ORGANIC LETTERS

2001
Vol. 3, No. 17
2693−2696

Synthesis and Biological Activity of Novel Epothilone Aziridines†

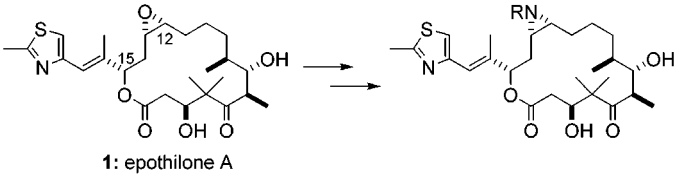
Alicia Regueiro-Ren,*,‡ Robert M. Borzilleri, Xiaoping Zheng, Soong-Hoon Kim, James A. Johnson, Craig R. Fairchild, Francis Y. F. Lee, Byron H. Long, and Gregory D. Vite

Divisions of Discovery Chemistry and Oncology Drug Discovery, The Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000

alicia.regueiroren@bms.com

Received June 13, 2001

ABSTRACT



1: epothilone A

A series of 12α,13α-aziridinyl epothilone derivatives were synthesized in an efficient manner from epothilone A. The final semisynthetic route involves a formal double-inversion of stereochemistry at both the C12 and C13 positions. All aziridine analogues were tested for effects on tubulin binding polymerization and cytotoxicity. The results indicate that the aziridine moiety is a viable isosteric replacement for the epoxide in the case of epothilones.

Epothilones are cytotoxic macrolides first isolated by Höfle and co-workers from myxobacterium *Sorangium cellulosum* strain 90.[1] This new class of microtubule-stabilizing natural products exhibits the same mechanism of action as paclitaxel.[2] More importantly, in cell culture, the epothilones are active against paclitaxel-resistant cell lines that express P-glycoprotein. Furthermore, epothilones have better solubility in water than paclitaxel. These features make them attractive complements to paclitaxel in the search for effective cancer therapies.

The ability of epothilones A and B to competitively inhibit [^{3}H]-paclitaxel binding to microtubules strongly suggests the existence of a common binding site.[2] Several publications[3] have appeared that attempt to define a common pharmacophore model to explain the similar biological activities observed for these two different classes of natural products. Of particular interest is the role of the epoxide in the epothilone family. The structure−activity relationship data generated from known synthetic epothilone derivatives[4] suggests that the epoxide moiety plays a conformational role by maintaining the structural rigidity necessary for interacting with the tubulin protein. The fact that the desoxy-epothilones **3** and **4** are only slightly less active than epothilones A (**1**)

† Dedicated to Professor Gilbert Stork on the occasion of his 80th birthday.

‡ Current address: Bristol Myers Squibb, Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

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

(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. (b) Kowalski, R. J.; Giannakakou, P.; Hamel, E. *J. Biol. Chem.* **1997**, *272*, 2534. (c) Wolff, A.; Technau, A.; Brandner, G. *Int. J. Oncol.* **1997**, *11*, 123.

(3) (a) 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. (b) He, L.; Jagtap, P. G.; Kingston, D. G. I.; Shen, H.-J.; Orr, G. A.; Horwitz, S. B. *Biochemistry* **2000**, *39*, 3972. (c) 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. (d) Wang, M.; Xia, X.; Kim, Y.; Hwang, D.; Jansen, J. M.; Botta, M.; Liotta, D. C.; Snyder, J. P. *Org. Lett.* **1999**. (e) Mineno, T.; Jayenda, J. B.; Woolfrey, J. R.; Buolamwini, J. K.; Mitchell, M. A. 3D-QSAR Studies of Taxoids and Analogues. In *Abstracts of Papers*, 217th National Meeting of the American Chemical Society, Anaheim, CA, March 21−25, 1999; American Chemical Society: Washington, DC, 1999; no. 135.

10.1021/ol016273w CCC: $20.00
Published on Web 07/26/2001

and B (**2**), together with our own results for the related 12α,13α-cyclopropyl analogues,[5] prompted us to investigate other bioisosteres for the epoxide (Figure 1). Hence, we set

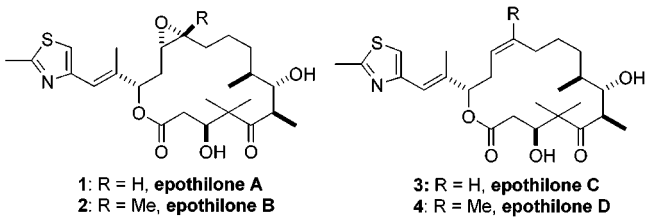


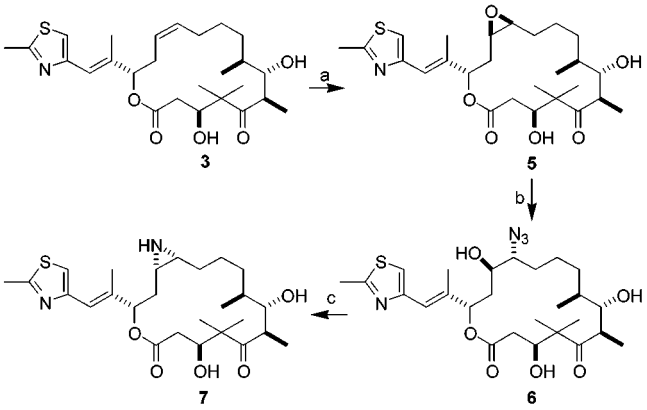
Figure 1. Epothilones A−D.

out to synthesize 12α,13α-aziridinyl analogues of epothilone A to determine if these new compounds would maintain biological activity and perhaps provide advantages over the oxiranyl analogues. In addition, the parent aziridine **7** would permit access to a variety of *N*-substituted derivatives, which may aid in the understanding of drug−tubulin interaction and assist in the rational design of more potent chemotherapeutic agents.[6]

We initially attempted to prepare the 12α,13α-aziridinyl analogues by direct aziridination of the corresponding C12,C13-olefin via a nitrene cycloaddition to the α-face of **3**. Unfortunately, when epothilone C (**3**)[5] or its bis-silyl ether protected derivative were subjected to standard aziridination procedures,[7] only trace amounts of the corresponding aziridines were detected.

Alternatively, we envisioned that a double-inversion of the stereochemistry at C12 and C13 of **1** would also furnish the desired parent aziridine **7**. Our attention was thus directed toward the synthesis of the diastereomeric 12*β*,13*β*-epoxide **5** (Scheme 1). Epoxidation of **3** with methyl(trifluoromethyl)-

Scheme 1. Synthesis of Epothilone A Aziridine **7** via Diastereomeric Epoxide **5**[a]



[a] (a) CF_3COCH_3, oxone, $NaHCO_3$, EDTA, CH_3CN, 20%; (b) NaN_3, NH_4Cl, EtOH, 65 °C, 55%; (c) Ph_3P, THF, 60 °C, 75%.

dioxirane gave the desired 12*β*,13*β*-epoxide **5** and epothilone A (**1**) in a 1:3 ratio and a combined yield of 80%. Attempts to alter and improve this ratio in favor of desired epoxide **5** were unsuccessful.[8] Nevertheless, nucleophilic ring opening of **5** with NaN_3/NH_4Cl in ethanol afforded azido alcohol **6** regioselectively in 55% yield. Triphenylphosphine reduction of the azide moiety of **6** followed by in situ ring closure[9] provided the desired aziridine **7** in 70% yield.[10]

Because our objective was to synthesize several *N*-substituted aziridinyl analogues, we devised a modified synthetic route that would avoid the nonselective epoxidation reaction and provide access to gram quantities of aziridine **7**. Thus, **1** was converted to the bis-triethylsilyl ether by treatment with TESCl in the presence of Hunig's base (Scheme 2). Epoxide opening using $MgBr_2 \cdot OEt_2$ at low temperature (−20 to −5 °C) afforded predominantly bromohydrin **8**, along with minor amounts of the C12*β*-Br/C13α-OH in 45% yield (67% yield based on recovered starting material) and >20:1 regioselectivity.[11] Nucleophilic substitution of the bromide by azide in DMF gave azido alcohol **9**

(4) (a) Meng, D.; Su, D.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.; Chou, T.; He, L.; Horowitz, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 2733. (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. (c) Su, D.; Meng, D.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.; Chou, T.; He, L.; Horowitz, S. B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 757; *Angew. Chem.* **1997**, *109*, 775. (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. (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; *Angew. Chem.* **1997**, *109*, 2181. (f) Nicolaou, K. C.; Ninkovic, S.; Finlay, M. R. V.; Sarabia, F.; Li, T. *Chem. Commun.* **1997**, 2343. (g) Nicolaou, K. C.; Roschangar, F.; Vourloumis, D. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2014; *Angew. Chem.* **1998** *110*, 2120.

(5) Johnson, J. A.; Kim, S.-H.; Bifano, M.; DiMarco, J.; Fairchild, C.; Gougoutas, J.; Lee, F.; Long, B.; Tokarski, J.; Vite, G. *Org. Lett.* **2000**, *2*, 1537.

(6) For a preliminary account of the work described herein and additional studies on the epothilones, see: (a) Vite, G.; Borzilleri, R.; Fooks, C.; Johnson, J. A.; Kim, S.-H.; Leavitt, K.; Mitt, T.; Regueiro-Ren, A.; Schmidt, R.; Zheng, P.; Lee F. Epothilones A and B: Springboards for the synthesis of promising antimitotic agents. In *Abstracts of Papers*, 219th National Meeting of the American Chemical Society, San Francisco, CA, March 26−30, 2000; American Chemical Society: Washington, DC, 2000; no. 286. (b) Borzilleri, R. M.; Kim, S.-H.; Regueiro-Ren, A.; Vite, G. D. PCT Int. Appl. WO0057874, 2000.

(7) (a) Evans, D. A.; Bilodeau, M. T.; Faul, M. M. *J. Am. Chem. Soc.* **1994**, *116*, 2742. (b) Banks, M. R.; Cadogan, J. I. G.; Gosney, I.; Hodgson, P. K. G.; Langride-Smith, P. R. R.; Millar, J. R. A.; Taylor, A. T. *Tetrahedron Lett.* **1994**, *35*, 9067. Seno, M.; Namba, T.; Kise, H. *J. Org. Chem.* **1978**, *43*, 3345.

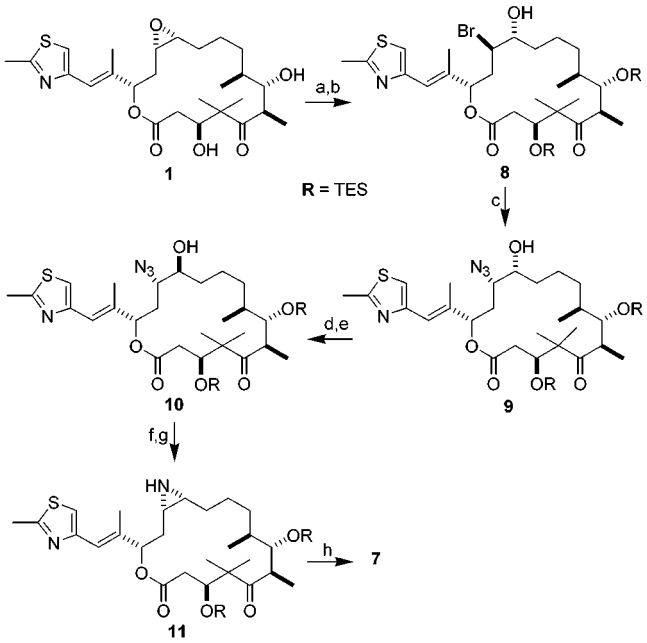
(8) Other reaction conditions attempted: low temperature; solvent variation (THF, CH_2Cl_2, MeOH); and alternative dioxirane source (trifluoroacetone) had no effect on the diastereoselectivity. Other oxidation methods, such as *m*-CPBA and DMDO, generated the *N*-oxide and/or the C16−C17 epoxide as side products.

(9) Ittah, Y.; Sasson, Y.; Shahak, I.; Tsarrom, S.; Blum, J. *J. Org. Chem.* **1978**, *43*, 4271.

(10) Analytical data for aziridine **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 1 H), 6.52 (s, 1 H), 5.46-5.44 (m, 1 H), 4.08- 4.04 (m, 1 H), 3.69-3.68 (m, 1 H), 3.20-3.17 (m, 1 H), 2.57 (s, 3 H), 2.44-2.38 (m, 1 H), 2.34-2.30 (m, 1 H), 1.95-1.93 (br s, 4 H), 1.82-1.78 (m, 2 H), 1.71-1.53 (m, 2 H), 1.46-1.31 (m, 4 H), 1.27 (s, 3 H), 1.23−1.01 (m, 2 H), 0.99 (d, 3 H, J = 6.9 Hz), 0.91 (s, 3 H), 0.81 (d, 3 H, J = 6.9 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 220.3, 171.2, 165.1, 152.4, 136.8, 119.2, 115.9, 76.5, 66.1, 75.4, 52.5, 45.0. 38.1, 34.6, 34.2, 30.1, 29.9, 26.1, 24.9, 22.9, 19.3, 17.6, 16.4, 15.4, 14.3; MS (ESI$^+$) 493.2 $(M + H)^+$.

(11) Higher reaction temperatures afforded a 1:1 ratio of the bromohydrins along with other side products.

Scheme 2. Synthesis of Epothilone A Aziridine **7** via Bromohydrin **8**[a]

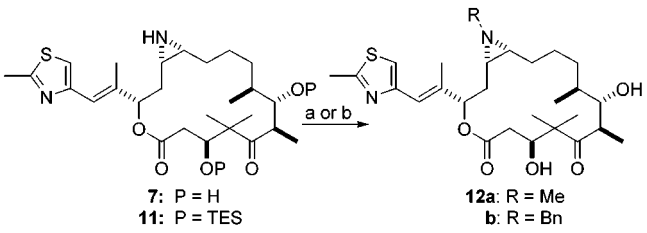


[a] (a)TESCl, DIPEA, DMF, 90%; (b) $MgBr_2-Et_2O$, CH_2Cl_2, −20 to −5 °C, 45% (<2% of the C12β-Br,C13α-OH regioisomer); (c) NaN_3, DMF, 48 h, 42 °C, 60%; (d) *p*-NBA, DEAD, Ph_3P, 99%; (e) NH_3, MeOH, 88%; (f) MsCl, Et_3N, CH_2Cl_2, 98%; (g) Me_3P, THF/H_2O, 98%; (h) 10% TFA/CH_2Cl_2, −10 °C, 90%.

in 60% yield. Inversion of the stereochemistry at the C12 position was carried out using *p*-nitrobenzoic acid under Mitsunobu conditions.[12] Ester hydrolysis provided **10** in quantitative yield. Azido alcohol **10** was converted to the corresponding mesylate, and subsequent reduction with trimethylphosphine and in situ cyclization afforded the 12α,13α-aziridine **11** in 96% yield.[13] Removal of the TES groups using trifluoroacetic acid in CH_2Cl_2 furnished **7** in 90% yield.

The *N*-alkyl aziridinyl analogues **12a** and **12b** were prepared either from **7** or **11** using methyl sulfate/proton sponge[14] or benzyl bromide/K_2CO_3, respectively (Scheme 3). Removal of the silyl ether protecting groups in the case

Scheme 3. Synthesis of *N*-Alkylated Epothilone A Aziridines **12a,b**[a]



[a] (a) P = H: Me_2SO_4, proton sponge, THF, 31%. (b) P = TES: BnBr, K_2CO_3, 18-crown-6, THF then 10% TFA in CH_2Cl_2, 66% over two steps.

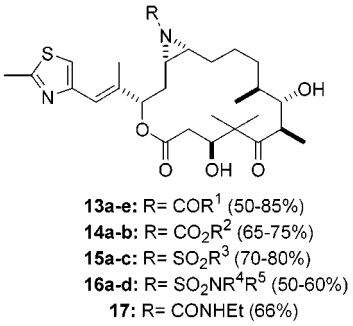
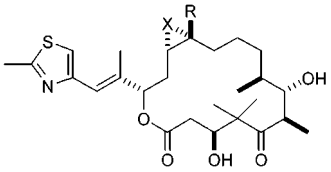


Figure 2. *N*-Acyl (**13a−e**), *N*-carbonyl (**14a,b**), *N*-sulfonyl (**15a−c**), *N*-sulfonylureas (**16a−d**), and *N*-carbamoyl (**17**) aziridine derivatives of epothilone A.

of **12b** was carried out using standard TFA/CH_2Cl_2 conditions.

The *N*-acyl (**13a−e**), *N*-alkoxycarbonyl (**14a,b**), *N*-alkylsulfonyl (**15a−c**), and *N*-sulfonylureido (**16a−d**) derivatives were synthesized uneventfully from unprotected aziridine **7** using Hunig's base and the corresponding acid chlorides (Figure 2). Urea **17** was prepared by treatment of **7** with ethyl isocyanate in ethyl acetate.

Table 1. In Vitro Data for Aziridine Analogues of Epothilone A



	X	R	tubulin[a] $EC_{0.01}$ (μM)	HTC-116[b] IC_{50} (nM)
taxol			4.6	2.3
1	O	H	2.0	4.4
2	O	Me	1.8	0.8
7	NH	H	14	2.7
12a	NCH_3	H	2.6	0.13
12b	$NCH_2C_6H_5$	H	35	23
13a	$NCOCH_2CH(CH_3)_2$	H	3.5	41
13b	$NCOCH_2N(CH_3)_2$	H	>1000	
13c	$NCOCH_3$	H	1.2	0.9
13d	$NCOC_6H_5$	H	1.4	3.0
13e	NCO-(2-thiophene)	H	1.2	1.6
14a	$NCOOCH_2CH_3$	H	1.2	7.7
14b	$NCOOC_6H_5$	H	1.1	1.8
15a	NSO_2CH_3	H	14	2.1
15b	$NSO_2CH_2CH_2CH_3$	H	9.4	94
15c	NSO_2-(2-thiophene)	H	4.0	30
16a	NSO_2NH_2	H	28	36
16b	NSO_2NHCH_3	H	2.3	7.8
16c	$NSO_2N(CH_3)_2$	H	1.0	0.2
16d	$NSO_2N(CH_2CH_2)_2O$	H	460	160
17	$NCONHCH_2CH_3$	H	>1000	

[a] Assay performed using method described in ref 14a. [b] HCT-116 cell line cytotoxicity assay performed using method described in ref 14b.

The biological activity of selected compounds are shown in Table 1.[15] The 12α,13α-aziridinyl epothilone A analogues were tested for effects on tubulin polymerization and cytotoxicity. The cytotoxicity of the parent aziridine **7** (IC_{50} = 2.7 nM) is comparable to that of epothilone A (**1**) (IC_{50} = 4.4 nM), although **7** is less efficient in the tubulin polymerization assay. Some of the novel analogues, e.g., methylaziridine **12a** and dimethylsulfonylurea **16c**, show improved in vitro activity over epothilones A (**1**) and B (**2**). *N*-Alkylaziridines **12a,b**, amides **13a**−**e**, *N*-sulfonylamides **15a**−**c**, and *N*-sulfonylureas **16a**−**d** present diverse binding affinity and cytotoxicity depending on the *N*-substituent. In general, aryl and small alkyl substituents appear to have favorable effect on both biological activities when compared with the parent aziridine **7**. Compounds such as **13b** and **16d** with basic substituents show poor activity in the tubulin polymerization assay. Interestingly, carbamates **14a,b** are well tolerated, but the related urea **17** is not.

In summary, we have described the efficient synthesis of a 12α,13α-aziridinyl epothilone via double inversion of stereochemistry at both C12 and C13 centers. Further substitution on the nitrogen allows access to a diverse group of substituted aziridinyl analogues that have been evaluated for in vitro biological activity. The results demonstrate that the aziridines are viable isosteric alternatives for the epoxide in the epothilone family. The comparable activities of these analogues indicate that the role of the epoxide in the context of tubulin binding is largely conformational. Importantly, the tubulin binding site can accommodate a variety of substituents at the C12−C13 position. Changes in the size and/or lipophilicity of the *N*-substituent produce substantial variation in the biological activities of these derivatives, and fine-tuning of these physicochemical parameters is necessary to improve both tubulin-binding affinity and cytotoxicity.

Supporting Information Available: Experimental procedure for the preparation of compounds **7**−**11** and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL016273W

(12) Martin, S. F.; Dodge, J. A. *Tetrahedron Lett.* **1991**, *32*, 3017.

(13) Aziridine **11** could also be prepared directly from azido alcohol **10** by reduction and in situ ring closure with trimethylphosphine, but only in 30% yield.

(14) Choi, D.; Yoo, B.; Colson, K. L.; Martin, G. E.; Kohn, H. *J. Org. Chem.* **1995**, *60*, 3391.

(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. Effective concentration ($EC_{0.01}$) is defined as the interpolated concentration capable of inducing an initial slope of 0.01 OD/min rate and is calculated using the formula $EC_{0.01}$ = concentration/slope. $EC_{0.01}$ values are expressed as the mean with standard deviation obtained from three different concentrations. (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).