Pergamon

Tetrahedron Letters, Vol. 38, No. 24, pp. 4273-4276, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00877-0

Synthesis and Evaluation of C-*Seco* Paclitaxel Analogues[1]

Giovanni Appendino,[a]* Bruno Danieli,[b]* Jasmin Jakupovic,[c] Emanuela Belloro[a], Giovanni Scambia[d] and Ezio Bombardelli[e]

a: Dipartimento di Scienza e Tecnologia del Farmaco via Giuria 9, 10125 Torino, Italy

b: Dipartimento di Chimica Organica e Industriale, e Centro CNR di Studio sulle Sostanze Organiche Naturali, via Venezian 21, 20133 Milano, Italy

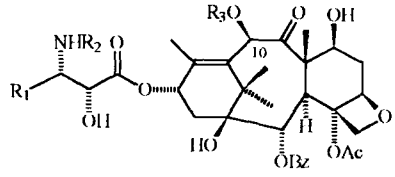
c: Institut für Organische Chemie, Technische Universität, Straße des 17. Juni 135, 10623 Berlin, Germany

d: Istituto di Clinica Ostetrica e Ginecologica, Università Cattolica del Sacro Cuore, Largo Gemelli 8, 00168 Roma, Italy

e: Indena S.p.A., viale Ortles 12, 20141 Milano, Italy

Abstract: Starting from 7,9-diTes-10-dehydro C-*seco*baccatin III (**4a**), C-*seco* analogues of paclitaxel retaining biological activity were synthesised. © 1997 Elsevier Science Ltd.

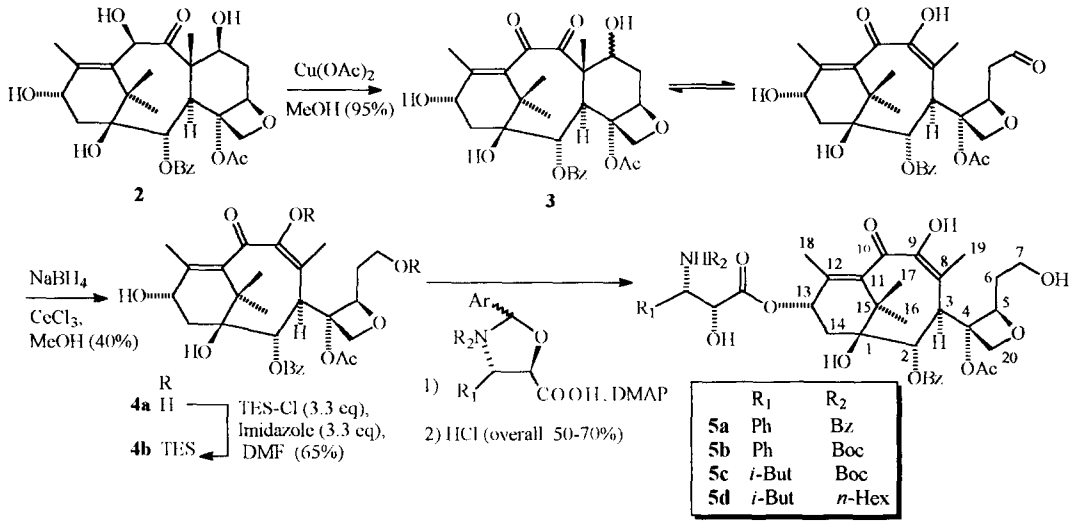
Over the past few years, considerable attention has been given to the synthesis of chemical analogues of paclitaxel (=Taxol®) (**1a**).[2] Biological analogues, that is compounds structurally unrelated to paclitaxel but sharing its recognition site, have also been discovered (epothilones[3a,b], discodermolide[3c]) All active chemical analogues described to date are derived from natural baccatins, and have the same tetracyclic core of paclitaxel.[2] Indeed, structure-activity studies have highlighted the important scaffolding role of the diterpenoid moiety of the natural product for an optimal interaction between the ester groups at C-2, C-4, C-13 and their complementary binding sites on tubulin.[2] The design of totally synthetic and simplified analogues of paclitaxel has so far been hampered by the lack of understanding of the critical molecular topology underlying its biological activity.[4] Furthermore, most libraries of analogues were obtained by deliberate modification at a single position. When multiple changes were considered, only positions known to tolerate a wide variety of substituents were considered. Thus, the effect of combined modifications is still largely unexplored, even though the most clinically useful analogue obtained to date (docetaxel = Taxotere®) (**1b**) combines, in terms of the structure-activity database of paclitaxel, a favourable modification on the aminoacidic side chain (*N*-Boc *vs* *N*-Bz) with an unfavourable modification on the terpenoid core (C-10 deacetylation).[5]



	R_1	R_2	R_3
1a	Ph	Bz	Ac
1b	Ph	Boc	H
1c	i-But	Boc	Ac

We now report the discovery of a novel class of analogues, some of which retain anticancer activity despite the opening of ring C, an operation having *per se* a detrimental effect on the activity of paclitaxel. The opening of the convex taxane core and a decreased number of stereogenic carbons make these compounds structurally simpler than all the other chemical analogues reported to date.

The synthesis of the C-*seco* analogues is outlined in Scheme 1. The chemoselective oxidation of 10-deacetylbaccatin III (**2**) followed by the reductive trapping ($NaBH_4$-$CeCl_3$) of the *seco*-aldehyde in retroaldol equilibration with **3** gave the seco taxane **4a** in overall 38 % yield.[6] The reductive trapping step was plagued by the competitive reduction of the α-diketone system, affording 10-epi-10-deacetylbaccatin III (15-20%)[6], 10-epi-10-deacetylbaccatin V (5-7%) and 9βH-9-dihydro-10-epi-10-deacetylbaccatin V (3-5%) as side products. The effect of various additives and reducing agents was investigated, but no substantial improvement in the yield of **4a** could be achieved.[7] Treatment of **4a** under the conditions used for the silylation of 10-deacetylbaccatin III (TES-Cl, pyridine)[8] gave a complex mixture. However, after considerable experimentation, a protocol affording the unstable 7,9-diTES derivative **4b** as the major reaction product (65 %) was eventually found.[9] As major by-products, the 7-TES- (5%), 9-TES- (4%) and 7,9,13-triTES- (15%) derivatives were obtained. Coupling of **4b** with *N*-benzoyl- and *N*-Boc (4*S*,5*R*)-2-(2,4-dimethoxyphenyl)-4-phenyl-5-oxazolidinecarboxylic acids,[10] gave, after acidic deprotection, **5a**[11] and **5b**[12], the C-*seco* analogues of paclitaxel and docetaxel, respectively.



Scheme 1. Synthesis of the C-seco taxoids **5a-d**

The citotoxicity of **5a,b** was tested towards normal- (MDA-MB231) and adriamycin-resistant (MCF-7 ADRr) breast tumour cells. **5a** showed a much reduced activity on both cell lines, especially on MDA-MB231 cells ($IC_{50}/IC_{50paclitaxel} > 110$, see Table 1), an observation consistent with the results reported for other paclitaxel analogues having a modified connectivity of ring C.[13] However, the decrease of activity for the C-*seco* analogue of docetaxel (**5b**) was not so marked (Table 1, entries 2 and 6), especially when compared with the data for 10-dehydrodocetaxel[14](Table 1, entry 4). This prompted us to explore the effect of further changes in the side-chain, and **5c**[15] and **5d**[16] were synthesised from the corresponding side-chain oxazolidine precursors.[10] **5c** and its corresponding taxane derivative (**1c**)[17] showed comparable nanomolar IC_{50} values against MDA-MB231 cells, and had similar tubulin assembly properties.[18] Also the C-*seco* compound of the 3'-dephenyl-3'-isobutyl *N*-debenzoyl, *N*-hexanoyl series (**5d**) retained considerable activity, and was even more active than paclitaxel on the adriamycin resistant cell line (Table 1, entry 8).

Taken together, these data suggest that structure-activity relationships within antitumour taxoids might be more complicated than expected, since compounds modified on the aminoacidic side chain show different structure-activity databases. On the other hand, they also show that, given a proper side-chain, cytotoxic activity comparable to that of paclitaxel can still be observed in compounds with a topological and stereochemical less complicated terpenoid core. The prospect of obtaining totally synthetic simplified variants of paclitaxel is as yet unrealised, but the activity of **5c** and **5d** points to the possibility to achieve this goal.

Table 1

Entry	Compound	IC_{50} (nM) (MDA-MB321)	IC_{50} (nM) (MCF-7 ADRr)
1	Paclitaxel (**1a**)	2.5	2,600
2	Docetaxel (**1b**)	0.8	700
3	**1c**	1.3	80
4	10-dehydrodocetaxel	3.1	5,000
5	**5a**	280	>10,000
6	**5b**	25	>5,000
7	**5c**	5.0	6,000
8	**5d**	33	1,000

REFERENCES AND NOTES

1. Part XXX in the series: The Chemistry and Occurrence of Taxane Derivatives. For part XXIX, see: Barboni, L.; Lambertucci, C.; Appendino, G.; Gabetta, B. *Phytochemistry.* **1997** (in press)
2. Chen, S.-H.; Farina, V. "Paclitaxel (Taxol®) Chemistry and Structure-Activity Relationships" in *The Chemistry and Pharmacology of Taxol® and its Derivatives* Farina, V. Ed.; Elsevier, 1995, pp. 165-253.
3. a) Bollag, D.M.;McQueney, P.A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C.M. *Canc. Res.* **1995**, *55*, 2325-2333. b) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567-1569. c) ter Haar, E.; Kowalski, R.J.; Hamel, E.; Lin, C.M.; Longley, R.E.; Gunasekera, S.P.; Rosenkranz, H.S.; Day, B.W. *Biochemistry* **1996**, *35*, 243-250.
4. a) Winkler, J.D.; Axelsen, P.H. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2963-2966. b) ter Haar, Rosenkranz, H.S.; Hamel, E.; Day, B.W. *Bioorg. Med. Chem.* **1996**, *4*, 1659-1671.
5. Compared to paclitaxel, 10-deacetylpaclitaxel is more than 20 times less cytotoxic on KB cells (McLaughlin, J.L.; Miller, R.W.; Powell, R.G.; Smith Jr., C.R. *J. Nat. Prod.* **1981**, *44*, 312-319), and *ca* 3 times less cytotoxic on J777.2 cells (Kingston, D.G.I.; Samaranayake, G.; Ivey, C.A. *J. Nat. Prod.* **1995**, *53*, 1-12). Deacetylation at C-10 caused a decreased cytotoxicity also in taxoids of the 3'-dephenyl-3'-alkyl type (Ojima, I.; Slater, J.C.; Michaud, E.; Kuduk, S.C.; Bounaud, P.-Y.; Vrignaud, P.; Bissery, M.-C.; Veith, J.M.; Pera, P.; Bernacki, R.J. *J. Med. Chem.* **1996**, *39*, 3889-3896). Interestingly, paclitaxel and 10-deacetylpaclitaxel have instead a similar affinity for tubulin (Guéritte-Voegelein, F.; Guénard, D.; Lavelle, F.; Le Goff, M.-T.; Mangatal, L.; Potier, P. *J. Med. Chem.* **1991**, *34*, 992-998).
6. Appendino, G.; Jakupovic, J.; Cravotto, G.; Enriù, R.; Varese, M.; Bombardelli, E. *Tetrahedron Lett.* **1995**, 3233-3236.
7. Parameters explored included alternative reducing agents (DIBAL, Red-Al®, KBH_4, $LiBH_4$, $Ca(BH_4)_2$, *n*-Bu_4NBH_4), alternative additives (no additive, $NiCl_2$) and solvents (EtOH, *i*-propanol).
8. Denis, J.-N.; Greene, A.E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917-5919.
9. To a solution of **4a** (542 mg, 1 mMol) in dry DMF (15 mL), imidazole (233 mg, 3.3 mMol, 3.3 mol. equiv.) and TES-Cl (551 µl, 495 mg, 3.3 mMol, 3.3 mol. equiv.) were added. After stirring at room temp. for 50 min., the reaction was worked up by pouring into a suspension of celite® (*ca* 500 mg) in water (*ca* 100 ml). The slurry was filtered (sintered glass funnel), and the cake was washed with water (2 x 50 mL) to remove DMF and then with EtOAc (2 x 25 ml) to recover the reaction products. After drying (Na_2SO_4) and evaporation, the residue was purified by CC (20 g silica gel) with a hexane-EtOAc gradient (9:1→ 8:2) to give, in order of elution, 132 mg (15%) of the 7,9,13-triTES derivative, 500 mg (65%) of the 7,9-diTES derivative (**4b**), 22 mg (5%) of the 7-TES derivative and 18 mg (4%) of the 9-TES derivative.
10. Johnson, R.A.; Nidy, E.G.; Dobrowolski, P.J.; Gebhard, I.; Qualls, S.J.; Wicnienski, N.A.; Kelly, R.C. *Tetrahedron Lett.* **1994**, 7893-7896.

11. ^{1}H NMR (400 MHz, $CDCl_3$, 60 °C): δ 8.08 (AA', *o*-Bz), 7.58 (C, *p*-Bz), 7.48 (BB', *m*-Bz), 7.43 (C, *p*-Ph), 7.41 (A,A'-*o*-Ph), 7.37 (B,B', *m*-Ph), 7.12 (d, J=9.5 Hz, NH), 6.45 (br s, 9-OH), 6.23 (ddq, J=10.0, 7.0, 1.5 Hz, H-13), 5.90 (dd, J=10.0, 3.0 Hz, H-3'), 5.63 (br d, J=9.0 Hz, H-2), 5.22 (br d, J=11.5 Hz, H-5), 5.18 (br d, J=8.0, H-20a), 4.82 (d, J=3.0 Hz, H-2'), 4.30 (br d, J=9.0 Hz, H-3), 4.29 (br d, J=8.0 Hz, H-20b), 3.89 (br ddd, J=11.0, 6.0, 6.0 Hz, H-7a), 3.72 (br ddd, J=11.0, 6.5, 6.0 Hz, H-7b), 2.85 (br dd, J=16.0, 7.0 Hz, H-14a), 2.59 (br m, H-6a), 2.39 (br dd, J=16.0, 10.0 Hz, H-14b), 2.10 (m, H-6b), 1.91 (br s, OAc), 1.89 (br s, H-19), 1.81 (d, J=1.5 Hz, H-18), 1.26 (s, H-17), 1.11 (s, H-16).
12. ^{1}H NMR (400 MHz, $CDCl_3$, 60°C): δ 8.05 (AA', *o*-Bz), 7.60 (C, *p*-Bz), 7.48 (BB', *m*-Bz), 7.41 (A,A'-*o*-Ph), 7.37 (B,B', *m*-Ph), 7.29 (C, *p*-Ph), 6.45 (s, 9-OH), 6.23 (ddq, J=10.0, 7.0, 1.5 Hz, H-13), 5.64 (br d, J=9.0 Hz, H-2), 5.49 (d, J=9.5 Hz, NH), 5.32 (dd, J=10.0, 3.0 Hz, H-3'), 5.26 (br d, J=11.5 Hz, H-5), 5.16 (d, J=8.0, H-20a), 4.67 (d, J=3.0 Hz, H-2'), 4.35 (br d, J=9.0 Hz, H-3), 4.29 (d, J=8.0 Hz, H-20b), 3.86 (ddd, J=11.0, 6.0, 6.0 Hz, H-7a), 3.70 (ddd, J=11.0, 6.5, 6.5 Hz, H-7b), 2.80 (br dd, J=16.0, 7.0 Hz, H-14a), 2.52 (m, H-6a), 2.44 (br dd, J=16.0, 10.0 Hz, H-14b), 2.18 (s, 1-OH), 2.10 (m, H-6b), 1.93 (s, OAc), 1.86 (br s, H-19), 1.85 (d, J=1.5 Hz, H-18), 1.31 (s, BOC), 1.28 (s, H-17), 1.12 (s, H-16).
13. a) Liang, X.; Kingston, D.G.I.; Long, B.H.; Fairchild, C.A.; Johnston, K.A. *Tetrahedron Lett.* **1995**, 7795-7798. b) Bouchard, H.; Pulicani, J.-P.; Vuilhorgne, M.; Bourzat, J.-D.; Commerçon, A. *Tetrahedron Lett.* **1994**, 9713-9716.
14. Prepared by oxidation of **1b** ((5 mol. equiv. $Cu(OAc)_2$, MeOH, 24 h, 54% 7β-epimer, 23% 7α–epimer).
15. ^{1}H NMR (400 MHz, $CDCl_3$, 60 °C): δ 8.06 (AA', *o*-Bz), 7.59 (C, *p*-Bz), 7.47 (BB', *m*-Bz), 6.43 (br s, 9-OH), 6.22 (ddq, J=10.0, 7.0, 1.5 Hz, H-13), 5.63 (br d, J=9.0 Hz, H-2), 5.25 (br d, J=11.0 Hz, H-5), 5.13 (br d, J=8.0, H-20a), 4.69 (d, J=10.0 Hz, NH), 4.39 (br d, J=9.0 Hz, H-3), 4.30 (br d, J=8.0 Hz, H-20b), 4.25 (d, J=3.0 Hz, H-2'), 4.18 (dddd, J=10.0, 10.0, 5.0, 3.0 Hz, H-3'), 3.87 (ddd, J=11.0, 6.0, 6.0 Hz, H-7a), 3.72 (ddd, J=11.0, 6.0, 6.0 Hz, H-7b), 2.83 (br dd, J=16.0, 7.0 Hz, H-14a), 2.49 (br m, H-6a), 2.47 (br dd, J=16.0, 10.0 Hz, H-14b), 2.10 (m, H-6b), 1.94 (br s, OAc), 1.90 (br s, H-19), 1.89 (d, J=1.5 Hz, H-18), 1.72 (m, H-5'), 1.61 (ddd, J=14.0, 10.0, 6.0 Hz, H-4'a), 1.44 (ddd, J=14.0, 8.0, 5.0 Hz, H-4'b), 1.31 (s, BOC), 1.27 (s, H-17), 1.12 (s, H-16), 1.00 (d, J=6.0 Hz, H-6'), 0.99 (d, J=6.0 Hz, H-7'). ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.1 (s, C-10), 173.3 (s, C-1'), 169.0 (s, 4-OAc), 167.4 (s, Bz), 155.8 (s, BOC), 148.8 (s, C-9), 142.1 (s, C-11), 136.8 (s, C-12), 133.8 (d, *p*-Bz), 129.7 (d, *m*-Bz), 129.3 (*i*-Bz), 128.9 (d, *o*-Bz), 124.4 (s, C-8), 87.2 (d, C-5), 86.1 (s, C-4), 80.3 (s, C-1), 79.9 (s, BOC), 74.8 (d, C-2), 74.8 (t, C-20), 73.4 (d, C-2'), 70.5 (d, C-13), 59.7 (t, C-7), 51.4 (d, C-3'), 44.4 (d, C-3), 42.9 (s, C-15), 40.8 (t, C-4'), 37.5 (t, C-6), 36.5 (t, C-14), 28.1 (q, BOC), 24.9 (d, C-5'), 24.7 (q, C-17), 23.1 (q, C-6'), 22.1 (q, C-7'), 22.1 (q, 4-OAc), 21.2 (q, C-16), 14.8 (q, C-18), 14.4 (q, C-19).
16. ^{1}H NMR (400 MHz, $CDCl_3$, 60 °C): δ 8.09 (AA', *o*-Bz), 7.59 (C, *p*-Bz), 7.48 (BB', *m*-Bz), 6.42 (br s, 9-OH), 6.22 (ddq, J=9.0, 7.0, 1.5 Hz, H-13), 5.99 (d, J=9.5 Hz, NH), 5.63 (br d, J=9.0 Hz, H-2), 5.22 (br d, J=11.5 Hz, H-5), 5.15 (br d, J=8.0, H-20a), 4.54 (dddd, J=9.5, 9.5, 5.5, 3.0 Hz, H-3'), 4.33 (br d, J=9.0 Hz, H-3), 4.30 (br d, J=8.0 Hz, H-20b), 4.29 (d, J=3.0 Hz, H-2'), 3.89 (br ddd, J=11.0, 6.0, 6.0 Hz, H-7a), 3.70 (br ddd, J=11.0, 7.0, 6.0 Hz, H-7b), 2.82 (br dd, J=16.0, 7.0 Hz, H-14a), 2.52 (br m, H-6a), 2.15 (br s, 1-OH), 2.06 (br m, H-6b), 2.43 (br dd, J=16.0, 9.0 Hz, H-14b), 2.09 (t, J=8.0 Hz, 2-Hex), 1.93 (br s, OAc), 1.90 (br s, H-19), 1.89 (d, J=1.5 Hz, H-18), 1.61 (ddd, J=14.0, 9.5, 5.5 Hz, H-4'a), 1.51 (m, H-4'b), 1.51 (m, 3-Hex), 1.70 (m, H-5'), 1.28 (s, H-17), 1.22 (m, 4-Hex and 5-Hex), 1.13 (s, H-16), 1.00 (d, J=6.0 Hz, H-6',H-7'), 0.83 (t, J=6.5Hz, 6-Hex).
17. Ojima, I.; Duclos, O.; Kuduk, S.D.; Sun C.-M.; Slater, J.C.; Lavelle, F.; Veith, J.M.; Bernacki, R.J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2631-2634.
18. The ED_{50}**5c**/ED_{50}**1c** in the tubulin assembly assay was 1.8.

(*Received in UK* 26 *March* 1997; *revised* 25 *April* 1997; *accepted* 2 *May* 1997)