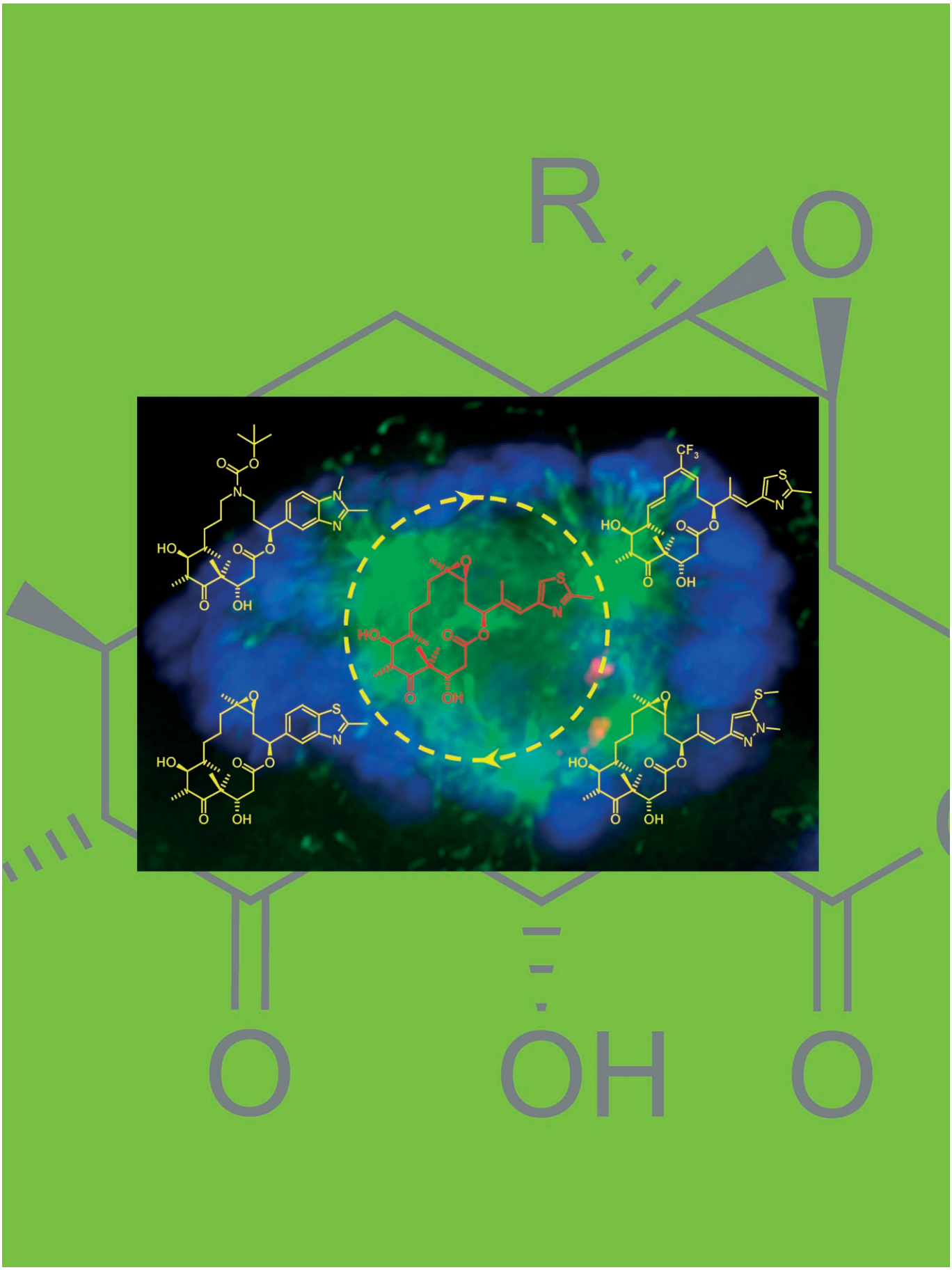

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The Chemistry and Biology of Epothilones—The Wheel Keeps Turning

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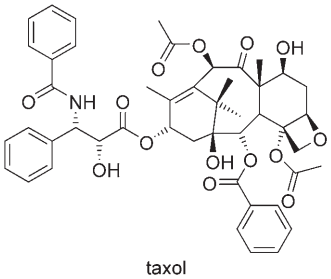
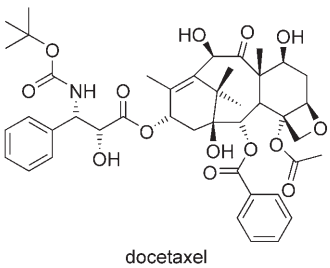
Introduction

Dynamic microtubules are among the most successful targets for anticancer drugs, and many microtubule-active compounds are currently in advanced stages of preclinical development or are already in clinical trials.[1] Microtubule-interacting agents can be grouped into two distinct functional classes, namely compounds which stabilize microtubules under normally destabilizing conditions ("microtubule-stabilizing agents") and those which inhibit the assembly of tubulin heterodimers into microtubules ("tubulin polymerization inhibitors").[2] While the use of the latter in cancer therapy dates back to the early 1960s, the introduction of microtubule-stabilizing agents into clinical practice constitutes a comparably recent development, which has occurred only within the last 15 years. The first microtubule-stabilizing agent to obtain FDA approval was taxol (paclitaxel, Taxol®)[1] in 1992, followed by the closely related analogue docetaxel (Taxotere®) in 1996.[4]

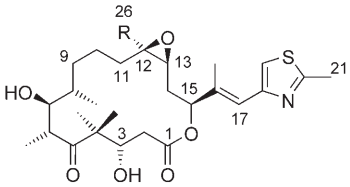


taxol

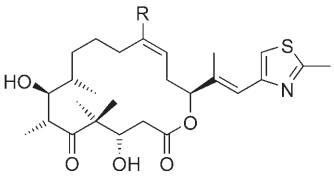


docetaxel

More than a decade passed after the elucidation of taxol's mode of action by Horwitz and co-workers in 1979[5] until other (that is, "non-taxane"-type) compounds acting through a similar "taxol-like" mechanism were identified by Bollag et al. at Merck Research Laboratories in 1995.[6] Interestingly, these compounds were the known natural products epothilone A (Epo A) and B (Epo B), which had already been discovered in 1987[7] by Reichenbach, Höfle, and co-workers at the "Gesellschaft für Biotechnologische Forschung" (GBF) in Braunschweig, Germany as bioactive components of extracts of the cellulose-degrading myxobacterium *Sorangium cellulosum Soce90* in a screen for new antifungal agents.[8,9]



R = H: epothilone A
R = Me: epothilone B



R = H: epothilone C
R = Me: epothilone D

The compounds were termed "epothilones" by Reichenbach and Höfle to reflect their basic structural features, which include an *epo*xide moiety, a *thi*azole-containing side chain, and a single keto*(ne)* function. While Epo A and Epo B are the major products originally isolated from fermentation broths of *Sorangium cellulosum Soce90*, numerous other related members of this class of natural products have since been discovered as minor components of the fermentation of myxobacteria, including, for example, epothilones C (Epo C) and D (Epo D).[10–12]

[1] *The name Taxol® is a protected trademark of Bristol–Myers Squibb (BMS) and it refers to the drug paclitaxel in its final clinical formulation. Paclitaxel is identical to the compound "taxol" as it was first reported in the scientific literature in 1971 by Wani et al.[3] As this occurred long before the drug Taxol® was introduced to the market, we prefer to maintain the name taxol for the compound throughout this article. This should not be considered as an infringement of the BMS trademark.*

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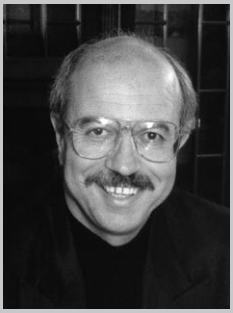
Epo A and B were also recognized shortly after their initial isolation to be potent inhibitors of human cancer cell proliferation, but their mechanism of action remained unexplored until their rediscovery by Bollag et al.[6] Subsequent in-depth profiling by the Merck group[6] as well as by Hamel and co-workers[13] established that both compounds potently induce tubulin polymerization in vitro and are able to stabilize microtubules under normally destabilizing conditions. While epothilones thus exert their antiproliferative activity through the same mechanism of action as taxol, there are distinct differences between the two classes of compounds with respect to their ability to inhibit the growth of multidrug-resistant (MDR) cancer cell lines.[6,13–15] In particular, epothilones, unlike taxol, have proven to be very poor substrates for the phosphoglycoprotein 170 (P-gp) efflux pump and thus retain almost full activity against P-gp-overexpressing, taxol-resistant cells.[6,13–15] In addition, Epo A and B are active against taxol-resistant cells, whose decreased sensitivity to taxol is caused by particular tubulin mutations.[16] Apart from these superior biological features, the increased water solubility of epothilones over taxol[17] enables their administration without the use of problematic clinical formulation vehicles such as Cremophor® EL, which in the case of Taxol® is believed to be responsible for some of the drug's clinical side effects.[4]

Professor Karl-Heinz Altmann studied chemistry at the University of Mainz in Germany, and he holds a PhD in organic chemistry from the University of Basel in Switzerland. After 13 years of drug-discovery-related research in the pharmaceutical industry (Novartis), he was appointed to Full Professor of Pharmaceutical Sciences at the Swiss Federal Institute of Technology (ETH) in Zürich in 2003. Professor Altmann's research interests are at the interface between chemistry and biology, with a particular focus on the chemical synthesis of biologically active natural products and their synthetic and semisynthetic analogues.

Dr. Bernhard Pfeiffer obtained his diploma from the University of Basel in 1997 and received his PhD in 2002 from the University of Zürich under the guidance of Professor J. A. Robinson. He then spent a year as a postdoctoral fellow at the Institute of Molecular Bioscience at the University of Queensland (Australia) under the supervision of Professor D. Fairlie. This was followed by another year as a research fellow in the group of Professor K.-H. Altmann at the Swiss Federal Institute of Technology (ETH) in Zürich. He is currently a research associate at the Institute of Pharmaceutical Sciences at the ETH.

Benjamin A. Pratt was born in Boston, Massachusetts in 1977. He received his BA degree with high honors in chemistry at Dartmouth College in 2000 where he worked under the direction of Professor Peter A. Jacobi on the synthesis of chlorin ring systems. In 2000, he began work as a scientist in the Department of Process Research and Development at Rhodia ChiRex in Boston. In the fall of 2003, he moved to San Diego and began his PhD studies under the guidance of Professor K. C. Nicolaou at The Scripps Research Institute, where his research has focused on the synthesis of highly active epothilone B analogues and other natural products.

Dr. Stellios Arseniyadis was born in Athens, Greece in 1975 and studied chemistry at the Université d'Orsay–Paris XI and the Université René Descartes–Paris V. He completed his PhD in 2002 with Dr. C. Mioskowski at the Université Louis Pasteur (Strasbourg, France) and joined Rhodia ChiRex (Boston, USA) for an 18-month compulsory military service. After an initial postdoctoral fellowship with Professor A. C. Spivey at Imperial College in London, he joined Professor K. C. Nicolaou's group at The Scripps Research Institute (La Jolla, USA), where he was engaged in the synthesis of new epothilone B analogues and the total synthesis of vannusal A. He currently holds a permanent researcher position in the group of Professor J. Cossy at CNRS (Paris, France), where his research interests include the development of new methods in the field of asymmetric synthesis and their application in natural product synthesis.



Professor K. C. Nicolaou, born in Cyprus and educated in England and the United States, is currently Chairman of the Department of Chemistry at The Scripps Research Institute, where he holds the Darlene Shiley Chair in Chemistry and the Aline W. and L. S. Skaggs Professorship in Chemical Biology. He is also Professor of Chemistry at the University of California, San Diego and Director of the Chemical Synthesis Laboratories at Biopolis, A*STAR, Singapore. The impact of his career on chemistry, biology, and medicine flows from his contributions to chemical synthesis, described in hundreds of publications and numerous patents. His dedication to chemical education is reflected in his training of hundreds of graduate students and postdoctoral fellows. His Classics in Total Synthesis series, which he coauthored with his students Erik J. Sorensen and Scott A. Snyder, is used as a teaching tool and source of inspiration for students and practitioners of the art of chemical synthesis all around the world.

Through the discovery of their taxol-like mechanism of action (a rare and unusual attribute at that time), epothilones immediately turned into highly attractive targets for total synthesis and important lead structures for anticancer drug discovery. Following the elucidation of their absolute configuration by Höfle et al. in 1996 (which was based on a combination of X-ray crystallography and chemical degradation studies),[17] extensive chemistry efforts ensued, which were directed at the total synthesis of natural epothilones, but also the design and synthesis of a wide variety of analogues for biological testing and SAR studies. These efforts are reflected in the significant number of total syntheses of epothilones and their analogues (for reviews, see refs. [18–23]), and in a vast body of literature available on the SAR of this compound class (reviewed in [7, 18, 19, 24–27]).

Besides generating an impressive volume of information on the structural variables governing the biological activity of epothilones, a more tangible result of this work is the fact that at least seven epothilone-derived agents are currently undergoing clinical evaluation in humans as potential anticancer drugs.[28–32] Apart from the natural product Epo B, which is being developed by Novartis (EPO906, patupilone; for preclinical studies, see refs. [15, 33–35]; for clinical studies, see refs. [36–38]), the group of compounds in clinical development includes BMS-247550 (ixabepilone, the lactam analogue of Epo B (BMS); for preclinical studies, see ref. [39]; for clinical studies, see refs. [40–46]), Epo D (deoxyepothilone B, KOS-862 (Kosan/Roche); for preclinical studies, see refs. [47–49]; for clinical studies, see refs. [50–52]), BMS-310705 (C21-amino-Epo B (BMS)),[24,53,54] ABJ879 (C20-desmethyl-C20-methylsulfanyl-Epo B (Novartis)),[55] 9,10-didehydro-Epo B (KOS-1584 (Kosan/Roche)), and a synthetic analogue, ZK-EPO, which is developed by Schering AG in Berlin and which is currently in phase II clinical trials.[56] The most advanced compounds so far are EPO906 and BMS-247550, which are both in phase III trials.

Over the course of the last few years, significant advances have also been made in the elucidation of epothilone biosynthesis (for recent reviews, see refs. [7, 57]) and the development of heterologous expression systems for epothilones and individual derivatives.[7,58,59] A detailed discussion of these achievements, however, is beyond the scope of this review article (with a few specific exceptions) as is the enormous amount of clinical data that have emerged from the various clinical trials. For these areas the reader is referred to a number of excellent recent review articles dealing with the corresponding subject (see references cited above). Likewise, this article does not recount the entire body of information that has accumulated on the biology, chemistry, and SAR of epothilones over the last decade, a large part of which has been reviewed previously.[7,15,18–27] Rather, this article focuses on the most recent developments (since 2003/2004) in the chemistry and biology of epothilones, with particular emphasis on new SAR data and the results of structural studies. Lastly, it should be noted that since the discovery of the microtubule-stabilizing properties of epothilones in 1995, a growing number of additional natural products have been identified as microtubule stabilizers, including discodermolide,[60] eleutherobin,[61] laulimalide,[62] peloruside,[63] and dictyostatin.[64] These discoveries have provided a whole new range of diverse lead structures for anticancer drug discovery, but none of them has yet been investigated and exploited to the same extent as epothilones.

Biochemistry and Pharmacology of Epo B

The basic biology and pharmacology of Epo B (as the most potent and most widely studied natural epothilone) have been summarized in a number of previous review articles.[7,15,18,24–27,65,66] As highlighted in the Introduction, the biological effects of Epo B are based on its ability to bind to microtubules, which is associated with a profound alteration of the intrinsic stability and the dynamic properties of these supramolecular structures. Epo B has been demonstrated in cell-free in vitro systems to prevent the disassembly of preformed microtubule polymers under normally depolymerizing conditions (high concentrations of Ca^{2+} or low temperatures).[13] On the other hand, Epo B, like taxol, promotes tubulin polymerization (to form microtubule polymers) in the absence of either guanosine triphosphate (GTP) and/or microtubule-associated proteins (MAPs), at temperatures significantly below 37 °C as well as in the presence of Ca^{2+}.[6,13] The latter effect, that is, the induction of tubulin polymerization, is often used as a biochemical readout for a quantitative assessment of the interaction of microtubule-stabilizing agents with tubulin. Epo A polymerizes tubulin with about the same potency as taxol, while Epo B is an even more efficient tubulin-polymerizing agent (for example, the EC_{50} values for the polymerization of microtubule protein by Epo A, Epo B, and taxol have been determined to be 1.12, 0.67, and 1.88 μM, respectively[67]). It should be mentioned, however, that the exact magnitude of tubulin polymerization in vitro (polymerization rates, extent of tubulin polymer formation) strongly depends on the assay conditions employed. Factors such as the biological source and purity of the protein, as well as the concentration of microtubule-stabilizing buffer components or the reaction temperature are very important and can strongly affect the absolute and relative potencies of individual compounds in the polymerization assay.[68] Epothilones have been shown to be able to displace [^{3}H]taxol from microtubules with efficiencies similar or superior to those of unlabelled taxol or docetaxel.[6,13] As demonstrated by kinetic experiments, the inhibition of taxol binding by epothilones occurs in a competitive fashion (with apparent K_i values of 1.4 μM (Epo A) and 0.7 μM (Epo B)[6,13]), which indicates that the binding sites for taxol and epothilones on microtubules are largely overlapping or might even be identical. This notion has recently been confirmed by structural studies on a complex between Zn^{2+}-stabilized tubulin polymer sheets and Epo A.[69] Recently, the binding constants of Epo A and B toward stabilized microtubules in vitro at 37 °C were determined using a fluorescence-based displacement assay to be $2.93\times10^7\ M^{-1}$ (Epo A) and $6.08\times10^8\ M^{-1}$ (Epo B).[70]

Treatment of cultured cells (RAT1, HeLa, Hs578T, Hs578Bst, PtK_2) with high concentrations of epothilones (10^{-6}–10^{-4} M) resulted in the formation of characteristic, extensive microtubule bundles (through lateral association of microtubules) through-

out the cytoplasm of interphase cells.[6,13] These microtubule bundles arise independent of the centrosome, thus indicating that epothilones can override the microtubule-nucleating activity of the centrosome. In line with its effects on tubulin polymerization in vitro (that is, in an ex-cellular context), the prevention of cold-induced depolymerization of microtubules by epothilones has also been demonstrated in cells.[25]

Treatment of human cancer cells with low nanomolar concentrations of Epo B leads to profound growth inhibition and subsequent cell death. As illustrated by the data summarized in Table 1, the rank order of growth inhibitory activity of Epo A, Epo B, and taxol parallels their ability to promote tubulin polymerization in vitro, with Epo B being a more potent antiproliferative agent than Epo A, which in turn is about equipotent with taxol. Similar to taxol, Epo B treatment produces abnormal mitotic spindles, causing cell-cycle arrest in mitosis and leading to apoptotic cell death.[6,13] However, contrary to previous assumptions, Horwitz and co-workers have recently shown that G2/M *arrest* is not an absolute prerequisite for growth inhibition and the induction of cell death in epothilone-treated A549 cells.[71,72] Instead, low concentrations of Epo B (and also taxol or discodermolide) produce a large aneuploid cell population in the *absence* of a mitotic block. These cells, which are arrested in the G1 phase of the cell cycle and which will eventually undergo apoptosis, arise from abnormal mitosis after formation of multipolar spindles. In contrast, higher drug concentrations lead to a protracted mitotic block from which the cells exit without division, thus forming tetraploid G1 cells.[72] Based on these results it is clear that the *entry* of cells into mitosis is a fundamental prerequisite for cell killing by microtubule-stabilizing agents, while cell death itself does not necessarily require prior mitotic arrest.

Table 1. Inhibition of human carcinoma cell lines by taxol, Epo A, and Epo B.[67]

Cell line	IC_{50} [nM]		
	Taxol	Epo A	Epo B
HCT-116 (colon)	2.79	2.51	0.32
PC-3M (prostate)	4.77	4.27	0.52
A549 (lung)	3.19	2.67	0.23
MCF-7 (breast)	1.80	1.49	0.18
NCI/ADR[a,b]	9105	27.5	2.92
KB-31 (cervix)	2.31	2.1	0.19
KB-8511[b,c]	533	1.9	0.19

[a] Multidrug-resistant cell line. [b] Multiple resistance mechanisms/MDR. [c] P-gp overexpression/MDR.

One of the most striking observations on the cellular activity of epothilones is the fact that cancer cell growth inhibition occurs at dramatically lower concentrations than those required for the induction of tubulin polymerization in vitro. This raises questions about potential alternative or at least additional targets (other than tubulin) that might be involved in the cellular effects of epothilones. This apparent discrepancy has been resolved, however, by careful uptake experiments in HeLa cells,[15,67] the results of which have shown that Epo A and B, like taxol,[74] accumulate several hundredfold inside cells over external medium concentrations. As a consequence, nanomolar medium concentrations of epothilones in cellular experiments translate into micromolar intracellular concentrations, which are thus within the concentration range required for the induction of tubulin polymerization in vitro. Similar findings have been reported by the Schering group for a close analogue of Epo B in MCF-7 cells.[75]

Notwithstanding the findings on intracellular accumulation, the growth inhibitory effect of epothilones (and also other microtubule-interacting agents) is generally believed to be a result of the suppression of microtubule dynamics and not a consequence of an overall increase in microtubule polymer mass due to massive induction of tubulin polymerization.[76] Indeed, using time-lapse microscopy in MCF-7 cells stably transfected with GFP–α-tubulin (GFP = green fluorescent protein), Kamath and Jordan[77] recently demonstrated that the inhibition of the dynamics of interphase microtubules by Epo B occurs in a concentration-dependent manner and correlates well with the extent of mitotic arrest (G2/M block). Thus, dynamicity (the length grown and shortened by an individual microtubule divided by its total lifespan) was decreased by more than 60% at the IC_{50} for mitotic arrest after 20 h (3.5 nM Epo B), whereas microtubule dynamics remained unaffected at concentrations not leading to a G2/M block (0.2 nM Epo B). These data provide a direct demonstration of the suppression of cellular microtubule dynamics by Epo B, although it is still unclear how effects on *interphase* microtubules relate to the dynamics of *spindle* microtubules (which could not be measured) and thus to mitotic arrest. Likewise, it remains to be established how mitotic entry and the associated rearrangement of the entire microtubule network can occur from a largely static state of this network. It appears likely, however, that mitotic signals lead to profound changes in microtubule dynamics, which may cause the dynamics of spindle microtubules to be suppressed less efficiently than is the case for interphase microtubules.

As is generally the case for anticancer drugs, the cellular response to microtubule-stabilizing agents can be modulated by adaptive changes of the cell that lead to acquired drug resistance. Alternatively, cells may be inherently protected from the antiproliferative effects of cytotoxic agents by a variety of mechanisms. As already indicated in the Introduction, Epo A and B show very low (if any) susceptibility to P-gp-mediated drug efflux and thus, unlike taxol or other standard cytotoxic anticancer agents, retain almost full antiproliferative activity against the corresponding multidrug-resistant cell lines in vitro (Table 1).[6,13–15] In contrast to the absence of P-gp-mediated drug expulsion, recent discoveries from various laboratories demonstrate that cancer cells can become resistant to epothilones through alternative mechanisms such as tubulin mutations. For example, Giannakakou et al.[78] produced an Epo A-resistant cell line, 1A9/A8, in which Thr 274, which maps to the taxol/epothilone binding site on tubulin,[69,79] is mutated to Ile. Similar findings were reported by Wartmann et al.,[25] who isolated an epothilone-resistant subline of the KB-31 cervix carcinoma cell line (termed KB-31/C5), which carries a single point

mutation in the HM40 β-tubulin gene (Thr 274 Pro), and which makes these cells 14-fold resistant to Epo A and 3.4-fold resistant to Epo B. Consistent with the a shared binding site between epothilones and taxol, both 1A9/A8 as well as KB-31/C5 cells are cross-resistant to taxol, albeit to varying degrees.

More recently, Horwitz and co-workers[80] generated three different Epo-resistant cell lines, A549B40, HeLa.EpoA9, and HeLa.EpoB1.8, each of which is characterized by a specific β-tubulin mutation, namely Gln 292 Glu in A549B40 cells, Pro 173 Ala in HeLa.EpoA9 cells, and Tyr 422 Cys in HeLa.EpoB1.8 cells. A549.EpoB40 cells are 95-fold resistant to Epo B and also exhibit marked cross-resistance with other microtubule-stabilizing agents, with the notable exception of discodermolide. Apart from their altered response to microtubule-stabilizing agents, A549.EpoB40 cells also exhibit hypersensitivity towards tubulin polymerization inhibitors, and this behavior may provide a clue as to the effects of the Gln 292 Glu mutation on microtubule stability (see below). In a more recent study, Horwitz and co-workers reported on the further selection of A549B40 cells, which has produced a new cell line, A549.EpoB480,[81] which is ~900-fold resistant to Epo B. In addition to the βGln 292 Glu mutation, this EpoB480 line exhibits mutations of β60 from Val to Phe and of α195 (α-tubulin) from Leu to Met. βVal60 is located at the end of the H1–S2 loop, which is assumed to be involved in M-loop contacts between protofilaments. Mutations of this amino acid could thus affect lateral contacts between protofilaments and lead to less stable microtubules. This hypothesis is in line with the observation that EpoB480 cells are not only hypersensitive to the action of vinblastine, but are also dependent on Epo B for survival.[81] The βGln 292 Glu mutation was also identified by Verrills et al.[82] in combination with a second mutation at position 231 of β-tubulin (Thr 231 Ala) in a highly resistant subline of the human T cell acute leukemia cell line CCRF-CEM (termed dEpoB300), which had been selected with Epo D. dEpo300 cells are 307-fold resistant to the selecting agent Epo D and exhibit 77- and 467-fold cross-resistance with Epo B and taxol, respectively.

In summary, all tubulin mutations identified to date in epothilone-resistant cells are found in regions of the tubulin structure, which can be assumed to be important for tubulin polymerization and/or microtubule stability. Some of these may additionally affect drug binding. These observations suggest that β-tubulin mutations in epothilone-resistant cancer cells may not only (or not even primarily) affect drug–target interactions, but they may also (or alternatively) affect intrinsic microtubule stability such that microtubules become hypostable.[25,80,81] This hypothesis, which had been previously formulated by Cabral and Barlow based on observations made with taxol-resistant CHO cells,[83] is consistent with the fact that all the above cell lines are hypersensitive to tubulin-depolymerizing drugs such as vincristine or colchicine. It should be noted, however, that tubulin mutations, which have been shown to occur in (non-P-gp-expressing) taxol-resistant 1A9PTX22 cells, do not lead to epothilone resistance, at least not to the same extent (1A9PTX10) as for taxol.[16] This finding may point to different key interactions between tubulin and taxol or epothilones, respectively, although binding occurs in the same protein pocket. Whether or not the emergence of tubulin mutations could lead to the development of epothilone resistance at the clinical level remains to be seen. In the case of Taxol® or Taxotere® the clinical significance of this resistance mechanism has not been established.

The preclinical in vivo effects of Epo B have been investigated in some detail by the research groups at Novartis and at the Memorial Sloan–Kettering Cancer Center. These experiments have led to different conclusions with regard to the therapeutic window offered by the compound in the treatment of tumor-bearing animals. The Sloan–Kettering group found Epo B to be associated with considerable toxicity, while having only limited effects on tumor growth.[47] In their view, Epo B might simply be too toxic to become a clinically useful anticancer agent. In contrast to these findings, studies by the Novartis group have demonstrated potent antitumor activity of Epo B in a number of drug-sensitive human tumor models (nude mice) upon iv administration,[15] in spite of the compound's limited plasma stability in rodents. 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, Epo B was found to be a potent inhibitor of tumor growth in P-gp-overexpressing multidrug-resistant human tumor models. Regressions were observed in two such models (KB-8511 (cervix carcinoma)[15] and HCT-15 (colon carcinoma)[36]), where tumors were either poorly responsive or completely nonresponsive to treatment with Taxol®. In general, therapeutic effects could be achieved at tolerated dose levels, but significant body weight loss was observed in several experiments (which was generally reversible after cessation of treatment), indicating a relatively narrow therapeutic window. The disparate results of in vivo experiments by the Sloan–Kettering and Novartis groups may be related to differences in the experimental setups, such as tumor models, formulation, and/or dosing regimens; therefore, the data reported by the two groups may not necessarily be incompatible.

Chemistry and Structure–Activity Relationships

Synthesis of natural epothilones

Since the first disclosure of their absolute configuration in 1996[17] more than 30 total syntheses of Epo A or B have been reported in the literature (for reviews, see refs. [18–23]; for specific examples of most recent work, see refs. [84–90]). The following section provides a brief overview on the basic concepts for the total synthesis of natural epothilones.

Most early approaches to the synthesis of natural epothilones were based on three different macrocyclization paradigms (Figure 1), namely the formation of a C12–C13 double bond through ring-closing metathesis (RCM; **A**), ring closure through intramolecular ester bond formation (**B**), or the formation of the C2–C3 bond through intramolecular aldol reaction (**C**; reviewed in refs. [18, 19]). Pioneering work in the area of

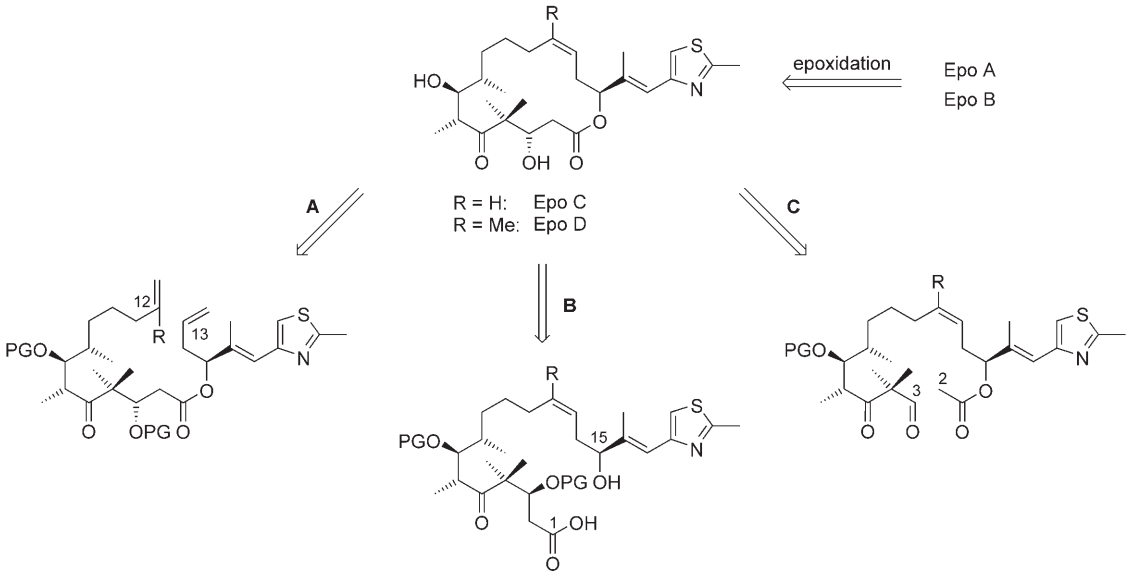


Figure 1. First-generation strategies for the total synthesis of epothilones (PG = protecting group).

epothilone total synthesis was performed by the research groups of Nicolaou,[91,92] Danishefsky,[93,94] and Schinzer.[95,96]

In all three paradigms ring closure is followed by removal of the O3 and O7 protecting groups and subsequent epoxidation of the olefinic C12–C13 double bond. Deviations from these general themes are Mulzer's second-generation synthesis of Epo B, which involves an early installment of the epoxide moiety in an acyclic precursor[97,98] or the olefin metathesis of an epoxide-containing diene substrate, an approach first introduced by Sun and Sinha.[99] While macrolactonization-based strategies have continued to play an important role in epothilone chemistry, the two other strategies have been used only infrequently in recent years, largely due to the lack of selectivity of the aldol reaction for the formation of the C2–C3 bond and the unsatisfactory *E*/*Z* ratios obtained in RCM at C12–C13. At the same time an elegant and efficient alternative to olefin metathesis at C12–C13 has been devised by Fürstner and co-workers, who employed ring-closing *alkyne* metathesis to establish the C12–C13 bond,[100] followed by selective reduction to the desired *Z* olefin. Alternative RCM-based cyclization strategies for the synthesis of Epo B have involved the formation of a C9–C10 or a C10–C11 double bond and subsequent regioselective reduction,[101,102] an approach which is discussed in more detail below.

Epothilone analogues

The largest part of the synthetic work on epothilones and a host of synthetic analogues for SAR studies has originated from the research groups of Nicolaou (for example, ref. [18]) and Danishefsky (for example, ref. [19]). Important contributions to the understanding of the epothilone SAR have also come from the research groups at Novartis (Altmann and co-workers, now at the ETH in Zürich)[67,103] and Schering[104] as well as the collaborative efforts between Höfle and co-workers at the GBF (for example, see refs. [105,106]) and the group at BMS (for example, see ref. [107]). This work has involved the biological investigation of a range of fully synthetic epothilone analogues as well as many semisynthetic derivatives, which have been a particular focus of the GBF–BMS collaboration. Much of the SAR data that have emerged from this research have been summarized in several excellent review articles[7,15,18,19,24–27] and are discussed here only in abbreviated form.

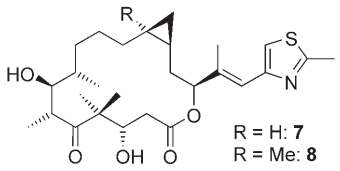
C12–C13 modifications

Much of the early SAR work on epothilones was directed at the epoxide moiety at C12–C13 of the macrolactone ring (reviewed in refs. [7,15,18,19,24–27]), and one of the most important results that emerged from those early studies was the fact that "deoxyepothilones" (Epo C and D) possess potent biological activity similar to that of the epoxide-containing parent compounds.[91c,93b,108–110] Thus, Epo D is equipotent to Epo B as an inducer of tubulin polymerization, it inhibits tumor cell growth with low nanomolar IC_{50} values, and, like Epo B, it retains full activity against P-gp-overexpressing multidrug-resistant cells (IC_{50} (KB-8511): 1.44 nM versus 0.19 nM for Epo B[15]). Extensive in vivo profiling by the Sloan–Kettering group has also shown the compound to be a highly effective antitumor agent in vivo, which potently inhibits the growth of different types of solid tumors in mouse models of human cancer.[47,48] Based on these promising preclinical findings, the compound was promoted to clinical development status and it is currently undergoing phase II clinical trials.[50–52]

In response to the increasing demand of material required for the extensive preclinical profiling of Epo D, Danishefsky and co-workers have continuously optimized their synthetic strategies to the compound, and these efforts have resulted in a highly efficient second-generation approach to Epo D (Scheme 1), which implicitly also provides improved synthetic access to Epo B[111] (for Danishefsky's first-generation approach

to Epo B, see refs. [93,94]). Key steps of this improved approach to Epo D are 1) the aldol reaction between ketone **2** and aldehyde **1**, which proceeds with ~5.5:1 selectivity, 2) the Suzuki–Miyaura coupling of terminal olefin **3** with vinyl iodide **4**, and 3) the highly selective Noyori reduction of the C3 keto group in **5**. The overall optimization process also included the development of efficient routes for the synthesis of the individual building blocks **1**, **2**, and **4**.

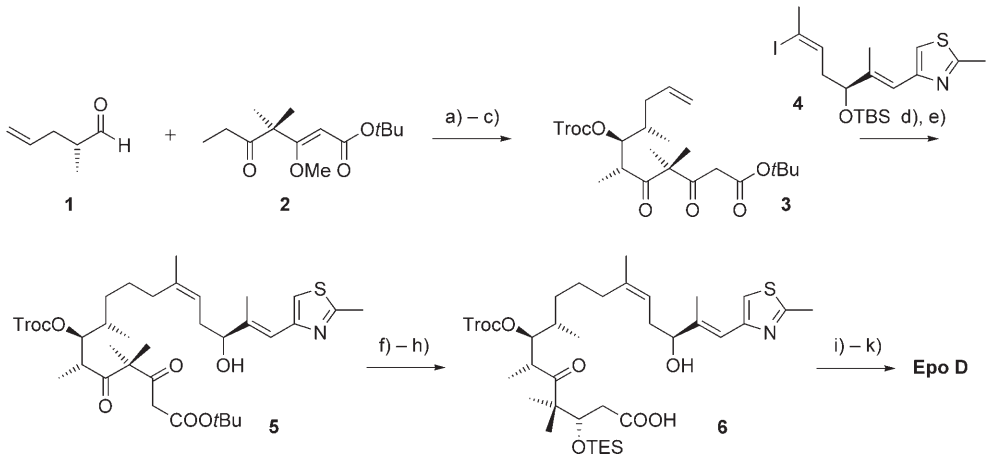
The above data on Epo C and D, which had demonstrated that efficient microtubule stabilization and the potent inhibition of human cancer cell growth by epothilone-type macrolides does not depend on the presence of an epoxide moiety, were subsequently reinforced by the investigation of cyclopropane-based analogues of Epo A and B (**7** and **8**, respectively).



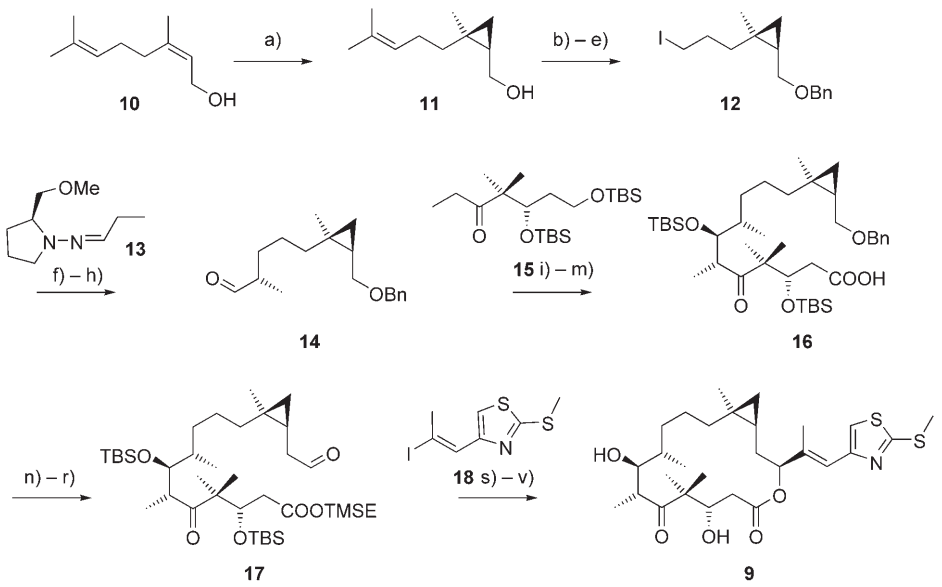
Thus, the replacement of the epoxide ring in Epo A or B by a cyclopropane moiety is well tolerated, and the corresponding analogues are essentially equipotent with the epoxide-derived natural products[112,113] (for example, IC_{50} values against the human colon carcinoma cell line HCT-116 are 1.4 nM for **7** and 0.7 nM for **8**, compared with 4.4 nM and 0.8 nM for Epo A and Epo B, respectively).[112] These results strongly suggest that the epoxide moiety, rather than acting as a reactive electrophile or hydrogen bond acceptor, may simply have a conformational role and serve to stabilize the proper bioactive conformation of the macrolactone ring. Moreover, Buey et al.[70] have recently shown that the replacement of the epoxide oxygen atom by a methylene group in Epo A or B leads to an entropy-driven increase in the binding to stabilized microtubules. As an extension of their work on C12–C13-cyclopropane-based epothilone analogues, Nicolaou and co-workers have also investigated the corresponding C12–C13 cyclobutyl derivative of Epo A. This compound proved to be less potent than **7**, but the magnitude of the activity loss appears to be dependent on the cell line.[113b]

Cyclopropyl epothilones were first prepared by the BMS group through semisynthesis from fermentatively produced Epo A or B (via deoxygenation to Epo C and D, respectively, and subsequent cyclopropanation).[112] Highly efficient synthetic routes to these analogues were subsequently developed by Nicolaou and co-workers, which also provided access to structures incorporating additional modifications (apart from the epoxide→cyclopropane exchange; see below).[113,114] As an example, Scheme 2 summarizes Nicolaou's synthesis of cyclopropyl-Epo B analogue **9**, which relies on the early installment of the cyclopropane moiety through Charette cyclopropanation[115] of *cis*-geraniol **10**. The resulting cyclopropane **11** was elaborated into iodide **12**, which served as an electrophile in the alkylation of the (−)-SAMP hydrazone **13** under Enders conditions.[117] The alkylation product was converted into aldehyde **14**, which was then connected to the C1–C6 building-block **15** by aldol reaction. Oxidation and homologation of the aldol product gave aldehyde **17**, which was submitted to Nozaki–Hiyama–Kishi coupling[118,119] with vinyl iodide **18**. Finally, macrolactonization under Yamaguchi conditions[120] followed by deprotection and isomer separation furnished the desired cyclopropane epothilone **9**. As indicated above, this route has been used for the construction of a variety side-chain-modified analogues of 12,13-cyclopropyl-Epo B, which can be obtained through Nozaki–Hiyama–Kishi coupling with different side chain vinyl iodides.[116] The biological activity of analogue **9** is discussed in more detail below.

A second intriguing feature of the early epothilone SAR associated with the C12–C13 region was the retention of potent

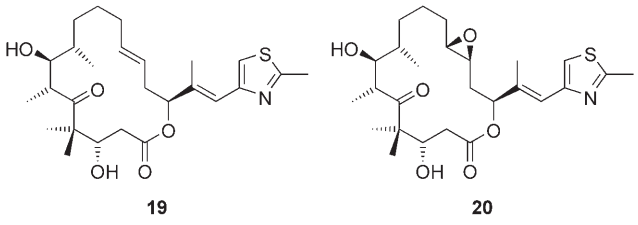


Scheme 1. Ref. [111b]: a) LDA, −120 °C, 50–60%. b) Troc-Cl, py, 0 °C. c) *p*-TSA, acetone, 87% (2 steps). d) (1) 9-BBN; (2) **4**, Cs_2CO_3, $[Pd(dppf)_2Cl_2]$, Ph_3As, DMF/H_2O, ~75%. e) 0.5 M HCl/MeOH, 85%. f) $[RuCl_2((R)\text{-binap})_2][Et_3N]$, H_2, 8300 kPa, HCl/MeOH, 82–88% (>98:2 d.r.). g) TESOTf, 2,6-lutidine, −78 °C→RT. h) 0.1 M HCl/MeOH, 70–77% (2 steps). i) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, DMAP, 78%. j) SmI_2, NiI_2 (cat.), −78 °C, 90–95%. k) HF·py, 0 °C, 98%. LDA = lithium diisopropyl amide, Troc = 2,2,2-trichloroethoxycarbonyl, TSA = toluene sulfonic acid, 9-BBN = 9-borabicyclononane, dppf = 1,1-bis(diphenylphosphino)ferrocene, DMF = *N*,*N*-dimethylformamide, binap = 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl, TES = triethylsilyl, Tf = triflate, DMAP = 4-dimethylaminopyridine.



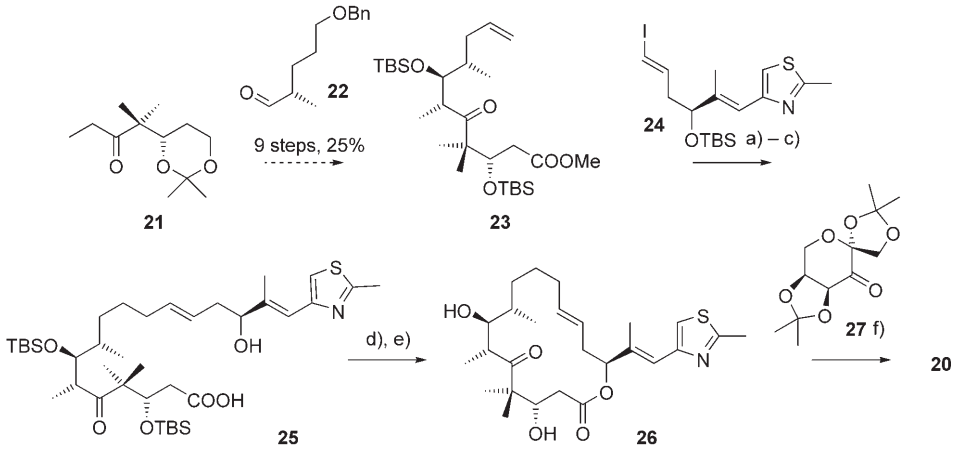
Scheme 2. Ref. [116]: a) 80%, 95% *ee*.[112a,115] b) NaH, BnBr, DMF, 0 °C→25 °C, 12 h, 100%. c) O_3, CH_2Cl_2/MeOH (4:1), −78 °C; then $NaBH_4$, −78 °C→25 °C, 1 h, 83%. d) MsCl, Et_3N, CH_2Cl_2, 25 °C, 1 h. e) NaI, acetone, 25 °C, 12 h, 91% (2 steps). f) LDA, **13**, THF, 0 °C, 6 h; then **12**, −98 °C→−10 °C, 14 h, 87%. g) MeI, reflux, 3 h. h) 3 M HCl/pentane (1:1), 25 °C, 3 h, 91% (2 steps). i) LDA, **15**, THF/Et_2O (1:1), −78 °C, 1 h; then −40 °C, 0.5 h; then **14** at −78 °C, 5 min, 80%. j) TBSOTf, 2,6-lutidine, CH_2Cl_2, −20 °C, 1 h. k) HF·py, py/THF (1:2), 0 °C, 8 h, 86% (2 steps). l) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, 5 min; then Et_3N, −78 °C→0 °C. m) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH/THF/H_2O (4:2:1), 25 °C, 1 h. n) TMSE-OH, EDC, DMAP, DMF, 25 °C, 12 h, 73% (3 steps). o) $Pd(OH)_2$/C (10 wt%; 10%), H_2, EtOH/EtOAc (1:1), 25 °C, 2 h, 89%. p) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then addition of alcohol; then Et_3N, −78 °C→0 °C, 99%. q) $MeOCH_2PPh_3Cl$, *n*BuLi, THF, 0 °C, 1 h; then addition of aldehyde, −78 °C→0 °C, 2 h, 79%. r) PPTS, dioxane/H_2O (9:1), 70 °C, 12 h, 81%. s) $CrCl_2$, $NiCl_2$, 4-*t*Bu-py, **18**, DMSO. t) TBAF, THF, 0 °C→25 °C, 45% (2 steps). u) (1) Et_3N, 2,4,6-$Cl_3C_6H_2C(O)Cl$, THF, 0 °C; 1 h; then DMAP, toluene, 75 °C, 3 h; (2) separation of isomers, 32%. v) CF_3COOH (20% *v*/*v*), CH_2Cl_2, 71%. TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate, DMSO = dimethyl sulfoxide, TMSE = 2-(trimethylsilyl)ethyl, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, DMAP = 4-dimethylaminopyridine, PPTS = pyridinium *p*-toluene sulfonate, TBAF = tetra-*n*-butylammonium fluoride.

biological activity by the nonnatural 12,13-*trans* analogues.[91c,108,109,110] Thus, *trans*-Epo C (**19**) was reported to be only slightly less active than Epo C,[91c,108,109,110] and *trans*-Epo A was shown by Nicolaou et al.[91c] to be virtually equipotent with Epo A on an ovarian (1A9) and a breast cancer (MCF-7) cell line. However, this was only true for one of the two possible *trans*-epoxide isomers (the other one was several hundredfold less active), and the absolute stereochemistry of this active isomer remained unassigned. To establish a basis for further exploitation of the interesting biological properties of the *trans*-Epo A scaffold in general and to address the problem of the absolute stereochemistry of the active epoxide isomer in particular, the Novartis group devised a stereoselective synthesis of such *trans*-epothilones A (Scheme 3).[121] Key steps of the synthesis comprise 1) an aldol reaction between the Schinzer ketone **21**[96,122] and aldehyde **22** (>20:1 selectivity), 2) construction of the C11–C12 bond through Suzuki–Miyaura coupling of olefin **23** and vinyl iodide **24**, 3) Yamaguchi macrolactonization of seco acid **25**, and finally, 4) the stereo- and regioselective epoxidation of **26** under conditions developed by Shi and co-workers,[123] which involve the use of fructose derivative **27** as the epoxidation catalyst and source of chiral induction.[121] As a result of this work, the configuration of the active *trans*-Epo A isomer was established as 12*S*,13*S* (structure **20**; IC_{50} value against the human cervix carcinoma cell line KB-31: 1.01 nM), while the 12*R*,13*R* isomer is at least 500-fold less active in cell proliferation assays (IC_{50} (KB-31): 523 nM).[121]

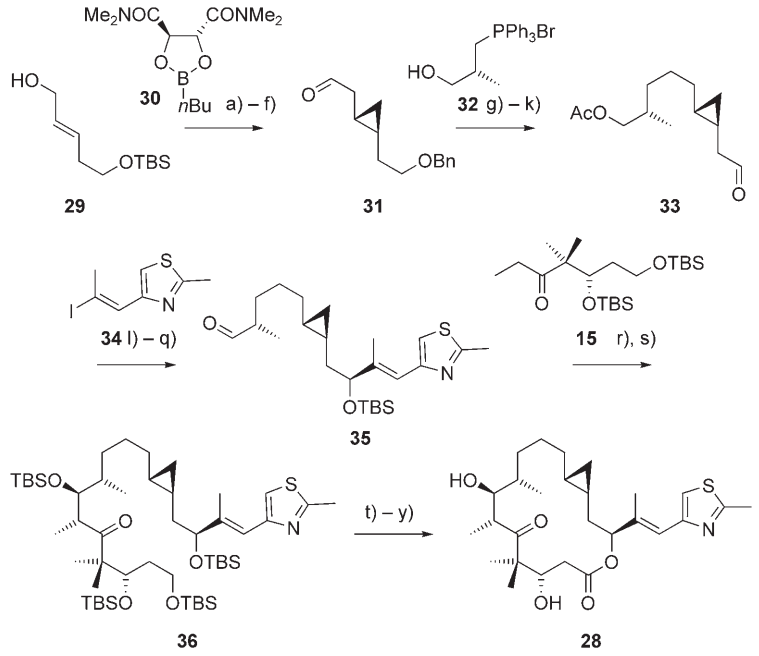


Hybrid analogues incorporating a *trans*-12,13-cyclopropane moiety were subsequently designed by the Nicolaou group at TSRI, including a number of side-chain-modified variants. The general strategy for the synthesis of such *trans*-12,13-cyclopropyl-Epo A analogues is illustrated in Scheme 4 for the synthesis of *trans*-12,13-cyclopropyl-Epo A **28**. As for the synthesis of 12,13-cyclopropyl-Epo B analogues (see Scheme 2), the strategy for the preparation of **28** is based on an early installment of the cyclopropyl moiety, in this case through stereoselective cyclopropanation of allylic alcohol **29**. Likewise, a high degree of selectivity in the crucial aldol step (**35**→**36**) was again ensured through the use of ketone **15**. In contrast to all prior epothilone syntheses, the side chain of **28** was introduced in its entirety in a single step through Nozaki–Kishi coupling between aldehyde **33** and vinyl iodide **34**. While the coupling reaction was nonselective, this deficiency could be corrected through oxidation of the diastereomeric mixture to the C15 ketone and subsequent stereoselective reduction to the desired *S* alcohol. 12,13-Cyclopropane-based analogues of *trans*-Epo A and side-chain-modified variants thereof (see below) proved to possess highly potent biological activity, which in some cases is similar to that of Epo B.[113b,114] In contrast, analogues of *trans*-Epo B are generally less potent than the corresponding *cis* isomers.[114]

In addition to changes in the epoxide structure itself, a variety of modifications of the C26 methyl group in Epo B and Epo D have been investigated. Several of the corresponding analogues have been shown to exhibit potent biological activi-



Scheme 3. Ref. [121]: 9 steps, 25%. a) (1) olefin **23**, 9-BBN, THF, RT; (2) **24**, Cs_2CO_3, $[PdCl_2(dppf)_2]$, Ph_3As, DMF, −10 °C→RT, 63%. b) LiOH, *i*PrOH/H_2O (4:1), 50 °C, 85%. c) TBAF, THF, 64%. d) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, DMAP, THF/toluene, 61%. e) CF_3COOH/CH_2Cl_2, 91%. f) Oxone®, **27** (30 mol%), Bu_4NHSO_4 (cat.), K_2CO_3, MeCN/DME/0.05 M $Na_2B_4O_7{\cdot}10H_2O$ in 4×10^{-4} M Na_2EDTA (2:1:2), RT, 1 h, 27% (~8:1 d.r.; 50% based on recovered starting material). DME = 1,2-dimethoxyethane, EDTA = ethylenediamine tetraacetic acid.

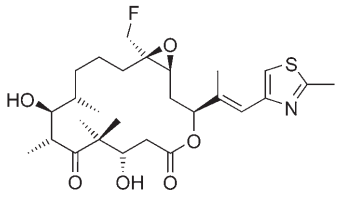


Scheme 4. a) **30**, DME, Et_2Zn, CH_2I_2, CH_2Cl_2, 98% (>90% *ee*). b) Et_3N, $SO_3{\cdot}py$, CH_2Cl_2/DMSO (4:1), 0 °C. c) $MeOCH_2PPh_3Cl$, NaHMDS, THF, −40 °C→25 °C, 81% (2 steps). d) TBAF, THF, 25 °C. e) NaH, BnBr, THF/DMF (5:1), 0 °C→25 °C. f) HCl (cat.), acetone/H_2O (9:1), 50 °C. g) **32**, NaHMDS, TMSCl, THF, 58% (4 steps). h) $(NCO_2K)_2$, HOAc, MeOH/py, 25 °C. i) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C. j) $Pd(OH)_2/C$ (20%), H_2 (100 kPa), EtOAc/EtOH (1:1), 25 °C, 98% (3 steps). k) DMP, CH_2Cl_2, 0 °C→25 °C. l) **34**, $CrCl_2$, $NiCl_2$ (cat.), DMSO, 25 °C, 91% (2 steps). m) DMP, CH_2Cl_2, 0 °C→25 °C, 83%. n) (−)-DIPCl, Et_2O, −15 °C→25 °C, 84%. o) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 91–100%. p) DIBAL, CH_2Cl_2, −78 °C, 93–96%. q) DMP, CH_2Cl_2, 25 °C. r) **15**, LDA, THF, −78 °C, 4 min, 70%. s) TBSOTf, 2,6-lutidine, CH_2Cl_2, −25 °C→25 °C, 94%. t) HF·py, py, 0 °C→25 °C. u) DMP, $NaHCO_3$, CH_2Cl_2, 25 °C. v) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*BuOH/H_2O (4:1), 25 °C. w) TBAF, THF, 25 °C. x) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, 0 °C; then DMAP, toluene, 75 °C, 53% (5 steps). y) 25% CF_3COOH/CH_2Cl_2, 25 °C, 73%. NaHMDS = sodium hexamethyldisilazide, TMS = trimethylsilyl, DMP = Dess–Martin periodinane, DIPCl = diisopinocampheyl chloroborane, DIBAL = diisobutylaluminum hydride.

ty, provided the substituents at C26 are of limited size[124] (see also ref. [109]). Of particular interest among these analogues is 26-fluoro-Epo B **37**, whose in vitro antiproliferative activity is equivalent to that of Epo B[124] and which also exhibits potent in vivo antitumor activity.[125,126]

All of the C12–C13 modifications discussed so far retain a carbon–carbon bond between positions 12 and 13 and thus preserve the polyketide character of the macrocycle. In contrast, the Novartis research group has also investigated a different family of analogues characterized by a disruption of the regular polyketide backbone structure of the macrolactone ring through the replacement of C12 by a nitrogen atom. The first generation of these analogues contained an *N*-methyl or *N*-ethyl C13(O)–N12 amide bond (**38a** and **39b**), which was conceived as a polar isostere of the C12–C13 *cis* double bond in Epo D (assuming a preference of the C–N partial double bond for a *cis* conformation). Unfortunately, analogues **38a** and **38b** were found to lack any tubulin polymerization or antiproliferative activity (IC_{50} values for growth inhibition were between 5 and 10 μM),[103] in spite of the fact that NMR studies with **38a** in DMSO/water mixtures demonstrated a clear preference for a *cis* conformation about the C13–N12 amide bond (*cis*/*trans* ratio ~4:1). The underlying reasons for this lack of biological activity were not elucidated, but subsequent data obtained for other (non-amide-based) structures suggested that increasing the steric bulk at C13 was generally associated with a significant decrease in potency. In light of these findings, Altmann and co-workers continued to explore the potential utility of nitrogen incorporation at position 12 of the macrocycle as a functional handle for further substitution, but without concomitant modification of C13.[127]

At the most straightforward level this approach involved simple acylation of N12, thus leading to amide- and carbamate-based analogues **39**, whose carbonyl oxygen atom could



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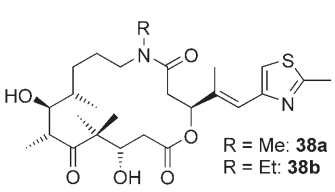
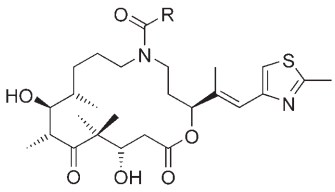


R = Me: **38a**
R = Et: **38b**



R = O*t*Bu: **39a**
R = OCH_2Ph: **39b**
R = OEt: **39c**
R = O*i*Pr: **39d**
R = Me: **39e**
R = Et: **39f**
R = *t*Bu: **39g**
R = Ph: **39h**

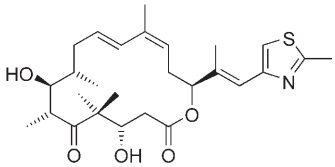
potentially assume the role of the epoxide oxygen in natural epothilones. These compounds have been termed "azathilones" by Altmann and co-workers[128] and they can retain significant antiproliferative activity against human cancer cells, with IC_{50} values <100 nM in the most favorable cases investigated so far (compounds **39a**, **39c**, and **39f**). In particular, *tert*-butyl carbamate **39a** proved to be only 15-fold less active than Epo A against the human cervix carcinoma cell line KB-31 (IC_{50} ~30 nM versus 2.1 nM for Epo A), making it roughly equipotent with Epo C.[127] Interestingly, analogues **39a**–**h** are generally less active against the P-gp-overexpressing multidrug-resistant KB-8511 line than the corresponding drug-sensitive KB-31 parental line, thus indicating that these compounds are (sometimes significantly) better P-gp substrates than natural epothilones. The structural basis for this phenomenon is not understood at this point, but the finding is in line with a more general tendency for polar epothilone analogues, such as compounds incorporating amide bonds or additional hydroxy groups, to exhibit increased resistance factors in the KB-31/KB-8511 cell line pair (that is, increased ratios of IC_{50} (KB-8511)/IC_{50} (KB-31); M. Wartmann, K.-H. Altmann, unpublished observations). Compound **39a** exhibits a rather moderate resistance factor of only ~3. The azathilone scaffold has been further explored by the Altmann group in combination with a particular side chain modification,[128] and these analogues are discussed in more detail below.

C9–C11 modifications

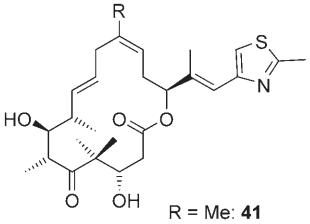
Initial studies on structural modifications in the C9–C11 trimethylene segment adjacent to the epoxide moiety had shown that analogues with enlarged or decreased ring size (based on the incorporation or removal of a methylene group) are all characterized by a substantial loss in activity.[109,129] Likewise, modifications designed to stabilize the purported bioactive conformation in this region, based on pharmacophore modeling, only led to inactive analogues.[67,104,130] More recently, however, a number of new epothilone analogues with modifications in the C9–C11 region have been described that are characterized by potent antiproliferative activity in vitro, some of which also show an improved in vivo pharmacological profile over Epo B and Epo D. These analogues are characterized by the presence of *trans* double bonds either between C10 and C11 or between C9 and C10. They have been obtained through both isolation from bacterial fermentation broths[10–12] and total chemical synthesis (spearheaded by the Danishefsky group).[101,102,131–134] For example, the heterologous expression of the modified epothilone polyketide synthase in *Myxococcus xanthus* yields epothilone 490 (**40**) as a minor fermentation product, whose in vitro antiproliferative activity is only 3–4-fold lower than that of the parent compound Epo D against a variety of human cancer cell lines.[11]

This finding has corroborated and extended earlier results reported by Höfle and co-workers for 10,11-dehydro-Epo C, which had been isolated as a minor fermentation product from cultures of the myxobacterium *S. cellulosum*.[10] Following the discovery of epothilone 490 (**40**) by researchers at Kosan,[11] the Danishefsky group skillfully adapted their second-generation synthesis of Epo D to provide sufficient quantities of the former for further investigation.[101] Unfortunately, in vivo studies with Epo 490 gave disappointing results, and the compound was abandoned owing to unfavorable pharmacokinetic properties.[102a,135] Even more intriguing than epothilone 490 proved to be its positional isomer *trans*-9,10-didehydro-Epo D (**41**) and the corresponding Epo B analogue **43**, both of which are generally more potent inhibitors of human cancer cell proliferation than the corresponding parent compounds Epo D and B, respectively.[102a,133–136]

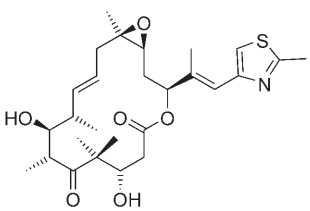
In light of the promising biological activity of these analogues, Danishefsky and co-workers developed a highly efficient route to *trans*-9,10-didehydro-Epo D (**41**), which is based on ring closure through RCM as the ultimate key step (Scheme 5).[102a,133,136] Other crucial steps in the buildup of the macrocycle include the formation of the stereocenters at C6/



epothilone 490 (**40**)



R = Me: **41**
R = CF_3: **42**



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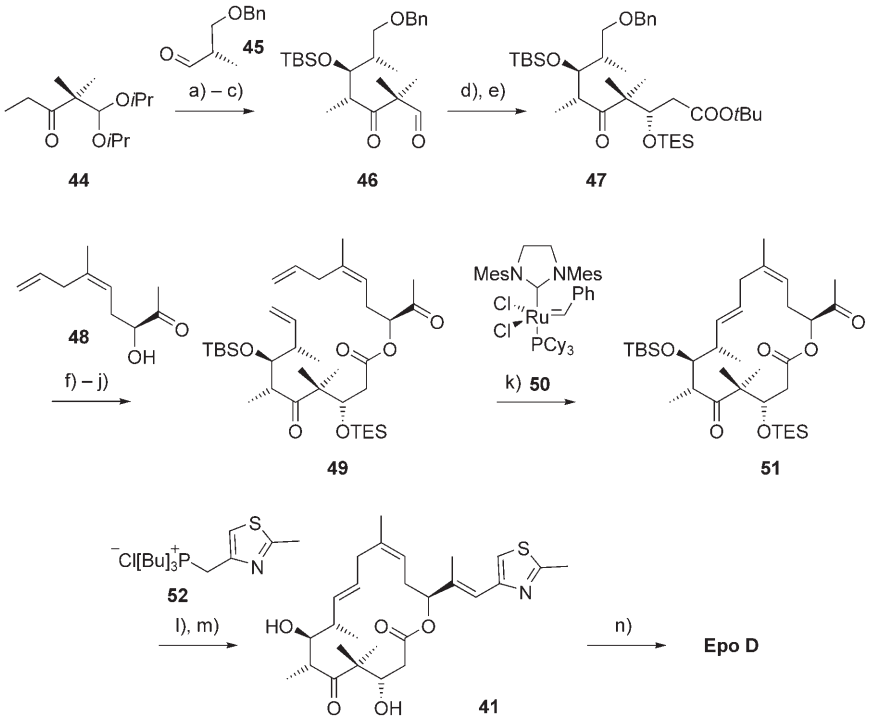
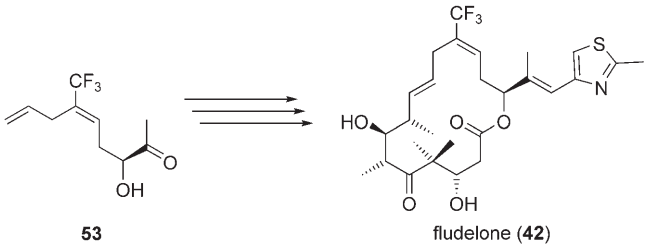


Scheme 5. Ref. [102a, 133]: a) LDA, THF, −90 °C, 78% (based on aldehyde **45**), 85:15 d.r. b) TBSOTf, 2,6-lutidine, CH_2Cl_2, −40 °C→20 °C, 97%. c) *p*-TsOH (cat.), THF/H_2O (4:1), reflux, 98%. d) AcO*t*Bu, LDA, $[CpTiCl(OR)_2]$ (R = 1,2:5,6-di-*O*-isopropylidine-α-L-glucofuranos-3-*O*-yl), Et_2O, −78 °C, 86% (> 20:1 d.r.). e) TESCl, DMF, 0 °C→RT, 98%. f) H_2, Pd/C (10%), EtOH, 83%. g) TPAP, NMO, CH_2Cl_2, 95%. h) $MePPh_3I$, *n*BuLi, THF, −78 °C→−5 °C, 78%. i) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C→RT. j) **48**, EDC, DMAP, CH_2Cl_2, 0 °C→RT, 81% (2 steps). k) **50**, toluene, 110 °C, 78% (for the desired *trans* isomer). l) **52**, KHMDS, THF, −78 °C→−20 °C, 76%. m) HF·py, THF, 97%. n) Refs. [133]: $TrisNH_2NH_2$, Et_3N, 1,2-dichloroethane, 50 °C, 91%. Ts = toluenesulfonyl, Cp = cyclopentadienyl, TPAP = tetra-*n*-propylammonium perruthenate, NMO = *N*-methylmorpholine-*N*-oxide, KHMDS = potassium hexamethyldisilazide.

C7 by an aldol reaction between ketone **44** and aldehyde **45**, and the highly selective (> 20:1) aldol reaction between aldehyde **46** and the titanium enolate of *tert*-butylacetate employing the Duthaler–Hafner reagent.[137] The RCM with triene **49** (employing the second-generation Grubbs catalyst **50**) proceeded with high selectivity to provide the desired 9,10-*trans* isomer in 78% yield. As **41** can be converted in high yield (91%) into Epo D, the route depicted in Scheme 5 also embodies a highly efficient new strategy for the synthesis of Epo D and Epo B.

Compound **41** possesses markedly increased antiproliferative activity relative to Epo D against the human leukemia cell line CCRF-CEM (IC_{50}: 0.9 nM for **41** versus 3.6 nM for Epo D).[102a, 133] Likewise, Epo B analogue **43** is more potent in vitro than Epo B, including activity against multidrug-resistant cell lines.[134] In contrast to **41**, the corresponding *cis* analogue, *cis*-9,10-didehydro-Epo D, has been reported by White et al.[138] to be ~30-fold less active than Epo D against the human cervix cancer cell line KB-31. (Note that the compound assumed to be *trans* in reference [138] was found later to be the *cis* isomer[139]). These experimental data are in agreement with recent findings from spectroscopic studies,[140] which suggest that the tubulin-bound structure of epothilones features *anti*-periplanar conformations about the C9–C10 and C10–C11 bonds. Both Epo D derivative **41**[102a, 133, 134, 141] as well as epoxide **43**[134] have been shown to exhibit potent in vivo antitumor activity in the human breast cancer model MX-1, and for **41** this effect has been specifically ascribed to a combination of enhanced antiproliferative activity and improved plasma stability in rodents.[133, 135] Unfortunately, **41** in these experiments was also associated with significant toxicity, which may represent a serious drawback in the development of the compound as a potential drug candidate.[136] Nevertheless, the compound (as KOS-1584) has been promoted to clinical development status and is currently undergoing phase I clinical trials.

As an extension of their work on **41**, which was driven by the desire to identify further improved epothilone analogues, Danishefsky and co-workers discovered that the C26-trifluoro analogue **42** (fludelone) showed a superior pharmacological profile over its parent compound **41**, with excellent in vivo antitumor activity in the absence of lethality or irreversible toxicity.[135, 141, 142] The synthesis of **42** follows the same synthetic route as that of **41**, except that the "C10–C15" fragment **48** is replaced by its corresponding trifluoro derivative **53**.[102a, 135]

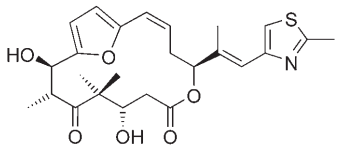


While fludelone exhibits similar in vitro antiproliferative activity to Epo D and thus is somewhat less active than **41**, the presence of the trifluoromethyl group leads to a substantial increase in metabolic stability in mouse plasma and also human liver microsomes.[141] Most importantly, however, fludelone exhibits exquisite in vivo antitumor activity in a variety of mouse models of human cancer with excellent tolerability.[135, 141] This includes models of human breast (MX-1), lung (A549), and colon (HCT-116) carcinomas as well as leukemia (CCRF)[141] and multiple myeloma (RPMI 8226 subcutaneous xenograft model and disseminated CAG MM model).[142] Sustained tumor remis-

sions ("cures") were observed upon treatment with fludelone in several experiments, and the compound proved to be clearly superior to both taxol and the nonfluorinated parent compound **41**. (At roughly equitoxic dose levels, the antitumor effects obtained with the latter were significantly less pronounced than those observed with fludelone).[141] Similar activity was observed against drug-sensitive and multidrug-resistant tumors (which were poorly responsive to Taxol®); likewise, similar effects against MX-1 xenografts were observed after iv or oral administration of equal doses of fludelone, thus indicating that the compound has high oral bioavailability.[141] As a general conclusion from the comprehensive in vivo evaluation of fludelone and other preclinical studies on Epo D and its derivatives, it has been suggested that "the epothilones lacking the 12,13-epoxide linkage, although less potent, seem to be displaying the more exploitable therapeutic indices".[141] This stands in marked contrast to the fact that the majority of compounds that have entered clinical trials still retain the epoxide moiety. However, independent of the general validity of the above hypothesis, the discovery of fludelone marks a major milestone in epothilone-based anticancer drug discovery and it represents the preliminary culmination of the extensive efforts of the Danishefsky group in this area. Although no official information is available on this issue, it seems clear that fludelone is bound to enter clinical development in the immediate future (if it has not done so already).

Danishefsky's work on unsaturated analogues has also led to the discovery that the insertion of an additional methylene group between C11 and C12 becomes possible in the presence of a *trans* double bond between C10 and C11 without substantial loss in the antiproliferative activity,[132] thus putting in perspective the earlier findings on the relationship between ring size and antiproliferative activity.

A different approach to restrict the conformational mobility of the epothilone macrocycle in the C9–C11 region has been reported by Schinzer et al.,[143] who designed and synthesized furano-Epo C analogue **54**. Compared with the parent compound Epo C, **54** exhibits ~20-fold decreased antiproliferative activity against the drug-sensitive cervix carcinoma cell line KB-31 (IC_{50} value of 519 nM versus 25 nM for Epo C). Activity is fully retained against the multidrug-resistant KB-8511 line (IC_{50} = 492 nM). Compound **54** is the only active epothilone analogue described so far that lacks a chiral center at C8.

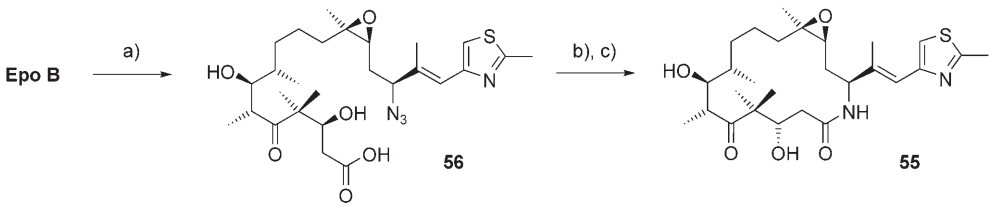


54

C1–C6 modifications

One of the most important modifications of the epothilone scaffold reported so far consists in the replacement of the bridging lactone oxygen atom by nitrogen, thus transforming a macrolactone into a macrolactam ring.[107,144] This strategy was spearheaded by the BMS group and has produced the most advanced clinical development compound identified so far (**55**, BMS-247550; Scheme 6). Compound **55** was reportedly conceived in order to overcome the limited stability of Epo B in rodent plasma which was thought to foreshadow future problems in humans.[144] On the other hand, Epo B possesses very potent in vivo antitumor activity in mice, despite its limited plasma stability.[15,34,36] In addition, Epo D has been shown to be significantly more stable in human than in rodent plasma,[145] thus indicating that lactone-based macrocycles (including Epo B itself) may not be associated with an inherent metabolic stability problem (see also ref. [141]). In fact, Epo B has recently been advanced to phase III clinical trials which indicates that the compound is sufficiently stable in humans to elicit promising therapeutic effects.

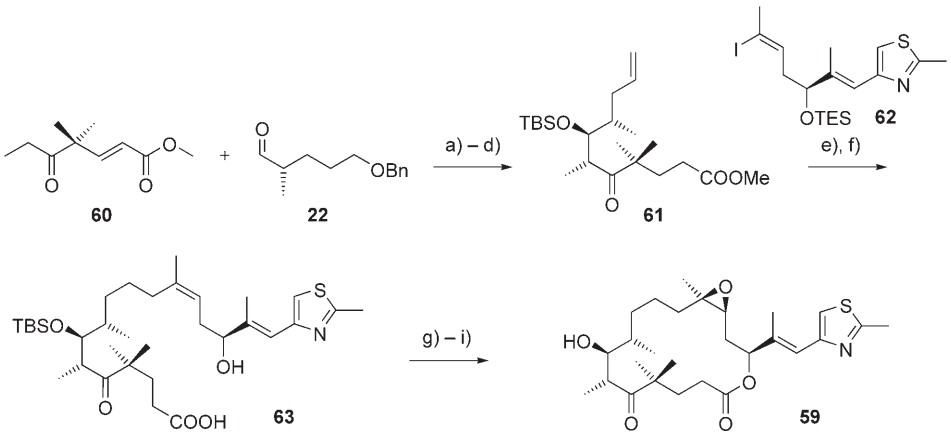
The BMS group has devised a highly original strategy for the preparation of epothilone lactams through semisynthesis (Scheme 6). The approach exploits the allylic nature of the ester group in the macrolactone ring, which allows the preparation of **51** in only three steps from Epo B.[144] Thus, the palladium-catalyzed opening of the lactone ring in Epo B produces azide **56** with complete retention of configuration. Subsequent reduction of the azide moiety to an amino group followed by intramolecular amide bond formation under standard conditions then furnishes the desired macrolactam **55**. As an alternative to this semisynthetic approach, the total synthesis of **55** has been reported by Danishefsky and co-workers[146] (see also ref. [147]). While BMS-247550 **55** is a potent inducer of tubulin polymerization, its antiproliferative activity is about one order of magnitude lower than that of Epo B[25,144] (IC_{50} values against the human colon carcinoma cell line HCT-116 are 3.6 nM and 0.42 nM, respectively, for **55** and Epo B[144]). Studies in Altmann's laboratory have also shown that the compound exhibits significantly decreased activity against multidrug-resistant cancer cell lines, thus indicating that **55** is a substrate for the P-gp efflux pump. For example, IC_{50} values of 2.85 nM and



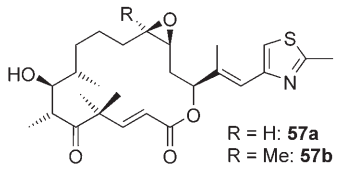
Scheme 6. Ref. [144]: a) $[Pd(PPh_3)_4]$, NaN_3, 45 °C, 60–70%. b) Me_3P, 71%. c) EDC, HOBt, 65%. HOBt = 1-hydroxybenzotriazole.

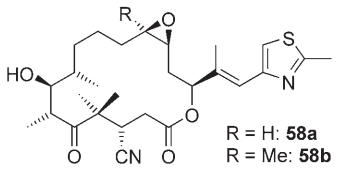
128 nM were observed against the drug-sensitive human cervix carcinoma cell line KB-31 and its P-gp-overexpressing multidrug-resistant KB-8511 variant, respectively.[25] Similar observations on the reduced activity of **55** against P-gp-overexpressing human cancer cell lines have also been reported by the Sloan–Kettering[146] and Schering[56] research groups.

The BMS group, in collaboration with Höfle's research group at the GBF, has also investigated the effect of modifications at the 3-position of the macrolactone ring. This has included 3-deoxy-2,3-didehydro derivatives **57a** and **57b**, which can be readily obtained from natural epothilones through bis-formylation at O3 and O7 followed by treatment with ammonia, as well as 3-deoxy-3-cyano derivatives **58a** and **58b**, which are also accessible through semisynthesis from Epo A or B.[148]



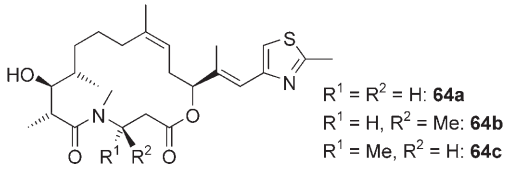
Scheme 7. Ref. [149]: a) LDA, −78 °C, 58%. b) TBSOTf, 2,6-lutidine, −10 °C, 82%. c) H_2, Pd/C, MeOH, 97%. d) *o*-NO_2-(C_6H_6)SeCN, Bu_3P, $NaHCO_3$, H_2O_2, RT, 69%. e) (1) 9-BBN, THF, RT; (2) **62**, Cs_2CO_3, [$PdCl_2(dppf)_2$], Ph_3As, DMF, −10 °C→RT, 55%. f) LiOH, *i*PrOH/H_2O (4:1), 60 °C, 98%. g) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, 0 °C. h) HF·py, THF, RT, 90% (2:1 mixture of isomers at C15). i) $MeReO_3$, H_2O_2/py/H_2O, RT, 72% (9:1 mixture of epoxide isomers).





Quite remarkably, analogues **57** retain most of the activity of the parent natural products, with **57b**, for example, being only fourfold less potent than Epo B against the human colon carcinoma cell line HCT-116. Likewise, the 3-deoxy-(3*S*)-cyano derivative **58a** exhibited only twofold decreased activity relative to Epo A, while the corresponding 3*R* isomer is significantly less active. What had not been investigated until very recently was the activity of the *saturated* 3-deoxy derivatives of Epo A and B, perhaps because they may have been expected to be significantly less potent than the α,β-unsaturated lactones **57**, owing to the lack of any direct conformational constraint about the C2–C3 bond. To address this issue, Altmann and co-workers recently synthesized 3-deoxyEpo B **59** as part of a more comprehensive program directed at the development of hypermodified epothilone analogues and, ultimately, of new scaffolds for microtubule inhibition. The synthesis of **59** followed an overall strategy that had been previously developed for the synthesis of *trans*-Epo A (**20**) as well as a new family of side-chain-modified epothilone analogues (Scheme 7).[149] Thus, formation of the C11–C12 bond was achieved through Suzuki–Miyaura coupling of vinyl iodide **62** with olefin **61**, which had in turn been obtained via the (poorly selective) aldol reaction of the α,β-unsaturated γ-keto ester **60** and aldehyde **22**. Saponification, accompanied by TES cleavage and subsequent Yamaguchi macrolactonization of **63**, followed by protecting group removal then provided 3-deoxyEpo D, which could be epoxidized with the $MeReO_3/H_2O_2$/pyridine/water system[150] to provide the desired target compound **59** with remarkable selectivity (9:1). Even more remarkable, perhaps, is the fact that 3-deoxyEpo B (**59**) retains highly potent biological activity, which is manifest in IC_{50} values for human cancer cell growth inhibition in the low nanomolar range. For example, the IC_{50} values of **59** against the human cervix carcinoma cell lines KB-31 and KB-8511 are 7.4 nM and 4.0 nM, respectively, versus 0.29 nM and 0.22 nM, respectively, for Epo B.[149] These findings clearly demonstrate that the presence of a 3-hydroxy group in epothilones is not a crucial requirement for potent biological activity, even in the absence of a hard conformational constraint about the C2–C3 bond, as it is still present in α,β-unsaturated lactones **57**.

As part of their general program on the synthesis and biological evaluation of aza-epothilones, that is, epothilone analogues in which one of the carbon atoms in the macrolide backbone is replaced by nitrogen, Altmann and co-workers have also investigated 4-aza analogues of Epo D, such as **64a–c**.[151] These analogues were inspired by the fact that one of the characteristic features of the tubulin-bound NMR structure



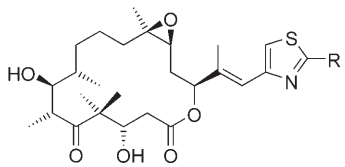
of Epo A[140] is the presence of a *syn*-periplanar conformation about the C4–C5 bond. The same geometry would be enforced in analogues of type **64**, provided that the amide bond between N4 and C5 would be present in a *cis* conformation. At the same time, preliminary modeling studies indicated that

the presence of a *cis* amide bond in this position should allow replacement of the C1–C4 segment by various types of β-amino acids without causing significant distortions in the bioactive conformation of the C5–O16 segment. Apart from these structural considerations, structures of type **64** also appeared attractive for chemical reasons, as they would lend themselves to an efficient combinatorial chemistry approach employing a single advanced intermediate (that is, a carboxylic acid encompassing the C21–C5 fragment (epothilone numbering), compound **69**, Scheme 8). As illustrated in Scheme 8 for analogues **64b**,**c**, the synthesis of 4-aza-Epo D analogues of type **64** involves the highly stereoselective aldol reaction between acyloxazolidinone **65** and aldehyde **22**, which under optimized conditions provided the desired aldol product as a single isomer in 90% isolated yield. The protected aldol product **66** was then converted into iodide **67**, which underwent smooth Negishi coupling with vinyl iodide **68**. Removal of the chiral auxiliary with LiOOH in THF then provided carboxylic acid **69** as the central key intermediate for the synthesis of epothilone analogues of type **64**. Coupling of this acid with racemic *N*-methyl-β-alanine (**70**) followed by selective removal of the TBS protecting group from O15, Yamaguchi macrolactonization, and finally cleavage of the O7 TBS group gave a mixture of the target compounds, which could be easily separated at this stage to provide **64b** and **64c** as single isomers.

So far, only a limited number of analogues of type **64** have been investigated, all of which were found to lack any meaningful tubulin polymerizing or antiproliferative activity.[151] However, a greater number of these structures (incorporating different types of α-, β- and γ-amino acids) will have to be investigated before a final conclusion on the (pharmaceutical) validity of this modification approach can be drawn. Based on the chemistry illustrated in Scheme 8, the synthesis of such additional analogues should be a straightforward undertaking.

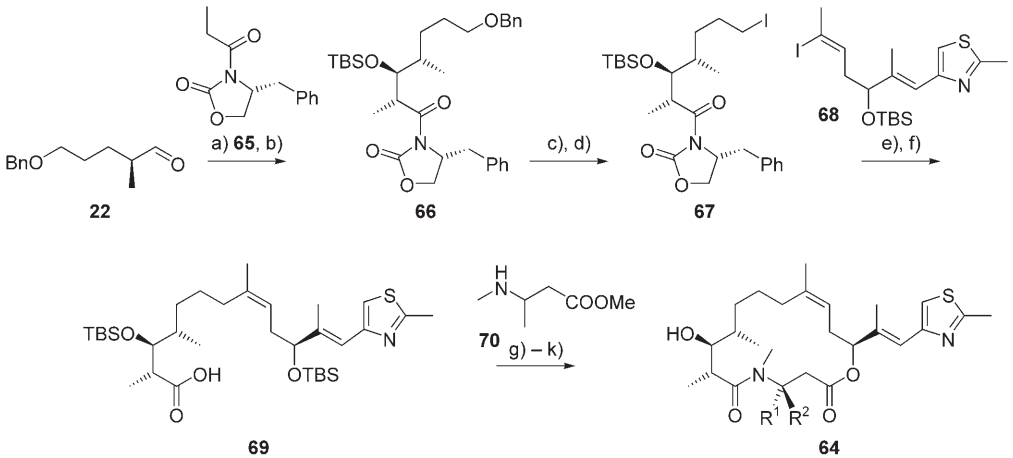
Side chain modifications

The unsaturated heterocycle-bearing side chain of epothilones is the most obvious site for structural alterations that could modulate the physicochemical and thus pharmacokinetic properties of the natural product leads. Therefore, it is not surprising that this part of the epothilone structure has been heavily targeted for SAR studies. Modifications of the epothilone side chain include the replacement of the thiazole ring by other heterocyclic structures[109,110,152] or simple phenyl groups,[109,153] modifications at the 2- and 4-positions of the thiazole ring,[110,114,154–157] and the synthesis of C16-desmethyl Epo B.[153,158] These studies have shown, for example, that an oxazole ring can be substituted for the natural thiazole heterocycle without any loss in biological potency[109,110] or that the removal of the allylic methyl group at C16 leads to only a minor decrease in activity.[67] Likewise, replacement of the 2-methyl group on the thiazole ring by relatively small substituents such as hydroxymethyl, aminomethyl, fluoromethyl, methylthio, or ethyl is well tolerated. In contrast, bulkier substituents can produce a substantial loss in potency.[25,110,155] Among these side-chain-modified analogues, C21-amino-Epo B (BMS-310705; **71**)[24,53,54] and 20-desmethyl-20-methylsulfanyl-Epo B (ABJ879; **72**)[55] have entered phase I clinical trials.



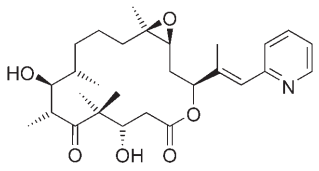
R = CH_2NH_2: BMS-310705 (**71**)
R = SCH_3: ABJ879 (**72**)

One of the most significant findings in the area of heterocycle modifications is the fact that pyridine-based analogue **73**



Scheme 8. Ref. [151]: a) **65**, Bu_2BOTf, Et_3N, CH_2Cl_2, 0 °C, 1 h; then addition of **22**, −78 °C, 3 h, 90%. b) TBSOTf, 2,6-lutidine, 0 °C→RT, 4 h, 91%. c) H_2, Pd/C (10%), MeOH, RT, 6 h, 82%. d) (1) MsCl, Et_3N, 0 °C, 30 min; (2) NaI, acetone, 50 °C, 3 h, 81% (2 steps). e) (1) Zn-Cu, 1,2-dibromoethane, TMSCl, DMA, TMSOTf; (2) **68**, [$Pd(PPh_3)_4$], benzene, 65 °C, 71%. f) LiOH, H_2O_2, THF/H_2O (4:1), RT, 3 h; 70%. g) **70**, HBTU, *i*Pr_2EtN, DMF, RT, 3 h, 72%. h) TBAF, HOAc, RT, 6 h, 65%. i) LiOH, THF/H_2O (7:1), RT, 6 h, 64%. j) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, RT, 30 min; then DMAP, toluene, RT, 1.5 h, 90%. k) (1) HF·py, MeCN, RT, 6 h, 86%; (2) flash chromatography, **64b**: 30%; **64c**: 30%. DMA = *N*,*N*-dimethyl acetamide, HBTU = *O*-benzotriazol-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, TBAF = tetrabutylammonium fluoride.

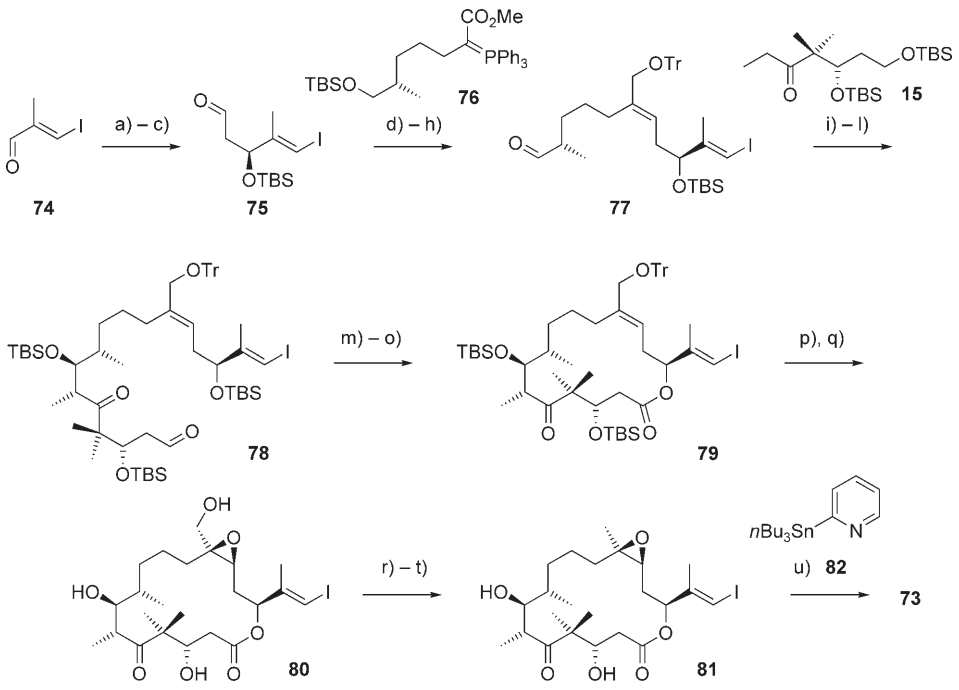
and methyl-substituted variants thereof are basically equipotent with Epo B,[152a] thus indicating that the presence of a 5-membered heterocycle is not an a priori requirement for biological activity. These pyridine-based analogues were investi-



73

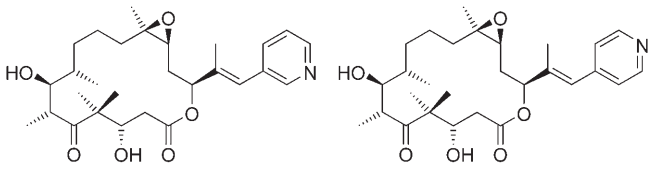
gated as part of the collaboration between Nicolaou's laboratory at TSRI and the Novartis group in Basel. Within the framework of these studies Nicolaou and co-workers have developed a highly efficient general approach to the synthesis of side-chain-modified epothilone analogues, which is based on vinyl iodide **81** as a central intermediate[152,153] (Scheme 9) and which is not limited to the synthesis of pyridine-containing analogues.[116,153] (For the use of vinyl iodide **80** (Scheme 9) in the synthesis of C26 and side-chain-modified analogues, see ref. [157].)

The construction of this precursor involves an early key asymmetric Brown allylboration of aldehyde **74** to establish the stereocenter at C15 (Scheme 9). The northern half of the epothilone scaffold is then generated with the desired olefin geometry through Wittig reaction of ylide **76** with aldehyde **75**. Subsequent functional group manipulations at C26 and C7 provide aldehyde **77**, which undergoes highly selective aldol coupling (d.r. > 10:1) with the lithium enolate of **15**. Cleavage of the primary TBS ether in the resulting aldol product followed by oxidation of the primary hydroxy group then yields aldehyde **78**. Oxidation of **78** with $NaClO_2$ produces the corresponding carboxylic acid, which undergoes smooth Yamaguchi macrolactonization to provide fully protected macrolactone **79**. Following removal of the protecting group at C26–O, the presence of the free allylic hydroxy group then allows stereoselective installment of the epoxide moiety through directed Sharpless asymmetric epoxidation (→**80**). Vinyl iodide **81** can be procured through conversion of the C26 hydroxy group of **80** to the iodide and subsequent reduction thereof with $NaBH_3CN$. This route addresses the major stereochemical issues associated with previous Epo B syntheses, such as the construction of the C12–C13 double bond with high *E* selectivity, the stereoselective formation of the C6–C7 aldol unit, and the stereoselective installment of the epoxide moiety. A particularly remarkable aspect of this route is the stability of the vinyl iodide moiety attached to C15, which proved to be sufficiently resistant to all reaction conditions to which it was exposed in the course of macrocycle construction. Compound **81** undergoes Stille-type cross-coupling with a variety of aromatic stannanes, such as **82**, thus providing convenient access to a broad range of epothilone analogues containing a variety of different heterocycles in place of the natural thiazole moiety.



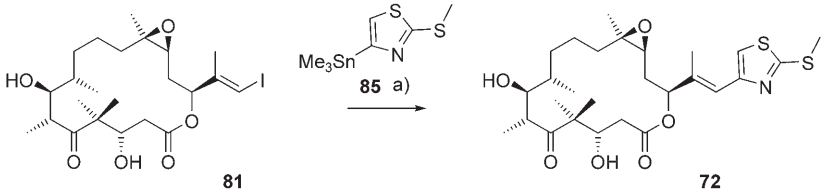
Scheme 9. Ref. [152, 157]: a) (+)-Ipc$_2$B(allyl), Et_2O, −100 °C, 91 %. b) TBSCl, imidazole, DMF, 0 °C→25 °C, 84 %. c) (1) OsO_4, NMO, THF/*t*BuOH/H_2O (5:5:1), 0 °C→25 °C, 89 %; (2) $NaIO_4$, MeOH/H_2O (2:1), 0 °C, 0.5 h, 92 %. d) **76**, benzene, reflux, 92 %. e) DIBAL, THF, −78 °C, 71 %. f) TrCl, DMAP, DMF, 80 °C, 94 %. g) HF·py, py/THF, 0 °C, 67 %. h) SO_3·py, Et_3N, DMSO/CH_2Cl_2 (1:1), 0 °C, 98 % (crude). i) **15**, LDA, THF, −78 °C→−40 °C; **77**, THF, −78 °C; AcOH, −78 °C→0 °C, 74 %. j) TBSOTf, 2,6-lutidine, CH_2Cl_2, −20 °C→0 °C, 90 %. k) HF·py, py/THF, 0 °C, 84 %. l) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C→0 °C, 98 %. m) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *t*BuOH/H_2O (9:2), 25 °C, 100 %. n) TBAF, THF, 0 °C→25 °C, 95 %. o) Et_3N, 2,4,6-$Cl_3C_6H_2C(O)Cl$, DMAP, THF, 84 %. p) HF·py (25 % *v*/*v*), THF, 0 °C→25 °C, 86 %. q) (+)-diethyl-L-tartrate, [Ti(*i*PrO)$_4$], *t*BuOOH, CH_2Cl_2, M.S. (4 Å), −30 °C, 2 h, 67 %. r) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C→25 °C. s) NaI, acetone, 25 °C, 75 % (2 steps). t) $NaBH_3CN$, DMPU, 45 °C, 70 %. u) **82**, [$PdCl_2(MeCN)_2$], DMF, 25 °C, 66 %. Ipc = isopinocampheyl, Tr = triphenylmethyl, DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone.

The investigation of pyridine-based epothilone analogues by the TSRI and Novartis groups has also included structures in which the nitrogen atom of the pyridine ring was moved to the *meta* and *para* positions relative to the attachment point of the vinyl linker between the heterocycle and the macrolactone ring (analogues **83** and **84**).[152a] These isomeric pyridine epothilones were found to be significantly less potent than **73**, which clearly illustrates that the presence of a nitrogen atom in the *ortho* position to the vinyl linker is of crucial importance for epothilone-like cellular activity (that is, single-digit nanomolar to sub-nanomolar IC_{50} values in the Epo B series). As **83** and **84**



also exhibit decreased activity in tubulin polymerization experiments (relative to **73**), the differences in cellular potency between **73**, **83**, and **84** were taken to reflect differences in the compounds' tubulin binding affinities. Additional support for this assumption came from subsequent structural studies on a complex between a two-dimensional tubulin polymer sheet and Epo A, which showed the thiazole nitrogen atom to be involved in a hydrogen bond with His 227 in β-tubulin.[69] However, recent work by Altmann and co-workers on a different family of side-chain-modified epothilone analogues has indicated that the situation may be more complex (see below).

Vinyl iodide **81** has also served as an intermediate in Nicolaou's synthesis of 20-desmethyl-20-methylsulfanyl-Epo B **72** (Scheme 10),[114] which has also been prepared by the Novartis group by means of

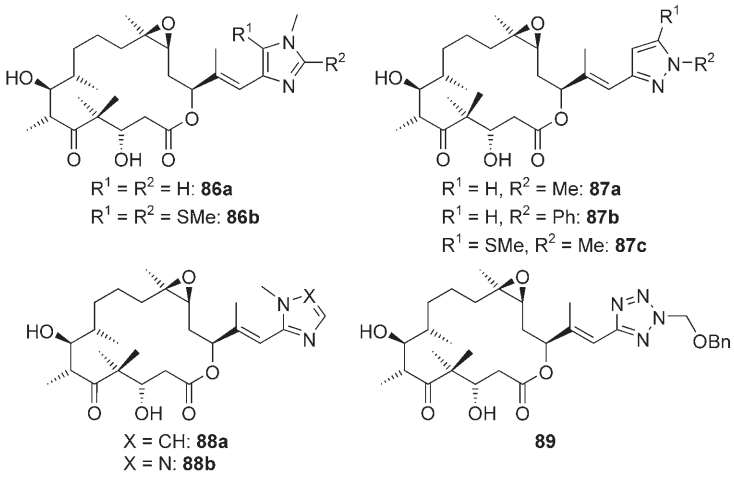


Scheme 10. Ref. [114]: a) **85**, $[Pd_2(dba)_3]\cdot CHCl_3$, CuI, $AsPh_3$, 25 °C, 72%. dba = dibenzylidene acetone.

semisynthesis.[55] As indicated above, compound **72** (as ABJ879) has entered Novartis-sponsored phase I clinical trials as a potential follow-up compound for EPO906.[55] ABJ879 induces tubulin polymerization in vitro with slightly higher potency than Epo B and taxol and it shows markedly higher antiproliferative activity. Thus, the average IC_{50} value for growth inhibition across a panel of drug-sensitive human cancer cell lines has been reported as 0.09 nM for ABJ879 versus 0.24 nM for Epo B and 4.7 nM for taxol.[55] Like Epo B, ABJ879 retains full activity against cancer cells overexpressing the drug efflux pump P-gp or harboring tubulin mutations.

Building on their highly efficient synthetic approach to side-chain-modified analogues of Epo B via the intermediacy of vinyl iodide **81** and motivated by the success of analogue ABJ879, the Nicolaou group has continued to explore the potential of heterocycle modifications for the development of further improved Epo B analogues. For example, to assess the effect of alternative sulfur-containing C20 substituents, 21-methylsulfanyl-Epo B and the acetate ester of 20-desmethyl-20-thiomethyl-Epo B were synthesized, but both compounds displayed slightly decreased antiproliferative activity compared with Epo B.

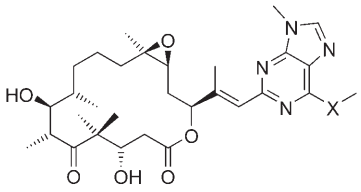
To expand the structural scope of side chain modifications based on 5-membered heterocycles beyond simple thiazole or oxazole ring systems, Epo B analogues incorporating functionalized imidazole, pyrazole, triazole, and tetrazole rings were investigated (analogues **86**–**89**).[152b] Rather surprisingly, imida-

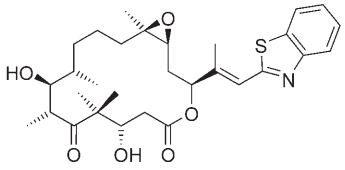


zole-containing side chains, such as in **86** and **88a** caused a substantial loss in activity, despite the fact that they are part of the side chains of eleutherobin or sarcodictyins, which are potent natural product microtubule inhibitors. Although earlier attempts at incorporating multiple nitrogen atoms into the heterocycle moiety (such as the change from a pyridine to a pyrimidine ring[25,103]) had led to decreased activity, the replacement of the imidazole moiety by a triazole or a tetrazole ring (analogues **88b** and **89**, respectively) still appeared to be of value, as it would allow one to probe the importance of the electron density of smaller rings for biological activity. While triazole derivative **88b** showed no appreciable activity, the tetrazole-based analogue **89**, in a surprising turn of events, proved almost equipotent to Epo B. This is particularly remarkable, as the analogous 2-substituted thiazole derivative is a hundredfold less active than **89** across the same panel of human cancer cell lines.[21] Highly potent antiproliferative activity was also observed for pyrazole-based analogues such as **87**. Compound **87a**, which incorporates an *N*-methyl pyrazole moiety and which may be regarded as a completely isosteric analogue of Epo B, proved to be 2–18-fold more potent than the latter, depending on the cell lines investigated (for example, the IC_{50} value of **87a** against the human cervix carcinoma cell line KB-31 is 0.19 nM versus 0.42 nM for Epo B). While phenylpyrazole derivative **87b** was slightly less potent than Epo B, quite intriguingly, it showed enhanced ac-

tivity against the Epo A-resistant 1A9/A8 cell line in comparison with the parental Epo A-sensitive 1A9 line. The most potent pyrazole-based analogue, however, proved to be the methylsulfanyl derivative **87c**, which was found to be significantly more active than Epo B in all cell lines investigated. For example, the compound shows 17-fold greater activity than Epo B against both the human ovarian carcinoma cell line 1A9 and its Epo A-resistant variant 1A9/A8 (IC_{50} values of 0.06 nM and 0.6 nM, respectively, versus 0.99 nM and 10.0 nM for Epo B), and its activity against the taxol-resistant 1A9/PTX10 line (IC_{50} = 0.1 nM) is 78-fold higher than that of Epo B. Based on these data, compound **87c** represents the most potent epothilone analogue identified to date.

To further probe the steric requirements of the epothilone binding pocket around the heterocycle moiety, a series of bicyclic aromatic thiazole replacements was synthesized and tested for antiproliferative activity.[152b] Analogues incorporating purine-based side chains such as **90a** and **90b** exhibit very potent growth inhibitory activity, with methylsulfanyl derivative **90a** being fourfold more potent than Epo B against both the 1A9/PTX10 and 1A9/A8 cell lines. Guided by previous data on Epo C analogues incorporating a thiazol-2-yl moiety (as opposed to the natural thiazol-4-yl group)[155] or a 2-quinoline moiety (Nicolaou et al., unpublished observations) as a thiazole replacement, benzothiazole analogue **91** was designed and synthesized.[152b] As for **90a**, this compound was found to be





up to fourfold more potent than Epo B. Taken together, these findings suggest that the side chain binding pocket on tubulin can accommodate significantly expanded heterocycles if the additional steric bulk is located in the northeastern quadrant of the structure. It is worth noting that this is contrary to the effects that have been observed for the incorporation of bulky substituents at the 2-position of the thiazole ring in Epo B, which have been reported to cause a substantial loss in biological potency.[25,155]

Again employing methodology which they had initially developed for the synthesis of analogues incorporating the natural 2-methylthiazole-based side chain, Nicolaou's group at TSRI has also investigated side-chain-modified variants of *cis*- and *trans*-cyclopropyl epothilones (Figure 2).[114,116] The synthesis of these hybrid structures was based on the strategies outlined in Schemes 2 and 4 for 12,13-cyclopropyl-Epo B and 12,13-*trans*-cyclopropyl-Epo A, respectively (or slight variants thereof for the corresponding *trans* and *cis* analogues), employing different heteroarylated vinyl iodides such as **18** (Scheme 2) or **92**. These studies revealed that *trans*-cyclopropane derivatives of Epo B generally exhibit significantly lower activity than the corresponding *trans*-Epo A derivatives (either cyclopropane- or epoxide-based); that is, in contrast to 12,13-*cis*-cyclopropyl epothilones, the presence of a methyl group at position 12 in the case of 12,13-*trans*-cyclopropyl epothilones is not beneficial, but leads to decreased activity.[114] As had also been observed for epoxide-based analogues, the combination of a 12,13-*cis*-cyclopropyl-Epo B macrocycle with substituted pyridines as thiazole replacements led to highly potent analogues.[116] Most significantly, the replacement of the epoxide ring in ABJ879 by a cyclopropane moiety produced an analogue (compound **9**, Scheme 2, Figure 2),[116] which binds to stabilized microtubules with 27.4-fold greater affinity than Epo B ($\Delta\Delta G_{35\,°C} = -8.2$ kJ mol^{-1}).[70] Furthermore, this compound has been found to be a more potent antiproliferative agent in vitro than either Epo B or, in some cases, ABJ879 (**72**) (IC_{50} values for growth inhibition of the human ovarian carcinoma cell line 1A9 are 0.3 (0.6) nM, 0.17 nM, and 0.10 nM for Epo B, **72**, and **9**, respectively).[116]

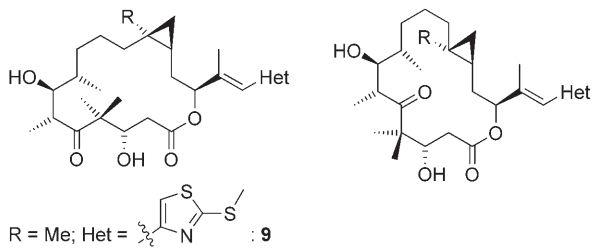
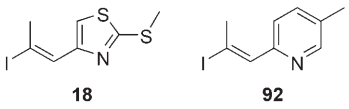
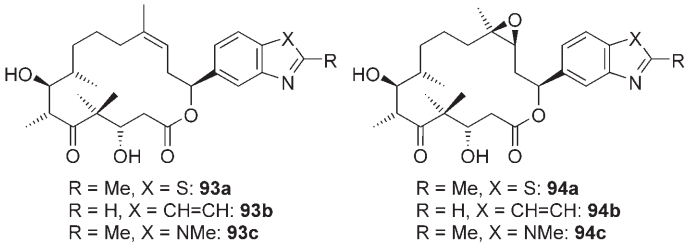


Figure 2. General structure of side-chain-modified variants of cyclopropyl epothilones as investigated by Nicolaou and co-workers.

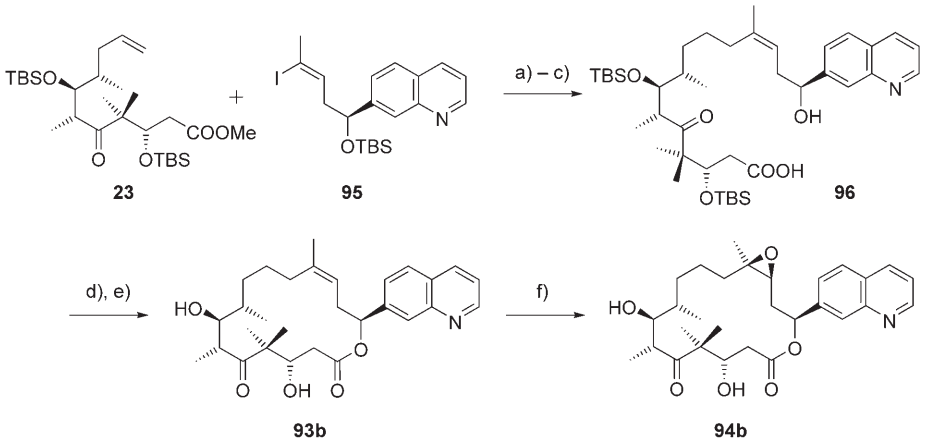


While the majority of side chain modifications in epothilones have involved changes in the structure of the heterocycle alone, the Novartis group has also pursued more far-reaching structural alterations, which are characterized by the rigidification of the entire side chain manifold[73,159] (structures **93** and **94**; see also ref. [104]). The design of these analogues



was guided by the results of solution NMR studies on the bioactive conformation of epothilones,[140] which had indicated a *transoid* arrangement of the C16–C17 double bond and the aromatic C18–N bond in the tubulin-bound state of Epo A (corresponding to a C16–C17–C18–N22 torsion angle of ~180°). The

synthesis of these structures (for example, **93b**/**94b**; Scheme 11) was based on the same general strategy that had been developed by the Novartis group for the synthesis of *trans*-Epo A (**20**)[121] and involved the construction of the C11–C12 bond through Suzuki–Miyaura coupling between the C1–C11 fragment **23** (see Scheme 3) and vinyl iodide **95**, for example, as one of the key steps. The resulting coupling product was elaborated into seco acid **96**, which was followed by Yamaguchi-type macrolactonization, cleavage of the O3 and O7 protecting groups, and epoxidation of the C12–C13 double bond. Epoxidation of **93b** was performed with the $MeReO_3$/H_2O_2 system[150] and proceeded with ~6:1 selectivity to give pure **94b** in 37% yield after chromatographic purification. Except for the epoxidation step, which was based on the use of dimethyldioxirane (DMDO) at −50 °C for the conversion of **93a** to **94a**, the same strategy was successfully applied to the synthesis of several related analogues incorporating benzothiazole-, benzoxazole-, and quinoline-type side chains.[73]



Scheme 11. Ref. [73]: a) (1) olefin **23**, 9-BBN, THF, RT, 4 h; (2) **95**, Cs_2CO_3, $[PdCl_2(dppf)_2]$, Ph_3As, DMF, −10 °C→RT, 16 h, 90%. b) LiOH, *i*PrOH/H_2O (4:1), 50 °C, 7 h, 84%. c) TBAF, THF, RT, 18 h, 84%. d) (1) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, 0 °C, 15 min; (2) dilution with toluene, addition to a solution of DMAP in toluene, 75 °C, 3 h, 70%. e) HF·py, THF, RT, 17 h, 73%. f) (1) $MeReO_3$, H_2O_2, py, CH_2Cl_2, RT, 17 h; (2) H_2, Ra-Ni, EtOH, RT, 37%.

In general, side-chain-modified analogues of this type are more potent inhibitors of human cancer cell proliferation than their respective parent compounds Epo D and B (Table 2).[73] However, as illustrated by the data summarized in Table 2, and for reasons poorly understood at this point, the potency increase conferred by side chain modifications of the above type is significantly more pronounced in the Epo D than the Epo B series. In addition, the enhancement in cellular activity observed for analogues **93a–c** and **94a–c** may not be related to more efficient interactions with the tubulin/microtubule system (relative to Epo D and B, respectively), and this point is discussed in more detail below.

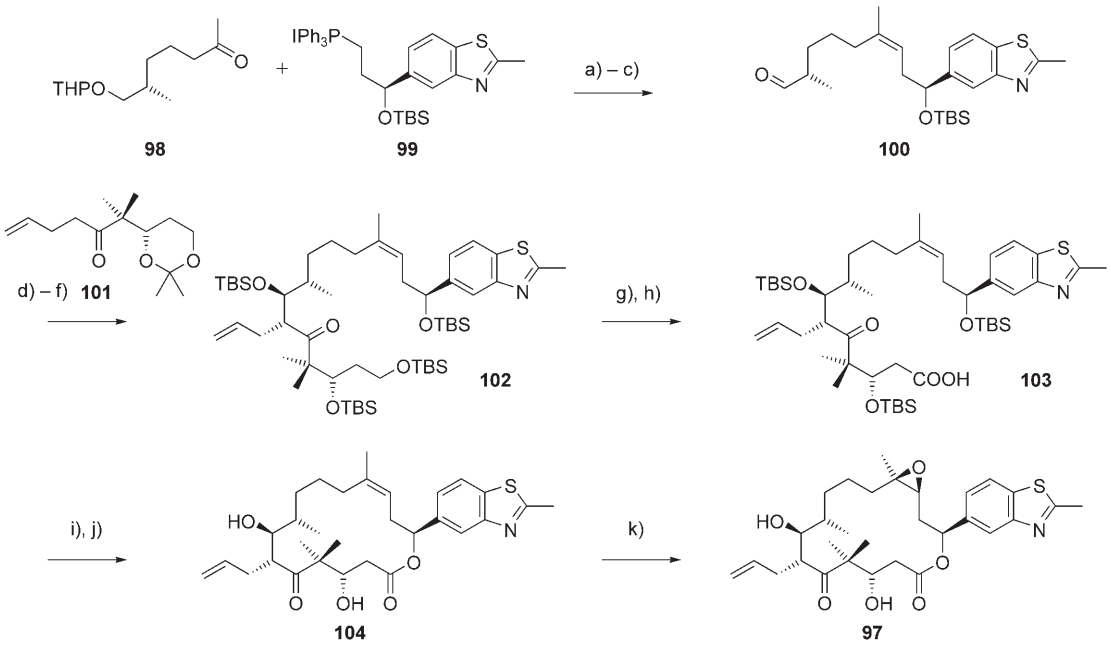
Table 2. Growth inhibition of human carcinoma cell lines by side-chain-modified epothilone analogues **93** and **94**.[73]

Compound	IC_{50} [nM]	
	KB-31	KB-5811
Epo D	2.70	1.44
93a	0.45	0.23
93b	0.59	0.38
93c	0.21	0.73
Epo B	0.19	0.18
94a	0.13	0.09
94b	0.11	0.10
94c	0.14	0.38

Side chain modifications of the type discussed above have been independently investigated by the research groups at Schering[56,104] and, more recently, at Kosan.[160] The work at Schering has, in fact, led to the identification of a C6-allylated analogue of compound **94a**, ZK-Epo (**97**), which is currently undergoing phase II clinical trials and which is the first fully synthetic epothilone analogue to have entered clinical studies.[56] As illustrated in Scheme 12, key steps in the synthesis of the compound include the formation of the C12–C13 double bond via Wittig reaction between ketone **98** and phosphonium salt **99** (which is nonselective), the stereoselective aldol reaction of ketone **101** (for which an efficient synthesis has been developed from pantolactone[161]) with aldehyde **100**, Yamaguchi macrolactonization of the seco acid derived from **103** through TBS removal from O15, and epoxidation of Epo D analogue **104** with DMDO at −78 °C. Fortunately, the mixture of 12,13-*cis*/*trans* isomers obtained in the Wittig step can be separated by chromatography, and the undesired *trans* analogue can be isomerized by irradiation at $\lambda > 280$ nm to a 3:2 mixture of *cis* and *trans* isomers.

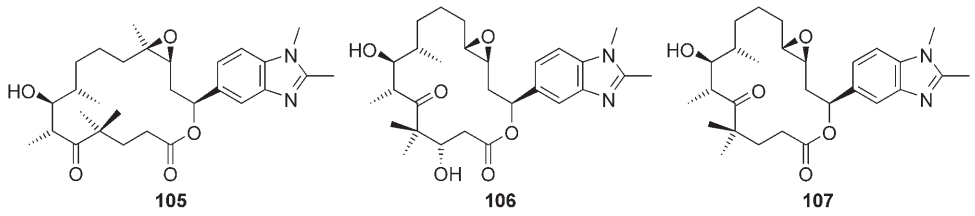
ZK-Epo (**97**) is reported to be more potent in vitro than Epo B against a variety of drug-sensitive cancer cell lines and to retain full activity against multidrug-resistant cancer cells (whereas Epo B exhibits somewhat decreased activity against certain types of MDR cell lines).[56] The compound also showed significant antitumor activity in a number of mouse xenograft models without inducing any profound body weight loss.[56] However, it should be noted that no tumor regressions were observed at these nontoxic doses.

Based on the significant increase in cellular potency associated with a dimethylbenzimidazole side chain within the structural framework of the Epo B/D macrocycle,[73,104,159] this modification has been further investigated by Altmann and co-workers as a potential potency-enhancing element in combination with a variety of macrocycle modifications.[162–164] (In addition, it should also be noted that among analogues **93** and **94** (and additional related structures not shown herein), those incorporating a benzimidazole moiety exhibit the most favorable solubility properties in aqueous medium.) These studies revealed

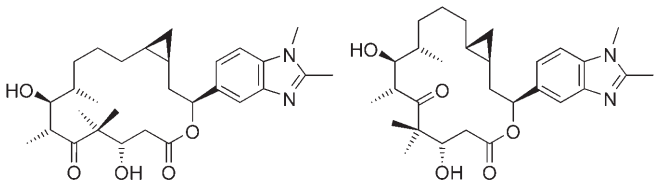


Scheme 12. Ref. [56]: a) NaHMDS, THF, 0 °C→RT, 83% (1:1 mixture of *Z* and *E* isomers). b) *p*-TsOH (cat.), EtOH, RT, 43% (86% for mixture of *Z* and *E* isomers). c) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C→0 °C, crude. d) **101**, LDA, $ZnCl_2$, THF, −70 °C, 64%. e) *p*-TsOH (cat.), EtOH, RT, 97%. f) TBSOTf, 2,6-lutidine, CH_2Cl_2, −70 °C→0 °C, 96%. g) CSA, CH_2Cl_2, MeOH, RT, 80%. h) (1) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C→0 °C, crude; (2) $NaOCl_2$, NaH_2PO_4, 2-methyl-2-butene, THF, H_2O, *t*BuOH, 0 °C→15 °C, 85%. i) (1) TBAF, THF, RT, crude; (2) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, 0 °C, 60%. j) HF·py, hexafluorosilicic acid, THF, RT, 87%. k) DMDO, acetone/CH_2Cl_2, −78 °C, 71% + 10% β-epoxide. DMDO = 3,3-dimethyldioxirane.

that the potency-enhancing effect of the dimethylbenzimidazole moiety is rather general in nature and extends to the corresponding analogues of Epo A and C,[162] of 3-deoxyEpo B (**105**), and also of *trans*-Epo A (**106**) and its 3-deoxy derivative (**107**).[149] For example, compound **106** is essentially equipotent to Epo B (which is the most potent natural epothilone) against drug-sensitive human cancer cell lines (IC_{50} values against the human cervix carcinoma cell line KB-31 are 0.25 nM and 0.29 nM for **106** and Epo B, respectively). Likewise, the modification can almost completely compensate for the loss in cellular potency caused by removal of the 3-hydroxy function in Epo B (compound **105**: IC_{50} (KB-31) = 0.58 nM versus 0.29 nM for Epo B). Lastly, the antiproliferative activity of compound **107**, in spite of significant structural deviations from the original natural product leads, is still similar to that of Epo A, Epo D, and taxol (IC_{50} (KB-31): 3.16 nM).[149] This latter analogue might, in fact, be considered the first representative of a new structural class of microtubule stabilizers whose overall pharmacological profile may be distinct from that of Epo A/B and more closely related analogues.



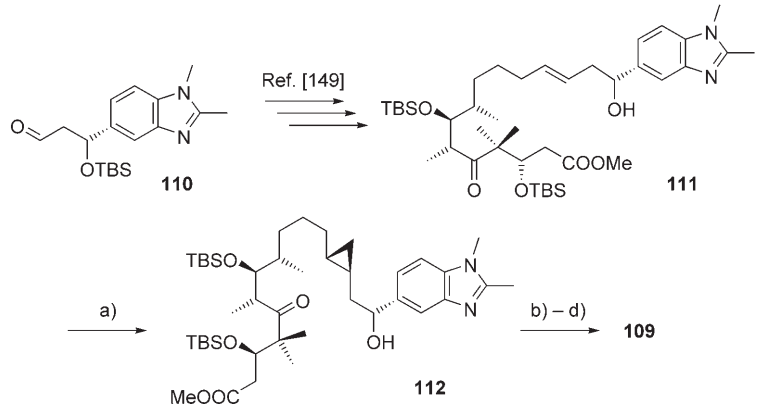
Whereas dimethylbenzimidazole-based epothilone analogues are generally more active against drug-sensitive human cancer cell lines than the corresponding derivatives incorporating the natural epothilone side chain, and also tend to be more water-soluble, they exhibit slightly increased susceptibility to P-gp-mediated drug efflux. For example, the IC_{50} values of compounds **105** and **106** against the P-gp-overexpressing human cervix carcinoma cell line KB-8511 are 1.89 nM and 1.36 nM versus 0.58 nM and 0.25 nM, respectively, for the drug-sensitive KB-31 line.[149] Similar effects on the activity against multidrug-resistant KB-8511 cells have also been observed for other types of (structurally unrelated) epothilone analogues with increased polarity over the corresponding parent compound (decreased Clog *P*).[149,162] Based on this observation, Altmann and co-workers attempted to overcome the P-gp susceptibility of benzimidazole-derived epothilone analogues through replacement of the epoxide oxygen atom by a more lipophilic methylene group, thus leading to side-chain-modified cyclopropane derivatives **108** and **109**.[163] The synthesis of **109** is summarized in Scheme 13 and includes the highly selective directed cyclopropanation of (15*R*!) homoallylic alcohol **111** as the very key transformation. To introduce the correct stereochemistry at C15, this route necessitated macrolactonization to be conducted under Mitsunobu conditions, which was associated with somewhat lower cyclization yields than the



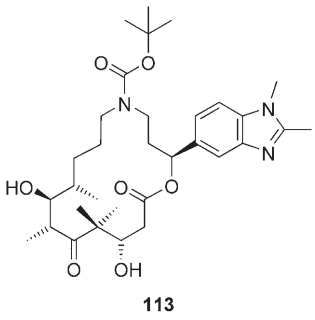
standard Yamaguchi approach. Nevertheless, the route depicted in Scheme 13 is still highly preferable over an alternative approach investigated that involved cyclopropanation of the *trans*-Epo C analogue corresponding to **109** in the last step of the synthesis.[163] The latter reaction was found to be completely nonselective under a variety of conditions, and the resulting 1:1 mixture of products proved to be very difficult to separate. Compounds **108** and **109** are equipotent with the corresponding epoxide-based analogues against the drug-sensitive KB-31 line and, quite remarkably, retain full activity against the multidrug-resistant KB-8511 line, thus validating the underlying hypothesis about the relationship between P-gp susceptibility and compound polarity.[163]

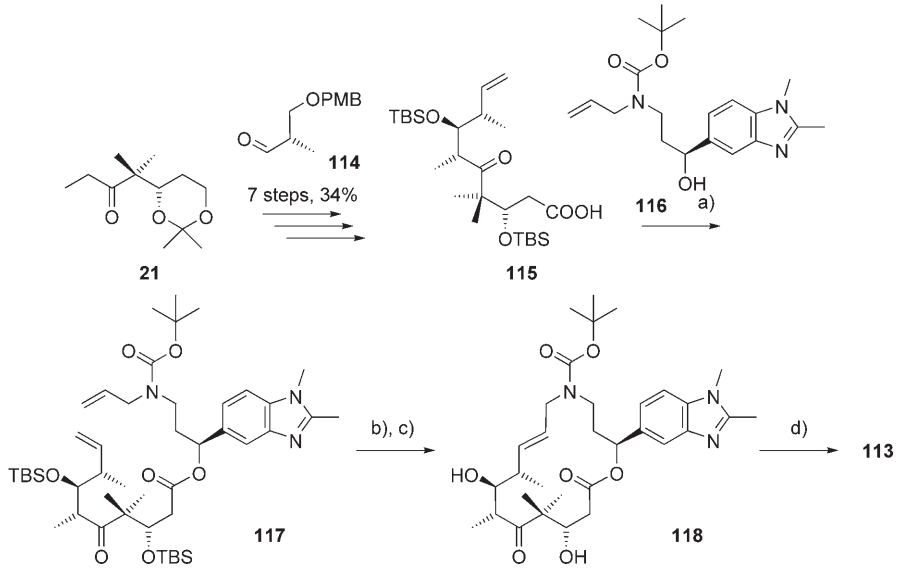
The potency-enhancing properties of a dimethylbenzimidazole side chain have also been exploited by Altmann and coworkers to enhance the activity of 12-aza-macrolide-based epothilone analogues (azathilones, see above).[128] Based on the available SAR data for this family of compounds, the most promising target for synthesis appeared to be analogue **113**, which is derived from azathilone **39a** (as the most potent "natural" derivative investigated so far) through replacement of the natural side chain by a dimethylbenzimidazole moiety.

Two synthetic routes to this compound have been developed and are summarized in Schemes 14 and 15.[128] In a first-generation approach, closure of the macrolactone ring was based on olefin metathesis between C9 and C10, which proceeded in high yield and with exclusive *trans* selectivity (Scheme 14). Unfortunately, the efficiency of this step was thwarted by somewhat unexpected difficulties in the reduction of the C9–C10 double bond (after removal of the TBS groups), which could only be achieved in a very modest yield of 31% under conditions that had been successfully employed in the reduction of **41** and **43** to Epo D and B, respectively, in high yield.[102,133,134] Although the amount of material obtained by this approach was sufficient for initial biological profiling, an alternative route to **113** was sought that would



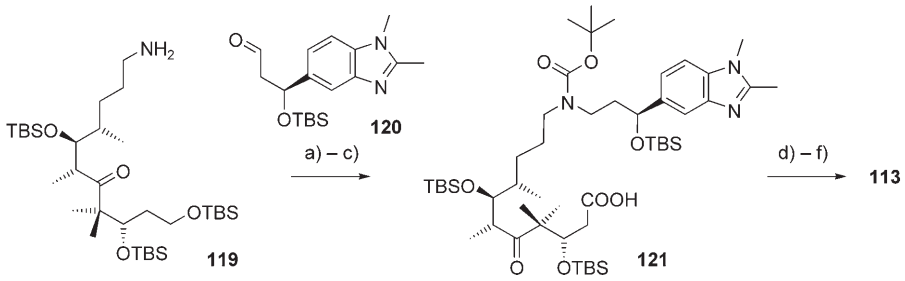
Scheme 13. Ref. [163]: a) (1) Et_2Zn, CH_2I_2, CF_3COOH, −13 °C, 30 min; (2) **111**, −13 °C, 20 min, 77%. b) LiOH, *i*PrOH/H_2O (4:1), 60 °C, 3 h, 91%. c) PPh_3, DIAD, toluene, −13 °C, 90 min, 46% (65% based on recovered starting material). d) HF·py, MeCN, RT, 2 h, 42% (after HPLC purification, 83% crude). DIAD = diisopropyl azodicarboxylate.





Scheme 14. Ref. [128]: a) **116**, DCC, DMAP, CH_2Cl_2, 0 °C, 15 min, RT, 15 h, 60%. b) 2nd-gen. Grubbs catalyst, CH_2Cl_2, reflux, 8 h, 85%. c) HF·py, py, THF, RT, 4 h, 70%. d) $(NCO_2K)_2$, AcOH, CH_2Cl_2, 31%. DCC = *N*,*N*′-dicyclohexylcarbodiimide.

enable access to larger quantities of material for eventual in vivo studies in a more efficient way. This second-generation approach was based on ring closure through Yamaguchi macrolactonization and involved construction of the C11–N12 bond through reductive amination between amine **119** and aldehyde **120** (Scheme 15). After in situ Boc protection, the coupling product was elaborated into **113** through selective cleavage of the primary TBS ether followed by oxidation of the resulting free hydroxy group to the carboxylic acid, removal of the O15 TBS group, Yamaguchi macrolactonization, and, finally, selective removal of the remaining TBS protecting groups on O3 and O7 with HF·pyridine.



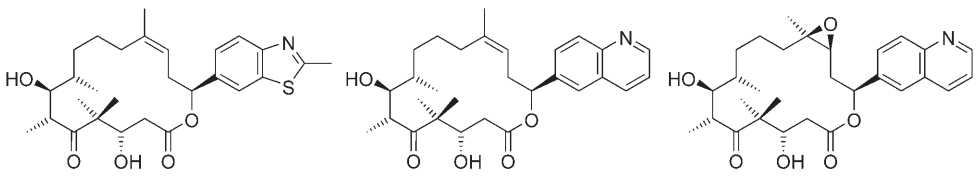
Scheme 15. Ref. [128]: a) (1) **120**, $NaBH(OAc)_3$, AcOH, M.S. (4 Å), RT, 2.5 h; (2) Boc_2O, TEA, THF, 0 °C, 45 min, 60% (2 steps). b) CSA, CH_2Cl_2/MeOH (1:1), 0 °C, 3 h, 80%. c) PDC, DMF, RT, 24 h, 50%. d) TBAF, THF, RT, 24 h. e) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, 0 °C, 20 min; then dilution with toluene and addition to a solution of DMAP in toluene, 75 °C, 1 h, 44% (2 steps). f) HF·py, py, THF, RT, 2.5 h; then preparative HPLC, 40%. Boc = *tert*-butoxycarbonyl, TEA = triethylamine, CSA = camphorsulfonic acid, PDC = pyridinium dichromate.

Azathilone **113** was found to be a highly active antiproliferative agent that inhibits the growth of different types of drug-sensitive human cancer cell lines with similar potency to Epo A. (For example, IC_{50} values of **113** against the human cancer cell lines A549 (lung), HCT-116 (colon), PC-3M (prostate), and KB-31 (cervix) are 1.9 nM, 1.6 nM, 2.3 nM, and 0.34 nM, respectively, versus 3.2 nM, 2.2 nM, 3.4 nM, and 2.15 nM for Epo A).[128] The replacement of the natural thiazolylvinyl side chain in **39a** by a dimethylbenzimidazole moiety thus results in a >60-fold increase in cellular potency, which significantly exceeds the effects that had been observed previously in the context of polyketide-based macrocycles (for which incorporation of a dimethylbenzimidazole side chain leads to a potency increase of 2–15-fold). In light of the profound structural differences between **113** and the original natural product lead Epo A, the activity of **113** is highly remarkable, and the compound may, in fact, be considered as a first representative of a new structural class of microtubule-stabilizing agents (**113** promotes microtubule assembly from soluble tubulin with the same efficiency as Epo A).[128] While epothilone analogues based on a 12-aza-macrolide core still retain many of the (two-dimensional) structural features of natural epothilones, their macrolactone ring is no longer based on a contiguous polyketide backbone. Analogues of this type have therefore been classified as "nonnatural natural products" by Altmann and coworkers.[128]

The importance of the *tert*-butyl group in **113** for biological activity was probed by its replacement with a less bulky and less lipophilic ethyl group. The resulting analogue proved to be somewhat less potent than **113**, but IC_{50} values for growth inhibition of drug-sensitive human cancer cell lines are still in the 10–20 nM range. Intriguingly, the presence of a *trans* double bond between C9 and C10 (compound **118**, Scheme 14) produced a dramatic loss of biological potency,[128] which is contrary to the effects of this modification within the structural framework of natural epothilones (see above). This finding may be indicative of differences in the tubulin-bound conformation between natural epothilones and the aza-macrolide-based azathilones, but further experimental work is necessary to either confirm or refute this hypothesis. Unfortunately, and in marked contrast to natural epothilones, the activity of **113** (as well as the corresponding ethyl carbamate) against the multidrug-resistant KB-8511 human cervix carcinoma cell line is significantly decreased, which indicates that the compound is highly susceptible to P-gp-mediated drug efflux. Efforts are currently underway to improve the activity of azathilone-type analogues against P-gp-overexpressing cells through the introduction of additional or alternate modifications.

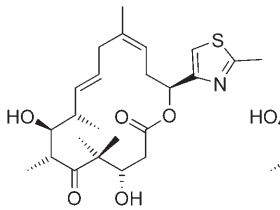
Epothilone analogues incorporating conformationally constrained side chains have also been used as templates for a more detailed investigation of the significance of the position of the side chain nitrogen atom.[164] In addition to analogues **93a** and **93b**/**94b**, this has involved the isomeric derivatives **122**–**124**.

The picture that has emerged from these studies with regard to the relationship between biological activity and nitrogen positioning in the epothilone side chain is rather complex and cannot be fully rationalized at this point. Thus, while both isomers within compound pairs **93a**/**122** and **93b**/**123** exhibit virtually identical effects on tubulin polymerization, they show very different antiproliferative activities, with the "natural" isomer (that is, the compound with the N atom *meta*



to the bond between the phenyl moiety and the macrolactone ring) being significantly (> 50-fold) more potent.[164] This would suggest the position of the side chain N atom to be highly relevant for cellular potency, but not for the interactions of these analogues with the tubulin/microtubule system. On the other hand, virtually no effect of the location of the N atom on biological activity was observed between epoxide-containing analogues **94b** and **124** either at the level of tubulin polymerization in vitro or at the level of cancer cell growth inhibition.[162] What seems to be clear from these data is that factors other than tubulin-polymerizing activity in vitro must have a pronounced impact on the cellular activity of Epo D analogues **93a**, **93b**, **122**, and **123**. However, further experiments are required to establish a mechanistic understanding of the observed differences in cellular potency and of their virtual disappearance upon conversion of **93b** and **123** to the corresponding epoxides.

A different family of side-chain-modified epothilone analogues that contain a (2-substituted) thiazol-4-yl moiety directly attached to C15 of the *trans*-9,10-didehydro-Epo D (**41**) macrocyclic core structure, has recently been reported by the research group at Kosan.[165] The design of these particular analogues was guided by the potency enhancement associated with either the incorporation of a *trans* double bond between C9 and C10 of the epothilone macrocycle (see above) or the presence of conformationally constrained side chains of the type discussed above, which have an aryl moiety directly attached to C15. Not unexpectedly, the simple removal of the vinyl linker between the thiazole moiety and the macrolactone ring in *trans*-9,10-didehydro-Epo D, leading to **125**, results in a significant loss in activity (IC_{50} value of 380 nM against the human breast carcinoma cell line MCF-7 versus 0.6 nM for **41**).[165] This finding confirms earlier data from the Danishefsky group that had shown the corresponding Epo D analogue to be substantially less potent than Epo D.[109] However, the activity loss resulting from the absence of a linker element between the thiazole moiety and the macrocycle could be compensated, at least to some extent, by the replacement of the methyl group at the 2-position of the thiazole ring with different aryl moieties such as in **126** and **127**. Analogues **126a**, **126b**, **126c**, and **127** exhibit IC_{50} values against the MCF-7 human breast cancer cell line of 40 nM, 52 nM, 34 nM, and 38 nM, respectively;[165] although the potency of these compounds is thus still > 50-fold lower than that of **41**, there is significant room for additional modifications of both heteroaryl moieties, which may well lead to further improvements in potency.



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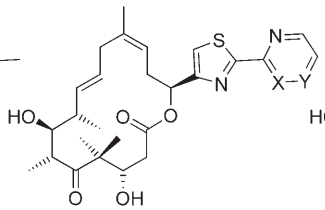
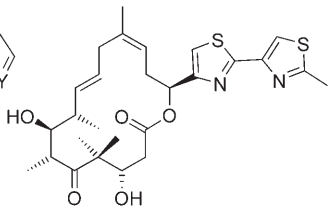


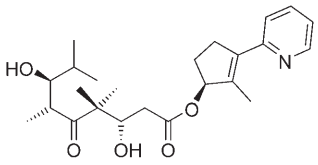
X, Y = CH: **126a**
X = N, Y = CH: **126b**
X = CH, Y = N: **126c**



127

Miscellaneous analogues

Although earlier attempts to design grossly simplified epothilone analogues (such as the removal of one complete half of the structure) had proven unsuccessful,[166a] Tillekeratne and co-workers[166b] recently reported a pair of new open-chain analogues, **128** and **129**, which lack the C10–C13 segment of the natural structure. Both compounds proved to be essentially inactive against all cell lines investigated.[166b]



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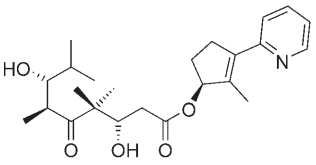

129

Conformational Studies and Pharmacophore Modeling

Ever since the discovery of the microtubule-stabilizing properties of epothilones in 1995, efforts have been made to develop a predictive pharmacophore model for this class of compounds in order to guide the design of improved and perhaps structurally simplified analogues. In the absence of any structural information on complexes between tubulin/microtubules and an epothilone-type molecule, early attempts on the development of an epothilone pharmacophore model[78,167–170] were generally based on the assumption of a common tubulin binding site between epothilones and taxol in conjunction with (low resolution) structural data on a complex between two-dimensional tubulin polymer sheets and taxol, which had been obtained by electron crystallography[79] (and which have undergone continuous computational refinement). Based on these premises, Giannakakou et al.[78] developed a model for the tubulin-bound conformation of natural epothilones that places the epoxide oxygen atom of epothilones in the same position as the oxetane oxygen in taxol, while the epothilone side chain is located in the same region as either the C3′-phenyl group or, alternatively, the C2-benzoyloxy moiety of taxol. Different conclusions were reached by Wang et al.[167] regarding the relative orientation of taxol and epothilones within the microtubule binding site. In their model, the position of the thiazole moiety in epothilones matches the position of the phenyl group of the C3′-benzamido substituent of taxol. Most significantly, the epothilone epoxide oxygen atom in this latter model is not involved in interactions with the protein, in line with the experimental data for cyclopropane-based epothilone analogues. A model similar to that of Wang et al. was recently proposed by Botta and co-work-

ers.[169] Although these computational models by their very nature are of limited accuracy, each is able to explain at least part of the published epothilone SAR and may thus provide some useful guidance for the design of new analogues. However, all of these models may need to be revisited in light of more recent structural data on the bioactive, tubulin-bound conformation of Epo A, which have been obtained by NMR spectroscopy on a soluble β-tubulin–Epo A complex[140] and by a combination of electron crystallography, NMR spectroscopic conformational analysis, and molecular modeling on a complex between Epo A and a Zn^{2+}-stabilized two-dimensional α,β-tubulin polymer sheet (solved at 2.89 Å resolution).[69] The latter study by Nettles et al.[69] has seriously called into question the assumption of a truly common pharmacophore between taxol and epothilones, which has been a central provision in all earlier modeling studies. Whereas both Epo A and taxol, according to Nettles et al., occupy the same gross binding pocket, the actual binding is mediated through different sets of hydrogen bonding and hydrophobic interactions for the two compounds. The bioactive conformation of Epo A as proposed by Nettles et al. differs from any of the computational models discussed above, but also from the structure derived by Carlomagno et al.[140] on the basis of transfer NOE experiments in solution (with soluble tubulin dimers and/or oligomers as receptors). In this context it should be noted that one important SAR feature of epothilones is not well explained by the structure of Nettles et al., namely the fact that formal dehydration across the C2–C3 bond (producing a *trans* double bond) is not associated with any major loss in tubulin-polymerizing or antiproliferative activity (see above). This finding suggests the C2–C3 torsion angle in the bioactive conformation of epothilones to be in the vicinity of 180°, which corresponds to the conformation in the NMR-derived structure, but is not the conformation suggested by the structure of Nettles et al. Both structural proposals emphasize the presence of a 180° torsion angle about the C17–C18 bond, thus locating the 27-methyl group and the thiazole nitrogen atom on opposite sides of the C16–C17 double bond. In fact, Nettles et al. proposed this nitrogen atom to be involved in a hydrogen bond with the protonated form of β-His 227. These conclusions are consistent with the observation that the antiproliferative activity of pyridine-based analogues of Epo B (thiazole ring replaced by a 2-, 3-, or 4-pyridyl moiety) decreases in the order 2-pyridyl ≫ 3-pyridyl ≈ 4-pyridyl, thus indicating a requirement for the nitrogen atom to be located in the position *ortho* to the attachment point of the linker between the heterocycle and the macrocyclic core (see above). However, as highlighted above in the discussion of Epo B and D analogues containing isomeric benzothiazole- or quinoline-based side chains, cellular effects may not be entirely reflective of the strength of ligand–tubulin interactions, and the interpretation of changes in cellular activity in terms of structural features of a ligand–tubulin complex could, in fact, be misleading.

It remains to be seen how the various pharmacophore models and structural proposals made on the basis of NMR or electron crystallography data will actually relate to the true (that is, derived from atomic resolution structural data, if they become available) bioactive conformation of epothilones (should there even be a single unique bioactive conformation). In the meantime, both the experimentally derived as well as the purely computational structures may serve as a useful basis for the design of new analogues.

Conclusions

The chemistry and biology of epothilones have been areas of significant interest and importance in anticancer drug discovery, ever since these compounds were established to inhibit human cancer cell growth through a "taxol-like" mechanism of action more than ten years ago. The ultimate objective of this research, be it basic or more applied, has been (and continues to be) the transformation of a promising family of natural product leads into a viable, clinically useful anticancer drug (or perhaps even a series of pharmacologically distinct clinical agents). On the way to this goal, the past decade has witnessed the design, synthesis, and pharmacological evaluation of hundreds of epothilone analogues and derivatives that have allowed the delineation of a comprehensive map of the structural elements associated with potent biological activity, and also those that are not well tolerated and should be avoided in the design of potent analogues. At the same time, our understanding of epothilone biology and pharmacology has grown much beyond the simple fact of gross microtubule stabilization and induction of tubulin polymerization. Most of the structure–activity work was conducted in the absence of structural information on tubulin/epothilone complexes and in an iterative process had to be guided by the activity of previously synthesized analogues. Progress in the organic and medicinal chemistry of epothilones was thus strongly linked to the rapid evaluation of newly synthesized compounds by biochemists, cell biologists, and pharmacologists. The (often promising) data thus generated have provided a basis for the design of new structures and, equally important, they have spurred the development of new and improved synthetic approaches, up to the point where the large-scale preparation of analogues for clinical trials has become reality. In this context it is also worth noting that most of the SAR information available for epothilones today is derived from the investigation of fully synthetic analogues that would not have been accessible through semisynthesis. More recently, structural proposals for the tubulin-bound bioactive conformation of Epo A have been advanced on the basis of solution NMR and electron crystallography data. Unfortunately, the conclusions derived from these structural studies have not led to a consistent and unique structural picture for the bioactive conformation of Epo A, and it remains to be seen to what extent this information will aid the future design of new and improved analogues.

Epothilone-based drug discovery research so far has delivered seven (publicly known) compounds which have entered clinical development. Two of these have already advanced to phase III trials, making it likely that the initial promise held by the natural product leads will soon have been transformed into new anticancer drugs and thus become manifest in clinical practice. While most of the analogues currently undergoing

clinical trials are either directly produced through fermentation or through semisynthesis from Epo B, it is worth noting that Schering AG is developing a compound that is entirely synthetic in nature and is produced through total chemical synthesis.

This well-filled clinical development pipeline is complemented by a number of more recent analogues, which are currently at various stages of preclinical profiling or development. Of particular note among these agents is fludelone, which exhibits a superb preclinical profile and which will hopefully enter clinical studies in humans in the very near future. On the other hand, highly potent new analogues with modified side chain structures have been discovered very recently as well as compounds such as azathilones, whose overall structural framework deviates significantly from that of the natural product leads and which may therefore be associated with distinctly different overall pharmacological profiles. Clearly, more work is required to establish the development potential of these more recent additions to the extensive collection of epothilone-derived structures. However, it is more than likely that the current group of clinical development compounds is not the end of the line in epothilone-based drug discovery and development. There is more to come.

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Keywords: antitumor agents · epothilones · natural products · structure–activity relationships · total synthesis

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