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SYNTHESIS OF 2-*O*-HETEROAROYL TAXANES: EVALUATION OF MICROTUBULE ASSEMBLY PROMOTION AND CYTOTOXICITY

Gunda I. Georg,* Geraldine C. B. Harriman, Syed M. Ali,
Apurba Datta and Michael Hepperle
Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045

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

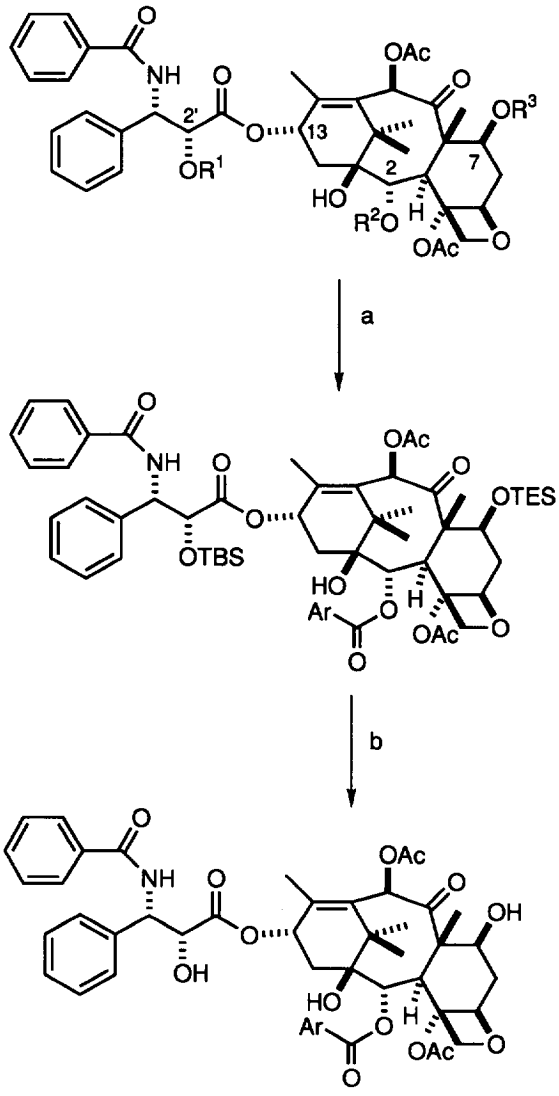
Abstract: A series of heterocyclic taxanes were synthesized and evaluated to investigate structure activity relationships at the 2-position of paclitaxel. The compounds were prepared through a regioselective deacylation, reacylation sequence of suitably protected paclitaxel. The heterocyclic substituted diterpenes were synthesized from paclitaxel in four steps in high overall yields. Evaluation of these analogues in a microtubule assembly assay and for their cytotoxicity against B16 melanoma cells illustrated that these compounds had diminished activity compared to paclitaxel.

The antitumor agent paclitaxel has proven to be effective in the treatment of a variety of cancers.[1,2] Isolated from the bark of the pacific yew,[3] this multifunctionalized diterpene offers an array of possible positions for modifications in order to improve its biological activity[2,4-7] as a promoter of microtubule polymerization.[8] Previous structure-activity studies in our laboratory on paclitaxel have centered on the C-13 *N*-benzoylphenylisoserine side chain.[9-15] In continuing our studies on the SAR of the diterpene moiety of paclitaxel,[15-18] we herein focus on the C-2 benzoate[19-22] of the diterpene moiety as an area of interest.

We have previously reported on the regioselective deacylation of 7-*O*-triethylsilylbaccatin III[23] and 2'-*O*-*tert*-butyldimethylsilyl-7-*O*-triethylsilylpaclitaxel[24] which described the selective debenzoylation at the 2-position utilizing potassium *tert*-butoxide. We have also reported on the chemistry of highly bioactive heterocycles substituted at the C-3' and 3'-nitrogen with both pyridine and furan functionalities.[25,26] We herein report on the utility of the versatile intermediate 2'-*O*-*tert*-butyldimethylsilyl-2-*O*-debenzoyl-7-*O*-triethylsilylpaclitaxel (**11**) in the synthesis of a variety of 2-*O*-heteroaroyltaxanes in a continued study on the effects of heterocyclic moieties on the biological activity of novel taxanes.

The syntheses of the 2-*O*-heteroaroyl taxanes (Scheme) were initiated by the selective protection of paclitaxel. We have shown that the 2'- and the 7-hydroxyl groups of paclitaxel must be suitably protected as a *tert*-butyldimethylsilyl ether (TBS) and a triethylsilyl ether (TES), **10**, respectively.[24] The protection of the 2'-hydroxyl as the corresponding *tert*-butyldimethylsilyl ether affords a bulky protecting group proximal to the C-1' carbonyl which prevents base promoted ester cleavage of the C-13 side chain. Protection of the 7-hydroxyl as the triethylsilyl ether serves to prevent epimerization at the 7-position via retroaldol chemistry.[6] Base induced removal of the 2-benzoate utilizing potassium *tert*-butoxide/H_2O was achieved in high yield (80%) and with high regioselectivity.[24] Acylation of 2'-*O*-*tert*-butyldimethylsilyl-2-*O*-debenzoyl-7-*O*-triethylsilylpaclitaxel (**11**), utilizing 1,3-dicyclohexylcarbodiimide (DCC, 12 equiv), *N*,*N*-dimethylaminopyridine (DMAP, 12 equiv)

Scheme



1 $R^1 = R^3 = H$, R^2 = benzoyl
10 R^1 = TBS, R^2 = benzoyl, R^3 = TES
11 R^1 = TBS, $R^2 = H$, R^3 = TES

12 Ar = 2-pyridyl
13 Ar = 3-pyridyl
14 Ar = 4-pyridyl
15 Ar = 2-thienyl
16 Ar = 3-thienyl
17 Ar = 2-furyl
18 Ar = 3-furyl
19 Ar = 2-*N*-methylpyrrole

2 Ar = 2-pyridyl (87%)
3 Ar = 3-pyridyl (55%)
4 Ar = 4-pyridyl (46%)
5 Ar = 2-thienyl (59%)
6 Ar = 3-thienyl (63%)
7 Ar = 2-furyl (96%)
8 Ar = 3-furyl (59%)
9 Ar = 2-*N*-methylpyrrole (39%)

a. Heterocyclic carboxylic acid (12 equiv), DCC (12 equiv), DMAP (12 equiv) in anhydrous toluene (0.05 M **11**), 60 °C, 3-5 h. b. Pyridinium hydrogen fluoride (excess), pyridine, RT, 3 h.

and the appropriate heterocyclic carboxylic acid (12 equiv), gave analogues **12-19**. The silyl protecting groups of heterocyclic taxanes **12-19** were subsequently removed in the presence of pyridinium hydrogen fluoride to provide **2-9** in good overall yields for the two step conversions from intermediate **11**.[27] (The yields for the two step conversions are given in brackets in the Scheme. The yields are not optimized.)

The heterocyclic taxanes **2-9** were evaluated in a microtubule assembly assay and also for their cytotoxicity against B16 melanoma cells. The effect of the heterocyclic taxanes on tubulin assembly proved to be quite varied. The thienyl taxanes **5** and **6** elicited microtubule assembly properties comparable to paclitaxel, while the pyridyl (**2**, **3**, and **4**), furyl (**7** and **8**) and *N*-methylpyrrole (**9**) substituted taxanes all displayed diminished microtubule assembly properties compared to that of paclitaxel. In the assay for B16 melanoma cytotoxicity all of the 2-*O*-heteroaroyl taxanes proved to be less cytotoxic than the parent.[21] We therefore concluded that the introduction of 2-heteroaroyl moieties at C-2 is detrimental to cytotoxicity.

Table. *In vitro* biological evaluation of heteroaromatic C-2 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
2	2.8	8.7
3	4.0	>36
4	7.3	>36
5	1.1	3.8
6	1.4	2.1
7	2.4	14
8	2.1	8.2
9	2.6	2.6

[a]For experimental procedures see ref. 10 [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.

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