Wedelolactone, a Naturally Occurring Coumestan, Enhances Interferon-γ Signaling through Inhibiting STAT1 Protein Dephosphorylation

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Background: IFN-γ inhibits tumor cell growth by activating STAT1.

Results: Wedelolactone specifically inhibited TCPTP, the major phosphatase of STAT1, prolonged IFN-γ-induced STAT1 phosphorylation, and enhanced the antitumor effect of IFN-γ.

Conclusion: Wedelolactone enhanced the antitumor effect of IFN-γ by inhibiting TCPTP-mediated STAT1 dephosphorylation.

Significance: We identified a novel antitumor drug candidate, a new target, and a new mechanism to develop novel anticancer therapeutics.

Signal transducers and activators of transcription 1 (STAT1) transduces signals from cytokines and growth factors, particularly IFN-γ, and regulates expression of genes involved in cell survival/death, proliferation, and migration. STAT1 is activated through phosphorylation on its tyrosine 701 by JAKs and is inactivated through dephosphorylation by tyrosine phosphatases. We discovered a natural compound, wedelolactone, that increased IFN-γ signaling by inhibiting STAT1 dephosphorylation and prolonging STAT1 activation through specific inhibition of T-cell protein tyrosine phosphatase (TCPTP), an important tyrosine phosphatase for STAT1 dephosphorylation. More interestingly, wedelolactone inhibited TCPTP through interaction with the C-terminal autoinhibition domain of TCPTP. We also found that wedelolactone synergized with IFN-γ to induce apoptosis of tumor cells. Our data suggest a new target for anticancer or antiproliferation drugs, a new mechanism to regulate PTPs specifically, and a new drug candidate for treating cancer or other proliferation disorders.

In addition to its roles in inflammation, STAT1 also functions as a tumor suppressor (2, 3). Loss of STAT1 signaling has been found in a large group of diverse tumors. STAT1 knockout mice suffer a higher spontaneous rate of malignancy. Deficiency in STAT1 signaling also allows tumor cells to escape from IFN-γ-dependent immunosurveillance. As a result, STAT1 null mice develop more frequent and rapidly growing tumors when exposed to chemical carcinogens (4). STAT1 is also required for the growth inhibition effects of IFN-γ (5, 6). STAT1 regulates a wide spectrum of genes involved in cell cycle control, apoptosis, angiogenesis, cell invasion, metastasis, and immune recognition (7). STAT1 can trigger the expression of proapoptotic genes, such as caspases 2, 3, and 7, while suppressing the expression of antiapoptotic genes such as BCL2L1 and BCL2 (8).

STAT1 is activated through phosphorylation on tyrosine 701, mainly by JAKs, leading to STAT1 dimerization, nuclear translocation, and activation of target gene expression. Activated STAT1 can be negatively regulated through protein tyrosine phosphatases (PTPs). T-cell protein tyrosine phosphatase (TCPTP) has been reported to be involved in the inactivation of STAT1 in IFN-γ-treated cells (9). Because TCPTP exists in two isoforms, a nuclear form and a cytoplasmic form, it was speculated that TCPTP was involved in the dephosphorylation of STAT1 in both the nucleus and cytoplasm (9).

TCPTP is linked to the development of several inflammatory disorders, including type 1 diabetes, Crohn’s disease, and rheumatoid arthritis (10, 11). Therefore, TCPTP regulators may serve as therapeutic agents. Efforts have been made to develop drugs against PTPs, but the highly conserved architectures of PTP active sites impede the development of selective PTP inhibitors (12). For example, TCPTP and PTP1B have a

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3 The abbreviations used are: PTP, protein tyrosine phosphatase; TCPTP, T cell protein tyrosine phosphatase; IKK, IκB kinase; GAS, interferon-gamma-activated sequence; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazo-lium bromide.
sequence identity of about 74% in their catalytic domains (13), although they clearly fulfill different biological functions (14–16), suggesting that the protein sequences outside of the catalytic domains play important roles in determining the specificity of the PTPs. Indeed, TCPTP itself was reported to be regulated by an autoinhibition mechanism (17). In vitro studies using proteolytically cleaved fragments of TCPTP have demonstrated that the catalytic activity of TCPTP is regulated by an intramolecular inhibition involving a carboxy terminal segment of the 45-kD form of TCPTP (17), indicating that the carboxy terminal domain of TCPTP has an important regulatory role.

Wedelolactone is a coumestan isolated from Eclipta prostrata L., a medicinal herb that has been used in the treatment of infective hepatitis in Indian snake venom poisoning in Brazil (18). A collective work of different groups has demonstrated that wedelolactone has multiple biological effects, including inhibition of IκB kinase (IKK) activity in NF-κB signaling (18), Na⁺,K⁺-ATPase activities (19), and phospholipase A (2) activity in snake venom (20). It has also been found to inhibit NS5B RNA polymerase activity, which is critical for hepatitis C virus replication (21), suggesting a potential hepatoprotective activity (22). Recently, wedelolactone has also been found to have antitumor effects by both in vitro and in vivo studies in a wide range of tumor types. The growth inhibition effects of wedelolactone on tumor cells were believed to be accomplished through its inhibition of IKK (23, 24), the androgen receptor (25, 26), or topoisomerase II (27).

We identified wedelolactone as an enhancer of STAT1 signaling through screening of a natural compound library. We found that wedelolactone prolonged IFN-γ-induced STAT1 tyrosine phosphorylation by targeting TCPTP and inhibiting STAT1 dephosphorylation. In doing so, wedelolactone synergistically enhanced IFN-γ-induced apoptosis of tumor cells in a STAT1-dependent manner. Our findings revealed a novel drug target, a novel mechanism to regulate PTPs, and a novel mechanism against cell proliferation. Wedelolactone, particularly in combination with IFN-γ, may be a new strategy to treat cancer and other proliferation-related diseases.

**EXPERIMENTAL PROCEDURES**

**General Reagents**—Wedelolactone was provided by Shangh hai Ambrosia Pharmaceuticals, Inc. The tyrosine phosphatase inhibitor sodium orthovanadate and sodium fluoride were purchased from Sigma Aldrich. Stock solution of sodium orthovanadate was constituted in H₂O at a concentration of 100 mm adjusted to pHi0, boiled until it became translucent, and then the pH was readjusted to 10. Chemicals, if not specified, were dissolved in dimethyl sulfoxide. In experiments where cells were treated with the various inhibitors, the same volumes of corresponding solvents were used as controls. Human IFN-γ was purchased from Shanghai Tongren Yaofang, Inc. IL-6 was from BD Biosciences, human IFN-α was from Peprotech, and recombinant human EGF was from Invitrogen.

**Cell Culture, Transfection, and Luciferase Gene Reporter Assay**—HepG2, WiDr, A431, and A549 cells were purchased from the ATCC. A HepG2 cell line stably transfected with an interferon-activated reporter gene was obtained from Dr. Xinyuan Fu of Indiana University. The cells, except HepG2, were maintained in DMEM (Invitrogen) supplemented with 10% fetal calf serum. HepG2 cells were cultured in α minimal essential medium (Invitrogen) with 10% fetal calf serum.

For transfection, cells were cultured to near confluence and transfected for 4 h with various plasmids using Lipofectamine 2000 (Invitrogen). Cells were trypsinized and cultured for 24 h after transfection. An equal number of cells was collected for various assays. The luciferase activity was measured with a luminometer using a luciferase assay system (Promega).

**DNA Extraction and RT-PCR**—Total cellular RNA was extracted from cells using TRIzol reagent (Invitrogen). Reverse transcription and PCR were performed as follows. Briefly, 5 μg of total cellular RNA from each sample was reverse-transcribed using Maloney murine leukemia virus reverse transcriptase (MBI). The first-strand cDNA was diluted 1:10 as the PCR template. The PCR r Taq polymerase mixture was prepared according to the instructions of the manufacturer (Takara). PCR primers for the different genes were designed as follows: SOCS1, 5′-CCCCCTCCAGATTGACC-3′ (sense) and 5′-AGGGATAGGAGCGTCCGAGT-3′ (antisense); IP-10, 5′-CCTCAGTCTCGAC-3′ (sense) and 5′-AGCAGGAGTGAAAGAC-3′ (antisense); IL-8, 5′-CAGTTTTGCGAGGGT-3′ (sense) and 5′-AACACTTGCTGAAA-3′ (antisense); and GAPDH, 5′-ACCACGAGCGAGG-3′ (sense) and 5′-TCCACACCTGTGTC-3′ (antisense).

**IFN-γ Receptor Binding Assay**—Suspended HepG2 cells were pretreated with vehicle or wedelolactone for 20 min and then incubated with FITC-IFN-γ (30,000 IU/ml) for another 70 min, followed by analysis using FACScan flow cytometric analysis (BD Biosciences).

**Antibodies and Immunoblotting Analysis**—The mouse monoclonal antibody to phosphorytosine (4G10) was obtained from Upstate Biotechnology. Anti-p-STAT1 (Tyr-701) antibody, anti-STAT1 antibody, anti-p-STAT3 (Tyr-705) antibody, anti-STAT3 antibody, anti-p-ERK (Thr-202/Tyr-204) antibody, anti-ERK antibody, anti-EGFR antibody, anti-p-JAK1 (Tyr-1022/1023) antibody, anti-p-JAK2 (Tyr-1007/1008) antibody, and anti-JAK1 antibody were purchased from Cell Signaling Technology, Inc. Anti-α-tubulin antibody and anti-JAK2 antibody were purchased from Santa Cruz Biotechnology, Inc. Anti-p-EGFR (Tyr-1068) was purchased from BioSource. All antibodies purchased commercially were used as recommended by the manufacturers.

For immunoblotting analysis, cells were lysed in 1× Laemmli sample buffer (Sigma Aldrich) and boiled for 5 min. Proteins were resolved by SDS-PAGE (8%), transferred to a nitrocellulose membrane, and blocked by incubation for 60 min at room temperature with 5% (w/v) nonfat dry milk in TBS containing 0.1% Tween 20 (TBST). All the membranes were incubated overnight at 4 °C with primary antibodies diluted in TBST with 5% BSA. The membranes were then washed and incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibodies (Promega). Immune complexes were detected by ECL (Pierce).
Wedelolactone Enhances IFN-γ/STAT1-mediated Promoter Activity—We used a cell-based promoter-reporter assay system, p-GAS-HepG2, which is a HepG2 cell line stably transfected with a STAT1-responsible promoter-reporter (p-GAS-luciferase) plasmid (see “Experimental Procedures”), to identify compounds that enhance IFN-γ/STAT1 signaling. We screened about 1000 traditional Chinese medicinal herb extracts using the assay and identified a natural compound, wedelolactone, from E. prostrata L. as a potent IFN-γ/STAT1 signaling enhancer (Fig. 1). Treatment of the p-GAS-HepG2 cells with wedelolactone increased the IFN-γ-induced luciferase reporter activity in a dose- and time-dependent manner (Fig. 1, a and b). The EC_{50} of the enhancement was about 20 μM, and the maximum enhancement was about 6- to 7-fold that of the control (Fig. 1a). The induction time course indicated that both the magnitude and the time course of the IFN-γ-induced luciferase activity were increased (Fig. 1b).

To ensure that the increased luciferase activity by wedelolactone was indeed due to the increased STAT1 activity, we analyzed the effects of wedelolactone on the expression of three endogenous IFN-γ-induced STAT1 target genes: IP-10, SOCS1, and IL-8. The mRNA levels of all three genes we tested were increased by wedelolactone treatment (Fig. 1c). These data collectively demonstrate that wedelolactone enhances IFN-γ/STAT1 signaling.

Wedelolactone Does Not Affect the Binding of IFN-γ to Its Receptor—To investigate the mechanisms of wedelolactone in enhancing the IFN-γ/STAT1 signaling, we first tested whether wedelolactone played a role in the ligand-receptor interaction. We increased the concentration of IFN-γ in the presence or absence of the same concentration of wedelolactone (Fig. 2a). Wedelolactone alone had very little effect on luciferase activity, suggesting that wedelolactone did not act as a ligand (supplemental Fig S1). Maximum luciferase activity was achieved when the concentration of IFN-γ reached 1000 IU/ml, suggesting saturation of IFN-γ receptor. Wedelolactone had similar enhancing effects on different concentrations of IFN-γ, suggesting that wedelolactone does not affect the binding of IFN-γ to its receptor (Fig. 2a). To confirm this, we examined the effects of wedelolactone on the binding of IFN-γ to its receptor directly by using the FITC-labeled IFN-γ, which allowed us to detect IFN-γ binding using flow cytometry. Wedelolactone treatment had no effect on the binding of IFN-γ to cells (Fig. 2b), although...
the FITC-labeled IFN-γ seemed to have a relatively low binding affinity. These results suggest that the enhancing effect of wedelolactone on the IFN-γ-induced luciferase activity may be not through affecting IFN-γ-receptor interactions.

**Wedelolactone Enhances IFN-γ/STAT1 Signaling by Prolonging STAT1 Tyrosine Phosphorylation**—We next analyzed the effects of wedelolactone on tyrosine phosphorylation of JAK1, JAK2, and STAT1, which are the upstream signaling events in response to the IFN-γ stimulation. Wedelolactone did not affect the tyrosine phosphorylation of JAK1 or JAK2 (Fig. 2c), nor the initial phosphorylation of STAT1 (c), but significantly prolonged the duration of STAT1 tyrosine phosphorylation. The IFN-γ-induced phosphorylation of STAT1 was a transient event that began to decrease 1–2 h after stimulation (Fig. 2, c and d). The STAT1 phosphorylation was sustained more than 8 h in the presence of wedelolactone (Fig. 2d). The effect of wedelolactone on STAT1 phosphorylation was also dose-dependent (Fig. 2e), consistent with its effect on the STAT1 promoter activity (Fig. 1a). It appeared that wedelolactone did not affect the IFN-γ-induced phosphorylation of STAT1 but prevented or slowed down its decrease after stimulation.

**Wedelolactone Specifically Enhances IFN-γ Signaling**—To address whether the enhancing effect of wedelolactone is specific for IFN-γ signaling, we tested the effects of wedelolactone on IFN-α, IL-6, or EGF signaling. HepG2 cells were stimulated with IFN-α, IL-6, or EGF in the presence or absence of wedelolactone. There were no obvious effects of wedelolactone on IFN-α-induced STAT1/3 tyrosine phosphorylation (Fig. 3a), IL-6-induced STAT3/ERK phosphorylation, or
the level of GP130 (b), nor on EGF-induced EGFR/ERK phosphorylation (c). These data strongly suggest that the enhancing effect of wedelolactone is specific on IFN-γ signaling.

**Wedelolactone Enhances IFN-γ Signaling through Inhibition of STAT1 Dephosphorylation**—Because slowdown of the deduction of STAT1 phosphorylation can be achieved either through activating its kinases or inhibiting its phosphatases, we performed a pause-chase experiment to distinguish between these two possibilities. HepG2 cells were stimulated with IFN-γ for 30 min followed by addition of a general kinase inhibitor, staurosporine, to inhibit further phosphorylation of STAT1 by its kinases (28). Wedelolactone, as well as the general PTP inhibitor vanadate, could still prolong the duration of STAT1 phosphorylation in the presence of staurosporine (Fig. 4a), sug-
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suggesting that wedelolactone acts through inhibiting STAT1 dephosphorylation rather than enhancing its phosphorylation.

To clarify that wedelolactone is indeed a STAT1 phosphatase inhibitor, we analyzed the effects of wedelolactone on STAT1 in vitro dephosphorylation using an in vitro dephosphorylation assay (29). The substrate cell lysate containing the phosphorylated STAT1 was prepared by treating HepG2 cells with IFN-γ. In parallel, the enzyme cell lysate was prepared from the untreated HepG2 cells. Equal amounts of the substrate cell lysate and the enzyme cell lysate were mixed together to allow in vitro dephosphorylation. STAT1 dephosphorylation could be observed after 30 min of incubation. Wedelolactone dose-dependently inhibited STAT1 in vitro dephosphorylation (Fig. 4b). Taken together, these results demonstrate that wedelolactone serves as a PTP inhibitor in the regulation of IFN-γ signaling.

To address whether wedelolactone is a general PTP inhibitor, we analyzed the effects of wedelolactone on general protein tyrosine phosphorylation in HepG2 cells and compared them with those of a general PTP inhibitor, vanadate. Vanadate increased the general tyrosine phosphorylation level in HepG2 cells as expected, whereas wedelolactone had no obvious effects on general tyrosine phosphorylation but significantly enhanced STAT1 phosphorylation, demonstrating that wedelolactone is not a general tyrosine phosphatase inhibitor (Fig. 4c).

We also examined the effects of wedelolactone on protein dephosphorylation in vitro using whole cell lysates and paranitrophenyl phosphate as the substrate. Vanadate again inhibited the in vitro phosphatase activities of the whole cell lysate in a dose-dependent manner, whereas wedelolactone had no effect (Fig. 4d). These results collectively indicate that wedelolactone is not a general PTP inhibitor, in contrast to vanadate.

Wedelolactone-enhanced STAT1 Phosphorylation Is TCPTP-dependent, and Wedelolactone Is a Specific TCPTP Inhibitor—

TCPTP has been reported as the major phosphatase involved in STAT1 dephosphorylation in IFN-γ signaling (9). We then examined the role of TCPTP in wedelolactone-enhanced STAT1 phosphorylation. We overexpressed TCPTP in p-GAS-HepG2 cells (Fig. 5a) and analyzed the effects of TCPTP overexpression on IFN-γ-induced luciferase activity in the presence or absence of wedelolactone. Overexpression of TCPTP reduced IFN-γ-induced luciferase activity, as expected, and decreased the enhancing effects of wedelolactone (Fig. 5a), suggesting that an increased level of TCPTP can counterbalance wedelolactone-enhanced IFN-γ signaling.

To confirm the role of TCPTP in mediating the effects of wedelolactone, we performed reciprocal experiments by knocking down TCPTP expression in p-GAS-HepG2 cells using TCPTP-specific siRNA (Fig. 5b). Luciferase activity was increased in TCPTP, knocking down p-GAS-HepG2 cells compared with the mock control. The effects of wedelolactone were almost completely abolished in TCPTP-deficient cells, strongly suggesting that wedelolactone targets TCPTP to modulate IFN-γ-induced STAT1 phosphorylation (Fig. 5b). The efficiency of overexpression and knocking down TCPTP were evaluated with Western blot analysis (Fig. 5c).

Similar results in STAT1 dephosphorylation were obtained by knocking down TCPTP in HepG2 cells (Fig. 5d). The dephosphorylation of STAT1 was inhibited by knocking down TCPTP, and the enhancing effects of both of wedelolactone and vanadate on STAT1 phosphorylation were reduced profoundly (Fig. 5c), again suggesting that TCPTP is the target of wedelolactone in enhancing IFN-γ-induced STAT1 phosphorylation.

To confirm that Wedelolactone is indeed a TCPTP inhibitor, we examined the effect of wedelolactone on TCPTP activity in vitro using purified enzymes. Wedelolactone efficiently inhibited the phosphatase activity of the full-length TCPTP (amino acids 1–387) in vitro (Fig. 5e) although it had no obvious effects on the activities of two control phosphatases: leukocyte antigen-related (LAR) and SHP-2 (e).
Interestingly, wedelolactone had much less of an effect on inhibiting the phosphatase activity of the TCPTP catalytic fragment (amino acids 2–346) (Fig. 5e), suggesting that the C-terminal domain of the TCPTP is required for wedelolactone to inhibit the catalytic activity of the TCPTP. Moreover, the inhibition of wedelolactone on the full-length TCPTP was not complete and reached a plateau after a 10 μM concentration of wedelolactone (Fig. 5e). These data strongly suggest that wedelolactone is a specific inhibitor of TCPTP and that it specifically interacts with the C-terminal domain of TCPTP.

**DISCUSSION**

STATs mediate important signals from cytokines, growth factors, and the extracellular matrix to regulate diverse biological activities. STAT phosphorylation is a nodal point for regulating STAT-mediated signaling. The collective work of multiple research groups has uncovered complex molecules and mechanisms regulating STATs phosphorylation, including kinases (32), phosphatases (9, 33, 34), conformational reorientation (35, 36), and scaffold proteins (37). We know a great deal about the function and regulation of the kinases and phosphorylation of STATs and have developed various compounds to target the kinases that inhibit STAT signaling for treating STAT signaling-related diseases (38–40). However, we know very little about the function and regulation of the phosphatases and dephosphorylation of STATs. There have been no compounds developed to target the dephosphorylation of STATs.

**Wedelolactone Synergizes with IFN-γ to Induce Tumor Cell Death**—IFN-γ has been known to induce apoptosis and cell cycle arrest in certain tumor cells (30). We therefore examined whether prolonged STAT1 tyrosine phosphorylation by wedelolactone could enhance the ability of IFN-γ to induce cell death or cell cycle arrest in tumor cells. Wedelolactone synergized with IFN-γ, to different extents, to induce cell death in the hepatocarcinoma cell line HepG2 (Fig. 6a), the colon cancer cell line WiDr (b), and the epidermoid carcinoma cell line A431NS (c) but had no effect on the hepatocarcinoma cell line 97H (d) (31), which lacks the STAT1 protein (e). These data suggest that wedelolactone enhances the antitumor activity of IFN-γ in a STAT1-dependent manner.
Here we report the identification of wedelolactone, a natural compound from *E. prostrata* L. that interfered with the dephosphorylation of STAT1. Wedelolactone specifically prolonged IFN-γ-induced STAT1 phosphorylation by inhibiting the TCPTP-dependent dephosphorylation of STAT1 and synergized with IFN-γ in inducing cell death among certain STAT1-expressing tumor cells. Our results suggest that wedelolactone acts as a TCPTP-specific inhibitor to inhibit the dephosphorylation of STAT1 after IFN-γ stimulation and enhances the biological effects of IFN-γ.

The transient activation of STAT1 after IFN-γ stimulation has been noticed before (28), but the biological significance of the regulation of the duration of STAT1 phosphorylation has not been revealed. STAT1 regulates hundreds of genes with diverse biological activities. The level and duration of the STAT1 activation may have a significant impact on the biological outcome of STAT1-mediated signaling. Indeed, inhibition of STAT1 dephosphorylation by wedelolactone facilitated the antitumor function of IFN-γ in several tumor cell lines (Fig. 6), demonstrating the biological significance of the duration of STAT1 phosphorylation.

IFN-γ is a multifunctional cytokine that is involved in many diseases, including cancer (41–43), tuberculosis (44), hepatitis (45), and rheumatoid arthritis (46). IFN-γlb was approved for the treatments of chronic granulomatous disease and adult T cell leukemia (47). Much effort has been made to explore its therapeutic potential to treat cancer. It has been shown that certain genotoxic agents could sensitize cells in response to very low doses of either interferon-α or γ through activation of STAT1 (48). Enhanced STAT1 phosphorylation caused by a mutation in the SH2 domain of STAT2 promoted type I IFN-induced apoptosis (49). Therefore, increased STAT1 phosphorylation appears to be a valid strategy to induce the cell death of tumor cells, and wedelolactone is a unique chemical modulator.
of IFN-γ signaling to enhance the biological functions of the cytokine in clinical use.

TCPTP (gene name PTPN2) was originally cloned from a human peripheral T cell cDNA library. Human PTPN2 is located within chromosomal region 18p11.2–11.3, where it is thought to be associated with susceptibility to three autoimmune diseases: Crohn’s disease, rheumatoid arthritis, and type 1 diabetes. TCPTP is also involved in many signaling pathways, including EGF signaling, IFN-γ signaling, and cell cycle regulation. Efforts have been made to develop specific small molecule inhibitors for TCPTP. However, the highly conserved catalytic domains of PTPs remain a challenge for developing specific small molecule inhibitors for PTPs. It has been reported that TCPTP can be regulated through conformational changes mediated by the extracellular matrix. In vitro studies using a proteolytically cleaved fragment of TCPTP have suggested that the catalytic activity of TCPTP is regulated by an intramolecular inhibition involving a carboxy terminal segment of TCPTP. In this regard, our finding that wedelolactone specifically inhibited the phosphatase activity of full-length TCPTP but not that of the C terminus-deleted TCPTP suggests that wedelolactone may interact with amino acid residues within the C-terminal regulatory domain of TCPTP to influence the catalytic activity of TCPTP. Further detailed understanding of the interactions of wedelolactone with TCPTP will uncover a new strategy to develop specific TCPTP inhibitors. Wedelolactone holds enormous potential to be a novel drug candidate for the treatment of various TCPTP-related diseases.

Compared with its effects on IFN-γ signaling, Wedelolactone only showed a mild effect on IFN-α-induced phosphorylation of STAT1 (Fig. 3a). This result suggests that the inactivation of STAT1 after IFN-α and IFN-γ stimulation are distinct. IFN-α treatment leads to STAT1 and STAT2 heterodimer formation, whereas IFN-γ treatment results in STAT1 homodimer formation. The conformation of phosphorylated STAT1 has been shown to be a crucial factor in facilitating enzyme-substrate interaction during its dephosphorylation (36). Therefore, dephosphorylation of STAT1 in these two specific dimers could be regulated by distinct mechanisms. Wedelolactone could interfere with the dephosphorylation of the STAT1 homodimer but not with the STAT1/STAT2 heterodimer.

Several studies have shown that wedelolactone alone could induce growth arrest and apoptosis in several cancer cell lines through targeting IKK (23, 24), the androgen receptor (25, 26), or topoisomerase II (27), which is consistent with our observations (Figs. 6, a–c). However, Wedelolactone could achieve a
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higher efficacy in inducing grow inhibition of tumor cells when combined with IFN-γ, confirming that the STAT pathway is an important target of wedelolactone in inhibiting tumor cell growth.

In conclusion, we identified wedelolactone as a potent enhancer of IFN-γ/STAT1 signaling. Wedelolactone specifically enhances IFN-γ-induced STAT1 phosphorylation by inhibiting STAT1 phosphatase TCPTP, possibly interacting with the C-terminal autoinhibitory domain of TCPTP. In doing so, wedelolactone facilitates IFN-γ to induce tumor cell death. Our results revealed a new strategy to target TCPTP and IFN-γ/STAT1 signaling and uncovered a new drug candidate to treat cancer and other proliferation-related diseases.

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