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Taxol Photoaffinity Label: 7-(*p*-Azidobenzoyl)taxol Synthesis and Biological Evaluation

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***Abstract**: An efficient semi-synthetic approach utilizing the appropriately functionalized (3R,4S)-3-hydroxy-4-phenyl-2-azetidinone (**4**) and 7-(p-azidobenzoyl)baccatin III (**3**) is described which leads to the targeted biologically active taxol photoaffinity label **6**.*

Taxol (**1**), a naturally occurring diterpene isolated from the bark of the Pacific yew,[1,2] exhibits remarkably high anticancer/antitumor activity.[3,4] Now in clinical trials in the United States, it appears to be one of the most exciting antitumor agents recently developed.[4-7] Taxol elicits its biological activity through an unique mechanism of tubulin assembly promotion and subsequent polymer stabilization effects which ultimately leads to cell death.[8] Although these effects are well documented, little is known about the precise mode of binding or topography of the taxol binding site on tubulin.[8,9]

Photoaffinity labels have been utilized extensively in the determination of binding orientations of organic molecules to biologically important receptors.[10] Aryl azides are an important group of photoaffinity labels and are convenient to prepare. Utilizing semi-synthetic methodology developed in our laboratories toward the synthesis of taxol and taxol analogues,[11-13] we report the semi-synthesis of a novel taxol photoaffinity label. Other reports of approaches to[14] and semi-synthesis[15] of taxol photoaffinity labels have appeared recently in the literature. Reports of minor decreases in biological activity of taxol derivatives possessing substituents at the C-7 hydroxyl group[15-17] had prompted us to pursue the synthesis of the 7-(*p*-azidobenzoyl)taxol affinity label.

The aryl azide located at the C-7 hydroxyl group of the molecule should allow, through labeling studies, the characterization of the binding site of taxol in tubulin. These studies may provide information for the elucidation of the precise mechanism of binding of taxol to microtubules.

7-(*p*-Azidobenzoyl)taxol (**6**) was prepared by utilizing both the availability of the naturally occuring taxane, 10-deacetyl baccatin III,[18] and the precedented synthesis of the C-13 phenylisoserine side chain via the ester enolate-imine condensation of (3*R*,4*S*)-3-hydroxy-4-phenyl-2-azetidinone previously reported from our laboratory.[12] 7-(*p*-Azidobenzoyl)baccatin III (**3**) (**Scheme 1**) was prepared by esterification of baccatin III (**2**)[18,19] with *p*-azidobenzoic acid in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP). The DCC/DMAP esterification takes advantage of the relative reactivity of the C-7 hydroxyl group in comparison to the C-13 hydroxyl group and the virtually unreactive C-1 hydroxyl group.[20] The esterification proceeded in 53% yield with no diesterification evident. A diastereomeric mixture of the ethoxyethyl (EE) protected (3*R*,4*S*)-3-hydroxy-4-phenyl-2-azetidinone **4**[11,13] was coupled[21] to baccatin III derivative **3** in the presence of pyridine and DMAP to afford taxol derivative **5** in 55% yield. Quantitative deprotection of the coupled product **5** by acid hydrolysis using 0.5% HCl in EtOH at 0 °C lead to photoaffinity label **6**.[22]

BIOLOGICAL EVALUATION[17]

The ability of the photoaffinity analogue **6** to promote microtubule assembly *in vitro* was compared to that of taxol. The concentration of the analogue which produced 50% stimulation of assembly (ED_{50}) was about 20-fold higher (15 μM) than that of taxol (0.7 μM). The effect of the taxol derivative **6** on the proliferation of B16 melanoma cells in culture was also examined. The ED_{50} value of **6** (concentration which inhibits cell proliferation by 50%) was about 30-fold higher (957 nM) than the ED_{50} value for taxol (31.5 nM). These data contrast with another photoaffinity analogue of taxol, 7-(*p*-(1-azi-2,2,2-trifluoroethyl)benzoyl)taxol,[15] which was found to be about one third as active in stabilizing microtubules against cold-depolymerization. The difference between **6** and 7-(*p*-(1-azi-2,2,2-trifluoroethyl)benzoyl)taxol could be due to a difference in bulkiness and orientation of the groups on the C-7 phenyl ring.

The reduced ability of derivative **6** to induce microtubule assembly and to inhibit melanoma cell proliferation may be explained by the bulkiness of the azidobenzoyl photoaffinity label at the C-7 hydroxyl group, indicating that it may interact with tubulin functional groups of the polypeptide backbone, making it potentially useful for photolabeling studies.

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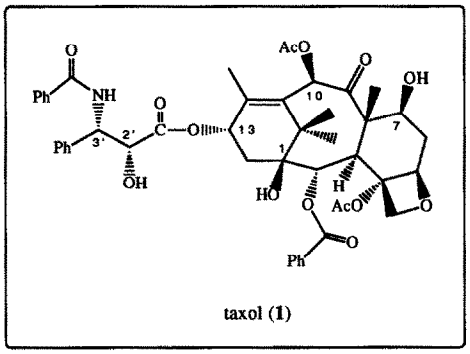
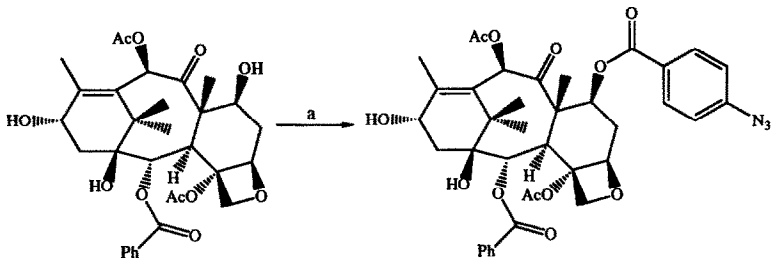
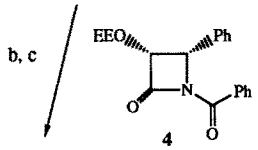


taxol (1)

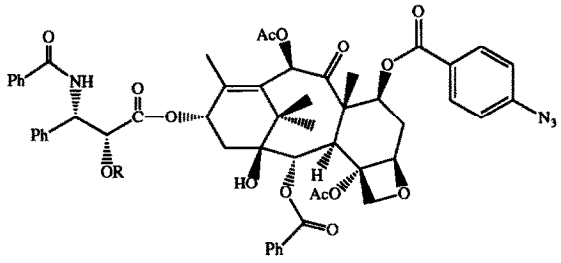
Scheme 1



baccatin III (2) 3



4



5 R = EE
6 R = H

(a) *p*-azidobenzoic acid (1.5 equiv.), DCC (2 equiv.), DMAP (1 equiv.), CH_2Cl_2, 24 h. (b) 4 (5 equiv.), DMAP (1 equiv.), pyridine, 24 h. (c) 0.5% HCl/EtOH, 0 °C, 48 h.

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