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# Epothilones as Lead Structures for the Synthesis-Based Discovery of New Chemotypes for Microtubule Stabilization

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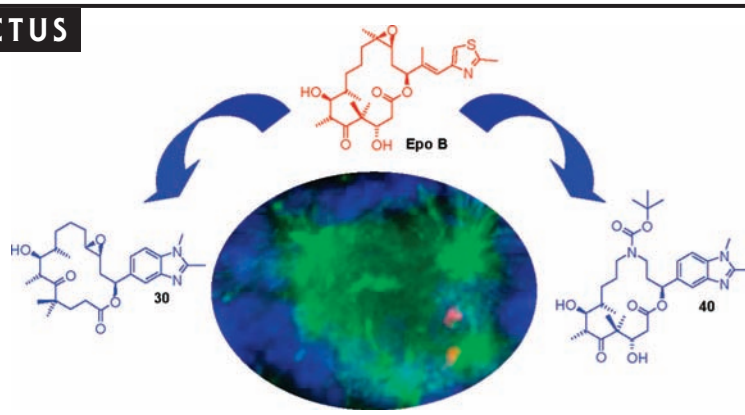
## CONSPECTUS




Epothilones are macrocyclic bacterial natural products with potent microtubule-stabilizing and antiproliferative activity. They have served as successful lead structures for the development of several clinical candidates for anticancer therapy. However, the structural diversity of this group of clinical compounds is rather limited, as their structures show little divergence from the original natural product leads. Our own research has explored the question of whether epothilones can serve as a basis for the development of new structural scaffolds, or chemotypes, for microtubule stabilization that might serve as a basis for the discovery of new generations of anticancer drugs. We have elaborated a series of epothilone-derived macrolactones whose overall structural features significantly deviate from those of the natural epothilone scaffold and thus define new structural families of microtubule-stabilizing agents. Key elements of our hypermodification strategy are the change of the natural epoxide geometry from *cis* to *trans*, the incorporation of a conformationally constrained side chain, the removal of the C3-hydroxyl group, and the replacement of C12 with nitrogen. So far, this approach has yielded analogs **30** and **40** that are the most advanced, the most rigorously modified, structures, both of which are potent antiproliferative agents with low nanomolar activity against several human cancer cell lines *in vitro*. The synthesis was achieved through a macrolactone-based strategy or a high-yielding RCM reaction. The 12-aza-epothilone ("azathilone" **40**) may be considered a "non-natural" natural product that still retains most of the overall structural characteristics of a true natural product but is structurally unique, because it lies outside of the general scope of Nature's biosynthetic machinery for polyketide synthesis. Like natural epothilones, both **30** and **40** promote tubulin polymerization *in vitro* and at the cellular level induce cell cycle arrest in mitosis. These facts indicate that cancer cell growth inhibition by these compounds is based on the same mechanistic underpinnings as those for natural epothilones. Interestingly, the 9,10-dehydro analog of **40** is significantly less active than the saturated parent compound, which is contrary to observations for natural epothilones B or D. This may point to differences in the bioactive conformations of *N*-acyl-12-aza-epothilones like **40** and natural epothilones. In light of their distinct structural features, combined with an epothilone-like (and taxol-like) *in vitro* biological profile, **30** and **40** can be considered as representative examples of new chemotypes for microtubule stabilization. As such, they may offer the same potential for pharmacological differentiation from the original epothilone leads as various newly discovered microtubule-stabilizing natural products with macrolactone structures, such as laulimalide, peloruside, or dictyostatin.

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## 1. Introduction

Microtubules are dynamic subcellular structures that are central to a multitude of cellular processes, including the maintenance of cell shape, cell motility, intracellular transport, and cell division. Upon entry of a cell into mitosis, the microtubule network undergoes a fundamental rearrangement, which produces the mitotic spindle as the structural scaffold for chromosome alignment and subsequent chromatid separation. In light of this fundamental role in cell division, it is not surprising that the modulation of microtubule function has emerged as an important mechanistic principle in cancer chemotherapy, with a number of microtubule-interacting agents being routinely employed in clinical practice[1] and many others being evaluated in clinical trials or undergoing advanced preclinical development.[2] Anticancer drugs targeting the tubulin/microtubule system include the tubulin polymerization inhibitors vinblastine, vincristine, and vinorelbin, as well as the microtubule stabilizers taxol (paclitaxel, Taxol) and docetaxel (Taxotere).[1] The distinction between tubulin polymerization inhibitors and microtubule stabilizers (which also promote tubulin polymerization from soluble tubulin under otherwise nonpolymerizing conditions) is based on clearly discernible differences between these groups in their effects on the polymerization state of tubulin *in vitro* (i.e., in experiments with isolated tubulin or microtubule protein), but the antiproliferative activity of both types of compounds in cells at low concentrations appears to arise from the suppression of microtubule dynamics (in the absence of measurable changes in tubulin polymer mass).[3] Microtubule-interacting agents (or tubulin modulators) are classical cytotoxics acting on a ubiquitous cellular target and therefore are associated with a number of sometimes severe clinical side effects. Nevertheless, and despite the emergence of many "cancer-specific" targets over the last 20 years, research into new (improved) tubulin modulators still represents an important part of modern anticancer drug discovery and development. Because the major types of solid human tumors (breast, lung, prostate, ovarian, and colon), which represent the vast majority of cancer cases today, are multicausal in nature, it is increasingly recognized that their treatment with "mechanism-based" agents alone is unlikely to be successful. Instead, improved treatment strategies are likely to involve combinations of, for example, signal transduction inhibitors with new and better cytotoxic drugs.

The first compound ever discovered to stabilize microtubules was the plant-derived diterpenoid taxol,[4] whose successful development into an effective anticancer drug arguably

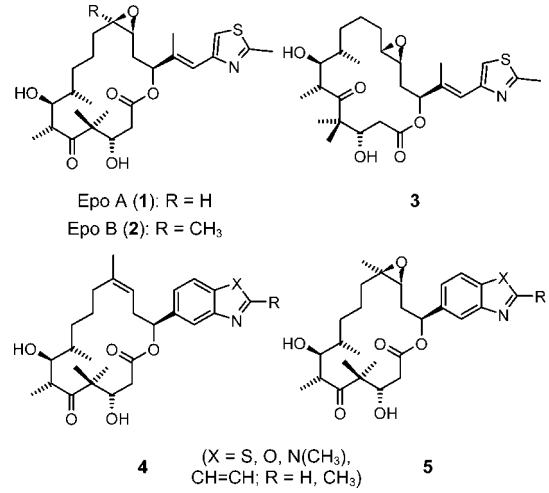



**FIGURE 1**

represents one of the major milestones in the history of cancer chemotherapy.[5] Following the elucidation of taxol's mechanism of action in 1979,[4] it took 16 years until the bacterial natural products epothilones A and B (whose antiproliferative effects on human cancer cells had been known for several years prior,[6] Figure 1) were established as the first non-taxane-based microtubule stabilizers in 1995 by Bollag et al. at Merck Research Laboratories.[7] In addition, and in marked contrast to taxol, epothilones were found to exhibit very low, if any, susceptibility to P-glycoprotein (P-gp)-mediated drug efflux, thus leading to virtually equivalent growth inhibitory activity against drug-sensitive and multidrug-resistant human cancer cells *in vitro* and *in vivo*. Based on these attributes, epothilones have been widely pursued as lead structures for the development of a new generation of non-taxane-derived microtubule stabilizers for cancer treatment.[8] These efforts have included the synthesis and semisynthesis of several hundred analogs for the investigation of structure–activity relationships (SAR), and most importantly, they have led to the discovery of at least six compounds that have entered clinical trials in humans (in addition to the natural product epothilone B (Epo B) itself).[8] One of these compounds (ixabepilone) has recently been approved by the FDA for clinical use in humans (http://www.cancer.gov/cancertopics/druginfo/fda-ixabepilone). However, the structural diversity represented by this group of clinical compounds is rather limited because most of them exhibit little structural divergence from the original natural product leads. While such small structural changes can in fact suffice to produce meaningful favorable changes in overall pharmacological profile (at least at the preclinical level), the availability of more structurally diverse analogs would still be highly desirable because they may offer a higher *a priori* potential for pharmacological distinction from the starting nat-

ural product. In general, structural diversity in the field of microtubule stabilizers has significantly increased over the past decade through the discovery of a series of structurally distinct natural products from a variety of sources,[9] but with one exception (discodermolide),[10] these compounds (or analogs thereof) are still at the stage of preclinical evaluation.

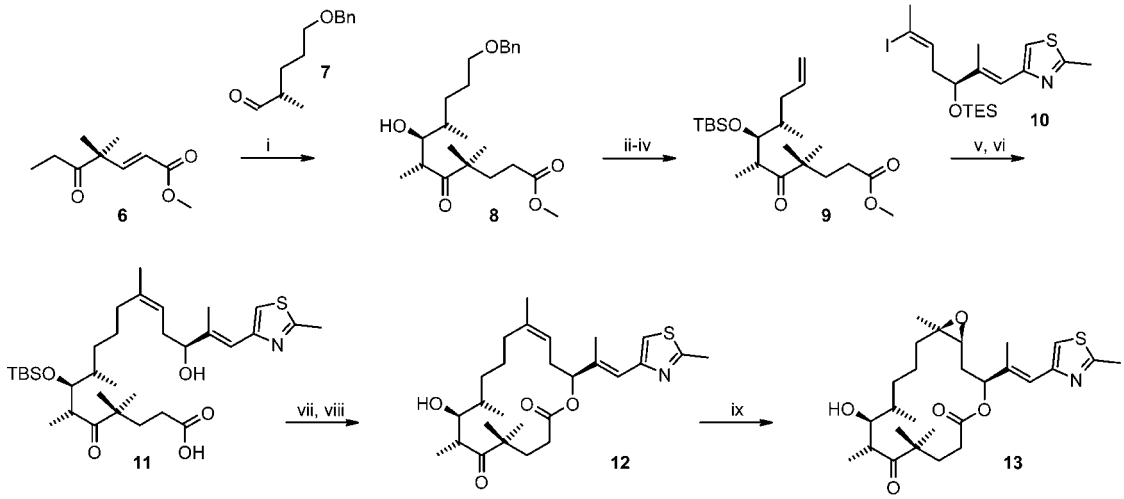
Natural products have been the most productive source of potent microtubule stabilizers by far,[9] and the screening of large natural products collections or of libraries of natural-product-related compounds obtained through diversity-oriented[11] or biology-oriented[12] synthesis may uncover new templates for microtubule stabilization in the future. Alternatively, such templates may be evolved from existing microtubule stabilizers, such as epothilones, through extensive structural modification (and perhaps simplification), provided such modifications do not produce an unacceptable loss in the desired biological activity. Thus, our own research in the area of natural-product-based microtubule stabilizers has been directed toward the evolution of hypermodified epothilone analogs (i.e., molecules with very limited, if any, structural similarity to the original epothilone scaffold) that would ultimately represent new chemotypes for microtubule inhibition. In a very general sense, this strategy could be viewed as the synthesis-based equivalent to the discovery of a new natural product with a specific biological activity. In this Account, we will highlight some of the structural changes of the epothilone scaffold that we have investigated within this conceptual framework and that have led to analog structures that may be regarded as prototypical examples of new templates for microtubule stabilization and, at a more general level, as "non-natural" natural products. A large part of this work was based on two specific SAR features for epothilones that we had previously elucidated in our laboratory and that involve (i) the effect of changing the geometry of the C12,C13 epoxide moiety from *cis* to *trans* and (ii) the rigidification of the C15 side chain through incorporation of a phenyl linker between the heterocycle and the macrolactone ring. Thus, we have demonstrated that 12*S*,13*S*-*trans*-Epo A (**3**) (Figure 1) has virtually equipotent (if not slightly more potent) effects on tubulin assembly and cancer cell growth *in vitro* as natural Epo A (**1**), while the corresponding 12*R*,13*R* isomer is several hundred fold less active.[13] Rigidification of the C15 side chain as in analogs of type **4** or **5** (Figure 1) generally leads to enhanced cellular potency; among the limited set of modifications investigated, this effect was most pronounced for a dimethyl-benzimidazole side chain (Figure 1 , X = $N(CH_3)$, R = $CH_3$),[14] which was thus selected as the standard structure in the design of hypermodified epothilone analogs. In addition to this side chain modification and the incorporation of a *trans* epoxide moiety into the macrocycle, a third strategic entry point into the creation of potent hypermodified epothilone analogs was the removal of the hydroxyl group in the 3-position of the epothilone scaffold, which would also lead to structural simplification and improved synthetic accessibility. This modification will be discussed in more detail in the following.

## 2. 3-Deoxyepothilones

While the biological properties of 3-deoxyepothilones (with a C−C single bond between C2 and C3) had not been evaluated prior to our own work, a number of related modifications had been described in the literature. In particular, it had been demonstrated by the BMS group that dehydration across the C2−C3 bond (leading to *trans*-3-deoxy-2,3-didehydro epothilones) is well tolerated as is the replacement of the (3*S*)-hydroxyl by a (3*S*)-cyano group.[15] The BMS group's findings on *trans*-3-deoxy-2,3-didehydro epothilones are suggestive of an *anti*-periplanar conformation of the C2−C3 bond in the tubulin-bound state of epothilones, which has also been proposed by Carlomagno et al. on the basis of NMR transfer NOE experiments.[16] Given the conformational constraints imposed by the cyclic structural framework of epothilones, we surmised that the preference for an antiperiplanar conformation about the C2-C3 bond might not critically depend on the presence of a hydroxyl group at C3 (even without desaturation between C2 and C3), especially in light of the sterically demanding quaternary nature of C4. In order to provide experimental support for this hypothesis, we embarked on the synthesis and biological evaluation of 3-deoxyEpo B (**13**),[17] a compound that had not been previously described in the literature. Key transformations in the synthesis of **13** (Scheme 1) comprised the Suzuki−Miyaura coupling of olefin **9** with vinyl iodide **10**, Yamaguchi macrolactonization of seco acid **11**, and finally epoxidation of the C12−C13 double bond in deprotected macrolactone **12**. Unexpectedly, macrolactonization was accompanied by partial epimerization at C15, which later necessitated purification of **12** by preparative HPLC (in order to remove the minor isomer).[17]

Intriguingly, 3-deoxyEpo B (**13**) showed potent tubulin-polymerizing and antiproliferative activity, despite the absence of a hard conformational constraint between C2 and C3, such as a C=C double bond.[17] The cellular activity of the compound against the human cervical cancer cell line KB-31 is only *ca*. 25-fold lower than that of Epo B ($IC_{50}$-values of 7.4 nM and 0.29 nM for **13** and Epo B, respectively (Table 1)) and

**SCHEME 1**[a]




[a] Conditions: (i) LDA, −78 °C, 2 h, 58% (dr = 2.5/1). (ii) TBSOTf, 2,6-lutidine, −10 °C, 1 h, 82%. (iii) $H_2$/Pd-C, MeOH, 1 h, 97%. (iv) *o*-$NO_2$PhSeCN, $Bu_3P$, $NaHCO_3$, $H_2O_2$, RT, 2 h, 69%. (v) (a) Olefin **9**, 9-BBN, THF, RT, 2 h (solution A); (b) Solution A added to mixture of $Cs_2CO_3$ (2 equiv), $PdCl_2(dppf)_2$ (0.2 equiv), $Ph_3As$ (0.2 equiv), vinyl iodide **10**, DMF, −10 °C→ RT, 16 h, 55%. (vi) LiOH (6 equiv), *i*-PrOH/$H_2O$ 4/1, 60 °C, 3 h, 98%. (vii) 2,4,6-$Cl_3C_6H_2C(O)Cl$, $Et_3N$, THF, 0 °C, 15 min, then diluted with toluene and added to a solution of DMAP in toluene, RT, 1 h, 80% (2/1 mixture of epimers). (viii) HF·pyridine, THF, RT, 6 h, 90% (2/1 mixture of isomers; pure **12** obtained through purification by preparative HPLC). (ix) $MeReO_3$, $H_2O_2$/pyridine/$H_2O$, RT, 90 min, 72% (9/1 mixture of epoxide isomers; pure **13** obtained through purification by preparative HPLC).

**TABLE 1.** Tubulin-Polymerizing and Antiproliferative Activity of Natural and Modified Epothilones

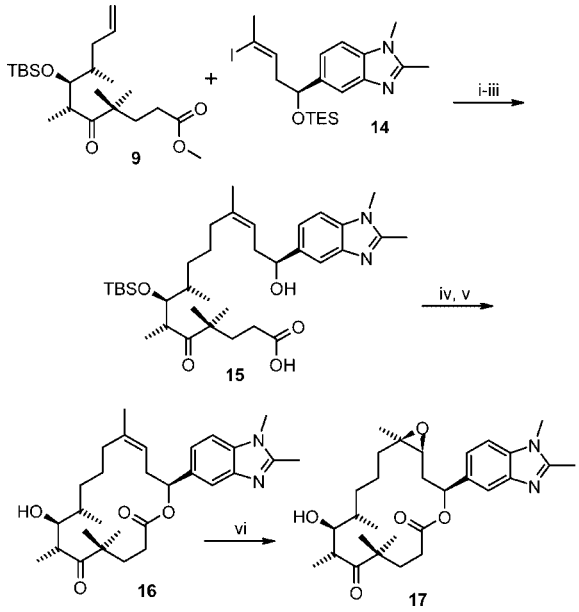
| compound | % tubulin polymerization[b] | $IC_{50}$ KB-31 (nM)[d] | $IC_{50}$ KB-8511 (nM)[d] |
|---|---|---|---|
| Epo A | 62.6 | 2.15 ± 0.07 | 1.91 ± 0.07 |
| Epo B | 95.8 | 0.29 ± 0.05 | 0.22 ± 0.05 |
| Epo C[a] | nd[c] | 24.9[e] | 9.9[e] |
| Epo D[a] | 81.0 | 2.70 ± 1.31 | 1.44 ± 0.78 |
| taxol | 53.7 | 3.68 ± 0.68 | 805 ± 144 |
| **13** | 92.2 | 7.4 ± 2.2 | 4.0 ± 1.6 |
| **12** | 78.7 | 114 ± 7 | 74 ± 3 |
| **17** | nd[c] | 0.58[f] | 1.89[f] |
| **16** | nd[c] | 4.99 ± 2.15 | 5.75 ± 3.24 |
| **25** | 95 | 0.25 ± 0.02 | 1.36 ± 0.36 |
| **28** | nd[c] | 0.17 ± 0.04 | 0.13 ± 0.02 |
| **30** | 82.0 | 3.16 ± 0.55 | 7.60 ± 2.44 |
| **40** | nd[c] | 0.34 ± 0.15 | 222 ± 48 |

[a] Epo C = 12,13-deoxyepothilone A; Epo D = 12,13-deoxyepothilone B. [b] Induction of polymerization of bovine brain tubulin by 5 $\mu$M test compound relative to the effect of 25 $\mu$M Epo B, which gave maximal polymerization (100% value). [c] nd = not determined. [d] $IC_{50}$ values for growth inhibition of the human cervical carcinoma cell lines KB-31 and KB-8511, respectively. KB-8511 is a P-glycoprotein 170 (P-gp170)-overexpressing multidrug-resistant subline of the KB-31 line. Cells were exposed to compounds for 72 h. Numbers are means of ≥3 independent experiments (±SD). [e] Single determination. [f] Average values for two determinations. Individual values: 0.54/0.62 (KB-31) and 1.61/2.16 (KB-8511). Data are from refs 17 (**12**, **13**, **16**, **17**, **30**), 19 (**25**), 21 (**28**), and 25 (**40**).

is thus comparable with that of taxol, Epo A, or *trans*-3-deoxy-2,3-didehydro-Epo B. Compound **13** also retains activity against multidrug-resistant KB-8511 cells. The reduction in antiproliferative activity is more pronounced for deoxy compound **12**, which is *ca*. 40-fold less potent than Epo D (12,13-deoxyEpo B) (Table 1).

While these data were highly encouraging, the removal of the 3-hydroxyl group was meant to represent only one element in our overall strategy toward hypermodified epothilone analogs, which were envisioned to incorporate additional modifications that would not further diminish biological potency. Based on our previous work on conformationally constrained side chain modifications, this quest led to the design of analog **17**,[17] which combines the 3-deoxyEpo B scaffold with a potentially potency-enhancing dimethyl-benzimidazole side chain. The synthesis of this analog is summarized in Scheme 2 and again proceeds through Pd(0)-catalyzed Suzuki−Miyaura coupling of olefin **9** with an appropriate vinyl iodide (i.e., **14**) as a key step. As in the synthesis of **13**, the coupling product was converted into the corresponding seco acid **15** by ester saponification and selective TES removal from O−C15. Yamaguchi macrolactonization with **15** followed by deprotection of O−C7 and epoxidation of the resulting 3-deoxyEpo D analog **16** then led to analog **17** as the final target structure. Again, macrolactonization produced a side product, which based on spectroscopic data was identified as the C15-*R* epimer and which was separated by flash chromatography at the stage of the free 12,13-deoxy compound **16** (i.e., after TBS removal from O−C7). The epoxidation of **16** with catalytic $MeReO_3$ and $H_2O_2$/pyridine in $CH_2Cl_2$[18] proceeded with >10/1 selectivity (85% yield for the mixture of isomers), and pure **17** was obtained in 54% isolated yield after purification by preparative HPLC. In contrast to **16** , the epoxidation of the corresponding Epo C analog (3-deoxyEpo C; Epo C = 12,13-deoxyEpo A) under identical conditions proved to be much less selective (2/1 selectivity in favor of the

**SCHEME 2**[a]




[a] Conditions: (i) (a) Olefin **9**, 9-BBN, THF, RT, 2 h (solution A); (b) solution A added to a mixture of $Cs_2CO_3$ (2 equiv), $PdCl_2(dppf)_2$ (0.2 equiv), $Ph_3As$ (0.2 equiv), vinyl iodide **14** (1 equiv), DMF, 0 °C→ RT, 2 h, 77%. (ii) LiOH (6 equiv), *i*-PrOH/$H_2O$ 4/1, 60 °C, 3 h, 90%. (iii) TBAF, THF, RT, 24 h, 75%. (iv) 2,4,6-$Cl_3C_6H_2C(O)Cl$, $Et_3N$, THF, 0 °C, 15 min, then diluted with toluene and added to solution of DMAP in toluene, RT, 1 h, 85% (10/1 mixture of epimers). (v) HF·pyridine, $CH_3CN$, RT, 3 h, 54% (single isomer; 87% for mixture of epimers). (vi) $MeReO_3$, $H_2O_2$/pyridine/$H_2O$, RT, 30 min, 64% (10/1 mixture of epoxide isomers).

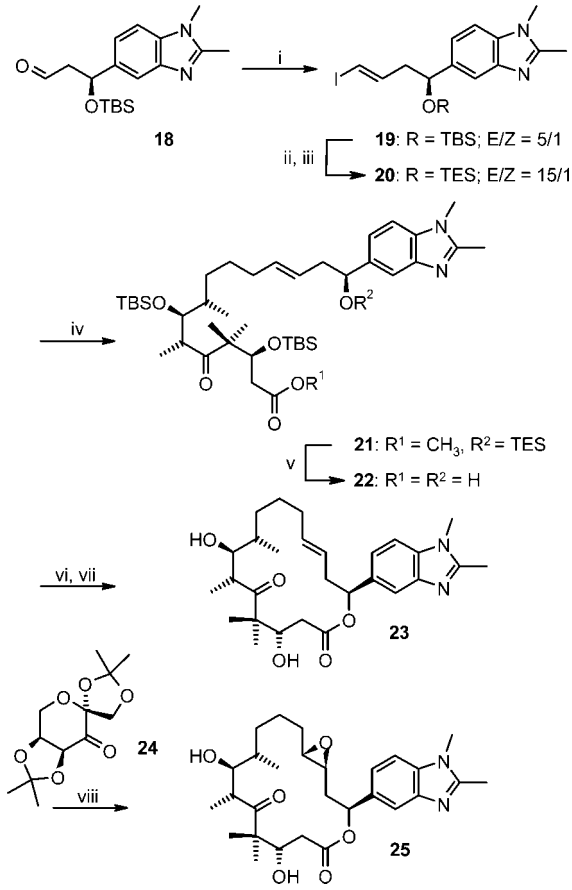
desired epoxide isomer) and the (12*R*,13*S*)-epoxide could only be obtained in 16% isolated yield (after purification by preparative TLC).[17]

The benzimidazole side chain in **17** leads to a significant potency increase over **13**, such that the activity of **17** against the drug-sensitive KB-31 line becomes comparable with that of Epo B, which is the most potent natural epothilone (Table 1).[17] Thus, the side chain modification can largely compensate for the removal of the 3-hydroxyl group from the natural epothilone core structure. In contrast to the drug-sensitive KB-31 cells, a smaller activity difference between **17** and **13** is observed for the multidrug-resistant KB-8511 line; in fact, compound **17** shows a small (3−4-fold), but clearly detectable activity differential between the sensitive and the resistant cell lines, indicating that it may be a weak substrate for the P-gp efflux pump. We have made similar observations for other benzimidazole-based epothilone analogs,[14,19] and reduced activity against KB-8511 cells has also been noted for a variety of structurally unrelated (i.e., non-benzimidazole-based) analogs, if they were more polar (reduced clogP) than the corresponding parent epothilone (M. Wartmann, K.-H. Altmann, A. Flörsheimer, unpublished data). This point will be addressed in more detail below. Similar conclusions as discussed here for compound **17** can also be derived for the benzimidazole-based analogs of 3-deoxyEpo D (**16**; Table 1)), 3-deoxyEpo A, and 3-deoxyEpo C.

## 3. *trans*-Epothilone A analogs

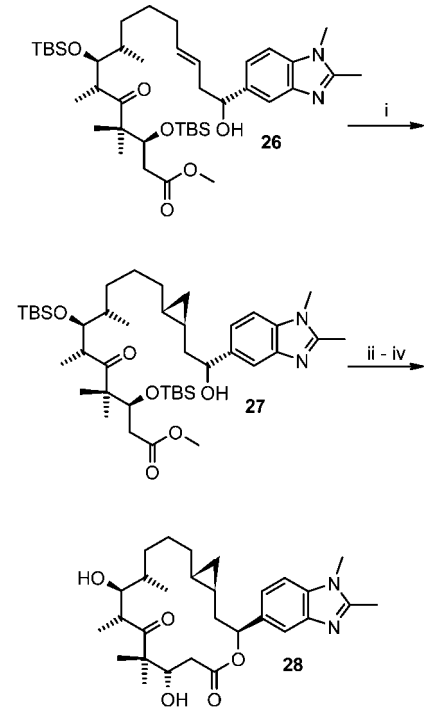
While compounds **13** and **17** deviate from natural epothilones in the structure of the core macrocycle and the heterocycle-bearing side chain, they are still characterized by a *cis* configured epoxide moiety at positions 12 and 13, which is a major structural hallmark of the natural products. On the other hand, synthetic (12*S*,13*S*)-*trans* Epo A (but not the corresponding (12*R*,13*R*)-isomer) is an equally potent tubulin polymerization inducer and cancer cell growth inhibitor as Epo A (*vide supra*),[13] which suggested that the *trans*-Epo A macrocycle could serve as a basic template for the development of new chemotypes for microtubule inhibition. In this context, it was important to understand whether the combination of the *trans*-Epo A scaffold with modifications that had proven to enhance potency or be reasonably well tolerated in *cis*-epoxide-based structures would still lead to potent analogs or whether the SAR pattern of *trans*- and *cis*-epothilones might in fact be different. In a first step, this question was addressed by the replacement of the natural epothilone side chain in *trans*-Epo A with a dimethyl-benzimidazole moiety, leading to compound **25** (Scheme 3) as an initial target structure for synthesis and biological evalution.[19] As illustrated in Scheme 3, the synthesis of **25** according to the strategy employed for **13** and **17** required *trans*-vinyl iodides **19** or **20** as the coupling partner for the Suzuki−Miyaura coupling with olefin **9**. These intermediates were obtained by Takai reaction with aldehyde **18**, which produced an inseparable 5/1 mixture of (TBS-protected) *E*- and *Z*-vinyl iodides (**19**). However, conversion of **19** into the corresponding mixture of free alcohols enabled the isolation of the desired *E*-isomer through careful crystallization in almost pure form (*E*/*Z*-ratio >15; 43% yield). Reprotection of the free homoallylic hydroxyl group provided TES-ether **20**, which underwent smooth Suzuki−Miyaura coupling with olefin **9** to produce **21**. Subsequent ester saponification was accompanied by concurrent loss of the TES group on O−C15 and the resulting seco acid **22** was converted into *trans*-Epo C analog **23** through Yamaguchi macrolactonization and TBS-ether cleavage. Treatment of **23** with an excess of oxone in the presence of 0.8 equiv of ketone **24**[20] produced an 8/1 mixture of epoxide isomers (70% yield after flash chromatography), from which **25** was isolated in pure form and 53% overall yield through preparative HPLC.[19] The yield of the epoxidation step (**23**→**25**) significantly exceeded the yield

**SCHEME 3**[a]




[a] Conditions: (i) $CHI_3$, $CrCl_2$ (8 equiv), dioxane/THF 6/1, 62%. (ii) CSA, $CH_2Cl_2$/MeOH, crystallization from $CH_2Cl_2$/hexane/MeOH, 43% (92% for 5/1 mixture of *E*/*Z* isomers before crystallization). (iii) TES-Cl, imidazole, DMF, 0 °C, 90 min, 97%. (iv) (a) Olefin **9**, 9-BBN, THF, RT, 2 h (solution A); (b) addition of solution A to a mixture of $Cs_2CO_3$, $PdCl_2(dppf)_2$, $Ph_3As$, vinyl iodide **20**, DMF, −10 °C → RT, 16 h, 55%. (v) LiOH (6 equiv), *i*-PrOH/$H_2O$ 4/1, 50 °C, 7 h, 78%. (vi) 2,4,6-$Cl_3C_6H_2C(O)Cl$, $Et_3N$, THF, 0 °C, 15 min, then dilution with toluene and addition to solution of DMAP in toluene, RT, 1 h, 93%. (vii) HF·pyridine, THF, RT, 8 h, 80%. (viii) Oxone, ketone **24**, $Bu_4N(HSO_4)$ (cat), $K_2CO_3$, MeCN/DME/0.05M $Na_2B_4O_7 \cdot 10H_2O$ in $4 \times 10^{-4}$ M $Na_2EDTA$ 1/1/1.3, 0 °C, 3 h, 70%.

**SCHEME 4**[a]




[a] Conditions: (i) (a) $Et_2Zn$ (9 equiv), $CH_2I_2$ (9 equiv), $CF_3COOH$ (9 equiv), −13 °C, 30 min; (b) + **26**, −13 °C, 20 min, 77%. (ii) LiOH, *i*-PrOH/$H_2O$ 4/1, 60 °C, 3 h, 91%. (iii) $PPh_3$, DIAD, toluene, −13 °C, 90 min, 40% (57% based on recovered SM). (iv) HF·pyridine, $CH_3CN$, RT, 2 h, 42% (after HPLC purification, 83% crude).

obtained for the conversion of *trans*-Epo C into *trans*-Epo A (**3**) under similar conditions (13%).[13]

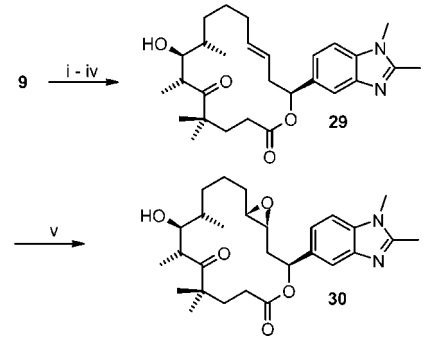
*trans*-Epo A analog **25** is a potent inducer of tubulin polymerization, and it exhibits high antiproliferative activity against the drug-sensitive human cervical cancer cell line KB-31 (Table 1).[19] Significantly, compound **25** is a more potent inhibitor of KB-31 cell growth than the parent compound *trans*-Epo A (**3**) ($IC_{50}$ values of 0.25 and 2.15 nM, respectively (Table 1)); in fact, the activity of **25** against this drug-sensitive cell line is comparable with that of Epo B as the most potent natural epothilone, which further attests to the activity-enhancing effect of the dimethyl-benzimidazole moiety. In contrast to KB-31 cells, a significantly different picture emerged for the P-gp-overexpressing, multidrug-resistant KB-8511 cell line, where **25** (like **17** and other dimethyl-benzimidazole-based epothilone analogs) showed reduced growth inhibitory activity (compared with its effects against the drug-sensitive KB-31 line). This finding suggests that **25** may be a (moderate) substrate for the P-gp efflux pump, which seems not to be the case for *trans*-Epo A (**3**) itself.[13] It should be noted, however, that benzimidazole-based analog **25** is still far less susceptible to P-gp-mediated efflux than taxol, for which the activity differential between KB-8511 and KB-31 cells is *ca*. 230-fold.

As indicated above, reduced activity against the P-gp-overexpressing KB-8511 cell line had been observed in our laboratory for a variety of epothilone analogs with enhanced polarity, including Epo B analog **17** (Table 1). Based on this observation, we speculated that the activity of **25** (and other benzimidazole-based epothilone analogs) against P-gp-overexpressing cells might be restored through appropriate adjustments in compound polarity, and we felt that in the case of **25** this could possibly be achieved by the isosteric replacement of the epoxide moiety with a cyclopropane ring. This type of modification had previously been shown to be well tolerated in natural Epo A and B[8] and was predicted to produce a change in clogP from 2.87 for **25** to 4.15 for the corresponding cyclopropyl analog **28** (Scheme 4).

At the synthetic level, the most straightforward approach to target structure **28** would consist in the direct cyclopropanation of the corresponding 12,13-deoxy precursor **23** (Scheme 3), and this reaction was investigated under a variety of conditions. Cyclopropanation of **23** could indeed be achieved with $Et_2Zn/CH_2I_2$ in the presence of equimolar amounts (relative to $Et_2Zn$ and $CH_2I_2$) of $CF_3CO_2H$, but a large excess (120 equiv) of cyclopropanating agent was necessary to drive the reaction to completion.[21] In addition, these conditions were associated with a complete lack of stereoselectivity and both cyclopropane isomers were formed in a 1/1 ratio. Other approaches to convert **23** into **28** either gave no conversion ($Et_2Zn/CH_2I_2/Cl_3PhOH$ or $CH_2N_2/Pd(OAc)_2$) or only led to the formation of degradation products ($CHBr_3$/NaOH/$BnEt_3NCl$).[21] The stereoselective incorporation of the cyclopropane moiety in analog **28** could finally be accomplished through the directed cyclopropanation of homoallylic alcohol **26** (Scheme 4), an approach that was suggested by prior work on the *cis*-cyclopropane isomer of **28**.[21] Thus, the treatment of **26** with 9 equiv of $Et_2Zn/CH_2I_2/CF_3CO_2H$ for 20 min produced cyclopropane **27** in 77% yield and essentially as a single isomer (Scheme 4).[21] It should be noted that the C15 stereocenter in **26** and **27** exhibits the "non-natural" *R* configuration, which is required for the cyclopropanation to provide the desired absolute configuration at C12,C13. Seco ester **27** could be elaborated into *trans*-Epo A analog **28** through ester saponification with LiOH in *i*PrOH/$H_2O$, Mitsunobu macrolactonization (which enables the concomitant inversion of stereochemistry at C15), and deprotection with HF·pyridine in 17% overall yield (including HPLC purification of the final product; Scheme 4).[21] The *cis*-cyclopropane isomer of **28** could be obtained in an analogous way, and both compounds were found to be extremely potent antiproliferative agents, which inhibit the growth of the drug-sensitive human cervical carcinoma cell line KB-31 with sub-nanomolar $IC_{50}$ values (Table 1; data for the *cis*-isomer not shown).[21] Thus, **28** ($IC_{50} = 0.17$ nM) inhibits the growth of drug-sensitive KB-31 cells with at least the same potency as the corresponding epoxide **25** ($IC_{50} = 0.25$ nM), and it is clearly more potent than its epoxide-based parent compound *trans*-Epo A (**3**). Most importantly, however, **28** retains *full* activity against the P-gp-overexpressing KB-8511 variant of the KB-31 line ($IC_{50} = 0.13$ nM), which is in marked contrast to the situation with epoxide-based analog **25**. The data indicate that **28** is significantly less susceptible to P-gp-mediated drug efflux than **25**, and the same applies to the corresponding pair of *cis*-cyclopropane/epoxide isomers.[21]

The potent biological activity of analogs **25** and **28** suggested that further modification of these structures by the removal of the C3-hydroxyl group would likely produce new hypermodified epothilone analogs that would retain significant biological activity. So far, this suggestion has been experimentally addressed with epoxide-based analog **30**, which was obtained from **9** via Suzuki–Miyaura coupling with vinyl iodide **20**, elaboration of the coupling product into deoxy analog **29**, and final epoxidation of the C12–C13 *E* double bond (Scheme 5).[17] As for the transformation of **23** to **25** (Scheme 3), the epoxidation step involved the use of catalyst **24**[20] and provided **30** as single isomer in 65% isolated yield after simple flash chromatography (86% based on recovered starting material).

**SCHEME 5**[a]




[a] Conditions: (i) (a) Olefin **9**, 9-BBN (0.8 equiv), THF, RT, 2 h (solution A); (b) addition of solution A to a mixture of $Cs_2CO_3$ (1.5 equiv), $PdCl_2(dppf)_2$ (0.1 equiv), $Ph_3As$ (0.2 equiv), vinyl iodide **20** (Scheme 3, 1 equiv), DMF, −10 °C→ RT, 16 h, 58%. (ii) LiOH (6 equiv), THF/$H_2O$ 7/1, RT, 24 h, 76%. (iii) 2,4,6-$Cl_3C_6H_2C(O)Cl$, $Et_3N$, THF, 0 °C, 15 min, then diluted with toluene and added to a solution of DMAP in toluene, RT, 1 h, 71%. (iv) HF·pyridine, THF, RT, 6 h, 94%. (v) Oxone, ketone **24** (0.8 equiv), $Bu_4N(HSO_4)$ (cat), $K_2CO_3$, MeCN/DMM/0.05M $Na_2B_4O_7 \cdot 10\ H_2O$ in $4 \times 10^{-4}$ M $Na_2EDTA$ 1/1/1.3, 0 °C, 3 h, 65% (86% based on recovered starting material; single isomer).

Quite remarkably, the tubulin-polymerizing and antiproliferative activity of **30** is virtually identical with that of Epo A or taxol, despite its harboring major structural alterations relative to the native epothilone scaffold (Table 1).[17] As for analog **25**, the growth inhibitory activity of **30** is slightly lower against the multidrug-resistant KB-8511 cell line than the taxol-sensitive KB-31 line. However, as demonstrated above, this shortcoming may be correctable through the replacement of the epoxide ring by a cyclopropane moiety, and studies along these lines are currently in progress in our laboratory.

## 4. 12-Aza-epothilones

As an alternative to the iterative modification approach described above, which does not alter the polyketide character of the core macrocycle, our efforts to develop new structural templates for microtubule stabilization have also included the design of different types of *aza*-epothilones. These analogs are characterized by the replacement of a backbone car-

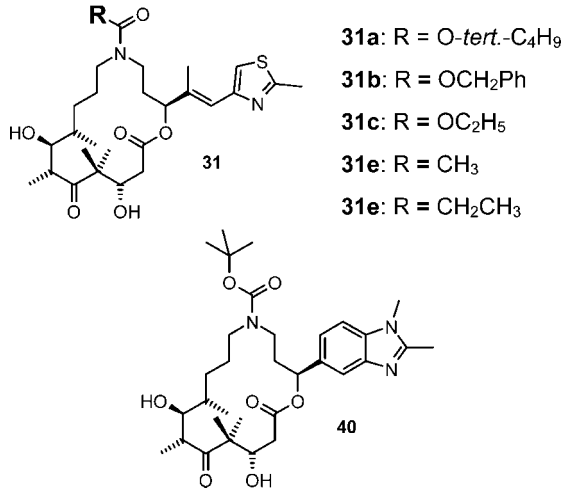
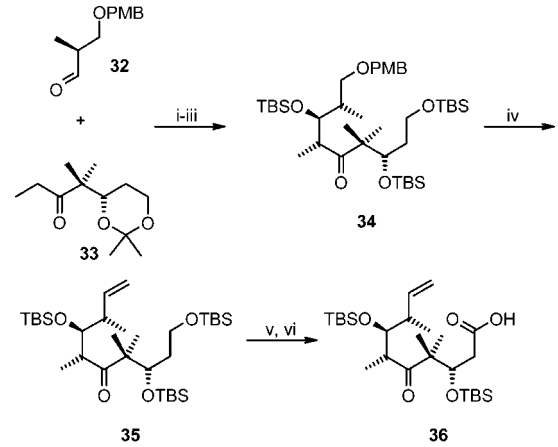



**FIGURE 2**

bon atom with nitrogen, which disrupts the regular polyketide structure of the macrocyclic backbone. As a result such aza-epothilones may be appropriately described as "non-natural" natural products: They still retain most of the (two-dimensional) structural features of the natural product lead, but at the same time they are structurally unique, because they are outside of the general scope of Nature's biosynthetic machinery for polyketide biosynthesis, which is not programmed for the incorporation of single nitrogen atoms in a regular polyketide backbone.[22] Among different classes of aza-epothilones investigated,[23–25] 12-aza analogs of type **31** (termed "azathilones"; Figure 2) proved to be of particular interest, because they can retain much of the antiproliferative activity of natural epothilones, depending on the nature of the acyl substituent attached to the backbone nitrogen atom.[23] The most potent analog identified so far (out of a limited collection of structures with varying N-substituents; Figure 2) is *tert*-butyl carbamate **31a**, whose antiproliferative activity is still in the 100 nM range (i.e., only 15–50-fold lower than the activity of Epo A). Considering the absence of any hard conformational constraint from the Northern part of the macrocycle, these results were highly intriguing; at the same time the question arose whether and how the activity of azathilone **31a** could possibly be improved, such as to approach the potency range of natural epothilones. Based on the significant potency enhancement associated with a dimethyl-benzimidazole side chain attached to polyketide-based, epothilone-derived macrocycles, the replacement of the natural epothilone side chain in **31a** with a dimethyl-benzimidazole moiety presented itself as an obvious approach toward the creation of highly potent new azathilones, thus leading to compound **40** as an initial target for total synthesis and biological investigation (Figure 2).

**SCHEME 6**[a]

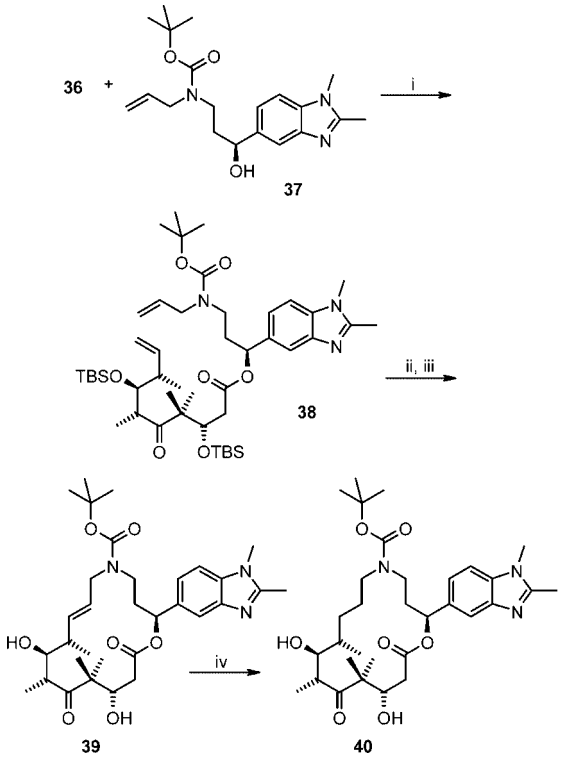



[a] Conditions: (i) **33**, LDA, −78 °C, 5 h, then addition of **32**, −90 °C, 75 min, 76%, dr = 8/1. (ii) PPTS, MeOH, RT, 20 h, 86%. (iii) (a) TBSOTf, 2,6-lutidine, −78 °C → RT, 1.5 h; (b) flash chromatography, 76% (single isomer). (iv) (a) $H_2$/Pd−C, MeOH, RT, 20 h; (b) TPAP, NMO, 4-Å MS, $CH_2Cl_2$, RT, 1 h; (c) $MePPh_3Br$, LiHMDS, THF, 0 °C, 1.5 h, 79% (three steps). (v) CSA (1.0 equiv), $CH_2Cl_2$/MeOH 1/1, 0 °C, 1 h, 87%. (vi) PDC (11 equiv), DMF, RT, 64 h, 85%.

Our synthesis of **40** was based on macrocycle formation through ring-closing olefin metathesis (RCM) between C9 and C10. Apart from the high chemical efficieny generally associated with RCM-based macrocyclizations,[26] this strategy offered the additional advantage that it would also provide the corresponding 9,10-didehydro analog of **40** (as the immediate cyclization product). Because previous work by Danishefsky and co-workers had shown the *E*-9,10-didehydro analogs of Epo B and D to be even more active in cancer cell proliferation assays than the respective saturated parent compounds,[27] it was of interest to assess this modification in the context of an aza-macrolide scaffold.

The RCM-based synthesis of azathilone **40** is summarized in Schemes 6 and 7 and involved three key strategic steps, namely, (i) the stereoselective aldol reaction between aldehyde **32** and ketone **33** (dr = 8:1) (Scheme 6), (ii) esterification of carboxylic acid **36** with the unsaturated alcohol **37**, and (iii) RCM with diene **38** (Scheme 7). While the treatment of diene **38** with the first generation Grubbs catalyst[26] gave no measurable conversion, the use of the dihydroimidazol-2-ylidene-based second generation catalyst[26] produced the cyclic olefin in excellent yield (85%) and with exclusive *E* selectivity. A similar degree of selectivity had been observed previously for the RCM-based cyclization of the analogous diene **41** (Figure 3), which incorporates the natural epothilone side chain. The efficiency of the cyclization reaction was somewhat thwarted by difficulties encountered in the subsequent reduction of the 9,10-double bond, which proved to be extremely sluggish under all experimental conditions investigated (thus leading to low yields and also side reactions such

**SCHEME 7**[a]




[a] Conditions: (i) DCC (1.2 equiv), DMAP (0.3 equiv), $CH_2Cl_2$, 0 °C, 15 min, RT, 15 h, 60%. (ii) 2nd generation Grubbs catalyst (0.15 equiv, incremental addition), $CH_2Cl_2$, reflux, 8 h, 85%. (iii) HF·pyridine, pyridine, THF, RT, 4 h, 70%. (iv) $KO_2C{-}N{=}N{-}CO_2K$ (excess), AcOH, $CH_2Cl_2$, refl., 31%; pure **40** obtained through purification by preparative HPLC.

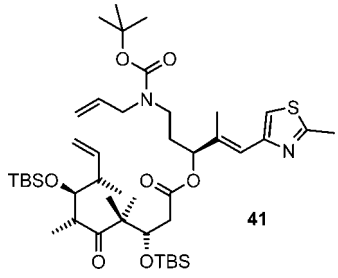



**FIGURE 3**

as reductive ester cleavage with $H_2$/Pd−C without reduction of the double bond). The only viable approach for the transformation of **39** into **40** involved the use of *in situ* generated diimide, which had been successfully employed in the transformation of 9,10- and 10,11-didehydroEpo D, respectively, into Epo D (12,13-deoxyEpoB)[27] and which produced **40** in 31% yield from **39** after purification by preparative HPLC.

Azathilone **40** was found to be a highly potent antiproliferative agent that inhibits the growth of different types of drug-sensitive human cancer cell lines (KB-31, A549, HCT-116, PC-3M) with low nanomolar $IC_{50}$ values (Table 1; $IC_{50}$-values against the A549, HCT-116, and PC-3 M cell lines are 1.9, 1.6, and 1.3 nM, respectively, vs 3.2, 2.2, and 3.4 nM for Epo A). The antiproliferative activity against drug-sensitive human tumor cells is thus comparable to that of Epo A. Likewise, **40** induces tubulin polymerization *in vitro* with similar potency to Epo A ($EC_{50}$ values for the induction of tubulin polymerization of 3.9 and 4.6 $\mu$M for **40** and Epo A, respectively),[25] which strongly suggests that inhibition of human cancer cell proliferation by **40**, as for natural epothilones, is a consequence of interference with microtubule functionality. This view is further corroborated by the fact that treatment of cancer cells with **40** results in cell cycle arrest at G2/M, which mirrors the effects on the cell cycle observed upon treatment with Epo A or B.[25]

Compound **40** is >60-fold more potent against drug-sensitive human cancer cells than the corresponding parent azathilone **31a** (Figure 2); this potency increase dramatically exceeds the potency-enhancing effects previously observed for the dimethyl-benzimidazole side chain in combination with polyketide-based macrocycles (2−15-fold).[17] Perhaps even more intriguing is the fact that the 9,10-didehydro analog **39** is significantly less potent than the fully saturated azathilone **40**, both at the level of tubulin polymerization and in cellular activity.[25] This finding is in strong contrast with effects observed for Epo B and D (*vide supra*),[27] and it may be indicative of differences in the bioactive conformation between azathilone-type analogs and natural epothilones. NMR studies to determine the bioactive conformation of **40** are currently ongoing in our laboratory.

Unfortunately, azathilone **40** is significantly less potent against the multidrug-resistant cervical carcinoma cell line KB-8511 than the drug-sensitive KB-31 line, which indicates that **40** is a substrate for the P-gp efflux pump (Table 1). However, as illustrated above for polyketide-based epothilone analogs, the susceptibility to P-gp-mediated drug efflux may be modulated through adjustments in compound lipophilicity, and this strategy will also be explored for lead structure **40**.

## 5. Conclusions

In the first part of this Account, we have shown how the iterative modification of an appropriate natural product scaffold can lead to highly potent analogs with significantly altered structural features relative to the natural product lead. As such, **30** and related compounds can be considered as representative examples of a new chemotype for microtubule stabilization, which may offer the same potential for pharmacological differentiation from the original epothilone leads as various newly discovered microtubule-stabilizing natural products with macrolactone structures, such as laulimalide, peloruside, or

dictyostatin.[9] While this assumption will have to be substantiated by future pharmacological studies, it should be noted that preliminary similarity analyses on the basis of Tanimoto coefficients have indicated **30** to be structurally equally dissimilar from Epo A or B as are laulimalide or peloruside.[28] Similar conclusions apply for **40** and related 12-aza-epothilones.[28] While the conception of these aza-macrolides is closely connected to the structure of natural epothilones (hence the name "azathilones"), given the degree of structural divergence from the natural epothilone template, they may be considered as members of a distinct group of "non-natural" natural products with unique structural features. Work currently ongoing in our laboratory aims at a better understanding of the SAR associated with structures of type **30** and **40**, which may not parallel the SAR associated with natural epothilones (as indicated by the activity difference between **40** and **39**). At the same time, more extensive biological profiling of selected analogs is required *in vitro* and *in vivo*, in order to establish whether compounds such as **30** or **40** are in fact pharmacologically distinct from Epo A and B.

## BIOGRAPHICAL INFORMATION

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## FOOTNOTES

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28 Tanimoto coefficients for relevant compound pairs (calculated with the Molinspiration toolkit): **17**/Epo A, 0.422; **25**/Epo A, 0.423; **30**/Epo A, 0.423; **31a**/Epo A, 0.729; **40**/Epo A, 0.371; laulimalide/Epo A, 0.444; peloruside/Epo A, 0.471; peloruside/laulimalide, 0.736; Epo A/Epo B, 0.996. We are indebted to Dr. Peter Ertl for performing these calculations.