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Synthesis and Biological Evaluation of Highly Potent Analogues of Epothilones B and D

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Abstract—A series of new epothilone B and D analogues incorporating fused hetero-aromatic side chains have been prepared. The synthetic strategy is based on olefin **3** as the common intermediate and allows variation of the side-chain structure in a highly convergent and stereoselective manner. Epothilone analogues **1a–d** and **2a–d** are more potent inhibitors of cancer cell proliferation than the corresponding parent epothilones B or D. © 2000 Elsevier Science Ltd. All rights reserved.

Cancer represents one of the major causes of mortality in first world countries and the search for better anticancer drugs represents one of the most difficult and important challenges in modern drug discovery. Perhaps the most important anticancer drug developed over the past decade is Taxol® (paclitaxel),[1] which acts as a microtubule stabilizer[2] and inhibits cancer cell proliferation through interference with microtubule dynamics.[3] More recently, the natural products *epothilones* have been identified as a new class of microtubule stabilizing agents (Fig. 1).[4,5a]

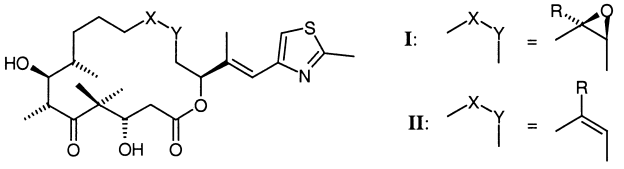

Figure 1. Structures of epothilones A (**I**, R = H), B (**I**, R = CH_3), C (**II**, R = H), and D (**II**, R = CH_3). Epothilones C and D are also known as deoxyepothilone A and deoxyepothilone B, respectively.

Like paclitaxel, these compounds exhibit potent antiproliferative activity on drug-sensitive cancer cell lines, but in contrast to paclitaxel, and also other standard cytotoxic anticancer drugs, they are equally active on multidrug-resistant human cancer cells overexpressing the P-gp 170 efflux pump.[5] Epothilones B[6] and D[5c,7] (Fig. 1) have also been reported to possess potent in vivo antitumor activity in experimental animal models. These findings have triggered a host of activities in the chemical community directed at the design and synthesis of novel epothilone analogues, which has led to a rather comprehensive understanding of the structure–activity relationship for this class of natural products.[8] However, with one exception,[9] no epothilone analogues have been described so far which exhibit more potent antiproliferative activity than their natural counterparts. In this communication we report on a new class of epothilone B and epothilone D analogues in which the natural (2-(2-methyl-thiazol-4-yl)-1-methyl)-ethenyl side-chain has been replaced by a number of benzo-heterocyclic moieties (Fig. 2) and which have generally shown more potent antiproliferative activity than the corresponding natural epothilones.

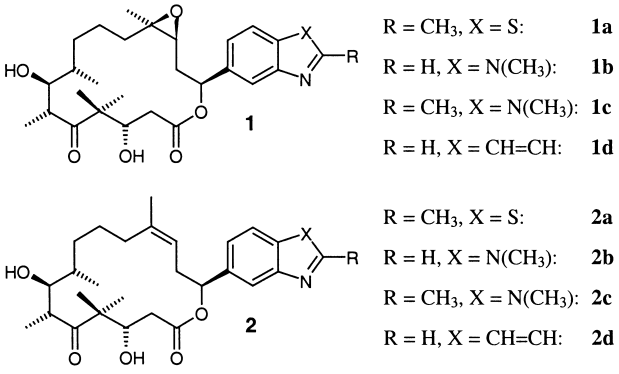

Figure 2. Epothilone analogues investigated in this study.

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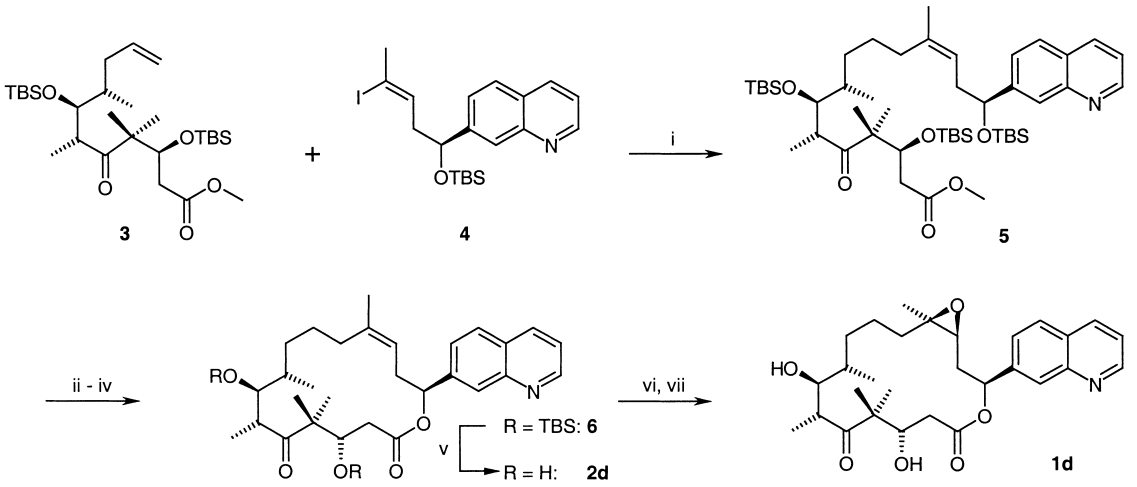
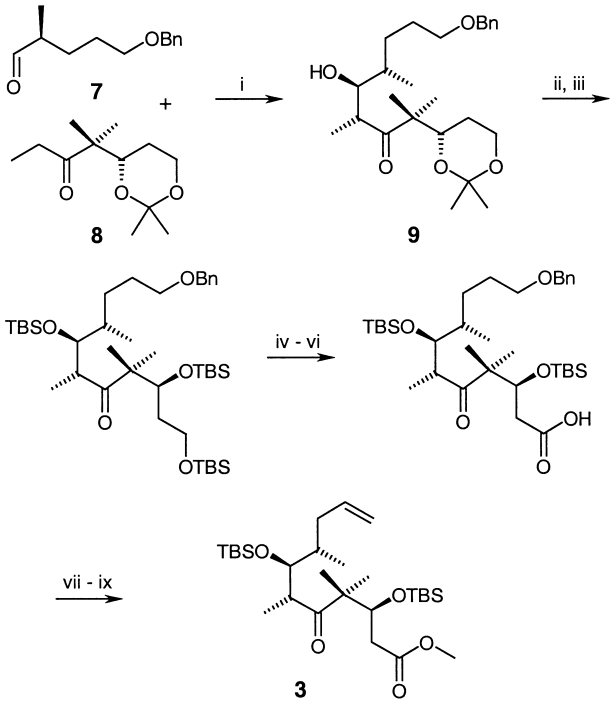


Scheme 1. (i) (a) Olefin **3** (1.25 equiv), 9-BBN, THF, rt, 4 h; (b) add to mixture of Cs_2CO_3 (1.5 equiv), $PdCl_2(dppf)_2$ (0.1 equiv), Ph_3As (0.2 equiv), vinyl iodide **4** (1 equiv), DMF, −10 °C→rt, 16 h, 90%; (ii) LiOH (6 equiv), *i*-PrOH/H_2O 4/1, 50 °C, 7 h, 84%; (iii) TBAF (3 equiv), THF, rt, 18 h, 84%; (iv) (a) 2,4,6-$Cl_3C_6H_2C(O)Cl$, Et_3N, THF, 0 °C, 15 min; (b) dilute with toluene, add to solution of DMAP in toluene, 75 °C, 3 h, 70%; (v) HF·pyridine, THF, rt, 17 h, 73%; (vi) $MeReO_3$, H_2O_2, pyridine, CH_2Cl_2, rt, 17 h; (vii) H_2, Ra-Ni, EtOH, rt, 37% (2 steps).

Our highly convergent general strategy for the synthesis of these analogues is exemplified in Scheme 1 for quinoline-based structures **1d** and **2d**. Alkyl-Suzuki coupling between olefin **3** and vinyl iodide **4**[10] provided the fully protected *seco*-acid **5** as a single double bond isomer and in excellent yield (90%). Ester saponification followed by selective deprotection of the 15-hydroxyl group with TBAF gave the free *seco*-acid, which under Yamaguchi conditions[11] smoothly cyclized to the protected macrolactone **6** in 70% yield. Removal of the TBS protecting groups with HF·pyridine then provided epothilone D analogue **2d** (73%). Epoxidation of the olefinic double bond with $MeReO_3/H_2O_2$[12] proceeded with ca. 6/1 selectivity in favor of the desired epoxide isomer, but was slightly complicated by the fact that *N*-oxidation of the quinoline moiety was faster than epoxidation. **2d** was thus first converted to the doubly oxidized species; selective reduction of the *N*-oxide with H_2/Ra-Ni then provided the desired epothilone B analogue **1d** in 37% yield for the two-step sequence from **2d**.

The syntheses of olefin **3** and vinyl iodide **4** are summarized in Schemes 2 and 3, respectively. Briefly, the key step in the preparation of **3** (Scheme 2) consists of the highly diastereoselective aldol reaction between aldehyde **7**[13] and ketone **8**[14] which provided the desired aldol diastereoisomer **9** in 82% yield after FC. Following simple protecting group manipulations and functional group transformations olefin **3** was finally obtained in 20% overall yield for the nine-step sequence from aldehyde **7**.

Preparation of **4** (Scheme 3) was based on the diastereoselective aldol reaction between acetyl sultam **10** and aldehyde **11**[15] as the key step.[16] The resulting aldol product was obtained as a 5/1 mixture of diastereoisomers, which, after silylation of the secondary hydroxyl group, could be separated by FC to provide the desired diastereoisomer **12** in 57% yield for the two-step sequence from aldehyde **11**. Direct reduction of **12** to aldehdye **13** and subsequent olefination with $[Ph_3CHICH_3]^+I^-$[17] gave vinyl iodide **4** (35% from **12**).



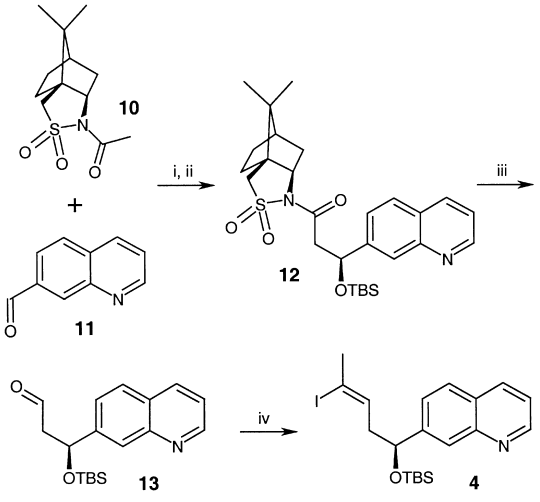
Scheme 2. (i) (a) **8** (2 equiv), LDA, THF, −78 °C, 80 min; (b) +**7** (1 equiv), −78 °C, 75 min, 82%; (ii) PPTS, MeOH, rt, 22 h, 83%; (iii) TBS-OTf, lutidine, CH_2Cl_2, rt, 84%; (iv) CSA, MeOH/CH_2Cl_2, 0 °C, 1 h, 80%; (v) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 93%; (vi) $NaClO_2$, *iso*-butene, NaH_2PO_4, THF, *tert*–BuOH, H_2O, rt, 4 h, 93%; (vii) DCC, DMAP, MeOH, CH_2Cl_2, −20 °C→rt, 4 h, 71%; (viii) H_2, Pd-C, MeOH, rt, atm. pressure, 1 h, 80%; (ix) (a) 2-NO_2-PhSeCN, Bu_3P, rt, 1 h; (b) $NaHCO_3$, 30% H_2O_2, rt, 24 h, 88%.

Table 1. Induction of tubulin polymerization and antiproliferative activity for compounds **1a–d** and **2a–d** in comparison to epothilones A, B, D

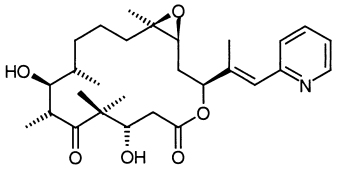
Cpd[a]	R	X	% Tubulin polymerization[b]	IC_{50} KB-31[c,e] (nM)	IC_{50} KB-8511[d,e] (nM)
1a	CH_3	S	83	0.13 ± 0.02	0.09 ± 0.02
1b	H	$N(CH_3)$	97	0.13 ± 0.01	0.46 ± 0.03
1c	CH_3	$N(CH_3)$	99	0.14 ± 0.01	0.38 ± 0.10
1d	H	CH=CH	78	0.11 ± 0.01	0.10 ± 0.02
2a	CH_3	S	76	0.45 ± 0.03	0.23 ± 0.03
2b	H	$N(CH_3)$	86	0.46 ± 0.09	0.91 ± 0.10
2c	CH_3	$N(CH_3)$	94	0.21 ± 0.01	0.73 ± 0.04
2d	H	CH=CH	90	0.59 ± 0.04	0.38 ± 0.03
	Epothilone A[f]		69	2.15 ± 0.07	1.91 ± 0.07
	Epothilone B[f]		90	0.19 ± 0.05	0.18 ± 0.01
	Epothilone D[f]		83	2.70 ± 0.76	1.44 ± 0.46
	Paclitaxel		49	2.92 ± 0.18	626 ± 32

[a]Cf. Fig. 2.
[b]Induction of polymerization of porcine brain-derived microtubule protein by 2 μM of test compound relative to the effect of 25 μM epothilone B, which gave maximal polymerization (85% of protein input). Tubulin polymerization was determined using a modified version of the centrifugation assay described in ref 18.
[c]Concentration required to inhibit the growth of paclitaxel-sensitive human epidermoid carcinoma cells KB-31 by 50% (72 h exposure).
[d]Concentration required to inhibit the growth of paclitaxel-resistant human epidermoid carcinoma cells KB-8511 by 50% (72 h exposure). KB-8511 is a P-gp 170 overexpressing paclitaxel-selected subline of the KB-31 parental line.
[e]Data are presented as mean ± standard error of the mean of at least three independent experiments.
[f]Cf. Fig. 1.

The biological activities of epothilone B analogues **1a–d** and epothilone D analogues **2a–d** are summarized in Table 1. Antiproliferative activity was assessed against the drug-sensitive human epidermoid cancer cell line KB-31 and a P-gp-overexpressing, paclitaxel-resistant subline KB-8511.[19] All *epothilone B* analogues (epoxides **1a–d**) are more potent inhibitors of KB-31 cell proliferation than epothilone B itself; **1a** and **1d** are also more active against KB-8511 cells. In addition, quinoline derivative **1d** is more potent than a pyridine-based analogue (pyridine-epothilone B **14**[9]), containing a simple 2-pyridyl moiety in place of the 2-methyl thiazole ring in natural epothilones (IC_{50}s for **14** are 0.30 nM and 0.30 nM against KB-31 and KB-8511 cells, respectively[9]).



Scheme 3. (i) (a) Et_3B, CF_3SO_3H, hexane, rt, 20 min, 40 °C, 10 min; (b) + Hünig's base, 0 °C; (c) + **10**, −5 °C, 5 min; (d) + **11**, −78 °C, 4 h, 82% (5/1 mixture); (ii) TBS-Cl, imidazole, DMF, 40 °C, 16 h, 70%. (iii) DIBAL-H, CH_2Cl_2, −78 °C, 6 h, 79%; (iv) (a) $[Ph_3PCHICH_3]^+I^-$, NaHMDS (0.95 equiv), THF, −78 °C, 30 min, −15 °C, 20 min; (b) + **13** (0.8 equiv), −78 °C, 40 min, 44%.



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The increase in growth inhibitory activity conferred by the benzo-heterocyclic side-chains is more pronounced for the *epothilone D* analogues **2a–d**, which inhibit the growth of KB-31 and KB-8511 cells up to 13-fold more potently than epothilone D. In addition, all epothilone D analogues are clearly more potent than epothilone A. It should be noted, however, that differences in cellular activity do not directly correlate with the tubulin polymerization data shown in Table 1, which define the ability of a compound to induce tubulin polymerization in vitro at a fixed concentration (2 μM) relative to the effect of 25 μM epothilone B. However, these data provide only a gross measure for tubulin affinity and in order to gain a better understanding of the relationship between the induction of tubulin polymerization in vitro and antiproliferative activity at the cellular level, we have determined accurate EC_{50}-values for in vitro tubulin polymerization for a series of selected compounds. Thus, the concentrations required to induce 50% polymerization of porcine brain-derived microtubule protein (EC_{50}) are 0.67 μM for epothilone B, 1.12 μM for epothilone A, 0.88 μM for epothilone D, and 0.93 μM for compound **2a**. These data suggest that at least for **2a** the improved antiproliferative activity over epothilone D is not the result of more pronounced effects on tubulin/microtubules, but may rather be related to parameters such as cellular uptake or intracellular processing.

In summary, we have developed a highly convergent route for the synthesis of epothilone B analogues **1a–d** and epothilone D analogues **2a–d**. Several of these

compounds are more potent inhibitors of human cancer cell growth than the respective parent epothilone and this is particularly true in the epothilone D series.[20] Given the fact that epothilone D has been suggested to be a promising anticancer drug candidate with a potentially improved therapeutic index over epothilone B (due to the absence of the epoxide moiety as the purported source of unspecific toxicity),[5c,7] our improved epothilone D analogues could represent interesting candidates for anticancer drug development. We are currently in the process of evaluating the in vivo antitumor activity of these compounds and the results of these studies will be reported in due course.

Acknowledgements

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