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Synthesis of isotopically labeled epothilones

Thota Ganesh^c, Peggy J. Brodie^a, Abhijit Banerjee^d, Susan Bane^b, and David G. I. Kingston^{a,*}

^aDepartment of Chemistry and the Virginia Tech Center for Drug Discovery, M/C 0212, Virginia Tech, Blacksburg, Virginia 24061, USA

^bDepartment of Chemistry, State University of New York, Binghamton, NY 13902, USA

Abstract

The epothilones, including epothilones B and D, are macrocyclic lactones which have potent cytotoxicities and which promote the polymerization of tubulin to microtubules by binding to and stabilizing the tubulin polymer. They have a very similar mechanism of action to paclitaxel (Taxol®). The determination of the microtubule-binding conformation of the epothilones is an important piece of information in designing improved analogs for possible clinical use, and internuclear distance information that will assist the determination of this conformation can be obtained by REDOR NMR studies of microtubule-bound epothilones with appropriate stable isotope labels. Analogs of epothilone B and epothilone D with [²H₃] and [¹⁹F] labels were prepared from an advanced precursor for potential use in REDOR NMR studies to determine internuclear distances in tubulin-bound ligand.

Keywords

deuterium; epothilones; bioactivity; fluorine; tubulin

Introduction

The epothilones, including epothilones A (1) and B (2), are a class of macrocyclic natural products derived from the myxobacterium *Sorangium cellulosum* strain So ce90.¹ Their excellent antiproliferative activity, coupled with their similar tubulin polymerization activity to paclitaxel (Taxol™),² generated significant interest in their cancer chemotherapeutic potential. Not only do epothilones enhance tubulin assembly into microtubules and stabilize the tubulin polymer much more effectively than paclitaxel, but they are more water-soluble than taxoid drugs, and unlike the taxoid drugs they do not suffer from drug resistance associated with Pgp-over expression.³ As a result numerous epothilone analogs have been synthesized, new ones have been isolated from natural sources,⁴ and structure activity relationships (SAR) have been developed.^{5–9} Notable epothilone analogs that have superior bioactivity to the parent epothilones (Figure 1) are 26-fluoroepothilone B (3),¹⁰ epothilone D (4),¹¹ and 9,10-dehydro-26-trifluoromethylepothilone D (fludelone, 5).¹² The clinical drug ixabepilone (7),^{13–14} developed from epothilone B, has greater metabolic stability than

*Correspondence to: D. G. I. Kingston. dkingston@vt.edu.

^cCurrent address: Department of Pharmacology, Emory School of Medicine, Atlanta, GA 30322, USA.

^dCurrent address: Nalco Water India Ltd, Panchshil, Magarpatta Road, Pune, MH 411028, India

Conflict of Interest

The authors did not report any conflict of interest.

epothilone B and also the commercial advantage of patentability. The bioactivities and clinical development of the epothilones have been reviewed.^{15–16}

Not only do epothilones act as tubulin-polymerization agents in the same way that paclitaxel does, but they also compete for the same binding site on the tubulin polymer and displace [³H]-paclitaxel from it.¹⁷ These findings indicate that paclitaxel and the epothilones share a common binding site, but the activity differences between the compounds suggest that critical aspects of the pharmacophore, and particularly its three-dimensional shape, differ in significant ways.

Knowledge of the molecular conformation of a ligand and of its binding site can assist in the development of analogs with the necessary three-dimensional features to bind to the protein target, but with minimal structural complexity. Although the X-ray structures of epothilones A and B have been published,¹ they do not necessarily define the structure of the molecule in the bound state on tubulin.

Several groups have made proposals for the tubulin-binding conformation of the epothilones. The Taylor group investigated the conformational properties of epothilones by computational and 2D NMR methods. They concluded that epothilones A and B prefer two distinct conformations in the regions C₁-C₈ and C₁₁-C₁₅ respectively,^{18–20} and this work proposed a conformation of epothilone on the tubulin polymer similar to the original X-ray crystal structure conformation.¹ This conformation was supported by a study of the interaction between epothilone A and tubulin by solution NMR,²¹ of epothilone B and tubulin by solid state NMR,²² and finally by an important recent paper reporting the high-resolution crystal structure of αβ-tubulin in complex with epothilone A.²³

The resulting conformation differs significantly from that proposed by the Downing and Snyder groups on the basis of electron crystallography of Zn-stabilized tubulin sheets,²⁴ and this explains in part why conformationally locked epothilones designed based on this model showed either reduced bioactivity^{25–27} or essentially unchanged activity.²⁸

We had elected to approach the question of the microtubule-binding conformation of epothilone and tubulin through a REDOR NMR study, similarly to the previous studies we made for paclitaxel.^{29–30} REDOR (Rotational Echo Double Resonance) NMR is a solid state NMR technique that can be used to solve the biological structures of protein-bound substrates by providing accurate inter-nuclear distances.³¹ It requires the use of labeled analogs of the ligand, using labels such as ²H, ¹⁹F, and ¹³C, and provides information on internuclear distances between the labeled atoms. In the case of epothilone analogs bound to microtubules, it would provide an experimental test of the previously proposed epothilone models.^{18–20} The recent paper describing the structure of epothilone A in complex with αβ-tubulin,²³ published after the synthetic work was complete, lessens the importance of this study, so this study reports the synthesis of the two highly active labeled epothilone analogs **11** and **14**.

26-Fluoroepothilone B (**3**) and 26-fluoroepothilone D (**6**) were found have similar levels of tubulin polymerization activity compared with their parent analogs epothilone B and D respectively,⁹ so their tubulin-binding mode is presumably the same as that of epothilones B and D. 26-Fluoroepothilone B was found to have superior *in vivo* activity against human prostate cancer in nude mice when compared with paclitaxel,⁹ and the CD₃ label at the C-21 position would not be expected to affect this activity in any significant way.

The synthesis of the labeled epothilone D analog **11** was achieved as shown in Scheme 1. The advanced precursor **8**, prepared by a known macrolactonization strategy,^{32–33} was used as the starting material. Stannane **9** was prepared from 2,4-dibromothiazole by the two-step

procedure described previously, substituting trideuteromethyl triflate for the methyl triflate use previously.³³ Stille coupling of the iodotriol **8** and stannane **9** furnished compound **10**, which was subjected to diethylaminosulfur trifluoride (DAST) at low temperature to provide epothilone analog **11** with labels at the C-21 and C-26 positions.

Epothilone B analog **14** was prepared by a similar route (Scheme 1). Epoxidation of lactone **8** by Sharpless asymmetric epoxidation as described previously³² gave the epoxide intermediate **12**, which was coupled with stannane **9** to give compound **13**. Treatment of **13** with DAST yielded analog **14**.

The antiproliferative activities of the labeled epothilone analogs **11** and **14** were determined towards the A2780 ovarian cancer and PC-3 prostate cancer cell lines (Table 1). Gratifyingly, the labeled analog **11** was about 1.8 times more potent than epothilone D, and the analog **14** was about 13 times more potent than epothilone D against the A2780 cell line. The microtubule binding properties of the labeled molecules were also measured. Compound **11** is 1.5 to 2-fold less effective than **14** as an inducer of tubulin assembly, which is consistent with the differences in the binding constants for the two molecules (Table 1).

Experimental

General experimental procedures

Optical rotations were recorded on a Perkin-Elmer 241 Polarimeter. ¹H and ¹³C NMR spectra were obtained on Varian Unity 400 or JEOL Eclipse 500 spectrometers in CDCl₃ at 400 and 100 MHz or at 500 and 125 MHz, respectively. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. High-resolution FAB mass spectra were obtained on a JEOL HX110 Dual Focusing Mass Spectrometer. Reagents and solvents were purchased from commercial sources and were used without further purification. Silica gel column chromatography was performed using flash silica gel (32 – 63 μ m). Preparative thin-layer chromatography (PTLC) separations were carried out on 500 or 1000 μ m thin layer chromatography plates. All reactions were carried out under a nitrogen atmosphere unless otherwise noted.

Compound 8— $[\alpha]_D -32$ °(c 0.2, CHCl₃), literature³² value $[\alpha]_D -32.1$ (c 0.2, CHCl₃)

Synthesis of thiazole 9

- a. To a solution of 2,4-dibromothiazole (0.83 g, 3.42 mmol) in ether (16 mL) was added dropwise *n*-BuLi (1.6 M, 2.3 mL, 3.76 mmol, 1.1 eq) at -78°C for 5 min. After complete addition, the reaction mixture was stirred for 45 min. CD₃SO₂CF₃ (0.77 mL, 6.9 mmol, 2eq) was added and stirred for 45 min. Sat. NaHCO₃ was added to quench the reaction, followed by water, and the mixture was extracted with ether (3 $\times$ 20 mL). The combined organic extracts were dried and evaporated. The resultant crude mixture was purified by chromatography over silica gel, eluting with 2% ether in hexane, to furnish 2-methyl-d₃-4-bromothiazole (230mg, 37% based on recovered starting material). ¹H NMR (400 MHz): δ 6.99 (s, 1H), ¹³C NMR (100 MHz): δ 167.3, 124.2, 116.3, 18.36 (m).
- b. . To a solution of the above compound (200 mg, 1.1 mmol) in ether (8 mL) was added dropwise *n*-BuLi (1.6 M, 0.76 mL, 1.22 mmol, 1.1 eq) at -78°C and the resulting solution was stirred for 15 min at -78°C . Tributyltin chloride (0.357 mL, 1.32 mmol, 1.2 eq) was added and the reaction was stirred for 2 h. Hexane was added and the mixture was filtered through a short plug of silica gel, eluting with 30% ethyl acetate in hexane. Evaporation of the solvent and purification of the resulting crude product on silica gel column, eluting with 3.8% ethyl acetate in

hexane, gave **9** (258 mg, 60% yield). ^1H NMR (400 MHz): δ 7.12 (s, 1H), 1.50 (m, 6H), 1.28 (m, 6H), 1.06 (m, 6H), 0.86 (t, J = 7 Hz, 9H). ^{13}C NMR (100 MHz): δ 166.0, 159.5, 124.9, 28.9, 27.2, 13.6, 10.0. HRFABMS: Calcd for $\text{C}_{16}\text{H}_{29}\text{D}_3\text{NSSn}$ ($M+1$), 393.1466; Found 393.14456 (Δ -5.0).

Synthesis of 10—To a solution of compound **8** (40 mg, 0.074 mmol) and $\text{Pd}(\text{AcCN})_2\text{Cl}_2$ (10 mg) was added a degassed solution of stannane **9** (105 mg, 0.20 mmol, 4.5 eq) in DMF (1 mL) at room temperature, and the resulting solution was stirred for 36 h. The reaction mixture was then filtered through a short plug of silica gel, eluting with ethyl acetate. The ethyl acetate was concentrated and the resulting residue was purified by preparative TLC on silica gel, eluting with 30–50% ethyl acetate in hexane, to provide **10** (26.8 mg, 70%). $[\alpha]_{\text{D}}^{25}$: -71° (c 1.3, CHCl_3). ^1H NMR (500 MHz): δ 6.95 (s, 1H), 6.63 (s, 1H), 5.41 (t, J = 7.3 Hz, 1H), 5.24 (d, J = 8.5 Hz, 1H), 4.29 (d, J = 10.5 Hz, 1H), 4.06 (d, J = 13 Hz, 1H), 4.03 (m, 1H), 4.00 (d, J = 13 Hz, 1H), 3.66 (bs, 1H), 3.14 (d, J = 6.9 Hz, 1H), 3.04 (bs, 1H), 2.66 (m, 1H), 2.44 (dd, J = 14.6, 11.2 Hz, 1H), 2.36 (dd, J = 13, 4.8 Hz, 1H), 2.24 (m, 2H), 2.11 (m, 1H), 2.01 (s, 3H), 1.75 (s, 3H), 1.66 (m, 1H), 1.33 (s, 3H), 1.33–1.30 (m, 1H), 1.09 (d, J = 6 Hz, 3H), 1.03 (s, 3H), 0.98 (d, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz): δ 220.1, 170.4, 165.1, 152.0, 142.0, 138.8, 121.8, 119.2, 115.7, 78.2, 74.2, 71.9, 66.2, 54.0, 41.8, 39.8, 38.2, 31.9, 31.8, 28.0, 25.4, 23.0, 17.7, 16.2, 15.9, 13.5. HRFABMS: Calcd for $\text{C}_{27}\text{H}_{39}\text{D}_3\text{NO}_6\text{S}$ ($M+1$), 511.2921; Found 511.2891 (Δ -5.9).

Synthesis of 11—To the solution of allyl alcohol **10** (16 mg, 0.032 mmol) in dichloromethane (1 mL) was added (diethylamino)sulfur trifluoride (DAST) (4.6 μL , 0.035 mmol, 1.09 eq) and the resulting solution was stirred for 5–10 min. Saturated NaHCO_3 solution (1.5 mL) was added to quench the reaction and the product was extracted with dichloromethane (3×10 mL). The combined dichloromethane solution was dried and concentrated. The resulting residue was purified by preparative TLC on silica gel, eluting with 30% ethyl acetate in hexane to provide **11** (10 mg, 63%). $[\alpha]_{\text{D}}^{25}$: -65° (c 0.4, CHCl_3). ^1H NMR (400 MHz): δ 6.96 (s, 1H), 6.59 (s, 1H), 5.33 (m, 1H), 5.24 (d, J = 8 Hz, 1H), 4.82 (dd, J = 57.4, 10.4 Hz, 1H, CH_2F), 4.68 (dd, J = 57.2, 10.8 Hz, 1H, CH_2F), 4.29 (d, J = 10.4 Hz, 1H), 3.70 (m, 1H), 3.58 (bs, 1H), 3.19 (q, J = 7 Hz, 1H), 3.02 (bs, 1H), 2.69 (m, 1H), 2.45 (dd, J = 14.4, 10.8 Hz, 1H), 2.35 (m, 2H), 2.26 (dd, J = 14.8, 2.8 Hz, 1H), 2.18 (m, 1H), 2.08 (s, 3H), 1.78 (m, 2H), 1.34 (s, 3H), 1.18 (d, J = 6.4 Hz, 3H), 1.06 (s, 3H), 1.0 (d, J = 6.8 Hz, 3H). ^{13}C NMR (125 MHz): δ 220.7, 170.3, 165.6, 151.3, 139.6, 138.2, 126.0, 118.9, 115.7, 86.9, 85.6, 78.2, 73.9, 72.1, 53.7, 41.6, 39.6, 38.0, 32.2, 31.7, 27.7, 25.2, 23.0, 17.7, 16.11, 15.8, 13.2. ^{19}F NMR (376 MHz): δ -79.48 (t, J = 50 Hz). HRFABMS: Calcd for $\text{C}_{27}\text{H}_{38}\text{D}_3\text{NO}_5\text{FS}$ ($M+1$), 513.2878; Found 513.2898 (Δ +3.9 ppm).

Compound 12—Compound **12** was prepared from **8** as reported.³² $[\alpha]_{\text{D}}^{25}$: -30° (c 0.125, CHCl_3), literature value $[\alpha]_{\text{D}}^{25}$: -32° (c 0.3, CHCl_3).

Synthesis of 13—A similar procedure to that described to prepare **10** from **8** was employed to convert **12** to **13** (17.6 mg, 60%). $[\alpha]_{\text{D}}^{25}$: -51.8° (c 0.83, CHCl_3). ^1H NMR (400 MHz): δ 6.97 (s, 1H), 6.60 (s, 1H), 5.42 (dd, J = 7.4, 3.2 Hz, 1H), 4.20 (bs, 2H), 3.75 (dd, J = 12.8, 3.2 Hz, 2H), 3.55 (dd, J = 12.2, 6.4 Hz, 1H), 3.32 (m, 1H), 3.14 (dd, J = 7, 4.8 Hz, 1H), 2.64 (bs, 1H), 2.54 (dd, J = 14, 9.6 Hz, 1H), 2.36 (dd, J = 13, 2.4 Hz, 1H), 2.09 (m, 1H), 2.07 (s, 3H), 2.00–1.98 (m, 2H), 1.86 (m, 1H), 1.70 (m, 1H), 1.42 (m, 3H), 1.35 (s, 3H), 1.16 (d, J = 6.8 Hz, 3H), 1.06 (s, 3H), 1.0 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz): δ 220.3, 170.4, 165.1, 151.5, 137.3, 119.5, 116.0, 77.2, 76.3, 74.2, 72.6, 63.8, 63.4, 57.2, 53.0, 43.0, 39.1, 36.4, 31.3, 30.8, 28.2, 21.9, 21.2, 19.5, 17.1, 15.9, 13.7. HRFABMS: Calcd for $\text{C}_{27}\text{H}_{39}\text{D}_3\text{NO}_7\text{S}$ ($M+1$), 527.2870; Found 527.28711 (Δ +0.2).

Synthesis of 14—A similar procedure to that described to convert **10** to **11** was used to convert **13** to **14** (50%). $[\alpha]_D$: -35° (c 0.04, CHCl_3). ^1H NMR (500 MHz): δ 6.98 (s, 1H), 6.60 (s, 1H), 5.42 (dd, $J = 7.4, 2.75\text{Hz}$, 1H), 4.45 (dd, $J = 47.6, 10.3\text{Hz}$, 1H, CH_2F), 4.20 (dd, $J = 47.6, 10.3\text{Hz}$, 1H, CH_2F), 4.20 (bs, 1H), 4.10 (m, 1H), 3.76 (d, $J = 3.4\text{Hz}$, 2H), 3.29 (dd, $J = 6.6, 4.3\text{Hz}$, 1H), 2.99 (dd, $J = 7, 5\text{Hz}$, 1H), 2.60 (bs, 1H), 2.55 (dd, $J = 13.7, 10.3\text{Hz}$, 1H), 2.36 (dd, $J = 14, 3\text{Hz}$, 1H), 2.12 (m, 1H), 2.09 (s, 3H), 1.96 (m, 2H), 1.73 (m, 1H), 1.61 (bs, 1H), 1.56 (m, 1H), 1.47 (m, 1H), 1.36 (s, 3H), 1.24 (m, 1H), 1.16 (d, $J = 6.6\text{Hz}$, 3H), 1.07 (s, 3H), 0.99 (d, $J = 6.8\text{Hz}$, 3H). ^{13}C NMR (125 MHz): δ 220.6, 170.5, 165.6, 151.6, 137.1, 119.8, 116.2, 85.9, 84.6, 76.3, 73.9, 72.7, 61.7, 57.5, 53.2, 42.8, 39.1, 36.4, 31.3, 31.0, 29.7, 27.4, 21.8, 21.4, 19.5, 17.1, 16.0, 13.5. HRFABMS: Calcd for $\text{C}_{27}\text{H}_{38}\text{D}_3\text{FNO}_6\text{S}$ ($M+1$), 529.2827; Found 529.2799 (Δ -5.3ppm).

Biological data

The affinity of the epothilones for GMPCPP-stabilized microtubules was measured by competition binding with fluorescent paclitaxel.³⁴ Measurements of efficacy of the ligands as promoters of microtubule assembly and of cytotoxicity were performed as previously described.^{34–35}

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- 1** R = H, X = O; Epo A
2 R = Me, X = O; Epo B
3 R = CH₂F, X = O
7 R = Me, X = NH; Ixabepilone

- 4** R = Me; Epo D
5 R = CF₃, 9,10-dehydro; Fludelone
6 R = CH₂F

Figure 1.
Structures of epothilones A, B, and D, and selected synthetic Analogs.

Scheme 1.

Synthesis of fluorine and deuterium labeled epothilones B and D.

Table 1

Bioactivities of the labeled epothilone analogs.

Cmpd	IC ₅₀ , A2780 (μM) ^a	ED ₅₀ , (μM) ^b	Ccr (μM) ^c	Ka (*10 ⁻⁷ , M ⁻¹) ^d
EpoD (4)	0.116 ± 0.005	0.44 ± 0.02	0.66 ± 0.04	19.1 ± 0.4
10	0.412 ± 0.005	ND	ND	ND
13	0.296 ± 0.009	ND	ND	ND
11	0.062 ± 0.01	0.61 ± 0.06	1.49 ± 0.06	11.1 ± 0.3
14	0.0087 ± 0.002	0.46 ± 0.06	0.79 ± 0.03	28.0 ± 1.4

^aIC₅₀ to the A2780 ovarian cancer cell line.^bED₅₀ for tubulin assembly^cCritical concentration for tubulin assembly^dAffinity constant for binding to GMPCPP-microtubules