 24 | 18 | 11 |
| A-549     | 55            | 56           | 86           | 13 | 32 | 3  |

Data are representative of at least two independent experiments.

* Asterisks represent a difference from control that is significant on the p < 0.05 level.
GGTI-298 and lovastatin, but not FTI-277, induce p21WAF RNA in a p53-deficient cell line. Calu-1 cells were treated with vehicle (lane 1), 30 μM lovastatin (lane 2), 5 μM FTI-277 (lane 3), or 15 μM GGTI-298 (lane 4) for 48 h, and total RNA was isolated as described under “Experimental Procedures.” Expression of p21WAF and cyclophilin mRNA was analyzed by Northern blot analysis. Data are representative of at least two independent experiments.

![Figure 3](image)

**Figure 3.** GGTI-298 and lovastatin, but not FTI-277, induce p21WAF RNA in a p53-deficient cell line. Calu-1 cells were treated with vehicle (lane 1), 30 μM lovastatin (lane 2), 5 μM FTI-277 (lane 3), or 15 μM GGTI-298 (lane 4) for 48 h, and total RNA was isolated as described under “Experimental Procedures.” Expression of p21WAF and cyclophilin mRNA was analyzed by Northern blot analysis. Data are representative of at least two independent experiments.

![Figure 4](image)

**Figure 4.** GGTI-298 Concentration-response effects on cell cycle events. Calu-1 cells were treated with GGTI-298 (0–15 μM), and the cells were processed and analyzed for Rap1A processing, p21WAF and cyclin D1 and E expression, and percentage of cells in the G0/G1 phase of the cell cycle as described under “Experimental Procedures.” Data are representative of two independent experiments.

GGTI-298 paralleled those required to induce p21WAF and to inhibit Rap1A processing (Fig. 4).

Table II

| Cell line | Control | FTI-277 | GGTI-298 |
|-----------|---------|---------|-----------|
| Calu-1    |         |         | +         |
| Panc-1    |         |         | +         |
| Colo-357  |         |         | +         |
| T-24      |         |         | +         |
| A-253     |         |         | +         |
| SKBr3     |         |         | +         |
| MDAMB-231 |         |         | +         |
| HT1080    | +       | +       | +         |
| A-549     | +       | +       | +         |

GGTI-298 can induce p21WAF, we analyzed by Western blotting the protein levels of p21WAF before and after treatment with GGTI-298 in the human tumor panel described in Table I. All human tumors evaluated except HT-1080 and A-549 expressed low background levels of p21WAF (Table II). In all seven tumors that expressed low levels of p21WAF, GGTI-298 induced the expression of p21WAF (Table II). In HT-1080 and A-549 cells, that started out with high basal levels of p21WAF, no further increase in p21WAF expression levels was seen (Table II).

**DISCUSSION**

The results described in this manuscript clearly demonstrate that inhibition of protein geranylgeranylation induces only a G1 block, whereas inhibition of protein farnesylation can result either in a G1 block, a G2/M enrichment, or no effect on cell cycle distribution. The results also demonstrate that GGTI-298 but not FTI-277 induces the cyclin-dependent kinase inhibitor, p21WAF. Several important conclusions can be drawn from these results. First, in T-24 and HT-1080 cells, either farnesylated or geranylgeranylated proteins can be involved in the G1 to S phase transition of the cell cycle. This is different from NIH 3T3, Rat-1, Calu-1, A-253, Panc-1, Colo 357, SKBr3, MDAMB-231, and A-549 cells where only geranylgeranylated proteins appear to be involved in the G1/S transition. Second, both GGTI-298 and FTI-277 caused G1 arrest in T-24 cells, yet only GGTI-298 induced p21WAF. Thus, in these cells, a farnesylated protein must be involved in the G1 to S phase transition, but this protein is not involved in the regulation of p21WAF induction. Third, the fact that FTI-277 induced an enrichment in G2/M phase suggests that at least in some human tumors (i.e., A-549, Colo 357, and Calu-1), farnesylated proteins may play an important role in this phase transition of the cell cycle.

Several geranylgeranylated and farnesylated proteins are possible candidates for the observed effects of FTI-277 and GGTI-298 on cell cycle distribution. For example, the Rho family of small G-proteins that are geranylgeranylated such as RhoA and Rac1 have been implicated in the G1 to S transition of the cell cycle (39). Since geranylgeranylation of these proteins is important for their cellular localization and function, inhibition of this posttranslational modification by GGTI-298 would be expected to prevent cells from entering S phase and cause their accumulation in the G1 phase of the cell cycle. Furthermore, constitutive activation of RhoA and Rac1 in their GTP-locked forms has been shown to result in transformation, and dominant negative forms of these proteins partially re-
verse malignant transformation by Ras (40, 41). We have recently shown that GGTI-298 inhibits the growth of Ras-transformed NIH 3T3 cells, consistent with the suggestion that RhoA and Rac1 act downstream of Ras and giving further support to their importance in the mechanism of action of GGTI-298. Possible farnesylated targets for the actions of FTI-277 on cell cycle distribution are the Ras proteins. K-Ras is an unlikely target since its prenylation is resistant to FTI-277 (4, 34). Even at concentrations as high as 50 μM, FTI-277 does not inhibit K-Ras prenylation in human tumors (8). This is due to alternative prenylation by GTTase-I of K-Ras when cells are treated with FTase inhibitors (4–7). Consistent with this is our finding that K-Ras prenylation is inhibited in human tumors only when co-treated with FTI-277 and GGTI-298 (8). Thus, K-Ras is not expected to play a role in FTI-277 effects on cell cycle distribution since, alone, FTI-277 is able to induce G1 block or G2/M enrichment. N- and H-Ras, on the other hand, may play a role since their prenylation is inhibited at concentrations (5 μM) that affect cell cycle distribution (8). Alternatively, other farnesylated proteins such as lamins or RhoB may also be important (42).

The fact that FTI-277 has different effects on the cell cycle in different cell lines is intriguing and suggests that farnesylated proteins may be involved in both G1/M and G2/M phase transitions of the cell cycle. The results also suggest that the ability of FTI-277 to affect the cell cycle may be dependent on other genetic alterations that the tumor harbors. For example, the levels of cyclins, cyclin-dependent kinases, their inhibitors, or the status of tumor suppressor genes such as p53 and Rb, that are all involved in cell cycle regulation, may determine whether an inhibitor of protein farnesylation results in G1 block or G2/M enrichment. Whether the ability of FTI-277 to inhibit tumor growth depends on its cell cycle effects is not clear. We do know that FTI-277 inhibits the tumor growth in nude mouse xenografts of Calu-1 cells (13), A-549, and Panc-1 cells2 and the growth on soft agar of Calu-1, A-549, Colo 357, MDA-MB-231, and SKBr3.3 These results would argue against a requirement of G1 block or G2/M enrichment for the ability of FTI-277 to inhibit tumor growth.

Seven out of the nine human tumor cell lines used in this study expressed low levels of p21^{WAF} that were induced by GGTI-298. Since GGTI-298 also induced a G1 block in these cells, the results suggest that GGTI-298 induces a G1 block through an induction of p21^{WAF}, which in turn could inhibit cyclin-dependent kinases. Consistent with this is our recent observation that GGTI-298 induces hypophosphorylation of Rb, a substrate for cyclin-dependent kinases (data not shown). However, GGTI-298 also induces a G1 block without further increases of the level of p21^{WAF} in the two out of nine human tumor cell lines that express a high basal level of p21^{WAF}. This implies that GGTI-298 can induce a G1 block by a mechanism that does not involve p21^{WAF} induction, at least in human tumor cells that express high basal levels of p21^{WAF}.

Unlike FTI-277, which can block cells in G1 or induce G2/M enrichment, lovastatin blocks cells primarily in the G1 phase (43). The ability of lovastatin to block cells in G1 appears to be due to its ability to inhibit protein geranylgeranylation rather than farnesylation. Indeed, several recent reports have shown that the lovastatin-induced G1 block can be released by GGOH (44, 45, 46), implicating geranylgeranylated proteins in the effects of lovastatin. Furthermore, we have also shown that lovastatin-induced apoptosis in A-549 (11) and smooth muscle4 can be overcome by GGOH. Similarly, the ability of lovastatin to induce nitric-oxide synthase-2 is also dependent on inhibition of protein geranylgeranylation since it is reversed by GGOH but not FOH (16).

Besides its ability to inhibit tumor growth and affect cell cycle in some cells, FTI-277 has little effect on other cellular events. In contrast, GGTI-298 causes profound changes in cell physiology. For example GGTI-298, but not FTI-277, inhibits PDGF- and EGF-induced tyrosine phosphorylation of their receptors (10), enhances induction of nitric-oxide synthase-2 (16) and apoptosis (11). Whether these effects are related to the ability of GGTI-298 to induce p21^{WAF} is not known. This induction of p21^{WAF}, however, is p53-independent. Since p21^{WAF} is a major negative regulator of the G1 to S transition, further development of GTTase-I inhibitors may in the future lead to antineoplastic agents that not only act as general cytotoxic agents but possibly restore p53-dependent growth inhibitory responses in cells lacking functional p53.

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