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7-*O*-ACYLPACLITAXEL ANALOGUES: POTENTIAL PROBES TO MAP THE PACLITAXEL BINDING SITE

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Abstract: The synthesis and biological evaluation of several 7-*O*-acylpaclitaxel analogues as potential photoaffinity, electrophilic, and fluorescent probes are described. © 1997 Elsevier Science Ltd.

The antitumor agent paclitaxel (**1**) , isolated from the bark of the pacific yew (*Taxus brevifolia*), is currently approved by the FDA for treatment of refractory ovarian and metastatic breast cancers.[2] Encouraging preliminary results from ongoing clinical trials suggest that paclitaxel will be useful in a variety of other cancers in the future.[3] Paclitaxel has been shown to promote the formation and hyperstabilization of microtubules[4] and to inhibit microtubule dynamics.[5] At low concentration the drug induces mitotic block in cells by stabilizing spindle microtubules.[6]

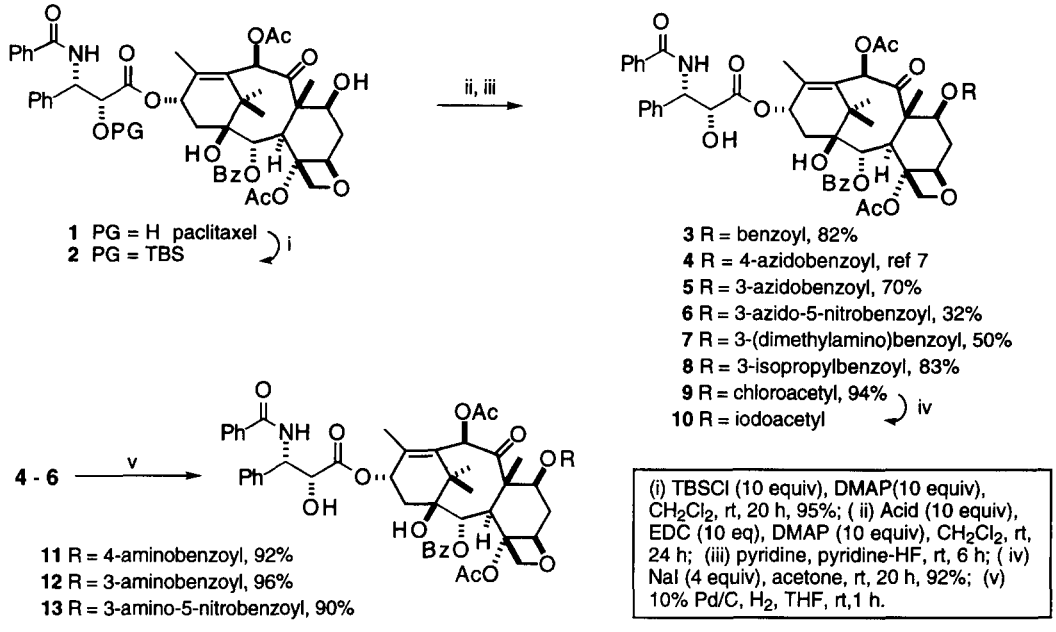
Little is known about paclitaxel's mechanism of action at the molecular level. However, some recent investigations, such as the preparation of photoaffinity analogues[7-15] and subsequent photolabeling experiments,[12-19] fluorescent probes,[20-23] and electron crystallography[24] have begun to shed light on the binding site of paclitaxel on microtubules. Based on these results and other previous experience in this area, we have synthesized a variety of 7-*O*-acylpaclitaxel analogues that may prove valuable in further elucidation of the paclitaxel binding site.

Chemistry

The common intermediate 2'-*O*-(*tert*-butyldimethylsilyl)paclitaxel (**2**) was prepared from paclitaxel, *tert*-butyldimethylsilyl chloride (TBSCl) and 4-dimethylaminopyridine (DMAP) in excellent yield by a modification of the literature method (Scheme).[25,26] Acylation of **2** was achieved through condensation of the appropriate acid in the presence of the dehydrating agent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and DMAP. The noncommercial 3-azidobenzoic,[27] 3-azido-5-nitrobenzoic,[27] and 3-isopropylbenzoic[28] acids were prepared as described previously. Subsequent silyl deprotection with pyridine-hydrogen fluoride gave the paclitaxel analogues **3-8**. The aminobenzoyl analogues **11**, **12**, and **13** were prepared from the corresponding azidobenzoyl intermediates **4**, **5**, and **6**, respectively, by catalytic hydrogenation. Although 7-*O*-iodoacetylpaclitaxel (**10**) could be synthesized from **2** and iodoacetic acid as described above, it was contaminated by a second 7-*O*-acylpaclitaxel product (1:1 by NMR), which could not be removed by chromatography. From the mass spectrum (FAB) of the contaminated product **10**, we noted a typical chlorine substituent pattern (930 and 932 *m/z*; 2:1) indicating 7-*O*-chloroacetylpaclitaxel (**9**) as the probable impurity. This can be reasonably explained by the large excess of chloride (from EDC) in the reaction mixture resulting in

halogen exchange of either iodoacetic acid or the 2'-*O*-(*tert*-butyldimethylsilyl)-7-*O*-iodoacetylpaclitaxel intermediate. Confirmation of the contaminant was achieved by synthesis of **9**, which was subsequently subjected to sodium iodide under Finkelstein conditions to give pure **10**.

Scheme



Results

Paclitaxel structure-activity relationships have demonstrated that modification at O7 is generally well tolerated.[29] Previously reported 7-*O*-benzoylpaclitaxel (**3**, $ED_{50}/ED_{50(paclitaxel)}$ = 1.7) and the related O7 photoaffinity analogue, 7-*O*-(4-(1-azi-2,2,2-trifluroethyl)benzoyl)paclitaxel ($ED_{50}/ED_{50(paclitaxel)}$ = 3.4), exhibited only slightly reduced activity compared to paclitaxel in an assay that measures microtubule stabilizing properties.[14] Thus, we were surprised that our initial attempts at O7 photoaffinity analogues,[7,8] for example 7-*O*-(4-azidobenzoyl)paclitaxel (**4**, Table), possessed greatly diminished activity in both the microtubule assembly and B16 cytotoxicity assays that we routinely use to evaluate paclitaxel analogues.[21] Although as a rule, the microtubule stabilization and assembly assays produce results of comparable magnitude when expressed as an $ED_{50}/ED_{50(paclitaxel)}$ ratio, we sought to confirm the results by synthesizing and reevaluating **3** with the assembly assay and for cytotoxicity against B16 melanoma cells.[30] The results (Table) show that compound **3** also has good activity in the assembly assay. We rationalize the results for **4** by hypothesizing that the linear structure of the 4-azidobenzoate results in an unfavorable steric interaction at the microtubule binding site. Consistent with this interpretation, the 4-aminobenzoyl **11** was found to be a relatively active analogue in the assembly and B16 cytotoxicity assays (Table). Similar results were obtained with O7 *meta*-substituted benzoyl analogues. Locating the azido moiety at the meta position resulted in photoaffinity analogues **5** and **6**, possessing extremely poor assembly-promoting properties, whereas the 3-aminobenzoyl analogues **12** and **13** have assembly-promoting properties similar to the parent 7-*O*-benzoyl analogue **3**.

Table. Biological evaluation of substituted 7-*O*-benzoylpaclitaxel analogues

compound	microtubule assembly $ED_{50}/ED_{50(paclitaxel)}$	B16 melanoma cytotoxicity $ED_{50}/ED_{50(paclitaxel)}$
1	1.0	1.0
3	4.1	9.7
4[7]	21	30
5	>24	-
6	>24	-
7	22	-
8	>32	-
9	1.6	0.92
10	3.3	3.7
11	1.7	17
12	1.5	7.1
13	4.4	16

Interestingly, 7-*O*-(5-azido-2-nitrobenzoyl)-paclitaxel was reported to be relatively active in the initial slope method (0.033 AU/min; paclitaxel = 0.008 AU/min).[10,30] Based on our findings we propose that the activity of this compound arises from the conformational modifying *ortho*-nitro substituent.

The 3-(dimethylamino)benzoyl analogue **7** has significantly reduced microtubule assembly properties. However, its activity is sufficient to be useful in fluorescent studies to probe the environment of tubulin-bound paclitaxel.[31] To provide further structure-activity information for the *meta*-substituted benzoates we prepared the 3-isopropylbenzoyl bioisostere **8**, which was again inactive.

We have also synthesized an electrophilic paclitaxel label. The 7-*O*-iodoacetate **10** possesses slightly reduced assembly-promoting properties and B16 cytotoxicity compared to paclitaxel. The synthetic intermediate 7-*O*-chloroacetylpaclitaxel (**9**) was the most active compound examined.[32]

In conclusion, we have examined a number of analogues intended for use as biological probes. We have established that there is a rather strict steric requirement for 7-*O*-benzoate substitution. All of the aminoaryl analogues (**7**, **11-13**) examined are useful for fluorescent studies. Among the azido substituted analogues only 4-substituted analogue **4** holds some promise as a photoaffinity label, whereas the 3-substituted arylazides **5** and **6** do not. The 7-iodoacetyl analogue **10** will be examined as a probe to map the paclitaxel binding site through electrophilic labeling.

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References and Notes

1. Present address: University of Missouri-Kansas City, Division of Pharmaceutical Sciences, Kansas City, MO 64110-2499.
2. For recent monographs, see: *Taxol® Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, 1995. *The Chemistry and Pharmacology of Taxol® and Its Derivatives*; Farina, V., Ed.; Elsevier: Amsterdam, 1995. *Taxane Anticancer Agents: Basic Science and Current Status*; Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M., Eds.; American Chemical Society: Washington, DC, 1995; ACS Symposium Series 583.
3. 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. T.; Ojima, I.; Vyas, D. M., Eds.; American Chemical Society: Washington, DC, 1995; ACS Symposium Series 583; pp 31-57.
4. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature, (London)* **1979**, *277*, 665.
5. Derry, W. B.; Wilson, L.; Jordan, M. A. *Biochemistry* **1995**, *34*, 2203.
6. Jordan, M. A.; Wendell, K.; Gardiner, S.; Derry, W. B.; Copp, H.; Wilson, L. *Cancer Res.* **1996**, *56*, 816.
7. Georg, G. I.; Harriman, G. C. B.; Himes, R. H.; Mejillano, M. R. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 735.
8. Georg, G. I.; Harriman, G. C. B.; Park, H.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 487.
9. 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.
10. Carboni, J. M.; Farina, V.; Rao, S.; Hauck, S. I.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1993**, *36*, 513.
11. 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.
12. Combeau, C.; Commerçon, A.; Mioskowski, C.; Rousseau, B.; Aubert, F.; Goeldner, M. *Biochemistry* **1994**, *33*, 6676.
13. Dasgupta, D.; Park, H.; Harriman, G. C. B.; Georg, G. I.; Himes, R. H. *J. Med. Chem.* **1994**, *37*, 2976.
14. Rimoldi, J. M.; Kingston, D. G. I.; Chaudhary, A. G.; Samaranayake, G.; Grover, S.; Hamel, E. *J. Nat. Prod.* **1993**, *56*, 1313.
15. Swindell, C. S.; Heerding, J. M.; Krauss, N. E.; Horwitz, S. B.; Rao, S.; Ringel, I. *J. Med. Chem.* **1994**, *37*, 1446.
16. Rao, S.; Horwitz, S. B.; Ringel, I. *J. Natl. Cancer Inst.* **1992**, *84*, 785.
17. 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.
18. Rao, S.; Orr, G. A.; Chaudhary, A. G.; Kingston, D. G. I.; Horwitz, S. B. *J. Biol. Chem.* **1995**, *270*, 20235.
19. Loeb, C.; Combeau, C.; Ehret-Sabatier, L.; Breton-Gilet, A.; Faucher, D.; Rousseau, B.; Commerçon, A.; Goeldner, M. *Biochemistry* **1997**, *36*, 3820.
20. Dubois, J.; Le Goff, M.-T.; Gueritte-Voegelein, F.; Guenard, D.; Tollon, Y.; Wright, M. *Bioorg. Med. Chem. Lett.* **1995**, *3*, 1357.
21. Sengupta, S.; Boge, T. C.; Georg, G. I.; Himes, R. H. *Biochemistry* **1995**, *36*, 11889.
22. Souto, A. A.; Acuna, A. U.; Andreu, J. M.; Barasoain, I.; Abal, M.; Amat-Guerri, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2710.
23. Guy, R. K.; Scott, Z. A.; Sloboda, R. D.; Nicolaou, K. C. *Chem. Biol.* **1996**, *3*, 1021.
24. Nogales, E.; Wolf, S. G.; Khan, I. A.; Ludueña, R. F.; Downing, K. H. *Nature, (London)* **1995**, *375*, 424.
25. Georg, G. I.; Ali, S. M.; Boge, T. C.; Datta, A.; Falborg, L.; Park, H.; Mejillano, M.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 259.
26. Georg, G. I.; Harriman, G. C. B.; Ali, S. M.; Datta, A.; Hepperle, M.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 115.
27. Georg, G. I.; Boge, T. C.; Park, H.; Himes, R. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 615.
28. Campbell, B. N.; Spaeth, E. C. *J. Am. Chem. Soc.* **1959**, *81*, 5933.
29. For recent reviews on structure-activity relationships, see: Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol® Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, 1995; pp 317-375. Chen, S. H.; Farina, V. In *The Chemistry and Pharmacology of Taxol® and Its Derivatives*; Farina, V., Ed.; Elsevier: Amsterdam, 1995; pp 165-253.
30. For a discussion of the various tubulin assays, see ref 29.
31. The details of this study have been published: Sengupta, S.; Boge, T. C.; Liu, Y.; Hepperle, M.; Georg, G. I.; Himes, R. H. *Biochemistry* **1997**, *36*, 5179.
32. It was recently reported that compound **9** irreversibly binds to tubulin. Ciomei, M. *Abstracts of Papers*, American Association of Cancer Research Meeting, Washington, DC, 1996; Abstract 1455.

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