Bioorganic & Medicinal Chemistry Letters, Vol.2, No.4, pp. 295-298, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

Novel Biologically Active Taxol Analogues: Baccatin III 13-(*N*-(*p*-Chlorobenzoyl)-(2'*R*,3'*S*)-3'-phenylisoserinate) and Baccatin III 13-(*N*-Benzoyl-(2'*R*,3'*S*)-3'-(*p*-chlorophenyl)isoserinate)

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(*Received* 9 *January* 1992)

Key words: Taxol analogues; synthesis; β-lactams; microtubule assembly; cytotoxicity

***Abstract**: Two novel taxol analogues, baccatin III 13-(N-(p-chlorobenzoyl)-(2'R,3'S)-3'-phenylisoserinate) (2) and baccatin III 13-(N-benzoyl-(2'R,3'S)-3'-(p-chlorophenyl)isoserinate) (3) were synthesized from 7-triethylsilyl baccatin III (4) and N-acyl 3-ethoxyethyloxy-4-aryl-2-azetidinones 5 and 6 in two steps and excellent overall yield. Both derivatives demonstrated activity in the microtubule assembly assay and cytotoxicity against B16 melanoma cells comparable to taxol.*

Taxol (**1**), isolated[1] from the stem bark of *Taxus brevifolia*[2] is currently considered a most exciting lead in cancer chemotherapy, possessing excellent antitumor activity against several forms of cancer.[3] Activity against advanced cisplatin refractory ovarian cancer has been established.[4,5] In vitro studies on taxol (**1**) have revealed a new and unique mechanism of action, blocking cell replication in HeLa cells and fibroblast cells.[6] It has been shown that taxol (**1**) promotes the assembly of stable microtubules, which cannot be depolymerized by calcium ion, cold or microtubule disassembling drugs.[7]

Unfortunately, taxol is available only in small quantities from natural sources, which are threatened by extinction should taxol be used clinically for cancer chemotherapy.[8] However, a semi-synthetic approach toward taxol synthesis and analogue development can be achieved through the utilization of 10-deacetyl baccatin III, a more readily available taxol-related natural product.[9] Since 10-deacetyl baccatin III is obtained from a regenerable source, the leaves of *Taxus baccata*, harvest does not threaten the survival of the species.

Recently, two different approaches were reported for the efficient conversion of 10-deacetyl baccatin III and baccatin III to taxol (**1**). Both methods involve the coupling of 7-triethylsilyl baccatin III (**4**) to either *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine[9] or an appropriately protected 3-hydroxy-4-phenyl-2-azetidinone.[10,11] Results by us[12,13] and others[9,14-17] have provided practical approaches toward the synthesis of *N*-benzoyl-(2*R*,3*S*)-3-phenylisoserine and optically active 3-hydroxy-4-phenyl-2-azetidinones, thus facilitating the semi-synthesis of taxol and its analogues.

Structure-activity studies of taxol[18] and 10-deacetyl taxol derivatives revealed that both the diterpene part of the molecule and the C-13 *N*-benzoyl-3'-phenylisoserine side chain are essential for cytotoxicity.[19] More detailed structure-activity studies[20-24] demonstrated that molecular simplifications at the *N*-benzoyl-3'-phenylisoserine side chain typically lead toward derivatives with reduced cytotoxic properties. However, replacement of the *N*-benzoyl group of the 3'-phenylisoserine side chain was tolerated very well. One derivative, taxotere, possessing a *N*-*t*-BOC group instead of the *N*-benzoyl group at the side chain, was found to be even more active than taxol.[23]

We now wish to report the synthesis[25] and biological evaluation of two novel taxol analogues **2** and **3** which possess *p*-chloro substituents at the phenyl rings of the *N*-benzoyl-3'-phenylisoserine side chain. Coupling of *N*-acyl β-lactams **5** and **6** with 7-triethylsilyl baccatin III (**4**)[26] (**Scheme 1**) was achieved[11] in the presence of 4-dimethylaminopyridine (DMAP) in pyridine as the solvent in 91% and 89% yield to form the taxol derivatives **7** and **8** respectively. Acidic hydrolysis[9] resulted in the removal of both the triethylsilyl and the ethoxyethyl protecting groups to afford the desired taxol analogues **2** and **3** in 90% and 92% yield respectively.

The β-lactams **5** and **6**, necessary for the coupling to **4**, were obtained in two steps from β-lactams **9** and **10** (**Scheme 2**). The asymmetric synthesis[27] of β-lactams of type **9** and **10** via the ester enolate-imine cyclocondensation reaction was recently described by us.[12] Removal of the silyl protecting group at the C-3 hydroxyl group of β-lactams **9** and **10**, followed by protection with ethyl vinyl ether (EVE) was achieved (**11**, **12**) in good yields by standard methodology.[28] Acylation of β-lactams **11** and **12** with *p*-chlorobenzoyl chloride and benzoyl chloride respectively, triethylamine, and a catalytic amount of DMAP in dichloromethane as the solvent produced β-lactam **5** in 96% yield and β-lactam **6** in 90 % yield.

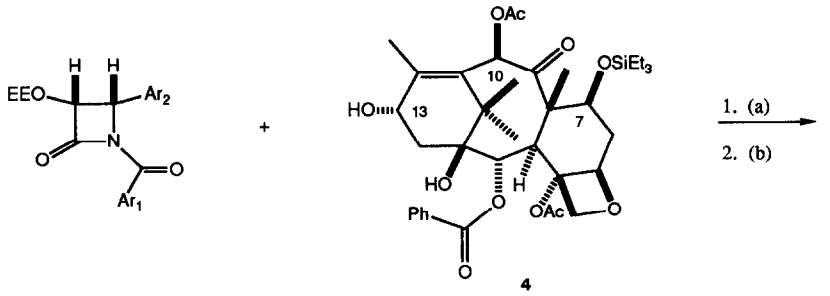
The novel taxol analogues **2** and **3** were examined[29] in comparison with taxol for their ability to promote microtubule assembly (10 μM tubulin concentration[30]). The concentrations of taxol (**1**), analogues **2** and **3** which produced a 50% effect (ED_{50}) were determined and found to be 0.7, 1.7, and 1.3 μM respectively.

The cytotoxicity of the new analogues **2** and **3** was also tested[29] in comparison with taxol (**1**) against B16 melanoma cells in culture. The concentrations of the compounds which produced 50% inhibition of proliferation after 40 h (ED_{50}) are 28 nM for taxol (**1**), 43 nM for derivative **2**, and 61 nM for analogue **3**.

Thus it was found that the new taxol analogues **2** and **3** have activity in the microtubule assembly assay and against B16 melanoma cells, which is comparable to taxol (**1**).

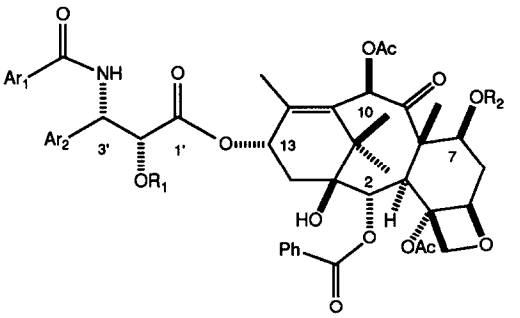
Acknowledgements: Financial assistance from the National Institutes of Health (CA 52790) and the American Cancer Society (IN-115K) is acknowledged. A mixture of taxol (**1**) and cephalomannine was provided to us for these studies by the National Cancer Institute. We would like to thank Ms. Jeanne Ellermeier for her excellent technical assistance, Charles Burke for performing some of the tubulin assembly assays, and Dr. David Vander Velde for his expert assistance with some NMR experiments.

Scheme 1



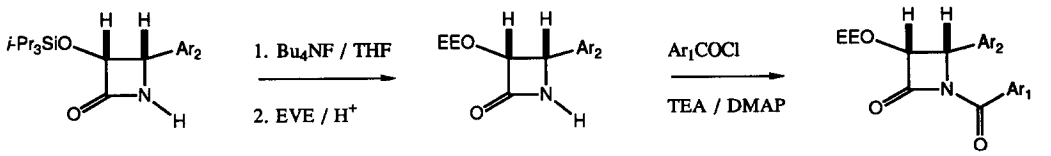
5 Ar_1 = *p*-chlorophenyl, Ar_2 = phenyl
6 Ar_1 = phenyl, Ar_2 = *p*-chlorophenyl

(a) ß-lactam (5 equiv), pyridine, 4-dimethylaminopyridine (1 equiv.), 25 °C, 24 h;
(b) EtOH / HCl (0.5%), 0 °C, 4 days



1 Ar_1, Ar_2 = phenyl; R_1, R_2 = H; taxol (1)
2 Ar_1 = *p*-chlorophenyl; Ar_2 = phenyl; R_1, R_2 = H
3 Ar_1 = phenyl; Ar_2 = *p*-chlorophenyl; R_1, R_2 = H
7 Ar_1 = *p*-chlorophenyl; Ar_2 = phenyl; R_1 = ethoxyethyl; R_2 = tricthylsilyl
8 Ar_1 = phenyl; Ar_2 = *p*-chlorophenyl; R_1 = ethoxyethyl; R_2 = triethylsilyl

Scheme 2



9,10 **11,12** **5, 6**

5, 9, 11 Ar_2 = phenyl; **6, 10, 12** Ar_2 = *p*-chlorophenyl

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