Synthesis and biological properties of C12,13-cyclopropyl-epothilone A and related epothilones

KC Nicolaou, M Ray V Finlay, Sacha Ninkovic, N Paul King, Yun He, Tianhu Li, Francisco Sarabia and Dionisios Vourloumis

Background: The epothilones are natural substances that are potently cytotoxic, having an almost identical mode of action to Taxol™ as tubulin-polymerization and microtubule-stabilizing agents. The development of detailed structure–activity relationships for these compounds and the further elucidation of their mechanism of action is of high priority.

Results: The chemical synthesis of the C12,13-cyclopropyl analog of epothilone A and its C12,13-*trans*-diastereoisomer has been accomplished. These compounds and several other epothilone analogs have been screened for their ability to induce tubulin polymerization and death of a number of tumor cells. Several interesting structure–activity trends within this family of compounds were identified.

Conclusions: The results of the biological tests conducted in this study have demonstrated that, although a number of positions on the epothilone skeleton are amenable to modification without significant loss of biological activity, the replacement of the epoxide moiety of epothilone A with a cyclopropyl group leads to total loss of activity.

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Introduction

A number of recent publications [1–9] have described total syntheses of the novel microtubule-stabilizing natural products epothilones A (**1**) and B (**2**) (Figure 1) [10–12]. As the epothilones have impressive anti-tumor properties [11–12], it was deemed important to engage in further chemical biology studies within the class. Accordingly, we and others have devoted considerable efforts to the design, chemical synthesis and study of epothilones [13–23]. In this article, we describe the chemical synthesis of the C12,13-cyclopropyl analog of epothilone A and its C12,13-*trans*-isomer and their biological evaluation in tubulin-polymerization and certain cytotoxicity assays. In addition, we disclose the biological properties of a series of new epothilone analogs, the synthesis of which is described elsewhere [19,24].

For some time we have been intrigued about the biological role of the C12,13 epoxide moiety of the epothilones. Previous studies [2,6,13–16,20] have demonstrated that, although analogs lacking the C12,13 epoxide show high levels of induction of tubulin polymerization, they lack the potent cellular cytotoxicity of their epoxidized counterparts. In order to probe more fully the biological significance of the epoxide oxygen, we initiated a program directed at the synthesis and study of the C12,13-*cis*-cyclopropyl-epothilone A (**3**) and its C12,13-*trans*-cyclopropane isomer (**4**; Figure 1).

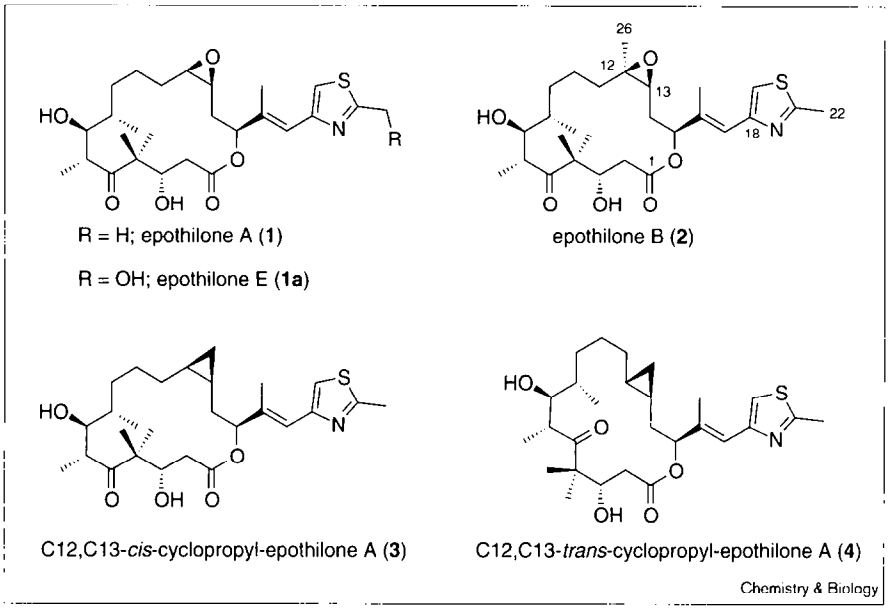
Results and discussion

The synthesis of the cyclopropane analogs **3** and **4** required some rather unusual chemistry. A wide range of methods have been described in the literature for the transformation of allylic alcohols to the corresponding cyclopropyl systems, several in either diastereoselective or enantioselective fashion (for a recent overview see [25]). Initial efforts employing either these methods or the classic Simmons–Smith procedure proved disappointing, however, when attempted on the previously prepared [20,22] macrocyclic substrate **5** (Figure 2).

In the light of these discouraging results, a new approach was devised. Previous studies [26,27] have shown that cyclopropanes may be prepared from γ-hydroxypropyl stannanes by elimination of the hydroxyl and stannyl moieties. We therefore envisaged that if we could prepare the γ-hydroxypropyl stannane systems **10** and **11** (Figure 2) then alcohol derivatization and subsequent acid-catalyzed formation of a carbocation could trigger spontaneous cyclization to the required cyclopropanes **12** and **13** respectively (Figure 2). It was further anticipated that the required stannanes could be prepared from allylic alcohol **9**, which in turn would be derived from the macrocylic epoxide system **6** (Figure 2).

Thus, as shown in Figure 2, subjecting allylic alcohol **5** [20,22] to Katsuki–Sharpless epoxidation conditions [28]

Figure 1



Structures of epothilones A (1), B (2), E (1a) and cyclopropyl analogs 3 and 4.

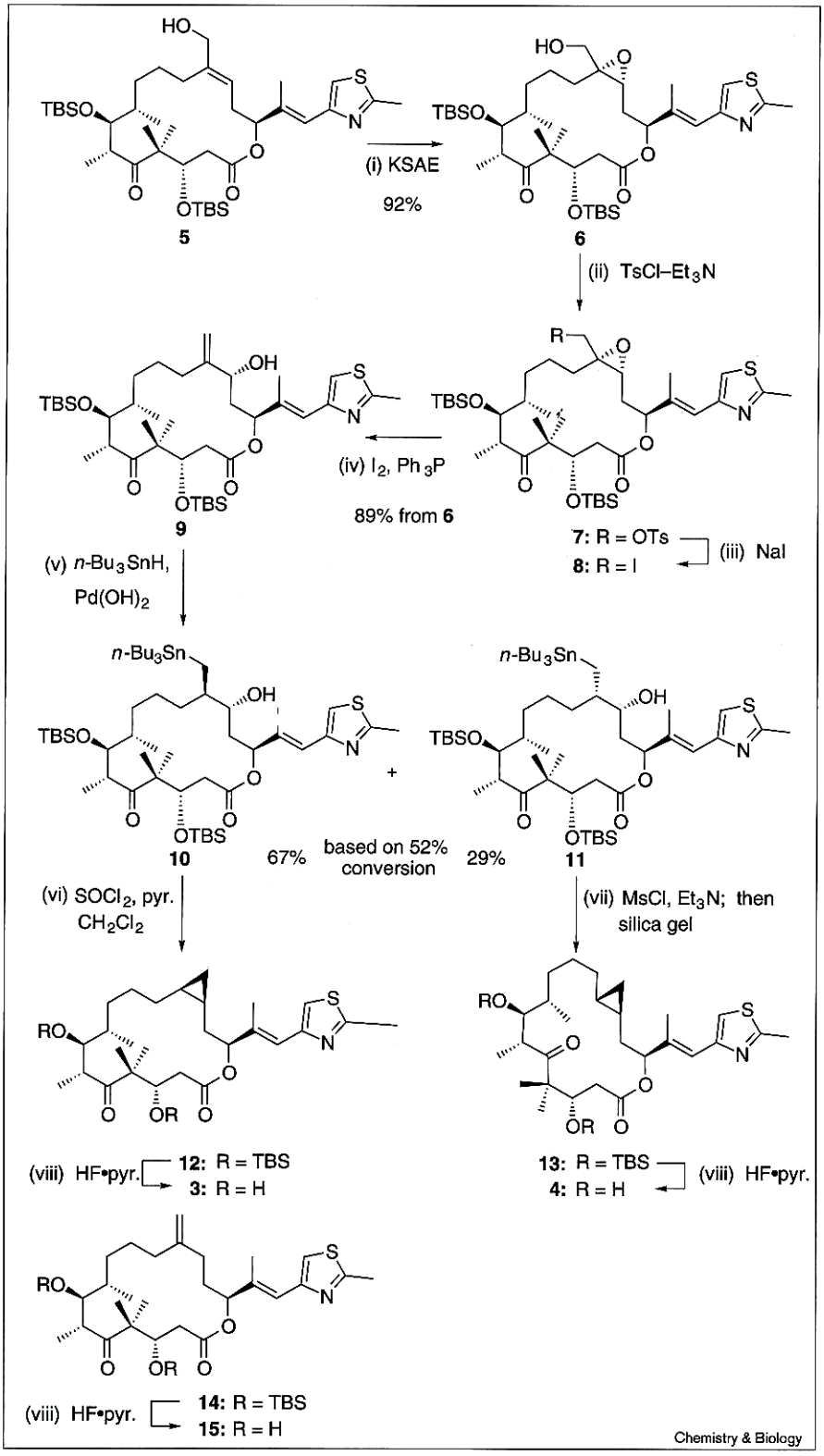
provided epoxy alcohol **6** in 92% yield and as a single diastereoisomer (as judged by ^{1}H nuclear magnetic resonance (NMR) analysis). Tosylation of the primary alcohol also proceeded smoothly to afford tosylate **7**. Subsequent treatment of **7** with sodium iodide in acetone gave the iodide **8** that, upon *in situ* treatment with triphenylphosphine and a catalytic amount of iodine [29], rapidly rearranged to allylic alcohol **9** (89% over three steps). The latter compound (**9**) was then exposed to tri-*n*-butyltin hydride in the presence of catalytic amounts of $Pd(OH)_2$ [30] to afford the stannanes **10** and **11** (96% yield based on ~ 52% conversion) albeit with modest diastereoselectivity (**10**:**11**; ~ 2:1). It was expected that, although elaboration of the C12-(*R*)-diastereoisomer **10** would lead to the *cis*-cyclopropane **12**, the isomeric stannane **11** could permit access to the equally interesting C12,13-*trans*-cyclopropane system **13**. Thus, treatment of **10** with thionyl chloride and pyridine in dichloromethane at –78°C, followed by warming to room temperature over five hours [26], promoted the required elimination, leading to an inseparable mixture of **12** and elimination product **14**. Desilylation (HF•pyr./THF) then allowed separation of the two components, providing C12,13-*cis*-cyclopropyl-epothilone A (**3**; 20% yield for two steps) and elimination product **15** (62% yield for two steps). In an analogous fashion, stannane **11** was converted efficiently to cyclopropane system **4**. Thus, following mesylation of the secondary hydroxyl group in **11**, exposure to silica gel facilitated ring closure, generating **13** in excellent yield (89%). Finally, desilylation as before (HF–pyr./THF) afforded C12,13-*trans*-cyclopropyl-epothilone A (**4**) in 90% yield. In both cases (**3** and **4**) the stereochemistry of the cyclopropane moiety was established by detailed ^{1}H NMR experiments (^{1}H ^{1}H correlation spectroscopy (COSY) and nuclear Overhauser effect correlation spectroscopy (NOESY)).

The tubulin-assembly and cytotoxicity data against certain tumor cell lines for cyclopropyl analogs **3** and **4**, together with those of a number of other epothilone analogs prepared recently in these laboratories [19,24], are shown in Table 1. Examination of entries 1 and 2 clearly shows that replacement of the epoxide moiety with a cyclopropane system has a profound effect on both the tubulin-polymerization and cytotoxic properties of the molecules. In order to more fully comprehend this drastic reduction in potency, we resorted to computational chemistry techniques to examine the conformations of **3** as compared to the parent epothilone A (**1**). We suspected that the partial sp^2 character of the 'banana bonds' of the cyclopropyl ring was possibly leading to distortion of the normal conformation of the epothilone framework, thereby preventing the molecule from adopting the required shape for binding to tubulin. As shown in Figure 3, the substitution of an epoxide for a cyclopropane moiety does indeed cause rather drastic changes to the minimum-energy conformation of epothilone A (**1**). The significant differences in the ^{1}H NMR spectra of compounds **1** and **3** were also in support of the drastic conformational changes imposed on the epothilone A skeleton by the cyclopropane ring. Similarly, the C12,13-*trans*-cyclopropyl-epothilone analog **4** was found to be devoid of any tubulin-polymerization and cytotoxicity properties as compared to its epoxide counterpart (**16**) and epothilone A (**1**) itself (see Table 1, entries 1–4).

A number of additional trends are apparent from examination of the remaining data in Table 1. Although analogs

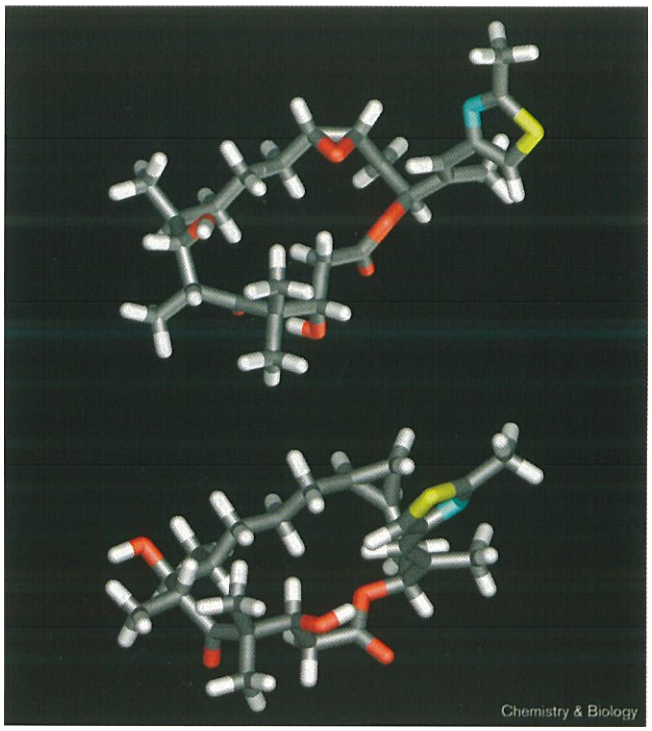
Figure 2

Stereoselective synthesis of C12,13-*cis*-cyclopropyl-epothilone A (**3**) and C12,13-*trans*-cyclopropyl-epothilone A (**4**). Reagents and conditions: (i) 0.5 equivalents of (−)-diethyl-D-tartrate, 0.4 equivalents of $Ti(i\text{-}OPr)_4$, 2.0 equivalents of *t*-BuOOH, CH_2Cl_2, −30°C, 2 h, 92%; (ii) 1.5 equivalents of tosyl chloride, 3.0 equivalents of Et_3N, 0.1 equivalents 4-DMAP, CH_2Cl_2, 0→25°C; (iii) 5.0 equivalents of NaI, acetone, reflux 2 h; (iv) 0.1 equivalents of I_2, 1.5 equivalents of Ph_3P, acetone/DMF, 89% from **6**; (v) 1.5 equivalents of *n*-Bu_3SnH, 0.1 equivalents of $Pd(OH)_2$, THF, 67% of **10**, 29% of **11** based on 52% conversion; (vi) 4.0 equivalents of $SOCl_2$, 8.0 equivalents of pyridine, CH_2Cl_2, −78→25°C, 5 h; (vii) 2.1 equivalents of mesyl chloride, 4.2 equivalents of Et_3N, CH_2Cl_2, 10 min, 89%; (viii) 30% HF•pyr. (by volume) in THF, 0→25°C, 24 h, 20% of **3** (over two steps), 90% of **4**, 62% of **15** (over two steps). 4-DMAP, dimethylaminopyridine; DMF, *N,N*-dimethylformamide; THF, tetrahydrofuran; pyr., pyridine.



without the epoxide moiety showed tubulin-binding activity, for the most part they displayed very low levels of cytotoxic activity against the tumor cell lines examined. The trends discussed below, therefore, are based on

Figure 3



Computer-generated minimum-energy structures of epothilone A (above) and C12,13-cyclopropyl epothilone A (below). Carbon, gray; hydrogen, white; oxygen, red; nitrogen, blue; sulfur, yellow. Molecular dynamics and minimization calculations (CV Force Field) were performed on an SGI Indigo-2 workstation using the program Insight II (Biosym Technologies Inc., San Diego, CA). Pictures were created with AVS software (AVS Inc., Waltham, MA) and locally developed modules running on a DEC Alpha 3000/500 with a Kubota Pacific Denali graphics card.

levels of tubulin polymerization. As expected, epothilone B type analogs (entries 36–52) generally had higher levels of activity than those of epothilone A (**1**; entry 1) and related analogs (entries 5–35). In comparing nonepoxidized substrates (entries 6–35), the C12,13-*cis* systems generally showed higher levels of tubulin polymerization than the corresponding C12,13-*trans* systems (compare entries 9–13 with 24–28).

Some more specific trends also became evident on comparing the C12,13-*cis*-olefins (entries 6–20). The presence and position of the nitrogen atom in the sidechain heterocycle seems to be important. Compound 26 (entry 15), in which the nitrogen atom is in its normal position adjacent to C18 but the sulfur atom of the thiazole has been relocated, still showed good activity. Compound **25** (entry 14), in which the nitrogen atom has been moved, was inactive, however. These trends were mirrored in the cases of the C12,13-*trans*-olefins (entries 29 and 30). A similar effect can be seen with the pyridine analogs **30** and **45** (entries 19 and 34). Previously synthesized pyridine-containing analogs in which the nitrogen atom was adjacent to C18 displayed good levels of activity [16], whereas **30** and **45** showed low levels of tubulin polymerization. Clearly, altering the position of the nitrogen by one atom has severe implications on activity. Entries 16–17 and 31–32, in which the thiazole of the epothilones had been replaced by either a furan or thiophene system, demonstrate that complete removal of the nitrogen leads to a considerable loss of tubulin-polymerization activity. Substitution of the five-membered heterocycle with a six-membered carbocyclic moiety (entries 18 and 33) resulted in analogs with low activity. As can be seen from entries 20 and 35, removal of the heterocycle altogether resulted in essentially complete loss of activity.

Modification at C22 (entries 6–13) seems well tolerated, provided the substituent is not too sterically demanding. For example, hydroxymethylene (**17**), fluoromethylene (**19**) and thiomethylether (**22**) compounds showed reasonable activity, whereas the larger acetate (**18**), ethoxythiazole (**21**), long-chain acetate (**23**) and piperidine (**24**) derivatives were somewhat less active. A similar trend was seen in the C12,13-*trans* systems (entries 21–28). Alteration at C26 (entries 36–52) seemed to be fairly well tolerated with high levels of activity being shown by the fluoromethylene olefins **52–55**, fluoromethylene epoxides **59** and **60**, and the ethyl epoxides **61–63**. The C26- hydroxy olefins **46–51** and C26-hydroxy epoxides **56–58** were somewhat less active, however.

Significance

The success of taxol as a therapeutic agent epitomizes the value of tubulin-polymerization microtubule-stabilizing agents in the fight against cancer. The similar mode of action and improved potency of the epothilones, particularly against taxol-resistant tumor cell lines, has made them of particular importance as potential anti-cancer drugs, especially in cases where taxol fails. A greater understanding of the structural requirements of the epothilones for biological activity should facilitate their further development as potential anti-cancer agents. In this study, the biological activities of a structurally diverse set of modified epothilones have been investigated and several useful trends noted. The biological action of the epothilones seems particularly sensitive to the location of basic heteroatoms in the sidechain and to the relative steric bulk of sidechain substituents. Furthermore, additional alterations at C26 may be tolerated resulting in analogs possessing varying degrees of activity. An important conclusion from this work was the finding that substitution of the epoxide moiety of epothilone A by a cyclopropyl group leads to total loss of activity, presumably due to drastic conformational changes imposed by this substitution.

Table 1

Tubulin-assembly and cytotoxicity data for epothilone analogs.

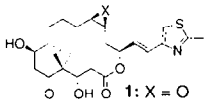
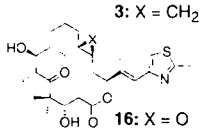
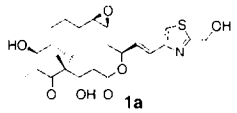
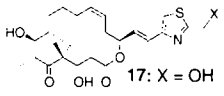
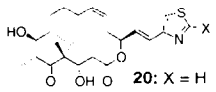
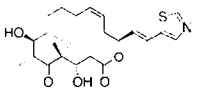
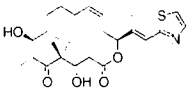
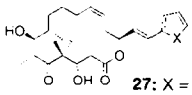
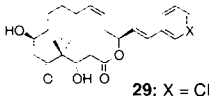
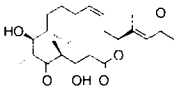
Entry	Compound	Reference	Induction of tubulin assembly (%)	IC_{50} (nM) Parental 1A9	IC_{50} (nM) Taxol resistant PTX10	IC_{50} (nM) Taxol resistant PTX22
1	 **1:** X = O	[1–11]	76	2.2	20	5.9
2	**3:** X = CH_2	–	2	>100	>100	>100
3	 **16:** X = O	[6,7,16]	92	2.0	18	3.0
4	**4:** X = CH_2	–	2	>100	>100	>100
5	 **1a**		52	>100	50	20
6	 **17:** X = OH	[19]	34	>100	>100	>100
7	**18:** X = OAc	[24]	3	50	>100	>100
8	**19:** X = F	[24]	57	>100	>100	>100
9	 **20:** X = H	[19]	50	>100	>100	>100
10	**21:** X = OEt	[24]	3	>100	>100	>100
11	**22:** X = SMe	[19]	92	9	22	28
12	**23:** X = $(CH_2)_5OAc$	[19]	2	>100	>100	>100
13	**24:** X = 	[19]	18	>100	>100	>100
14	 **25**	[19]	2	>100	>100	>100
15	 **26**	[19]	63	10	28	25
16	 **27:** X = O	[19]	4	>100	>100	>100
17	**28:** X = S	[19]	6	>100	>100	>100
18	 **29:** X = CH	[19]	16	>100	>100	>100
19	**30:** X = N	[19]	13	>100	>100	>100
20	 **31**	[24]	1	>100	>100	>100

Table 1 (cont'd)

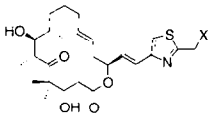
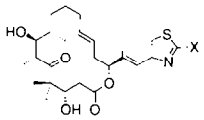
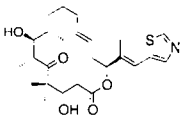
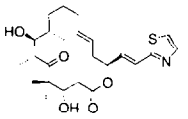
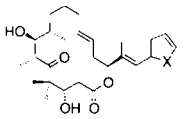
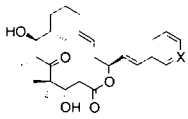
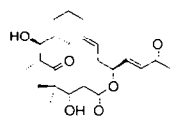
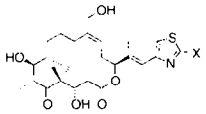
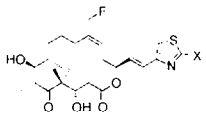
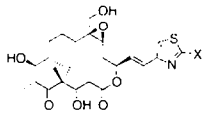
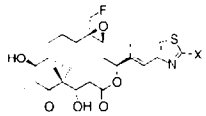
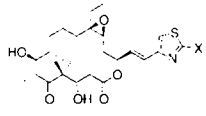
Entry	Compound	Reference	Induction of tubulin assembly (%)	IC_{50} (nM) Parental 1A9	IC_{50} (nM) Taxol resistant PTX10	IC_{50} (nM) Taxol resistant PTX22
						
21	**32:** X = OH	[19]	40	>100	>100	>100
22	**33:** X = OAc	[24]	2	>100	>100	>100
23	**34:** X = F	[24]	55	>100	>100	>100
						
24	**35:** X = H	[19]	41	20	>100	45
25	**36:** X = OEt	[24]	2	>100	>100	>100
26	**37:** X = SMe	[19]	71	15	>100	20
27	**38:** X = $(CH_2)_5OAc$	[19]	0	>100	>100	>100
28	**39:** X =	[19]	5	>100	>100	>100
						
29	**40**	[19]	2	>100	>100	>100
						
30	**41**	[19]	57	>100	70	>100
						
31	**42:** X = O	[19]	0	>100	>100	>100
32	**43:** X = S	[19]	2	>100	>100	>100
						
33	**44:** X = CH	[19]	26	>100	>100	>100
34	**45:** X = N	[19]	2	>100	>100	>100
						
35	**46**	[24]	1	>100	>100	>100

Table 1 (cont'd)

Entry	Compound	Reference	Induction of tubulin assembly (%)	IC_{50} (nM) Parental 1A9	IC_{50} (nM) Taxol resistant PTX10	IC_{50} (nM) Taxol resistant PTX22
						
36	**46**: X = CH_2F	[24]	11	>100	>100	>100
37	**48**: X = OMe	[24]	25	75	>100	>100
38	**49**: X = $CHCH_2$	[24]	48	>100	>100	>100
39	**50**: X = CH_2CH_3	[24]	58	>100	>100	>100
40	**51**: X = CH_2OH	[24]	14	>100	>100	>100
						
41	**52**: X = CH_2F	[24]	80	80	>100	>100
42	**53**: X = OMe	[24]	80	10	90	>100
43	**54**: X = $CHCH_2$	[24]	92	1.2	11	>100
44	**55**: X = CH_2CH_3	[24]	97	2.0	15	>100
						
45	**56**: X = CH_2F	[24]	6	>100	>100	>100
46	**57**: X = OMe	[24]	9	>100	>100	>100
47	**58**: X = CH_2CH_3	[24]	39	>100	>100	>100
						
48	**59**: X = CH_2F	[24]	92	0.54	2.8	1.5
49	**60**: X = OMe	[24]	91	0.40	1.2	2.5
						
50	**61**: X = CH_2F	[24]	91	5.5	10	8.8
51	**62**: X = OMe	[24]	93	10	29	15
52	**63**: X = CH_2CH_3	[24]	95	0.12	0.35	0.14

Materials and methods

Chemical synthesis

Further details for the synthesis of compounds **3** and **4** and selected physical data for the compounds shown in Figure 2 are included in the Supplementary material. Details of the synthesis and physical properties of compounds shown in Table 1 can be found in the given references.

Tubulin polymerization and cytotoxicity assays

Tubulin polymerization was determined by the filtration-colorimetric method, developed by Bollag *et al.* [12]. Purified tubulin (1 mg/ml) was incubated at 37°C for 30 min in the presence of each compound (20 μM) in MEM buffer [(100 mM 2-(N-morpholino)ethanesulfonic acid, pH 6.75, 1 mM ethylene glycol bis(β-aminoethyl ether), *N, N, N', N'*-tetraacetic acid, and 1 mM $MgCl_2$]; the mixture was then filtered to remove unpolymerized tubulin using a 96-well Millipore Multiscreen Durapore hydrophillic 0.22 μm pore size filtration plate; the collected polymerized tubulin was stained with amido black solution and quantified by measuring absorbance of the dyed solution on a Molecular Devices Microplate Reader. The growth of all cell lines was evaluated by quantitation of the protein in 96-well plates as described previously [31]. Briefly, 500 cells were seeded in each well of the plates and incubated with the various concentrations of the epothilones at 37°C in a humidified 5% CO_2 atmosphere for four days. After cell fixation with 50% trichloroacetic acid, the optical density corresponding to the quantity of proteins was measured in 25 mM NaOH solution (50% methanol: 50% water) at a wavelength of 564 nm. The IC_{50} was defined as the dose of drug required to inhibit cell growth by 50%.

Supplementary material

Details of the synthesis and analytical data for the cyclopropane analogs **3** and **4** are available with this paper on the internet.

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