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SAR and pH Stability of Cyano-Substituted Epothilones

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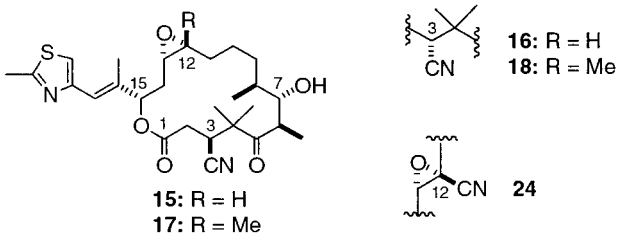
ABSTRACT



3-Cyano epothilones 15–18 are the only examples of non-hydroxy C-3-substituted analogues. Their tubulin binding affinity and cytotoxicity provide meaningful structure–activity relationship information on the dependence of C-1/C-3 conformation upon activity. 12-Cyano epothilone 24 has improved pH stability over epothilone B, and its activity further supports the hypothesis that C-12 stereochemistry is not critical for tubulin affinity.

Epothilones (Figure 1) are potent cytotoxic agents that, like the taxanes, bind to tubulin.[1] In contrast to their similar molecular target, epothilones are active against several taxane-resistant cell lines, both in vitro and in vivo.[2] The activity against taxane-resistant cell lines and their unique structure have garnered extensive research effort from academic and pharmaceutical research groups.

The structure−activity relationship (SAR) of various epothilone analogues suggests that epothilones do not tolerate broad structural changes.[1−3] One challenge has been the introduction of small changes that maintain both tubulin affinity and cytotoxicity while also providing meaningful SAR information and improved physicochemical properties. Accordingly, we report our studies on cyano-substituted

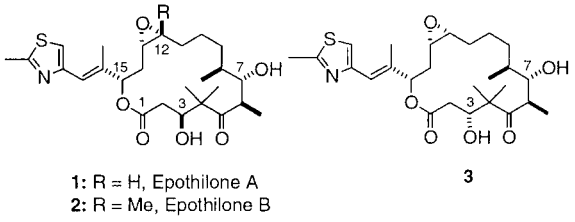


Figure 1. Structures of natural and C-3 epimeric epothilones.

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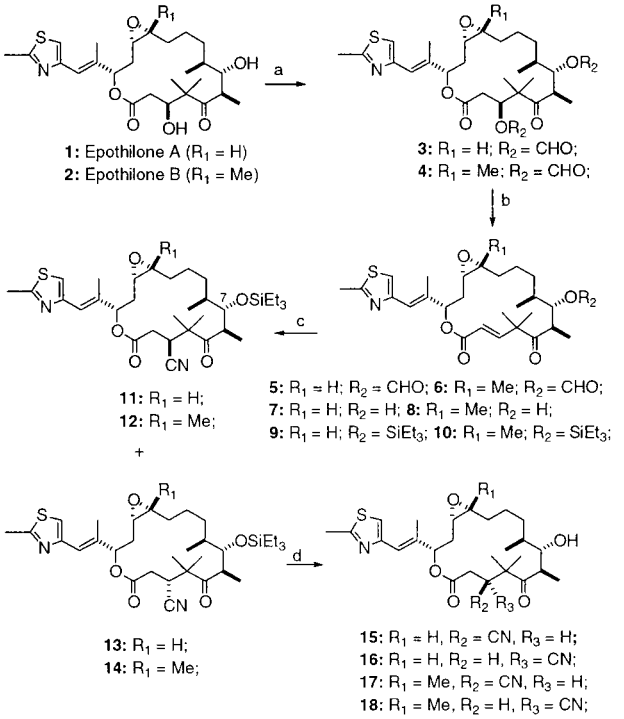
epothilones, which are active in vitro, provide relevant SAR information, and demonstrate improved chemical stability.

Previous studies have established that, in general, changes in the 16-membered macrolide framework lead to loss of tubulin affinity. For instance, stereochemical inversion[4] and ring contraction/expansion[5] reduce the tubulin binding affinity.

Specifically, the C-3 diastereomer (3*R*) of epothilone A, **3**, has been reported to be inactive in both tubulin polymerization and cytotoxicity assays.[6] It has been proposed that a hydrogen bond between the C-3 hydroxyl hydrogen and the C-1 lactone carbonyl may play an important role as a conformational constraint. Presumably, the stereochemical dependence of this interaction explains the lack of activity for the C-3 diastereomer.[7] Additional support comes from the activity of conjugated lactones **7** and **8** (Scheme 1), which

Scheme 1. Synthesis of 3-Cyano Epothilones[a]



[a] Reagents and conditions: (a) HCO_2H, TEA, DMAP, H_2O. (b) DBU, CH_2Cl_2; then NH_3, MeOH (yield from **1** and **2**: 73% for R1 = H; 95% for R1 = Me); $ClSiEt_3$, TEA, DMAP, CH_2Cl_2, 45 °C (96% for R1 = H; 90% for R1 = Me). (c) KCN, 18-C-6, DMF, 38% for R1 = H; 65% for R1 = Me). (d) AcOH/THF/H_2O, (83% for R1 = H; 41% for R1 = Me).

are active in the tubulin polymerization assay (Table 1).[8] Both compounds lack the C-3 hydroxyl group (hydrogen bonding is not possible), yet tubulin affinity is maintained perhaps because the C2−C3 olefin plays an analogous role

Table 1. Tubulin Polymerization and Cytotoxicity Data of Selected Epothilone Analogues

compound	tubulin[a] $EC_{0.01}$ (μM)	HCT-116[a,b] IC_{50} (nM)
paclitaxel	4.6	2.3
1	2.2	4.4
2	1.2	0.8
7	10.0	39.4
8	1.5	3.4
15	15	63
16	583	>200
17	1.2	8.4
18	103	
19	>1000	
24	2.5	4.1

[a] Assay performed using the method described in ref 8. [b] Cytotoxicity was assessed in HCT-116 human colon carcinoma cells.

of rigidifying the C1−C3 backbone.[3a] Though a conformational preference may exist between C-1 and C-3, herein evidence is shown that the aforementioned hydrogen bonding may not play a critical role. The corresponding 3-cyano analogues **15** and **17**, where the 3-hydroxyl group has been replaced with a cyano group, are active in both tubulin polymerization and cytotoxicity assays.[9]

The synthesis of 3-cyano epothilone analogues was achieved via Michael addition of cyanide to 2,3-unsaturated epothilones as shown in Scheme 1. Bis- formylation of **1** and **2** provided the corresponding 3,7-formates **3** and **4**, respectively. Selective base-catalyzed elimination of the C-3 formate followed by ammoniolysis of the 7-formate afforded 2,3-unsaturated lactones **7** and **8**,[9] which were converted to silyl ethers **9** and **10**, respectively. Michael addition to the enoate using KCN gave 1:1 mixtures of C-3 diastereomers **11/13** and **12/14**, which were separated by flash chromatography. Deprotection with acetic acid in THF/H_2O gave analogues **15**−**18**. These compounds constitute the first examples of Michael addition of a nucleophile to 2,3-unsaturated epothilones.

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(9) The synthesis of **7** and **8** was first disclosed by Höfle and co-workers in patent literature (DE 19639456.2).

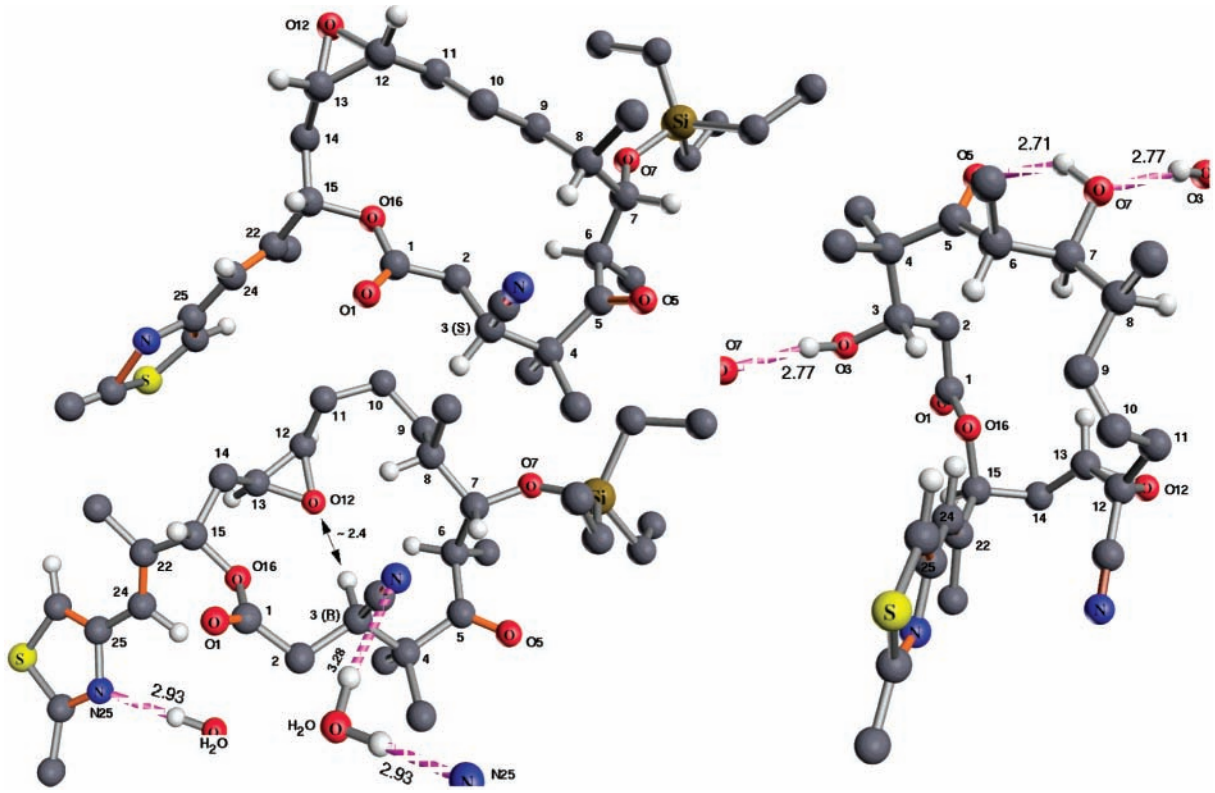

Figure 2. Solid state conformations and H-bonding (dashed bonds) in **11** (top left), **13** (monohydrate, bottom left), and **24** (right).[11]

The relative stereochemistry was first assigned using NOE experiments[10] and later confirmed by a single-crystal X-ray analysis. As shown in Figure 2, the backbone conformation of the diastereomers is grossly different, with analogue **11** having a conformation similar to epothilone A and B.[11] The 3-cyano analogues **15**−**18** show the same configurational dependence in activity as observed in natural epothilones. As shown in Table 1, compounds **16** and **18** with the unnatural (3*R*)-configuration are inactive, while analogues **15** and **17** with the natural (3*S*)-stereochemistry are active. Importantly, the cyano group cannot form a hydrogen bond with the C-1 carbonyl group, suggesting that a hydrogen bond between the C-3 hydroxyl group and the C-1 carbonyl group in the natural epothilones, if present, is not critical for intrinsic biological activity.

In an extension of this work, we investigated the effects of installing a cyano group elsewhere in the molecule. While epothilone B is active in vivo and presently in phase II clinical trials,[12] one possible limitation of epothilone B is the lack of stability at low pH, which may be a critical issue for eventual investigation of oral dosing regimens.[13] As shown in Figure 3, epothilone B (**1**) undergoes rapid, acid-catalyzed hydrolysis to diol **19** with concomitant loss of

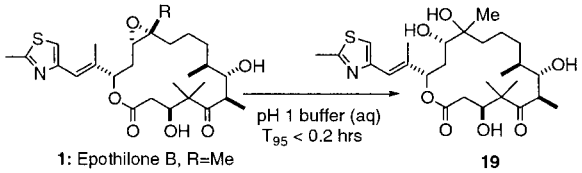

Figure 3. Acid-catalyzed epoxide opening of Epothilone B.[14]

(10) The NOE pattern between epothilone A and B analogues with the same relative stereochemistry was remarkably similar. NOE effects between H-3 and H-6, H-3 and H-8, as well as H-3 and the adjacent methyl with a lower chemical shift could be observed for all (3*S*)-diastereoisomers.

(11) H-bond distances designate the O−O or O−N separation (Å). Methyl and methylene hydrogens have been omitted from the figure. Data were recorded at −50 °C for single crystals of **11** and **13** from *n*-heptane/AcOEt. **11:** a = 15.0598(7), b = 6.6164(3), c = 17.6048(8) Å, β = 94.088(2)°, $P2_1$, Z = 2, R = 0.065, R_w = 0.091 for 2713 observed intensities ($I > 3\sigma(I)$), mp = 111−120 °C; **13 (monohydrate):** a = 10.1816(3), b = 8.8874-(3), c = 20.5177(8) Å, β = 101.836(2)°, $P2_1$, Z = 2, R = 0.067, R_w = 0.094 for 2793 observed intensities ($I > 3\sigma(I)$), mp = 79−98 °C. Data were recorded at +25 °C for single crystals of **24** (from cooled melt at 140 °C): a = 18.675(2), b = 8.5059(7), c = 17.495(1) Å, $P2_12_12_1$, Z = 4, R = 0.059, R_w = 0.065 for 1256 observed intensities ($I > 3\sigma(I)$), mp = 158−161 °C. Only one position is shown for some rotamerically disordered ethyls of the triethylsilyl group of **11**. Crystallographic data (excluding structure factors) for the above compounds have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-190177, -190176, and -190178, respectively. Copies of the data can be obtained free of charge upon application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: (+44)1223-336-033. E-mail: deposit@ccdc.cam.ac.uk).

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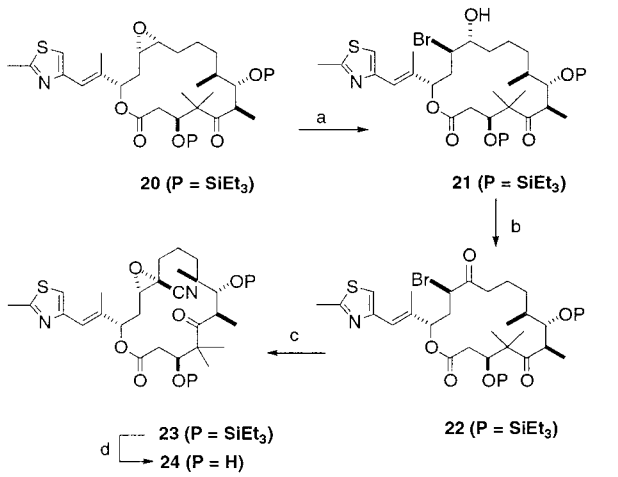
(13) Stomach pH is estimated to be between 1 and 3. T_{95} refers to 5% degradation of compound in pH 1 buffer as determined by HPLC analysis.

biological activity.[14] An electron-withdrawing R group at C-12 may slow the S_N1 solvolysis. A cyano group would be ideal since it is small and electron-withdrawing. Therefore, we set out to prepare an epothilone B analogue in which the 12-methyl group is replaced by a 12-cyano group.

Scheme 2. Synthesis of 12-Cyano Epothilones[a]



[a] Reagents and Conditions: (a) $MgBr_2-Et_2O$, CH_2Cl_2, from −20 to −5 °C, 45%. (b) PCC, py, CH_2Cl_2, 92%; (c) KCN, 18-C-6, THF, 49%; (d) 10% TFA/CH_2Cl_2, −10 °C, 98%.

As shown in Scheme 2, regioselective ring-opening of **20** with $MgBr_2{\cdot}OEt_2$ afforded bromohydrin **21**, as previously described.[15] Oxidation of the secondary alcohol with PCC provided ketone **22**. In the final stages of the synthesis, the pending C-13 stereochemistry is set by the configuration of the bromine substituent, barring any neighboring group participation from the lactone carbonyl.[16] However, the pending C-12 configuration is dependent on the stereochemical course of addition to the prochiral 12-ketone. In the event, cyanide addition[17] to bromoketone **22** occurred in a facial selective mode that, upon ring closure by intramolecular displacement of the bromide, afforded the trans-like cyanoepoxide **23**. Silyl group deprotection with TFA in dichloromethane provided **24**, the structure of which was confirmed by X-ray crystallography (Figure 2).[11] Although cyanide addition from the opposite face would have given the desired cis-like cyanoepoxide resembling epothilone B, there is ample literature precedent for α-face-selective additions to epothilones having sp^2 hybridization at C-12.[16,18] Interestingly, compound **24** displays excellent activity in both in vitro assays[19] and shows improved pH stability (pH 1 buffer (aq), T_{95} = 11 h) relative to epothilone B.[20] Furthermore, the activity of **24** supports the recent hypothesis[21] that the nature of the C-12 stereochemistry is not absolutely critical for tubulin affinity.

In summary, several epothilone analogues with cyano-substitution are active in vitro, provide useful SAR information, and show improved chemical stability. The 3-cyano analogues **15**−**18** are the only examples of non-hydroxy, 3-substituted analogues. Only the analogues **15** and **17** with the natural (3*S*)-configuration are active in vitro. Since chemical stability of epothilones may be an important issue in their clinical development, a 12-cyano epothilone **24** was prepared. Analogue **24** has improved pH stability over epothilone B, and its in vitro potency further supports the observation that C-12 stereochemistry is not crucial for biological activity.

Acknowledgment. The authors thank Stella Huang for assistance with NOE experiments and Michael Witkus for pH stability studies.

Supporting Information Available: Experimental procedures for the preparation of compounds **9**−**18** and **20**−**24** and characterization for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) The improved pH stability may be due to the presence of the nitrile group as we anticipated, although the altered epoxide geometry of **24** cannot be ruled out.

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