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Semisynthesis and Biological Activity of Taxol Analogues: Baccatin III 13-(*N*-benzoyl-(2'*R*,3'*S*)-3'-(*p*-tolyl)isoserinate), Baccatin III 13-(*N*-(*p*-toluoyl)-(2'*R*,3'*S*)-3'-phenylisoserinate), Baccatin III 13-(*N*-benzoyl-(2'*R*,3'*S*)-3'-(*p* -trifluoromethylphenyl)isoserinate), and Baccatin III 13-(*N*-(*p*-trifluoromethylbenzoyl)-(2'*R*,3'*S*)-3'-phenylisoserinate)

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Abstract: *The semisynthesis of the four novel taxol analogues baccatin III 13-(N-benzoyl-(2'R,3'S)-3'-(p-tolyl)isoserinate) (**2**), baccatin III 13-(N-(p-toluoyl)-(2'R,3'S)-3'-phenylisoserinate) (**3**), baccatin III 13-(N-benzoyl-(2'R,3'S)-3'(p-trifluoromethylphenyl)isoserinate) (**4**), and baccatin III 13-(N-(p-trifluoromethylbenzoyl)-(2'R,3'S)-3'-phenylisoserinate) (**5**) from 7-triethylsilyl baccatin III (**6**) and the N-acyl-3-ethoxyethyl-4-aryl-2-azetidinones (**11-14**) is described. Derivatives **2**, **3**, and **5** demonstrated activity comparable to taxol (**1**) in the microtubule assembly assay and cytotoxicity against B16 melanoma cells. Derivative **4**, however, was found to be an unstable product.*

Taxol (**1**), a complex diterpene plant product is isolated[1] in small quantities from the stem bark of *Taxus brevifolia*.[2] It was found to have significant activity [3] against a wide variety of cancer cell lines and xenografts of human tumors in mice and hence was selected for clinical trials in 1977.[4] Currently, taxol is in phase II and phase III clinical trials in the United States.[5] Activity against cisplatin refractory ovarian cancer has been established.[6] Promising results have also been reported for the treatment of breast cancer[7] and potentially for the treatment of lung cancer.[8] In vitro studies have revealed that taxol has a new and unique mechanism of action, promoting the assembly of stable microtubules, which cannot be depolymerized by calcium ions, cold or microtubule disassembling drugs.[9,10]

Unfortunately, taxol (**1**) is obtained in very low yields from the very slow growing Pacific yew trees.[11] However, 10-deacetyl baccatin III, which is a more readily available taxol precursor, and which can be obtained from a regenerable source,[12] can be coupled to either *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine[12,13] or an appropriately protected 3-hydroxy-4-phenyl-2-azetidinone.[14-17]

The access to 10-deacetyl baccatin III and baccatin III has not only allowed for the semisynthesis of taxol but also of potent taxol analogues such as taxotere[13] and other analogues with modified *N*-benzoyl-3'-phenylisoserine side chains.[13,18,19]

Recently, we reported on the first semisynthesis and biological evaluation of two taxol analogues[15,16] with substituted phenyl rings at the C-13 *N*-benzoyl-(2'*R*,3'*S*)-3'-phenylisoserine side chain of taxol (**1**), utilizing baccatin III as a precursor and coupling it with *N*-acyl-ß-lactams following the Holton methodology.[14]

In continuation of our studies on the evaluation of substituent effects at the C-13 phenylisoserine side chain, utilizing the Topliss method,[20] we now wish to report on the synthesis and biological evaluation of four novel taxol analogues **2-5** which possess *p*-methyl and *p*-trifluoromethyl substituents at the phenyl rings of the *N*-benzoyl-3'-phenylisoserine side chain.

Coupling of *N*-acyl-ß-lactams **11**, **12**, **13** and **14** (5 equiv.) with 7-triethylsilyl baccatin III (**6**)[21] at 25 °C in the presence of 4-dimethylaminopyridine (DMAP) and pyridine for 12-24 h gave taxol derivatives (**7-10**) in moderate to good yields (**Scheme 1**). Deprotection of both triethylsilyl and ethoxyethyl protecting groups with 0.5% HCl/ethanol at 0 °C,[12] afforded the desired taxol analogues baccatin III 13-(*N*-benzoyl-(2'*R*,3'*S*)-3'-(*p*-tolyl)isoserinate) (**2**), baccatin III 13-(*N*-(*p*-toluoyl)-(2'*R*,3'*S*)-3'-phenylisoserinate) (**3**), baccatin III 13-(*N*-benzoyl-(2'*R*,3'*S*)-3'-(*p*-trifluoromethylphenyl)isoserinate) (**4**) and baccatin III 13-(*N*-(*p*-trifluoromethylbenzoyl)-(2'*R*,3'*S*)-3'-phenylisoserinate) (**5**) in excellent yields (82-93%).[22]

The optically active triisopropylsilyl protected 3-hydroxy-4-aryl-azetidinones **15**, **16**, and **17** were synthesized in excellent enantiomeric excesses (96, 96, and 91 % ee respectively)[23] via the ester enolate-imine cyclocondensation reaction recently described by us.[15,16,23] The bulky triisopropylsilyl protecting group had to be replaced by a sterically less hindered ethoxyethyl group to obtain good yields in the coupling of ß-lactams **11-14** to 7-triethylsilyl baccatin III (**6**).[15,16] Therefore the ß-lactams **15-17** were desilylated[24] with tetrabutyl ammonium fluoride in tetrahydrofuran or 40% HF in acetonitrile at 25 °C,[25] followed by reprotection with ethylvinyl ether (EVE) and catalytic amounts of *p*-toluenesulfonic acid[26] (0 to 25 °C) to obtain ß-lactams **18-20** in good yields (70-90%). Acylation of the ß-lactams **18-20** with either benzoyl chloride, *p*-toluoyl chloride, or *p*-trifluoromethylbenzoyl chloride and triethylamine with catalytic amounts of DMAP (0 to 25 °C) in dichloromethane[17] gave the corresponding *N*-acyl-ß-lactams **11-14**[25] (**Scheme 2**).

The novel taxol analogues **2**, **3**, **4**, and **5** were tested for their ability to promote microtubule assembly in vitro (10 μM tubulin concentration [27]) and for their cytotoxicity against B16 melanoma cells,[16,28] as compared to taxol (**1**).

Table 1. Activity of taxol (1) and taxol derivatives 2, 3, and 5 in the tubulin assembly assay and their cytotoxicity against B16 melanoma cells

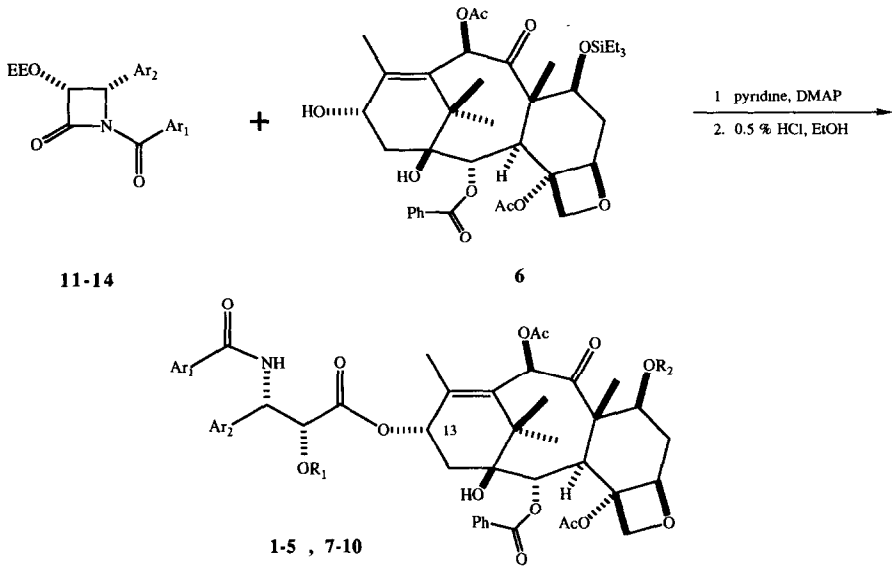
Compound	microtubule assembly ED_{50} / ED_{50} (taxol)[a]	B16 melanoma ED_{50} / ED_{50} (taxol)[b]
1 taxol	1.0	1.0
2 NSC 651196	2.4	3.0
3 NSC 651195	1.6	1.4
5 NSC 653244	6.0	17.7

[a] ED_{50} is the concentration which causes polymerization of 50 % of the tubulin present in 15 min at 37 °C. ED_{50} / ED_{50} (taxol) gives the activity as a ratio in comparison with taxol (taxol ED_{50} = 0.7 to 0.8 μM).

[b] ED_{50} refers to the concentration which produces 50% inhibition of proliferation after 40 h incubation. ED_{50} / ED_{50} (taxol) gives the activity as a ratio in comparison with taxol (taxol ED_{50} = 23 to 34 nM)

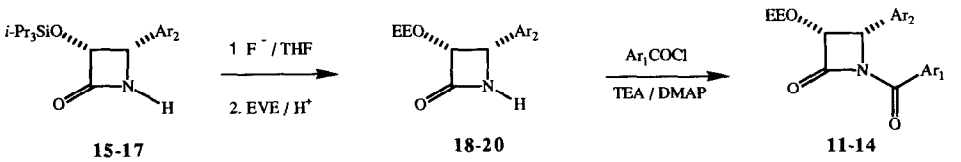
It was found that taxol analogues **2**, **3**, and **5** had activity (**Table 1**) in the microtubule assembly assay and against B16 melanoma cells, which was comparable to taxol (**1**). However, analogue **4** showed no activity in the microtubule assembly assay and no cytotoxicity against B16 melanoma cells. A further investigation of this surprising result revealed that analogue **4** had decomposed during the time it was made and characterized spectroscopically and its biological evaluation (about two weeks). Identical decomposition products were observed from a sample stored at freezer temperature and from a sample kept in methanol at -70 °C. Inspection of the HPLC trace[22] of the decomposition products demonstrated the total absence of analogue **4** in a mixture of several products, one of which probably is baccatin III. The ^1H NMR spectrum also indicated a mixture of several decomposition products and the absence of taxol analogue **4**. The instability of **4** can obviously be traced to the 3'-(*p*-trifluoromethylphenyl) group of **4**. It is of note that the corresponding (*p*-trifluoromethylphenyl) group containing β-lactams desilylated **17** and derivative **13** also displayed instability during synthetic procedures and purification.[25]

Scheme 1



	Compound	yield %
1	Ar_1, Ar_2 = phenyl; R_1, R_2 = H; taxol	-
2	Ar_1 = phenyl; Ar_2 = *p*-tolyl; R_1, R_2 = H (NSC 651196)	93
3	Ar_1 = *p*-tolyl; Ar_2 = phenyl; R_1, R_2 = H (NSC 651195)	90
4	Ar_1 = phenyl; Ar_2 = *p*-trifluoromethylphenyl; R_1, R_2 = H (NSC 654374)	88
5	Ar_1 = *p*-trifluoromethylphenyl; Ar_2 = phenyl; R_1, R_2 = H (NSC 653244)	82
7	Ar_1 = phenyl; Ar_2 = *p*-tolyl; R_1 = ethoxyethyl; R_2 = triethylsilyl	87
8	Ar_1 = *p*-tolyl; Ar_2 = phenyl; R_1 = ethoxyethyl; R_2 = triethylsilyl	80
9	Ar_1 = phenyl; Ar_2 = *p*-trifluoromethylphenyl; R_1 = ethoxyethyl; R_2 = triethylsilyl	54
10	Ar_1 = *p*-trifluoromethylphenyl; Ar_2 = phenyl; R_1 = ethoxyethyl; R_2 = triethylsilyl	41

Scheme 2



15, 18 Ar_2 = phenyl
16, 19 Ar_2 = *p*-tolyl
17, 20 Ar_2 = *p*-trifluoromethylphenyl

11 Ar_1 = phenyl Ar_2 = *p*-tolyl
12 Ar_1 = *p*-tolyl Ar_2 = phenyl
13 Ar_1 = phenyl Ar_2 = *p*-trifluoromethylphenyl
14 Ar_1 = *p*-trifluoromethylphenyl Ar_2 = phenyl

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