Peroxisome Proliferator-Activated Receptor γ-Independent Ablation of Cyclin D1 by Thiazolidinediones and Their Derivatives in Breast Cancer Cells

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ABSTRACT

In light of the clinical relevance of targeting cyclin D1 in breast cancer, we have investigated the mechanism underlying the effect of the peroxisome proliferator-activated receptor-γ (PPARγ) agonists troglitazone and ciglitazone on cyclin D1 repression. We obtain evidence that the ability of high doses of troglitazone and ciglitazone to repress cyclin D1 is independent of PPARγ activation. PPARγ-inactive troglitazone and ciglitazone analogs 5-[4-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl-methoxy)-benzylidene]-2,4-thiazolidinedione (Δ2-TG) and 5-[4-(1-methyl-cyclohexylmethyl)-benzylidene]-thiazolidine-2,4-dione are able to facilitate cyclin D1 ablation with potency similar to that of troglitazone and ciglitazone in MCF-7 cells. Reverse transcription-polymerase chain reaction shows that the mRNA level of cyclin D1 remains unaltered in drug-treated cells, indicating the repression is mediated at the post-transcriptional level. Moreover, the ablative effect of these agents is specific to cyclin D1, in that the expression levels of many other cyclins and cyclin-dependent kinases examined remain unchanged after drug treatment. Our data indicate that troglitazone- and Δ2-TG-induced cyclin D1 repression is mediated via proteasome-facilitated proteolysis because it is inhibited by different proteasome inhibitors, including N-carbobenzoxy-L-leucinyl-L-leucinyl-L-norleucinal (MG132), lactacystin, and epoxomicin, and is preceded by increased ubiquitination. The dissociation of these two pharmacological activities (i.e., PPARγ activation and cyclin D1 ablation) provides a molecular basis to use Δ2-TG as a scaffold to develop a novel class of cyclin D1-ablative agents. Therefore, a series of Δ2-TG derivatives have been synthesized. Among them, 5-[4-(6-allyoxy-2,5,7,8-tetramethyl-chroman-2-yl-methoxy)-benzylidene]-2,4-thiazolidinedione represents a structurally optimized agent with potency that is an order of magnitude higher than that of Δ2-TG in cyclin D1 repression and MCF-7 cell growth inhibition.

Cyclin D1 represents an important downstream effector of diverse proliferative and transforming signaling pathways, including those mediated by β-catenin (Shutman et al., 1999), estrogen receptor α (ERα) (Lukas et al., 1996; Wilcken et al., 1997; Prall et al., 1998), Her-2/Neu (Lee et al., 2000), nuclear factor-κB (Joyce et al., 1999; Henry et al., 2000), Rac (Westwick et al., 1997), Ras (Albanese et al., 1995), Src (Lee et al., 1999), signal transducer and activator of transcription (Bromberg et al., 1999; Matsumura et al., 1999), and Wnt (D’Amico et al., 2000). In mammary cells, transcriptional activation of cyclin D1 in response to these mitogenic signals leads to G1/S progression and increased proliferation. Cyclin D1 overexpression has been implicated in oncogene-induced mammary tumorigenesis; it has been noted in more than 50% of primary breast carcinomas correlating with poor prognosis (McIntosh et al., 1995; Kenny et al., 1999). In addition to activating cyclin-dependent kinases (CDKs) and sequestering of CDK inhibitors in the G1/S transition, the function of many other cyclins and cyclin-dependent kinases examined remain unchanged after drug treatment. Our data indicate that troglitazone- and Δ2-TG-induced cyclin D1 repression is mediated via proteasome-facilitated proteolysis because it is inhibited by different proteasome inhibitors, including N-carbobenzoxy-L-leucinyl-L-leucinyl-L-norleucinal (MG132), lactacystin, and epoxomicin, and is preceded by increased ubiquitination. The dissociation of these two pharmacological activities (i.e., PPARγ activation and cyclin D1 ablation) provides a molecular basis to use Δ2-TG as a scaffold to develop a novel class of cyclin D1-ablative agents. Therefore, a series of Δ2-TG derivatives have been synthesized. Among them, 5-[4-(6-allyoxy-2,5,7,8-tetramethyl-chroman-2-yl-methoxy)-benzylidene]-2,4-thiazolidinedione represents a structurally optimized agent with potency that is an order of magnitude higher than that of Δ2-TG in cyclin D1 repression and MCF-7 cell growth inhibition.

ABBREVIATIONS: ER, estrogen receptor; CDK, cyclin-dependent kinase; PPAR, peroxisome proliferator-activated receptor; PGJ2, 15-deoxy-D12,14-prostaglandin J2; J8, N-carbobenzoxy-L-leucinyl-L-leucinyl-L-norleucinal; Δ2-TG, 5-[4-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl-methoxy)-benzylidene]-2,4-thiazolidinedione; Δ2-CG, 5-[4-(1-methyl-cyclohexylmethyl)-benzylidene]-thiazolidine-2,4-dione; Δ2-TG-6, 5-[4-(6-allyoxy-2,5,7,8-tetramethyl-chroman-2-yl-methoxy)-benzylidene]-2,4-thiazolidinedione; DMEM, dimethyl sulfoxide; GSK, glycogen synthase kinase; DMEM, Dulbecco’s modified Eagle’s medium; PBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; ELISA, enzyme-linked immunosorbent assay; TBST, Tris-buffered saline (W9620); RT, reverse transcriptase; PCR, polymerase chain reaction; SB216763, 3-[2,4-dichlorophenyl]-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione; GW9662, 2-chloro-5-nitro-N-phenylbenzamide.
cyclin D1 as a CDK-independent activator of ERα is especially noteworthy (Neuman et al., 1997; Zwijnen et al., 1997; McMahon et al., 1999; Lamb et al., 2000). Cyclin D1 overexpression confers resistance to antiestrogens in breast cancer cells (Musgrove et al., 2001; Hui et al., 2002) and represents a negative predictive factor for tamoxifen response (Stendahl et al., 2004). Together, these findings suggest that an anti-cyclin D1 therapy might be highly specific for treating human breast cancer (Yu et al., 2001).

Peroxisome proliferator-activated receptor γ (PPARγ) agonists, including 15-deoxy-Δ12,14-prostaglandin J2 (PGJ2) and thiazolidinediones, have been shown to down-regulate cyclin D1 expression as part of the mechanism for causing cell-cycle arrest and growth inhibition in breast cancer cells (Wang et al., 2001; Yin et al., 2001; Lapillonne et al., 2003; Qin et al., 2003, 2004). Two distinct PPARγ-dependent mechanisms have been reported to account for PGJ2γ and ciglitazone-mediated cyclin D1 repression. First, PGJ2γ treatment could lead to the sequestration of p300, a coactivator protein, thereby preventing the transcriptional activation of the cyclin D1 promoter (Wang et al., 2001). Second, PGJ2γ and ciglitazone could activate proteasome-mediated degradation of cyclin D1 (Qin et al., 2003).

In this study, we obtained evidence that the ability of troglitazone and ciglitazone to down-regulate cyclin D1 and, to a lesser extent, ERα was independent of PPARγ activation. We demonstrate that PPARγ-inactive troglitazone and ciglitazone analogs could facilitate proteasome-mediated proteolysis of cyclin D1 in a manner similar to that of their parent thiazolidinediones. The dissociation of these two pharmacological activities provides a molecular basis upon which to develop a novel class of cyclin D1-ablative agents. The proof of principle is illustrated by a troglitazone analog with efficacy that is an order of magnitude higher than that of troglitazone in cyclin D1 repression and MCF-7 cell growth inhibition.

Materials and Methods

Reagents. Troglitazone, ciglitazone, MG132, lactacystin, and SB216763 were purchased from Sigma-Aldrich (St. Louis, MO). Rosiglitazone and pioglitazone were prepared from the respective commercial tablets by solvent extraction, followed by recrystallization or crystallization. Epoxomicin was a kind gift from Dr. Y.-P. Chen (KCL, Taiwan). Thiazolidinedione derivatives were verified by proton nuclear magnetic resonance, high-resolution mass spectrometry, and elemental analysis. These agents and thiocarbamide were dissolved in DMSO and added to cells in medium with a final DMSO concentration of 0.1%. Rabbit antibodies against p-glycogen synthase kinase (GSK) and mouse anti-cyclin D1 or anti-ubiquitin (sc-8030) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Mouse monoclonal anti-actin was purchased from Sigma-Aldrich (St. Louis, MO). Mouse monoclonal anti-actin was purchased from Sigma-Aldrich (St. Louis, MO). Rabbit antibodies against p-glycogen synthase kinase (GSK) and mouse anti-cyclin D1 or anti-ubiquitin (sc-8030) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Cell Culture. ER-positive MCF-7 and ER-negative MDA-MB-231 breast cancer cells were obtained from the American Type Culture Collection (Manassas, VA) and were maintained in DMEM/Ham’s F-12 medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified incubator containing 5% CO2.

Cell Viability Analysis. The effect of individual test agents on cell viability was assessed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay in six replicates. Cells were seeded and incubated in 96-well, flat-bottomed plates in DMEM/Ham’s F-12 media with 10% FBS for 24 h and were exposed to various concentrations of test agents dissolved in DMSO (final DMSO concentration, 0.1%) in 5% FBS-supplemented DMEM/Ham’s F-12 medium. Controls received DMSO vehicle at a concentration equal to that of drug-treated cells. The medium was removed and replaced by 200 μl of 0.5% MTT in 10% FBS-containing RPMI 1640 medium, and cells were incubated in the CO2 incubator at 37°C for 2 h. Supernatants were removed from the wells, and the reduced MTT dye was solubilized at 200 μl/well DMSO. Absorbance at 570 nm was determined on a plate reader.

Analysis of PPARγ Activation. The analysis was carried out by using a PPARγ transcription factor ELISA kit (Active Motif, Carlsbad, CA), in which an oligonucleotide containing the peroxisome proliferator response element was immobilized onto a 96-well plate. PPARs contained in nuclear extracts bind specifically to this oligonucleotide and are detected through an antibody directed against PPARγ. In brief, MCF-7 cells were cultured in DMEM/Ham’s F-12 medium supplemented with 10% FBS and treated with DMSO vehicle or individual test agents, 10 μM each, for 48 h. Cells were collected, and nuclear extracts were prepared with a Nuclear Extract kit (Active Motif Inc., Carlsbad, CA). Nuclear extracts of the same protein concentration from individual treatments were subject to the PPARγ transcription factor ELISA according to the manufacturer’s instruction.

Western Blot Analysis. MCF-7 or MDA-MB-231 cells were seeded in 10% FBS-containing DMEM/Ham’s F-12 medium for 24 h and treated with various agents as indicated. After individual treatments for 24 h, both the incubation medium and adherent cells in T-25 or T-75 flasks were scraped and collected by centrifugation at 2000g for 10 min. The supernatants were recovered, placed on ice, and triturated with 20 to 50 μl of a chilled lysis buffer (M-PER Mammalian Protein Extraction Reagent; Pierce, Rockford, IL), to which was added 1% protease inhibitor cocktail (set III; EMD Biosciences, Inc., San Diego, CA). After a 30-min incubation on ice, the mixture was centrifuged at 16,100g for 3 min. Two microliters of the supernatant was taken for protein analysis using the Bradford assay kit (Bio-Rad, Hercules, CA); to the remaining solution was added the same volume of 2× SDS-polyacrylamide gel electrophoresis sample loading buffer (100 mM Tris-HCl, pH 6.8, 4% SDS, 5% mercaptoethanol, 20% glycerol, and 0.1% bromphenol blue). The mixture was boiled for 10 min. Equal amounts of proteins were loaded onto 10% SDS-polyacrylamide gels. After electrophoresis, protein bands were transferred to nitrocellulose membranes in a semidyry transfer cell. The transblotted membrane was blocked with Tris-buffered saline (TBS)/0.1% Tween 20 (TBST) containing 5% nonfat milk for 90 min, and the membrane was incubated with the appropriate primary antibody in TBST/5% nonfat milk at 4°C overnight. After washing three times with TBST for a total of 45 min, the transblotted membrane was incubated with goat anti-rabbit or anti-mouse IgG-horseradish peroxidase conjugates (diluted 1:1000) for 1 h at room temperature and washed four times with TBST for a total of 1 h. The immunoblots were visualized by enhanced chemiluminescence.

Coimmunoprecipitation/Western Blot. MCF-7 cells were cultured in 10% FBS-containing DMEM/Ham’s F-12 medium in 75-mm plates for 24 h. Cells were treated with DMSO vehicle, 30 μM troglitazone, or 20 μM Δ2-TG in 5% FBS-containing DMEM/Ham’s F-12 medium for another 20 h. Cells were rinsed with phosphate-buffered saline at room temperature, scraped off the flask, transferred into centrifuge tubes, and centrifuged at 2000g for 10 min to pellet the cells. The pellet was resuspended in ice-cold 0.5 ml of radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, and 1% protease inhibitor cocktail) and gently mixed on an orbital shaker at 4°C for 15 min, followed by centrifugation at
Results

Effect of Thiazolidinediones on Cyclin D1 Down-regulation Is Independent of PPARγ. Three lines of evidence suggest that thiazolidinedione-mediated cyclin D1 down-regulation in breast cancer cells was independent of PPARγ activation. First, we assessed the effect of troglitazone on cyclin D1 expression in two breast cancer cell lines: ER-positive MCF-7 and ER-negative MDA-MB-231. Among many genotypic differences, these two cell lines exhibit differential PPARγ expression; i.e., PPARγ expression in MDA-MB-231 cells was at least an order of magnitude higher than that of MCF-7 cells (Fig. 1A). Despite this discrepancy, MCF-7 cells showed a higher degree of susceptibility to troglitazone-mediated cyclin D1 down-regulation compared with the PPARγ-rich MDA-MB-231 cells (B).

Second, we assessed the effect of four different thiazolidinediones (i.e., troglitazone, ciglitazone, rosiglitazone, and pioglitazone) on intracellular cyclin D1 in MCF-7 cells. Among them, troglitazone and ciglitazone at high doses were effective in reducing cyclin D1 and ERα levels (Fig. 2, B and C). In contrast, rosiglitazone and pioglitazone lacked appreciable effects at comparable concentrations (data not shown), although these two agents are more active than troglitazone and ciglitazone in PPARγ activation.

![Fig. 1. Effect of troglitazone on cyclin D1 down-regulation in breast cancer cells is irrespective of PPARγ expression levels.](https://example.com/fig1.png)

![Fig. 2. Pharmacological evidence that the effect of troglitazone and ciglitazone on cyclin D1 down-regulation is dissociated from PPARγ activation.](https://example.com/fig2.png)
Third, we examined the effect of GW9662, a potent PPARγ antagonist (Leesnitzer et al., 2002; Seargent et al., 2004), on troglitazone-mediated cyclin D1 repression in MCF-7 cells. Even at concentrations 3 orders of magnitude higher than the IC_{50} in PPARγ binding, GW9662 had no appreciable effect on cyclin D1 expression and did not prevent troglitazone-mediated cyclin D1 down-regulation (Fig. 1C).

Separation of the Cyclin D1-Ablative Effect from the PPARγ Agonist Activity. To further discern the role of PPARγ in thiazolidinedione-induced cyclin D1 ablation, we synthesized the unsaturated derivatives of troglitazone and ciglitazone [i.e., Δ2-TG and Δ2-CG (Fig. 2A)], both of which were inactive in PPARγ activation according to a PPARγ transcription factor ELISA (right). The effects of troglitazone, ciglitazone, and their Δ2-counterparts on the expression of cyclin D1 and ERα in MCF-7 cells were analyzed by Western blotting.

As shown, Δ2-TG and Δ2-CG, although devoid of PPARγ activity, reduced the expression levels of cyclin D1 and ERα in MCF-7 cells in a dose-dependent manner with potency higher than that of troglitazone and ciglitazone (Fig. 2B). For example, the minimum concentration required for the complete ablation of cyclin D1 was 30 μM for both Δ2-TG and Δ2-CG, compared with 40 and 50 μM for troglitazone and ciglitazone, respectively. In contrast, the effect of these agents on ERα lagged behind that of cyclin D1, requiring substantially higher concentrations to achieve the same extent of repression.

Figure 3A depicts the time course of cyclin D1 down-regulation by 40 μM troglitazone and 30 μM Δ2-TG in MCF-7 cells. Both agents achieved complete ablation at 24 h after treatment. However, semiquantitative PCR shows that the mRNA level of cyclin D1 remained unaltered after 24 h exposure (Fig. 3), suggesting that troglitazone- and Δ2-TG-induced cyclin D1 ablation was mediated at the post-transcriptional level.

To examine whether the ablative effect of troglitazone- and Δ2-TG was unique to cyclin D1, we assessed the expression levels of cyclins D2, D3, A, B, and E, and CDKs 2 and 4 in MCF cells treated with different doses of troglitazone- and Δ2-TG (Fig. 4). Although cyclin D2 and CDK4 showed a slight decrease in their expression levels, among these cell cycle-regulating proteins no appreciable effect was observed with the other cyclins and CDKs, indicating that the ablative effect was highly specific.

Troglitazone and Δ2-TG Facilitate Proteasome-Mediated Proteolysis of Cyclin D1. Pursuant to the report that the effect of PGJ2 and ciglitazone on cyclin D1 repression was attributable to proteasome-mediated degradation (Choi et al., 1997; Qin et al., 2003), we tested the effect of three proteasome inhibitors (MG132, lactacystin, and epoxomicin) on troglitazone and Δ2-TG-facilitated cyclin D1 ablation in MCF-7 cells. As shown in Fig. 5, all three proteasome inhibitors were effective in rescuing the drug-induced cyclin D1 repression.

Because proteasome-facilitated proteolysis of cyclin D1 is preceded by ubiquitination (Coqueret, 2002), we examined the formation of ubiquitinated cyclin D1 in MCF-7 cells after exposure to Δ2-TG for 24 h. Signals were quantitated by densitometry and normalized against β-actin measurements (bottom). Each data point represents mean ± S.D. (n = 3).

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blotting with either cyclin D1 or ubiquitin antibodies (Fig. 6). As shown, whereas cyclin D1 expression was diminished in troglitazone- and Δ2-TG-treated MCF-7 cells (left; IP, anti-cyclin D1; WB, anti-cyclin D1), the extent of ubiquitination of cyclin D1 increased as indicated by a complex ladder of ubiquitinated cyclin D1 bands (right; IP, anti-cyclin D1; WB, anti-ubiquitin).

Evidence indicates that cyclin D1 ubiquitination could be facilitated by either a GSK-3β-dependent or -independent pathway. In the GSK-3β-dependent pathway, CDK-bound cyclin D1 undergoes GSK-3β-mediated phosphorylation, followed by translocation to the cytoplasm, where it undergoes proteasomal degradation (Diehl et al., 1997, 1998). Otherwise, free cyclin D1 can be ubiquitinated independently of GSK-3β, although the exact mechanism remains elusive (Germain et al., 2000). In this study, we obtained two lines of evidence to exclude the involvement of GSK-3β in troglitazone- and Δ2-TG-facilitated cyclin D1 degradation. First, the GSK-3β phosphorylation level remained unaltered in troglitazone- and Δ2-TG-treated MCF-7 cells (Fig. 7A). Second, cotreatment with the selective GSK-3β inhibitor SB216763 could not rescue troglitazone- or Δ2-TG-induced cyclin D1 ablation (B).

Development of Novel Δ2-TG-Derived Cyclin D1-Ablative Agents. The findings described above prompted the notion that Δ2-TG could be used as a scaffold to develop novel cyclin D1 ablative agents. Therefore, a series of Δ2-TG derivatives was synthesized, and the derivatives’ respective activities in ablating cyclin D1 in MCF-7 cells were examined. Among more than 20 derivatives tested, Δ2-TG-6 represented a structurally optimized agent with potency an order of magnitude higher than that of Δ2-TG. This increase was attributed to an additional allyl moiety on the terminal hydroxyl function of Δ2-TG (Fig. 8A). As shown, Δ2-TG-6 reduced cyclin D1 levels at concentrations as low as 2.5 μM compared with >20 μM for Δ2-TG (B). Like its parent molecule, the effect of Δ2-TG-6 on cyclin D1 ablation could be blocked by the proteasome inhibitor MG132 (C). In line with its enhanced ability in cyclin D1 ablation, Δ2-TG-6 exhibited significantly higher potency than Δ2-TG in inhibiting MCF-7 cell proliferation (IC50, 8 versus 55 μM) (D).
Discussion

A variety of mechanisms has been proposed to account for the ability of various antiproliferative agents to ablate cyclin D1 expression. These include transcriptional repression of the cyclin D1 promoter (flavopiridol and PGJ2) (Carlson et al., 1999; Wang et al., 2001), calpain-mediated proteolytic degradation (lovastatin and actinomycin D) (Choi et al., 1997), and proteasome-facilitated proteolysis (retinoic acid and various PPARγ agonists) (Langenfeld et al., 1997; Wang et al., 2001; Lapillonne et al., 2003). From a clinical perspective, this drug-induced cyclin D1 repression not only contributes to the inhibition of breast cancer cell proliferation but can also overcome drug resistance by sensitizing breast cancer cells to apoptotic signals emanating from Akt inhibition (Wu et al., 2002). Thus, an urgent need exists to develop potent cyclin D1- ablative agents that are effective in the therapeutically attainable range (≤5 μM) for the treatment and/or prevention of breast cancer.

Of the aforementioned agents, the PPARγ agonists troglitazone and ciglitazone represent attractive molecules for this drug discovery effort. Thus, we first investigated the mechanism underlying troglitazone- and ciglitazone-mediated cyclin D1 down-regulation. Several lines of evidence suggest that the effect of troglitazone and ciglitazone on cyclin D1 is independent of PPARγ activation. First, this cyclin D1- ablative effect was not noted with the more potent PPARγ agonists rosiglitazone and pioglitazone at comparable concentrations and could not be rescinded by the PPARγ antagonist GW9662. Second, despite significantly higher PPARγ expression, MDA-MB-231 cells were less susceptible to troglitazone-mediated cyclin D1 ablation. Third, Δ2-TG and Δ2-CG, although devoid of PPARγ activity, were able to mediate cyclin D1 ablation with slightly higher potency than that of troglitazone and ciglitazone. Furthermore, troglitazone and Δ2-TG exhibit the same mechanism in down-regulating cyclin D1 in MCF-7 cells. Our data indicate that both agents facilitated proteasomal proteolysis via a GSK-3β-independent mechanism. Two lines of evidence suggest that ERα might play a role in the thiazolidinedione-promoted degradation of cyclin D1. First, the cyclin D1 ablation was accompanied by a decrease in ERα expression in MCF-7 cells (Fig. 2). Second, the ERα-negative MDA-MB-231 cells were more resistant to the cyclin D1- ablative effect of troglitazone (Fig. 1).

This thiazolidinedione-mediated down-regulation of cyclin D1 and ERα is reminiscent of that of the histone deacetylase inhibitor trichostatin A (Alao et al., 2004). Trichostatin A has been shown to repress cyclin D1 and ERα expression, in part through the up-regulation of Skp2/p45, a regulatory component of the Skp1/Cullin/F-box complex implicated in the ubiquitination of cyclin D1 (Alao et al., 2004). Involvement of Skp2 in thiazolidinedione-mediated cyclin D1 ablation is currently under investigation.

The separation of cyclin D1 ablation from PPARγ provides a rationale to use the structure of Δ2-TG as a platform to carry out lead optimization. The proof of principle for this premise was Δ2-TG-6, a close structural analog that exhibited potency that was an order of magnitude higher than that of troglitazone and Δ2-TG in facilitating cyclin D1 repression and inhibiting MCF-7 cell proliferation. The clinical relevance of these small-molecule cyclin D1 ablative agents in breast cancer therapy/prevention is multifold. First, cyclin D1 ablation provides specific protection against breast carcinogenesis (Yu et al., 2001). Second, in light of the role of cyclin D1 overexpression in antiestrogen resistance, cyclin D1 ablation may help overcome the resistance. Third, the synergistic interaction between flavopiridol and trastuzumab in inhibiting breast cancer cell proliferation was attributable, in part, to the reduction of cyclin D1 expression (Wu et al., 2002). These agents may sensitize cells to the antiproliferative action of either CDK inhibitor or Her-2/Akt inhibition. Therefore, structural modifications of Δ2-TG-6 to further enhance its cyclin D1- ablative potency constitute the current focus of this investigation.

References


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