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Discovery and development of the epothilones: a novel class of antineoplastic drugs

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Abstract

The epothilones are a novel class of antineoplastic agents possessing anti-tubulin activity. The compounds were originally identified as secondary metabolites produced by the soil-dwelling myxobacterium *Sorangium cellulosum*. Two major compounds, epothilone A and epothilone B, were purified from the *S. cellulosum* strain So ce90 and their structure was identified as 16 member macrolides. Initial screening with these compounds revealed a very narrow and selective antifungal activity against the zygomycete, *Mucor hiemalis*. In addition, strong cytotoxic activity against eukaryotic cells, mouse L929 fibroblasts and human T-24 bladder carcinoma cells was observed. Subsequent studies revealed that epothilones induce tubulin polymerization and enhance microtubule stability. Epothilone-induced stabilization of microtubules was shown to cause arrest at the G2-M transition of the cell cycle and apoptosis. The compounds are active against cancer cells that have developed resistance to taxanes due to acquisition of β-tubulin overexpression or mutations and against multidrug resistant cells that overexpress P-glycoprotein (P-gp) or multidrug resistance associated protein (MRP). Thus, epothilones represent a new class of antimicrotubule agents with low susceptibility to key tumor resistance mechanisms.

More recently, a range of synthetic and semi-synthetic epothilone analogs have been produced to maximize pharmacokinetic and antitumor properties. Various

epothilone analogs have demonstrated activity against many tumor types in preclinical studies and several compounds have been, and still are evaluated in clinical trials. This article reviews the identification and early characterization of the epothilones and the current status of research and development of these novel antitumor agents.

Introduction

The development of novel antitumor agents has significantly improved the prognosis and survival of patients with various forms of cancer. However, the effectiveness of current treatment modalities is often limited by intrinsic or acquired tumor resistance, which results in disease progression in the majority of cases. Many of the most effective antineoplastic agents currently in use were derived from natural sources. For example, the vinca alkaloid, vinblastine, was obtained from the Madagascar periwinkle plant *Catharanthus roseus;* anthracyclines are fermentation products of the soil bacterium *Streptomyces peucetius* var. *caesius*, and the pacific yew tree is the original source of the taxanes. However, what all of these compounds have in common is that tumors invariably become resistant to their inhibitory activities, frequently due to reduced intracellular concentrations of the antineoplastic agent.[1-6] This limitation drives a continuing search to identify new agents that will overcome mechanisms of tumor resistance and minimize toxicity.

Although rational drug design and screening of synthetic combinatorial libraries have been used with some success, one of the most promising approaches to identify new biologically active agents is to tap the huge reservoir of natural compounds. The significant contributions that microtubule-targeting agents, such as the vinca alkaloids and the taxanes,[7] have made to cancer chemotherapy prompted several pharmaceutical companies to begin the search for new

compounds with a similar mechanism of action in extracts of plants and micro-organisms. In the 1980s, investigation into the products of a soil-dwelling myxobacterium, *Sorangium cellulosum*, led to the identification of a new class of compound: the epothilones. These 16-member macrolides were originally selected for their antifungal properties, but were subsequently identified as a new class of highly active microtubule-stabilizing agents. Various synthetic and semi-synthetic analogs of the epothilones have shown activity against a wide range of tumor types including multi-drug resistant disease. This review focuses on the early identification and characterization of the epothilones and the current evaluation of these compounds as antineoplastic agents.

1. Myxobacteria

The myxobacteria are unique micro-organisms with unparalleled properties.[8] Myxobacteria are relatively large (0.9–1.0 x 3–6 μm) rod-shaped bacteria (Figure 1) that move by gliding or creeping along surfaces. They are strictly aerobic, and are found in soil, decaying organic material, on tree bark and in fresh water. One of their most notable social behaviors is the formation of multicellular fruiting bodies (Figure 2), containing dormant myxospores. In times of nutrient deprivation, tens of thousands of cells move toward discrete aggregation sites within the swarm colony (Figure 3), where they form a raised mound and from this develops a fruiting body. Within the maturing fruiting body, the rod-shaped cells shorten and fatten. The resultant myxospores are resistant against

desiccation, UV-radiation, mechanical stress and elevated temperatures, thus helping the organism to survive unfavorable environmental conditions. Myxospores germinate when a nutrient source becomes available.[9] Most relevant to the oncologist is the fact that they frequently produce secondary metabolites with cytotoxic activity.[10-12] It is from one of these organisms that the epothilones were isolated as described below.

[Figure 1. *Sorangium cellulosum* vegetative cells **– to appear near here]**

[Figure 2. Myxobacterial fruiting body **– to appear near here]**

[Figure 3. *Sorangium cellulosum*, section of a swarm colony **– to appear near here]**

2. Identification of Epothilones

The epothilones were first obtained from cellulose-degrading *Sorangium cellulosum*, strain So ce90, isolated in 1985 at the Gesellschaft für Biotechnologische Forschung in Braunschweig, Germany. After adaptation of the strain to homogeneous growth in suspension, an antifungal activity was identified from the culture broth of So ce90 with selectivity against the zygomycete, *Mucor hiemalis.*[13] Following the isolation of the active compounds, it was found that the strain excreted substantial amounts of highly cytotoxic spirangiens; in addition, much lower quantities (around 2 mg/L) of epothilones A and B were produced, [13,14] such that the cytotoxicity observed in the screening was likely due to the

presence of structurally distinct spirangiens.[13-15] The antineoplastic activity of the epothilones became fully apparent when they were purified in 1987. In August of that year, the structures of epothilones A and B (Figure 4) were established as 16-membered macrolides[16] and the structures of their biosynthetic precursors, epothilones C and D (Figure 4) were determined shortly thereafter.[17,18] So far, no other myxobacterium, and indeed no other organism have been found to produce epothilones.

[Figure 4. A. Structure of natural epothilones A–D, derived from *Sorangium cellulosum.* **– to appear near here]**

Initial screening assays with purified epothilones A and B demonstrated inhibition of the plant pathogenic fungi *Pythium infestans*, *Plasmopara viticola* and *Phytophtora infestans*. Bacteria were not inhibited but strong cytotoxic activity was observed against mouse L929 fibroblasts and human T-24 bladder carcinoma cells.[13] However, due to lack of interest of pharmaceutical companies in simply cytotoxic compounds at that time, the mode of action and possible applications in oncology were not pursued.

3. Mechanism of Action of Epothilones

Following identification of their cytotoxic activity, the epothilones were shown to bind to β-tubulin subunits with high affinity.[19-23] When bound to tubulin,

epothilones stimulated its polymerization and stabilized the resulting microtubule structures.[19-21] These effects were also observed under conditions that would normally prevent tubulin polymerization or destabilize microtubules, such as low temperatures (0–25°C), high calcium concentrations, the absence of guanosine 5′-triphosphate (GTP), the absence of microtubule-associated proteins (MAPs) or dilution of tubulin below the critical concentration required for spontaneous microtubule formation.[19]

The microtubule cytoskeleton is an effective target for antineoplastic agents. The vinca alkaloids inhibit the assembly of tubulin into microtubules and prevent formation of the mitotic spindle.[24] The taxanes stimulate tubulin polymerization, thus enhancing the formation and stability of microtubules.[25-27] Both agents disrupt the dynamic states of microtubule growth and shrinkage that is necessary for proper regulation of cellular functions, including mitosis and meiosis, maintenance of cell shape and intracellular trafficking of macromolecules and organelles. [28-30]

The epothilones were shown to suppress microtubule dynamics. They induce microtubule bundling and formation of multipolar spindles within cells.[19,31-34] The end result of the stimulation of microtubule polymerization is arrest at the G_2/M-transition of the cell cycle and subsequent cell death via apoptosis.[19,35-36] While this mechanism of tubulin binding by the epothilones appears to be similar to that of paclitaxel, there are some important differences in the properties of these two

classes of agents. First, epothilones bind to various β-tubulin isotypes including βIII tubulin, the overexpression of which is associated in vivo and clinically with intrinsic and acquired resistance to the taxanes.[37-40] Second, while paclitaxel-induced apoptosis has been reported to occur independently of caspase activation,[41,42] apoptosis induced by epothilones and analogs is associated with activation of caspase 3 and additional caspases in a variety of cell types.[43-46]

4. Biological Effects of Epothilones

In agreement with experiments performed on isolated tubulin, studies on a range of human cancer cell lines have demonstrated that treatment with natural epothilones leads to profound growth inhibition and death of cancer cells. There is a dramatic reduction in the effective concentrations of epothilones required for cellular effects compared to those observed using isolated tubulin. This is consistent with a several hundred–fold accumulation of epothilones within cells.[47] HeLa cells, for example, accumulate 4.2 and 2.6 μM epothilone A and B, respectively, within 2 hours in the presence of 10 nM drugs in the medium; and at a higher drug exposure (above 100 nM) the epothilones reach saturation levels of 17 and 26 μM, which corresponds well with the intracellular tubulin concentration of approximately 25 μM.

Consistent with studies using isolated tubulin, epothilone B was found to be more potent than epothilone A in vitro [48], and both epothilones demonstrated stronger

activity than paclitaxel against a panel of tumor cell lines (Table 1). Although conflicting results were seen when the epothilones were tested in vivo, [49-52] potent antitumor activity has been demonstrated for epothilone B in several drug-sensitive human tumor cell models, including lung, breast, colon and prostate.[52]

[Table 1. IC_{50} values of epothilones A and B and paclitaxel in human cancer cell lines **– near here]**

5. Reduced Susceptibility to Multidrug Resistance

One important feature of the epothilones is that they display reduced susceptibility to multiple mechanisms of tumor resistance. A major cause of intrinsic and acquired tumor resistance is the overexpression of efflux pumps such as P-gp and MRP, of which many common chemotherapeutic agents are substrates.[1-6] Epothilones, by contrast, have low affinity for these efflux pumps; consequently, most multidrug resistant tumor cell lines, including those that are resistant to paclitaxel, remain sensitive to epothilones.[19,31]

As mentioned above, epothilones are also able to overcome tumor resistance due to certain mutations in β-tubulin [31] and changes in tubulin isotype composition, as demonstrated by the activity of ixabepilone against Pat-21 breast cancer cells, which are characterized by a loss of βII tubulin isotype and overexpression of βIII tubulin.[53] A comparison between paclitaxel and epothilone

A/B IC_{50} values in paclitaxel-resistant cell lines versus their parental cell lines shows that while paclitaxel resistance increased by a factor of 22 to 19167, the resistance to epothilone B rose only 1.67 to 5.0-fold (Table 2).[19,32,49,54]

[Insert Table 2. IC_{50} values of epothilones A and B, and paclitaxel in resistant and parental (nonresistant) cell lines **– near here]**

While the in vitro experiments summarized above demonstrated potent antineoplastic properties of the epothilones, translation to in vivo antitumor efficacy was not always satisfactory. This was due to the poor metabolic stability and unfavorable pharmacokinetic properties of the natural epothilones. Lactone hydrolysis is the main pathway of epothilone B metabolism in mice [55]; epothilones with a lactone are rapidly metabolized in murine plasma, with half-lives of approximately 20 minutes.[56] In dogs, however, the half-life is more than 5 hours.[56] In rodents, the degradation rates of the natural epothilones were found to be as follows: epothilone A, 0.50 n mol/min.mg serum protein; epothilone B, 1.02 nmol/min/mg protein; and epothilone D, 1.20 nmol/min.mg seruim protein (BMS, data on file). The differences in metabolism between species may be due to differences in the activity of plasma and tissue esterases; however, the data demonstrate the poor metabolic stability of the natural epothilones. This realization led to the development of epothilone analogs with more favorable metabolic and pharmacokinetic profiles.

6. Epothilone Analogs

A vast array of semi-synthetic and synthetic epothilone analogs have been synthesized in efforts to improve upon the antitumor activity of the natural epothilones.[57,58] With seven stereogenic centers in a 16-membered macrolide, the total synthesis of epothilones, although challenging, appeared to be far less difficult than that of paclitaxel.[59] Of the synthetic and semi-synthetic analogs, the most promising are ixabepilone (BMS-247550, a lactam analog of epothilone B),[31] BMS-310705 (C21-amine of epothilone B),[60,61] dehydelone (KOS-1584; 9,10-didehydroepothilone D),[62] and ZK-EPO (synthetic epothilone B analog (Figure 5).[63,64] BMS-247550, KOS-1584, sagopilone (=ZK-EPO), as well as patupilone (EPO906; natural epothilone B) are currently in clinical development. In addition, although not yet in clinical development, the epothilone analogs fludelone (26-trifluoro-(E)-9,10-dehydro-12,13-desoxy-epothilone B) and ABJ879 (methylthioepothilone B) (Figure 5) have shown promise in a range of preclinical xenograft models.[65-68]

[Figure 5. Structure of synthetic and semi-synthetic epothilones in development **– to appear near here]**

The semi-synthetic and synthetic analogs benefit from improved pharmacokinetic properties compared with the natural epothilones, for example, the half-life of ixabepilone in mice is 13 hours following IV administration of 6 mg/kg and 16

hours following IV administration of 10 mg/kg (Bristol-Myers Squibb, data on file). Similarly, the half-life of dehydelone (KOS-1584) is approximately three-fold that of the natural epothilone D (KOS-862).[62] The degradation rate of ixabepilone is also lower compared with natural epothilone D, viz, 0.01 n mol/min.mg serum protein versus 1.02 n mol/min.mg serum protein (Bristol-Myers Squibb, data on file). Unlike the natural epothilones, data from early clinical trials demonstrated good metabolic stability and availability of epothilone analogs (Table 3).

7. Conclusions

The epothilones, originally identified as selective antifungal agents, are a family of macrolides specifically produced by the myxobacterium *Sorangium cellulosum*. Although it is unclear what role the epothilones play in the life cycle of this organism, their high toxicity toward eukaryotic cells suggests that they may help to protect the bacterium's ecological niche against competitors and predators, such as fungi, soil protozoa and nematodes. Alternatively, the bacterium may utilize the compounds to secure access to essential nutrients like nitrogen and phosphorus, in its nutrient-poor environment.

Further characterization of the epothilones has demonstrated strong in vitro and in vivo cytotoxic activity toward tumor cells. The biological actions of the epothilones are mediated by induction of tubulin polymerization, microtubule stabilization, cell cycle arrest and apoptosis. Other antimicrotubule agents, such

as the taxanes, have been widely and successfully used as chemotherapeutic agents for many years. However, the therapeutic benefit of these drugs has been limited by their susceptibility to tumor cell resistance mechanisms. Cells that overexpress efflux pumps such as P-gp, encoded by the *mdr* gene resist the cytotoxic effects of taxanes. In addition, cells that lose expression of the tubulin βII isoform (the target of taxanes) and overexpress βIII tubulin have also demonstrated a taxane resistant phenotype. Unlike the taxanes, the epothilones have demonstrated antineoplastic activity in cell lines and in vivo human xenograft models characterized by P-gp and βIII tubulin overexpression.

The comparatively simple structure of the epothilones is amenable to synthesis, and a multitude of semi-synthetic and synthetic analogs have been generated since their initial discovery. The compounds have demonstrated notable antineoplastic activity in a broad range of tumor types, including metastatic tumors. Thus, the epothilones constitute a novel class of antineoplastic agents possessing antitubulin activity and low susceptibility to key tumor resistance mechanisms. Clinical trials are currently ongoing with various natural epothilones and synthetic analogs to examine the efficacy and safety of these compounds in the treatment of cancer.[69-73]

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Table 1. IC_{50} values (nM) of epothilones A and B and paclitaxel in human cancer cell lines.

	Cell line						
	HCT-116 (colon)	**PC-3M (prostate)**	**A549 (lung)**	**MCF-7 (breast)**	**MCF-7/ADR (breast)**	**KB3-1 (epidermoid)**	**KB-8511 (epidermoid)**
Epothilone A	2.51	4.27	2.67	1.49	27.5	2.1	1.9
Epothilone B	0.32	0.52	0.23	0.18	2.92	0.19	0.19
Paclitaxel	2.79	4.77	3.19	1.80	9105	2.31	533

ADR = Adriamycin (doxorubicin)

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Table 2. IC_{50} values of epothilones A and B and paclitaxel in paclitaxel-resistant and parental (non-resistant) cell lines

Paclitaxel-resistant cell line (Parental line)	**Epothilone A IC_{50} value**	**Relative resistance[a]**	**Epothilone B IC_{50} value**	**Relative resistance**	**Paclitaxel IC_{50} value**	**Relative resistance**
KBV-1, MDR epidermoid line (KB3-1)[19]	160 nM (13 nM)	12.3	58 nM (15 nM)	3.9	23 μM (1.2 nM)	19,167
SW620AD-300, MDR colon carcinoma (SW620)[32]	3 nM (2 nM)	1.5	0.3 nM (0.1 nM)	3.0	250 nM (0.2 nM)	1250
KB-8511, human epidermoid cancer, P-gp overexpressing (KB-31)[54]	Not measured	–	0.12 nM (0.19 nM)	0.6	Not measured	–
1A9 PTX22, ovarian carcinoma with β-tubulin mutation (1A9)[32]	3 nM (2 nM)	1.5	0.1 nM (0.06 nM)	1.7	43 nM (2 nM)	21.5
CCRF-CEM/VBL human leukemia (CCRF-CEM)[49]	20 nM (3 nM)	6.7	1 nM (0.2 nM)	5.0	4.14 μM (2 nM)	2070

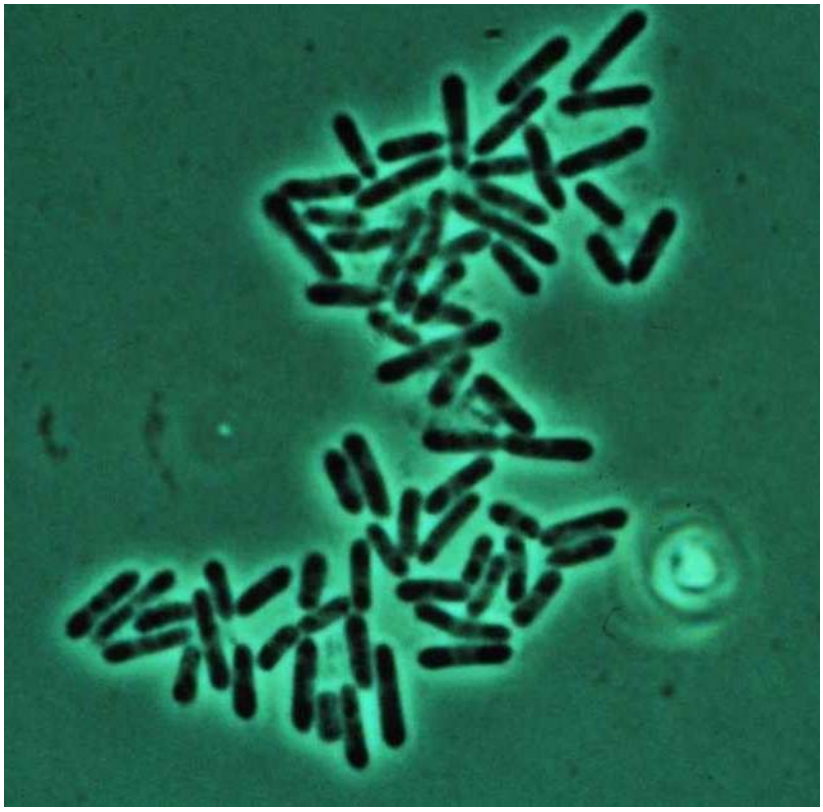
[a]Relative resistance obtained by dividing the IC_{50} value of the resistant line by the IC_{50} value of the parental line; **MDR** = multidrug resistant; **P-gp** = P-glycoprotein; **VBL** = vinblastine

Table 3. Phase 1 pharmacokinetic parameters of epothilone analogs in cancer patients[69]

Phase I Trial Setup			Pharmacokinetic Measurements			
Epothilone or Analog	**Total No. of Patients**	**Dosing**	**Doses Selected for Phase II/III**	**No. of Patients at Phase II/III Dose**	**Half-life (hrs)**	**Steady State Volume of Distribution**
Epothilone D (KOS-862)	38	1 hr infusion 9 to 185 mg/m^2 Q 3 weeks	120 mg/m^2	31	10	95 $\pm$ 39
BMS-247550 (ixabepilone)	40	1 hr infusion 7.4 to 59.2 mg/m^2 Q 3 weeks	40 mg/m^2	14	35	826
BMS-310705	59	15 min infusion 0.6 to 70 mg/m^2 Q 3 weeks	40 mg/m^2	16	42	443

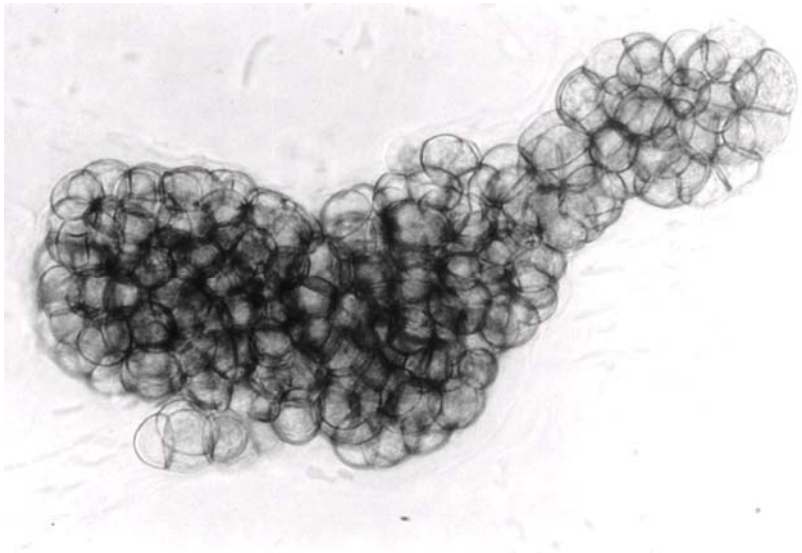
Q = every

Figure 1. *Sorangium cellulosum*, vegetative cells.



Phase contrast microscopy, 1550x. Individual cells measure 0.9-1.0 x 3-6 μm.

Figure 2. *Sorangium cellulosum*, fruiting body consisting of tiny sporangioles.



Phase contrast microscopy, 460x. The fruiting body measures 275 x 100 µm.

Figure 3. *Sorangium cellulosum*, section of a swarm colony.

The migrating cells pack together into massive radial veins. 25x (width at margin 2.2 mm).

Figure 4. **Structure of natural epothilones A–D, derived from *Sorangium cellulosum.***

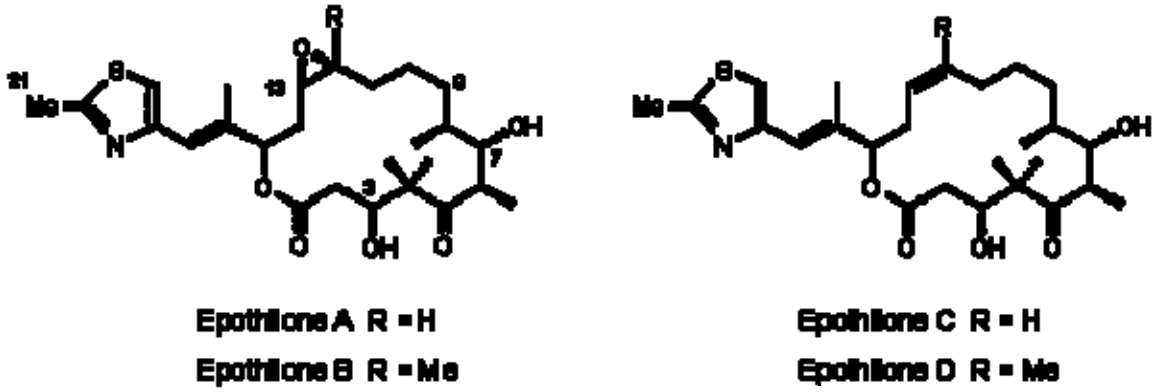
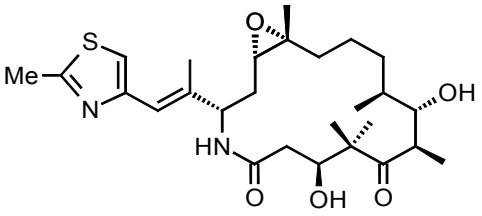
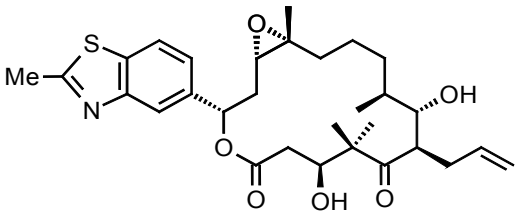


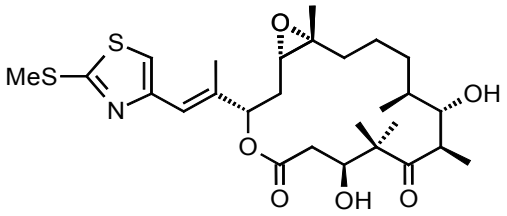
Figure 5. **Structure of synthetic and semi-synthetic epothilones in development**



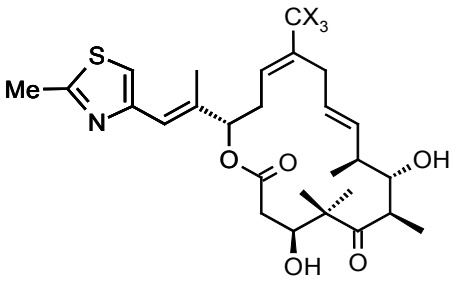
Epothilone B-lactam (Ixabepilone)
Bristol-Myers Squibb



Sagopilone (ZK-EPO)
Schering AG



Methylthioepothilone B (ABJ879)
Novartis Pharma



Dehydelone (KOS-1584, X = H)
Kosan Biosciences

Fludelone (X = F)
Sloan Kettering Cancer Res. Center