Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 6, pp. 615-620, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00083-6

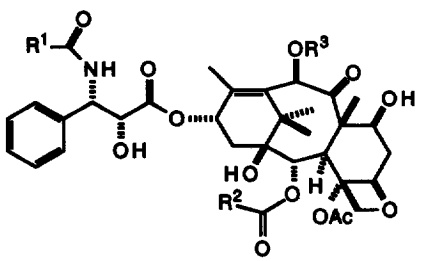
PACLITAXEL AND DOCETAXEL PHOTOAFFINITY LABELS

Gunda I. Georg,* Thomas C. Boge, and Haeil Park
Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045

Richard H. Himes
Department of Biochemistry University of Kansas, Lawrence, KS 66045

Abstract: The synthesis and *in vitro* evaluation of four novel 3-azidobenzoyl photoaffinity analogues of paclitaxel and docetaxel is detailed. Due to their good to excellent ability to stimulate the assembly of microtubules they have potential as photoaffinity probes.

Paclitaxel (**1**)[1] has been recently approved by the FDA for the treatment of cisplatin refractory ovarian cancer[2] and metastatic breast cancer.[3] In addition, reports from Phase II clinical trials suggest that paclitaxel and its semisynthetic analogue docetaxel (**2**) may hold promise for the treatment of other tumors (e.g. non-small cell lung cancer).[4] It is believed that the predominant mechanism of cytotoxicity results from the ability of the antitumor taxanes to promote the assembly of unusually stable microtubules leading to arrested cell division.[5,6] Paclitaxel and docetaxel are unique among the "spindle poisons" in this respect. Other members of this class (e.g. the vinca alkaloids) are inhibitors of microtubule formation.[7]



1 $R^1 = R^2$ = phenyl, R^3 = Ac (paclitaxel)
2 R^1 = *tert*-butoxy, R^2 = phenyl, R^3 = H (docetaxel)
3 R^1 = 3,5-[$^3H_2$]-4-azidophenyl, R^2 = phenyl, R^3 = Ac
4 R^1 = 3-azidophenyl, R^2 = phenyl, R^3 = Ac
5 R^1 = 3-azido-5-nitrophenyl, R^2 = phenyl, R^3 = Ac
6 R^1 = phenyl, R^2 = 3-azidophenyl, R^3 = Ac
7 R^1 = *tert*-butoxy, R^2 = 3-azidophenyl, R^3 = Ac

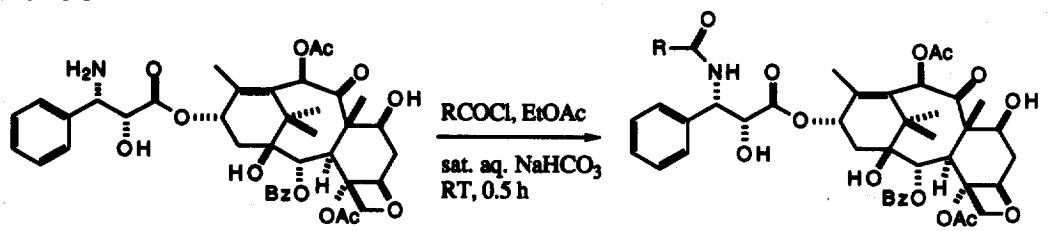
The exact details of the paclitaxel binding site have not yet been fully elucidated. However, paclitaxel does not bind to sites previously identified for colchicine nor the vinca alkaloids.[8] Based on microtubule labeling experiments with [^{3}H]paclitaxel, it was proposed that the paclitaxel binding site is on the β-subunit of the tubulin dimer.[9] Since this work, we[10-12] and others[13-19] have reported efforts to find suitable paclitaxel photoaffinity labels.[20] Successful photolabeling of microtubules was achieved with *N*-(3,5-[$^3H_2$]-4-azidobenzoyl)-*N*-debenzoylpaclitaxel (**3**).[18] Consistent with their previous finding, Horwitz and coworkers disclosed that the covalent microtubule adduct is within 31 amino acids residues from the *N*-terminal of the β-subunit.[18,21] We have also studied photoaffinity label **3**. In our experiments both the α- and β-subunits were labeled, although about 80% of the labeling was found in the β-subunit.[12] Thus, the paclitaxel binding site may overlap the tubulin subunits. Similar results were reported by Combeau *et al.* for the *N*-methyl derivative of **3**.[19]

Low microtubule assembly properties were found for 7-(4-azidobenzoyl)paclitaxel[10] and nonspecific radiolabeling was reported for a 7-diazirin paclitaxel analogue.[16]

This communication describes the identification of potential candidates for microtubule photolabeling experiments which will investigate the initial event of paclitaxel's mechanism of action at the molecular level.

Our success with **3** led us to consider its meta isomer *N*-(3-azidobenzoyl)-*N*-debenzoylpaclitaxel (**4**). We expect that the dissymmetric substitution pattern in **4** will provide finer detail of the tubulin binding pocket for the *N*-benzoyl moiety and possibly increase photolabeling of the α-subunit of tubulin. The synthesis of **4** was achieved in 79% yield by Schotten-Baumann acylation[22] of *N*-debenzoylpaclitaxel (**8**) with 3-azidobenzoyl chloride (Scheme 1).

Scheme 1

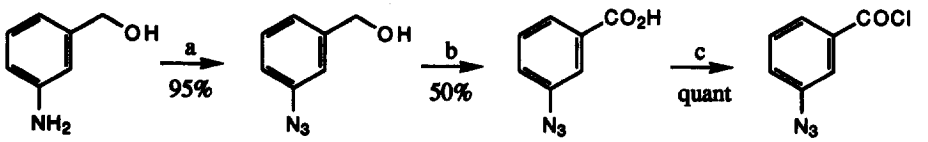


8

4 R = 3-azidophenyl
5 R = 3-azido-5-nitrophenyl

3-Azidobenzoic acid was prepared in three steps from 3-aminobenzyl alcohol (Scheme 2).[23] Standard diazotization of 3-aminobenzyl alcohol with subsequent treatment of the resulting diazonium salt with sodium azide (95%),[24] followed by oxidation of the alcohol with $KMnO_4$ (50%) yielded 3-azidobenzoic acid. 3-Azidobenzoyl chloride was prepared from the acid and thionyl chloride and used without purification for the acylation. The details for the synthesis of **8** were recently reported by us.[12]

Scheme 2

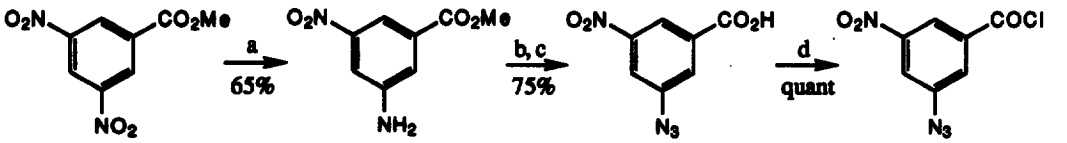


(a) HCl/H_2O, $NaNO_2$, NaN_3, 0 °C; (b) $KMNO_4$,benzene/H_2O, Bu_4NBr, RT, 3 h;
(c) $SOCl_2$, 65 °C, 3 h.

Encouraged by the excellent biological data for **4** (Table), we selected *N*-(3-azido-5-nitrobenzoyl)-*N*-debenzoylpaclitaxel (**5**, Scheme 1) as a target. The presence of the nitro group allows photolysis of the azide to the highly reactive nitrene intermediate at longer wavelength. Photoaffinity label **5** was prepared from **8** and 3-azido-5-nitrobenzoyl chloride in 53% yield under Schotten-Baumann conditions (Scheme 1). 3-Azido-5-nitrobenzoyl chloride was prepared from methyl 3,5-dinitrobenzoate in four steps and in good yields[23] (Scheme

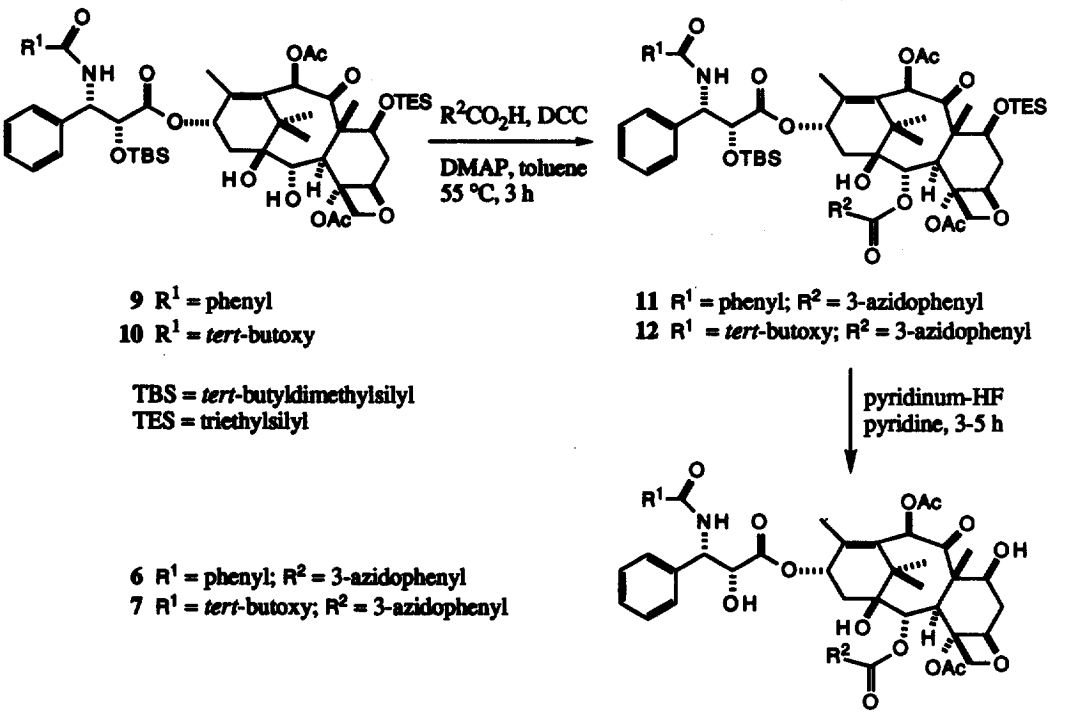
3). Methyl 3,5-dinitrobenzoate was selectively reduced under hydrogen transfer conditions by a known method[25] to yield methyl 3-amino-5-nitrobenzoate. Hydrolysis of the ester (LiOH, THF/H_2O) was performed prior to azide[24] formation to give 3-azido-5-nitrobenzoic acid in 75% yield. 3-Azido-5-nitrobenzoyl chloride was generated from 3-azido-5-nitrobenzoic acid with cyanuric chloride.[26] The crude acid chloride was used in the acylation of **8**. To the best of our knowledge, the synthesis of 3-azido-5-nitrobenzoic acid has not been reported before. Since this derivative is easily available by the route described in Scheme 3 and can be photolysed at relatively long wavelength, it holds promise to be useful in other photoaffinity labeling studies.

Scheme 3



(a) Ref. 25; (b) LiOH, THF/H_2O, reflux, 2 h; (c) HCl/H_2O, $NaNO_2$, NaN_3, 0 °C; (d) cyanuric chloride, Et_3N, acetone, RT, 3 h.

Scheme 4



9 R^1 = phenyl
10 R^1 = *tert*-butoxy

TBS = *tert*-butyldimethylsilyl
TES = triethylsilyl

11 R^1 = phenyl; R^2 = 3-azidophenyl
12 R^1 = *tert*-butoxy; R^2 = 3-azidophenyl

6 R^1 = phenyl; R^2 = 3-azidophenyl
7 R^1 = *tert*-butoxy; R^2 = 3-azidophenyl

Substitution of a photoactivatable group for the 2-benzoyl moiety of paclitaxel and a docetaxel derivative has also been achieved. We have previously reported the two-step preparation of 2-debenzoyl paclitaxel derivative **9** in 70 to 80% yield from paclitaxel and the corresponding docetaxel analogue **10** in 89% yield.[27] Acylation of **9** with excess 3-azidobenzoic acid, dicyclohexylcarbodiimide (DCC) and *N*,*N*-dimethylaminopyridine (DMAP) and subsequent removal of the protecting groups from intermediate **11** provided **6** (78%) (Scheme 4). Photoaffinity label **6** has been prepared before by a very similar route.[17] We have also prepared 2-(3-azidobenzoyl) docetaxel analogue **7** (Scheme 2) from 2-debenzoyl docetaxel derivative **10**.

The photoaffinity analogues were evaluated in a microtubule assembly assay and for their cytotoxicity against B16 melanoma cells (Table). Excellent activity in both assays was observed for **4**, **6** and **7**, all of which contain the 3-azidobenzoyl moiety. The 3-azido-5-nitrobenzoyl derivative **5** was three times less active than paclitaxel in the microtubule assembly assay and about 17 times less cytotoxic against B16 melanoma cells. Interestingly, **6** has been reported to be 150 times more active than paclitaxel in P388 murine leukemia cells,[17] a result we did not observe in our B16 melanoma cytotoxicity assay (Table). To investigate whether the discrepancy between cell line data were due to cell-selectivity or perhaps poor sensitivity in the B16 melanoma assay, we also tested **6** in the P388 cell line. Although **6** was quite cytotoxic to P388 cells ($ED_{50}/ED_{50(paclitaxel)}$ = 0.08), we were unable to corroborate the activity noted previously.[17] However, a twelve-fold activity of **6** against P388 compared to paclitaxel is consistent with the report that **6** was at least ten-fold more active against five other unspecified cancer cell lines.[17] Consistent with an earlier report,[17] we found that **6** promoted assembly of tubulin at low temperatures, a property also shown by paclitaxel but with less efficiency.

Table. *In vitro* biological evaluation of paclitaxel and docetaxel photoaffinity analogues.[28]

compound	microtubule assembly[a] $ED_{50}/ED_{50(paclitaxel)}$	B16 melanoma cytotoxicity[a] $ED_{50}/ED_{50(paclitaxel)}$
1 (paclitaxel)	1.0	1.0
2 (docetaxel)	0.45	0.41
3[11]	2.0	4.6
4	0.81	2.6
5	3.0	17.4
6	0.40	0.65 (0.08)[b]
7	1.2	0.37

[a]Data reported relative to paclitaxel = 1.0. [b]P388 cell cytotoxicity.

In conclusion, four photoaffinity analogues have been prepared. Since all of them demonstrated good to excellent ability to induce the formation of microtubules, they are potential candidates for microtubule photolabeling experiments.

Acknowledgments: Financial support from the National Institutes of Health (CA 55141 and CA55160) is acknowledged. Support is also acknowledged from the Scientific Education Partnership of Marion Merrell Dow for a postdoctoral fellowship awarded to Thomas C. Boge. We are grateful for the excellent technical assistance of Ms. Jeanne Ellermeier. Paclitaxel was provided to us for these studies by the National Cancer Institute and Hauser Chemical Research, Inc.

References and Notes

(1) For reviews on the chemistry, biology and clinical activity of paclitaxel see: *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. C.; Ojima, I.; Vyas, D. M., Eds.; ACS Symposium Series No. 583; American Chemical Society: Washington, DC, 1995.

(2) McGuire, W. P.; Rowinsky, E. K.; Rosenshein, N. B.; Grumbine, F. C.; Ettinger, D. S.; Armstrong, D. K.; Donehower, R. C. *Ann. Intern. Med.* **1989**, *111*, 273.

(3) Holmes, F. A.; Walters, R. S.; Theriault, R. L.; Forman, A. D.; Newton, L. K.; Raber, M. N.; Buzdar, A. U.; Frye, D. K.; Hortobagyi, G. N. *J. Natl. Cancer Inst.* **1991**, *83*, 1797.

(4) For review: Holmes, F. A.; Kudelka, A. P.; Kavanagh, J. J.; Huber, M. H.; Ajani, J. A.; Valero, V. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. C., Ojima, I. , Vyas, D. M., Eds.; ACS Symposium Series No. 583; American Chemical Society: Washington, DC, 1995; pp 31-57.

(5) Manfredi, J. J.; Horwitz, S. B. *Pharmacol. Ther.* **1984**, *25*, 83.

(6) Horwitz, S. B. *Trends Pharm. Sci.* **1992**, *13*, 134.

(7) Hamel, E. *Pharmacol. Ther.* **1992**, *55*, 31.

(8) Kumar, N. *J. Biol. Chem.* **1981**, *256*, 10435.

(9) Rao, S.; Horwitz, S. B.; Ringel, I. *J. Natl. Cancer Inst.* **1992**, *84*, 785.

(10) Georg, G. I.; Harriman, G. C. B.; Himes, R. H.; Mejillano, M. R. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 735.

(11) Georg, G. I.; Harriman, G. C. B.; Park, H.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 487.

(12) Dasgupta, D.; Park, H.; Harriman, G. C. B.; Georg, G. I.; Himes, R. H. *J. Med. Chem.* **1994**, *37*, 2976.

(13) Chatterjee, A.; Williamson, J. S.; Zjawiony, J. K.; Peterson, J. R. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 91.

(14) Carboni, J. M.; Farina, V.; Rao, S.; Hauck, S. I.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1993**, *36*, 513.

(15) Swindell, C. S.; Heerding, J. M.; Krauss, N. E.; Horwitz, S. B.; Rao, S.; Ringel, I. *J. Med. Chem.* **1994**, *37*, 1446.

(16) Rimoldi, J. M.; Kingston, D. G. I.; Chaudhary, A. G.; Samaranayake, G.; Grover, S.; Hamel, E. *J. Nat. Prod.* **1993**, *56*, 1313.

(17) Chaudhary, A. G.; Gharpure, M. M.; Rimoldi, J. M.; Chordia, M. D.; Gunatilaka, A. A. L.; Kingston, D. G. I.; Grover, S.; Lin, C. M.; Hamel, E. *J. Am. Chem. Soc.* **1994**, *116*, 4097.

(18) Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1994**, *269*, 3132.

(19) Combeau, C.; Commerçon, A.; Mioskowski, C.; Rousseau, B.; Aubert, F.; Goeldner, M. *Biochemistry* **1994**, *33*, 6676.

(20) Horwitz, S. B.; Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. T., Ojima, I. , Vyas, D. M., Eds.; ACS Symposium Series No. 583; American Chemical Society: Washington, DC, 1995; pp 154-161.

(21) Georg, G. I.; Harriman, G. C. B.; Vander Velde, D. G.; Boge, T. C.; Cheruvallath, Z. S.; Datta, A.; Hepperle, M.; Park, H.; Himes, R. H.; Jayasinghe, L. In *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I., Chen, T. C., Ojima, I. , Vyas, D. M., Eds.; ACS Symposium Series No. 583; American Chemical Society: Washington, DC, 1995; pp 217-232.

(22) Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Harriman, G. C. B.; Hepperle, M.; Park, H.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 335.

(23) The yields are not optimized.

(24) Smith, P. A. S.; Brown, B. B. *J. Am. Chem. Soc.* **1951**, *73*, 2438.

(25) Terpko, M. O.; Heck, R. F. *J. Org. Chem.* **1980**, *45*, 4992.

(26) Venkataraman, K.; Wagle, D. R. *Tetrahedron Lett.* **1979**, 3037.

(27) Georg, G. I.; Ali, S. M.; Boge, T. C.; Datta, A.; Falborg, L.; Himes, R. H. *Tetrahedron Lett.* **1994**, *35*, 8931. For the preparation of the 2'-TES analogue of **9** in 43% yield see reference 17.

(28) For experimental procedures see: Georg, G. I.; Cheruvallath, Z. S.; Himes, R. H.; Mejillano, M. R.; Burke, C. T. *J. Med. Chem.* **1992**, *35*, 4230.

(*Received in USA* 18 *January* 1995; *accepted* 8 *February* 1995)