

BMS-247550: A Novel Epothilone Analog with a Mode of Action Similar to Paclitaxel but Possessing Superior Antitumor Efficacy

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ABSTRACT

BMS-247550, a novel epothilone derivative, is being developed by Bristol-Myers Squibb Company (BMS) as an anticancer agent for the treatment of patients with malignant tumors. BMS-247550 is a semisynthetic analogue of the natural product epothilone B and has a mode of action analogous to that of paclitaxel (i.e., microtubule stabilization). *In vitro*, it is twice as potent as paclitaxel in inducing tubulin polymerization. Like paclitaxel, BMS-247550 is a highly potent cytotoxic agent capable of killing cancer cells at low nanomolar concentrations. Importantly, BMS-247550 retains its antineoplastic activity against human cancers that are naturally insensitive to paclitaxel or that have developed resistance to paclitaxel, both *in vitro* and *in vivo*. Tumors for which BMS-247550 demonstrated significant antitumor activity encompass both paclitaxel-sensitive and -refractory categories, i.e., (a) paclitaxel-resistant: HCT116/VM46 colorectal (multidrug resistant), Pat-21 breast and Pat-7 ovarian carcinoma (clinical isolates; mechanisms of resistance not fully known), and A2780Tax ovarian carcinoma (tubulin mutation); (b) paclitaxel-insensitive: Pat-26 human pancreatic carcinoma (clinical isolate) and M5076 murine fibrosarcoma; and (c) paclitaxel sensitive: A2780 ovarian, LS174T, and HCT116 human colon carcinoma. In addition, BMS-247550 is p.o. efficacious against preclinical human tumor xenografts grown in immunocompromised mice or rats. Schedule optimization studies indicate that BMS-247550 is efficacious when administered frequently (every 2 days \times 5) or intermittently (every 4 days \times 3 or every 8 days \times 2). These efficacy data demonstrate that BMS-247550 has the

potential to surpass Taxol[®] in both clinical efficacy and ease of use (i.e., less frequent treatment schedule and/or oral administration).

INTRODUCTION

The epothilones represent a family of 16-membered ring macrolides that were originally isolated by Holfe and Reichenbach in 1992 from the fermentation broth of the myxobacterium *Sorangium cellulosum* (1). Interest in the potential use of an epothilone as an anticancer agent was kindled after the report by Bollag *et al.* (2) that two closely related natural fermentation products, epothilone A and B, had a mode of action similar to paclitaxel, i.e., microtubule stabilization.

Since its introduction in 1993, Taxol[®] has established itself as one of the most active antineoplastic agents against a wide spectrum of malignancies, including ovarian, breast, lung, and head and neck cancers and Kaposi's sarcoma. Despite the success of Taxol[®], there remains considerable room for improvement. For example, a majority of initially responsive patients eventually develop resistance, and not all cancers respond to treatment with the taxanes; diseases such as colorectal cancers or melanoma are known to be innately resistant.

The novel epothilone chemotype represents an attractive alternative to the taxanes, based upon their activities against various paclitaxel-resistant cancer cell lines, encompassing both the MDR and tubulin mutation modes of resistance (2, 3). However, despite their impressive *in vitro* activities, preliminary studies with epothilones A and B in *in vivo* models of cancer revealed that both compounds had only modest *in vivo* antitumor activity (4). This was shown to be attributable to their poor metabolic stability, unfavorable pharmacokinetic characteristics and narrow therapeutic window. Therefore, we initiated an epothilone analogue program to optimize the *in vivo* antitumor efficacy and therapeutic index of this chemical class. Over 300 semisynthetic analogues were made and tested in various *in vitro* and *in vivo* systems (5, 6). From these efforts, BMS-247550, a lactam analogue of epothilone B (Fig. 1), emerged as the most efficacious epothilone in a battery of *in vivo* preclinical chemotherapy studies, out-performing paclitaxel in each of the paclitaxel-resistant tumor models tested. Here we report in full the *in vitro* and *in vivo* antineoplastic activities of BMS-247550.

MATERIALS AND METHODS

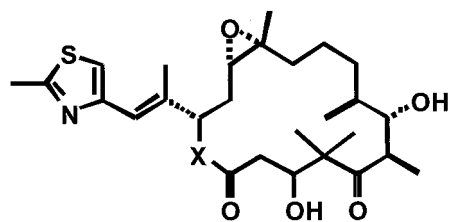
Chemicals. BMS-247550 was conceived and first synthesized in the Oncology Chemistry Department at Bristol-

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² The abbreviations used are: MDR, multidrug-resistance; MTD, maximum tolerated dose; OD, optimal dose; TVDT, tumor volume doubling time; LCK, log cell kill.



Epothilone B; X=O
BMS-247550; X=NH

Fig. 1 Chemical structures of epothilone B and BMS-247550.

Myers Squibb Pharmaceutical Research Institute (6). Unless specified, chemicals and solutions used for the maintenance of cell culture were obtained from Life Technologies, Inc. (Grand Island, NY). Sterile tissue culture ware was obtained from Corning, NY. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) at the highest grade available.

Drug Administration. *In vitro* studies used BMS-247550 dissolved in 100% DMSO and serially diluted in media/10% fetal bovine serum. For administration of BMS-247550 to rodents, two different excipients were used: (a) ethanol:water (1:9, v/v); and (b) Cremophor:ethanol:water (1:1:8, v/v). BMS-247550 was first dissolved in ethanol or a mixture of Cremophor:ethanol (50:50). Final dilution to the required dosage strength was made <1 h before drug administration. For parenteral administration (i.v.), dilution was made with water so that the dosing solutions contained the specified excipient composition described above. For oral administration (p.o.), the dilution was made with 0.25 M sodium phosphate buffer (pH 8.0) at a ratio of 30:70, v/v. Paclitaxel was dissolved in a 50:50 mixture of ethanol and Cremophor and stored at 4°C. Final dilution of paclitaxel was performed immediately before drug administration using NaCl solution (0.9%). Fresh preparation of paclitaxel was necessary to avoid undesired precipitation. The volumes of all compounds injected were 0.01 ml/g body weight of mice, and 0.005 ml/g body weight of rats.

Tumor Cell Lines. HCT116 human carcinoma cell lines and HCT116/VM46 cells, a MDR variant (7), were maintained in McCoy's 5A medium and 10% heat inactivated fetal bovine serum. A2780 human ovarian carcinoma cells and A2780Tax cells, obtained from Dr. Antonio Fojo (National Cancer Institute, Bethesda, MD), were maintained in IMEM and 10% heat-inactivated fetal bovine serum (Life Technologies, Inc.). All other cell lines were maintained in RPMI 1640 with 10% heat inactivated fetal bovine serum. Other cell lines with acquired resistance included human ovarian carcinoma A2780/DDP-S (parental) and A2780/DDP-R (cisplatin; Ref. 8) and colon HCT116(VP)35 (etoposide; Ref. 7). Additional cell lines that were studied included human ovarian carcinoma (OVCAR-3), human breast carcinoma (MCF-7 and SKBR-3), human prostate carcinoma (LNCAP and PC-3), human colon carcinoma (Caco-2, LS174T, and MIP), human lung carcinoma (A549, LX-1), human squamous cell carcinoma (A431), human leukemias (CCRF-CEM, HL-60, and K562), human fibroblasts (HS27), adult bovine aortic endothelial-bFGF-dependent

growth, mouse lung carcinoma (M109), and mouse lung fibroblast from p53 knockout mouse.

Growth Inhibition Assay. The *in vitro* cytotoxicity was assessed in tumor cells by a tetrazolium-based colorimetric assay, which takes advantage of the metabolic conversion of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfonyl)-2H-tetrazolium, inner salt) to a reduced form that absorbs light at 492 nm (9). Cells were seeded 24 h before compound addition. After a 72-h incubation at 37°C with serially diluted compound, MTS, in combination with the electron coupling agent phenazine methosulfate, was added to the cells. The incubation was continued for 3 h, and then the absorbency of the medium at 492 nm was measured with a spectrophotometer to obtain the number of surviving cells relative to control populations. The results are expressed as mean cytotoxic concentrations (IC₅₀s). The IC₅₀s are expressed visually as a mean bar graph that plots the difference between the log IC₅₀ for each cell line and the log of the mean of all of the cell line IC₅₀s. Bars projecting to the right depict cells more sensitive than average and bars to the left indicate those less sensitive than average.

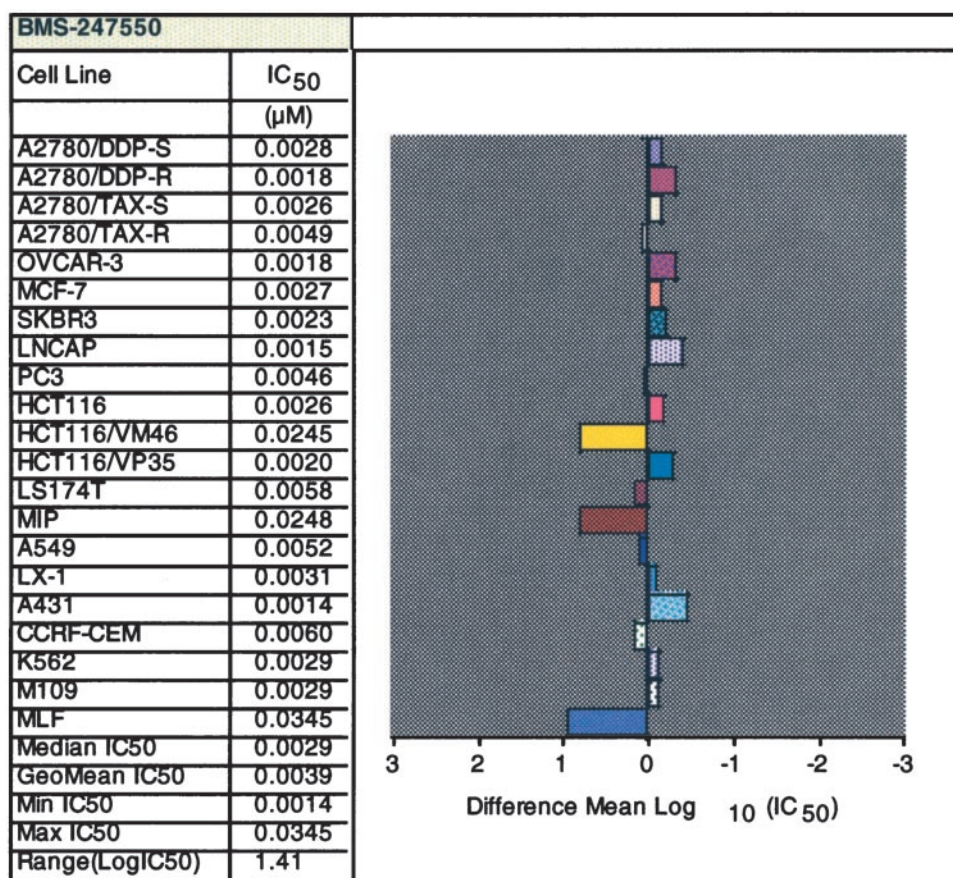
Clonogenic Cell Colony-formation Assay. The potency with which BMS-247550 and paclitaxel kill clonogenic tumor cells (cells that are able to divide indefinitely to form a colony) *in vitro* was evaluated by a colony formation assay. The concentration needed to kill 90% of clonogenic cancer cells (IC₉₀) was determined.

Tubulin Polymerization Assay. The potency with which BMS-247550 and paclitaxel polymerize tubulin isolated from calf brain was evaluated by published techniques (10, 11). Briefly, different concentrations of paclitaxel or BMS-247550 in polymerization buffer [0.1 M MES, 1 mM EGTA, 0.5 mM MgCl₂ (pH 6.6)] were added to tubulin in polymerization buffer at 37°C in microcuvette wells of a Beckman (Beckman Instruments) Model DU 7400 UV spectrophotometer. A final microtubule protein concentration of 1.0 mg/ml and compound concentrations of generally 2.5, 5.0, and 10 μM were used. Initial slopes of absorbance (A_{280 nm}) change, measured every 10 s, were calculated by the software program accompanying the instrument. Effective concentration (EC_{0.01}) was defined as the interpolated concentration capable of inducing an initial slope of 0.01 A_{280 nm}/min rate and was calculated using the formula: EC_{0.01} = concentration/slope. EC_{0.01} values are expressed as the mean with SD obtained from three different concentrations.

Flow Cytometry. Cell cycle distribution was evaluated by flow cytometry using standard DNA staining procedures. Briefly, HCT116 cells from cultures were collected by trypsinization after 1, 2, 4, 8, 16, and 24 h exposure to 7.5 nM of BMS-247550. Cells were pelleted and fixed in 80% ethanol at -20°C. After an overnight storage at -20°C, cells were rehydrated with PBS buffer and DNA stained by incubation with propidium iodide (5 μg/ml) in 0.1% RNase for 15–30 min. Fluorescence-activated cell sorter acquisition was performed using the FACS Calibur instrument (Becton Dickinson, San Jose, CA), and analysis was done using Cellquest and Modfit software (Becton Dickinson).

Animals. All rodents were obtained from Harlan Sprague Dawley Co. (Indianapolis, Indiana), and maintained in an ammonia-free environment in a defined and pathogen-free colony. Immunocompromised rodents were housed in cages obtained

Fig. 2 Cytotoxicity spectrum of BMS-247550 against a panel of tumor cell lines. The mean bar graph, on the right, graphically depicts the difference between the log of the individual cell line IC₅₀ values relative to the mean log of all of the IC₅₀ values. Right-projecting bars, cells; left projecting bars, resistant cell lines. Mean IC₅₀ = 3.9 nM. ND, not done.



from Thoren Caging Systems (Hazleton, PA), which provides positive individual ventilation to each cage. The animal care program of Bristol-Myers Squibb Pharmaceutical Research Institute is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

In Vivo Antitumor Testing. The following human tumors were used: ovarian carcinoma A2780, A2780Tax (established from cells obtained from Dr. Antonio Fojo, Medicine Branch, National Cancer Institute), and Pat-7 (established from an ovarian tumor biopsy from a patient who had developed resistance to Taxol[®]; provided by Dr. Thomas Hamilton, Fox Chase Cancer Center, Philadelphia, PA); HCT116, HCT116/VM46, and LS174T colon carcinomas; Pat-21 breast carcinoma (established from a breast tumor biopsy from a patient who failed Taxol[®] therapy; provided by Dr. William Hait, Cancer Institute of New Jersey, New Brunswick, NJ); and Pat-26 pancreatic carcinoma (from a liver metastasis biopsy provided by Dr. John Hoffman, Fox Chase Cancer Center). Pat-7, Pat-21, and Pat-26 xenografts were established directly from primary tumor biopsies by s.c. xenotransplantation into whole-body irradiated nude mice without any intervening *in vitro* cell-culturing steps. In addition, the innately paclitaxel-insensitive murine fibrosarcoma M5076 was also used.

The human tumor xenografts were maintained in BALB/c nu/nu nude mice. M5076 was maintained in C57BL/6 mice. Tumors were propagated as s.c. transplants in the appropriate

mouse strain using tumor fragments obtained from donor mice. Tumor passage occurred biweekly for murine tumors and approximately every 2–8 weeks for the various human tumor lines. With regard to efficacy testing, M5076 tumors were implanted in (C57Bl/6 × DBA/2)F1 hybrid mice, and human tumors were implanted in nude mice. All tumor implants for efficacy testing were s.c.

The required number of animals needed to detect a meaningful response (6–8) were pooled at the start of the experiment, and each was given a s.c. implant of a tumor fragment (~50 mg) with a 13-gauge trocar. For treatment of early-stage tumors, the animals were again pooled before distribution to the various treatment and control groups. For treatment of animals with advanced-stage disease, tumors were allowed to grow to the predetermined size window (tumors outside the range were excluded), and animals were evenly distributed to various treatment and control groups. Treatment of each animal was based on individual body weight. Treated animals were checked daily for treatment related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again after the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provided a measure of treatment-related toxicity.

Tumor response was determined by the measurement of tumors with a caliper twice a week until the tumors reached a

predetermined target size of 0.5 or 1.0 g. Tumor weights (mg) were estimated from the formula:

$$\text{Tumor weight} = (\text{length} \times \text{width}^2) \div 2$$

The MTD is defined as the dose level immediately above which excessive toxicity (*i.e.*, more than one death) occurred. The MTD was frequently equivalent to the OD. Activity is described at the OD. Treated mice dying before having their tumors reach target size were considered to have died from drug toxicity. No control mice died bearing tumors less than target size. Treatment groups with more than one death caused by drug toxicity were considered to have had excessively toxic treatments, and their data were not included in the evaluation of a compound's antitumor efficacy.

Tumor response end point was expressed in terms of tumor growth delay ($T - C$), defined as the difference in time (days) required for the treated tumors (T) to reach a predetermined target size compared with those of the control group (C).

To estimate tumor cell kill, the (tumor volume doubling time) TVDT was first calculated with the formula: $\text{TVDT} = \text{Median time (days) for control tumors to reach target size} - \text{Median time (days) for control tumors to reach half the target size}$ and $\text{LCK} = (T - C) \div (3.32 \times \text{TVDT})$.

A tumor is defined as "cured" when there is no detectable disease at the time of study termination (day >75 days post-tumor implantation); the interval between study termination and the end of drug treatment always exceeded 10 times the TVDT of any particular tumor type.

Group sizes typically consisted of eight mice in all treatment and control groups. Statistical analyses of response data were carried out using Gehan's generalized Wilcoxon test (12).

RESULTS

Cytotoxicity against Cancer Cells *in Vitro*. BMS-247550 has a broad spectrum of activity against a panel of tumor cell lines *in vitro* (Fig. 2). Of the 21 cell lines tested, the IC_{50} values were in the range of 1.4–34.5 nM. Significantly, BMS-247550 seemed to overcome to a large extent the two main mechanisms of resistance to paclitaxel, *i.e.*, MDR resistance attributable to P-glycoprotein overexpression (exemplified by HCT116/VM46) and β -tubulin mutation (exemplified by A2780Tax). Additional demonstration of the ability of BMS-247550 to overcome paclitaxel resistance is illustrated in Fig. 3. BMS-247550 and paclitaxel were similarly potent in killing clonogenic cells in the two sensitive tumor cell lines (HCT116 and A2780). However, against the three cell lines that had developed resistance to paclitaxel (HCT116/VM46, A2780Tax, and Pat-7), BMS-247550 was more effective than paclitaxel, almost completely retaining its cytotoxic potency against these resistant cell lines as compared with the sensitive lines.

Mechanism of Cytotoxicity—Tubulin Polymerization.

The cytotoxic activities of the epothilones, like those of the taxanes, have been linked to the stabilization of microtubules, which results in mitotic arrest at the G_2 -M transition. In this regard the potency BMS-247550 is similar to those of its two natural analogues, epothilone A and B, but was ~2.5-fold more potent than paclitaxel (Table 1).

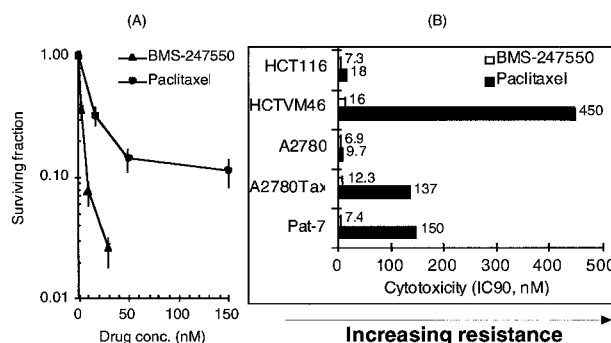


Fig. 3 BMS-247550 retained its anticancer cytotoxicity against tumor types that had developed resistance to paclitaxel. A, clonogenic cell survival of Pat-7 ovarian carcinoma cells after a 16-h exposure to BMS-247550 or paclitaxel. B, comparative cytotoxic potency (IC_{90}) of BMS-247550 and paclitaxel in five human tumor lines: HCT116 human colon carcinoma, HCT116/VM46 (MDR-resistant variant derived from HCT116), A2780 human ovarian cancer, A2780Tax (paclitaxel-resistant variant, attributable to a mutation in β -tubulin), and Pat-7 human ovarian cancer (derived from a patient who had developed resistance to Taxol[®] monotherapy). IC_{90} , the concentrations of the agent required to reduce colony formation by 90%. IC_{90} s are shown next to the bar graphs.

Table 1 Tubulin polymerization potency of three epothilones relative to paclitaxel

Analog	Polymerization potency $\text{EC}_{0.01}$ (μM)	Ratio of polymerization Potency of analog: Paclitaxel
BMS-247550	3.5 ± 0.4	0.4
Epothilone A	2.0 ± 0.2	0.4
Epothilone B	1.8 ± 0.2	0.3
Paclitaxel	$8.5 \pm 1.4, 5.0 \pm 1.0, 6.0 \pm 0.7$	1.0

Mechanism of Cytotoxicity—Effects on Cell Cycle Progression. Similar to paclitaxel, BMS-247550 blocks cells in the mitotic phase of the cell division cycle. Moreover, the concentration of BMS-247550 needed to arrest cells in mitosis, as measured by flow cytometry, corresponds well to the concentration required to kill cells over the same treatment duration. Thus, as shown in Fig. 4, BMS-247550 at a concentration close to the IC_{90} (~7.5 nM, clonogenic cytotoxicity assay) almost completely blocks cells in mitosis as early as 8 h after the initiation of drug exposure (Fig. 4).

Antitumor Activity by Parenteral Administration. BMS-247550 was evaluated in a panel of eight human and murine tumor models, some of which were chosen because of their known, well-characterized resistance to paclitaxel (Table 2). In addition, three paclitaxel-sensitive models were included to gain a full assessment of the spectrum of antitumor activity of BMS-247550.

Paclitaxel-refractory Tumor Models

Pat-7 Human Ovarian Carcinoma Xenograft Model.

This tumor model was established directly from a tumor biopsy of an ovarian cancer patient by implantation of tumor fragments

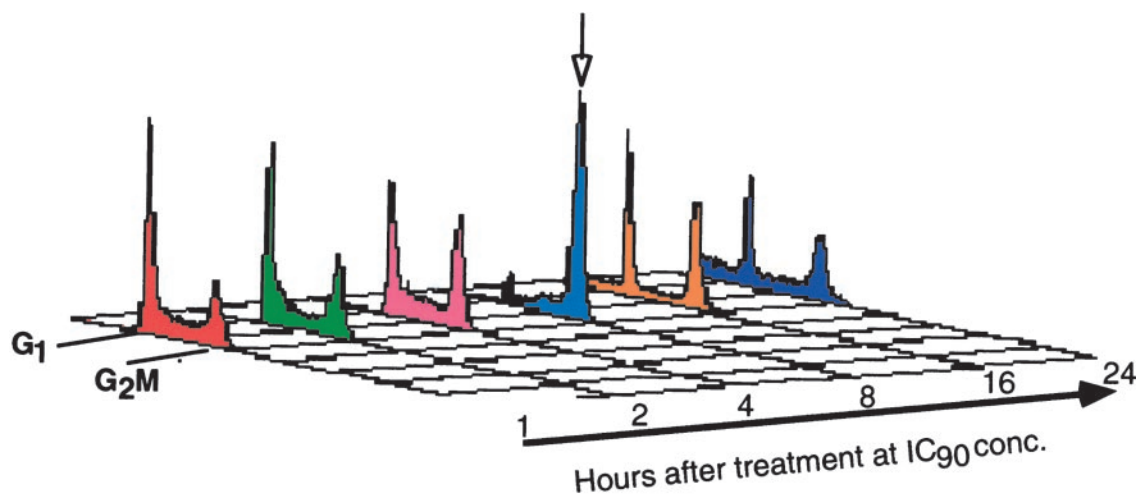


Fig. 4 Time course of the mitotic blockade induced by the incubation of HCT116 colon carcinoma cells in cell-culture media containing 7.5 nM (IC_{90}) BMS-247550. Arrow, position of the G_2M cell population.

Table 2 Tumor model characteristics

Tumor	Histology	Source	Paclitaxel sensitivity	Resistance mechanism(s)
Human				
Pat-26	Pancreatic	Biopsy	Insensitive	Unknown
Pat-7	Ovarian	Biopsy	Resistant ¹	MDR, ² MRP ³
A2780Tax	Ovarian	Cell line	Resistant	Tubulin mutation
HCT116/VM46	Colon	Cell line	Resistant	MDR
Pat-21	Breast	Biopsy	Resistant ¹	Unknown
A2780	Ovarian	Cell line	Sensitive	–
HCT116	Colon	Cell line	Sensitive	–
LS174T	Colon	Cell line	Sensitive	–
Murine				
M5076	Fibrosarcoma	Cell line	Insensitive	Unknown, non-MDR

¹ Clinical resistance to Taxol®.

² MDR, multidrug resistance attributable to P-glycoprotein overexpression.

³ MRP, multidrug resistance-related protein.

into whole-body irradiated nude mice. The donor patient was initially responsive to Taxol® treatment but ultimately developed resistance after nine courses of monotherapy with Taxol®. Before treatment with Taxol®, the patient also received numerous other chemotherapeutic agents including carboplatin, cyclophosphamide, VP-16, ifosfamide and Hexalen. Tumor biopsy was taken after development of Taxol® resistance.

BMS-247550 was administered to nude mice bearing staged tumors using a schedule of every 2 days \times 5. At its optimal dose, BMS-247550 was highly active in 3 different experiments, eliciting 2.9 ± 1.4 LCK (Table 3 and Fig. 5A). Concomitantly evaluated i.v. paclitaxel yielded a 0.8 ± 0.5 LCK at its OD and optimal schedule.

To evaluate the activity of BMS-247550 in a second species, Pat-7 was implanted into immunocompromised nude rats, and BMS-247550 was administered i.v. on a schedule of every 8 days \times 2 (Table 3). At the optimal dose of 3 mg/kg/injection, BMS-247550 was highly active, yielding >5 LCK and 4 of 6 cures. In comparison, paclitaxel produced 2.2 LCK at its optimal dose and no cures ($n = 6$).

A2780Tax Human Ovarian Carcinoma Xenograft (Mutated Tubulin). A2780Tax is a paclitaxel-resistant human ovarian carcinoma model, derived from the sensitive parent A2780 line by coincubation of cells with paclitaxel and verapamil (an MDR-reversal agent). Its resistance mechanism has been shown to be non-MDR-related and is attributed to a mutation in the gene encoding the β -tubulin protein (13).

BMS-247550 administered to mice bearing advanced-staged tumors on a schedule of every 2 days \times 5 yielded 2.5 LCK at its MTD (6.3 mg/kg/injection). In comparison, i.v. paclitaxel yielded 0.8 LCK at its MTD. BMS-247550 was significantly more active than paclitaxel in this test (Table 3).

HCT116/VM46 Human Colon Carcinoma Xenograft (Multidrug Resistant). HCT116/VM46 is an MDR-resistant colon carcinoma developed from the sensitive HCT116 parent line. Grown in nude mice, HCT116/VM46 has consistently demonstrated high resistance to paclitaxel (Table 3). In 12 consecutive studies, paclitaxel, when evaluated at its MTD, elicited low LCKs that ranged from 0–0.9 (median, 0.4 LCK).

BMS-247550 treatment of mice bearing staged HCT116/

Table 3 Preclinical antitumor activity of BMS-247550 and paclitaxel versus paclitaxel-resistant tumors

Tumor	Route, schedule	BMS-247550			Paclitaxel	
		OD ¹ (mg/kg)	LCK ^{2,3}	GD ^{3,4} (days)	LCK ^{2,4,5}	GD ^{3,4} (days)
Human tumors—in nude mice						
Pat-7	i.v. q2d×5	4.8–6.3	2.9 ± 1.4	29.9 ± 3.8	0.8 ± 0.5	9.7 ± 45
A2780Tax	i.v. q2d×5	6.3	2.5	23	0.8	7.5
HCT116/VM46	i.v. q2d×5	4.8–6.3	2.4	33	0.55	7.0
	i.v. q4d×3	16	2.0	26.8	ND ⁶	ND ⁶
Pat-21	i.v. q4d×3 (2 courses)	10	1.6	94.7	0.3	15.7
Pat-26	i.v. q4d×3	13	1.2	23.5	0.4	8.5
Human tumors—in nude rats						
Pat-7	i.v. q8d×2	3	>5 (4:6 cures)	>46	2.2 (No cures)	21.5 (No cures)
Murine tumor						
M5076	i.v. q4d×3	24	1.0	11.5	0.3	4

¹ OD, MTD.² LCK, gross LCK.³ LCKs and GDs are for OD or MTD, if inactive.⁴ GD, delay (in days) of tumor growth to specified volume (500 or 1000 mm³).⁵ LCK are for paclitaxel OD (dose ranged from 24–36 mg/kg, i.v. q2d×5), or highest dose tested, if inactive.⁶ ND, not determined.

VM46 tumors using a schedule of every 2 days × 5 produced significant antitumor effects. At its optimal dose (4.8–6.3 mg/kg/injection) in three separate studies, BMS-247550 yielded 2.1 ± 0.9 LCK. In contrast, concomitantly tested i.v. paclitaxel yielded only 0.5 ± 0.2 LCK.

Pat-21 Human Breast Carcinoma Xenograft. Pat-21 is an early-passage, Taxol[®]-resistant tumor model established from a primary tumor biopsy of a breast cancer patient with metastatic disease who was given, and failed to respond to, an experimental therapy consisting of five cycles of Taxol[®] in combination with the MDR reversal agent dexverapamil. Before Taxol[®] therapy, the patient also received chemotherapy consisting of Adriamycin, Cytosan, methotrexate, and 5-fluorouracil. Tumor biopsies were obtained after cessation of Taxol[®] therapy.

Pat-21 is a slow-growing tumor that doubles in volume approximately every 3 weeks. For antitumor efficacy evaluation, two courses of BMS-247550 or paclitaxel were administered to mice bearing Pat-21 tumors staged to ~100 mg (day 41 postimplantation). The two courses of treatment were separated by a 3-week interval. Each course consisted of three injections given every 4 days. Paclitaxel was completely inactive against this model, yielding 0.3 LCK at its MTD of 36 mg/kg/injection (Fig. 5B). In contrast, BMS-247550 was active, yielding 1.6 LCK at its optimal dose of 10 mg/kg/injection.

Pat-26 Human Pancreatic Carcinoma Model. Pat-26 is a human pancreatic carcinoma xenograft model established from a liver metastasis of a patient with metastatic pancreatic cancer. Biopsy was obtained at diagnosis and the patient had no previous therapy. Pat-26 is innately resistant to paclitaxel (Fig. 5C). Tested i.v. at its optimal schedule (every 2 days × 5), paclitaxel only produced 0.4 LCK of antitumor activity at its MTD of 36 mg/kg/injection. In contrast, BMS-247550 was active, yielding 1.2 LCK at its MTD (13 mg/kg/injection every 4 days × 3).

M5076 Murine Sarcoma Model. M5076 is a mouse fibrosarcoma that is inherently refractory to paclitaxel *in vivo*. Paclitaxel, tested i.v. on a schedule of every 2 days × 5 against unstaged s.c. implanted tumors, was inactive at its MTD of 36

mg/kg/injection, yielding 0.3 ± 0.1 LCK, in two separate experiments (Table 3). BMS-247550 administered every 4 days × 3 demonstrated improved antitumor activity, yielding 1.0 LCK at the MTD of 24 mg/kg/injection. Note that the conventional BDF1 mice used for the M5076 tumors were somewhat more tolerant to BMS-247550 than nude mice (MTDs of 24 mg/kg/injection versus 16 mg/kg/injection, respectively).

Paclitaxel-sensitive Tumor Models

A2780 Human Ovarian Carcinoma Model. A2780 is a fast-growing human ovarian carcinoma model that is highly sensitive to paclitaxel (Table 4). Nude mice bearing staged tumors were treated with BMS-247550 using the “paclitaxel-optimized schedule” of i.v. administration every 2 days for a total of 5 injections (every 2 days × 5). At the maximum tolerated dose (6.3 mg/kg/injection), BMS-247550 was highly active, yielding >4.8, 2, and 3.1 LCK in three separate experiments. Concomitantly tested i.v. paclitaxel, included in the first two studies, yielded 2 and 3.5 LCK, respectively, at its optimal dose.

HCT116 Human Colon Carcinoma Xenografts. HCT116 is a human colon carcinoma model that has been shown to be highly sensitive to paclitaxel *in vivo*. BMS-247550 administered to nude mice bearing staged (~100 mg) HCT116 tumors was highly active, producing >6.3 LCK and a large number of cures at three different treatment schedules, *i.e.*, every 2 days × 5 injections, every 4 days × 3 injections, and every 8 days × 2 injections (Table 4). However, these activities, though impressive, were comparable with but not superior to the historical results obtained for paclitaxel given at its OD and optimal schedule.

LS174T Human Colon Carcinoma Xenografts. LS174T is a human colon carcinoma model known to be sensitive to paclitaxel. BMS-247550, administered every 4 days × 3 produced 2.3 LCK at its MTD of 16 mg/kg/injection. In comparison, concomitantly tested i.v. paclitaxel yielded 2.0 LCK at its optimal regimen of 36 mg/kg/injection, administered every 2 days for 5 injections (Table 4).

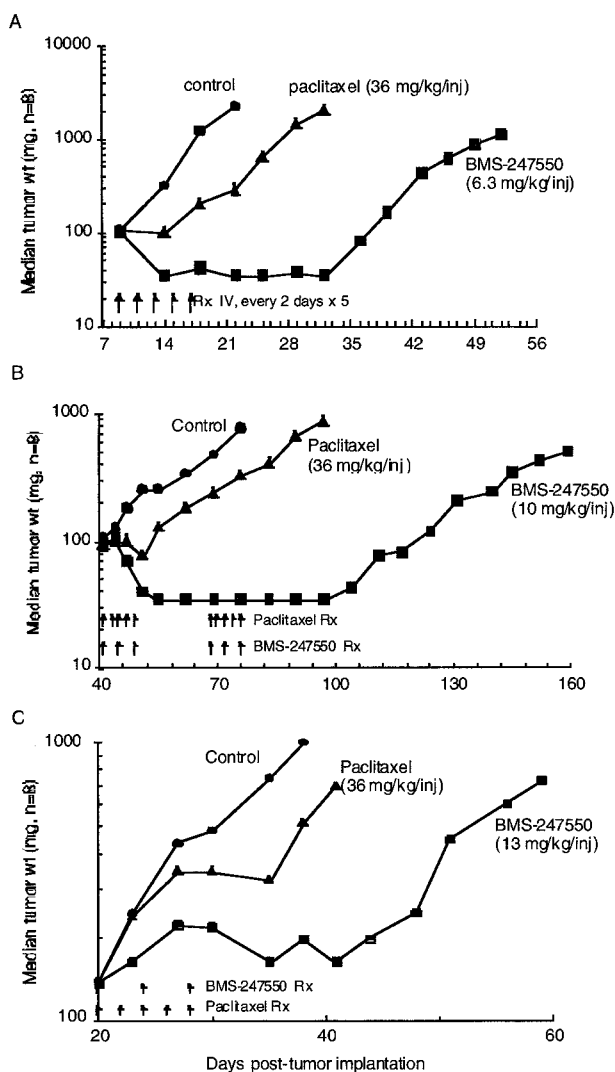


Fig. 5 Comparative antitumor activity of BMS-247550 and paclitaxel against three paclitaxel-resistant and -insensitive human cancer models. *A*, s.c. Pat-7 human ovarian carcinoma model. *B*, s.c. Pat-21 human breast carcinoma model. *C*, s.c. Pat-26 human pancreatic carcinoma model. Drugs were administered at the indicated doses represented by arrows on figures. Each datum point, the median tumor weight of eight mice.

Antitumor Activity by the Oral Route of Administration. Because BMS-247550 is more stable at neutral pHs than at low pHs, the evaluation of BMS-247550 by oral administration (p.o.) used a pH-buffering vehicle [0.25 M potassium phosphate (pH 8.0)]. Using a schedule of every 2 days \times 5, BMS-247550 was highly active p.o. against the Pat-7 human ovarian carcinoma model (Table 5). In two separate experiments p.o.-administered BMS-247550 yielded 3.1 and 2.5 LCK at its MTD (Fig. 6 and Table 5). In comparison, concomitantly tested i.v. paclitaxel produced 1.3 and 1.2 LCK, respectively, at its OD and optimal schedule. Paclitaxel is typically inactive when administered by the oral route.

In the HCT116 human colon carcinoma model, p.o.-administered BMS-247550 cured eight of eight mice when adminis-

tered at a dose of 90 mg/kg/administration every 2 days \times 5. Note that this degree of antitumor activity was equivalent to that achieved by the best concomitantly tested i.v. regimen (every 8 days \times 2; see Table 4) of this drug.

DISCUSSION

Among the various classes of anticancer agents developed in the past two decades, few have achieved the same degree of preclinical and clinical validation as the microtubule stabilizers, exemplified by agents such as Taxol[®] (14) and Taxotere (15). However, despite their impressive clinical successes, there remains considerable room for improvement, both in terms of efficacy and safety. Areas in which improvement can be made include: (a) a broader spectrum of antitumor activity to encompass those diseases not responsive to current taxanes (e.g., colon and melanoma); (b) activity in previously treated taxane-resistant tumors; (c) prolonging the duration of remission (increase survival) in taxane-responsive diseases; (d) less susceptibility to the development of drug resistance; (e) reduced toxicity; and (f) oral activity.

In recent years considerable academic and industrial research effort had been focused on the discovery of agents that act mechanistically like the taxanes but with improved biological or pharmaceutical characteristics. It is now clear that the microtubule stabilizing action of the taxanes is not unique to this chemotype. Three other new classes of natural products have recently been identified that share the same antimitotic mechanism of action as the taxanes, *i.e.*, eleutherobin (16, 17) and the structurally related sarcodictyins (18), discodermolide (19), and the epothilones (2). These agents were reported to possess potent cytotoxicity against tumor cells *in vitro*, and were shown to inhibit microtubule depolymerization and to competitively inhibit paclitaxel binding to microtubules (3, 20). These data suggest not only that these agents have a similar mechanism of action as paclitaxel, but also that they are likely bind to a common or overlapping binding site on the tubulin protein. Recent molecular modeling studies using wild-type and mutant tubulin from cell lines that had developed resistance to epothilone B and partial cross-resistance to paclitaxel lend additional support to the thesis that there is a common pharmacophore for the epothilones and the taxanes (21).

Chemically, the epothilones offer an unique advantage over the other chemotypes mentioned above; namely, the availability of a fermentation-based semisynthetic approach to analogue synthesis with an "unlimited" scale-up potential to support eventual clinical development. For this reason, after assessing the feasibility of the total synthesis approaches, we opted instead to focus our "analoging" effort on the semisynthetic route (6).

Despite the initial report of excellent *in vitro* antineoplastic activity for both epothilones A and B, additional experimentation in our laboratories revealed serious difficulties with the natural products. Both compounds lacked robust *in vivo* antitumor activity against human tumor xenografts in rodents. Subsequent studies demonstrated at least two reasons for the lack of activity *in vivo*: (a) metabolic instability in rodent plasma and liver microsomal preparations; and (b) potent toxicity to both

Table 4 Preclinical antitumor activity of BMS-247550 and paclitaxel versus paclitaxel-sensitive tumors

Tumor	Route, schedule	BMS-247550		
		OD ¹ (mg/kg)	Tumor response LCK (GD) ² (cures/total) Human tumors—in nude mice	Paclitaxel LCK (GD) ^{2,3}
A2780	i.v. q2d×5	6.3	>4.8 (>47.5)	2 (19.5)
	i.v. q2d×5	6.3	2 (17)	3.5 (35)
	i.v. q2d×5	4.8	3.1 (17.5)	ND ⁴
	i.v. q2d×5	6.3	2.4 (21.8)	ND ⁴
	i.v. q4d×3	16	>5.3 (47.5)	ND ⁴
HCT116	i.v. q2d×5	6.3	>6.3 (>69) (4:8 cures)	ND ⁴
	i.v. q4d×3	10	>6.3 (>69) (5:8 cures)	ND ⁴
	i.v. q8d×2	24	>6.3 (>69) (8:8 cures)	ND ⁴
LS174T	i.v. q4d×3	16	2.3 (25)	2.0 (22.3)

¹ OD, or MTD.² GD, delay in tumor growth (days) to target volume (500 or 1000 mm³).³ LCK are for optimal paclitaxel dose (dose ranged from 24–36 mg/kg, i.v. q2d×5) or highest dose tested, if inactive.⁴ ND, not done.

Table 5 Antitumor activity of oral BMS-247550 and i.v. paclitaxel

Tumor	Route, schedule	BMS-247550 (p.o.)		
		OD ¹ (mg/kg)	Tumor response LCK (GD) ² (cures/total)	Paclitaxel LCK (GD) ^{2,3}
Pat-7	p.o. q2d×5	60–80	3.1 (32.8)	1.3 (9.8)
HCT116	p.o. q2d×5	80	2.5 (29.3)	1.2 (13.5)
	p.o. q2d×5	90	>6.3 (>69) (7/8 cures)	ND ⁴

¹ OD, or MTD.² GD = delay in tumor growth (days) to target volume (500 or 1000 mm³).³ LCK are for optimal paclitaxel dose (dose ranged from 24–36 mg/kg, i.v. q2d×5) or highest dose tested, if inactive.⁴ ND, not done.

rodents and lower primates.³ Therefore, the primary objective of our etoposide analogue program was to identify metabolically stable analogues that retained their antineoplastic potency but with an improved safety profile. Our discovery effort culminated in the clinical development candidate BMS-247550, a lactam analogue of etoposide B. BMS-247550 was completely stable to esterase-mediated enzymatic degradation in rodent and human plasma as well as in liver microsomal preparations.

In vitro, BMS-247550 was as active as etoposide B in inducing cytotoxicity in a large panel of cancer cell lines (Fig. 2); it was equally potent in a microtubule stabilization assay (Table 1), and as effective as etoposide B in its ability to arrest proliferating tumor cells in mitosis (Fig. 4). In addition, like etoposide B, BMS-247550 retains its antineoplastic activity in cancer cells that have developed resistance to paclitaxel, whether through overexpression of the MDR P-glycoprotein or because of tubulin mutation (Figs. 2 and 3).

In vivo, BMS-247550 has clearly demonstrated antitumor activity that is superior to paclitaxel in both paclitaxel-resistant and -sensitive tumors. BMS-247550 was more efficacious than paclitaxel in all five paclitaxel-resistant tumors evaluated in this study (four human and one murine): *i.e.*, the clinically derived

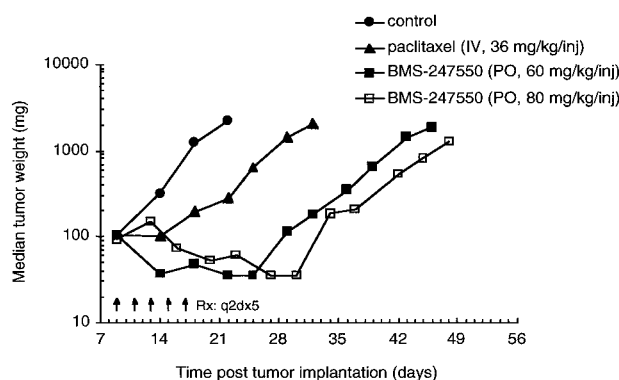


Fig. 6 Comparative antitumor activity of oral BMS-247550 and i.v. paclitaxel in the Pat-7 human ovarian carcinoma model. Compound was administered at the indicated doses every other day for a total of five administrations starting 8 days after tumor implantation (q2dx5). Each datum point, the median tumor weight of eight mice.

paclitaxel resistant Pat-7 ovarian carcinoma, the A2780Tax ovarian carcinoma that is resistant to paclitaxel because of tubulin mutations, the HCT116/VM46 MDR colon carcinoma, the clinically derived paclitaxel-resistant Pat-21 breast carcinoma, and the murine fibrosarcoma M5076. Against three paclitaxel-sensitive human tumor xenografts, BMS-247550 pro-

³ Bristol-Myers Squibb, unpublished results.

duced antitumor activity equivalent to paclitaxel: *i.e.*, A2780 human ovarian carcinoma, HCT116, and LS174T human colon carcinoma.

An additional advantage of BMS-247550 over the prototypical taxanes is its efficacy by oral administration, producing antitumor activity when given *p.o.*, that is equivalent to that produced by *i.v.* drug administration in two different human tumor xenografts (Fig. 6). The availability of an oral dosage form of BMS-247550 may be beneficial to patient care because of its lower cost and convenience, inasmuch as it may permit the patients to remain at home while taking their medications and avoid the necessity for venous access. In addition, with the recent demonstration of the utility of a metronomic (low dose, chronic) dosing regimen that selectively targets endothelial cells (22), the availability of an oral formulation will greatly improve the feasibility and cost effectiveness of such a dosing regimen.

In conclusion, the novel epothilone lactam analogue BMS-247550 demonstrated remarkable antitumor efficacy in a variety of paclitaxel-sensitive and -refractory preclinical cancer models. Given its broad preclinical activity spectrum and the ability to overcome multiple forms of paclitaxel-resistance, we look forward to the expeditious evaluation of this compound in clinical trials.

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Clinical Cancer Research

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