Chemical Synthesis and Biological Evaluation of cis- and trans-12,13-Cyclopropyl and 12,13-Cyclobutyl Epothilones and Related Pyridine Side Chain Analogues

K. C. Nicolaou,*† Kenji Namoto,‡ Andreas Ritzén,‡ Trond Ulven,‡ Mitsuru Shoji,‡ Jim Li,§ Gina D’Amico,† Dennis Liotta,¶ Christopher T. French,§ Markus Wartmann,∥ Karl-Heinz Altmann,⊥ and Paraskevi Giannakakou¶

Contribution from the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, Department of Chemistry and Biochemistry, University of California, San Diego, 9505 Gilman Drive, La Jolla, California 92093, Chemistry Department, Emory University, 1521 Pierce Drive, Atlanta, Georgia 30322, Winship Cancer Institute, Emory University School of Medicine, 1365-B Clifton Rd., Atlanta, Georgia 30322, and Novartis Pharma AG, TA Oncology Research, CH-4002, Basel, Switzerland

Received May 31, 2001

Abstract: The design, chemical synthesis, and biological evaluation of a series of cyclopropyl and cyclobutyl epothilone analogues (3–12, Figure 1) are described. The synthetic strategies toward these epothilones involved a Nozaki–Hiyama–Kishi coupling to form the C15–C16 carbon–carbon bond, an aldol reaction to construct the C6–C7 carbon–carbon bond, and a Yamaguchi macrolactonization to complete the required skeletal framework. Biological studies with the synthesized compounds led to the identification of epothilone analogues 3, 4, 7, 8, 9, and 11 as potent tubulin polymerization promoters and cytotoxic agents with (12R,13S,15S)-cyclopropyl 5-methylpyridine epothilone A (11) as the most powerful compound whose potencies (e.g. IC_{50} = 0.6 nM against the I.A9 ovarian carcinoma cell line) approach those of epothilone B. These investigations led to a number of important structure–activity relationships, including the conclusion that neither the epoxide nor the stereochemistry at C12 are essential, while the stereochemistry at both C13 and C15 are crucial for biological activity. These studies also confirmed the importance of both the cyclopropyl and 5-methylpyridine moieties in conferring potent and potentially clinically useful biological properties to the epothilone scaffold.

Introduction

With some members in clinical trials, the epothilones command special attention as potential anticancer agents of considerable promise. In addition to the several naturally occurring substances, an impressive array of epothilone analogues have been constructed and biologically evaluated.1,2 In a preliminary communication,3 we reported the chemical synthesis and biological data of 12,13-cyclopropyl and 12,13-cyclobutyl epothilones A (3–6, Figure 1) where the epoxide moiety of epothilone A (1, Figure 1) has been replaced by a small cycloalkane ring. Encouraged by the biological actions of 3 and 4 we have extended these investigations to other members of the 12,13-cycloalkane epothilone family (e.g. compounds 7–12, Figure 1). In this article we report the details of these endeavors including chemical synthesis and biological activities of all analogues shown in Figure 1. Interestingly, these investigations revealed that compounds 3 and 4 exhibit comparable potencies to epothilone A (1) in cytotoxicity studies, supporting the notion that the overall shape of the epothilone scaffold is a most signific-

1 The Scripps Research Institute and University of California, San Diego.
2 Emory University.
3 Winship Cancer Institute, Emory University School of Medicine.
4 Novartis Pharma AG.

design 12,13-cycloalkane thiazole epothilone analogues. The absolute configuration at C15 is R to excellent activity, while all the other analogues, where the absolute configuration varies, are virtually devoid of any cytotoxic activity. The most active analogue emerging from these endeavors was the 12,13-cyclopropyl 5-methylpyridine epothilone A (11), whose impressive cytotoxicity rivals that of epothilone B (2), the most potent naturally occurring epothilone.

Chemical Synthesis

Thiazole Epothilone Analogues. The chemical synthesis of the designed 12,13-cycloalkane thiazole epothilone analogues 3–8 was carried out according to a strategy derived from the retrosynthetic analysis shown in Scheme 1. Thus, a Nozaki–Hiyama–Kishi coupling, an aldol reaction, and a Yamaguchi lactonization were employed to disconnect the three strategic bonds as indicated, revealing building blocks 13–16, 17, and 18. The assembly and elaboration of these building blocks to the final targets was to follow the order shown in

### Scheme 1. Retrosynthetic Analysis and Key Fragments for Epothilone Analogues 3–8

- **Nozaki-Hiyama-Kishi coupling**
- **Aldol reaction**
- **Yamaguchi macro lactonization**

Figure 1. Epothilones prepared in this study.

to excellent activity, while all the other analogues, where the absolute configuration at C15 is R (5, 6, 10, and 12), are virtually devoid of any cytotoxic activity. The most active analogue emerging from these endeavors was the 12,13-cyclopropyl 5-methylpyridine epothilone A (11), whose impressive cytotoxicity rivals that of epothilone B (2), the most potent naturally occurring epothilone.
hydrolysis led to the homologated aldehyde, which reacted with the ylide derived from phosphonium salt 21 to afford olefin 31 in 58% yield for the four steps. Diimide reduction, acetylation, and hydrogenolysis furnished alcohol 32 (98% overall yield). Dess–Martin oxidation then yielded the desired aldehyde 14, which was not isolated, but rather used immediately for the subsequent Nozaki–Hiyama–Kishi coupling (vide infra).

The syntheses of the C12–C13-cyclobutyl aldehydes 15 and 16 were carried out as shown in Scheme 4. As these compounds are very closely related to the cyclopropane derivatives 13 and 14, a similar synthetic route was again followed. Thus, starting from the monoacetate 33, readily available through enzymatic group-selective saponification of the corresponding diacetate,15 cis-aldehyde 34 was prepared by Dess–Martin periodinane oxidation (95% yield), while the corresponding trans-aldehyde 39 was conveniently available by base-catalyzed epimerization of 34 (88% from 33). Following the route described for the cyclopropyl derivatives, 34 and 39 were homologated to 35 and 40, respectively, and the latter compounds were coupled with the chiral phosphane derived from enantiomerically pure phosphonium salt 21 and NaHMDS-TMSCl to yield olefins 36 and 41, respectively. Hydrogenation of the double bond using a platinum catalyst [olefin 36 was initially reduced with diimide because it was done in parallel with 22, where catalytic hydrogenation was not feasible, see discussion of 22 above; because the reduction was incomplete (see Supporting Information), further catalytic hydrogenation with Pt was necessary; it was later found that Pt hydrogenation alone worked for compound 41] followed by standard protecting group manipulations afforded alcohols 38 and 43, which were again homologated and protected, as summarized in Scheme 4, thus producing aldehydes 15 and 16, respectively.

The requisite vinyl iodide 17 was constructed from aldehyde 44 via a sequence involving (i) a modified Corey–Fuchs protocol13 with in situ methylation of the intermediate acetylenide via intermediates 45 (88%) and 46 (97%), (ii) stereoselective hydrostannylation14 (84%), and (iii) iodine–tin exchange (99%), as shown in Scheme 5. This sequence represents a significant improvement, regarding both simplicity and yields, over the preliminary route previously disclosed.13

With all the building blocks in hand, final assembly of epothilone analogues 3–8 could begin. The cyclopropyl analogues 3, 5, and 7 were synthesized as shown in Scheme 6. Aldehyde 13 was coupled with vinyl iodide 17 by the Nozaki–Hiyama–Kishi procedure employing CrCl3·NiCl2,5 furnishing a diastereomeric mixture of alcohols 48 (ca. 1:1 ratio, 56% yield, unoptimized). This mixture was taken through the sequence until chromatographic separation of the two isomers became feasible upon Yamaguchi macrolactonization (vide infra).4 Silylation of 48 (TBSOTf·2,6-lutidine, 100% yield) furnished silyl ether 49, which was deacetylated (DIBAL, 99% yield) to yield the advanced intermediate alcohol 50. In a similar way, trans-aldehyde 14 was coupled with iodide 17, but this time, oxidation (DMP) of the resulting mixture of epimeric secondary alcohols led to ketone 56 in 75% overall yield. Stereoselective reduction of 56 with (--)-DIPCl15 afforded alcohol 57 as a single stereoisomer (by 1H NMR spectroscopy) in 84% yield, thus demonstrating the flexibility of this route to generate either one

Reagents and conditions: (a) (COCl)2 (1.5 equiv) DMSO (2.0 equiv), Et3N (5.0 equiv), CH2Cl2, −78 °C; (b) MeOCH2PPh3Cl, NaHMDS (1.4 equiv), THF, −78 °C; (c) catalytic HCl, acetone:water 9:1, 50 °C, 0.5 h, 88%; (g) 20% Pd(OH)2/C, H2 (1 atm), EtOAc:EtOH 1:1 25 °C, 2 h, 76%; (h) TPAP (0.05 equiv), NMO (1.5 equiv), MS 4 Å, CH2Cl2, 25 °C, 1 h, 89%; (i) MeOCH2PPh3Cl (1.2 equiv), NaHMDS (1.1 equiv), THF, 0 °C, 71%; (j) catalytic HCl, acetone:water 9:1, 55–60 °C, 2 h, 87%. 4-DMAP = 4-(dimethylamino)pyridine, NaHMDS = sodium hexamethyldisilazide, NMO = N-methylmorpholine N-oxide, py = pyridine, TPAP = tetra-n-propylammonium perruthenate.

Reagents and conditions: (a) DME (2.2 equiv), Et3Zn (2.2 equiv), CH2Cl2 (4.4 equiv), 28 (1.2 equiv), CH2Cl2, 98% yield >90% ee; (b) Et3N (6.0 equiv), SO2-py (3.0 equiv), CH2Cl2·DMSO 4:1, 0 °C, 2 h; (c) MeOCH2PPh3Cl (1.5 equiv), NaHMDS (1.3 equiv), THF, −40−25 °C, 12 h, 81% over 2 steps; (d) TBAF (1.5 equiv), THF 25 °C, 2 h; (e) NaH (1.5 equiv), Br2 (2.0 equiv), THF·DMF 5:1, 0−25 °C, 10 h; (f) catalytic HCl, acetone:water 9:1, 50 °C, 5 h; (g) 21 (1.5 equiv), NaHMDS (2.8 equiv), TMSI (1.3 equiv), THF, 58% over 4 steps; (h) (NCO)K2 (20 equiv), HOAc (40 equiv), MeOH, py, 25 °C, 7 h; (i) iAcO (2.0 equiv), Et3N (5.0 equiv), 4-DMAP (0.1 equiv), CH2Cl2, 0 °C, 20 min; (j) 20% Pd(OH)2/C, H2 (1 atm), EtOAc·EtOH 1:1 25 °C, 6 h, 98% over 3 steps; (k) DMP (1.2 equiv), CH2Cl2, 0−25 °C, 40 min. 4-DMAP = 4-(dimethylamino)pyridine, DME = dimethoxyethane, DMP = Dess–Martín periodinane, NaHMDS = sodium hexamethyldisilazide, py = pyridine, TBAF = tetrabutylammonium fluoride, TMSI = chlorotrimethylsilane.


Scheme 3

Reagents and conditions: (a) (COCl)2 (1.5 equiv) DMSO (2.0 equiv), Et3N (5.0 equiv), CH2Cl2, −78 °C; (b) MeOCH2PPh3Cl, NaHMDS (1.4 equiv), THF, −78 °C; (c) catalytic HCl, acetone:water 9:1, 50 °C, 0.5 h, 88%; (g) 20% Pd(OH)2/C, H2 (1 atm), EtOAc·EtOH 1:1 25 °C, 2 h, 76%; (h) TPAP (0.05 equiv), NMO (1.5 equiv), MS 4 Å, CH2Cl2, 25 °C, 1 h, 89%; (i) MeOCH2PPh3Cl (1.2 equiv), NaHMDS (1.1 equiv), THF, 0 °C, 71%; (j) catalytic HCl, acetone:water 9:1, 55–60 °C, 2 h, 87%. 4-DMAP = 4-(dimethylamino)pyridine, NaHMDS = sodium hexamethyldisilazide, NMO = N-methylmorpholine N-oxide, py = pyridine, TPAP = tetra-n-propylammonium perruthenate.

Reagents and conditions: (a) (COCl)2 (1.5 equiv) DMSO (2.0 equiv), Et3N (5.0 equiv), CH2Cl2, −78 °C; (b) MeOCH2PPh3Cl, NaHMDS (1.4 equiv), THF, −78 °C; (c) catalytic HCl, acetone:water 9:1, 85% over 3 steps; (d) 21 (1.5 equiv), n-BuLi (3.0 equiv), THF, −78 °C, 78%. (e) (NCO)K2 (20 equiv), HOAc (40 equiv), MeOH, py, 25 °C, 48 h, 94%; (f) iAcO (1.1 equiv), Et3N (1.2 equiv), 4-DMAP (0.1 equiv), CH2Cl2, 25 °C, 0.5 h, 88%; (g) 20% Pd(OH)2/C, H2 (1 atm), EtOAc·EtOH 1:1 25 °C, 2 h, 76%; (h) TPAP (0.05 equiv), NMO (1.5 equiv), MS 4 Å, CH2Cl2, 25 °C, 1 h, 89%; (i) MeOCH2PPh3Cl (1.2 equiv), NaHMDS (1.1 equiv), THF, 0 °C, 71%; (j) catalytic HCl, acetone:water 9:1, 55–60 °C, 2 h, 87%. 4-DMAP = 4-(dimethylamino)pyridine, NaHMDS = sodium hexamethyldisilazide, NMO = N-methylmorpholine N-oxide, py = pyridine, TPAP = tetra-n-propylammonium perruthenate.

was employed to simultaneously create the C6 ketone yielding. The stage was now set for the stereoselective aldol coupling which of the previously described C1 bond and set the stereochemistry was assumed based on C6 ketone was protected as a TBS ether (57), the chirality of the reducing agent. Compound S (5.1 equiv), CH2Cl2, 25 °C, 2 h, then H2, Pt

to our optimized protocol.6c In this manner, aldols 51 (63%) and 60 (70%) were generated and isolated with complete control of the C6–C7 stereochemistry (as determined by 1H NMR spectroscopy). Protection of the secondary hydroxyl groups as the TBS ethers 52 and 61, followed by a two-step oxidation of the C1 position (liberated selectively by the action of HF-py) and selective cleavage of the C15 TBS ether (TBAF), afforded the hydroxy acids 53 and 62, respectively. Yamaguchi macrolactonization6d of 53 gave a 69% combined yield of the protected epothilone derivatives 54 and 55, which were chromatographically separated [54 (42%); 55 (27%)]. Analogously, macrolactonization of 62 yielded bis-silyl ether 63 (53% from 61 after 5 steps). Desilylation of 54, 55, and 63 with 20% TFA in CH2Cl2 finally afforded the desired epothilone analogues 3, 5, and 7, respectively. The 15S configuration of the trans analogue 7 was now further corroborated by comparison of the 1H NMR spectrum of 7 with those of the cis isomers 3 and 5, where the spectrum of 7 is more similar to that of 3 than to that of 5, particularly considering the signals from the protons attached to C2 and C15 (see Supporting Information).

The cis-cyclobutyl thiazole epothilones 4 and 6 were assembled in an analogous fashion, as summarized in Scheme 5. A Nozaki–Hiyama–Kishi coupling between cis-aldehyde 15 and the side chain vinyl iodide 17 afforded the secondary alcohol 64 (89% yield) as a 1:1 mixture of C15 epimers. Protective group manipulations and oxidation yielded, via intermediates 65 and 66, aldehyde 67, which smoothly underwent the stereoselective aldol coupling reaction with ketone 18, thus producing alcohol 68. Further manipulation of protecting groups and oxidation of the C1 position yielded hydroxy acid 72, which was cyclized by applying the Yamaguchi protocol to afford the two lactones 73 and 74. At this point, the C15 epimers 73 and 74 were chromatographically separated and deprotected to yield the desired cis-cyclobutyl epothilones 4 and 6, respectively, and in good overall yields.

The trans-cyclobutyl thiazole epothilone 8 was prepared by a similar sequence, as detailed in Scheme 5. Thus, after the Nozaki–Hiyama–Kishi coupling between aldehyde 16 and iodide 17, the resulting alcohol was oxidized to the corresponding enone 75, which was then stereo-selectively reduced with (−)-DIPCl15 to afford only the (1S)-epimer 76. The remaining steps followed the same sequence described for the cis compounds (see Scheme 7), and proceeded smoothly and in similar yields, affording the targeted trans-cyclobutyl epothilone 8.

Pyridine Epothilone Analogues. Some of the most active epothilone analogues prepared to date include within their structures a pyridine side chain as a replacement for the thiazole moiety of the naturally occurring substances.16 Given the very promising preliminary results with cyclopropane epothilone analogue 3, we reasoned that combining these two structural modifications might result in highly active compounds despite the absence of the epoxide oxygen. Such compounds (e.g., 9–12, Figure 1) may be metabolically more stable leading to longer in vivo lifetime and lower toxicity. In an effort to improve the

overall synthesis of these compounds, and to accommodate future preparation of other side chain-modified analogues via a convergent
Scheme 8

![Scheme 8](image)

Reagents and conditions: (a) 17 (1.5 equiv), CrCl₂ (12.6 equiv), NiCl₂ (0.13 equiv), DMSO, 25°C, 6 h, 91%; (b) DMP (1.2 equiv), NaHCO₃ (1.5 equiv), CH₂Cl₂, 25°C, 3 h; (c) NaClO₂ (5.0 equiv), 2-methyl-2-butene (7.5 equiv), THF, 25°C, 5 min, 84% for 2 steps; (d) TBSOTf (1.0 equiv), NaH₂PO₄ (2.5 equiv), Et₂O, CH₂Cl₂, 25°C, 2 h; (e) NaClO₂ (5.0 equiv), 2-methyl-2-butene (7.5 equiv), THF, 25°C, 5 min, 84% for 2 steps; (f) DMP (1.2 equiv), NaH₂PO₄ (2.5 equiv), Et₂O, CH₂Cl₂, 25°C, 1.5 h; (g) LDA (3.1 equiv), THF, 25°C, 3 h; then 4-DMAP (1.5 equiv), CrCl₂ (12.6 equiv), Et₃N (6.0 equiv), THF, 0°C, 15 min, 1.8; (h) LDA (3.1 equiv), THF, 0°C, 15 min, 1.8; (i) HF-py, py, THF, 0°C, 25°C, 3 h, 81%; (j) DMP (1.2 equiv), NaHCO₃ (1.5 equiv), CH₂Cl₂, 25°C, 2 h; (k) NaClO₂ (5.0 equiv), 2-methyl-2-butene (7.5 equiv), NaH₂PO₄ (2.5 equiv), 2-BuOH:H₂O 4:1, 25°C, 10 min; (l) TBSOTf (1.0 equiv), CH₂Cl₂, 0°C, 2 h, 79%; (m) Yamaguchi macrolactonization.

strategy, a slightly different scheme for their total synthesis was designed based on the retrosynthetic analysis shown in Scheme 9. The devised strategy for the construction of the pyridine cycloalkane epothilones (9–12) is similar to that utilized for the total synthesis of their thiazole counterparts except for the reversal of the coupling order of the fragments. Thus, the aldol reaction of building blocks 84 and 85 with ketone 18 will now precede the Nozaki–Hiyama–Kishi coupling with vinyl iodide 86.

The required building blocks 84 and 85 were prepared as shown in Scheme 10. A Wittig reaction between the ylide derived from the enantiomerically pure phosphonium salt 21 and NaHMDS-TMSCl and the commercially available aldehyde 87 (68% yield), followed by protection of the resulting alcohol 88 as its TBDPS ether (TBDPSCl-imid.), afforded alkenes 89 in 89% yield. Hydrogenation of the double bond in 89 with concomitant cleavage of the benzyl ether gave primary alcohol 90 in 75% yield. This compound (90) was then converted into the corresponding iodide (91) in 93% yield by exposure to Li/Ph₃P, Coupling of 91 with alkyne 92 (n-BuLi, 72% yield), followed by removal of the TBS group (BF₃-OEt₂) from the resulting alkyn 93, produced the propargylic alcohol 94 (89% yield). This compound was used as a common precursor to prepare both the cis- and the trans-cyclopropyl pyridine epothilone analogues (9–12). The synthesis of the cis series of compounds commenced with a nickel boride reduction of alkyn 94 to furnish cis olefin 95 in 95% yield (Scheme 10), while the corresponding trans alkyn (97) was prepared from the same intermediate (94) via reduction with LiAlH₄(OEt)₃ (83% yield). Charette cyclopropanation of 95 and 97 smoothly afforded the cyclopropanes 96 (99% yield) and 98 (93%) in >95% de, as judged by 1H NMR spectroscopic analysis of the corresponding Mosher esters. Subsequent benzylation of the primary hydroxy group, followed by removal of the silyl group at the other end of the molecule, led to the desired primary alcohols 84 and 85, respectively.

The requisite side chain vinyl iodide 86 was synthesized as shown in Scheme 11. A Sonogashira coupling of 5-methyl-2-bromopyridine 99 with propyne 19 yielded alkyne 100 (98% yield). This was then hydrostannylated, and the tin was exchanged for iodine (86% for two steps) by the same method as that employed to prepare the thiazole side chain precursor 107 (Scheme 5), thus yielding iodide 86 via stannane 101 (100% yield).

The final stages of the synthesis of the targeted pyridine analogues are depicted in Schemes 12 and 13. Oxidation of alcohols 84 and 85 with Dess–Martin periodinane was followed by the stereoselective aldol coupling with ketone 18 previously employed (vide supra). This coupling was performed according to our general procedure, yielding aldols 102 (75% yield) and 108 (89% yield) with a dr of ca. 10:1 (by 1H NMR spectroscopy) in both cases. Further elaboration of these compounds (102 and 108) involved TBS protection of their secondary alcohols, selective removal of the primary TBS group (H₂O-py), oxidation of the resulting primary alcohol (DMP; NaClO₃), and methylation of the so obtained carboxylic acid, leading to compounds

Scheme 9. Retrosynthetic Analysis and Key Fragments for Epothilone Analogs 9–12

![Scheme 9](image)

Hydrogenolysis of the benzyl ether from 104 and 110 was followed by oxidation of the resulting primary alcohols (105 and 111) to the corresponding aldehydes (DMP) and homologation to install the C15 carbon atom, thus yielding aldehydes 107 and 113 via enol ethers 106 and 112, respectively.

104 and 110, as shown in Scheme 12. Hydrogenolysis of the benzylic ether from 104 and 110 was followed by oxidation of the resulting primary alcohols (105 and 111) to the corresponding aldehydes (DMP) and homologation to install the C15 carbon atom, thus yielding aldehydes 107 and 113 via enol ethers 106 and 112, respectively.

The cis-aldehyde 107 was then subjected to the Nozaki–Hiyama–Kishi coupling with vinyl iodide 86 to yield methyl ester 114 (43%, unoptimized), which was hydrolyzed to the corresponding acid (115) in 76% yield (Scheme 13). The ester hydrolysis (114→115) was extremely slow, requiring 4 days for completion. When the same

**Scheme 10**

| Reagents and conditions: (a) NaHMDS (2.1 equiv), TMSCl (1.1 equiv), imidazole (2.0 equiv), DMF, 25 °C, 1 h, 89%; (b) TBDPSCI (1.1 equiv), imidazole (2.0 equiv), DMF, 25 °C, 1 h, 89%; (c) 10% Pd/C, H2 (1 atm), MeOH, 25 °C, 6 h, 98%; (d) PPh3, DMAP, I2, THF (9%); (e) n-BuLi (3.3 equiv), 3-(tert-butyldimethylsilyloxy)propyne (92), THF, -78°C, 2.5 h, 72%; (f) BF3·OEt2, CH2Cl2, 25 °C, 1.5 h, 89%; (g) NiCl2 (1.0 equiv), NaBH4 (1.0 equiv), EDA (3.0 equiv), H2 (1 atm), EtOH, 0 °C, 1 h, 95%; (h) LiAlH4 (1.0 equiv), MeOH (1.0 equiv), THF, 50 °C, 0.5 h, 83%; (i) DME (2.2 equiv), Et2Zn (2.2 equiv), CH2I2 (4.4 equiv), ent-28 (1.2 equiv), CH2Cl2, -15 °C to 25 °C, 6 h, 99% (96), 93% (98); (j) TBAF (5.0 equiv), THF, 25 °C, 4 h, 83% (85), 85% (85) over 2 steps. 4-DMAP = 4-(dimethylamino)pyridine, DME = dimethoxyethane, DMP = Dess-Martin periodinane, EDA = ethylenediamine, HMPA = hexamethylphosphoramide, NaHMDS = sodium hexamethyldisilazide, TBAF = tetrabutylammonium fluoride, TBAI = tetrabutylammonium iodide.

**Scheme 11**

| Reagents and conditions: (a) Pd(PPh3)2Cl2 (0.01 equiv), CuI (0.02 equiv), propyne (1 atm), DMF/i-Pr2NH, 25 °C, 3 h, 98%; (b) n-BuLi (4.0 equiv), (n-Bu3Sn)2 (4.0 equiv), CuCN (2.0 equiv), MeOH (110 equiv), THF, -10 °C, 15 h, 86%; (c) i2 (1.05 equiv), CH2Cl2, 25 °C, 5 min, 100%. 1104 and 110, as shown in Scheme 12. Hydrogenolysis of the benzylic ether from 104 and 110 was followed by oxidation of the resulting primary alcohols (105 and 111) to the corresponding aldehydes (DMP) and homologation to install the C15 carbon atom, thus yielding aldehydes 107 and 113 via enol ethers 106 and 112, respectively. The cis-aldehyde 107 was then subjected to the Nozaki–Hiyama–Kishi coupling with vinyl iodide 86 to yield methyl ester 114 (43%, unoptimized), which was hydrolyzed to the corresponding acid (115) in 76% yield (Scheme 13). The ester hydrolysis (114→115) was extremely slow, requiring 4 days for completion. When the same
Sequence was applied to the trans compound 113. It proved impossible to hydrolyze the corresponding methyl ester after the Nozaki–Hiyama–Kishi coupling. Clearly, another protecting group was needed for the C1 carboxylic acid, and we opted to try a trimethylsilylethyl (TMSE) ester instead of the methyl ester. In the event, the aldehyde 113 was reduced to the hydroxy ester 118 (NaBH₄, 72% yield), which could now be hydrolyzed to the corresponding hydroxy acid and protected (TMSE-OH, EDC, 4-DMAP), affording the TMSE ester 119 in 81% yield. Direct hydrolysis of aldehyde 113 was unsuccessful, which dictated the adoption of the above plan requiring reduction to the alcohol prior to hydrolysis. Reoxidation of 119 with Dess–Martin periodinane gave aldehyde 120 (93% yield), which smoothly underwent the Nozaki–Hiyama–Kishi coupling with 86 to furnish hydroxy ester 121 in 71% yield. Cleavage of the TMSE ester with TBAF now proceeded smoothly, affording hydroxy acid 122.

**Figure 2.** Cyclopropyl epothilones prepared in a previous study.²³

---

Figure 3. In vitro tubulin polymerization. Comparison of the abilities of epothilone A (1), cis-(15S)-CP-py-epo (9), trans-(15S)-CP-py-epo (11), and trans-(15R)-CP-py-epo (12) to induce tubulin polymerization in the absence of microtubule-associated proteins (MAPs). Polymerization reactions with epothilone B (2) and paclitaxel (Taxol) are included for comparison. In each assay 10 μM (1 mg/mL) purified rat brain tubulin in G-PEM buffer (80 mM PIPES, 1 mM GTP, 1 mM EGTA, 1 mM MgCl₂, pH 6.9) was mixed with 3 μM (curve 1) or 10 μM compound and polymerization at 25 °C was followed for 30 min. The optical density at 350 nm (absorbance 350) was then recorded at 15 s intervals for 30 min. Curves 0 are included as controls, showing tubulin polymerization reactions under similar conditions in the absence of the respective compound.
in high yield. Both the cis and trans isomers 115 and 122 were cyclized by using the Yamaguchi protocol (70% yield), after which the C15 epimers were chromatographically separated, yielding compounds 116, 117, 123, and 124. Desilylation of these compounds finally afforded the desired cyclopropyl epothilones 9–12 in excellent yields.

Table 1. Cytotoxicity of Epothilones 1 through 12 and Paclitaxel against 1A9 Human Ovarian Carcinoma Cells and β-Tubulin Mutant Cell Lines Selected with Paclitaxel or Epothilone A

<table>
<thead>
<tr>
<th>compound</th>
<th>1A9 IC₅₀ (nM)</th>
<th>A8 (β274) IC₅₀ (nM)</th>
<th>RR</th>
<th>PTX10 (β270) IC₅₀ (nM)</th>
<th>RR</th>
<th>PTX22 (β364) IC₅₀ (nM)</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>epothilone A (Epo A) 1</td>
<td>2.37 ± 0.433</td>
<td>117 ± 27.01</td>
<td>49.3</td>
<td>23.35 ± 1.85</td>
<td>9.9</td>
<td>5.21 ± 0.344</td>
<td>2.2</td>
</tr>
<tr>
<td>epothilone B (Epo B) 2</td>
<td>0.095 ± 0.007</td>
<td>2.14 ± 0.072</td>
<td>22.5</td>
<td>0.548 ± 0.156</td>
<td>5.8</td>
<td>0.163 ± 0.02</td>
<td>1.7</td>
</tr>
<tr>
<td>paclitaxel (Taxol)</td>
<td>1.77 ± 0.227</td>
<td>17.95 ± 3.08</td>
<td>10.14</td>
<td>52.75 ± 9.4</td>
<td>29.9</td>
<td>28.5 ± 2.75</td>
<td>16.1</td>
</tr>
<tr>
<td>cis-(15S)-CP-epo 3</td>
<td>1.60 ± 0.124</td>
<td>23.43 ± 4.29</td>
<td>14.6</td>
<td>10.9 ± 1.4</td>
<td>6.8</td>
<td>2.6 ± 0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>cis-(15S)-CB-epo 4</td>
<td>8.8 ± 0.00</td>
<td>196 ± 0.00</td>
<td>22.2</td>
<td>62 ± 0.00</td>
<td>7.1</td>
<td>20 ± 0.00</td>
<td>2.3</td>
</tr>
<tr>
<td>cis-(15R)-CP-epo 5</td>
<td>225</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
</tr>
<tr>
<td>cis-(15R)-CB-epo 6</td>
<td>180</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
</tr>
<tr>
<td>trans-(15S)-CP-epo 7</td>
<td>2.7 ± 0.100</td>
<td>48 ± 0.00</td>
<td>17.8</td>
<td>14.4 ± 0.00</td>
<td>5.3</td>
<td>3.7 ± 0.00</td>
<td>1.4</td>
</tr>
<tr>
<td>trans-(15S)-CB-epo 8</td>
<td>25.5 ± 1.50</td>
<td>&gt;300 (inactive)</td>
<td>&gt;11.7</td>
<td>146 ± 0.00</td>
<td>5.7</td>
<td>63 ± 0.00</td>
<td>2.5</td>
</tr>
<tr>
<td>cis-(15S)-CP-py-epo 9</td>
<td>1.40 ± 0.453</td>
<td>53.5 ± 14.57</td>
<td>38.2</td>
<td>8.15 ± 0.565</td>
<td>5.8</td>
<td>1.17 ± 0.94</td>
<td>0.84</td>
</tr>
<tr>
<td>cis-(15R)-CP-py-epo 10</td>
<td>&gt;300 (inactive)</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
</tr>
<tr>
<td>trans-(15S)-CP-py-epo 11</td>
<td>0.625 ± 0.175</td>
<td>9.5</td>
<td>15.2</td>
<td>3.49 ± 0.00</td>
<td>5.6</td>
<td>0.39 ± 0.00</td>
<td>0.63</td>
</tr>
<tr>
<td>trans-(15R)-CP-py-epo 12</td>
<td>&gt;300 (inactive)</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>&gt;300 (inactive)</td>
<td>na</td>
<td>nd</td>
<td>na</td>
</tr>
</tbody>
</table>

The antiproliferative effects of the tested compounds against the parental 1A9 and the paclitaxel- and epothilone-selected drug resistant clones (PTX10, PTX22, and A8, respectively) were assessed in a 72 h growth inhibition assay using the SRB (sulforhodamine-B) assay. IC₅₀ values for each compound are given in nM and represent the mean of 3–5 independent experiments ± standard error of the mean. Relative resistance (RR) is calculated as an IC₅₀ value for each resistant subline divided by that for the parental cell line (1A9). Data from ref 3. CP = cyclopropyl, CB = cyclobutyl, na = not applicable, nd = not determined, py = 5-methylpyridine side chain.

**Chemical Biology**

The biological activities of the synthesized epothilones were evaluated through cytotoxicity and tubulin polymerization assays. Cytotoxicity was first evaluated in a set of ovarian...
carcinoma cell lines, including a parental cell line (lA9) and three drug-resistant cell lines, namely the paclitaxel-resistant cell lines20 lA9/PTX10 and lA9/PTX22 and the epothilone-resistant cell line21 lA9/A8. These resistant cell lines harbor distinct acquired β-tubulin mutations which affect drug–tubulin interaction and result in impaired taxane and epothilone-driven tubulin polymerization. The results of these biological investigations are summarized in Table 1. Further cytotoxicity studies were undertaken using a set of human epidermoid cancer cell lines, including a parent cell line (KB-31) and a paclitaxel-resistant (due to P-gp overexpression).

In agreement with previous reports,3,4 we found that the cyclopropyl epothilone A (3) inhibits slightly more potently the lA9 and KB-31 cell growth than the parent compound epothilone A (1). The 155-cyclobutyl epothilone A (4) retains good activity but is less potent than either 1 or 3. It is noteworthy that the 15R-isomers (5 and 6) of both compounds are inactive at low concentrations against the parental sensitive lA9 and KB-31 cells. Interestingly, even the (12R,13S)-trans-substituted epothilones 7 and 8 showed good activity, again with the cyclopropyl analogue being the most potent. These results are in agreement with our previous report concerning trans-epoxide analogues of epothilones A and B.22 In another study,23 we found that (13R)-cyclopropyl epothilones 125 and 126 (see Figure 2), originally incorrectly assigned as (13S)-diastereomers, were inactive. Thus, we have now prepared and tested all four possible diastereomers of 12,13-cyclopropyl epothilone A, and on the basis of these results, it would appear that while the configuration at C12 has relatively little influence on the cytotoxicity, the 13S configuration is essential.

Remarkably, the trans-cyclopropyl pyridine analogue 11 showed outstanding activity against all of our cell lines, with IC₅₀ = 0.6 nM in the lA9 human ovarian carcinoma cell line. The cis analogue 9 was also highly active, but was a factor of 3 to 5 less active than 11. Again, the 15R isomeric analogues (10 and 12) were inactive.

It is noteworthy that the active compounds (3, 4, 7, 8, 9, and 11) display a similar cytotoxicity profile against the β-tubulin mutants compared to epothilone A (1) (see Table 1). In other words, these compounds lose some activity against the clones PTX10 (β270) and A8 (β274), suggesting that residues 270 and 274 are important for the binding of these analogues to tubulin. However, the most active analogue (11) still retains IC₅₀ < 10 nM for all of these cell lines. Furthermore, we found in the current study, and in agreement with previous reports,3,20,21 that the paclitaxel-selected clone PTX22 (β364) retains sensitivity to the epothilones, especially in the case of the most active analogues (9 and 11) where the relative resistance (RR) values are <1.

The cytotoxicity analysis was supplemented with data from two independent in vitro tubulin polymerization assays. In one assay, the fraction of tubulin polymerized into microtubules upon exposure to a given concentration of the respective compound was determined (see Table 2). In the other assay, tubulin polymerization kinetics upon exposure to the respective compounds was determined by using purified rat brain tubulin through measurement of the absorbance at 350 nm (see Figure 3). For this analysis, paclitaxel, epothilone A (1), and epothilone B (2) were used as controls while compounds 9, 11, and 12 were selected for in vitro analysis. Compound 12 had no in vitro activity consistent with the lack of cytotoxic activity for this compound (Table 1). Compounds 9 and 11 exhibited good in vitro activity although the maximum degree of tubulin polymerization induced by these compounds was smaller compared with that induced by epothilone A (1). However, the increased cytotoxic activity of compounds 9 and 11 relative to epothilone A (1) could potentially be explained by the faster kinetics of polymerization induced by compounds 9 and 11 [time to A₅₃₀ = 0.25 is <1 min for compounds 9 and 11, and 2 min for epothilone A (1)].

Finally, tubulin polymerization products of these compounds were examined by electron microscopy (Figure 4) to rule out the potential increase in absorbance due to the formation of nonmicrotubule polymers. As seen in Figure 4, all compounds tested induced the formation of microtubule polymers with the exception of compound 12 where no microtubules were observed.

**Table 2.** Tubulin Polymerization Potency a and Cytotoxicity b of Epothilones 1 through 12 and Paclitaxel against Human Epidermoid Cancer Cell Lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>% TP</th>
<th>KB-31</th>
<th>KB-8511</th>
</tr>
</thead>
<tbody>
<tr>
<td>epothilone A (Epo A) 1</td>
<td>69</td>
<td>2.15</td>
<td>1.91</td>
</tr>
<tr>
<td>epothilone B (Epo B) 2</td>
<td>90</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>paclitaxel (Taxol) 3</td>
<td>49</td>
<td>2.92</td>
<td>625</td>
</tr>
<tr>
<td>cis-(15S)-CP-epo 4</td>
<td>83</td>
<td>0.838</td>
<td>0.408</td>
</tr>
<tr>
<td>cis-(15S)-CB-epo 5</td>
<td>79</td>
<td>60.7</td>
<td>29.7</td>
</tr>
<tr>
<td>cis-(15R)-CB-epo 6</td>
<td>26</td>
<td>159.5</td>
<td>66.7</td>
</tr>
<tr>
<td>cis-(15S)-CS-epo 7</td>
<td>29</td>
<td>378</td>
<td>156</td>
</tr>
<tr>
<td>cis-(15S)-CP-py-epo 8</td>
<td>100</td>
<td>0.971</td>
<td>0.641</td>
</tr>
<tr>
<td>cis-(15R)-CP-py-epo 9</td>
<td>82</td>
<td>23.1</td>
<td>11.5</td>
</tr>
<tr>
<td>cis-(15S)-CP-py-epo 10</td>
<td>100</td>
<td>0.618</td>
<td>0.446</td>
</tr>
<tr>
<td>cis-(15R)-CP-py-epo 11</td>
<td>&lt;10</td>
<td>930</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>trans-(15S)-CP-epo 12</td>
<td>&lt;10</td>
<td>930</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

a % TP = percent tubulin polymerized after incubation of tubulin with a known concentration of compound (typically 3 µM). b Cytotoxicity toward human cancer cell lines as IC₅₀ values given in nM. KB-31: epidermoid Taxol-sensitive, KB-8511: epidermoid Taxol-resistant (due to P-gp overexpression).
Experimental Section

Full experimental details are provided as Supporting Information.

Acknowledgment. We thank Dr. Robert Apkarian (Emory) for assistance with electron microscopy, and Drs. D. H. Huang and G. Siuzdak (TSRI) for NMR and mass spectrometric assistance, respectively. Financial support for this work was provided by The Skaggs Institute for Chemical Biology, the National Institutes of Health (USA), and CaPCURE, with fellowships from the Naito Foundation, Japan (to M.S.), the STINT Scholar Program (to A.R.), the Norwegian Research Council (NFR) (to T.U.), and grants from Abbott, Amgen, ArrayBiopharma, Boehringer-Ingelheim, Glaxo, Hoffmann-La Roche, DuPont, Merck, Novartis, Pfizer, and Schering Plough.

Supporting Information Available: Full experimental details of all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.