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ORIGINAL ARTICLE

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Antitumor efficacy of 26-fluoroepothilone B against human prostate cancer xenografts

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Abstract *Background*: Epothilone compounds (e.g. epothilones A and B) represent a new structural class of microtubule inhibitors with the remarkable ability to inhibit tumor growth of multidrug-resistant cell lines at low nanomolar or even subnanomolar concentrations. Unfortunately, this therapeutic efficacy has only been achieved to date with a narrow therapeutic window. Hence, other structural analogs of compounds such as epothilone B are currently being synthesized in the hope that they will demonstrate equivalent antitumor efficacy with reduced systemic toxicity. *Purpose*: To evaluate the relative efficacy and toxicity of selectively modified epothilone compounds. *Methods*: Compounds were initially screened for relative cytotoxicity against the human prostate cancer cell lines PC3, LNCaP, MDA PCa 2a and MDA PCa 2b. Growth inhibitory IC_{50} values of 0.5 to 4 n*M* were obtained. From this initial screen, one epothilone compound, 26-fluoroepothilone B, was chosen for further evaluation against the growth of s.c.-implanted MDA PCa 2b- and PC3-derived prostate tumors in athymic nude mice. The compound was administered intravenously at 2, 5 and 10 mg/kg after the tumors had reached 300 mm^3. Two control groups were used: paclitaxel (40 mg/kg) and saline. *Results*: Following treatment with 10 mg 26-fluoroepothilone B/kg, there was a sustained decrease in tumor size for 30 days reaching a maximal reduction of 80% when compared with tumor growth in the saline control group. Sustained suppression (>20 days) of tumor growth was observed following the second drug injection. Although a maximal body weight loss of 30% occurred after the second injection, all mice completely regained their initial body weight in 20 days. A lower dose (2 mg/kg) produced a 58% maximal reduction in tumor size and a 20% body weight loss. Minimal inhibition of tumor growth, however, was obtained with paclitaxel at a maximally tolerated dose (40 mg/kg). Other epothilones tested were either less effective and/or more toxic than 26-fluoroepothilone B. This new fluorinated epothilone compound supports the growth of paclitaxel-dependent Tax-18 mutant CHO cells and produces microtubule bundles similar to those produced by paclitaxel, indicating that the two drugs share a similar mechanism of action. *Conclusion*: A new fluorinated epothilone compound, 26-fluoroepothilone B, has been described that stabilizes microtubule structures based on its support of growth of a mutant paclitaxel-dependent CHO cell line. Its antitumor activity against human prostate cancer in

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nude mice is superior to that of paclitaxel at equivalent toxic doses. Further research is required to determine optimal dosing strategies and to fully assess the compound's activity against other malignant diseases.

Keywords Epothilone · Prostate cancer · Paclitaxel · Drug development

Introduction

Epothilones represent a class of novel compounds initially discovered in an antifungal screening program [7, 8, 11]. They have now been shown to possess antitumor activity and a paclitaxel-like mechanism of action [1, 2, 23]. In an attempt to improve upon the overall activity and therapeutic index of epothilone compounds, a small series of modified drugs were synthesized and examined for their pharmacology against human prostate tumors in nude mice.

Localized prostate cancer has a favorable prognosis following treatment with radical prostatectomy or radiation therapy, but the prospect of cure diminishes significantly once the disease escapes beyond the confines of the prostate gland. While most patients with metastatic disease initially respond to androgen-deprivation therapy, patients eventually relapse with androgen-independent disease. Therapeutic options for treatment of androgen-independent prostate cancer recently increased with the demonstration that chemotherapy may be of benefit even in those patients with very advanced disease. Responses, however, are generally of short duration due to emergence of resistance [6, 9, 10, 25, 26]. Therefore, the development of new drugs that target androgen-independent prostate cancer is of critical importance.

Combination treatment regimens that include paclitaxel, a complex diterpene obtained from the Pacific yew (*Taxus brevifolia*), have recently been reported to be successful in treating hormone-refractory prostate cancer in the phase II setting [29]. A principal mechanism of cytotoxic action for paclitaxel, stabilization of microtubules leading to mitotic arrest, is now believed to be shared by the recently identified natural products epothilones A and B [1, 2, 7, 8, 14, 19, 23]. In this report we present initial cytotoxicity and antitumor efficacy data for a new epothilone compound, 26-fluoroepothilone B. Data suggesting the mechanism of action of 26-fluoroepothilone B are also presented.

New models of human prostate cancer, MDA PCa 2a, and MDA PCa 2b cell lines, and two widely used and well-characterized human prostate cancer cell lines, LNCaP and PC3, were used. MDA PCa 2a and MDA PCa 2b cell lines were recently established from a bone metastasis of a patient with androgen-independent prostate cancer [20, 21, 22]. These novel cell lines are androgen sensitive, express PSA and androgen receptors and grow well in vitro and in vivo. As such, they possess typical features of prostate cancer and represent clinically relevant models.

A small series of structurally related compounds including epothilone A, epothilone B, and 26-fluoroepothilone B (Fig. 1) were evaluated. The resulting IC_{50} data were subsequently used to select drugs and a range of doses for in vivo studies. Epothilone B and 26-fluoroepothilone B were evaluated for antitumor activity in vivo against tumors produced in athymic (nude) mice by s.c. inoculation of MDA PCa 2b cells. The relative ability of these compounds to interfere with microtubule structures was also investigated.

Materials and methods

Drugs

Paclitaxel was obtained from Handy Technology Laboratory (Houston, Tx.). All other compounds (see Table 1) were obtained from The Scripps Research Institute (La Jolla, Calif.). Company Code for 26-fluoroepothilone B: CGP85715 at Novartis Pharmaceuticals Inc. Stock solutions of each drug were made using DMSO at a concentration of 40 mg/ml. Working dilutions of all drugs were prepared by mixing stock solutions with ethanol; these were subsequently diluted in saline or cell growth medium as required for individual experiments.

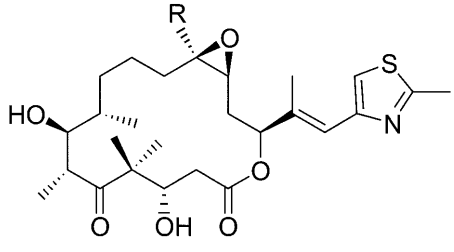
Cell lines

MDA PCa 2a and MDA PCa 2b cells [20] were propagated in BRFF-HPC1 medium (Biological Research Faculty and Facility, Janesville, Md.), 20% fetal bovine serum (Sigma Chemical Co., St Louis, Mo.) and gentamicin (50 μg/ml; GIBCO BRL, Gaithersburg, Md.). LNCaP and PC3 cells were obtained from the American Type Culture Collection (Manassas, Va.), and were routinely cultured in T medium [13] supplemented with 10% fetal bovine serum (Sigma).

Wildtype CHO subline 10001 [4] was grown in an alpha modification of minimal essential medium containing 5% fetal bovine serum, 50 U/ml penicillin, and 50 mg/ml streptomycin (all from GIBCO BRL). Tax-18, a paclitaxel-dependent and -resistant mutant cell line [3, 5], was grown in the same medium supplemented with 0.2 μg/ml paclitaxel. Cells were grown under a humidified atmosphere containing 5% CO_2 at 37°C.

In vitro drug screening

A cell viability assay was used to test the relative cytotoxicity of drugs in vitro. MDA PCa 2a, MDA PCa 2b or LNCaP cells were



Epothilone A, R = H
Epothilone B, R = CH_3
26-Fluoroepothilone B, R = CH_2F

Fig. 1 Structures of the fluorinated epothilone compound, 26-fluoroepothilone B, and of epothilones A and B

Table 1 Relative inhibition of tumor cell growth following treatment with paclitaxel and novel epothilone compounds. Data are presented as mean IC_{50} values (n*M*) from three separate experiments. Cells were exposed to drug continuously for 72 h prior to determination of cell viability and number by the MTT vital dye method

Drug	MDA PCa 2a	MDA PCa 2b	LNCaP	PC3
26-Fluoroepothilone B	2.8 ± 0.2	2.7 ± 0.4	1.2 ± 0.1	0.6 ± 0.1
Epothilone A	1.2 ± 0.1	5.1 ± 0.2	0.7 ± 0.1	3.7 ± 0.25
Epothilone B	0.7 ± 0.2	1.0 ± 0.01	0.2 ± 0.03	0.2 ± 0.02
Paclitaxel	4.0 ± 0.6	6.2 ± 0.5	1.6 ± 0.09	10.3 ± 0.4

seeded in 96-well plates at a density of 10,000 cells/well; PC3 cells were seeded at a density of 3500 cells/well. Cells were grown for 24 h prior to addition of drug. Drug dilutions were made in growth medium with fetal bovine serum to produce concentrations ranging from 10 to 7500 n*M*. Cells were continuously incubated with drug for 72 h. Each cytotoxicity assay was performed in triplicate. The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay was used to assess relative inhibition of cell growth.

Drug resistance

Plating efficiency was used to compare the relative sensitivities of wildtype CHO cells and Tax-18 to paclitaxel, epothilone B, and 26-fluoroepothilone B. Approximately 100–200 cells were seeded into replicate wells of 24-well tissue culture dishes containing growth medium with increasing concentrations of each drug. After 7 days the medium was removed and the cells were stained with 0.05% methylene blue as previously described [4].

Immunofluorescence

To evaluate the effects on organization of cellular microtubules, wildtype CHO cells were grown to approximately 50% confluence on glass coverslips and treated overnight (20 h) with ten times the minimal lethal dose of each drug (500 n*M* paclitaxel, 2 n*M* epothilone B and 8 n*M* 26-fluoroepothilone B). Cells were then rinsed in PBS, fixed in MeOH at –20°C for 10 min, rehydrated in PBS and incubated with a 1:100 dilution of α-tubulin-specific mouse monoclonal antibody DM1A (Sigma) for 1 h in a humid box at 37°C. Cells were then washed again in PBS and incubated for an additional hour in a 1:50 dilution of fluorescein-conjugated goat anti-mouse IgG (Sigma). Following further washing in PBS, coverslips were inverted cell-side down over 3 µl of mounting solution (90% glycerol, 10% PBS, 0.1% phenylenediamine, pH 8.7) and photographed with TMAX-400 film (Eastman Kodak, Rochester, N.Y.) using an Optiphoto microscope (Nikon, Melville, N.Y.) equipped with a PlanApo 60/NA 1.40 oil immersion objective.

Tumor generation

Male athymic (nude) mice at 6–8 weeks of age (Charles River Laboratory, Wilmington, Mass.) were injected s.c. with $1–3\times10^6$ MDA PCa 2b or PC3 cells. Animals were housed in the Athymic Animal Facility of the M.D. Anderson Cancer Center and manipulated under surgical aseptic conditions in a laminar flow hood. Cells to be injected into the mice were trypsinized, washed, counted, and resuspended at $1–3\times10^6$ cells/100 µl growth medium. MDA PCa 2b and PC3 cell lines were chosen for in vivo tumor growth because both are derived from a bone metastasis of prostate cancer [20, 21].

Tissue samples

Excised tumors were placed in neutral-buffered formalin. The tissue was then embedded in paraffin blocks from which 4-µm sections were cut and stained with hematoxylin and eosin. Both mitosis and apoptosis were scored in coded slides by microscopic examination at 400× magnification. The morphological features used for histopathological identification of apoptotic bodies have been previously described [16, 17, 18, 28]. Five fields of non-necrotic areas were randomly selected in each histological section, and in each field the number of apoptotic nuclei and cells in mitosis were recorded as numbers per 100 nuclei. The numbers of apoptotic bodies and mitotic figures were divided by the total number of cells counted, and the results expressed as a percentage.

Toxicity studies

Toxicity studies were performed with 26-fluoroepothilone B in male athymic mice. The analog 26-fluoroepothilone B (2, 5, and 10 mg/kg) was administered i.v. three times (7 days apart) to three mice per dose level. A control group was injected with saline. Body weight was measured on days 0, 7 and 14. Mice were killed on day 20. Necropsy was performed and tissues harvested (liver, heart, spleen, kidney, and testis) for histopathological evaluation.

Antitumor activity

The relative antitumor efficacy of 26-fluoroepothilone B was compared to that of paclitaxel against the growth of tumors produced by injection of MDA PCa 2b or PC3 cells. Tumor volume was calculated as length×width×height×0.5236 (the formula for an ellipsoid). Three separate experiments were performed.

Experiment 1. The relative antitumor activity of 26-fluoroepothilone B and paclitaxel against human prostate cancer tumor produced by MDA PCa 2b cells in nude mice was determined. Treatment (i.v.) was initiated when tumors reached an average size of 400 mm^3. Drug was administered i.v. on two separate days (4 days apart) at one of three dose levels (10, 5 or 2 mg/kg per dose). Saline or paclitaxel (40 mg/kg per injection), administered on the same schedule as 26-fluoroepothilone B, were used as controls. Tumor volume and body weight were recorded prior to initial drug administration and every 4 days thereafter.

Experiment 2. The antitumor activity of 26-fluoroepothilone B was evaluated in mice bearing human prostate PC3-derived tumor. Treatment was initiated when tumors reached an average size of 130 mm^3. Drug was administered i.v. three times (7 days apart) at one of two dose levels (5 or 10 mg/kg per dose). Saline was injected into control animals. Tumor volume and body weights were recorded prior to drug administration and every 4 days thereafter.

Experiment 3. The long-term (> 7 weeks) antitumor activity and short-term (7, 15, and 21 days) histopathological effects of 26-fluoroepothilone B against human prostate tumor produced by MDA PCa 2b cells in nude mice were determined. Treatment was initiated when tumors reached an average size of 200 mm^3. Drug was administered i.v. 7 days apart at 10 mg/kg per dose for a total of three doses. Saline was used in control mice. A group of 13 mice was used for drug treatment and a group of 6 for control treatment. Tumor volume and body weights were recorded prior to drug administration and every 4 days thereafter. Mice from the treatment group were killed on day 7 (four mice), day 14 (three mice), day 21 (one mouse) and on day 28 (one mouse). Tumor was processed for

histopathological evaluation. Four remaining mice from the treatment group were monitored over time. Control animals were injected with saline/DMSO/ethanol (1.9:0.05:0.05 v/v/v) solution.

Tumor growth delay was calculated as the mean time (days) for tumors in treated mice to double in size minus the mean time (days) for tumors in untreated (control) mice to double in size. The initial tumor size measurement was made 1 day after initiation of drug therapy.

Results

Cytotoxicity against human prostate tumor cell lines

The relative ability of the selected compounds to inhibit human prostate tumor cells was assessed using an MTT assay. Against the standard prostate cancer cell lines PC3 and LNCap, there was a wide range of relative cytotoxicity with IC_{50} values ranging from 0.2 to 5.1 n*M* (see Table 1). Of the compounds examined, epothilones clearly demonstrated the most potent cytotoxic activity. All cell lines tested had similar sensitivity to epothilones without regard to androgen sensitivity or derivation (lymph node or bone metastases).

26-Fluoroepothilone B retains a paclitaxel-like mechanism of action

The possibility that modifications introduced into the epothilone structure might have altered the drug's mechanism of action was assessed in two ways. The first made use of Tax-18, a paclitaxel-resistant cell line that requires the presence of paclitaxel for cell division [3]. Prior work has demonstrated that this paclitaxel-dependent phenotype can be rescued by any agent able to promote microtubule assembly [5]. Thus, these cells can serve as a biological assay for paclitaxel-like drugs that share a similar mechanism of action. The ability of wildtype and Tax-18 cells to grow in various concentrations of paclitaxel, 26-fluoroepothilone B and epothilone B is shown in Fig. 2. All three drugs were capable of rescuing the growth of Tax-18, and Tax-18 was more resistant to all of the drugs than were the wildtype cells. Although 26-fluoroepothilone B was approximately fourfold less potent than epothilone B, it retained a large increase in potency over paclitaxel. The ability of 26-fluoroepothilone B to promote the growth of Tax-18 clearly demonstrates that it can promote microtubule assembly in living cells.

A second assay made use of the well-known observation that paclitaxel induces microtubule bundle formation in treated cells. To determine whether 26-fluoroepothilone B induces similar bundles, wildtype CHO cells were treated overnight with paclitaxel, epothilone B, or 26-fluoroepothilone B at ten times the minimal lethal concentration. The cells were then fixed and stained for tubulin immunofluorescence (Fig. 3). All three drugs induced the formation of bundles in the microtubule network, further supporting the notion that epothilone B and 26-fluoroepothilone B share a common mechanism of action with paclitaxel.

In vivo testing of anticancer drug candidates for prostate cancer

Based on their cytotoxicities against human tumor cells in vitro, we selected 26-fluoroepothilone B and epothilone B for evaluation of their antitumor activity in vivo. Because epothilones and paclitaxel share a common mechanism of cytotoxic action, paclitaxel was selected as a positive control drug. The results of this experiment are shown in Fig. 4. 26-Fluoroepothilone B (at 2 mg/kg) was as effective an antitumor agent as paclitaxel (at its highest tolerated dose) against the MDA PCa 2b tumor. Higher doses of 26-fluoroepothilone B, however, were clearly superior to paclitaxel. Tumor regrowth was delayed until approximately 50 days following treatment with 26-fluoroepothilone B at 5 mg/kg and 60 days at a dose of 10 mg/kg. Epothilone B at doses of 5 and 10 mg/kg resulted in a high incidence of morbidity and death, demonstrating the extremely narrow therapeutic range of this compound. These doses have previously been found to be effective against human tumors in nude mice although sudden death is also a common finding [1]. Measured as tumor doubling time delay, paclitaxel produced a delay of 10.5 days which was equivalent to that from the low dose of 26-fluoroepothilone B. 26-Fluoroepothilone B produced tumor growth delays of 28 days at 5 mg/kg and approximately 56 days at 10 mg/kg.

26-Fluoroepothilone B was also found to be effective against human prostate PC-3 tumors in nude mice (Fig. 5). Triple injections of 26-fluoroepothilone B at 10 mg/kg produced a delay in tumor regrowth of approximately 45 days. Similarly, 26-fluoroepothilone B at a dose of 10 mg/kg was also effective against the human MDA PCa 2b tumor producing a delay in tumor regrowth of at least 50 days (Fig. 6).

Histopathology

MDA PCa 2b tumor tissues from mice in experiment 3 (Fig. 6) were submitted for examination of histopathology. In addition, slides were scored for relative mitotic and apoptotic indices (Fig. 7). These data demonstrate a low mitotic index in tumor tissue from control mice with only a modest level of background apoptosis. In contrast, by day 7 after the initial drug treatment with 26-fluoroepothilone B, the mitotic index moderately increased and the apoptotic index substantially increased. The mitotic index peaked on day 14 while the apoptotic index peaked on day 21 and had returned to control levels by day 67. Also noted on day 67 was a moderate coagulative necrosis within the tumor associated with mild hemorrhage and neutrophilic inflammation, suggesting a prolonged drug-mediated effect.

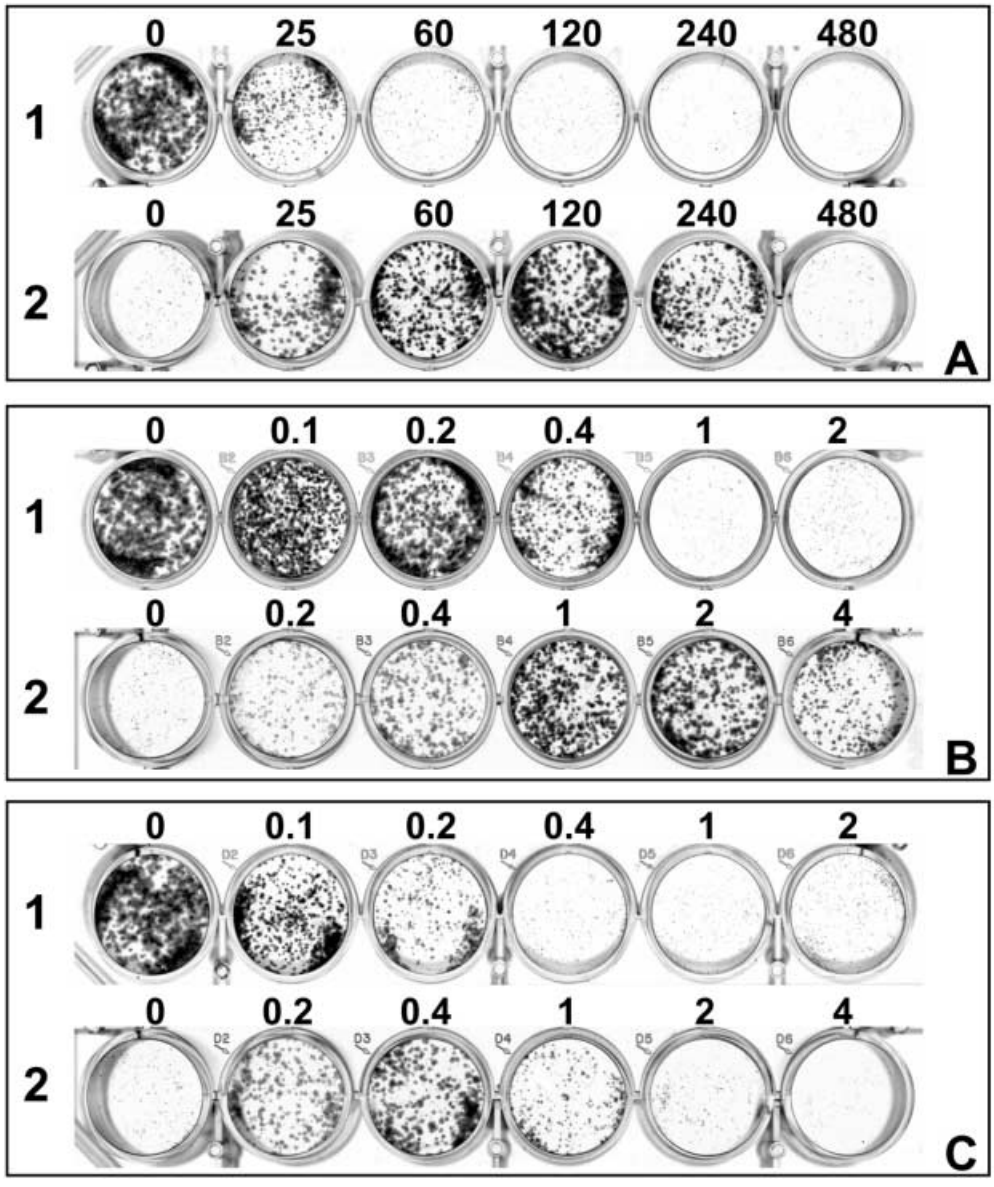


Fig. 2A–C 26-Fluoroepothilone B promotes the growth of a paclitaxel-dependent mutant CHO cell line. Wildtype (*1*) or Tax-18 (*2*) CHO cells were seeded into replicate wells of 24-well dishes in the presence of the indicated concentrations of drug (n*M*), allowed to grow for 7 days, and stained. The drugs used were paclitaxel (**A**), 26-fluoroepothilone B (**B**) and epothilone B (**C**). Compared to wild-type cells, Tax-18 is more resistant to the cytotoxic effects of all the drugs. Also note that all the drugs promote the growth of the mutant cells, indicating that they share a common mechanism of action

Discussion

It is now well-recognized that stabilization of cell microtubules inhibits their function and that this can contribute to inhibition of malignant cell growth. Although the structures of compounds that stabilize microtubules exhibit great heterogeneity, complex diterpenes such as paclitaxel and epothilones may nevertheless share common structural features [12, 24] and common mechanisms of action [7, 8, 12, 24, 27]. For example, these compounds have been shown to displace [^{3}H]paclitaxel from tubulin binding sites, lead to cell arrest in mitosis, cause formation of bundles of intracellular microtubules in nonmitotic cells, and induce the formation of hyperstable tubulin polymers [1, 2, 7, 8, 19]. Epothilone compounds, of which several have now been reported [7, 8, 11, 23], may offer several therapeutic advantages over paclitaxel; for example, they typically possess greater potency. In addition, epothilone compounds have been reported to retain activity against multiple drug-resistant cell lines as well as paclitaxel-resistant cell lines that do not have a MDR phenotype [1, 8, 11].

It is our belief that as a class the epothilones represent a potent chemical framework for compounds that might be useful for the treatment of cancer. We report here the structure and initial biological activity of 26-fluoroepothilone B, a novel C26 fluorinated epothilone derivative. The findings of the present studies demonstrate that 26-fluoroepothilone B has a potent cytotoxic effect against human prostate cancer cell lines that is independent of their relative androgen requirement. Furthermore, 26-fluoroepothilone B has excellent growth-inhibitory capabilities against two novel metastatic human prostate cancer cell lines.

The growth of a cell line that requires paclitaxel for stabilization of microtubule structure was rescued by 26-fluoroepothilone B suggesting that the drug shares a similar mechanism of action to paclitaxel and other epothilone compounds. Moreover, the ability of 26-fluoroepothilone B to produce microtubule bundles resembles that of epothilone B. It is interesting to note, however, that the effective concentration range rescuing the growth of paclitaxel-dependent cells is wider for 26-fluoroepothilone B than it is for epothilone B (Fig. 2). The fluorinated derivative more closely mimics paclitaxel in this regard and suggests that, like paclitaxel, it may possess a wider therapeutic index than epothilone B.

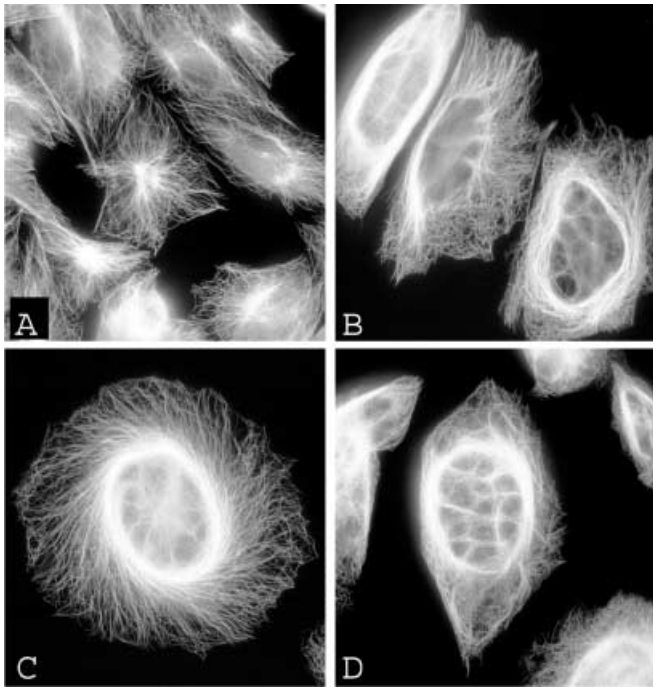


Fig. 3A–D Microtubule bundles induced by paclitaxel and epothilones. Wildtype CHO cells were either untreated (**A**) or treated overnight with 500 n*M* paclitaxel (**B**), 2 n*M* epothilone B (**C**), or 8 n*M* 26-fluoroepothilone B (**D**). Microtubules were stained with an antibody to α-tubulin and examined by indirect immunofluorescence. Note that all of the drugs induced formation of microtubule (×450)

Published studies of epothilone B indicate a useful level of antitumor activity but also question its worrisome toxicity and raise serious concerns about the tolerability of this drug [1, 7, 8]. In contrast to epothilone B, Chou et al. [7, 8] have shown that desoxyepothilone B shows excellent antitumor activity against several human tumors including MX-1, SK-OV-3 and the drug-resistant tumor MCF-7/Adr. Interestingly, their compound worked best when administered by the i.p. route. In the present study, we showed that the antitumor efficacy of 26-fluoroepothilone B against the human prostate MDA PCa 2b tumor cell line appears to be very promising. While paclitaxel at its maximal tolerated dose produced only minor inhibition of tumor growth, delays in tumor growth following injections of 26-fluoroepothilone B in excess of 50 days were typically observed. Tumor growth delay was associated with a relatively high apoptotic index in tumor tissue that peaked several weeks after initiation of drug therapy. In our experience [16, 17, 18, 28] sustained elevation of apoptotic indices weeks following drug treatment of tumored animals is impressive and unusual. The toxicity of 26-fluoroepothilone B at 5 and 10 mg/kg was con-

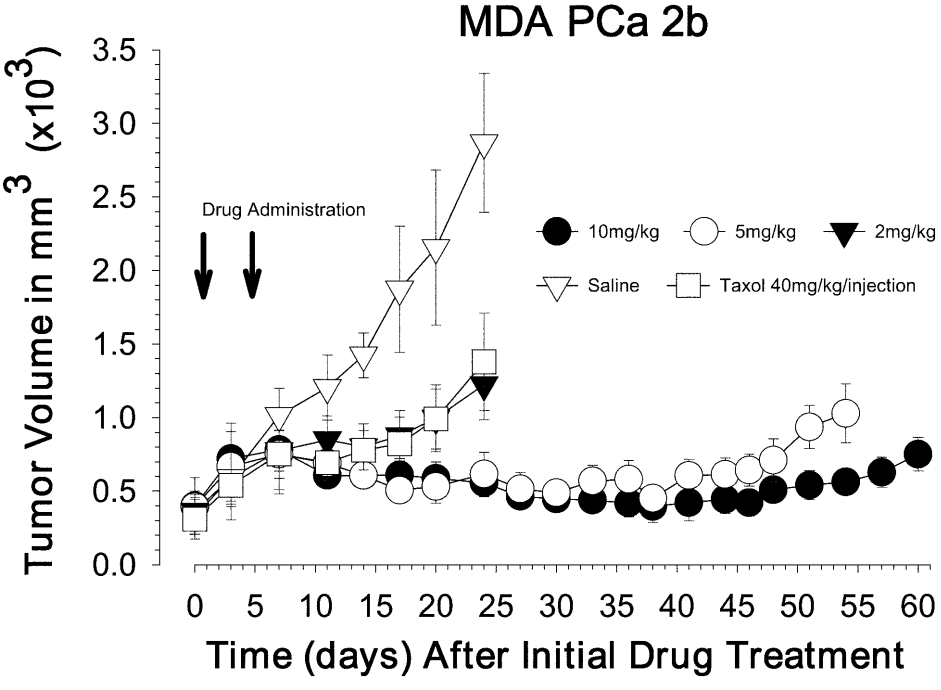


Fig. 4 Effect of 26-fluoroepothilone B on the growth of MDA PCa 2b cells in vivo (experiment 1). Nude mice with s.c. tumors were treated with drug once the tumor volume had reached 300–500 mm^3. Mice received two i.v. injections of 26-fluoroepothilone B 4 days apart at 10, 5 or 2 mg/kg per dose. Saline and paclitaxel (40 mg/kg per injection) served as controls. The data are presented as means ± SD (five mice per group)

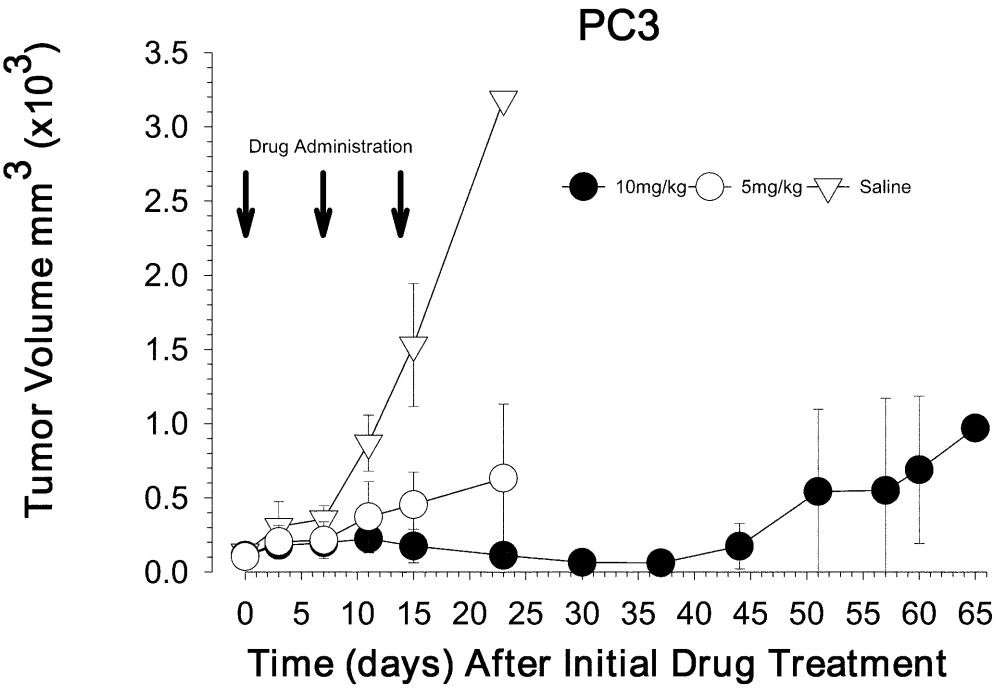


Fig. 5 Effect of 26-fluoroepothilone B on the growth of PC3 cells in vivo (experiment 2). Nude mice with s.c. PC3 tumors were treated with drug once the tumor volume had reached 60–200 mm^3. Mice received three i.v. injections of 26-fluoroepothilone B 7 days apart at 10 or 5 mg/kg per dose. Saline was injected into control mice. The data are presented as means ± SD (four mice per group)

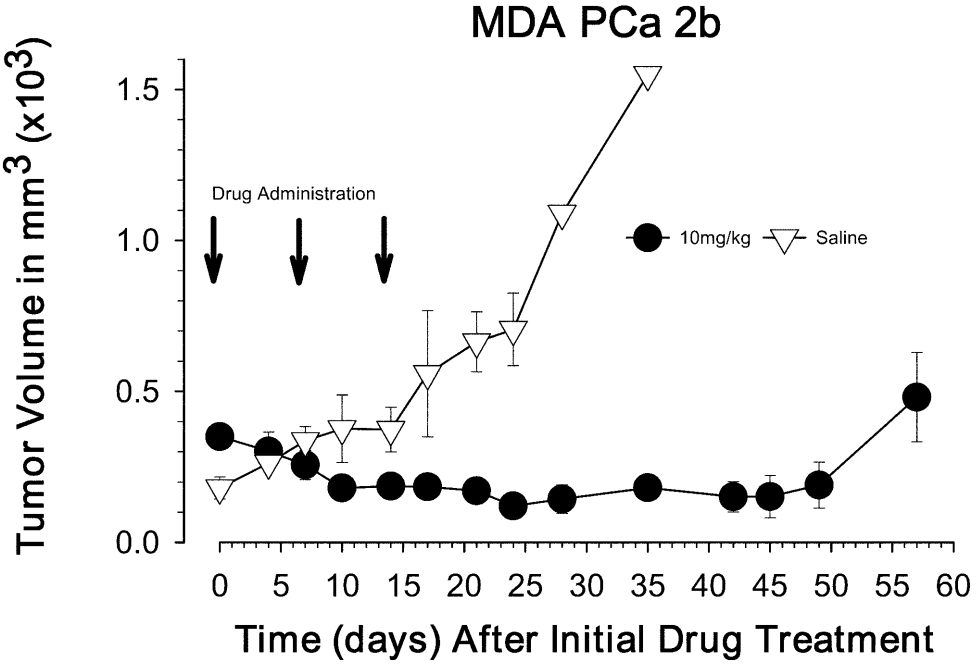


Fig. 6 Effect of 26-fluoroepothilone B on the growth of MDA PCa 2b cells in vivo (experiment 3). Nude mice with s.c. tumors were treated with drug once the tumors had reached 100–300 mm^3. Mice received three i.v. injections of drug 7 days apart at 10 mg/kg per dose. Saline/DMSO/ethanol (1.9:0.05:0.05 v/v/v) was injected into control mice. Mice from the drug-treated group were killed on days 7, 21, and 28, and tumor was processed for histopathological evaluation. Four remaining mice from the treated group were monitored over time for a total of 67 days. Tumor volume data in the control group are presented as means ± SD (five mice per group)

sidered acceptable with no drug-related deaths. Potent antitumor activity against PC3 human prostate cancer was also observed.

It must be pointed out that although we included paclitaxel as a control drug, it was not used in what may be considered to be the best vehicle and schedule of administration for this compound. Accordingly, the comparison of 26-fluoroepothilone B to paclitaxel reported here is not intended to address the ultimate promise of this particular epothilone derivative versus paclitaxel. Additional studies of formulation, route of administration and schedule of injections must also be performed with this new epothilone derivative. The results demonstrate, however, promising antitumor activity against established models of human prostate tumors (e.g. PC3) in nude mice. We showed that the compound has the ability to produce a prolonged alteration in prostate tumor mitotic and apoptotic indices that may extend to significant antitumor responses in humans.

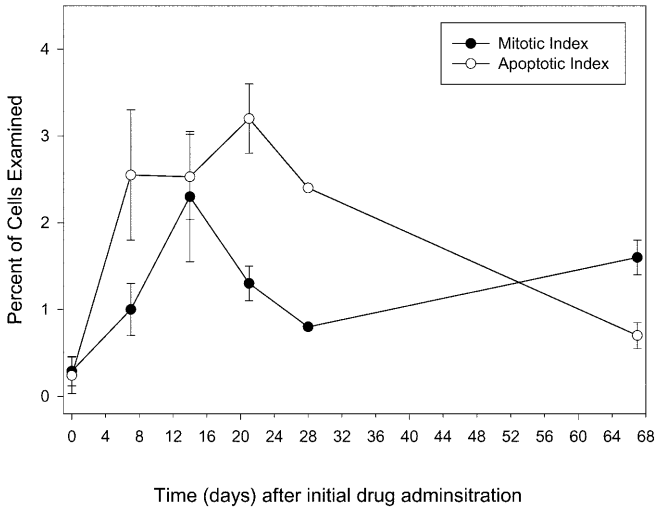


Fig. 7 Relative mitotic and apoptotic indices in MDA PCa 2b tumor tissue from mice in experiment 3 (see Fig. 6). Mice bearing MDA PCa 2b tumors were injected with 26-fluoroepothilone B, and the percentages of mitosis and apoptosis were scored from histopathological sections made from tumors at 0–67 days after initial drug treatment (see Methods). The data are presented as means ± SD

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