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Molecular Design and Chemical Synthesis of a Highly Potent Epothilone**

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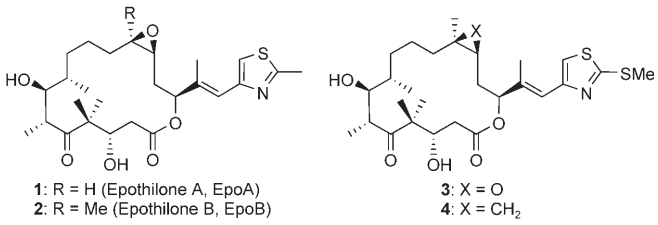
Ever since the initial discovery of the epothilones in 1996 (for example, EpoA (**1**) and EpoB (**2**), Figure 1),[1] synthetic chemists have been enamored with their structures from the perspectives of both synthesis and modification.[2] This rather intense and persistent interest is neither surprising nor without merit, for these naturally occurring substances have proven themselves challenging targets for synthesis, powerful tools in biology, and worthy drug candidates currently in clinical trials[3] as anticancer agents. Our structure–activity relationship (SAR) studies within the epothilone class[2c,d,f,4] have defined a narrow range of structural motifs that, when present, endow the epothilone molecule with biological activity and have resulted in the development of several potent epothilones such as the methylthio-EpoB analogues **3**[4c] and **4**.[4b] This model was more or less confirmed by a recent electron crystallographic and NMR-based conformational analysis[5] of a tubulin–EpoA complex that appears to accommodate most of the published SAR data. Herein, we report a new study based on this model that has led to the identification of the most potent epothilone reported to date, natural or designed.



1: R = H (Epothilone A, EpoA)
2: R = Me (Epothilone B, EpoB)
3: X = O
4: X = CH_2

Figure 1. Selected natural and designed epothilones.

From the several regions of the epothilone structure where modifications could be made with the potential to improve the activity of the molecule, we chose the heterocyclic ring-containing side-chain domain. Having previously established the importance of the basic nitrogen atom in its specific location,[4e] we set that condition as a structural requirement for any new designs and kept the rest of the structure of EpoB intact. These limits had the advantage that any potential drug candidate **I** that could emerge from the investigation could, in principle, be produced either by total synthesis or through semisynthesis from a degradation-derived advanced intermediate **II** and a heterocyclic stannane **III** by a palladium-catalyzed cross-coupling reaction such as the Stille reaction,[4c] for example, as indicated retrosynthetically in Figure 2.

Epothilones **5**–**21** (Figure 3) were designed within the structural constraints mentioned above and with certain further rationales. The beneficial effect of certain lipophilic substituents such as methyl and methylthio groups on tubulin binding and cytotoxicity did not escape our attention, and thus we introduced such moieties on several of these designs (compounds **5**–**20**). We also wanted to probe the effect of additional rings on the heterocyclic side chain (compounds **7**, **17**–**19**, and **20**) as well as the absence of a ring on the same side chain (compound **21**). Finally, we wished to challenge the ability of the tubulin receptor pocket to accommodate bulky halogen substituents such as those in compounds **10** and **12**.

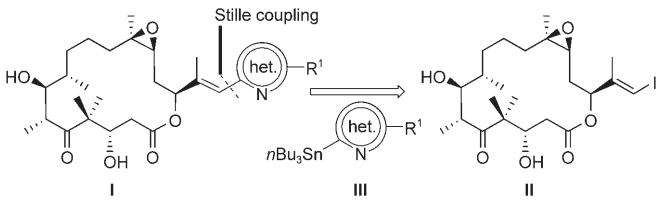


Figure 2. Designed EpoB analogues and retrosynthetic analysis.

For the synthesis of these epothilones according to the retrosynthetic analysis of Figure 2, the corresponding heterocyclic stannanes were required. They were prepared either as shown in Scheme 1 (for compounds **26a**–**b**, **28**, **30a**–**b**, **32**, **33**, **35a**–**c**, and **37a**–**b**) or by published procedures (**35b**,[6] **36**,[7] **38** and **39**,[8] **40**,[9] and **41** (commercially available)). The attachment of each heterocyclic moiety onto the epothilone scaffold was then carried out by Stille coupling[4c] of each stannane with vinyl iodide **22**[10] to afford the targeted epothilones **5**–**21** as shown in Scheme 2.

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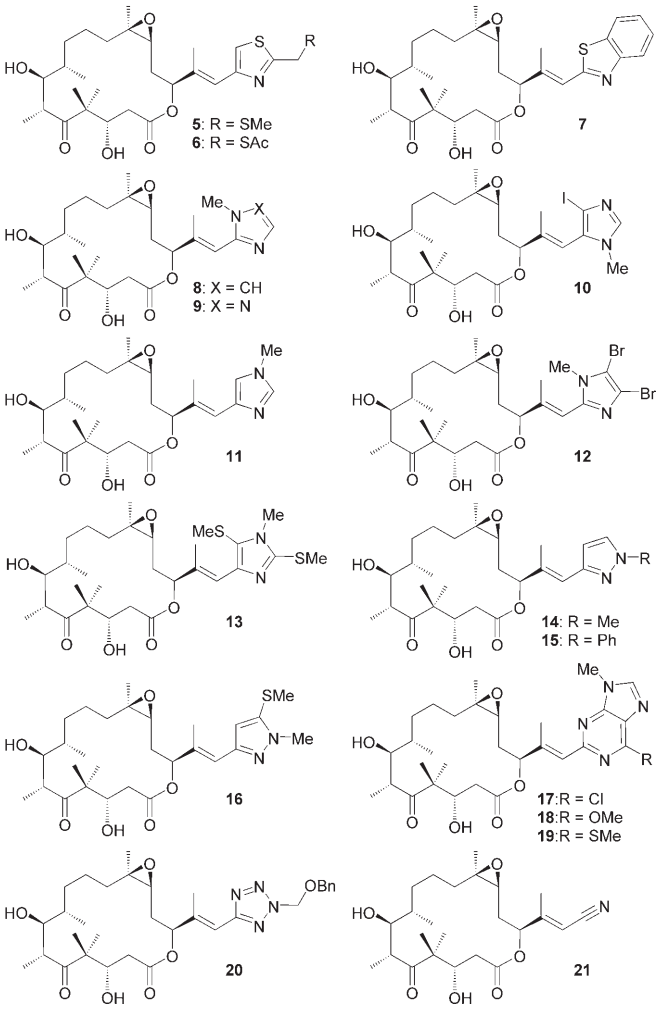
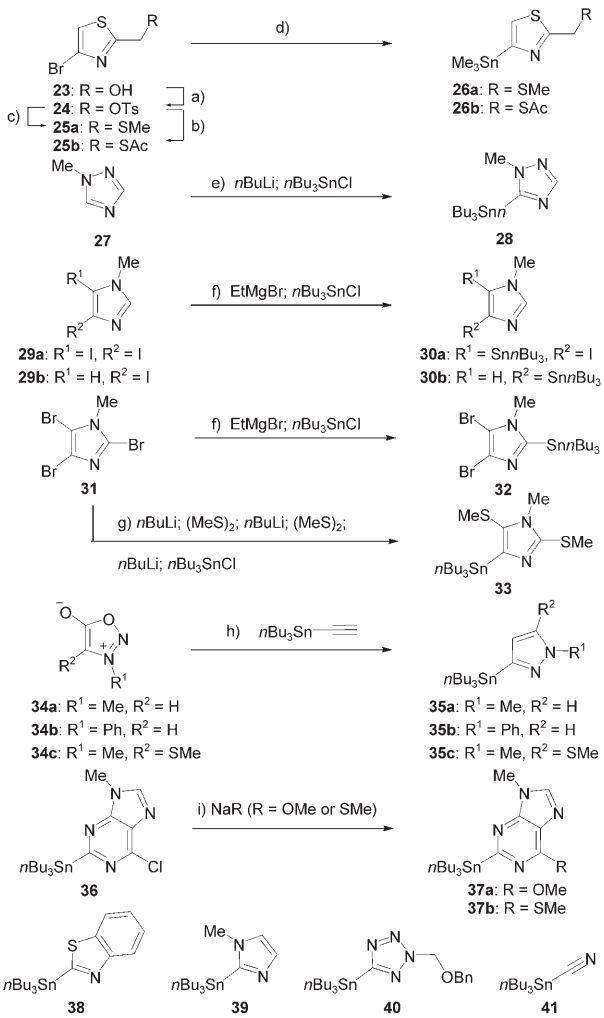


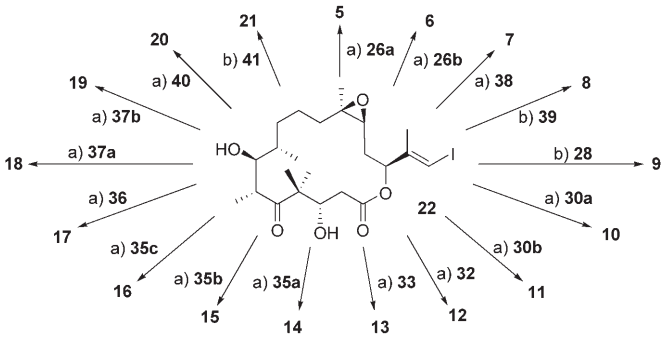
Figure 3. Newly designed and synthesized epothilones.



Scheme 1. Synthesis of stannane coupling partners; reagents and conditions: a) TsCl (1.5 equiv), Et_3N (3.0 equiv), DMAP (0.1 equiv), CH_2Cl_2, 25 °C, 1 h, 84%; b) thioacetic acid (1.1 equiv), Et_3N (1.1 equiv), CH_2Cl_2, 0→25 °C, 1 h, 78%; c) NaSMe (3.0 equiv), EtOH, 25 °C, 10 min, 97%; d) $(Me_3Sn)_2$ (7.0 equiv), $[Pd(PPh_3)_4]$ (0.1 equiv), toluene, 110 °C, 1 h, **25a**→**26a**: 95%, **25b**→**26b**: 63%; e) *n*BuLi (1.0 equiv), methylcyclohexane, THF, −78 °C, 10 h; then nBu$_3$SnCl (1.0 equiv), −78 °C, 1 h, 83%; f) EtMgBr (1.0 equiv), THF, 25 °C, 1.5 h; then nBu$_3$SnCl (1.1 equiv), 25 °C, 1 h, **29a**→**30a**: 74%, **29b**→**30b**: 79%; g) *n*BuLi (1.0 equiv), THF, −78 °C, 5 min; then $(MeS)_2$ (1.0 equiv), 5 min; then *n*BuLi (1.0 equiv), 15 min; then $(MeS)_2$ (1.0 equiv), 5 min; then *n*BuLi (1.0 equiv), 15 min; then nBu$_3$SnCl (1.0 equiv), −78 °C, 1 h, 83%; h) nBu$_3$SnCCH (1.5 equiv), xylenes, 138 °C, 6 h, **34a**→**35a**: 41%, **34b**→**35b**: 76%, **34c**→**35c**: 30%; i) NaOMe or NaSMe (3.0 equiv), *t*BuOK (2.0 equiv), *i*PrOH, 25 °C, 15 min, **37a**: 10%, **37b**: 89%. Bn = benzyl, DMAP = 4-dimethylaminopyridine, Ts = *p*-tolunesulfonyl.

The synthesized epothilones were tested in cytotoxicity assays for their ability to inhibit growth and were compared with either taxotere or taxol and either EpoA or EpoB. The results against several drug-sensitive and drug-resistant human cancer cell lines are summarized in Table 1. The cell lines include the parental drug-sensitive ovarian carcinoma line 1A9, its taxol-resistant subline 1A9/PTX10,[11] its EpoA-resistant subline 1A9/A8,[12] and the mutant variant that overexpresses the P-glycoprotein (Pgp) efflux pump (1A9/AD10). The taxol- and EpoA-resistant cell lines harboring distinct acquired β-tubulin mutations affect the drug–tubulin interaction and result in impaired taxane- and epothilone-driven tubulin polymerization. Further cytotoxicity studies were carried out with a pair of drug-sensitive parental human epidermoid carcinoma cell lines: KB-31 and its taxol-resistant Pgp-overexpression subline, KB-5811 (Table 1).

Compounds **5** and **6**, although less potent than EpoB, exhibited high potency against several of the cell lines tested, which reflects considerable tolerance of the receptor to the structural changes embodied within these molecules. The benzothiazole analogue **7** (Table 2) exhibited excellent cytotoxicity across the range of cell lines, and is more potent than EpoB. The significantly lower activities of the imidazole analogues **8**, **10**–**13**, and the triazole epothilone **9** point to difficulties of the *N*-methylimidazole moiety to fit into the tubulin receptor, whereas the substitution pattern and crowded nature of **13** underscores the requirement of the specific positioning of the basic nitrogen atom and the planarity of the conjugated side-chain system.[13] Replacement of the thiazole ring with a pyrazole moiety resulted in excellent biological activity as evidenced by



Scheme 2. Synthesis of epothilones **5**-**21**; reagents and conditions: a) $[Pd_2(dba)_3]\cdot CHCl_3$ (0.2 equiv), CuI (2.0 equiv), $AsPh_3$ (0.8 equiv), **26a**→**5**: 74%, **26b**→**6**: 40%, **38**→**7**: 55%, **30a**→**10**: 43%, **30b**→**11**: 68%, **32**→**12**: 76%, **33**→**13**: 79%, **35a**→**14**: 71%, **35b**→**15**: 66%, **35c**→**16**: 60%, **36**→**17**: 52%, **37a**→**18**: 39%, **37b**→**19**: 53%, **40**→**20**: 56%, (2.0 equiv each stannane), DMF, 25 °C, 3 h; b) $[Pd(PPh_3)_4]$ (0.2 equiv), CuI (2.0 equiv), **39**→**8**: 51%, **28**→**9**: 47%, **41**→**21**: 34% (2.0 equiv each stannane), DMF, 25 °C, 3 h. Yields are not optimized; dba = dibenzylidine-acetone, DMF = *N*,*N*-dimethylformamide.

compounds **14**–**16**, which provide interesting SARs. *N*-Methyl-substituted pyrazole epothilone **14** exhibited more potent cytotoxicity than EpoB overall, yet its *N*-phenyl counterpart **15** was somewhat less active. Compound **16**, with the extra *S*-methyl substituent (Table 2), proved to be the most potent of all compounds tested. Indeed, epothilone **16** exhibited a remarkable 17-fold increase in activity against both the 1A9 parental and the 1A9/A8 EpoA-resistant cell lines and an even more impressive 78-fold increase in potency against the taxol-resistant 1A9/PTX10 cell line, relative to the potency of naturally occurring EpoB. The action of **16** against the KB cell lines is also impressive, with a three to fivefold increase in potency relative to EpoB. Interestingly, however, despite a fourfold higher potency as a growth inhibitor against the PgP-overexpressing cell line A2780/AD10 over EpoB, **16** was not as potent as compound **14** (20-fold more potent than EpoB). The purine epothilones **17**–**19** showed similar potencies to those of EpoB across the entire range of cell lines tested and therefore represent a further expansion of the boundaries of steric bulk and electron cloud volume that can be tolerated by the tubulin receptor. Finally, the nitrile epothilone **21** was found to be devoid of any significant cytotoxicity against any of the cell lines tested, confirming the importance of the basic heterocyclic moiety of the epothilone structure.

The striking biological profile of the pyrazole epothilone **16** in relation to the profiles of EpoB and the potent analogues **3**,[4c] **4**,[4b] **7**, and **14** (Table 3) makes this analogue, to the best of our knowledge, the most potent epothilone reported to date. Compound **16** outperformed EpoB in all five cancer cell lines tested, exhibiting remarkably higher potency than EpoB against the parental ovarian cell line 1A9 (16.5-fold), its taxol-resistant variant 1A9/PTX10 (78.0-fold), and its EpoA-resistant mutant strain 1A9/A8 (16.7-fold). Given that EpoB (**2**) and analogue **3** have entered clinical trials[14] as anticancer agents, compound **16** merits further investigation as a potential drug candidate.

Table 1. Cytotoxicity of designed epothilones **5**–**21**.[a]

	1A9[b] IC_{50} [nM]	1A9/PTX10[c] IC_{50} [nM]	RR_p[d]	1A9/A8[e] IC_{50} [nM]	RR_p[d]	A2780/AD10[f] IC_{50} [nM]	RR_p[d]	KB-31[g] IC_{50} [nM]	KB-8511[h] IC_{50} [nM]	RR_p[d]
taxol	–	–	–	–	–	–	–	6.08 ± 1.19	1133 ± 74	186
taxotere	1.5 ± 1.1	32.0 ± 6.8	21.3	0.8 ± 0.2	0.5	> 150	–	–	–	–
EpoA	2.4 ± 2.6	5.1 ± 2.6	2.1	41.0 ± 7.3	17.1	29.4 ± 5.4	12.3	–	–	–
EpoB	0.99 ± 0.8	7.8 ± 4.6	7.9	10.0 ± 1.4	10.1	25.4 ± 8.6	25.7	0.42 ± 0.03	0.39 ± 0.02	0.9
5	3.6 ± 1.4	43.3 ± 12.0	12.0	18.6 ± 11.3	5.2	56.5 ± 23.3	15.7	1.38 ± 0.05	1.97 ± 0.25	1.4
6	5.4 ± 1.1	28.2 ± 9.3	5.2	49.8 ± 34.5	9.2	82.2 ± 49.6	15.2	1.18 ± 0.12	8.39 ± 1.37	7.1
7	0.19 ± 0.1	2.6 ± 2.5	13.7	0.7 ± 0.4	3.7	8.6 ± 3.3	45.3	0.21 ± 0.07	0.08 ± 0.04	0.4
8	95.5 ± 13.4	> 750	–	242	2.5	707	7.4	69.9 ± 5.31	> 1000	–
9	540	> 750	–	627	1.2	760	1.4	382 ± 49.4	> 1000	–
10	> 750	> 750	–	> 750	–	> 750	–	> 1000	> 1000	–
11	181 ± 81.6	> 750	–	150	0.8	1072	5.9	277 ± 122	> 1000	–
12	> 750	> 750	–	> 750	–	> 750	–	> 1000	> 1000	–
13	> 750	> 750	–	574	–	574	–	240 ± 34.0	247 ± 47.9	1.0
14	0.50 ± 0.3	1.7 ± 1.1	3.4	0.8 ± 0.3	1.6	1.3 ± 0.3	2.6	0.19 ± 0.01	0.25 ± 0.04	1.3
15	5.45 ± 3.7	25.8 ± 19.8	4.7	4.8 ± 3.6	0.9	25.5 ± 15.1	4.7	2.27 ± 0.16	1.62 ± 0.14	0.7
16	0.06 ± 0.04	0.1 ± 0.0	1.7	0.6 ± 0.5	10.0	6.4	107	0.09 ± 0.01	0.14 ± 0.01	1.6
17	3.7 ± 1.5	13.6 ± 7.3	3.7	4.0 ± 2.5	1.1	30.3 ± 17.7	8.2	1.62 ± 0.22	20.2 ± 2.14	12.5
18	3.7 ± 1.8	19.8 ± 10.6	5.4	2.2 ± 1.6	0.6	46.3 ± 33.2	12.5	0.15 ± 0.09	7.45 ± 0.81	49.7
19	1.0 ± 0.8	2.0 ± 1.5	2.0	2.8 ± 2.2	2.8	47.3 ± 36.5	47.3	0.36 ± 0.17	6.21 ± 0.86	17.3
20	4.0 ± 0.3	49.0 ± 7.0	12.3	16.8 ± 9.2	4.2	67.0 ± 9.8	16.8	0.57 ± 0.08	4.44 ± 0.69	7.8
21	> 750	> 750	–	> 750	–	> 750	–	346 ± 10.5	> 1000	–

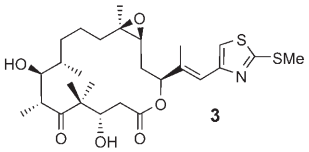
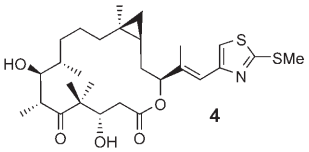
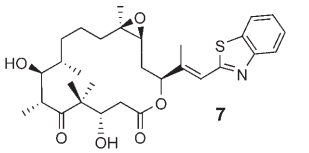
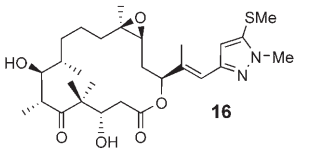
[a] Compounds were tested for their antiproliferative effects in a 72-h growth-inhibition assay by using the methylene blue (KB-31 and KB-8511) or sulforhodamine B (all other cell lines) staining methods.[4e,15] IC_{50} values for each compound represent the mean of 3–5 independent experiments ± standard error of the mean. [b] Human ovarian cancer cell line. [c] Taxol-resistant 1A9 cell line expressing a single acquired point mutation at β-tubulin position 270 (Phe→Val). [d] Relative resistance of the parental cell line (RR_p) is calculated as the IC_{50} value for each resistant cell line divided by that of the parental cell line. [e] EpoA-resistant cell line 1A9 expressing a single acquired point mutation at β-tubulin position 274 (Thr→Ile). [f] Drug-resistant clone of the human ovarian carcinoma A2780 cell line that overexpresses Pgp as a result of drug selection with adriamycin. [g] Human epidermoid cancer cell line. [h] Taxol-resistant (Pgp overexpression) KB cell line.

Table 2. Selected physical properties for compounds **7** and **16**.

Epothilone **7:** white foam; 55% yield; $R_f = 0.37$ (silica gel, EtOAc/hexanes 1:1); $[\alpha]_D^{32} = -10$ (DMSO, $c = 0.32$); IR (film): $\tilde{\nu}_{max} = 3441$ br, 2959, 2919, 2356, 1738, 1725, 1714, 1682, 1650, 1454, 1379, 1248, 1143, 1056, 1002, 973, 882, 761, 730 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.01$ (d, $J = 8.2$ Hz, 1H), 7.89 (d, $J = 8.0$ Hz, 1H), 7.51–7.47 (m, 1H), 7.41–7.37 (m, 1H), 6.93 (s, 1H), 5.54 (dd, $J = 6.0$, 3.7 Hz, 1H), 4.20 (d, $J = 9.3$ Hz, 1H), 3.97 (br s, 1H), 3.80 (t, $J = 4.3$ Hz, 1H), 3.38–3.31 (m, 1H), 2.83 (t, $J = 6.2$ Hz, 1H), 2.61 (dd, $J = 14.0$, 10.2 Hz, 1H), 2.50 (br s, 1H), 2.46 (dd, $J = 14.0$, 3.5 Hz, 1H), 2.32 (s, 3H), 2.13–2.06 (m, 1H), 2.05–1.98 (m, 1H), 1.77–1.66 (m, 1H), 1.59–1.32 (m, 6H), 1.40 (s, 3H), 1.29 (s, 3H), 1.18 (d, $J = 6.8$ Hz, 3H), 1.09 (s, 3H), 1.01 ppm (d, $J = 6.9$ Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 220.7$, 170.4, 164.6, 152.4, 144.8, 134.7, 126.5, 125.3, 122.8, 121.4, 119.4, 76.0, 74.5, 73.0, 61.3, 61.3, 53.0, 43.2, 38.9, 36.4, 31.7, 31.7, 30.5, 22.8, 21.2, 21.0, 20.1, 17.1, 17.0, 14.0 ppm; HRMS (ESI-TOF) calcd for $C_{30}H_{41}NO_6S^+$ [M+H$^+$]: 544.2727, found: 544.2743.

Epothilone **16:** white solid; 60% yield; $R_f = 0.82$ (silica gel, EtOAc); $[\alpha]_D^{32} = -28.3$ (CH_2Cl_2, $c = 0.53$); IR (film): $\tilde{\nu}_{max} = 3444$ br, 2966, 2931, 1738, 1732, 1694, 1682, 1469, 1455, 1381, 1371, 1284, 1266, 1250, 1148, 1056, 978, 736 cm^{-1}; ^1H NMR (600 MHz, CD_2Cl_2): $\delta = 6.41$ (s, 1H), 6.33 (s, 1H), 5.38 (dd, $J = 8.1$, 2.3 Hz, 1H), 4.20–4.15 (m, 1H), 3.84 (s, 3H), 3.72 (dd, $J = 7.8$, 4.0 Hz, 1H), 3.67 (d, $J = 5.6$ Hz, 1H), 3.29–3.24 (m, 1H), 2.77 (dd, $J = 7.9$, 4.3 Hz, 1H), 2.51 (br s, 1H), 2.48 (dd, $J = 14.3$, 10.3 Hz, 1H), 2.41 (s, 3H), 2.36–2.32 (m, 1H), 2.09–2.03 (m, 1H), 2.01 (s, 3H), 1.94–1.86 (m, 1H), 1.72–1.65 (m, 1H), 1.53–1.35 (m, 6H), 1.34 (s, 3H), 1.25 (s, 3H), 1.14 (d, $J = 6.8$ Hz, 3H), 1.04 (s, 3H), 0.99 ppm (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (150 MHz, CD_2Cl_2): $\delta = 220.7$, 170.5, 148.1, 137.2, 136.4, 118.1, 108.9, 76.7, 74.3, 73.2, 61.5, 61.2, 52.8, 43.1, 39.0, 36.5, 32.1, 31.9, 30.7, 22.8, 22.6, 21.2, 20.3, 18.8, 17.1, 15.7, 14.2, 13.9 ppm; HRMS (ESI-TOF) calcd for $C_{28}H_{44}N_2O_6S^+$ [M+H$^+$]: 537.2993, found: 537.2992.

Table 3. Relative activities of selected highly potent epothilones.

Epothilone	Cell line[a]	IC_{50}	EpoB	RA_{EpoB}[b]
 **3**	1A9	0.17	0.3	1.8
	1A9/PTX10	0.26	3.7	14.2
	1A9/A8	1.3	6.5	5.0
	KB-31	0.11	0.19	1.7
	KB-5811	0.07	0.18	2.6
 **4**	1A9	0.1	0.6	6.0
	1A9/PTX10	0.7	3.1	4.4
	1A9/A8	2.4	6.5	2.7
	KB-31	0.20	0.19	1.0
	KB-5811	0.12	0.12	1.0
 **7**	1A9	0.19	0.99	5.2
	1A9/PTX10	2.6	7.8	3.0
	1A9/A8	0.7	10.0	14.3
	KB-31	0.21	0.42	2.0
	KB-5811	0.08	0.39	4.9
 **16**	1A9	0.06	0.99	16.5
	1A9/PTX10	0.1	7.8	78.0
	1A9/A8	0.6	10.0	16.7
	KB-31	0.09	0.42	4.7
	KB-5811	0.14	0.39	2.8

[a] See Table 1 for definition of cell lines. [b] Relative activity: ratio of the IC_{50} value of EpoB to the IC_{50} value of the compound tested in the concurrent biological assay. This value represents the fold increase in potency of the tested compound compared with EpoB determined concurrently.

Keywords: analogues · antitumor agents · epothilones · molecular design · synthesis design

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