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SYNTHESIS OF BIOLOGICALLY ACTIVE 2-BENZOYL PACLITAXEL ANALOGUES

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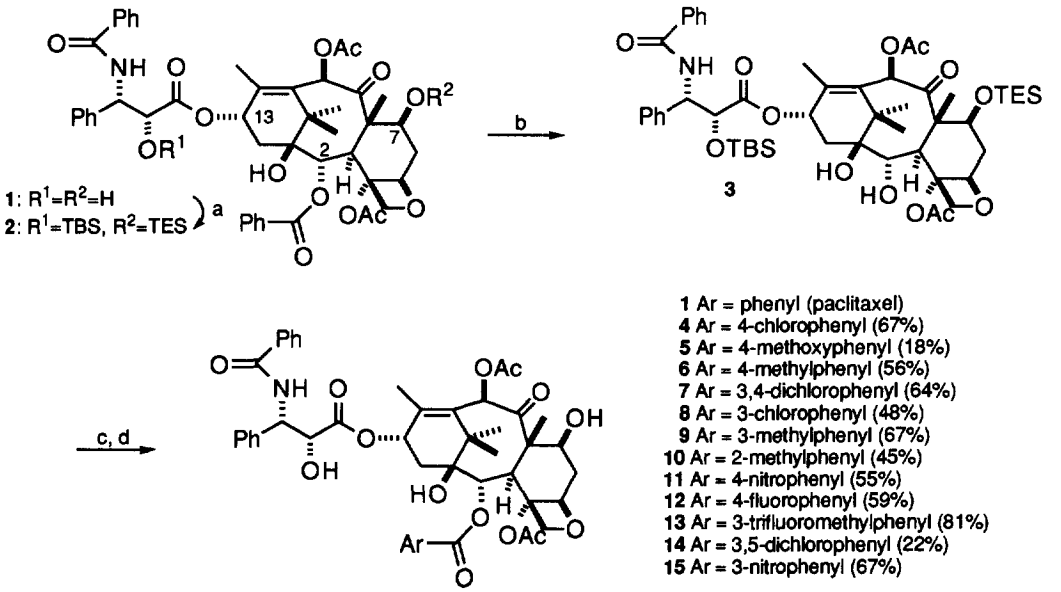
Abstract: The influence of aromatic substitution at the 2-benzoyl moiety of paclitaxel on biological activity was investigated, following the Topliss Operational Scheme. Twelve paclitaxel derivatives were synthesized and evaluated in a microtubule assembly assay and for cytotoxicity against B16 melanoma cells. Most of the analogues were found to possess biological properties similar to paclitaxel.

Paclitaxel (**1**), regarded as the most promising new anticancer agent developed in this decade, has been approved by the FDA for the treatment of refractory ovarian cancer and metastatic breast cancer.[1] In recent clinical studies paclitaxel has also shown promise for the chemotherapy of lung cancer, head and neck cancer and esophageal carcinomas.[1,2] The exciting anticancer activity of paclitaxel has stimulated significant efforts aimed at elucidating the influence of structural modifications on biological activity.[3-6] Structure-activity studies initially centered on modifications of the C-13 phenylisoserine side chain,[7] which is essential for biological activity.[8] Recent studies from several groups have provided information on the influence of functional groups of the diterpene moiety of paclitaxel on its cytotoxic activity.[3-6] These studies revealed that modifications at C-7 to C-10 are typically tolerated very well,[3-6] whereas structural changes at C-2 and C-4 can have a profound influence on bioactivity. For example, deletion of the 2-benzoate[9] and the 4-acetyl group[10,11] in paclitaxel leads to inactive compounds.

Since the 2-benzoate group is of vital importance for paclitaxel activity, we decided to systematically investigate the influence of aromatic substitution on the bioactivity of paclitaxel analogues,[12-14] utilizing the Topliss approach.[15,16] The Topliss operational scheme is a non-mathematical application of the Hansch analysis, designed to guide toward the most active analogue of a lead compound. Lipophilic, electronic and steric parameters of the substituents are taken into account.

For the preparation of the desired 2-benzoate analogues **4-15** (Table),[17] we developed a convergent synthetic scheme,[18] which allowed for their synthesis in two steps (Scheme)[19] from common intermediate **3** (2-debenzoyl-2'-*tert*-butyldimethylsilyl-7-(triethylsilyl)paclitaxel) via acylation at C-2, followed by removal of the silyl protecting groups at positions 2' and 7. Debenzoyl derivative **3**, can be obtained in high yield by treating 2'-*tert*-butyldimethylsilyl-7-(triethylsilyl)paclitaxel (**2**) with anhydrous KOH.[18]

Scheme



(a) (i) DMAP (10 equiv), TBSCl (10 equiv), CH_2Cl_2, rt, 12 h, then TESCl (10 equiv), DMAP (10 equiv), rt, 1 h: **2**, 93%. (b) *tert*-BuOK (1.4 equiv), water (1.2 equiv), THF, -40 to -15 °C, 24 to 48 h: **3**, 70-80%. (c) ArCOOH (20 equiv), DMAP (20 equiv), DCC or EDC (20 equiv), toluene, 60 °C, 4 to 48 h. (d) Pyridinium hydrofluoride, pyridine, 0 °C to rt, 4-6 h.

The first compound to be prepared in the Topliss operational scheme is the 4-chlorobenzoyl analogue **4**. Analogue **4** displayed activity similar to the parent compound in the microtubule assembly assay ($ED_{50}/ED_{50(paclitaxel)} = 0.79$) and in the cytotoxicity test against B16 melanoma cells ($ED_{50}/ED_{50(paclitaxel)} = 3.1$). We then prepared the next set of compounds of the Topliss tree, the 4-methoxy (**5**), the 4-methyl (**6**) and the 3,4-dichloro (**7**) analogues. Since the 4-chlorobenzoyl analogue **4** was similar in activity to the parent compound and neither derivative **5** nor **7** was significantly more active (Table) than paclitaxel we decided to follow the branch of the Topliss tree for equally active compounds and prepare 3-chloro derivative **8**. Compound **8** had microtubule assembly properties similar to paclitaxel ($ED_{50}/ED_{50(paclitaxel)} = 1.1$) and was slightly more cytotoxic ($ED_{50}/ED_{50(paclitaxel)} = 0.57$) than the parent compound. Since this compound was judged to possess biological properties similar to paclitaxel, the equally active branch of the Topliss tree was pursued again. No potency increase was observed for 3-methyl analogue **9** and 2-methyl derivative **10**. They demonstrated activity similar to paclitaxel (Table). A significant decrease of activity was, however, observed for the last two compounds of the Topliss tree, the 4-nitro (**11**) and the 4-fluoro (**12**) derivatives. The 4-nitro analogue **11** was essentially inactive[20] in the microtubule assembly assay ($ED_{50}/ED_{50(paclitaxel)} = >8.8$) and the 4-fluoro compound **12** had greatly reduced activity in this assay. Compound **12** had little cytotoxicity against B16 melanoma cells.[21]

While we pursued our studies on the influence of aromatic substitution at the C-2 benzoate moiety on biological activity, a report by Chaudhary *et al.* detailed that 3-chloro derivative **8** was 700 times more cytotoxic than paclitaxel against P388 murine leukemia cells and other 3-substituted 2-benzoyl paclitaxel analogues were found to be up to 800 times more cytotoxic than paclitaxel against P388 cells.[22] Prompted by this report, we decided to prepare analogues **13-15**, to be made according to the Topliss Scheme when the 3-chloro derivative is more active than the 4-methyl analogue. Biological evaluation of 3-trifluoromethyl (**13**), 3,5-dichloro (**14**) and 3-nitro (**15**) derivatives revealed biological activity similar to paclitaxel (Table). No significant increase of activity was observed in both assays.

Table. *In vitro* biological evaluation of substituted 2-benzoyl paclitaxel analogues.[a]

compound	microtubule assembly[b] $ED_{50}/ED_{50(paclitaxel)}$	B16 melanoma cytotoxicity[c] $ED_{50}/ED_{50(paclitaxel)}$
1	1.0	1.0 (1.0)[d]
4	0.79	3.1
5	6.0	22
6	1.6	5.1
7	0.45	2.7
8	1.1	0.57 (0.81)[d]
9	1.7	1.6
10	1.1	0.84
11	>8.8	n. d.
12	3.2	>36
13	0.64	2.7
14	0.98	1.8
15	1.3	0.73

[a]For experimental procedures see ref. 23. [b]ED_{50} is the concentration which causes polymerization of 50% of the tubulin present in 15 min at 37 °C. Data reported relative to paclitaxel = 1.0. [c]ED_{50} refers to the concentration which produces 50% inhibition of proliferation after 40 h of incubation. Data reported relative to paclitaxel = 1.0. [d]Cytotoxicity against P388 cells. ED_{50} refers to the concentration which produces 50% inhibition of proliferation after 40 h of incubation. Data reported relative to paclitaxel = 1.0

Since we had found that 3-chloro analogue **8** was similar to paclitaxel in the microtubule assembly assay and only about twice as active against B16 melanoma cells, we also tested **8** for cytotoxicity against P388 cells. With our assay we could not confirm the results by Chaudhary *et al.* who had found that **8** was 700 times more active against this cell line.[22] Our results indicated that 3-chloro analogue **8** was only slightly more active than paclitaxel against P388 cells (Table).

Chaudhary *et al.*[22] also reported that the same analogue (**8**) had the property of stimulating microtubule assembly at low temperature. We also observed this activity (Figure). The data presented in the figure demonstrate that at 15 °C analogue **8** was significantly more effective than paclitaxel when tested at the same concentration. At 37 °C the difference between the two compounds was minimal (Figure and Table). Electron microscopic examination of thin-sectioned pellets of the products formed failed to reveal striking differences between the two compounds. A mixture of microtubules and protofilament sheets were observed, with a greater percentage of microtubules present at 15 °C than at 37 °C. The apparent difference in microtubule binding between paclitaxel and **8** in response to temperature probably reflects differences in ΔS of binding.

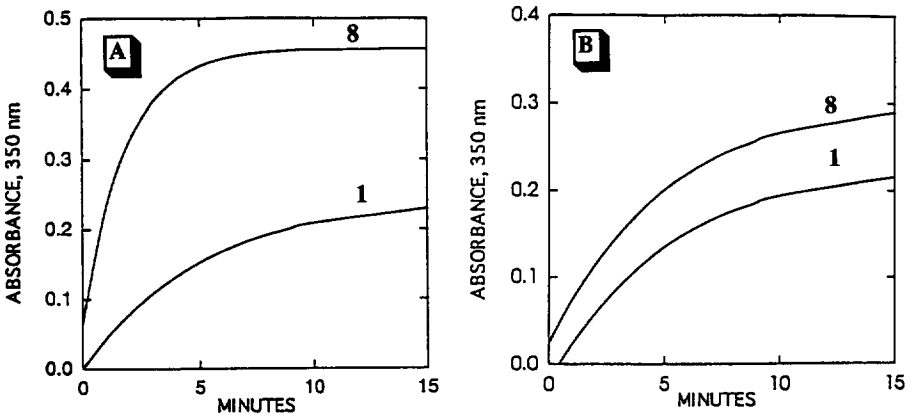


Figure. Assembly of tubulin in the presence of paclitaxel (**1**) and paclitaxel analogue **8**. Tubulin (10 μM) was incubated in 0.1 M Pipes, 1 mM MgSO4, 1 mM EGTA, and 0.5 mM GTP. Panel A, at 15 °C with 10 μM of each compound. Panel B, at 37 °C with 2 μM of each compound.

Summary:

The results of our studies led us to the conclusion that substitution at the 2-benzoyl moiety of paclitaxel does not result in any remarkable enhancement of biological activity in the microtubule assembly assay at 37 °C or in the cytotoxicity test against B16 melanoma cells. Likewise, we did not detect a significant difference between paclitaxel and 3-chloro analogue **8** in the cytotoxicity against P388 cells.

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Houston and Ms. Jeanne Ellermeier. Taxol was provided to us for these studies by the National Cancer Institute and Hauser Chemical Research, Inc.

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(17) All new analogues displayed spectroscopic properties and analytic data in agreement with their structures. The yields given in the Scheme are for the two step conversion of **3** to target compounds **4-15**. The yields are not optimized.

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(19) Abbreviations used in the Scheme: TBS (*tert*-butyldimethylsilyl), TES (triethylsilyl), DMF (*N,N*-dimethylformamide), rt (room temperature), THF (tetrahydrofuran), Ar (aryl), DMAP (4-dimethylaminopyridine), DCC (1,3-dicyclohexylcarbodiimide), EDC (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride).

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