



Design, Synthesis, and Biological Properties of Highly Potent Epothilone B Analogues**

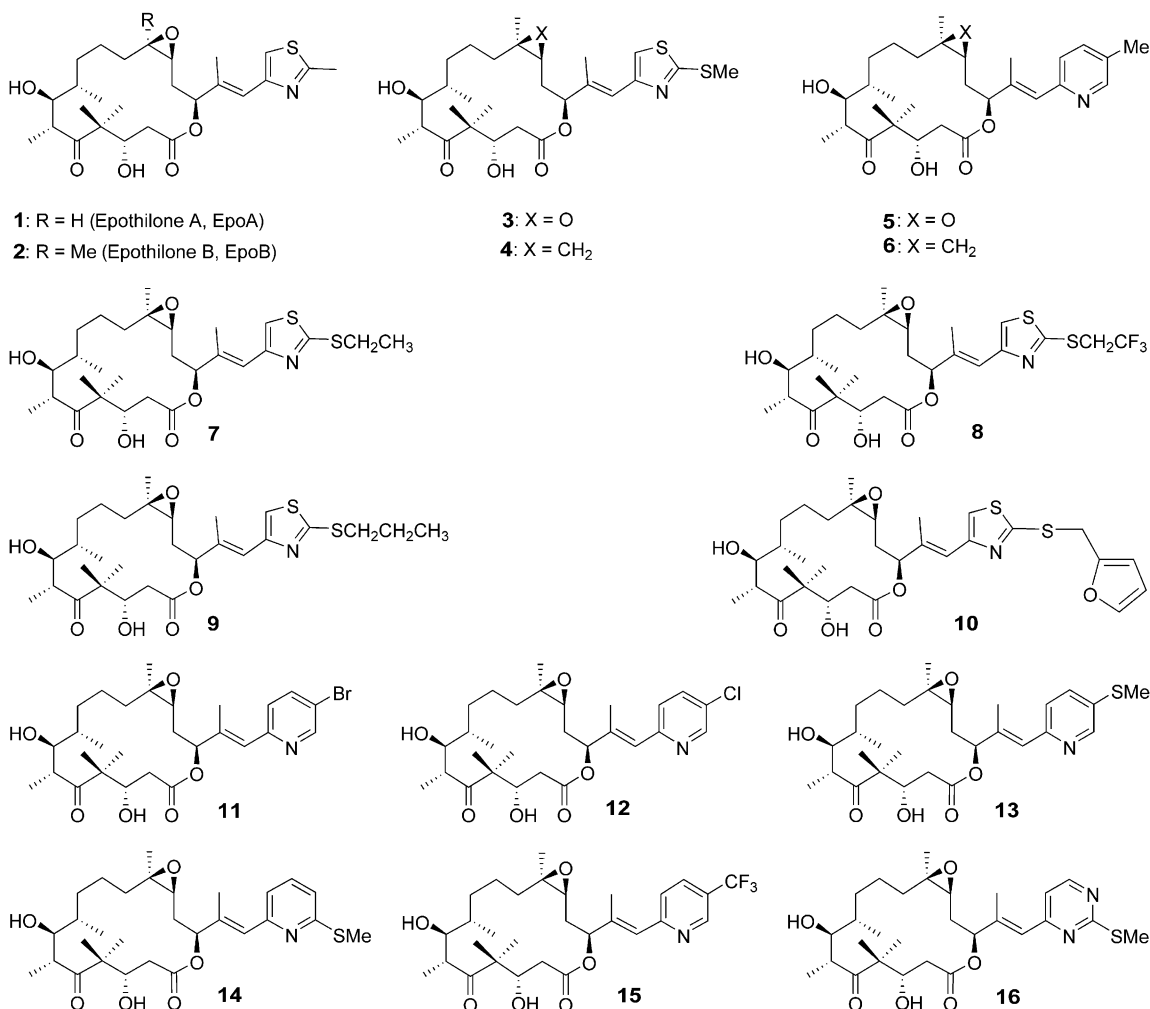
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Owing to their potent cytotoxicity against tumor cells, including taxol (paclitaxel)-resistant cell lines, the epothilones (for example, epothilone A (**1**) and epothilone B (**2**))^[1] continue to be the focus of intense chemical, biological, and clinical research efforts around the world.^[2,3] Following the findings that cyclopropane-,^[4] methylsulfanylthiazole-,^[2d,5] and pyridine-^[6]containing epothilone B derivatives (e.g. **3**^[5a] and **5**,^[6]) exhibit outstanding biological profiles as potential antitumor agents, we directed our attention toward the synthesis and evaluation of a small designed library of epothilone B analogues whose members are characterized by such structural motifs. Herein we report the details of these synthetic and biological investigations, which culminated in the discovery of 12,13-*cis*-cyclopropane methylsulfanyl epothilone B (**4**) as an extremely potent epothilone B analogue.

The design of the present focused epothilone library was based on the current knowledge of structure–activity relationships (SAR), specifically the facts that: 1) epothilone B (**2**) is considerably more potent than epothilone A (**1**), 2) a methylsulfanyl replacement for the methyl group on the thiazole moiety enhances the potency,^[2d,5] 3) a heterocycle (e.g. pyridine)^[6] replacement for the thiazole ring needs to maintain the proper position (adjacent to the point of

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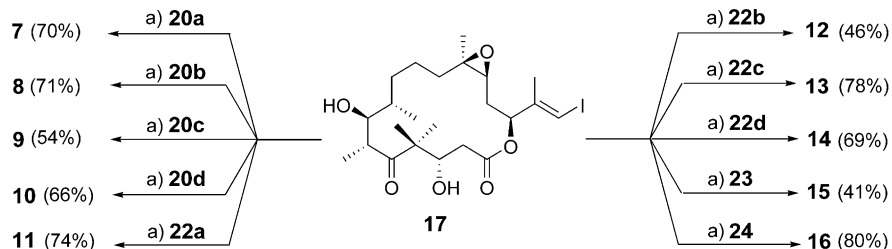
attachment to the mainframe) for the nitrogen for biological activity, and 4) a cyclopropane ring can replace the epoxide moiety without loss of activity.^[4] From these considerations, epoxythilones **4**, **6**, and **7–16** were considered as prime candidates for chemical synthesis and biological evaluation.

The designed epoxythilone analogues **7–16** were synthesized in a convergent manner from vinyl iodide **17**^[7] and the corresponding aromatic stannanes **20a–d**, **22a–d**, **23**, and **24** (Scheme 2) was carried out in the presence of [PdCl₂(MeCN)₂], CuI, and AsPh₃ in DMF at

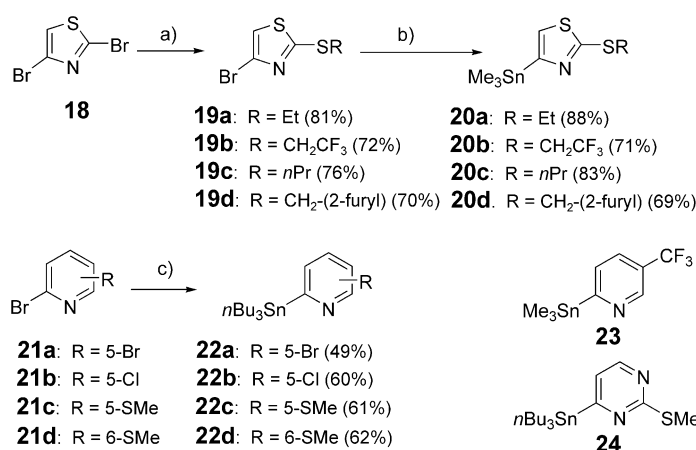
ambient temperature, leading directly to the desired epoxythilones **7–16** in the indicated yields. The required aromatic stannanes were prepared as summarized in Scheme 2. Thus, for the thiazole compounds **20a–d**, the commercially available 2,4-dibromothiazole (**18**) was treated with the corresponding thiol in the presence of NaH, leading first to the intermediate sulfides **19a–d** through replacement of the more reactive 2-bromide substituent. Subsequent coupling of these substrates with Me₃SnSnMe₃ in the presence of [Pd(PPh₃)₄] in toluene at 100 °C then gave the desired products **20a–d** by reaction of the second bromide residue. The pyridyl stannanes

22a–d were similarly synthesized from the readily available 2-bromopyridines **21a, b**,^[8] **c**,^[5a] and **d**,^[9] respectively, through a metal-halogen exchange (*n*BuLi) followed by quenching of the resulting 2-lithio derivatives^[10] with *n*Bu₃SnCl. Stannanes **23**^[11] and **24**^[12] were prepared from the respective halides according to the literature procedures.

The synthesis of cyclopropane epoxythilones **4** and **6** required the key aldehyde **39**, which was constructed from nerol (**25**) as shown in Scheme 3. Thus, Charrette asymmetric cyclopropanation^[4a, 5a, 13] of **25** in the presence of



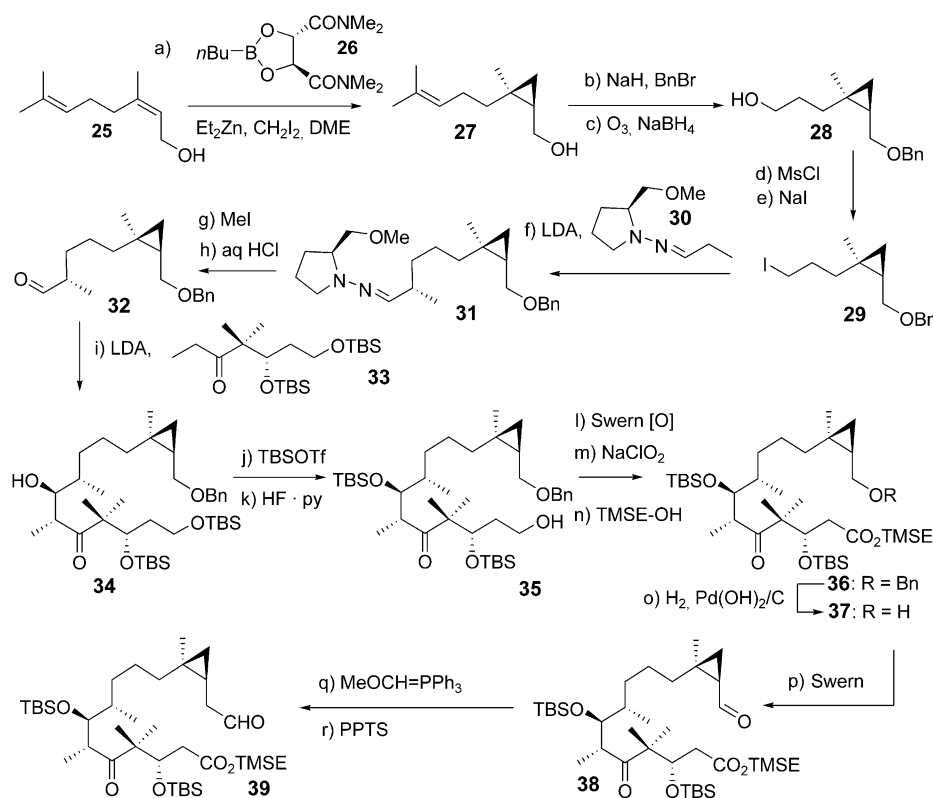
Scheme 1. Synthesis of **7–16**. Reagents and conditions: a) [PdCl₂(MeCN)₂] (0.5 equiv), CuI (2.0 equiv), AsPh₃ (1.0 equiv), **20a–d**, **22a–d**, **23–24** (2.5 equiv), DMF, 25 °C, 1–3 h, 41–80%. DMF = *N,N*-dimethylformamide.



Scheme 2. Preparation of **20a–d** and **22a–d**. Reagents and conditions:

a) NaH (3.0 equiv), RSH (3.0 equiv), *i*PrOH, 25 °C, 24 h, 70–81%;
 b) (Me₃Sn)₂ (5.0–10.0 equiv), [Pd(PPh₃)₄] (5 mol%), toluene, 100 °C, 1–3 h, 71–88%; c) *n*BuLi (1.1 equiv), diethyl ether, –78 °C, 1 h; then *n*Bu₃SnCl (1.2 equiv), –78 → –25 °C, 1 h, 49–62%.

ligand **26** furnished cyclopropane alcohol **27** in 80% yield and 95% *ee*. The hydroxy group in **27** was protected as a benzyl ether (NaH, BnBr, 100%) and the resulting product was subjected to ozonolysis (O₃, then NaBH₄), leading to primary alcohol **28** (83% yield). This alcohol was converted into the corresponding iodide **29** through mesylation (MsCl, Et₃N) and subsequent displacement of the intermediate mesylate with NaI (91% overall yield). Alkylation of (–)-SAMP hydrazone **30** with iodide **29** according to the procedure of Enders^[14] under the influence of LDA proceeded smoothly to afford hydrazone **31** (87% yield), whose cleavage (MeI, then aqueous HCl) led to aldehyde **32** (91% yield). The crucial aldol reaction between ketone **33**^[15] and aldehyde **32** in the presence of LDA proceeded smoothly and stereoselectively in THF/Et₂O (1:1) at –78 °C to afford the desired hydroxy ketone **34** in 80% yield (d.r. > 14:1). Protection of the secondary alcohol in **34** as a silyl ether (TBSOTf, 2,6-lutidine) followed by selective removal of the primary TBS group (HF·py) furnished primary



Scheme 3. Construction of **39**. Reagents and conditions: a) 80% yield, 95% *ee*;^[4a,5a,13] b) NaH (1.5 equiv), BnBr (1.2 equiv), DMF, 0 → 25 °C, 12 h, 100%; c) O₃, CH₂Cl₂/MeOH (4:1), –78 °C; then NaBH₄ (3.0 equiv), –78 → 25 °C, 1 h, 83%; d) MsCl (1.3 equiv), Et₃N (1.5 equiv), CH₂Cl₂, 25 °C, 1 h; e) NaI (3.0 equiv), acetone, 25 °C, 12 h (91% for two steps); f) LDA (1.4 equiv), **30** (1.3 equiv), THF, 0 °C, 6 h; then **29**, –98 → –10 °C, 14 h, 87%; g) MeI, reflux, 3 h; h) HCl (3 N)/pentane (1:1), 25 °C, 3 h (91% for two steps); i) LDA (2.4 equiv), **33** (2.3 equiv), THF/Et₂O (1:1), –78 °C, 1 h; then –40 °C, 0.5 h; then **32** at –78 °C, 5 min, 80%; j) TBSOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), CH₂Cl₂, –20 °C, 1 h; k) HF·py (1.8 mL mmol^{–1}), pyridine/THF (1:2), 0 °C, 8 h (86% for two steps); l) (COCl)₂ (1.2 equiv), DMSO (2.0 equiv), CH₂Cl₂, –78 °C, 5 min; then **35** (1.0 equiv), 20 min; then Et₃N (3.0 equiv), –78 → 0 °C; m) NaClO₂ (5.0 equiv), NaH₂PO₄ (3.0 equiv), 2-methyl-2-butene (75 equiv), *t*BuOH/THF/H₂O (4:2:1), 25 °C, 1 h; n) TMSE-OH (4.0 equiv), EDC (1.5 equiv), DMAP (0.1 equiv), DMF, 25 °C, 12 h (73% for three steps); o) Pd(OH)₂/C (10 wt%; 10%), H₂, EtOH/EtOAc (1:1), 25 °C, 2 h, 89%; p) (COCl)₂ (1.2 equiv), DMSO (2.0 equiv), CH₂Cl₂, –78 °C, 5 min; then **37** (1.0 equiv), 20 min; then Et₃N (3.0 equiv), –78 → 0 °C, 99%; q) MeOCH₂PPh₃Cl (3.0 equiv), *n*BuLi (2.8 equiv), THF, 0 °C, 1 h; then **38**, –78 → 0 °C, 2 h, 79%; r) PPTS (10.0 equiv), dioxane/water (9:1), 70 °C, 12 h, 81%. Bn = benzyl, Ms = methanesulfonyl, LDA = lithium diisopropyl amide, TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl, py = pyridine, DMSO = dimethyl sulfoxide, TMSE = 2-(trimethylsilyl)ethyl, EDC = 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide, DMAP = 4-dimethylaminopyridine, PPTS = pyridinium *para*-toluene sulfonate.

alcohol **35** (86% overall yield). The latter compound was then oxidized, and the resulting carboxylic acid was protected as a TMSE ester to afford **36** in 73% overall yield. Hydrogenolysis of the benzyl group within **36** (H_2 , 10% Pd(OH)₂/C) led to alcohol **37** (89% yield). Swern oxidation of **37** led to the corresponding aldehyde **38** (99% yield). Homologation of **38** through Wittig olefination (MeOCH₂PPh₃Cl, *n*BuLi, 79% yield) followed by acid hydrolysis (PPTS, 81% yield) of the resulting enol ether led to the targeted aldehyde **39**.

Following on our previously developed strategy^[4a] toward epothilone analogues, we subjected aldehyde **39** to a Nozaki–Hiyama–Kishi coupling^[16] reaction with vinyl iodides **40a**^[4a] and **40b**^[5a] followed by treatment with TBAF to afford the corresponding secondary alcohols **41** and **43** as mixtures (ca. 1:1) of the two epimers (at C15) in an unoptimized combined yield of 42–45% (Scheme 4). These mixtures were then cyclized under Yamaguchi conditions^[17] (2,4,6-trichlorobenzoyl chloride, Et₃N, DMAP, toluene, 0→75°C) to afford the desired 15*S* 16-membered lactones **42** (33% yield) and **44** (32% yield) together with their 15*R* epimers (ca. 1:1 ratio, chromatographically separated, silica gel).^[18] Finally the TBS groups were removed from **42** and **44** by the action of TFA, leading to epothilones **6** (48% yield) and **4** (Table 1, 71% yield) (unoptimized yields) as shown in Scheme 4.

The biological activities of the synthesized epothilones were evaluated through cell-growth-inhibition assays (cytotoxicity assays). Cytotoxicity was first evaluated in a set of ovarian carcinoma cell lines, including a parental cell line (1A9) and three drug-resistant cell lines, namely the taxol-resistant cell lines 1A9/PTX10 and 1A9/PTX22^[19] and the epothilone-resistant cell line 1A9/A8.^[20] These resistant cell lines harbor distinct acquired β-tubulin mutations that affect drug–tubulin interaction and result in impaired taxane and epothilone-driven tubulin polymerization. The results of

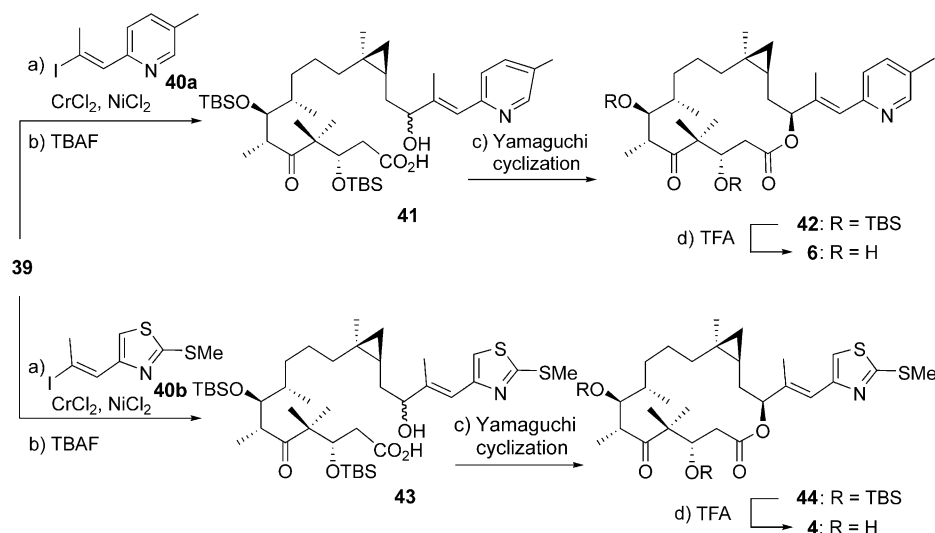
Table 1: Selected data for **4**.

<p>4: $R_f=0.19$ (silica gel, EtOAc/hexanes 3:7); $[\alpha]_D^{20}=-19.3$ ($c=0.14$, CH₂Cl₂); IR (film): $\bar{\nu}_{max}=3484$ br, 2932, 1729, 1459, 1375, 1249, 1043, 982, 733 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta=6.97$ (s, 1H), 6.47 (s, 1H), 5.25 (dd, $J=7.1, 5.7$ Hz, 1H), 4.04 (dd, $J=8.1, 3.0$ Hz, 1H), 3.91 (dd, $J=4.1, 4.1$ Hz, 1H), 3.23 (m, 1H), 2.69 (s, 3H), 2.52 (dd, $J=14.9, 8.4$ Hz, 1H), 2.46 (dd, $J=14.9, 2.6$ Hz, 1H), 2.11 (s, 3H), 2.04 (dd, $J=14.5, 4.0$ Hz, 1H), 1.72–1.66 (m, 1H), 1.62–1.44 (m, 4H), 1.36 (s, 3H), 1.35–1.22 (m, 2H), 1.17 (d, $J=7.5$ Hz, 3H), 1.16 (s, 3H), 1.15–1.04 (m, 1H), 0.99 (d, $J=7.0$ Hz, 3H), 0.97 (s, 3H), 0.48 (m, 1H), 0.40 (dd, $J=8.8, 3.9$ Hz, 1H), -0.11 ppm (br t, $J=4.6$ Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): $\delta=221.5, 171.1, 165.7, 152.9, 138.6, 120.1, 116.2, 82.0, 73.8, 73.2, 52.0, 42.9, 39.4, 36.5, 35.0, 33.2, 31.6, 24.6, 23.5, 22.54, 22.49, 21.1, 20.8, 19.4, 17.4, 16.8, 15.0, 13.2$ ppm; FTMS (MALDI): m/z calcd for C₂₈H₄₄NO₅S₂: 538.2655, found: 538.2632 [MH⁺]</p>

these biological investigations are summarized in Table 2. Further cytotoxicity studies were carried out on a set of human epidermoid cancer cell lines, including a parent cell line (KB-31) and a taxol-resistant (due to Pgp overexpression) cell line (KB-8511). The results of these studies are summarized in Table 3.

There is a general agreement in the relative potency of the substituted epothilone B analogues against the 1A9 human ovarian and the KB-31 human epidermoid cancer cells. Collectively, the results of these cytotoxicity assays revealed interesting information in terms of structure–activity relationships within the epothilone family. First, compounds **4** and **6**, in which the C12–C13 epoxide moiety is replaced by a cyclopropane ring, are the two most potent compounds of all the epothilone B analogues presented herein. This result reaffirms that the C12–C13 epoxide moiety is not necessary for biological activity, as previously noted.^[4] Compound **4** is

six times more active than the parent epothilone B (**2**) against the 1A9 human ovarian carcinoma cells (Table 2), which further confirms that the replacement of the methyl group on the thiazole side chain with a methylsulfonyl group leads to increased activity. This result is in agreement with our previous data on a similar substitution in epothilone B without replacement of the C12–C13 epoxide (i.e. compound **3**).^[5a] The latter compound (**3**) was about twice as active as the parent epothilone B, whereas compound **4** is sixfold more potent than epothilone B. This result makes compound **4** the most active epothilone B analogue against the 1A9 cell line synthesized to date and suggests that replacement of the epoxide by a cyclopropane moiety together with the replacement of the methyl substituent on the thiazole moiety with a methylsulfonyl group act synergistically, leading to the observed enhancement of biological activity. Interestingly, substitution of the methyl group of the thiazole ring with



Scheme 4. Synthesis of **4** and **6**. Reagents and conditions: a) CrCl₂ (10.0 equiv), NiCl₂ (0.2 equiv), 4-*t*BuPy (30 equiv), **40a** or **40b** (3.0 equiv), DMSO, 25°C, 24 h; b) TBAF (2.0 equiv), THF, 25°C, 2 h, 42% yield for two steps (**41**) or 45% yield for two steps (**43**); c) Et₃N (6.0 equiv), 2,4,6-trichlorobenzoyl chloride (2.4 equiv), **41** or **43**, THF, 0°C, 1 h; then DMAP (2.2 equiv), toluene, 75°C, 3 h, 33% (**42**) or 32% (**44**); d) TFA/CH₂Cl₂ (20% v/v), 25°C, 3 h, 48% (**6**) or 71% (**4**). TBAF = tetra-*n*-butylammonium fluoride, TFA = trifluoroacetic acid.

Table 2: Cytotoxicity of epothilones **4**, **6**, and **8–16** against 1A9 human carcinoma cells and β -tubulin mutant cell lines selected with taxol or epothilone A.^[a]

Compound	Cell Line						
	1A9	A8		PTX10		PTX22	
	IC ₅₀	IC ₅₀	RR	IC ₅₀	RR	IC ₅₀	RR
taxol	3.0 ± 0.4	10.1 ± 2.9	3.3	89.7 ± 9.0	29.5	53.4 ± 26.5	17.6
EpoA	2.4 ± 0.6	91.0 ± 10.0	38.7	34.2 ± 2.0	14.5	8.7 ± 2.2	3.7
EpoB	0.6 ± 0.3	6.5 ± 0.9	10.7	3.1 ± 0.5	5.2	0.8 ± 0.5	1.3
3 ^[b]	0.17 ± 0.8	1.3 ± 0.65	7.6	0.26 ± 0.11	1.5	0.25 ± 0.17	1.5
4	0.1 ± 0.0	2.4 ± 1.1	23.5	0.7 ± 0.3	6.5	0.6 ± 0.5	5.9
6	0.3 ± 0.1	10.4 ± 2.4	41.4	3.3 ± 1.2	13.2	1.3 ± 1.1	5.3
8	3.5 ± 0.7	18.4 ± 1.4	5.3	16.1 ± 2.1	4.6	3.8 ± 0.3	1.1
9	4.4 ± 2.4	42.9 ± 5.1	9.7	24.7 ± 4.9	5.6	5.2 ± 0.8	1.2
10	2.1 ± 0.8	16.0 ± 5.5	7.6	9.8 ± 1.4	4.7	2.9 ± 1.3	1.4
11	0.7 ± 0.2	11.1 ± 1.0	16.6	3.9 ± 0.4	5.8	0.3 ± 0.1	0.5
12	3.2 ± 0.1	31.9 ± 3.1	10.0	16.1 ± 4.1	5.1	3.2 ± 0.3	1.0
13	0.4 ± 0.1	11.6 ± 6.7	31.7	3.9 ± 1.1	10.5	2.1 ± 1.9	5.8
14	3.3 ± 0.2	27.7 ± 3.2	8.3	12.2 ± 7.4	3.7	6.6 ± 2.6	2.0
15	4.3 ± 0.4	83.0 ± 2.0	19.2	65.3 ± 11.9	15.1	9.6 ± 1.3	2.2
16	8.6 ± 1.2	32.3 ± 2.7	3.8	42.9 ± 10.3	5.0	9.6 ± 1.0	1.1

[a] The antiproliferative effects of the tested compounds against the parental 1A9 and the taxol- and epothilone-selected drug-resistant clones (PTX10, PTX22, and A8, respectively) were assessed in a 72 h growth-inhibition assay using the SRB (sulforhodamine-B) assay.^[22] IC₅₀ values for each compound are given in nM and represent the mean of three independent experiments ± standard error of the mean. Relative resistance (RR) is calculated as an IC₅₀ value for each resistant subline divided by that for the parental cell line (1A9). [b] Results taken from ref. [5a].

Table 3: Cytotoxicity (IC₅₀) of selected epothilones against the human epidermoid cell lines KB-31 and KB-8511.^[a]

Compound	KB-31	KB-8511
EpoB ^[b]	0.19	0.12
3 ^[b]	0.11	0.07
4	0.20	0.12
6	0.44	0.29
8	3.04	2.67
9	10.0	6.73
10	1.16	1.28
11	0.72	0.55
13	0.54	0.41
14	4.87	3.24
15	8.38	7.37
16	9.01	11.65

[a] The antiproliferative effects of the tested compounds were assessed in two human epidermoid cancer cell lines, including a parent cell line (KB-31) and a taxol-resistant (due to Pgp-overexpression) cell line (KB-8511). IC₅₀ values are given in nM. [b] Results taken from ref. [5a].

larger moieties (compounds **7–10**)^[21] led to diminished biological activity relative to epothilone B (Tables 2 and 3).

Among the epothilone B analogues with a side chain at C5 of the pyridine substituent, **11–13** and **15**, the methylsulfanyl analogue **13** is the most potent, followed by the bromo-substituted derivative **11** and the chloro-substituted system **12**. When the methylsulfanyl group is relocated from C5 of the pyridine ring (**13**) to C6 (**14**), loss of activity occurs as the IC₅₀ value drops from 0.4 nM (for **13**) to 3.3 nM (for **14**) (Table 2). Furthermore, replacement of the methylsulfanyl group at the C5 of the pyridine ring (**13**) with a trifluoromethyl group (**15**) results in a tenfold loss of activity. Finally, the least active of the synthesized epothilone B analogues is compound **16** in

which a pyrimidine side chain with a methylsulfanyl substituent has replaced the thiazole side chain of the parent compound.

Varying degrees of cross-resistance are obtained with the substituted epothilone B analogues against the taxol- and epothilone-resistant human ovarian carcinoma sublines (Table 2) ranging from 3- to 41-fold. These results suggest that the location of the tubulin mutations in these lines affects differentially the binding of each of the analogues to tubulin. Moreover, and in agreement with the original observations with the naturally occurring epothilones A and B, none of the epothilone B analogues tested herein appears to be a good substrate for the drug-efflux pump P-glycoprotein (Pgp). This is evident by the lack of cross-resistance of each of these analogues to the Pgp-expressing cell line KB-8511 (Table 3). In contrast, we have

previously shown that taxol—a known Pgp substrate—was 214-fold less active against KB-8511 cells than against their parent counterpart, non-Pgp-expressing KB-31 cells.^[5a]

In conclusion, we have constructed a number of rationally designed epoxide and cyclopropane epothilone B analogues with substituted side chains and evaluated their biological activities against a series of human cancer cell lines. Among the several bioactive analogues, the novel cyclopropyl epothilone B analogue **4** with a methylsulfanyl thiazole ring stands out as the most potent. This compound is six times more active than the naturally occurring epothilone B (**2**) and appears to be, together with its oxygen counterpart **3**, the most potent epothilone B analogue synthesized to date. Our previous structure–activity relationship studies,^[2c,4b,5a] together with the data presented herein, reconfirm that the epoxide oxygen atom is not required for biological activity within this class of small molecules and that the lipophilic methylsulfanyl group on the thiazole moiety considerably enhances the potency of these compounds. As epothilone **4** lacks the relatively reactive epoxide function of **2** and **3**, it may prove to be advantageous over the latter compounds with regard to stability and side effects and, therefore, may present a unique opportunity for clinical development.^[23]

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

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