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Synthesis, Structure Proof, and Biological Activity of Epothilone Cyclopropanes

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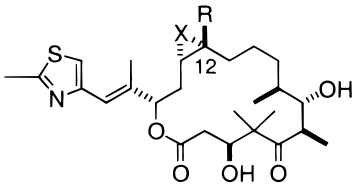
ABSTRACT



1: X=O, R=H; Epothilone A
2: X=O, R=Me; Epothilone B
8: X=CH_2, R=H
9: X=CH_2, R=Me

A semisynthetic route to epothilone cyclopropanes from epothilones A and B is described. Of significance, the deoxygenation of the 12,13-epoxide to give the corresponding olefin was achieved with high efficiency. The title compounds (8, 9) were active in both tubulin polymerization and cytotoxicity assays, which is in direct contrast to a previously published report. These results provide further evidence that the role of the 12,13-epoxide of epothilones is largely conformational and argue against some of the current pharmacophore models.

The epothilones A (**1**) and B (**2**), first isolated by Höfle and Reichenbach via fermentation of *Sorangium cellulosum*,[1] are a new class of cytotoxic agents that exert their cell-killing effects by the same mechanism as paclitaxel. Importantly, these new tubulin polymerization agents maintain their cytotoxic potency against paclitaxel-resistant cell lines.[2] Recent disclosures that both epothilones B (**2**) and D (**4**) display antitumor effects in murine tumor models further illustrate the importance of this new class of cytotoxic agents.[3] The tubulin binding affinities of several epothilone analogues derived from semisynthesis and total synthesis suggest that the epothilones have well-defined structural requirements for effective tubulin binding.[4] For instance, epothilones C and D (**3** and **4**), where the epoxide group is replaced with an olefin, show a slight loss of tubulin polymerization activity, suggesting that the role of the epoxide is largely conformational.[3,4] Subsequently, Nicolaou and co-workers reported a synthesis of the 12,13-cyclopropyl analogue of epothilone A (**8**) and found it to be inactive in a tubulin polymerization assay.[5] This apparent lack of activity is surprising given the structural similarity between oxiranyl and cyclopropyl rings. The present work describes the semisynthesis, unambiguous structural proof, and biological activity of 12,13-cyclopropyl epothilones. The data herein

(1) (a) Gerth, K.; Bedorf, N.; Höfle, G.; Irschitk, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560−563. (b) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1567−1569; *Angew. Chem.* **1996**, *108*, 1671−1673.

(2) (a) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. *Cancer Res.* **1995**, *55*, 2325−33. (b) Kowalski, R. J.; Giannakakou, P.; Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534−2541. (c) Wolff, A.; Technau, A.; Brandner, G. *Int. J. Oncol.* **1997**, *11*, 123−126.

(3) (a) Su, D.; Balog, A.; Meng, D.; Bertinato, P.; Danishefsky, S. J.; Zheng, Y.; Chou, T.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2093−2096; *Angew. Chem.* **1997**, *109*, 2178−2181. (b) Chou, T.; Zhang, X.; Balog, A.; Su, D.; Meng, D.; Savin, K. A.; Bertino, J. R.; Danishefsky, S. J.; *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642−9647. (c) Chou, T.; Zhang, X.; Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K. A.; Bertino, J. R.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15798−15802.

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demonstrate that 12,13-cyclopropyl epothilones analogues are, in fact, active in both tubulin polymerization and cytotoxicity assays and support the hypothesis that the role of the epoxide is largely conformational.

As a part of an ongoing effort to prepare more stable epothilone derivatives, the cyclopropyl analogue of epothilone A (**8**) was chosen as a target for synthesis. Molecular modeling studies indicated that the lowest energy conformations found for epothilone A (**1**) and the cyclopropyl analogue (**8**) were virtually identical (Figure 1).[6] Thus, in the absence

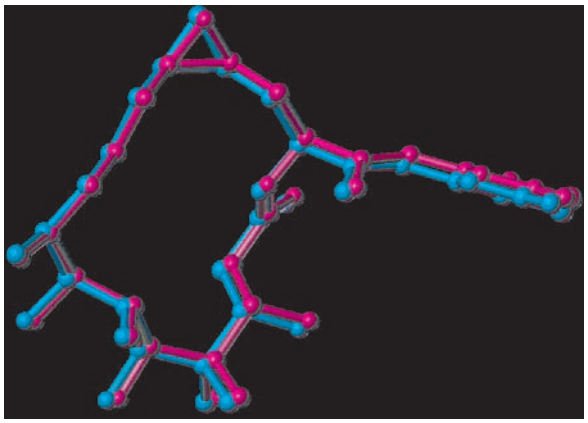
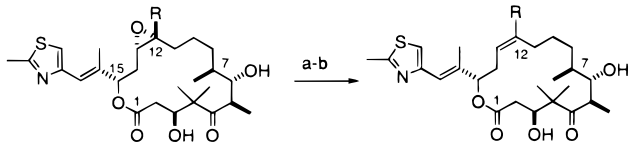


Figure 1. Superimposition of modeling-derived lowest energy conformations for epothilone A (magenta) and corresponding cyclopropyl analogue (cyan).[6a]

of any unfavorable nonbonded interactions between the tubulin and the cyclopropyl ring, it was expected that the cyclopropyl analogue would possess the desired biological activity.

The most direct route to 12,13-cyclopropyl analogues is the cyclopropanation of the corresponding olefins. Unfortunately, unlike epothilones A (**1**) and B (**2**), both epothilones C (**3**) and D (**4**) are isolated as minor metabolites from the fermentation process.[7] Thus, the first goal of this semisynthetic approach was the stereoselective conversion of the epoxide to the olefin (Scheme 1). Remarkably, the deoxy-

Scheme 1. Synthesis of Epothilones C (**3**) and D (**4**)

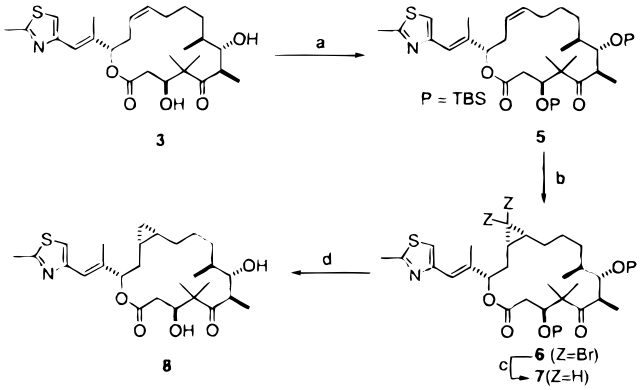


a) R = H: $TiCp_2Cl_2$, Mg(s), THF, 80%; b) R = Me: WCl_6, nBuLi, THF, 78%.

genation of *unprotected* **1** with Cp_2TiCl_2/Mg occurred in 80% yield to give **3** as a single olefin isomer.[8] The analogous reaction using unprotected **2** gave only trace amounts of **4**. However, the reduction of **2** using the procedure of Sharpless (WCl_6/*n*-BuLi) cleanly afforded **4** stereoselectively in 78% yield.[9]

The route to the cyclopropyl analogue of epothilone A (**8**) is shown in Scheme 2. As noted by Nicolaou and co-

Scheme 2. Synthesis of Epothilone A Cyclopropane **8**



a) TBS-OTf, 2,6- lutidine, CH_2Cl_2, 0 °C, 69%; b) benzyltriethylammonium chloride, 50% NaOH (aq), $CHBr_3$, 45 °C, 12%; c) Bu_3SnH, AIBN, C_6H_{12}, 70 °C, 76%; d) 20% TFA/ CH_2Cl_2, -15 °C, 90%.

workers for a related substrate, cyclopropanation of protected epothilone C (**5**) using Simmons−Smith or related conditions gave only trace amounts of the desired adduct.[5,10] However, treatment of **5** with NaOH/$CHBr_3$ under phase transfer conditions afforded dibromocyclopropane derivative **6** in 12% yield as a single diastereomer.[11] The stereochemistry

(4) (a) Meng, D.; Su, D.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.; Chou, T.; He, L.; Horwitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733−2734. (b) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, E. *Nature* **1997**, *387*, 268−272. (c) Su, D.; Meng, D.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.; Chou, T.; He, L.; Horwitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 757−759; *Angew. Chem.* **1997**, *109*, 775−777. (d) Balog, A.; Bertinato, P.; Su, D.; Meng, D.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.; Chou, T.; He, L.; Horwitz, S. B. *Tetrahedron Lett.* **1997**, *38*, 4529−4532. (e) Nicolaou, K. C.; Vourloumis, D.; Li, T.; Pastor, J.; Winssinger, N.; He, Y.; Ninkovic, S.; Sarabia, F.; Vallberg, H.; Roschangar, F.; King, P. N.; Finlay, R. M.; Giannakakou, P.; Verdier-Pinard, P.; Hamel, E. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2097−2103; *Angew. Chem.* **1997**, *109*, 2181−2187. (f) Nicolaou, K. C.; Ninkovic, S.; Finlay, M. R. V.; Sarabia, F.; Li, T. *Chem. Commun.* **1997**, 2343−2344. (g) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed.* **1998**, *37*, 2014−2045; *Angew. Chem.* **1998** *110*, 2120−2153.

(5) Nicolaou, K. C.; Finlay, R. M.; Ninkovic, S.; King, P. N.; He, Y.; Li, T.; Sarabia, F.; Vourloumis, D. *Chem. Biol.* **1998**, *5*, 365−372.

(6) (a) The modeling was performed on an SGI Octane workstation. The crystallographic coordinates of the X-ray structure of epothilone A[6b] were used as the starting geometry for modeling both epothilone A and the cyclopropyl analogue. Modeling details are included in the Supporting Information. (b) The authors want to thank Professor G. Höfle for kindly providing the X-ray coordinates for epothilone A (**1**). (c) A detailed conformation study of epothilone A has been published. Taylor, R. E.; Zajicek, J., *J. Org. Chem.* **1999**, *64*, 7224−7228.

(7) Reichenbach, H.; Hofle, G.; Gerth, K.; Steinmetz, H. PCT Int. Appl. 1998, WO9822461.

(8) Schobert, R.; Hoehlein, U. *Synlett* **1990**, *8*, 465.

(9) Sharpless, K. B.; Umbreit, M. A.; Nieh, M. T.; Flood, T. C. *J. Am. Chem. Soc.* **1972**, *94*, 6538.

of the cyclopropane of **6** was confirmed by single-crystal X-ray structure determination (Figure 2).[12] Despite the

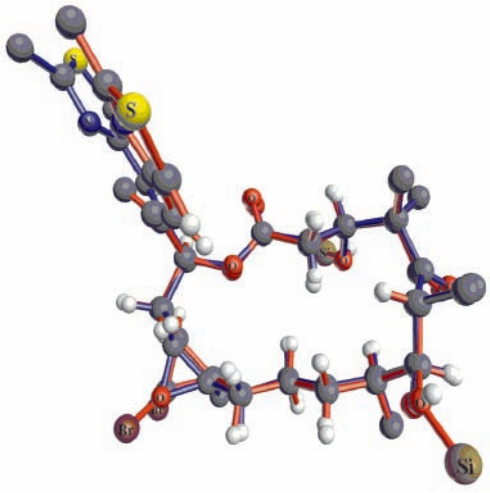
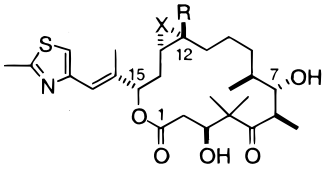


Figure 2. Solid state structure of dibromocyclopropane **6**,[12] superimposed with that of epothilone B.[1b] For clarity, methyl group hydrogens and alkyl groups attached to silicon are absent in the graphic.

presence of the bulky bromine atoms and silyl protecting groups, the solid state structure of **6** has a macrocyclic ring conformation that is similar to that of epothilone B.[1b,6c] The desired cyclopropane **8** was obtained after reduction and deprotection.[13] The route is short, stereoselective, and general. Other cyclopropyl analogues of epothilones, including the cyclopropyl analogue of epothilone B (**9**), were obtained by following the same route (Table 1). [14]

As shown in Table 1, all the cyclopropyl analogues, including the bulky dihalocyclopropanes **10** and **11**, are active

Table 1. In Vitro Data of Selected Epothilone Analogues



compd	X	R	tubulin[a] $EC_{0.01}$ (μM)	HCT-116[b] IC_{50} (nM)
Taxol			4.6	2.3
1	O	H	2.0	4.4
2	O	Me	1.8	0.8
3		H	3.9	63
4		Me	0.6	6.0
8	CH_2	H	1.4	1.4
9	CH_2	Me	2.1	0.7
10	CCl_2	Me	1.7	1.9
11	CBr_2	Me	1.6	3.8

[a] Assay performed using method described in ref 15a. [b] HCT-116 cell line cytotoxicity assay performed using method described in ref 15b.

in both the tubulin polymerization and cytotoxicity assays.[15] The comparable activities of the cyclopropyl analogues provide additional evidence that the role of the epoxide in the context of tubulin binding is largely conformational. Nonbonded interactions between the ether oxygen and tubulin protein, if present, do not appear to be significant or critical. Moreover, the potent cytotoxicity of the cyclopropyl analogues indicates that the epoxide is not necessary for cell activity, as suggested elsewhere.[4b−f,5]

Several pharmacophore models, published here and elsewhere, have been formulated to explain the interaction of epothilone and tubulin at an atomic level.[16] In particular, Giannakakou and co-workers recently suggested that the epoxide oxygen is involved in a productive binding interaction with tubulin based in part on the overlap with the oxetane ring of paclitaxel (computer modeling studies) and on the apparent lack of biological activity of the corresponding epothilone cyclopropane, as described by Nicolaou.[5,16c] Furthermore, Snyder and co-workers proposed a novel binding conformation for the epothilones where an internal hydrogen bond between the epoxide oxygen and the C3-hydroxyl group was a critical element.[16b] That the epothilone cyclopropanes are biologically active indicates that further refinement of the proposed models is required to adequately explain the currently available structure−activity data.

In summary, an efficient deoxygenation of epothilone 12,13-epoxides and stereospecific cyclopropanation of the resulting olefins has led to the preparation of epothilone

(10) The following cyclopropanation conditions were attempted on both TBS-protected epothilones C and D: Et_2Zn, 1,2-dichloroethane, ICH_2Cl; Zn−Ag, CH_2I_2, Et_2O; Bu_3Al, CH_2I_2, hexanes; CH_2N_2, $Pd(OAc)_2$, Et_2O; CH_2N_2, $Cu(acac)_2$, Et_2O; CH_2I_2, *hν*, dichloroethane; N_2CCO_2Et, $Rh_2(OAc)_4$, CH_2Cl_2. With reactive protocols, such as $Et_2Zn-ICH_2Cl$, cyclopropanation of C16−17 olefin was also observed.

(11) De Frutos, M. P.; Fernandez, M. D.; Fernandez-Alvarez, E.; Bernabe, M. *Tetrahedron* **1992**, *48*, 1123. The related Seyferth reagent, phenyl-(tribromomethyl)mercury, gave the C12−13 cyclopropane but in lower yield (Seyferth, D. *Acc. Chem.* **1972**, *5*, 65).

(12) (a) Single crystals of **6** were obtained from *n*-hexane: −43 °C, a = 15.888(3), b = 29.582(4), and c = 9.680(1) Å, $P2_12_12_1$, Z = 4, R = 0.046, R_w = 0.051 for 2586 observed intensities ($I > 3\sigma(I)$), mp = 175−180 °C. Crystallographic data (excluding structure factors) for **6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC#140777. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223−336−033; email: deposit@ccdc.cam.ac.uk).

(13) While compound **8** was previously reported by Nicolaou and co-workers (ref 5), the published ^{1}H NMR data are not in agreement with ours. This discrepancy was communicated to Professor Nicolaou, and he has informed us that the cyclopropanes in his 1998 article were erroneously assigned. A forthcoming publication from his laboratory will address the corresponding structural reassignments.

(14) In general, cyclopropanation of **4**, versus **3**, occurred in higher yield (∼30%), as expected. Dichlorosubstituted cyclopropane **10** was obtained using $CHCl_3$ as solvent.

(15) (a) Tubulin polymerization assays were performed as described in Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1991**, *34*, 1176−1184. (b) Cytoxicity was assessed in HCT-116 human colon carcinoma cells by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2*H*-tetrazolium, inner salt) assay as reported in Riss, T. L.; Moravec, R. A. *Mol. Biol. Cell* **1992**, *3 (Suppl.)*, 184a (abstract #1067).

(16) (a) Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S. B.; Kuduk, S. D.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4256−4261. (b) Wang, M.; Xia, X.; Kim, Y.; Hwang, D.; Jansen, J. M.; Botta, M.; Liotta, D. C.; Snyder, J. P.; *Org. Lett.* **1999**, *1*, 43−46. (c) Giannakakou, P.; Gussio, R.; Nogales, E.; Downing, K. H.; Zaharevitz, D.; Bollbuck, B.; Poy, G.; Sackett, D.; Nicolaou, K. C.; Fojo, T. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2904−2909.

cyclopropanes of unambiguous structural identity. The potent biological activity of these derivatives brings into question some of the early suppositions in this field: (1) the importance of nonbonded interactions between the epoxide oxygen and tubulin; (2) the importance of an intramolecular, trans-annular hydrogen bonding of the epoxide oxygen contributing to the biologically active conformation of epothilone; and (3) the difference in hydrophobicity between an olefin (12,13-desoxyepothilones) and an epoxide (epothilones) to account for differences in cytotoxicity. Clearly, further work in synthetic chemistry, computational chemistry, and structural biology are needed to improve our understanding of the effects of structure and conformation on biological activity for this exciting class of natural products.

Supporting Information Available: Experimental details of thepreparation of **5**−**8**. A detailed description of molecular modeling methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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