Epothilone B and its Analogs – A New Family of Anticancer Agents

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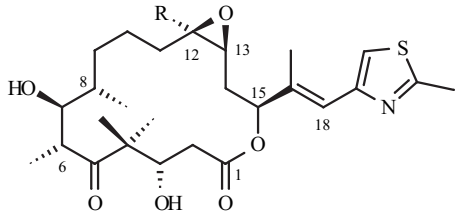
Abstract: Epothilones are naturally occurring 16-membered macrolides with the ability to promote tubulin polymerization *in vitro* and to stabilize preformed microtubules against Ca^{2+}- or cold-induced depolymerization. In contrast to paclitaxel (Taxol®) epothilones are also active *in vitro* against multidrug-resistant cancer cell lines as well as cell lines whose paclitaxel-resistance is derived from specific β-tubulin mutations. Based on their attractive *in vitro* biological profile epothilones have turned into important lead structures in anticancer drug discovery and hundreds of analogs and derivatives of epothilone A and B have been prepared and biologically characterized over the past four years. A number of compounds, including natural epothilone B, deoxyepothilone B, and epothilone B lactam (BMS-247550) have also been reported to exhibit profound *in vivo* antitumor activity in animal models. Apart from providing a brief summary of the SAR that has emerged from the above *in vitro* studies, this minireview will largely focus on the biology and chemistry of those analogs for which *in vivo* antitumor activity has been reported in the literature. Two of these compounds, natural epothilone B and epothilone B lactam (BMS-247550) have advanced to clinical studies in humans.

I. INTRODUCTION

Cytotoxic agents have a long-standing history as anticancer drugs [1]. The antitumor activity of most of these compounds is based on the inhibition of cell proliferation and consequent induction of cell death by apoptosis. At the molecular level, a variety of different targets are involved in the antiproliferative action of different classes of cytotoxic agents [1]. For a prominent subgroup of anticancer drugs, including members of the taxane family such as paclitaxel (Taxol®) or docetaxel (Taxotere®), cytotoxic activity is based on their interference with microtubule functionality [2].

Microtubules are vital to the performance of many critical cellular functions, particularly mitosis, and also play an important role in the maintenance of cell shape and intracellular transport. They are present within the cell in dynamic equilibrium with tubulin heterodimers and many of their unique functional properties are the result of their ability to polymerize and depolymerize in response to critical physiological messages in the cell, including those related to cell cycle progression [3]. Both paclitaxel and docetaxel preferentially bind to and stabilize microtubules [3,4], thereby shifting the dynamic equilibrium between tubulin dimers and microtubules towards polymerization.

Paclitaxel and its analogs for more than a decade were the only microtubule depolymerization inhibitors known in the literature [5] until in 1993 a second class of cytotoxic natural products was discovered by Reichenbach and Höfle in Germany [6], which were later demonstrated by a group at Merck Research Laboratories to possess a paclitaxel-like mechanism of action [7]. Based on their molecular structure these compounds, which are produced as secondary metabolites by various types of myxobacteria, have been named "Epothilones" by Reichenbach and Höfle [6b]. The major products isolated from fermentations of the myxobacterium *Sorangium cellulosum Sc 90* are epothilone A and epothilone B, which differ by the absence or presence of a methyl group at the trisubstituted epoxide moiety (Fig. **1**); however, numerous related structures have been isolated as minor components from fermentations of myxobacteria since the original discovery of epothilones [8]. The relative and absolute stereochemistry of epothilone B was established by a combination of X-ray crystallography and chemical degradation studies and correlation of the degradation products with structures of known stereochemistry [9].



R = H: Epothilone A
R = CH_3: Epothilone B

Fig. (1).

In contrast to paclitaxel (Taxol®) epothilones are potent growth inhibitors of multidrug-resistant cancer cell lines [7,10,11] and they have been shown to be active *in vitro* against cell lines whose paclitaxel-resistance is derived from specific tubulin mutations [12]. Epothilones have also been quoted as being significantly more water-soluble than paclitaxel [9]. This should allow the use of formulation vehicles less problematic than Cremophor, which in the case

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of Taxol® is believed to contribute to the drug's clinical side-effects [13]. The very attractive *in vitro* profile of epothilones in combination with their *relatively* simple structures (at least in comparison with paclitaxel) has led to widespread interest in these natural products throughout the scientific community. Since the first disclosure of their absolute stereochemistry in 1996 [9], several total syntheses of epothilones have been published in the literature [for reviews *cf.* [14-17]) and the methodology developed in the course of those studies has also been exploited for the synthesis of a host of analogs [14,15]. Unlike the situation with paclitaxel, where a practical total synthesis was (and still is) clearly out of reach, hundreds of analogs of epothilones have been generated by chemical synthesis on a scale sufficient for extensive *in vitro* profiling and SAR studies (*vide infra*). In addition, even the large-scale production of such compounds for clinical studies is likely to be feasible [15,18].

The chemistry and biology of epothilones have been extensively discussed in recent review articles [11,14,15,19] and it is not the intent of this minireview to simply repeat these discussions under a different label. Thus, this article will only provide a *general* outline of the *in vitro* SAR features of epothilones as they have emerged from extensive studies in academic as well as industrial research laboratories. The major focus of the discussion, however, will be on those analogs for which *in vivo* antitumor data are available in the literature, which allows a more meaningful discussion of their therapeutic potential than simple *in vitro* antiproliferative activity.

II. EPOTHILONE SAR – A GENERAL OUTLINE

As indicated above, the chemistry of epothilones has been extensively explored, resulting in hundreds of analogs and a wealth of SAR information being generated for this class of natural products over the last four years. Most of the synthetic analogs have originated from the groups of Danishefsky (*cf*., e.g., [15]) and Nicoloau (*cf*., e.g., [14]) and to a lesser extent also the groups at Novartis [20,21] and Schering AG [22], while semisynthetic work has been primarily reported by the groups at the "Gesellschaft für Biotechnologische Forschung" in Braunschweig, Germany (GBF; *cf*., e.g., [23,24]) and Bristol Myers Squibb (BMS; *cf*., e.g., [25]). The most pertinent features of the epothilone SAR that have emerged from this research can be summarized as follows (Fig. **2**):

(i) The presence of a C12-C13 epoxide moiety is not an absolute requirement for efficient microtubule stabilization and potent antiproliferative activity, which decreases only about 10 - 30-fold upon reduction of the epoxide moiety to a *cis* olefin ("Deoxyepothilones"; (Fig. **2A**, R = H, CH_3) [26-30]. Likewise, the replacement of the oxirane ring by a cyclopropane [31-33] or variously N-substituted aziridine [34] moieties is generally well tolerated or can even lead to enhanced potency.

(ii) Modification of the 26-methyl group in epothilone B through the replacement of one hydrogen by relatively small and apolar substituents such as F, Cl, CH_3, or C_2H_5 (Fig. **2B**, n = 1, X = CH_2F, CH_2Cl, C_2H_5, n-C_3H_7, Y = CH_3, Z = S), produces analogs which are only slightly less potent *in vitro* than epothilone B itself [28,35].

(iii) Ring contraction or expansion *via* the removal of existing or the incorporation of additional CH_2-groups in the C9-C11 region (Fig. **2B**, n = 0, 2, 3, X = H, Y = CH_3, Z = S) both cause a substantial loss in biological potency [28,36].

(iv) Removal of the C8 methyl group [37], simultaneous inversion of stereochemistry at C7 and C8 [30] as well as inversion of stereochemistry at C3 [30] all lead to reduced biological activity.

(v) The presence of the allylic methyl group at C-16 is not required for potent biological activity and 16-desmethylepothilone B (Fig. **2B**, n = 1, X= CH_3, Y = H, Z = S) is virtually equipotent with epothilone B (*cf*. [38]). Likewise, the replacement of the thiazole ring either by an oxazole (Fig. **2B**, n = 1, X = CH_3, Y = CH_3, Z = O) [28,30] or various pyridine moieties [22,39] is well tolerated. Epothilone (A or B)-like activity in these heterocycle-modified analogs requires the presence of an aromatic nitrogen atom *ortho* to the attachment point of the linker between the heterocycle and the macrocyclic skeleton.

(vi) The ester group in epothilones can be replaced by a secondary lactam moiety with only a *ca*. 10-fold reduction in *in vitro* potency [40,41].

In terms of structure-based design of epothilone analogs, it should be noted that no structural data on either tubulin alone or a tubulin/microtubule-epothilone complex are available at the atomic level. The electron-crystallographic structure of a complex between two-dimensional tubulin-polymer sheets and docetaxel has been reported recently, but the level of resolution does not exceed 3.7 Å [42]. In the

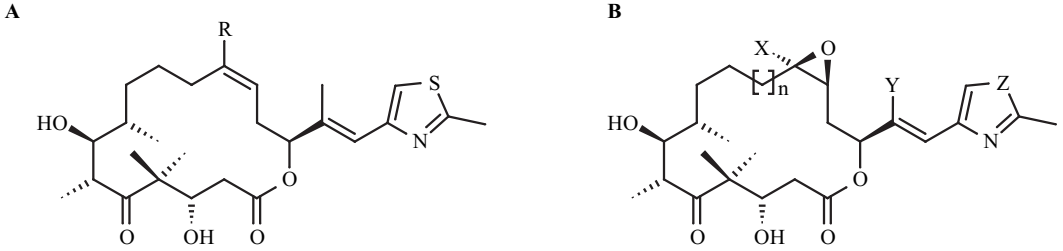


Fig. (2).

absence of precise structural information, different pharmacophore models for epothilones have been advanced recently [43,44,45]. One of these models is based on an energy-refined model of the 3.7 Å density map of docetaxel bound to β-tubulin in conjunction with a limited set of SAR data for epothilone-resistant cell lines harboring specific β-tubulin mutations [43]. While all of these pharmacophore models claim to adequately accommodate the published SAR for epothilones, the postulated bioactive conformations are significantly different from each other and no successful application of either model for the design of potent new analogs has been reported so far.

III. EPOTHILONE B

Epothilone B is able to induce polymerization of tubulin dimers into microtubules *in vitro* and to stabilize preformed microtubules against cold- or Ca^{2+}-induced depolymerization [7,10]. It is a competitive inhibitor of paclitaxel binding to tubulin polymer and is able to displace [^{3}H]-paclitaxel from microtubules with an efficiency similar or superior to that of unlabelled paclitaxel or docetaxel (K_i = 0.71 μM) [7,10]. These findings strongly suggest that the microtubule binding sites of paclitaxel and epothilone B are largely overlapping or even identical. Exposure of human cells to epothilone B leads to aberrant spindle formation during mitosis, mitotic arrest at the G2/M transition and eventually apoptotic cell death [7,10,11]. EC50s for induction of mitotic arrest correlate well with those for cytotoxicity (cell death) [7].

The antiproliferative activity of epothilone B against a series of drug-sensitive cancer cell lines *in vitro* is summarized in Table 1. The data demonstrate that the compound is a potent inhibitor of cell growth in a variety of human cancer cell lines, including those derived from all major types of solid human tumors. Epothilone B generally exhibits higher potency than paclitaxel (3 - 20-fold). As indicated above, epothilone B, in contrast to paclitaxel, is equally cytotoxic to drug-sensitive and multidrug-resistant cells overexpressing the P-glycoprotein efflux pump [7,10,11]. This is illustrated by the data summarized in Table 2, which compares the antiproliferative activity of both compounds against the human epidermoid carcinoma cell line KB-31 and a P-glycoprotein overexpresseing subline thereof, KB-8511. Epothilone B also retains activity against a cell line where resistance to palitaxel is mediated by a mutation in the β-tubulin gene (1A9PTX-10; Table 2).

Table 1. IC50-Values [nM] for Growth Inhibition of Human Carcinoma Cell Lines by Epothilone B in Comparison to Paclitaxel. (Data from [11] and M. Wartmann, Unpublished)

Cell Line	Epothilone B	Paclitaxel
A549 (Lung)	0.19	3.75
T-24 (Bladder)	0.25	4.40
ZR-75-1 (Breast)	0.64	3.60
MCF-7 (Breast)	0.42	2.53
BT-20 (Breast)	0.13	1.83
MDA-MB-231 (Breast)	0.12	0.59
Du145 (prostate)	0.8	4.3
PC-3M (prostate)	3.8	6.7
HCT-116 (colon)	0.42	1.96
A-431 (Epidermoid)	0.26	1.66

The *in vivo* effects of epothilone B have been investigated in some detail by a group at the Sloan-Kettering Cancer Center. In a first set of studies [28] the activity of the compound was characterized in drug-sensitive as well as multidrug-resistant xenograft models of human leukemia (CCRF-CEM and CCRF-CEM/VBL (MDR)) in CB-SCID mice. Administration was intraperitoneal (i.p.) or intravenous (i.v.) and significant growth inhibition (60% - 86%) was observed in both models for dosing regimens of 0.7 mg/kg/day (x 4), or 1.5 or 3 mg/kg/week (x 3), although the weekly dosing regimens in some cases were also associated with mortalities. In another set of experiments epothilone B when given i.p. to non-tumor bearing nude mice daily at 0.6 mg/kg for 4 administrations surprisingly was found to be lethal to all animals treated [46]. Toxicity was also observed in efficacy experiments based on a q2d (i.e. every other day) treatment schedule (0.3 mg/kg or 0.6 mg/kg) and only limited effects on tumor growth were observed against human MX-1 breast or SKOV-3 ovarian tumors in nude mouse xenograft models. These data have led to the conclusion that epothilone B might simply be too toxic to become a clinically useful anticancer agent [46].

In contrast to these findings studies in our own laboratory have clearly demonstrated that epothilone B possesses potent antitumor activity in a number of drug-sensitive human tumor models (nude mice) when

Table 2. Antiproliferative Effect of Epothilone B on Pairs of Drug-Sensitive/Drug-Resistant Human Carcinoma Cell Lines (IC50-Values [nM]). Values in Parentheses Indicate Relative Resistance, i. e., IC50 (Resistant Line)/IC50 (Parental Line). (Data fom [11] and M. Wartmann, Unpublished)

Cell line		Epothilone B	Paclitaxel
KB-31	(Epidermoid, parental)	0.71	2.9
KB-8511	(Epidermoid, MDR)[a]	0.89 (1.25)	994 (343)
1A9	(Ovarian, parental)	3.54	5.11
1A9PTX-10	(Ovarian, tubulin mutation)[b]	18.4 (5.20)	82.5 (16.1)

[a]P-glycoprotein overexpressing. [b]Phe270Val mutation in β-tubulin [12].

administered i. v. either at a single dose of 4 - 6 mg/kg or weekly at 3 - 4 mg/kg (2 - 3 administrations) [11]. Activity was observed for models encompassing all four major types of solid human tumors (lung, breast, colon, prostate) and was manifest either as profound growth inhibition (stable disease) or significant tumor regression. In addition, epothilone B was found to be a potent inhibitor of tumor growth in P-gp-mediated multidrug-resistant human tumor models. Regressions were observed in two such models (KB-8511 (epidermoid carcinoma) and HCT-15 (colon carcinoma)), where tumors were either poorly responsive or completely non-responsive to treatment with Taxol®. Treatment with epothilone B in these experiments was associated with body weight loss of 10 - 20% at nadir and occasionally also mortalities, indicating a relatively narrow therapeutic window. However, in general, therapeutic effects could be achieved at tolerated dose levels and recovery of body weight occurred after termination of treatment.

When trying to reconcile our results with those obtained by the Sloan-Kettering group it should be noted that there is no basis for a meaningful comparison, as experimental conditions deviate substantially between our two groups with regard to tumor models, formulation, and/or dosing regimens. The results of our preclinical evaluation of (fermentatively produced) epothilone B finally resulted in the initiation of clinical trials with the compound [11] and these trials are currently ongoing. No *in vivo* data have been published for epothilone A.

IV. EPOTHILONE B LACTAM – BMS-247550

The replacement of the lactone oxygen by nitrogen has emerged as one of the most important strategies in epothilone-based anticancer drug discovery and has led to the identification of the development compound BMS-247550, the lactam analog of epothilone B (Fig. **3A**) [25,40]. Lactam-based analogs of epothilones were conceived as metabolically more stable alternatives to the lactone-based natural products [25,40], which exhibit limited metabolic stability in rodent plasma. This is in line with our own findings in mice and rats, but it should be noted that in spite of its short plasma half-life epothilone B shows potent antitumor activity in a variety of nude mouse human tumor models and the same is true for deoxyepothilone B (*vide infra*). In addition, deoxyepothilone B has been demonstrated to be significantly more stable in human than in rodent plasma [47]. This is in line with our own studies on epothilone B and clearly is a result of the well known difference in plasma esterase activity between humans and rats or mice.

BMS-247550 is a potent inducer of tubulin polymerization, but its antiproliferative activity is *ca*. 10-fold lower than that of epothilone B (Table 3). What is more striking, however, is the activity differential between the drug sensitive KB-31 cell line and its P-gp overexpressing multidrug-resistant KB-8511 variant, which clearly indicates that BMS-247550 is a substrate for the P-gp efflux pump.

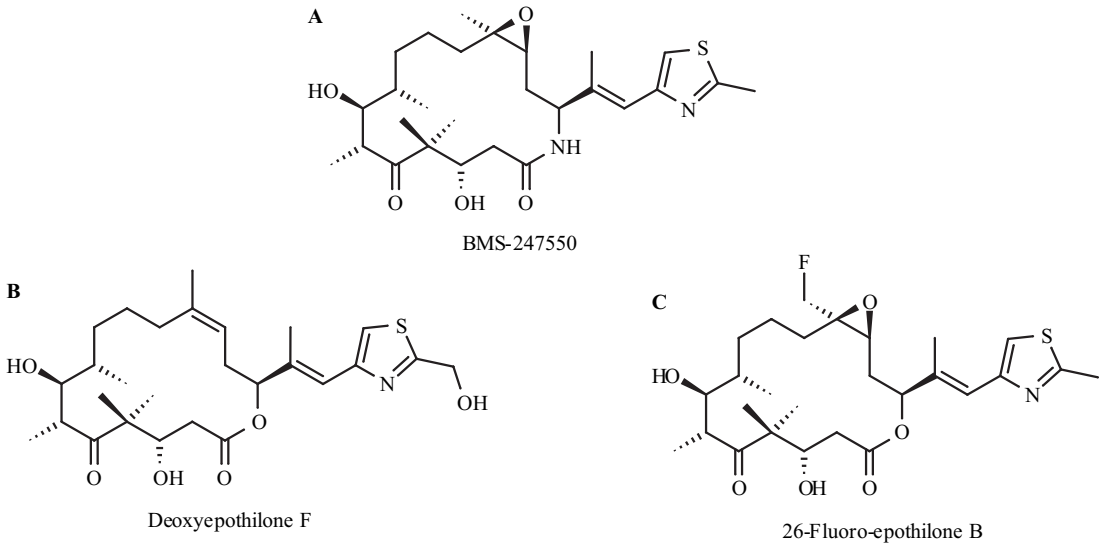
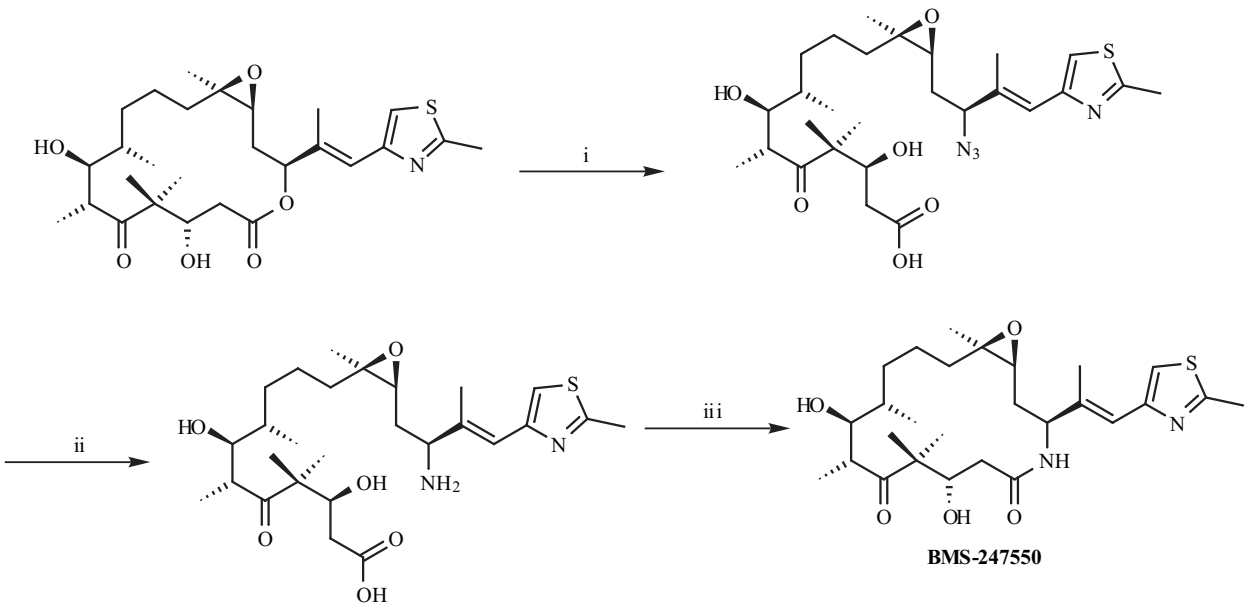


Fig. (3).

Table 3. Induction of Tubulin Polymerization and Inhibition of Human Cancer Cell Growth by Epothilone B Lactam BMS-247550

Compound	% Tubulin Polymerization[a]	Growth Inhibition (IC50 [nM]) Cell Line			
		KB-31[b]	KB-8511[b]	HCT-116[c]	CCRF-CEM[d]
Epo B	84	0.19	0.19	0.42	0.35[e]
BMS-247550	89[b]	2.85	128	3.6	2.1

[a]Induction of polymerization of porcine brain microtubule protein (tubulin with microtubule-associated proteins (MAPs)) by 2 μM of test compound relative to the effect of 25 μM of epothilone B, which gave maximal polymerization (M. Wartmann, unpublished). [b]M. Wartmann, unpublished. [c]Ref. [40]. [d]Ref. [48]. [e]Ref. [46].



Scheme 1. [40]: i: $Pd(Ph_3)_4$, NaN_3, 45°, 60-70%. ii: Me_3P, 71%. iii: EDCI, HOBt, 65%

Similar observations have been made by Stachel *et al.* [48], who have reported resistance factors of 1423 and 81.4 for BMS-247550 in the drug-sensitive/multidrug-resistant cell lines pairs CCRF-CEM / CCRF-CEM/VBL_{100} and CCRF-CEM / CCRF-CEM/Taxol, respectively (*vs*. resistance factors of 6.1 and 3.1, respectively, for epothilone B). Recent data published by Lee and co-workers at BMS [49] suggest that BMS-247550 has antitumor activities similar to that of paclitaxel in Taxol®-sensitive tumor models (i.e. A2780 human ovarian carcinoma, HCT116 and LS174T human colon carcinomas) when each drug is given at its optimal dose. BMS-247550 was shown to be superior to paclitaxel in Taxol®-resistant tumor models (i.e. Pat-7 and A2780Tax human ovarian carcinomas, Pat-21 human breast carcinoma, Pat-26 human pancreatic carcinoma, M5076 murine sarcoma). Furthermore, the compound showed remarkable antitumor activity against Pat-7 ovarian and HCT-116 colon carcinoma xenografts following oral administration [49].

BMS-247550 can be produced by semi-synthesis from epothilone B in a higly effective three-step sequence developed by the BMS group [40] (Scheme 1). This involves Pd(0)-catalyzed lactone opening and concomitant introduction of an azide group at C-15 (which occurs with complete retention of configuration), reduction of the azide to an amino group, and finally EDCI/HOBt-mediated macrolactamization. All three steps can also be carried out in a single reactor in 20-25% yield.

V. DEOXYEPOTHILONE B AND DEOXYEPOTHILONE F

One of the most intriguing features of the epothilone SAR consists in the fact that the 12,13-epoxide moiety of epothilones A and B is not essential for tubulin/microtubule-related effects *in vitro*, with the corresponding deoxy analogs (epothilones C and D; Fig. **2A**; R = H and CH_3, respectively) being equally potent inducers of tubulin polymerization as epothilones A and B, respectively [26 – 30]. In contrast, at the cellular level removal of the epoxide oxygen does cause a significant decrease (*ca*. 10 - 30-fold) in antiproliferative activity [26 – 30] (Table 4). The underlying reasons for this discrepancy have not been elucidated, but it is worth emphasizing that the ability of epothilone analogs to affect tubulin polymerization in a test tube does not always directly correlate with antiproliferative activity at the cellular level and we have observed this phenomenon for a variety of analogs other than deoxyepothilones A or B.

It should also be noted that the activity differential between epothilone A and B is retained in the deoxy

Table 4. Growth Inhibition of Human Cancer Cell Lines by Deoxyepothilones A and B in Comparison with Epothilones A and B and Paclitaxel (IC50s [nM])

Cell line	Paclitaxel	Epo A	Epo B	Deoxyepo A	Deoxyepo B
KB-31[a]	12	2	1.2	40	24
HCT-116[b]	2.3	3.2	0.42	64	6.5
CCRF-CEM[c]	2.1	2.7	0.35	22	9.5
KB-31[d]	2.3	2.1	0.19	25	2.7

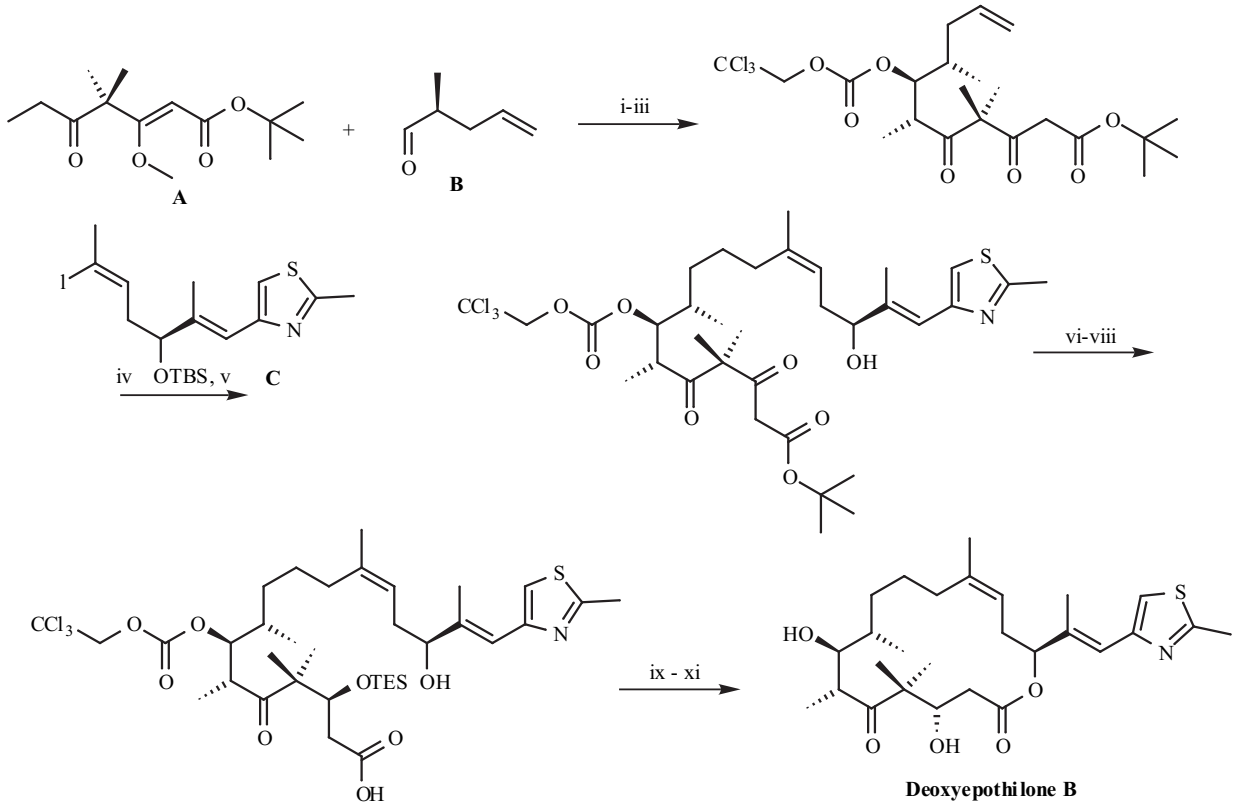
[a]Data from [50]. [b]Ref. [40]. [c]Ref. [46]. [d]Ref. [11] and M. Wartmann, unpublished data for compounds prepared by Prof. K. C. Nicolaou.

variants, and as a general rule, with all other structural features being equal, analogs with a methyl group on C-12 are usually more potent than their unsubstituted counterparts. As for epothilones A and B the corresponding deoxy variants are equally effective growth inhibitors of drug-sensitive and multidrug-resistant cells, which is in marked contrast to epothilone B lactam BMS-247550 (*vide supra*).

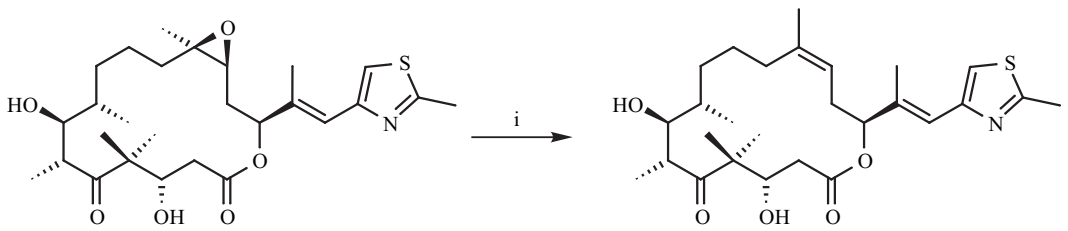
Deoxyepothilone B has been extensively characterized *in vivo* by the group at the Sloan-Kettering Cancer Center. In a first series of experiments the compound was shown to be more efficacious and less toxic than epothilone B or paclitaxel when all agents were administered i.p. to nude mice bearing human mammary carcinoma MX-1 xenografts [46]. Efficacy could also be demonstrated for the i.v. route of administration when employing a specifically optimized dosing regimen (30 mg/kg, 6 h infusion, q2d x 5) [51]. Under these particular conditions the toxicity and efficacy of deoxyepothilone B was comparable to that of paclitaxel when tested against MX-1 breast carcinoma and HT-29 colon xenograft tumors, while being far superior to paclitaxel when tested against two multidrug-resistant models, MCF-7/Adr and CCRF-CEM/paclitaxel. Therapeutic effects ranged from tumor stasis to complete tumor regressions at the end of the treatment period. This is in spite of the fact that deoxyepothilone B exhibits a very short half life in rodent plasma, similar to what has been reported for the parent compound epothilone B (*vide supra*) [40]. In human plasma the compound is significantly more stable (*in vitro*) [47], thus indicating that plasma stability is unlikely to be limiting for therapeutic applications of deoxyepothilone B (and also epothilone B) in humans.

More recently the Sloan-Kettering group has reported *in vivo* data for deoxyepothilone F (Fig. **3B**), which was found to exhibit comparable efficacy as deoxyepothilone B [48,52]. However, the compound is significantly more water-soluble than deoxyepothilone B, which could make it a more attractive drug development candidate. Employing a 6 h continuous i.v. infusion regimen for both compounds, deoxyepothilone F was found to have significantly superior antitumor effects over BMS-247550 in a CCRF-CEM as well as a MX-1 tumor model [47,48]. However, it should be kept in mind that while the 6 h continuous infusion schedule may be optimal for deoxyepothilones B and F, this must not be the case for BMS-247550, thus rendering the interpretation of these comparisons problematic.

The material employed by the Sloan-Kettering group in their profiling of deoxyepothilones B and F was produced by total synthesis (for recent examples *cf*. [18,53,54], for a review *cf*. [15]). Several syntheses of deoxyepothilone B have been reported in the literature as the penultimate intermediate on the way to epothilone B, but only Danishefsky`s group at Sloan-Kettering has embarked on the extensive optimization of the synthesis of this compound [18,53,54]. The chemistry developed by Danishefsky and co-workers in this process could well be suitable for the production of material on large scale. The most advanced

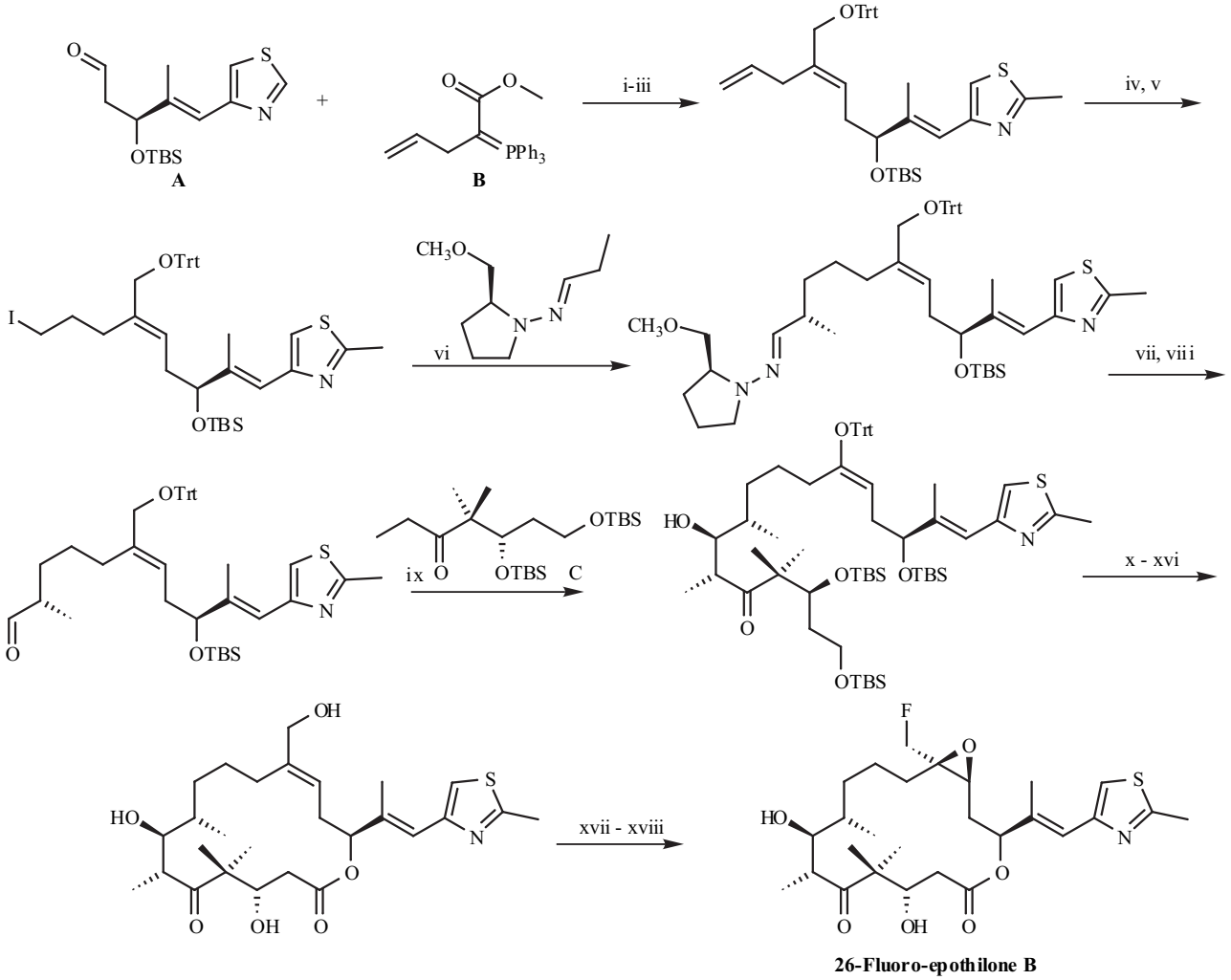


Scheme 2. [54]: i: LDA, -120°, 50-60%. ii: Troc-Cl, pyridine, 0°. iii: *p*-TSA, acetone, 87% (2 steps). iv: 9-BBN; Cs_2CO_3, $Pd(dppf)_2Cl_2$, Ph_3As, DMF, H_2O, *ca*. 75%. v: 0.5 M HCl/MeOH, 85%. vi: $[RuCl_2((R)\text{-BINAP})_2,Et_3N]$, H_2, 1200 psi, MeOH, HCl, 82-88% (> 95% de). vii: TESOTf, 2,6-lutidine, -78°→RT. viii: 0.1 N HCl/MeOH, 70-77% (2 steps). ix: 2,4,6-trichlorobenzoylchloride, Et_3N, DMAP, 78%. x: SmI_2, cat. NiI_2, -78°, 90-95%. xi: HF x pyridine, 0°, 98%.



Scheme 3. [31]: i: WCl_6, n-BuLi, 78%.

version of their synthesis of deoxyepothilone B is summarized in Scheme 2 [53,54]. The key steps in the synthesis are the diastereoselective aldol reaction between aldehyde **B** and ethyl ketone **A** (de ≈ 5.5/1), the B-alkyl-Suzuki coupling between the aldol product derived from **A** and **B** with **C**, the highly selective reduction of the keto group on C3 using a Noyori catalyst, and finally macrolactonization under Yamaguchi conditions. The synthesis comprises between 23 and 25 total steps from readily (although not necessarily commercially) available starting materials with 13 steps for the longest linear sequence. The evaluation of an alternative strategy which employs a highly selective aldol reaction to establish the chiral center at C3 late in the synthesis has recently been reported [55]. In addition, an improved synthesis for the critical vinyl iodide **C** has recently been reported by Chappell *et al.*, which in combination with the chemistry summarized in Scheme 2 (or ref. [55]) is believed to provide the basis for the production of deoxyepothilone B on a scale that will support clinical trials with this compound [18].



Scheme 4. [63]: i: Benzene, refl., 95%. ii: DIBAL, -78°, 98%. iii: Trt-Cl, DMAP, 70°, 99%. iv: a. 9-BBN, 0°; b. NaOH, H_2O_2, 94%. v. I_2, imidazole, Ph_3P, 0°, 90%. vi: a. LDA, 0°, 14h; b. iodide in THF, -100° → -20°, 66%. vii: Monoperoxyphthalic acid magnesium salt, 0°, 91%. viii: DIBAL, -78°, 97%. ix: a. LDA, 0°; b. aldehyde, -78°, 85% (3:1 mixture of diastereoisomers). x: TBSOTf, 2,6-lutidine, 92%. xi: HF x pyridine, 0° → 25°, 74%. xii: $(COCl)_2$, DMSO, Et_3N, -78° → 0°, 99%. xiii: $NaOCl_2$, 2-methyl-2-butene, 25°, 99%. xiv: TBAF, 25°, 89%. xv: 2,4,6-trichlorobenzoylchloride, Et_3N, 0°, then addition of DMAP, 75°, 75%. xvi: HF x pyridine, 0° → 25°, 78%. xvii: (+)-Diethyltartrate, Ti(*i*-OPr)$_4$, t-BuOOH, -30°, 76% (d.e. > 95%). xviii: DAST, -78°, 65%.

It should be noted that deoxyepothilone B, at least on small scale, can also be accessed in excellent yield from natural epothilone B (produced by fermentation) in a one-step process developed by the BMS group [31] (Scheme 3).

Apart from these synthetic and semi-synthetic approaches a third alternative for the production of deoxyepothilone B consists in the cloning and heterologous expression of the gene cluster of the polyketide synthase for epothilones. The DNA sequence for this gene cluster has recently been elucidated by different laboratories [56-59] and the small scale expression of deoxyepothilone B in actinomycetes (which are not the natural host or producing organism for epothilones, but easier to handle than myxobacteria) has been accomplished [60]. Whether this approach will indeed prove to be effective at a practical level remains to bee seen.

VI. 26-FLUORO-EPOTHILONE B

In vivo data have recently been reported by Newman and co-workers for a 26-modified analog of epothilone B, 26-fluoro-epothilone B (Fig. **3C**) [61]. This compound exhibits *in vitro* antiproliferative activity which is equivalent to that of epothilone B [35] and in a human prostate xenograft model in nude mice was found to have significantly better antitumor activity than paclitaxel when administered at equitoxic doses [61]. No comparison with epothilone B was included in this work, but data from our own laboratory indicate that the *in vivo* profile of 26-fluoro-epothilone B is similar to that of epothilone B itself [62]. However, more extensive studies will be required in order to determine whether a difference may exist between these two compounds with regard to their therapeutic potential.

26-Fluoro-epothilone B is a fully synthetic analog of epothilone B and the synthesis of this compound is summarized in Scheme 4 [63]. Starting from thioacetamide (→ **A**), 4-bromo-1-butene (→ **B**), and isobutyraldehyde (→ **C**) the synthesis comprises a total of 34 steps with 24 steps for the longest linear sequence. Although Nicolaou *et al*. have recently reported an improved route to the hydroxymethyl precursor of 26-fluoro-epothilone B [64], among the compounds discussed in this article this analog is probably the most difficult one to access.

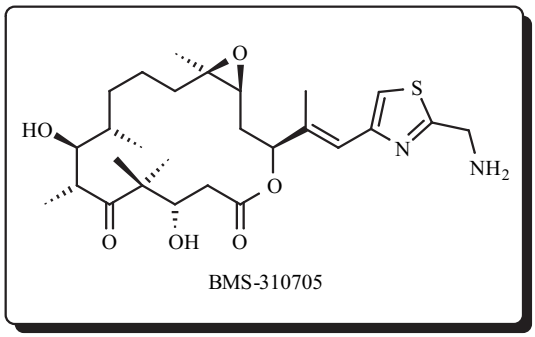
VII. CONCLUSIONS

Epothilones A and B represent a new class of microtubule depolymerization inhibitors, which at least *in vitro* are not subject to the same limitations with regard to P-gp-mediated drug resistance as paclitaxel (Taxol®). Epothilone research so far has produced two clinical development compounds (epothilone B, which is in Phase II trials by Novartis and BMS-247550 which is undergoing Phase II/III trials) and one pre-clinical candidate which appears likely to enter clinical trials in the near future (deoxyepothilone B (epothilone D)). All three compounds are structurally similar and they all are virtually equipotent inducers of tubulin polymerization *in vitro*. However, deoxyepothilone B as well as BMS-247550 exhibit significantly lower antiproliferative activity than epothilone B. Furthermore, distinct differences exist between epothilone B and deoxyepothilone B on one hand and BMS-247550 on the other with regard to their activity against P-gp-overexpressing multidrug-resistant cell lines, where BMS-247550 shows significantly reduced activity (relative to its effect on drug-sensitive lines). In spite of these findings, all three compounds have been shown to exhibit potent *in vivo* antitumor activity in animal models, including multidrug-resistant tumor models that are characterized by elevated P-gp levels. The efficacious dosing regimens employed in these *in vivo* experiments differ significantly between epothilone B, BMS-247550, and deoxyepothilone B and had to be specifically optimized for each compound.

Although it is still too early to judge the true clinical potential of any epothilone-based anticancer agent, the available preclincical and clinical data for the most advanced compounds of this class are highly encouraging. Epothilone-derived anticancer drugs may thus become part of the therapeutic armamentarium in the fight against cancer in the not too far future.

NOTE ADDED IN PROOF

Since the completion of this manuscript at the end of 2001 additional developments have occurred in the epothilone field which could not be captured in this review article. Most importantly, clinical trials have been initiated with deoxyepothilone B (Epo D; Sloan-Kettering/Kosan /Roche) and also with C21-amino epothilone B (BMS-310705; BMS: Vite, G.; Höfle, G.; Bifano, M.; Fairchild, C.; Glaser, N.; Johnston, K.; Kamath, A.; Kim, S.-H.; Leavitt, K.; Lee, F.-Y.; Leibold, T.; Long, B.; Peterson, R.; Raghavan, K.; Reguerio-Ren, A. Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, April 7-11, 2002).



BMS-310705

In addition, significant progress has been made with regard to the heterologous expression of epothilones in microorganisms other then myxobacteria, in particular for deoxyepothilone B (epothilone D; Arslanian, R. L.; Parker, C. D.; Wang, P. K.; McIntire, J. R.; Lau, J.; Starks, C.; Licari, P. J. *J. Nat. Prod.* **2002**, *65*, 570-572).

As regards the differences in *in vitro* antiproliferative activity between epothilone B and deoxyepothilone B (in spite of their comparable effects on tubulin polymerization) a recent study by the Schering group suggests that this a

consequence of less pronounced intracellular accumulation of deoxyepohtilone B (Lichtner, R. B.; Rotgeri, A.; Bunte, T.; Buchmann, B.; Hoffmann, J.; Schwede, W.; Skuballa, W.; Klar, U. PNAS **2001**, *98*, 11743-11748).

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