

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogado
ALVARO CORREA ORDOÑEZ

ASUNTO	Radicación	08 125 426
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	111201
	Folios	10

Patente de Invención



Patente de Modelo de Utilidad

Vía Nacional

Vía PCT (Fase Nacional)



Capítulo I



Capítulo II

No. Solicitud Internacional

PCT/US2007/069736

Fecha de Presentación Internacional

25/Mayo/2006

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 109 a 220	25/Noviembre/2008
Reivindicaciones:	Folios 221 a 223	25/Noviembre/2008
Prioridad:	US 60 808 366	25/05/2006

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

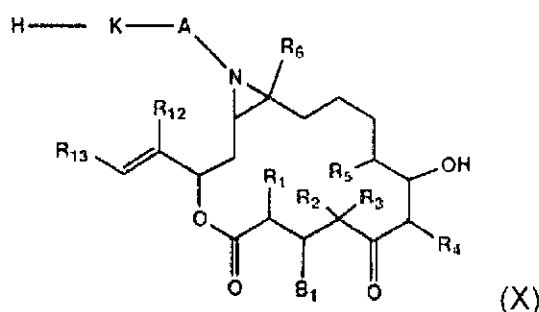
3. Objeto de la invención

Título de la solicitud: "Compuestos de Aziridinil-Epotilona".

El problema planteado en esta solicitud consiste en la necesidad de proporcionar compuestos que pueden utilizarse en el tratamiento del cáncer para la preparación

de conjugados para administrar un fármaco dirigido, especialmente folatos.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula (X) y composiciones que lo contienen



El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C 07 D 491/04, A 61 K 31/427 // 31/396, A 61 P 35/00.

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicación 9 no es patentable, de acuerdo con el Art 14.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

- La reivindicación 1 no es concisa porque emplea términos como alquilo, alquenoilo y cicloalquilo para estos sustituyentes se solicita establecer el número de átomos que los componen, por otra parte, es necesario aclarar cuáles son los heteroátomos y cuántos átomos son los que hacen parte de los radicales heterocicloalquilo y heteroarilo.
- En la reivindicación 2 se solicita aclarar cuáles heteroátomos y de qué tamaño son los sustituyentes heteroarilo descritos y definir el número de átomos de carbono del radical alquilo inferior.
- En la reivindicación 10 se describe un procedimiento pero este no se define mediante características como alguna etapa ó alguna condición específica en que debiera realizarse. Se sugiere, entonces, definir la invención por sus características esenciales como son las etapas y condiciones fisicoquímicas bajo las que se llevo a cabo.

6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de Presentación de la Solicitud de Prioridad: Mayo 25 de 20016 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	US2005002942	Vitamin receptor binding drug delivery conjugates	06/01/2005
D2	US6399638	12,13-modified epothilone derivatives	01/06/2002
D3	WO02098868	Epothilone Derivatives	12/12/2002
D4	Organic Letters 2001, Vol. 3, No. 17, Pág. 2693-2696	Synthesis and Biological Activity of Novel Epothilone Aziridines	13/06/2001

D1

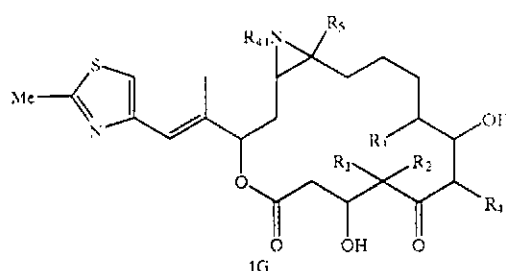
http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=2005002942A1&KC=A1&FT=D&ND=&date=20050106&DB=&locale=en_EP

Divulga (Pág.24, Párrafo 0205, líneas 9-11) la combinación de vitaminas conjugadas con folatos y describe la aplicación de epotilones para el tratamiento del cáncer junto con otros compuestos (Pág. 24, párrafo 0208, lín.19).

D2

http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=6399638B1&KC=B1&FT=D&ND=&date=20020604&DB=&locale=en_EP

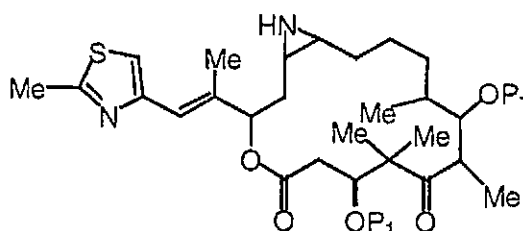
Divulga (Col 8, estructura 1G) derivados de epotilones - aziridinas



D3

http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=02098868A1&KC=A1&FT=D&ND=&date=20021212&DB=&locale=en_EP

Divulga (Pág. 31, figura 18F) derivado de epotilones - aziridinas



D4

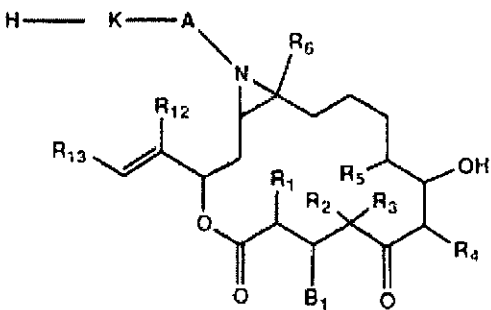
<http://www.sinab.unal.edu.co:2139/action/doSearch?action=search&searchText=Synthesis+and+biological+activity+of+novel+epothilone+aziridines&qsearchArea=searchText>

Divulga (Pág. 2695, Tabla 1) estudios in vitro de derivados de aziridina, análogos de epotilona A.

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en derivados de

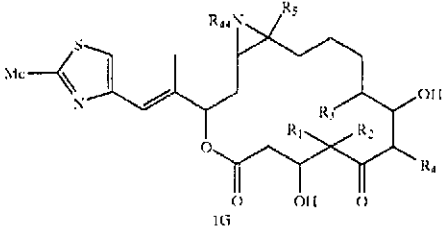


Útiles para el tratamiento del cáncer.

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2/D3/D4
Derivados de Aziridinil – Epotilonas 		Derivados de Aziridinil – Epotilonas
Conjugación de los Folatos	Conjugación de vitaminas, como el folato para la liberación de fármacos (Pág.24, Párrafo 0205, líneas 7-11).	

Los documentos D1 y D2, D3, D4 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulgan el producto



La diferencia entre la invención, definida en la reivindicación 1 y el producto de D2, D3, D4 consiste en que en D1 se menciona de manera general el uso de epotilones (Pág. 24, párrafo 0208, lín.19) junto con vitaminas, por ejemplo ácido fólico (Pág.23, párrafo 204, línea 1) para mejorar la liberación del medicamento (Pág.24, párrafo 205, lín.6-11).

La diferencia entre la invención, definida en la reivindicación 1 y el producto de D2 consiste en que en D2, D3, D4 se describen derivados de epotilones – aziridina, moléculas similares al taxol y empleadas en el tratamiento del cáncer pero no se menciona el uso de combinaciones que puedan mejorar su efectividad, adicionalmente en el documento D4 se evalúa el efecto sobre la actividad por la modificación de radicales sobre el “N” de la aziridina¹.

El efecto que logra el incluir folatos es que aumenta la permeabilidad de las células sobre las cuales actúa el agente anti cancerígeno, por lo cual incrementa su actividad.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: “cómo modificar las composiciones de epotilones – aziridina, conocidos en D2 para incrementar la actividad del medicamento”.

Sin embargo, la utilización de los folatos para aumentar la actividad, ya está divulgada en el documento D1 que se refiere a las combinaciones de vitaminas, como el folato para mejorar la actividad de una gran variedad de principios activos, entre ellos los epotilones (Pág. 24, Párrafo {0205}, lín.7-11; párrafo {0208}, lín.18).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría folatos de D1 en la composición de D2 para llegar así al objeto de la reivindicación 1 y 10 de la solicitud. Por lo cual se considera obvia.

Conclusión:
La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

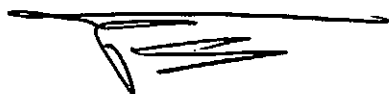
Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US2005002942 (A1)	1 – 11	Nivel Inventivo
D2	US6399638 (B1)	1 – 11	Nivel Inventivo
D3	WO02098868 (A1)	1 – 11	Nivel Inventivo
D4	Organic Letters 2001, Vol. 3, No. 17, Pág. 2693-2696	1 – 11	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

¹ “Synthesis and biological activity of novel epothilone aziridines”, Organic Letters 2001, Vol. 3, No. 17, Pág. 2695, table 1.
Expediente 08-125426 1er Art 45

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante para (60) días y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha

27 JUN. 2012

Elaborado por: Juan Pablo Ramos Piñeros 
Revisado por: Consuelo Leguizamón Leguizamón 

Synthesis and Biological Activity of Novel Epothilone Aziridines[†]

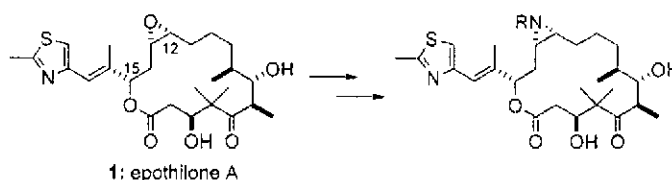
Alicia Regueiro-Ren,^{*,†} Robert M. Borzilleri, Xiaoping Zheng, Soong-Hoon Kim, James A. Johnson, Craig R. Fairchild, Francis Y. F. Lee, Byron H. Long, and Gregory D. Vite

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ABSTRACT



A series of 12 α ,13 α -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 [³H]-paclitaxel binding to microtubules strongly suggests the

existence of a common binding site.³ Several publications³ 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 derivatives⁴ 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.

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(1) (a) Gerth, K.; Bedford, N.; Höfle, G.; Irshick, H.; Reichenbach, H. *J. Antibiot.* **1996**, *49*, 560. (b) Höfle, G.; Bedford, N.; Steinmetz, H.; Schoenburg, 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.; Knopel, 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.; Bollback, 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.; Woolfey, 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.

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

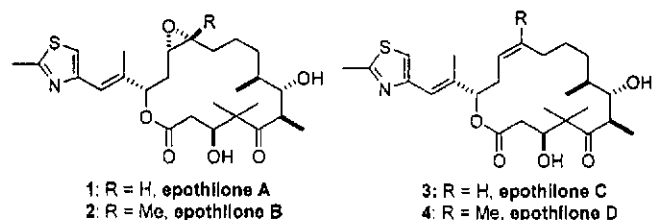


Figure 1. Epothilones A–D.

out to synthesize 12 α ,13 α -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 12 α ,13 α -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

(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.; Winsinger, N.; Pastor, J.; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Yang, Z.; Li, T.; Giannakakou, P.; Hamel, F. *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.; Horowitz, S. B. *Tetrahedron Lett.* 1997, 38, 4529. (e) Nicolaou, K. C.; Vourloumis, D.; Li, T.; Pastor, J.; Winsinger, N.; He, Y.; Ninkovic, S.; Sarabia, F.; Vallberg, H.; Roschangar, F.; King, P. N.; Finlay, R. M.; Giannakakou, P.; Verdier-Pinard, P.; Hamel, F. *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, 2011. *Angew. Chem.* 1998, 110, 2120.

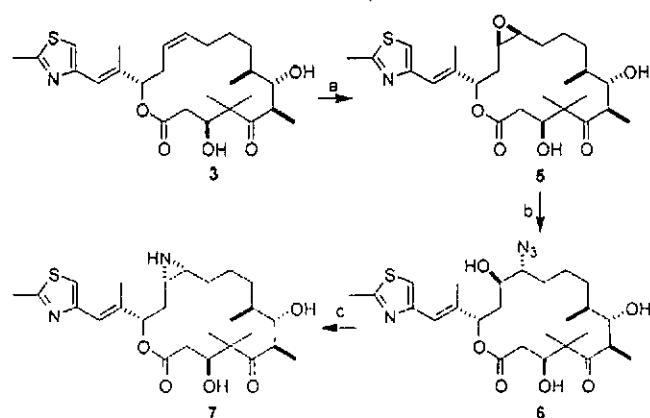
(5) Johnson, J. A.; Kim, S.-H.; Bufano, M.; DiMarco, J.; Fairchild, C.; Gougoulis, J.; Lee, F.; Long, B.; Tokarski, J.; Vite, G. *Org. Lett.* 2000, 2, 1537.

(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.; Min, T.; Regueiro-Ren, A.; Schmidt, R.; Zheng, P.; Lee, F. *Epothilones A and B: Springboards for the synthesis of promising antitumor 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.; Langridge-Smith, P. R. R.; Millar, J. R. A.; Taylor, A. I. *Tetrahedron Lett.* 1994, 35, 9067. Seno, M.; Namba, T.; Kise, H. *J. Org. Chem.* 1978, 43, 3345.

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) CF₃COCH₃, oxone, NaHCO₃, EDTA, CH₃CN, 20%; (b) NaN₃, NH₄Cl, EtOH, 65 °C, 55%; (c) Ph₃P, 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 NaN₃/NH₄Cl in ethanol afforded azido alcohol 6 regioselectively in 55% yield. Triphenylphosphine reduction of the azide moiety of 6 followed by in situ ring closure 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·OEt₂ at low temperature (−20 to −5 °C) afforded predominantly bromohydrin 8, along with minor amounts of the C12 β -Br-C13 α -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

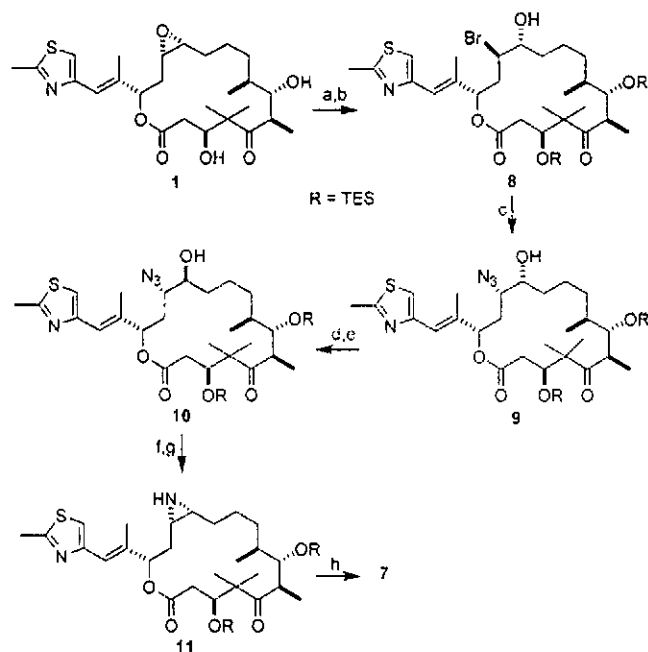
(8) Other reaction conditions attempted: low temperature, solvent variation (THF, CH₂Cl₂, MeOH); and alternative dioxirane source (trifluoroacetone) had no effect on the diastereoselectivity. Other oxidation methods, such as *m*-CPBA and DMSO, generated the γ -oxide and/or the C16–C17 epoxide as side products.

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

(10) Analytical data for aziridine 7: ¹H NMR (400 MHz, CDCl₃) 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), 2.14–2.38 (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); ¹³C NMR (300 MHz, CDCl₃) 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

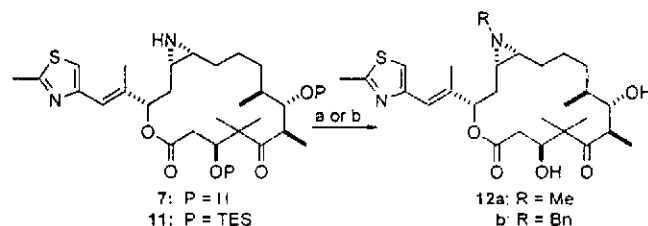


^a (a) TESC1, DIPEA, DMF, 90%; (b) MgBr₂·Et₂O, CH₂Cl₂, -20 to -5 °C, 45% (<2% of the C12β-Br, C13α-OH regioisomer); (c) NaN₃, DMF, 48 h, 42 °C, 60%; (d) *p*-NBA, DEAD, Ph₃P, 99%; (e) NH₃, MeOH, 88%; (f) MsCl, Et₃N, CH₂Cl₂, 98%; (g) Me₃P, THF/H₂O, 98%; (h) 10% TFA/CH₂Cl₂, -10 °C, 90%.

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 CH₂Cl₂ furnished **7** in 90% yield.

The *N*-alkyl aziridiny1 analogues **12a** and **12b** were prepared either from **7** or **11** using methyl sulfate/proton sponge¹⁴ or benzyl bromide/K₂CO₃, 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: Me₂SO₄, proton sponge, THF, 31%. (b) P = TES: BnBr, K₂CO₃, 18-crown-6, THF then 10% TFA in CH₂Cl₂, 66% over two steps.

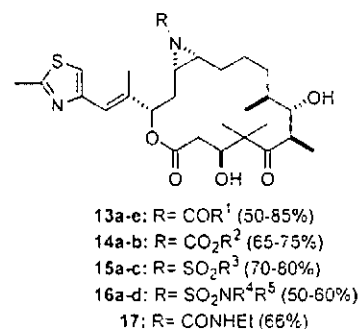


Figure 2. *N*-Acyl (**13a-e**), *N*-carbonyl (**14a,b**), *N*-sulfonyl (**15a-c**), *N*-sulfonylureas (**16a-d**), and *N*-carbamoyl (**17**) aziridine derivatives of epothilone A.

of **12b** was carried out using standard TFA/CH₂Cl₂ 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 ₅₀ (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 ₂ C ₆ H ₅	H	35	23
13a	NCOCH ₂ CH(CH ₃) ₂	H	3.5	41
13b	NCOCH ₂ N(CH ₃) ₂	H	> 1000	
13c	NCOCH ₃	H	1.2	0.9
13d	NCOC ₆ H ₅	H	1.4	3.0
13e	NCO-(2-thiophene)	H	1.2	1.6
14a	NCOOCH ₂ CH ₃	H	1.2	7.7
14b	NCOOC ₆ H ₅	H	1.1	1.8
15a	NSO ₂ CH ₃	H	14	2.1
15b	NSO ₂ CH ₂ CH ₂ CH ₃	H	9.4	94
15c	NSO ₂ -(2-thiophene)	H	4.0	30
16a	NSO ₂ NH ₂	H	28	36
16b	NSO ₂ NHCH ₃	H	2.3	7.8
16c	NSO ₂ N(CH ₃) ₂	H	1.0	0.2
16d	NSO ₂ N(CH ₂ CH ₂) ₂ O	H	460	160
17	NCONHCH ₂ CH ₃	H	> 1000	

^a Assay performed using method described in ref 14a. ^b HTC-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 12 α ,13 α -aziridinyl epothilone A analogues were tested for effects on tubulin polymerization and cytotoxicity. The cytotoxicity of the parent aziridine **7** (IC₅₀ = 2.7 nM) is comparable to that of epothilone A (**1**) (IC₅₀ = 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–c**, *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 12 α ,13 α -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>.

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(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*, 3591.

(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 (EC₅₀) is defined as the interpolated concentration capable of inducing an initial slope of 0.01 OD/min rate and is calculated using the formula EC₅₀ = concentration/slope. EC₅₀ values are expressed as the mean with standard deviation obtained from three different concentrations. (b) Cytotoxicity was assessed in HCT-116 human colon carcinoma cells by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium, inner salt) assay as reported in Riss, T. L.; Moravec, R. A. *Mol. Biol. Cell* **1992**, *3*(Suppl), 184a (abstract 1067).