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NEW PHOTOAFFINITY ANALOGS OF PACLITAXEL

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Abstract: Two new photoreactive paclitaxel analogs bearing [$^3H_2$]-3-(4-benzoyl)phenylpropanoyl group as the photophore as well as radiolabeling unit at the 7 and 10 positions, respectively, are developed. These new photoreactive analogs showed excellent preliminary results on the photoaffinity labeling of tubulin and P-glycoprotein.

Isolated from the bark of the western yew tree *Taxus brevifolia*, paclitaxel is a powerful therapeutic drug for cancer chemotherapy.[1] It exhibits remarkably high cytotoxicity and strong antitumor activity against different cancers resistant to existing anticancer drugs.[2,3] In late 1992, paclitaxel was approved by the FDA for the treatment of advanced ovarian cancer and, in 1994, for the treatment of breast cancer. It is currently in clinical trials for other types of tumors, with encouraging results.[4]

In contrast to other common anticancer drugs, paclitaxel elicits its biological activity through a rather unique mechanism.[5] By promoting tubulin assembly and stabilizing the microtubules formed, the drug blocks the cell cycle at the mitotic stage, thus inducing cell death. On the molecular level, however, its mechanism of action is still uncertain. In order to discover paclitaxel binding site on microtubules, different techniques have been employed, including electron crystallography,[6] the use of photoaffinity analogs,[7-19] and of fluorescent probes.[20-21] Recent studies utilizing 3'-(4-azidobenzamido)paclitaxel and 2-(3-azidobenzoyl)paclitaxel as photoaffinity analogs have identified the N-terminal 31 amino acids and amino acids 217-231 of β-tubulin, respectively, as two domains of the binding site.[14,18] To further characterize the binding site, new photoaffinity analogs with high specificity have to be developed.

In that respect, we previously reported the synthesis of a paclitaxel analog with 3-(4-benzoylphenyl)propanoyl photoprobe at the 3'-position on the side chain (3'-BzDC-paclitaxel, Figure 1).[22]

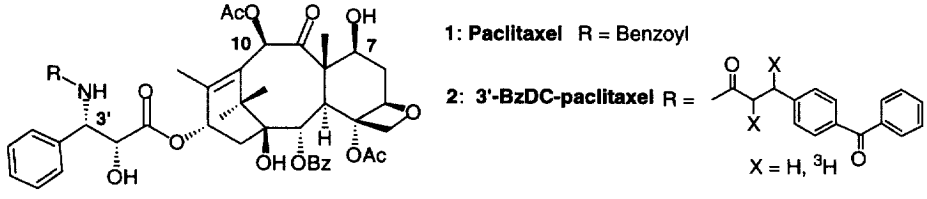

Figure 1. Structures of Paclitaxel (**1**) and 3'-BzDC-Paclitaxel (**2**)

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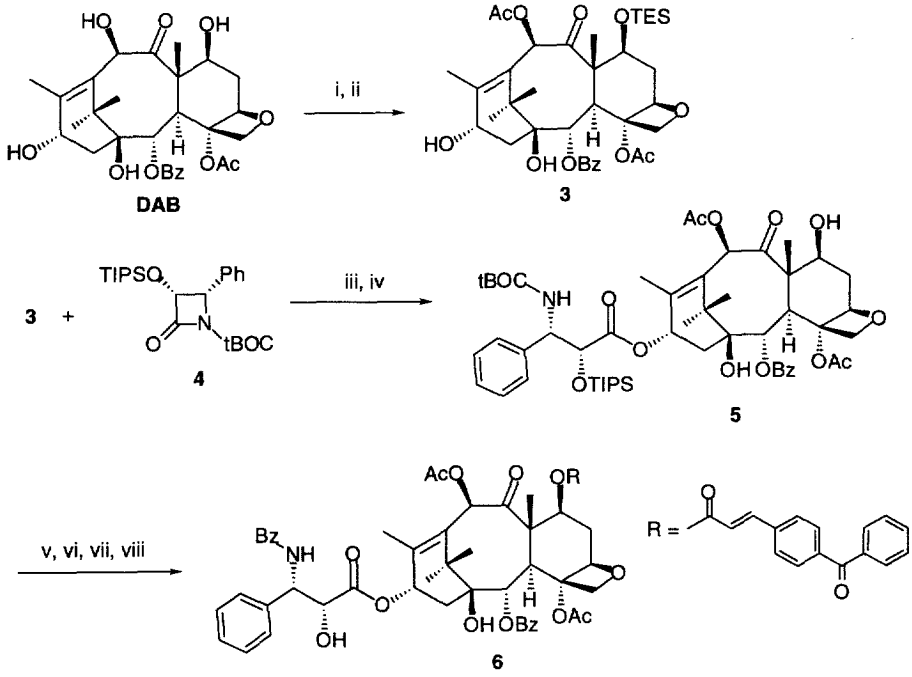
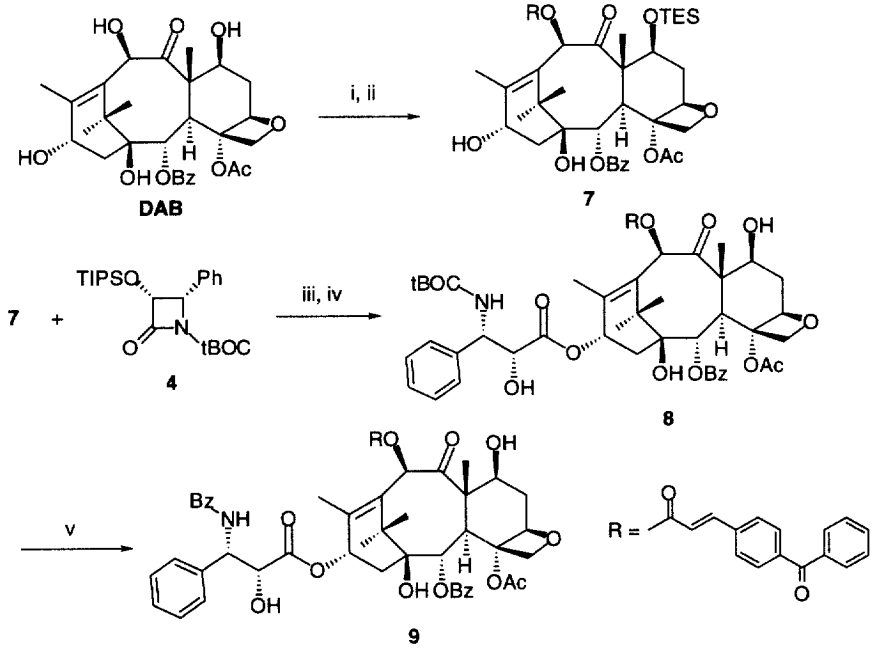
[$^3H_2$]-BzDC-paclitaxel demonstrated excellent photoincorporation into β-tubulin as well as P-glycoprotein, a membrane glycoprotein responsible for the multidrug resistance phenotype in cancer cells. Encouraged by these results, we have synthesized two other photoaffinity analogs with the same benzophenone probe attached at the 7- and 10-positions on the baccatin moiety. By moving the probe around the scaffold, we thus envision to elucidate the three dimensional map of paclitaxel binding site on microtubules and P-glycoprotein.

The methodology applied to the synthesis of the two new photoreactive paclitaxel analogs involves the coupling of an enantiomerically pure β-lactam, *N*-*t*BOC-3-triisopropylsiloxy-4-phenylazetidin-2-one (**4**), with a suitably protected baccatin, following the standard procedure developed in these laboratories.[23] As shown in Scheme 1, the selective protection of the hydroxyl at the 7-position with triethylsilyl chloride and acetylation at the 10-position afforded 7-TES-baccatin **3**. Coupling with β-lactam **4** and selective removal of the 7-TES group with 0.1 *N* HCl provided 10-acetyl-2'-TIPS-docetaxel **5**. The photoprobe was introduced at the 7-position by condensation of the 4-benzoylcinnamic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC.HCl) and DMAP. The TIPS group at the 2'-position was then deprotected with hydrogen fluoride in pyridine/acetonitrile, and the *t*BOC group was removed with trifluoroacetic acid. Finally, benzoylation of the 3'-amino group by a Schotten-Baumann procedure afforded 7-modified paclitaxel analog **6**.[24]

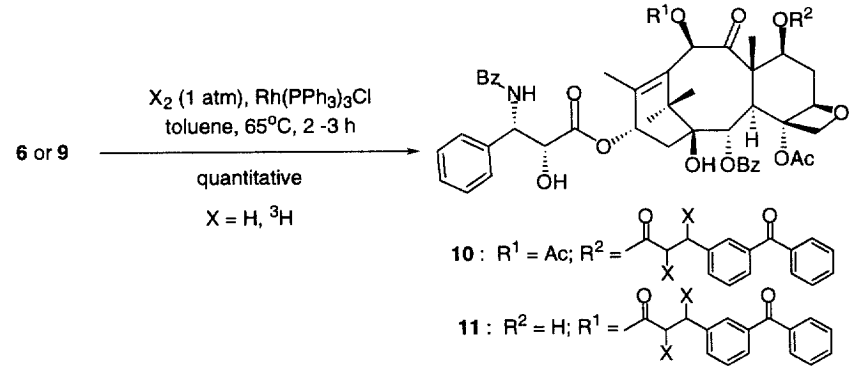


Scheme 1. (i) TESCl, imidazole, DMF, room temperature, 5 h, 81 %; (ii) AcCl, LiHMDS, THF, -40 °C, 30 min, 91 %; (iii) LiHMDS, THF, -40 °C, 30 min, 67 %; (iv) 0.1 N HCl, EtOH, room temperature, 19 h, 67%; (v) RCO_2H, EDC.HCl, DMAP, CH_2Cl_2, room temperature, 1.5 h, 75 %; (vi) HF-Pyridine, pyridine/CH_3CN, 0°C to room temperature, 16 h, 88%; (v) TFA, CH_2Cl_2, 0 °C, 15 min, then BzCl, $NaHCO_3$, EtOAc, room temperature, 30 min, 67%.

The synthesis of the 10-modified analog follows a similar sequence of reactions (Scheme 2). After protecting 7-OH by a TES group, the photoprobe was introduced selectively at the 10 position using an activated ester of 4-benzoylcinnamic acid to give compound **7**. Coupling to β-lactam **4** and removal of silyl protecting groups at 7 and 2' positions with hydrogen fluoride in pyridine/acetonitrile afforded 10-modified docetaxel analog **8**. The 3'-amino group was subsequently deprotected and subjected to benzoylation to give 10-modified paclitaxel analog **9**.[25]



Scheme 2. (i) TESCl, imidazole, DMF, room temperature, 5 h, 81%; (ii) *N*-hydroxyphthalimide ester of RCO_2H, LiHMDS, THF, -40 °C, 30 min, 95%; (iii) LiHMDS, THF, -40 °C, 30 min, 79%; (iv) HF-Pyridine, pyridine/CH_3CN, 0 °C to room temperature, 14 h, 91%; (v) TFA, CH_2Cl_2, 0 °C, 15 min, then BzCl, $NaHCO_3$, EtOAc, room temperature, 30 min, 61%.



Scheme 3. Hydrogenation/tritiation of compounds **6** and **9**.

Both analogs **6** and **9** were hydrogenated in the presence of Wilkinson's catalyst, $RhCl(PPh_3)_3$, to give the desired photoreactive analogs, 7-BzDC-paclitaxel (**10**)[26] and 10-BzDC-paclitaxel (**11**) (Scheme 3).[27] This method offers a significant advantage of incorporating radioactivity at the end of the synthesis by replacing hydrogen gas with tritium gas. The tritiation of **6** (or **9**) was carried out using at 65 °C and ambient pressure of pure $^3H_2$ gas in toluene to give the corresponding radioactive analog [$^3H_2$]-**10** (or [$^3H_2$]-**11**) in nearly quantitative yield. TLC purification of the product provided [$^3H_2$]-**10** and [$^3H_2$]-**11** in a pure form for photoaffinity labeling experiments.[28]

The photoaffinity analogs, **10** and **11**, were evaluated for their cytotoxicity in human tumor cell lines, ovarian carcinoma A121, non-small cell lung carcinoma A549, colon carcinoma HT-29, mammalian breast carcinoma MCF7, and doxorubicin-resistant mammalian breast carcinoma MCF7-R as shown below.[29] 7-Modified analog **10** displayed only slightly lower activity than that of paclitaxel, as expected from previous SAR studies, while several fold weaker activity was observed for 10-modified analog **11**.

Taxoid	A121[a] (ovarian)	A459[a] (NSCL)	HT-29[a] (colon)	MCF7[a] (breast)	MCF7-R[a] (breast)
paclitaxel	6.1	3.6	3.2	1.7	300
10	8.8	13	6.5	2.4	450
11	32.8	40.8	40.8	20.7	1541

[a]The concentration of compound that inhibits 50% (IC_{50}, nM) of the growth of human tumor cell line after 72 h of drug exposure according to the method developed by Skehan et al.[30]

It is worth mentioning that the two analogs were found to behave differently as compared to paclitaxel in the microtubule assembly assay (i.e., these analogs do not promote the polymerization of tubulin to microtubules, like paclitaxel does) but *the analogs do inhibit the disassembly process if microtubules are pre-formed (!)*[31] Preliminary study with tritiated analogs **10** and **11** confirmed their incorporation to the β-tubulin subunit.[31] The results will be published elsewhere.

Photoaffinity labeling experiments were successfully carried out on P-glycoprotein with the two tritiated analogs **2** and **10**.[32] Two different domains of photoincorporation were identified, depending on the locale of the photoprobe on the paclitaxel skeleton.[32] The results together with competitive binding study with cold material strongly imply that there is a specific binding site for paclitaxel on P-glycoprotein.

In summary, two new photoaffinity analogs of paclitaxel have been prepared, along with their tritiated form. Photoaffinity labeling experiments were successfully performed on P-glycoprotein. Photoaffinity labeling experiments with tubulin are currently underway and will be reported elsewhere.

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24. Characterization data for 7-(4-benzoylcinnamoyl)paclitaxel (**6**): White solid; mp 178-181 °C; $[\alpha]^{20}_D$ -16.7 (c 0.18, CH_2Cl_2); IR (KBr disk) 3419, 2955, 1723, 1654, 1508, 1452, 1372, 1261, 1244, 1160, 1071, 1023, 709 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.19 (br s, 6 H), 1.86 (s, 3 H), 1.89 (s, 4 H), 2.07 (s, 3 H), 2.35 (m, 2 H), 2.38 (s, 3 H), 2.70 (m, 1 H), 3.75 (d, *J* = 4.9 Hz, 1 H), 3.97 (d, *J* = 6.8 Hz, 1 H), 4.20 (d, *J* = 8.4 Hz, 1 H), 4.32 (d, *J* = 8.4 Hz, 1 H), 4.80 (m, 1 H), 4.96 (d, *J* = 8.6 Hz, 1 H), 5.67 (m, 2 H), 5.80 (d, *J* = 8.7 Hz, 1 H), 6.18 (t, *J* = 8.1 Hz, 1 H), 6.32 (s, 1 H), 6.45 (d, *J* = 16.0 Hz, 1 H), 7.13 (d, *J* = 9.0 Hz, 1 H), 7.33-7.52 (m, 14 H), 7.59 (m, 4 H), 7.73 (m, 5 H), 8.11 (d, *J* = 7.3 Hz, 2 H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 10.90, 14.59, 20.61, 20.84, 22.53, 26.50, 33.44, 35.58, 43.21, 46.94, 54.90, 56.27, 71.92, 72.11, 73.21, 74.32, 75.10, 78.49, 81.01, 83.89, 120.48, 127.02, 127.05, 127.99, 128.28, 128.34, 128.67, 128.71, 128.94, 129.05, 129.96, 130.15, 130.45, 131.90, 132.58, 133.06, 133.65, 133.76, 137.32, 138.00, 138.33, 138.52, 140.40, 143.35, 165.36, 166.85, 166.98, 168.66, 170.36, 172.41, 195.97, 202.09. Anal. calcd for $C_{63}H_{61}NO_{16}$: C, 69.54; H, 5.65; N, 1.29. Found: C, 69.66; H, 5.55; N, 1.22.
25. Characterization data for 10-(4-benzoylcinnamoyl)-10-deacetylpaclitaxel (**9**): White solid; mp 174-177 °C; $[\alpha]^{20}_D$ -23.5 ° (c 0.59, CH_2Cl_2); IR (neat) 3503, 3010, 2961, 1715, 1651, 1601, 1520, 1487, 1448, 1315, 1272, 1166, 1070, 1025, 986, 842, 799, 710 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 1.21 (s, 3 H), 1.29 (s, 3 H), 1.70 (s, 3 H), 1.83 (s, 3 H), 1.91 (m, 1 H), 2.34 (m, 2 H), 2.39 (s, 3 H), 2.57 (m, 1 H), 2.64 (m, 1 H), 3.66 (m, 1 H), 3.83 (d, *J* = 7.0 Hz, 1 H), 4.20 (d, *J* = 8.4 Hz, 1 H), 4.30 (d, *J* = 8.4 Hz, 1 H), 4.46 (m, 1 H), 4.80 (m, 1 H), 4.96 (d, *J* = 8.3 Hz, 1 H), 5.70 (d, *J* = 6.9 Hz, 1 H), 5.80 (dd, *J* = 8.7, 2.1 Hz, 1 H), 6.25 (t, *J* = 8.8 Hz, 1 H), 6.43 (s, 1 H), 6.67 (d, *J* = 16.0 Hz, 1 H), 7.04 (d, *J* = 8.8

Hz, 1 H), 7.32-7.84 (m, 22 H), 8.13 (d, J = 7.3 Hz, 2 H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 9.58, 14.89, 22.00, 22.62, 26.98, 35.68, 43.19, 45.66, 55.05, 58.66, 72.25, 72.35, 73.17, 74.93, 75.81, 79.00, 81.14, 84.40, 119.15, 127.01, 128.10, 128.39, 128.70, 129.00, 129.09, 129.99, 130.18, 130.56, 131.95, 132.71, 133.10, 133.56, 133.71, 137.18, 137.71, 137.92, 139.03, 142.30, 145.33, 166.35, 166.95, 167.08, 170.38, 172.70, 195.87, 203.51. HRMS calcd for $C_{61}H_{59}NO_{15}Na$ (MNa^+): 1068.3782. Found: 1068.3825.

26. Characterization data for 7-[3-(4-benzoylphenyl)propanoyl]paclitaxel (**10**): White solid; mp 153-157 °C; $[\alpha]^{20}_D$ -50 (c 0.58, CH_2Cl_2); IR (KBr disk) 3434, 2945, 1736, 1655, 16.05, 1509, 1483, 1449, 1372, 1240, 1177, 1066, 1023, 703 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.16 (s, 3 H), 1.20 (s, 3 H), 1.74 (m, 1 H), 1.79 (s, 3 H), 1.83 (s, 3 H), 2.17 (s, 3 H), 2.34 (m, 2 H), 2.37 (s, 3 H), 2.4-2.8 (m, 3 H), 3.02 (m, 2 H), 3.75 (m, 1 H), 3.9 (d, J = 6.7 Hz, 1 H), 4.17 (d, J = 8.3 Hz, 1 H), 4.29 (d, J = 8.3 Hz, 1 H), 4.79 (m, 1 H), 4.91 (d, J = 9.0 Hz, 1 H), 5.57 (m, 1 H), 5.66 (d, J = 6.9 Hz, 1 H), 5.79 (dd, J = 9.1, 2.6 Hz, 1 H), 6.17 (t, J = 8.2 Hz, 1 H), 6.23 (s, 1 H), 7.13 (d, J = 8.9 Hz, 1 H), 7.3-7.6 (m, 16 H), 7.75 (m, 6 H), 8.1 (d, J = 7.2 Hz, 2 H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 10.78, 14.61, 20.78, 22.5, 26.5, 30.39, 33.33, 35.12, 35.54, 43.20, 46.95, 54.90, 56.13, 71.47, 72.06, 73.21, 74.25, 75.28, 76.37, 78.45, 80.98, 83.81, 127.02, 127.05, 128.19, 128.26, 128.66, 128.69, 128.93, 129.02, 129.92, 130.13, 130.35, 131.88, 132.19, 132.90, 133.64, 133.75, 135.48, 137.80, 138.00, 140.37, 145.95, 166.82, 166.95, 169.00, 170.33, 171.71, 172.37, 196.46, 201.84. Anal. calcd for $C_{63}H_{63}NO_{16}$: C, 69.41; H, 5.82; N, 1.28. Found: C, 69.30; H, 5.88; N, 1.13.

27. Characterization data for 10-[3-(4-benzoylphenyl)propanoyl]-10-deacetylpaclitaxel (**11**): White solid; mp 149-152 °C; $[\alpha]^{20}_D$ -39.1 (c 0.69, CH_2Cl_2); IR (KBr disk) 3419, 2934, 1723, 1654, 1608, 1522, 1449, 1373, 1276, 1142, 1070, 1025, 984, 704 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.12 (s, 3 H), 1.21 (s, 3 H), 1.68 (s, 3 H), 1.74 (s, 3 H), 1.87 (m, 1 H), 2.31 (m, 2 H), 2.38 (s, 3 H), 2.38 (s, 3 H), 2.46 (m, 1 H), 2.54 (m, 1 H), 2.89 (m, 2 H), 3.11 (t, J = 7.4 Hz, 2 H), 3.79 (m, 2 H), 4.18 (d, J = 8.3 Hz, 1 H), 4.29 (d, J = 8.3 Hz, 1 H), 4.39 (m, 1 H), 4.79 (m, 1 H), 4.93 (d, J = 7.9 Hz, 1 H), 5.67 (d, J = 7.0 Hz, 1 H), 5.76 (dd, J = 8.7, 2.37 Hz, 1 H), 6.22 (t, J = 9.0 Hz, 1 H), 6.26 (s, 1 H), 7.07 (d, J = 8.8 Hz, 1 H), 7.31-7.63 (m, 16 H), 7.75 (m, 6 H), 8.11 (d, J = 7.3 Hz, 2 H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 9.57, 14.78, 21.95, 22.58, 26.86, 30.73, 35.15, 35.64, 43.12, 45.61, 55.10, 58.56, 72.15, 72.20, 73.19, 74.91, 75.62, 79.0, 81.09, 84.35, 127.0, 127.04, 128.25, 128.67, 129.0, 129.11, 129.99, 130.18, 130.49, 131.95, 132.34, 133.0, 133.61, 133.70, 135.80, 137.66, 137.99, 142.17, 145.08, 166.95, 167.18, 170.33, 172.31, 172.75, 196.48, 203.58. Anal. calcd for $C_{61}H_{61}NO_{15}$: C, 69.90; H, 5.87; N, 1.34. Found: C, 69.79; H, 5.59; N, 1.34.

28. A solution of taxol analog **6** (or **9**) (2.0 mg, 2 μmol) in toluene (2 mL) was added to the reaction vessel containing $RhCl(PPh_3)_3$ (2.0 mg) which had been pre-treated with 100% tritium gas. The mixture was stirred for 3 h at 65 °C and ambient pressure of 100% tritium gas. After removal of excess tritium via a Toepler pump, any labile tritium was exchanged with isopropanol (3 x 3 mL) through co-evaporation of the solvents of the crude product. The crude product was dissolved in hexane-EtOAc (1:1 v/v) (10 mL), assayed (114 mCi, 95%), and purified by TLC on Silica Gel G using hexane-EtOAc (1:1 v/v) as the eluant to give [^{3}H]-**10** (or [^{3}H]-**11**). The specific activity of [^{3}H]-**10** (or [^{3}H]-**11**) is ca. 50 Ci/mmol.

29. The cytotoxicity assay of **10** and **11** were carried about by Dr. Ralph J. Bernacki and Ms. Paula Pera, Department of Experimental therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute.

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31. The tubulin binding assay as well as photoaffinity labeling experiments were performed in the laboratories of Professors Suzan B. Horwitz and George A. Orr, Albert Einstein College of Medicine.

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