# A Common Pharmacophore for Taxol and the Epothilones Based on the Biological Activity of a Taxane Molecule Lacking a C-13 Side Chain†

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*Received November 1, 1999; Revised Manuscript Received February 4, 2000*

ABSTRACT: Extensive structure−activity studies done with Taxol have identified the side chain at C-13 as one of the requirements for biological activity. Baccatin III, an analogue of Taxol lacking the C-13 side chain, has none of the biological characteristics of Taxol. Since 2-*m*-azido Taxol, a Taxol derivative with a *m*-azido substituent in the C-2 benzoyl ring, has greater activity than Taxol, we questioned whether 2-*m*-azido baccatin III might be active. 2-*m*-Azido baccatin III inhibited the proliferation of human cancer cells at nanomolar concentrations, blocked cells at mitosis, and reorganized the interphase microtubules into distinct bundles, a typical morphological change induced by Taxol. In contrast to 2-*m*-azido baccatin III, 2-*p*-azido baccatin III was similar to baccatin III, having no Taxol-like activity, further indicating the specificity and significance of the 2-*meta* position substituent. Molecular modeling studies done with the C-2 benzoyl ring of Taxol indicated that it fits into a pocket formed by His227 and Asp224 on $\beta$-tubulin and that the 2-*m*-azido, in contrast to the 2-*p*-azido substituent, is capable of enhancing the interaction between the benzoyl group and the side chain of Asp224. The observation that the C-13 side chain is not an absolute requirement for biological activity in a taxane molecule has enabled the development of a new common pharmacophore model between Taxol and the epothilones.

Taxol is an effective antitumor drug approved for the treatment of breast, ovarian, and lung carcinomas. The drug promotes microtubule assembly in vitro in the absence of GTP, and the microtubules assembled by Taxol are stable to depolymerization by cold or calcium, which readily depolymerize normal microtubules (*1*−*4*). In cells, Taxol treatment results in a reorganization of the microtubule cytoskeleton in interphase cells with the formation of bundles of stable microtubules and a block at the $G_2$/M phase of the cell cycle (*5*, *6*). Low concentrations of Taxol (30−100 nM) suppress microtubule dynamics in Caov-3 and A-498 cells (*7*). Analysis of cell death from Taxol is complex and has led to the idea that at different concentrations of drug, death may result from distinct mechanisms (*8*).

Extensive investigations have been made to unravel the nature of the Taxol−microtubule interaction. It is known that the binding site for Taxol exists in the microtubule polymer and that the drug binds specifically to microtubules with a stoichiometry approaching 1 mol of Taxol per mole of tubulin heterodimer (*9*, *10*). Photoaffinity labeling studies, using either radiolabeled Taxol or Taxol analogues bearing photoreactive groups at the C-2, C-3′, or C-7 positions, identified the $\beta$-tubulin subunit as the site of specific photoincorporation (*11*−*14*). Domain mapping experiments revealed that the C-3′ benzamido group makes contact with the N-terminal 1−31 amino acids (*12*) whereas the C-2 benzoyl group interacts with residues 217−233 of $\beta$-tubulin (*13*). Our most recent studies indicate that 7-(benzoyldihydrocinnamoyl)-Taxol (7-BzDC-Taxol) cross-links to Arg282 in $\beta$-tubulin (*14*).

Structure−activity relationship (SAR) studies have provided insight into the structural determinants that are important for the activity of Taxol. It is generally agreed that the A ring side chain at C-13, the C-2 benzoyl group, and an intact oxetane ring are all essential for both the cytotoxicity and stabilization of microtubules exhibited by Taxol (*15*−*17*). In addition, substituents on the C-2 benzoyl group have profound effects on the biological activity of Taxol. Taxol analogues possessing bulky substituents (e.g., $N_3$, CN, or $OCH_3$) at the *para* position are inactive, whereas the same substituents at the *meta* position result in considerable enhancement of microtubule polymerization compared to Taxol (*18*). 2-*m*-Azido Taxol, particularly, was found to be substantially more active than Taxol (*19*). Based on this information, we questioned whether debenzoyl-2-(*m*-azidobenzoyl)-baccatin III (2-*m*-azido baccatin III) (Figure 1), a Taxol analogue lacking the complete A ring C-13 side chain, but with a *m*-azido benzoyl at the C-2 position of the taxane ring, might be biologically active.

## MATERIALS AND METHODS

*Materials.* Taxol and [$^3$H]Taxol (dissolved in dimethyl sulfoxide and stored at −20 °C) were obtained from the Drug Development Branch, National Cancer Institute. 2-Deben-

† This research was supported in part by U.S. Public Health Service Grants CA77263 and CA83185 (to S.B.H.), CA69571 (to D.G.I.K.), and HD27569 (to G.A.O.), by the Bristol-Myers Squibb U.S. Pharmaceutical Group (to S.B.H.), and by Cancer Core Support Grant CA13330 (to Albert Einstein Comprehensive Cancer Center).

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10.1021/bi992518p CCC: $19.00 
Published on Web 03/18/2000

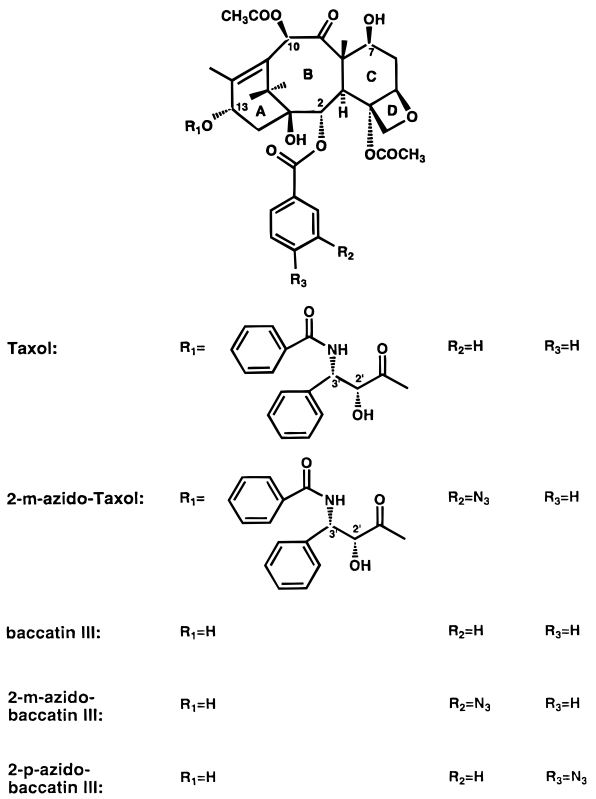


FIGURE 1: Molecular structures of Taxol, 2-*m*-azido Taxol, baccatin III, 2-*m*-azido baccatin III, and 2-*p*-azido baccatin III.

zoyl-2-(*m*-azidobenzoyl)-baccatin III (2-*m*-azido baccatin III) and 2-*p*-azido baccatin III were prepared from baccatin III as follows. Reaction of 10-deacetyl baccatin III with a 3-fold excess of chlorotriethylsilane (TESCl) and imidazole at room temperature for 1.5 h gave 7-TES-10-deacetyl baccatin III, which was acetylated at C-10 (acetyl chloride and pyridine, 0 °C, 36 h) to give 7-TES baccatin III. Silylation of 7-TES baccatin III (TESCl, imidazole, DMF, rt, 24 h) gave 7,13-di-TES baccatin III (67% overall yield). Removal of the 2-benzoyl group with Red-Al (*20*) afforded 2-debenzoyl-7,-13-di-TES baccatin III (66%), and this was acylated with either *m*- or *p*-azidobenzoic acid in the presence of dicyclohexylcarbodiimide and pyrrolidinopyridine in toluene (*18*, *21*) to give the corresponding 2-aroyl analogues. Deprotection of the 2-aroyl analogues (HF/pyridine, rt, 1.5 h) gave 2-*m*-azido baccatin III (58%) or 2-*p*-azido baccatin III (59%). The baccatin III analogues were chromatographically homogeneous, and had clean $^1$H NMR spectra consistent with their structures.

Microtubule protein (MTP) was prepared from calf brain by two cycles of temperature-dependent assembly−disassembly (*22*) and stored at −70 °C in MES buffer (0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$, pH 6.6) containing 6 M glycerol. The concentration of tubulin in MTP was approximately 85%. GTP and anti-mouse $\beta$-tubulin monoclonal antibody, T-4026, were obtained from Sigma Chemical Co.

*Tubulin Polymerization Assay.* Assembly and disassembly of microtubule protein (MTP) was monitored spectrophotometrically (UVIKON, Research Instruments Int., San Diego, CA) by recording changes in turbidity at 350 nm at 37 °C (*22*, *23*). MTP was diluted to 1 mg/mL in MES buffer containing 3 M glycerol. GTP and the compounds to be evaluated were added to MTP and incubated at 37 °C to follow their effect on microtubule assembly. To study microtubule depolymerization, the water bath temperature was switched from 37 to 4 °C after the assembly reaction had reached equilibrium. Aliquots were taken from each reaction and loaded on 300 mesh EM grids for electron microscopy studies that were made at a magnification of 8.3×, 20×, and 50×.

*Cells and Cytotoxicity Assays.* SKOV3, a human ovarian cancer cell line, SKVLB, a vinblastine-selected resistant cell line derived from SKOV3, A549, a human nonsmall cell lung cancer, and AT12, a Taxol-selected resistant cell line from A549, were used for experiments (*24*). SKOV3 and SKVLB were maintained in alpha-MEM plus ribonucleotides, deoxynucleotides, 15% fetal bovine serum, and 1% penicillin−streptomycin (Gibco Labs) at 37 °C in 5% $CO_2$; 1 $\mu$M vinblastine (Sigma Chemical Co.) was present in SKVLB cell culture medium. A549 and AT12 cells were grown in RPMI 1640 with 10% fetal bovine serum and 1% penicillin−streptomycin (Gibco Labs) at 37 °C in 7% $CO_2$. AT12 was maintained in 12 nM Taxol. SKVLB overexpressed P-glycoprotein, whereas the AT12 cells expressed no detectable P-glycoprotein (*24*). Cells were seeded into either 6-well plates or 96 well-plates at densities of $4 \times 10^4$ cells/well and $1 \times 10^4$ cells/well, respectively, and allowed to attach for 24 h, at which time drugs were added to each well and cells were incubated for an additional 72 h. Cell number was determined either by the MTS-based colorimetric method (Promega) or by counting with a Coulter counter. The $IC_{50}$ value is defined as the concentration of drug that inhibits cell growth by 50%. The results represent the average of three independent experiments done in duplicate.

*Immunofluorescence.* NIH3T3 cells were grown to subconfluency on glass coverslips in 35 mm plastic Petri dishes, and then incubated with drugs for 14 h. Cells were extracted with 0.5% Triton X-100 in microtubule stabilizing buffer (PEM: 100 mM PIPES, 2 mM EGTA, and 2 mM $MgCl_2$, pH 6.8) for 4 min, fixed in 3% formaldehyde in PEM for 40 min, blocked with 20% normal goat serum (NGS) for 30 min, and incubated with 1:100 $\beta$-tubulin monoclonal antibody for 1 h. FITC-conjugated goat anti-mouse IgG (Cappal)(1:200) was used as the secondary antibody. Samples were mounted on glass slides (Fisher) in 30% glycerol in PBS containing $\beta$-phenylenediamine (1 mg/mL) and observed with a Zeiss Axioskop microscope.

*Competition Studies.* Drug competition tests were performed using the method developed by Bollag et al. (*25*). In their paper, the rationale and experimental design for this competition assay were fully discussed. MTP (0.4 mg/mL) was incubated at 37 °C with 0.4 mM GTP and 7.5 nM Taxol for 20 min. One hundred nanomolar [$^3$H]Taxol (specific activity, 19.3 Ci/mmol) and the indicated concentration of the competing compound were added simultaneously to the microtubules. This concentration of tritiated Taxol binds to 2.5% of the available Taxol binding sites. These conditions induce the maximum microtubule assembly and provide an approximate constant number of Taxol binding sites over the range of drug concentrations used in the displacement assay (*25*). After 30 min of incubation at 37 °C, microtubules were collected by ultracentrifugation, and the pellet was washed twice with MES buffer containing 3M glycerol. Radioactivity was determined in a liquid scintillation counter.

*Cell Cycle Analysis.* A549 cells were grown to subconfluency and then incubated with the indicated concentration of drug for 24 h. Cells were trypsinized, collected by centrifugation, resuspended, and fixed in 70% ethanol at 4 °C for 1 h. After centrifugation, cells were washed twice with PBS and resuspended in 1 mL of PBS containing 20 $\mu$g/mL propidium iodide and 5 Kunitz units of DNase-free RNase A. The samples were incubated at 37 °C for 30 min and analyzed using a FACS flow cytometer.

To determine the mitotic index, cells were grown to subconfluency and treated with drug for approximately 15 h. Cytospin slides were prepared, and the samples were fixed and stained with $\beta$-tubulin monoclonal antibody. Cy3-labeled goat anti-mouse IgG (Jackson Immuno Research Co.) was used as the secondary antibody, and DNA was stained with Yo-Pro-1 (Molecular Probes). Samples were observed with a Zeiss Axioskop microscope. For each sample, more than 2000 cells were counted to determine the percentage of cells in mitosis.

## RESULTS

*2-m-Azido Baccatin III Promotes Tubulin Polymerization in the Absence of GTP and Stabilizes Microtubules Against Cold Treatment*. When 10 $\mu$M 2-*m*-azido baccatin III was added to MTP at 37 °C, microtubule assembly was induced in the absence of GTP (Figure 2A, trace 4). The microtubules formed were stable against normal destabilizing conditions, such as cold treatment. 2-*m*-azido Taxol (Figure 2A, trace 6) exhibited stronger activity than Taxol in the assembly assay, consistent with the results previously reported (*18*, *19*). After 60 min of incubation, 10 $\mu$M 2-*m*-azido baccatin III showed ~40−50% of the microtubule polymerizing activity demonstrated by 10 $\mu$M Taxol. Electron microscopy confirmed the formation of microtubules in the presence of 2-*m*-azido baccatin III, with morphology essentially identical to those formed with Taxol (data not shown). This activity is impressive since 10 $\mu$M baccatin III (Figure 2A, trace 1) and 10 $\mu$M 2-*p*-azido baccatin III (Figure 2A, trace 3), both of which lack the C-13 side chain, are essentially inactive in the same assay. The activity of 2-*m*-azido baccatin III is concentration-dependent as shown in Figure 2B. This in vitro tubulin assembly data indicated that 2-*m*-azido baccatin III was binding to microtubules.

*2-m-Azido Baccatin III Is Cytotoxic to Cells*. After observing the Taxol-like activities of 2-*m*-azido baccatin III in vitro, we examined the effect of the compound on cultured cell lines. Four cell lines were used for the experiments: SKOV3 (a human ovarian cancer cell line), SKVLB (a P-glycoprotein-expressing SKOV3-derived cell line maintained in 1 $\mu$M vinblastine), A549 (a human nonsmall cell lung cancer cell line), and AT12 (a non-P-glycoprotein-expressing A-549-derived cell line maintained in 12 nM Taxol). The results of these experiments are summarized in Table 1. For SKOV3 and A549, the $IC_{50}$ values for 2-*m*-azido baccatin III were 323 and 68 nM, respectively, while the values for Taxol were 7.5 and 2.7 nM, respectively. The data indicate that like Taxol, 2-*m*-azido baccatin III inhibits the proliferation of cultured cells. The cytotoxicity of 2-*m*-azido baccatin III is approximately 25−45-fold less than that of Taxol. These data are consistent with the in vitro microtubule polymerization activity of 2-*m*-azido baccatin III.

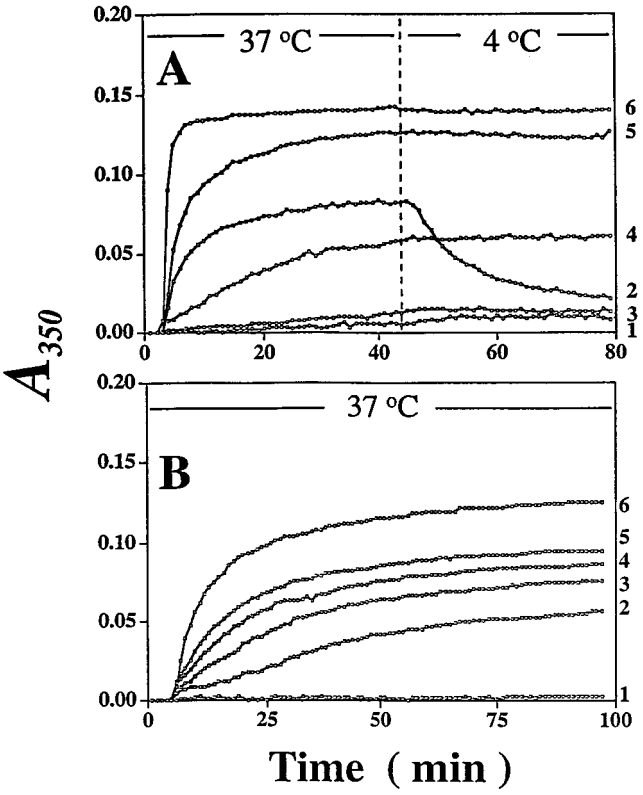


FIGURE 2: Effect of 2-*m*-azido baccatin III on in vitro microtubule assembly. MTP was diluted to 1 mg/mL in MES buffer containing 3 M glycerol. GTP and compounds to be evaluated were added to MTP and incubated at 37 °C to follow turbidity changes at 350 nM as an indication of microtubule mass. To study the stabilizing activity of compounds, the temperature in the cuvettes was switched from 37 to 4 °C after the assembly reaction had reached equilibrium. (A) 1, baccatin III, 10 $\mu$M; 2, GTP, 1 mM; 3, 2-*p*-azido baccatin III, 10 $\mu$M; 4, 2-*m*-azido baccatin III, 10 $\mu$M; 5, Taxol, 10 $\mu$M; 6, 2-*m*-azido Taxol, 10 $\mu$M. (B) 1, Control (DMSO); 2, 2-*m*-azido baccatin III, 10 $\mu$M; 3, 2-*m*-azido baccatin III, 20 $\mu$M; 4, 2-*m*-azido baccatin III, 30 $\mu$M; 5, 2-*m*-azido baccatin III, 40 $\mu$M; 6, Taxol, 10 $\mu$M.

Table 1: Cytotoxicity of 2-*m*-Azido Baccatin III in Taxol-Sensitive and -Resistant Cell Lines

| | $IC_{50}$ (nM)[a] | | | |
|---|---|---|---|---|
| compound | SKOV3 | SKVLB | A549 | AT12[b] |
| baccatin III | >1000 | >10000 | >1000 | <10000 |
| 2-*m*-azido baccatin III | 323 | 9700(30)[c] | 68 | 300(4.4) |
| Taxol | 7.5 | 7800(1040) | 2.7 | 22.8(8.4) |
| 2-*m*-azido Taxol | 0.5 | 550(1100) | 0.3 | 0.73(2.4) |

[a] $IC_{50}$, drug concentration that inhibits cell division by 50% after 72 h. [b] Cells were maintained in 12 nM Taxol during cross-resistance experiments. [c] Ratio of $IC_{50}$ for resistant cell lines (SKVLB and AT12) to that for sensitive cell lines (SKOV3 and A549).

Baccatin III showed no obvious inhibitory effects at concentrations up to 1 $\mu$M. It has been reported that 2-*m*-azido Taxol is more potent than Taxol (*18*, *19*). The obvious difference between baccatin III and 2-*m*-azido baccatin III suggested that the *m*-azido substitution on the C-2 benzoyl ring has an enhancing effect on biological activity. It is of interest to note that 2-*m*-azido baccatin III demonstrated considerably less cross-resistance to SKVLB than Taxol or 2-*m*-azido Taxol, suggesting that it is a poorer substrate for P-glycoprotein. The latter would be compatible with the reduced hydrophobicity of 2-*m*-azido baccatin III compared

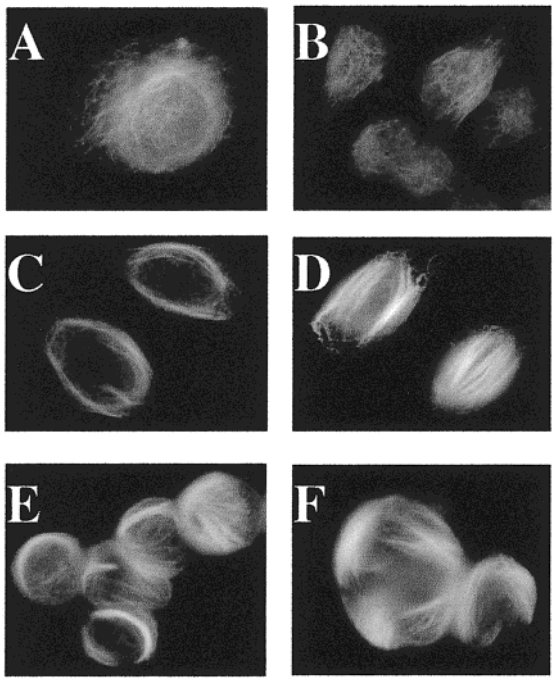


FIGURE 3: 2-*m*-Azido baccatin III induces microtubule bundle formation in cultured cells. NIH3T3 cells were treated with drugs for 14 h and then prepared for immunofluorescence. Microtubules were stained with anti-mouse $\beta$-tubulin and FITC-conjugated goat anti-mouse IgG. (A) Control (DMSO); (B) baccatin III, 10 $\mu$M; (C) 2-*m*-azido baccatin III, 1 $\mu$M; (D) 2-*m*-azido baccatin III, 10 $\mu$M; (E) Taxol, 1 $\mu$M; (F) 2-*m*-azido Taxol, 1 $\mu$M.

to Taxol. In the Taxol-resistant AT12 cell line that does not express P-glycoprotein, the resistance of all three compounds was similar.

*2-m-Azido Baccatin III Induces Microtubule Bundle Formation in Cultured Cells*. Microtubule bundle formation is an identifying feature of Taxol-treated cells. To confirm that 2-*m*-azido baccatin III possessed the same mechanism of action as Taxol in cells, the morphological changes that occurred in NIH3T3 cells incubated with 2-*m*-azido baccatin III were analyzed. As shown in Figure 3, exposure of the cells to 10 $\mu$M 2-*m*-azido baccatin III (Figure 3D) resulted in the formation of extensive microtubule bundles, which were similar to the changes induced by Taxol (Figure 3E) and 2-*m*-azido Taxol (Figure 3F). In contrast, baccatin III (Figure 3B) at 10 $\mu$M did not alter the intracellular microtubule network. This suggested that 2-*m*-azido baccatin III has the same mode of action as Taxol, i.e., binding to cellular microtubules and disrupting normal microtubule organization.

*2-m-Azido Baccatin III Arrests Cells at Mitosis and Increases the Mitotic Index of the Cell Population.* The disruption of normal microtubule function by Taxol, resulting in cell cycle arrest at the mitotic phase of the cycle, is well accepted. In FACS analysis studies, A549 cells treated with 5 $\mu$M 2-*m*-azido baccatin III for 15 h were arrested at mitosis (Figure 4). A549 cells, treated with either 5 $\mu$M baccatin III or 5 $\mu$M 2-*p*-azido baccatin III, could not be differentiated from control cells. The determination of the mitotic index (MI) in NIH3T3 cells incubated with 2-*m*-azido baccatin III further confirmed that this compound blocked the cells in mitosis (Table 2). MI is defined as the percentage of mitotic cells in the total cell population. In the presence of

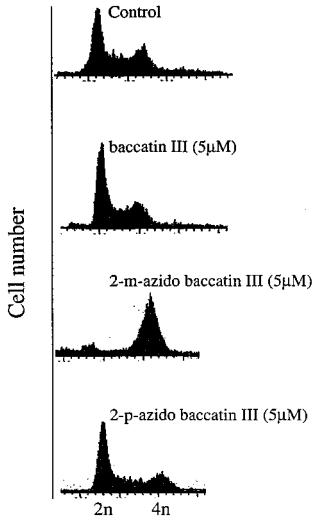


FIGURE 4: 2-*m*-Azido baccatin III arrests cells at the $G_2$/M phase of the cell cycle. A549 cells were exposed to drugs for 24 h prior to fixation and propidium iodide staining. Samples were analyzed with a FACS flow cytometer after the addition of a 5 $\mu$M sample of either baccatin III, 2-*m*-azido baccatin III, 2-*p*-azido baccatin III, or DMSO.

Table 2: Mitotic Index of Cells Exposed to 2-*m*-azido Baccatin III[a]

| conditions | no. of mitotic cells/ total no. of cells counted | mitotic index (%) |
|---|---|---|
| control | 96/2382 | 4.03 |
| baccatin III, 10 $\mu$M | 251/2454 | 10.23 |
| 2-*m*-azido Bac, 1 $\mu$M | 338/2370 | 14.26 |
| 2-*m*-azido Bac, 10 $\mu$M | 1827/2288 | 79.85 |
| Taxol, 1 $\mu$M | 1634/2325 | 70.28 |
| 2-*m*-azido Taxol, 1 $\mu$M | 1897/2375 | 79.87 |

[a] NIH3T3 cells were exposed to drugs for 15 h and then prepared as cytospin slides. The cells were stained with $\beta$-tubulin antibody and Cy3-labeled goat anti-mouse IgG. DNA was stained with Yo-Pro-1. Samples were examined with a Zeiss Axioskop microscope, and for each sample, more than 2000 cells were counted.

10 $\mu$M 2-*m*-azido baccatin III, the MI increased from 4% to 80%, suggesting that the majority of cells were blocked at mitosis.

*2-m-Azido Baccatin III Competitively Inhibits the Binding of [$^3$H]Taxol to Microtubules*. Previous studies have indicated that Taxol binds to specific sites on $\beta$-tubulin. To determine whether 2-*m*-azido baccatin III also binds to those Taxol-binding domains, drug competition studies were performed. At 100 $\mu$M, 2-*m*-azido baccatin III inhibited 50% of the binding of [$^3$H]Taxol to microtubules (Figure 5), being 10−15-fold less potent than Taxol. Baccatin III at concentrations up to 200 $\mu$M had no inhibitory effect on the binding of [$^3$H]Taxol to microtubules. These results indicated that 2-*m*-azido baccatin III binds to the same or overlapping sites as Taxol on the microtubule. However, in the absence of the C-13 side chain, the binding affinity is reduced.

## DISCUSSION

We report that 2-*m*-azido baccatin III, a Taxol analogue lacking the C-13 side chain but with a *m*-azido benzoyl group at the C-2 position, possesses all of the activities that are characteristic of Taxol. It promotes microtubule assembly in the absence of GTP, stabilizes microtubules, and competitively inhibits the binding of [$^3$H]Taxol to microtubule

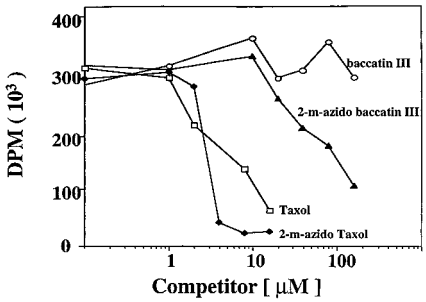


FIGURE 5: 2-*m*-Azido baccatin III competitively inhibits the binding of [$^3$H]Taxol to microtubules. Preformed microtubules (0.4 mg/mL) were incubated with 100 nM [$^3$H]Taxol and competing drug for 30 min at 37 °C. Microtubules were collected by centrifugation, and the radioactivity was determined with a liquid scintillation counter.

protein. The compound is cytotoxic and arrests cells at the mitotic phase of the cell cycle. Although 2-*m*-azido baccatin III is less potent than Taxol, the fact that it is biologically active reveals valuable information on the binding of Taxol to tubulin.

Previous SAR studies of Taxol and a series of analogues have demonstrated that in the absence of the C-13 side chain, baccatin III is an inactive compound (*16*). The removal of the C-2 benzoyl group also results in a loss of biological activity. Taxol analogues with substituents (e.g., $N_3$, CN, or $OCH_3$) at the *meta* position on the C-2 benzoyl group are more active than Taxol. The same substituents at the *para* position lead to a significant decrease in Taxol activity (*18*, *19*).

Photoaffinity labeling studies have disclosed information on the nature of the interactions between Taxol and tubulin. The photoreactive Taxol analogues, 3′-*p*-azido Taxol and 2-*m*-azido Taxol, photolabeled the N-terminal 31 amino acids and amino acid residues 217−233 of $\beta$-tubulin, respectively (*12*, *13*). The 7-benzophenone Taxol analogue photo-cross-linked to Arg282 in $\beta$-tubulin (*14*). A model of the $\alpha,\beta$-tubulin dimer fitted to a 3.7 Å density map has been obtained by Nogales and collaborators utilizing electron crystallography (*26*, *27*). In the model obtained from zinc-induced microtubule sheets, Taxotere was located at one side of $\beta$-tubulin that is believed to be in the microtubule lumen (*27*, *28*). The model places the taxane ring near the M loop and the C-2 benzoyl group near the H6 and the H6−H7 loop (*26*).

Using the coordinates published by Nogales and collaborators, we did molecular modeling studies to obtain information on the binding of 2-*m*-azido baccatin III to tubulin. The 2-*m*-azido baccatin III molecule was docked into $\beta$-tubulin by superimposing its baccatin III ring with the baccatin III core of the Taxotere molecule. This places the C-2 benzoyl ring into a pocket formed by His227 and Asp224 (Figure 6A). The C-2 benzoyl ring is packed against the imidazole ring of His227, and the *m*-azido group on the C-2 benzoyl ring extends over Asp224. This model is consistent with photoaffinity labeling data since both amino acids are part of the peptide labeled by [$^3$H]-2-*m*-azido Taxol. We suggest that the addition of an azido group at the *meta* position enhances the interaction between the C-2 ring of baccatin III and Asp224, therefore increasing the overall binding affinity of the taxane ring for $\beta$-tubulin. Asp224 is the most conserved residue of the Taxol binding domain in

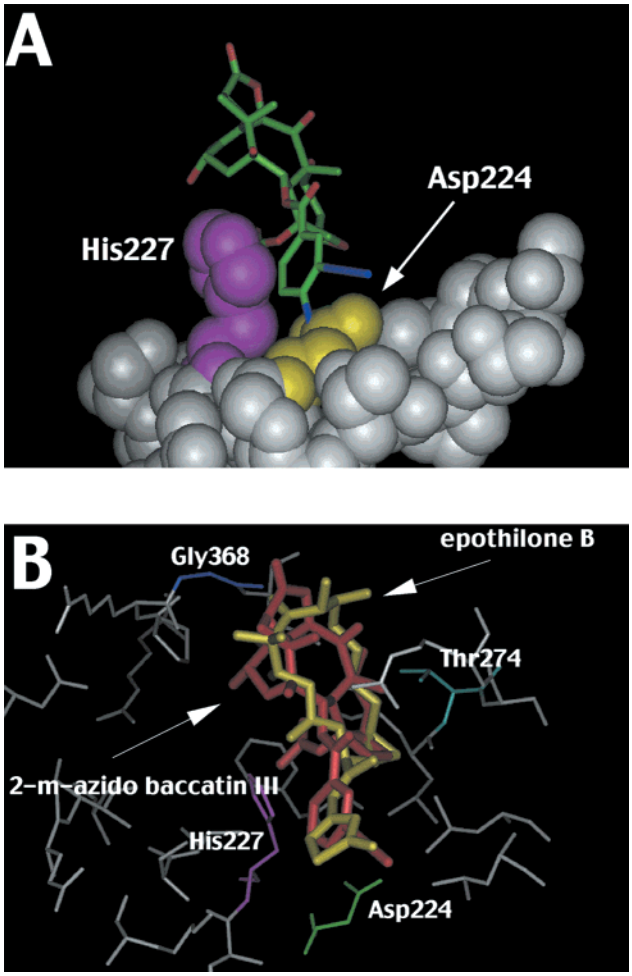


FIGURE 6: Molecular modeling studies indicate that His227 and Asp224 of $\beta$-tubulin are the key amino acid residues involved in the binding of the C-2 benzoyl side chain of Taxol to $\beta$-tubulin. Molecular modeling work was done with the Insight II (v.97.0) software (Molecular Simulations Inc.). Tubulin structure was taken from Nogales et al. (PDB file code: 1TUB) (*26*). The baccatin III molecule carrying both *m*- and *p*-azido groups was built by modifying the Taxotere X-ray structure (*39*) and docking it into the structure of $\beta$-tubulin through superimposing its baccatin III core with that of the Taxotere molecule in the tubulin structure. Amino acid residues 217−233 of $\beta$-tubulin are in white, and His227 and Asp224 are highlighted to show their proximity to the C-2 benzoyl ring (A). The coordinates for epothilone B were retrieved from the Cambridge Structural Database (Refcode: TIPFON). Epothilone B and the 2-*m*-azido baccatin III molecules were superimposed to show their structural similarities (B).

$\beta$-tubulin (*28*). Due to steric hindrance, an azido group at the *para* position on the C-2 benzoyl ring would not fit into the binding domain formed by His227 and Asp224. To strengthen this binding model, we synthesized 2-*p*-azido baccatin III and found that it was inactive both in the in vitro tubulin polymerization assay and in blocking cell cycle progression. It has been hypothesized that the increased activity of 2-*m*-azido Taxol is due to an enhanced interaction between the substituted C-2 benzoyl group and the C-13 side chain (*19*). It is difficult to explain within this hypothesis why the dramatic change in activity occurred when the azido group was moved from the *meta* position to the *para* position. Photoaffinity-labeled compounds with azido groups on the C-2 benzoyl and C-3′ benzamido rings interacted with distinct and distant peptide fragments on $\beta$-tubulin. We believe that Taxol binds to $\beta$-tubulin with

three major contacts: the taxane ring near the M-loop, the C-2 benzoyl ring in proximity to His227 and Asp224 as suggested by molecular modeling, and the C-13 benzamido group within the N-terminal 1−31 amino acids of $\beta$-tubulin. The binding of the C-2 benzoyl ring and C-13 side chains serve as "anchors" to secure and enhance the binding of the taxane ring. Removal of either side chain results in a loss of microtubule stabilization. In the case of 2-*m*-azido Taxol, the binding of the C-2 side chain led to an increase in activity compared to Taxol. For 2-*m*-azido baccatin III, the presence of the *m*-azido group partially compensated for the loss of the C-13 side chain and increased the binding affinity of the compound. The binding of the C-2 side chain is quite specific since an azido at the *para* position is inactive and actually inhibits the interaction of Taxol with the microtubule. This is understandable since in order to accommodate the *p*-azido to fit into the His227 and Asp224 binding pocket, a repositioning of the taxane ring is required.

Recently, several novel compounds that function like Taxol, such as the epothilones, discodermolide, and eleutherobin, have been discovered. Even though their chemical structures are unique, they are all similar to Taxol in their biological function (*25*, *29*−*33*). Attempts have been made to identify a common pharmacophore of these Taxol-like compounds, particularly between Taxol and the epothilones (*34*, *35*). This has been a difficult task due to the fact that the epothilones have one side chain compared to the two spatially distant C-2 and C-13 side chains of Taxol, both of which were thought to be necessary for activity. A model has been proposed in which the thiazole side chain of the epothilones is superimposed with the C-13 side chain of Taxol (*35*). Our observation that the C-13 side chain is not an absolute requirement for biological activity has important ramifications for the delineation of a common pharmacophore for the epothilones and taxane-based drugs. We propose an alternative model in which the thiazole side chain of the epothilones corresponds to the C-2 side chain of Taxol and the macrolide ring system overlaps with the taxane ring system (Figure 6B). Based on this model, alterations to the thiazole ring system may influence biological activity as has been observed with the 2-benzoyl group of Taxol. In fact, replacement of the thiazole with a phenyl group, shortening the linker between C-15 and the thiazole ring, depletion of the thiazole ring, or bulky substitutents on the thiazole ring all resulted in significant decreases in biological activity (*36*, *37*). Considering this model in the context of the SAR data available on eleutherobin, it has been reported that removal of the C-8 urocanic acid group resulted in a complete loss of activity (*38*). We propose that the 14-member fused ring system in eleutherobin, like the macrolide ring of the epothilones, overlays with the taxane ring of Taxol and that the urocanic acid side chain, like the thiazole side chain of the epothilones, mimics the C-2 side chain of Taxol. These similarities between Taxol, the epothilones and eleutherobin may allow them all to bind at the Taxol binding site in $\beta$-tubulin on the microtubule.

## ACKNOWLEDGMENT

We thank Drs. Steve Almo and Iwao Ojima for helpful discussions and comments.

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BI992518P