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CS1 is a novel topoisomerase IIα inhibitor with favorable drug resistance profiles

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ABSTRACT

DNA topoisomerase II (Topo II) is an essential nuclear enzyme and a validated target for anticancer agent screening. CS1, a novel 2-phenylnaphthalene, had potent cytotoxicity against nine tested tumor cell lines and showed 6–10-fold less toxicity against normal cell lines compared with etoposide. In addition, CS1 showed potential anti-multidrug resistance capabilities. kDNA decatenation, DNA relaxation and cleavage complex assays indicated that CS1 acted as a nonintercalating topoisomerase IIα (Topo IIα) inhibitor by stabilizing the DNA-Topo IIα cleavage complex. CS1 also induced DNA breaks in MDA-MB-231 cells evidenced by comet tails and the accumulation of γH2AX foci. The ability of CS1 in inducing DNA breaks mediated by Topo II resulted in G2/M phase arrest and apoptosis. Moreover, CS1 exhibited dramatic *in vivo* antitumor activity and lower toxicity compared with etoposide. This work supports the development of CS1 as a promising candidate for the treatment of cancer by targeting Topo IIα.

1. Introduction

DNA topoisomerase II (Topo II), an essential nuclear enzyme which plays roles in replication, transcription, recombination, and chromosome condensation and segregation, is a well-validated target of anti-cancer compounds [1,2]. Topo II inhibitors (such as etoposide) are among the most effective antitumor agents for the treatment of human cancers in clinic [3,4]. Of these agents, Topo II poisons are more efficient in chemotherapy by generating topoisomerase-mediated DNA breaks than other types of Topo II inhibitors. However, most Topo II poisons exhibit severe side effects including cardiotoxicity, multidrug resistance, and development of secondary malignancies such as leukemia [5–7]. Hence, finding novel small molecules targeting Topo II with low toxicity and overcoming these limitations is urgent.

Natural products *p*-terphenyls, mainly found in mycomycetes and actinomycetes, showed potent cytotoxicity against tumor cells [8,9]. In our previous study, eight synthetic *p*-terphenyl derivatives exhibited potent cytotoxicity against human breast carcinoma MDA-MB-435 cell line through topoisomerase inhibition [10]. Five *p*-terphenyls bearing glucuronic acid isolated from *Streptomyces* sp. LZ35 also inhibited topoisomerases [11]. Besides, a series of aryl-substituted naphthalenes showed anti-topoisomerase activity and potent antitumor activity against three tumor cell lines [12]. All these data indicate that the naphthalene and biaryl scaffold possess potential topoisomerase inhibition activity. Nevertheless, the specificity of targeting tumor cells as well as *in vivo* antitumor activity of these *p*-terphenyl derivatives and aryl-substituted naphthalenes is unsatisfactory (data not shown). To overcome these defects, a novel 2-phenylnaphthalene CS1 was designed and synthesized. In present study, the *in vitro* and *in vivo* antitumor activity, as well as antitumor mechanism of CS1 will be discussed.

2. Materials and methods

2.1. Materials

The chemical structure of CS1 (molecular weight 296) was shown in Fig. 1A. Etoposide and adriamycin were obtained from Sigma–Aldrich (USA).

2.2. Cell culture

All cancer cell lines otherwise indicated were obtained directly from American Type Culture Collection (ATCC, USA). Human umbilical vein endothelial cells HUVEC were kindly provided by Department of Pharmaceutics of Shandong University. The adriamycin-selected multidrug resistant (MDR) cells MCF-7/ADR and the human normal liver cell line HL7702 were kindly donated by Department of Pharmacology, School of Pharmaceutical Sciences,

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Shandong University. Human breast cancer cells MDA-MB-231, human cervix adenocarcinoma cancer cells HeLa, human prostate cancer cells PC3, human lung cancer cells A549, human colon cancer cells SW620 and human gastric cancer cells BGC-823 were maintained in DMEM (Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA). The human breast cancer cells MDA-MB-435 were maintained in a 1:1 mixture of DMEM and Ham's F-12 medium containing 10% FBS. Human umbilical vein endothelial cells HUVEC were kindly provided by Department of Pharmaceutics of Shandong University and maintained in the supplied endothelial cell medium with 1% endothelial cell growth supplement (ECGS), 5% FBS and 1% penicillin/streptomycin (P/S). MCF-7/ADR and HL7702 cells were cultured in RPMI-1640 supplemented with 10% FBS (Gibco, USA). All cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. Cell proliferation assay

The cytotoxicity of CS1 was evaluated by SRB assay as described previously [13]. The inhibition rate for each well was calculated as follows: $(A570_{\text{control cells}} - A570_{\text{treated cells}})/A570_{\text{control cells}} \times 100\%$. The IC_{50} value was calculated using software Prism 5 (GraphPad Software, Inc., USA).

2.4. Cell cycle, Annexin V-FITC apoptosis detection assay, immunoblotting and immunofluorescence

Cells were treated for the indicated time with CS1 or etoposide, followed by processing for flow cytometry, immunoblotting or immunofluorescence as described [14]. Apoptosis was quantified by flow cytometric analysis using Annexin V-FITC apoptosis detection kit (KeyGEN Biotech, China) according to the manufacturer's instructions. To examine DNA damage, γH2AX-S139 (Cell Signaling Technologies) primary antibody was used for immunoblotting and immunofluorescence.

2.5. shRNA and lentiviral vector-mediated silencing of Topo IIα in MDA-MB-231 cells

ShRNA and Lentiviral Vector-mediated silencing of Topo IIα was handled as described previously [14]. Primers used were as followings:

- 5′-t(GCTGATCAGATTGTGACTAAA)ttcaagaga(TTTAGTCACAATCTGATCAGC)ttttttc-3′ and
- 5′-tcgagaaaaaa(GCTGATCAGATTGTGACTAAA)tctcttgaa(TTTAGTCACAATCTGATCAGC)a-3′.

2.6. Single-cell gel electrophoresis

MDA-MB-231 cells were treated for 1 h or 4 h with CS1 or etoposide, DNA breaks were evaluated by neutral single-cell gel electrophoresis as described by Singh et al. [15] with slight modification. Briefly, a total of 600 cells were resuspended in 0.5% low melting point agarose. After solidification, the slides were lysed for 2 h in lysis buffer (2.5 mol/L NaCl, 10 mmol/L Tris, 100 mmol/L Na2EDTA, 1% sodium sarcosinate, 10% DMSO, and 1% Triton X-100, pH 8.5) at 4 °C. The slides were then placed for 20 min in ice-cold neutral buffer (1× TAE buffer, pH 8.5) at 4 °C to allow unwinding of DNA. Electrophoresis was carried out for 20 min at room temperature at 20 V (1 *V* /cm). The slides were

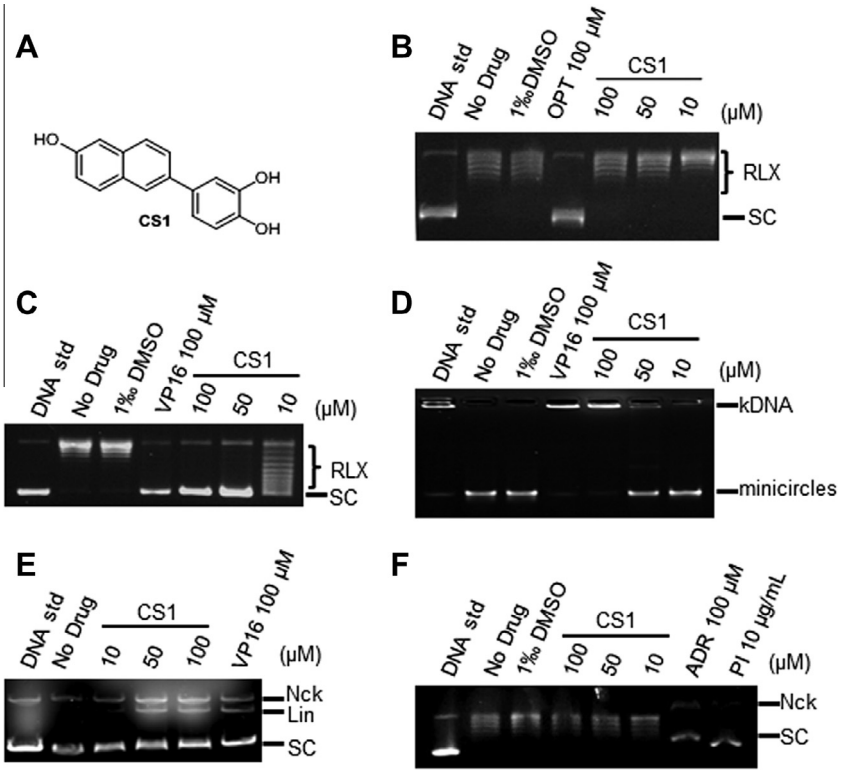

Fig. 1. CS1 inhibited the activity of Topo IIα. (A) The chemical structure of CS1. (B) CS1 did not inhibit Topo I-mediated supercoiled DNA relaxation. Hydroxycamptothecine (OPT) was used as a positive control. Negatively supercoiled pBR322 DNA (SC) and relaxed DNA (RLX) were shown. (C) CS1 inhibited Topo IIα-mediated supercoiled DNA relaxation. Etoposide (VP16) was employed as a positive control. (D) CS1 inhibited Topo IIα-mediated kinetoplast DNA (kDNA) decatenation to form minicircular DNA. (E) CS1 stabilized enzyme–DNA cleavage complex. The position of linear DNA (L) and nicked DNA (NCK) were indicated. (F) CS1 did not intercalate into DNA. pBR322 DNA was relaxed by 1 U Topo I before incubated with 100 μM adriamycin (ADR), 2 μg/mL propidium iodide (PI) or different concentrations of CS1. ADR and PI were used as the positive controls. The data were the representatives of three independent experiments.

stained for 5 min with acridine orange (10 μg/mL). Fluorescent comet patterns were examined with a fluorescence microscope (OLYMPUS, IX71, Japan) under 100x magnification.

2.7. Topo II-mediated supercoiled pBR322 relaxation, decatenation of kinetoplast DNA

DNA relaxation or kDNA decatenation assays were carried out using our previously reported protocols [10,12]. Briefly, pBR322 DNA (0.25 μg) or kDNA (0.20 μg) was incubated for 30 min with 1 unit of Topo IIα (Affymetrix, USA) at 37 °C in a total of 20 μL reaction buffer [10 mM Tris (pH 7.9), 50 mM KCl, 50 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 μg/mL of bovine serum albumin (BSA), 1 mM ATP]. The reaction was then stopped with 2 μL of 10% SDS. Electrophoresis was carried out for 1 h in a 0.8% agarose gel in 1× TAE at 5 *V*/cm. Further the gel was stained for 30 min with 0.5 μg/mL of ethidium bromide (EB) and destained for 30 min with distilled water and photographed.

2.8. DNA intercalation assay

This assay was performed in two steps as described [16]. First, 0.25 μg pBR322 DNA was relaxed for 30 min by 1 units Topo I at 37 °C in the absence of drug to fully relax the DNA. Then, the relaxed DNA was further incubated for 30 min at 37 °C in the presence of increasing concentrations of drugs. The DNA samples were subjected to electrophoresis under the same conditions as described above.

2.9. Stabilization of the cleavage complex

pBR322 DNA (0.25 μg) was incubated for 30 min with 1 unit of Topo IIα at 37 °C. The Topo IIα-mediated relaxation was stopped with 10% SDS, followed by digestion with proteinase K and further incubated for 1 h at 37 °C. The reaction was stopped with 2 μL 10% SDS. Electrophoresis was carried out in a 0.8% agarose gel in 1× TAE buffer containing 0.5 μg/mL EB and the gel was destained for 30 min with distilled water and photographed.

2.10. Tumor growth inhibition

Five-week-old female athymic nude mice (BALB/c-nu) were obtained from Slac Laboratory Animal (Shanghai, China). MDA-MB-231 cells (5×10^6 cells per animal) were injected into the right flank to generate orthotopic xenografts. The tumors were cut into fragments and the fragments were implanted into the right flank of nude mice. Two weeks after injection, tumors were palpable (about 100 mm^3) and mice were randomized into treatment and control groups (6 mice per group). The drugs were injected via caudal vein every other day at a dose of 20 mg/kg body weight, whereas the blank control group received an equal volume of physiological saline solution containing 1% DMSO, 10% ethanol and 20% PEG-400. During treatment, subcutaneous tumors were measured with a vernier caliper every three days. Tumor volume was calculated by the formula ($V = ab^2/2$, where *a* and *b* stand for the longest and shortest diameter respectively). After treated for two weeks with drugs, the animals were sacrificed and solid tumors were removed and weighted. The inhibition rate was calculated as [(averaged tumor weight of the control group − averaged tumor weight of drug-treated group)/averaged tumor weight of the control group] × 100%.

3. Results

3.1. CS1 inhibited the activity of Topo IIα by stabilizing enzyme–DNA cleavage complex

To evaluate the topoisomerase inhibition activity of CS1, the biochemical assays for Topo-mediated relaxation of supercoil DNA were performed. CS1 exhibited potent Topo IIα inhibition in a dose-dependent manner (Fig. 1C). In comparison, CS1 had no evident effect on Topo I-mediated pBR322 DNA relaxation (Fig. 1B). This inhibition was further confirmed by Topo IIα specific kinetoplast DNA (kDNA) decatenation assay (Fig. 1D). These data suggest that CS1 is a specific Topo II inhibitor.

Topo II poisons disrupt the catalytic cycle of the enzyme by stabilizing the enzyme–DNA cleavage complex, leading to the formation of linear DNA. In the cleavage complex assay, the gel was run in the presence of ethidium bromide (EB) in order to visualize the linear DNA. Consistent with the positive control etoposide, the linear form of the DNA was clearly observed in the case of CS1 at the concentrations of 50 μM and 100 μM (Fig. 1E). These observations indicate that CS1 acts as a Topo II poison. In addition, molecule docking indicate that CS1 may intercalate into DNA-Topo IIα pocket and inhibits the activity of Topo IIα (detailed in Fig. S1).

DNA intercalation is an important feature of some Topo II poisons. Adriamycin (ADR), mAMSA and daunorubicin act as Topo II poisons by intercalating into DNA or bind to the minor groove of DNA [17]. To determine whether CS1 inhibit Topo II as a DNA intercalator, the DNA intercalation assay was performed. DNA intercalator ADR and PI, changing the degree of supercoiling of relaxed DNA, were used as the positive controls. CS1 showed no intercalating effect on relaxed DNA up to 100 μM, (Fig. 1F). The results indicate that CS1 is a nonintercalative Topo IIα inhibitor.

3.2. CS1 inhibited in vitro cancer cell proliferation

To evaluate the antitumor effects of CS1, the *in vitro* anti-proliferative effects of CS1 were examined against nine tumor cell lines (Fig. 2A). CS1 displayed potent and comparable cytotoxicity. Interestingly, CS1 exhibited weak cytotoxicity against the normal cell line HUVEC with a higher IC_{50} (40.67 μM). To further evaluate the selectivity of CS1, a human normal hepatic cell line HL7702 [18] was applied. Etoposide, a well known Topo II inhibitor, exhibited equipotent toxicity to HepG2 and HL7702 cells, which showed 49.0% and 56.1% growth inhibition at 2.5 μM, respectively. CS1 caused 53.1% cell death at 5 μM in HepG2 cells, whereas 42.7% cell death was noted at 20 μM in HL7702 cells (Fig. 2B), in agreement with HUVEC cells. All the data indicate that CS1 possesses higher selectivity against tumor cells compared with etoposide.

Multidrug resistance is a severe side effect of most anticancer drugs [19]. To detect whether CS1 posseses anti-multidrug resistance capability, P-glycoprotein (P-gp) overexpressed tumor cell line MCF-7/ADR [20] was employed. This cell line displayed high degree of drug resistance to reference compounds including adriamycin and etoposide (resistance fold values were 426.99 and 17.15, respectively). Nevertheless, CS1 exhibited totally equipotent cytotoxicity in MCF-7 and MCF-7/ADR cells (Table 1). These data indicate that CS1 is not a substrate of P-gp pump and possesses potential anti-multidrug resistance capability.

To investigate whether the anti-proliferation of CS1 was related with Topo IIα inhibition, the *in vitro* anti-proliferative effects of CS1 were examined against MDA-MB-231 cells with shRNA targeted against Topo IIα (Fig. 2C). Strikingly, Topo IIα-knockdown cells were less sensitive to CS1, the cell growth inhibition of increasing concentrations (2.5–10 μM) of CS1 against wide type MDA-MB-231 cells were significantly higher than that of Topo

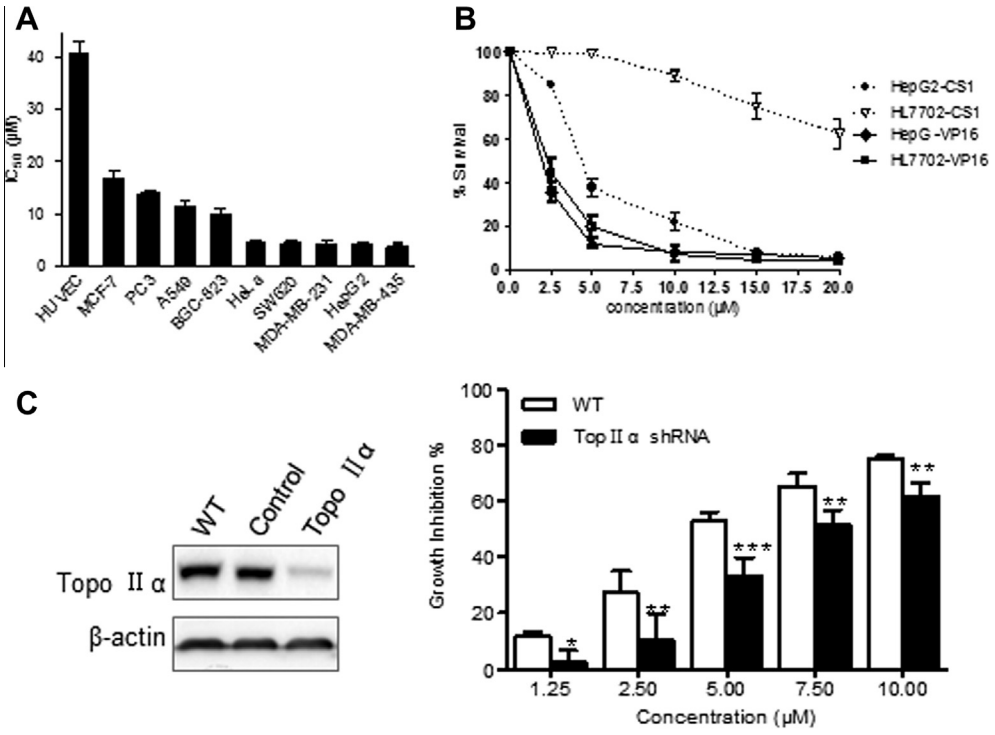

Fig. 2. CS1 inhibited *in vitro* tumor cell proliferation. (A) CS1 inhibited tumor cell proliferation. Sulforhodamine B (SRB) assay was carried out to determine the cytotoxicity of CS1. (B) The cytotoxicity of CS1 and etoposide (VP16) against tumor cells HepG2 and nontumor cells HL7702. (C) Topo IIα mediated the cytotoxicity of CS1 in MDA-MB-231 cells. MDA-MB-231 cells were transfected with negative control shRNA (control) or Topo IIα shRNA (Topo IIα), and the expression level of Topo IIα was evaluated by western blotting (left). The growth inhibition of wild type and Topo IIα shRNA transfected cells was measured by SRB assay (right).

Table 1
Cytotoxicity of CS1 and reference drugs against pairs of resistant and sensitive cell lines.

Compound	IC_{50} (μM)[a]		RF[b]
	MCF-7	MCF-7/ADR	
CS1	16.92 ± 1.43	18.88 ± 2.93	1.12
ADR	0.79 ± 0.68	337.32 ± 27.91	426.99
VP16	13.78 ± 2.43	236.38 ± 17.91	17.15

[a] IC_{50} values are presented as mean ± SD ($n = 3$).
[b] RF (resistance fold) = IC_{50} value of MCF-7/ADR cells/IC_{50} value of MCF-7 cells; VP16, etoposide; ADR, adriamycin.

IIα-knockdown cells ($P < 0.05$). As Topo IIα is essential for cell proliferation and cells cannot survival without Topo IIα, there was still a certain amount of Topo IIα in Topo IIα-knockdown MDA-MB-231 cells. In addition, Topo IIβ could partially substitute for Topo IIα in the Topo IIα-deficient cells [21]. Hence, the effect of Topo II depletion by shRNA on growth inhibition by CS1 was not great. However, these results suggested that Topo IIα is involved in CS1-induced antitumor activity (see Table 1).

3.3. CS1 induced DNA breaks, leading to cell cycle arrest and apoptosis

To confirm the impact of CS1 on the activity of Topo IIα *in vivo*, neutral single-cell gel electrophoresis were performed to detect whether CS1 leads to DNA breaks. MDA-MB-231 cells were exposed for the indicated time to 10 μM CS1 or 10 μM etoposide. Clear comet tails were observed in the presence of CS1 and etoposide, while no comet formation was detected in untreated cells, indicating the production of DNA breaks in those treated cells (Fig. 3A). Histone H2AX plays important role in maintaining genomic stability. In response to DNA double-stand breaks (DSB), H2AX is phosphorylated and participated in DSB signaling [22]. To further confirm whether CS1 induced DNA breaks, western blotting and immunofluorescence staining analysis were employed. Numerous cells presenting phosphorylated histone H2AX (γH2AX) foci were observed in CS1 treated cells (Fig. 3B), which was confirmed by western blotting analysis (Fig. 3C). These data indicate that CS1 indeed induces DNA breaks in MDA-MB-231 cells.

To study the drug effects on cell cycle progression, flow cytometry analysis was performed. MDA-MB-231 cells were treated for 24 h with CS1 at different concentrations. The cell number at the G2/M phase was dramatically increased from 20.65% to 76.57% after treatment with 10 μM. Meanwhile, a significant decrease of cells at G1 phase and a modest increase at the sub-G1 phase were observed. (Fig. 3D). These results show that CS1 induces cell cycle arrest at the G2/M phase accompanied by significant apoptosis.

To further evaluate the ability of CS1 to induce apoptosis, DNA fragmentations were assayed. CS1 triggered DNA fragmentations of MDA-MB-231 cells in a does-dependent manner (Fig. 3E). Annexin V/PI assay was applied to quantitate the apoptotic cells of MDA-MB-231 cells. As illustrated in Fig. 3F, the proportion of apoptotic cells increased from 7.44% to 39.57% when MDA-MB-231 cells exposed to 2.5–10 μM CS1. These data further confirm that CS1 can induce apoptosis in MDA-MB-231 cells.

3.4. CS1 inhibited tumor growth in vivo

As CS1 selectively inhibited cancer cell growth by inducing Topo IIα-mediated DNA damage which lead to G2/M phase arrest and apoptosis, we expected a strong *in vivo* antitumor activity and lower toxicity of CS1. In the human breast cancer MDA-MB-231 xenograft tumorigenesis model, CS1 at 20 mg/kg body weight by tail vein injection every other day for 2 weeks achieved tumor weight inhibition of 62.3% ($P < 0.001$) (Fig. 4A and B), which is equipotent to the positive control etoposide (20 mg/kg). However, the outstanding antitumor activity of etoposide was associated

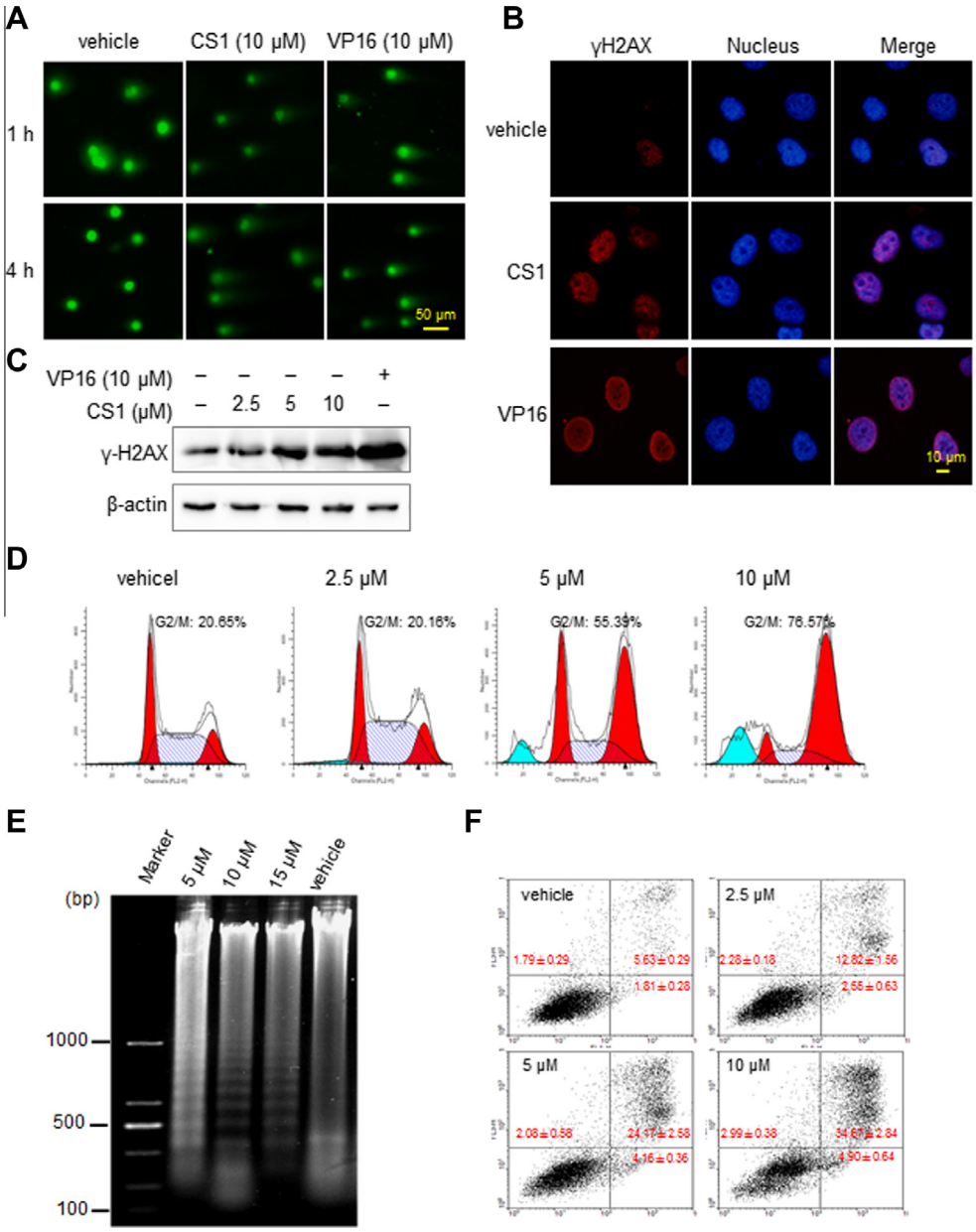

Fig. 3. CS1 induced DNA breaks, cell cycle arrest and apoptosis. (A) Representative images of CS1- or etoposide (VP16)-induced DNA damages. (B) Images of γH2AX foci generated in CS1 or VP16 treated cells. Nuclei was stained by Hoechst 33342 (blue), γH2AX was stained with γH2AX specific antibody (red). (C) Western blotting analysis for γH2AX protein. β-actin was used as a loading control. (D) Dose-dependent effects of CS1 on cell cycle distribution. (E) DNA fragmentation in CS1-treated MDA-MB-231 cells. Cells exposed to (5–15 μM) CS1 for 24 h were collected and the DNA fragments were detected by DNA agarose gel electrophoresis. (F) Flow cytometric analysis by Annexin-V/PI double-staining. CS1-treated cells were harvested and stained with Annexin V-FITC and PI, and then analyzed by flow cytometry to determine early or late apoptotic and necrotic cell population. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with toxicity. At the beginning of the study, the body weights of the mice in the three groups were around 24.2 g/mouse (Fig. 4C). With the treatment of etoposide for 8 days, the nude mouse body weights significantly decreased (16.9% lose), compared to the control, $P < 0.01$. In contrast, CS1 had no effect on the body weights, which is consistent with *in vitro* selective toxicity on cancer cells. All these data indicate that CS1 possesses potent *in vivo* antitumor activity with low toxicity.

4. Discussion

Although a large diversity of natural and synthetic Topo II inhibitors have been reported. Most topoisomerase inhibitors are efficient, however, the low selectivity against tumor cells and normal cells as well as other severe side effects including myelosuppression, leucopoenia, gastrointestinal toxicities, limit their clinical uses [23]. CS1, a synthetic 2-phenylnaphthalenoid with the goal to target Topo II, exhibits strong antitumor activity against various types of tumor cells. CS1 displayed comparable cytotoxicity to etoposide against MDA-MB-231 and HepG2 cell lines. However, CS1 is 6–10-fold less cytotoxic against HL7702 and HUVEC cells compared with etoposide. Although etoposide is slightly more potent against tumor cells than CS1, its high toxicity against normal cells limited its use in some way.

CS1 has a similar skeleton with biaryl compounds including *p*-terphenyls that showed various biological activities. We have

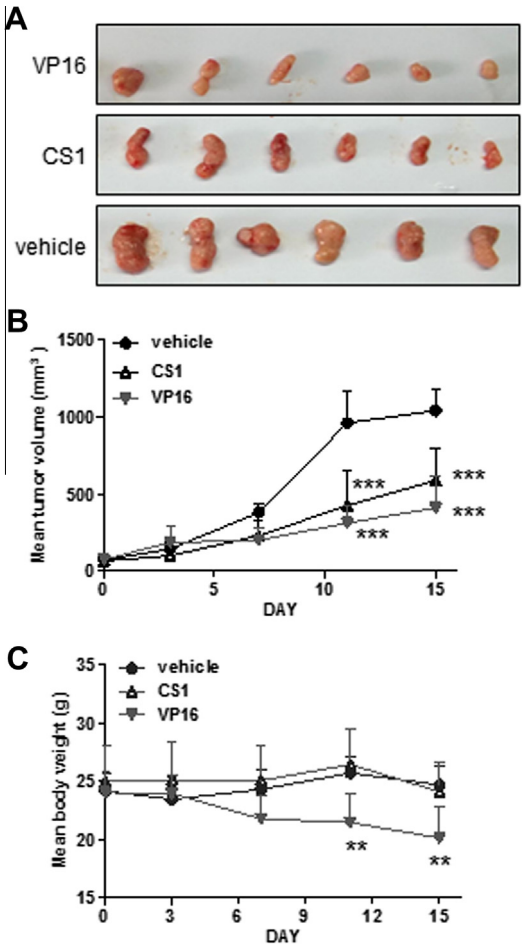

Fig. 4. CS1 suppressed the growth of xenograft tumors *in vivo*. Female nude mice implanted with MDA-MB-231 tumor fragments were randomized into treatment and control groups ($n = 6$). The nude mice were treated with CS1 or VP16 at the dose of 20 mg/kg body weigh every other day for two weeks. Tumor volume (B) and nude mouse body weights (C) were measured. $^{**}P < 0.01$, $^{***}P < 0.001$, the CS1 group or VP16 group versus the control group.

previously reported that natural *p*-terphenyl derivatives could induce apoptosis mediated by the generation of ROS [24]. However, ROS scavenger NAC was unable to decrease growth inhibition induced by CS1 (Fig. S2A). This data implicates that the cytotoxicity of CS1 is independent of the generation of ROS. Moreover, Wetzel et al. found that hydroxyphenylnaphthol inhibited 17β-hydroxysteroid dehydrogenase (17β-HSD1) and the estrogen receptors (ERs) α and β [25], which disrupt the balance of the formation of E2. We found that the combination of CS1 with increasing concentrations (1 nM–10 μM) of estradiol had no effect on the cytotoxicity of CS1 (Fig. S2B), suggesting that the cell growth inhibition of CS1 might be independent of E2. In this study, we found that Topo IIα-mediated pBR322 DNA relaxation and kDNA decatenation (Fig. 1C and D) were dramatically blocked by CS1. Moreover, Topo IIα-knockdown cells exhibited less sensitive to CS1 than that of wide type MDA-MB-231 cells (Fig. 2C). These data further evidence that CS1 inhibits cell growth by targeting Topo IIα.

Most of the current Topo II inhibitors, such as Adriamycin (ADR), etoposide and their analogs were the most effective antitumor agents for the treatment of human cancers. However, these inhibitors frequently elicit life-threatening toxicities and induce tumor multidrug resistance, which limit their usage. Moreover, resistance to Topo II inhibitors is frequently associated with the reduced expression of Topo II and overexpress of P-glycoprotein [26,27]. Surprisingly, our results demonstrat that CS1 is not a substrate for the P-glycoprotein and have the potency to overcome multidrug resistance. In addition, reduction of Topo IIα protein levels were sufficient to confer resistance to multiple classes of Topo II poisons [28]. In our study, we found that etoposide significantly downregulated the expression levels of proteins Topo IIα and Topo IIβ in HepG2 and HL7702 cells, which is consistent with previous reports [28]. However, CS1 did not decrease the levels of Topo IIα and Topo IIβ (Fig. S3), which suggests that CS1 may be less likely to induce drug resistance.

The high antitumor potential of CS1 is further confirmed *in vivo* using the human breast tumor xenograft model. Etoposide exerted high antitumor activity but also associated with toxicity. However, CS1 displays a marked antitumor activity without bodyweight lose. Although CS1 is slightly less potent than etoposide against tumor cells and tumor xenografts, it is much safer and more potent against multidrug resistant cells. In this regard, CS1 represents a new class of Topo II-targeting compounds with lower toxicity and potent anti-multidrug resistance activity.

In summary, our study demonstrat that CS1, a novel 2-phenylnaphthalene, is an interesting lead Topo II inhibitor. This compound displays broad-spectrum *in vitro* antitumor effects, low toxicity *in vivo* and potential anti-multidrug resistance capabilities. CS1 functions as a Topo II poison to stabilize Topo II/DNA complex, which leads to DNA damage, cell cycle arrest at G2/M phase and apoptosis. The biological properties of CS1 supports its potential as a promising candidate for treatment of cancer [29].

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.042.

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