Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 14, pp. 1941-1944, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00337-4

SYNTHESIS OF NOVEL TAXOL ANALOGS AND EVALUATION OF THEIR BIOLOGICAL ACTIVITIES

Paul A. Wender*, Daesung Lee and Tapan K. Lal
Department of Chemistry, Stanford University, Stanford, CA 94305

Susan B. Horwitz* and Srinivasa Rao
Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

Abstract: Two new taxol analogs **6** and **10** have been prepared from baccatin III (**1**) and taxol (**7a**), respectively. Like taxol, both compounds were found to promote microtubule formation and stabilization, although they were less active than taxol. Both **6** and **10** exhibited cytotoxicity against J774.2 cells; **6** was ~60-fold less active and **10** was ~15-fold less active. © 1997 Elsevier Science Ltd.

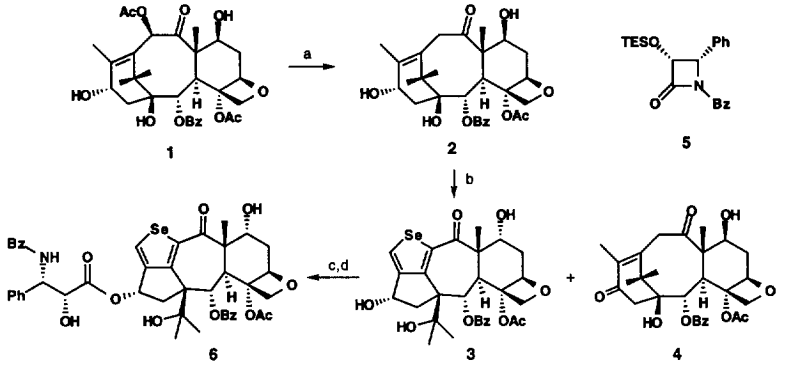
Taxol®[1] is a novel diterpenoid extracted from the bark of the western yew, *Taxus brevifolia*,[2] and a particularly significant new lead for the treatment of cancer.[3] Thus far, it has been approved by the FDA for the treatment of advanced ovarian and metastatic breast cancer and is currently in phase II and III clinical trials for lung and other cancers.[4] Adding to the medicinal significance of this compound is the finding that it operates through a novel mode of action involving facilitated assembly and stabilization of microtubules.[5] While the molecular basis for this action is not understood, advances in recent years have done much to define which structural features of taxol are required for activity.[6] Photoaffinity labeling studies with taxol analogs indicate that taxol binds to the N-terminal region of β-tubulin and a peptide containing β-tubulin amino acid residues 217-231.[7]

One of the principal goals associated with research in this area is the identification of the structural features of taxol required for its biochemical and physiological performance. Ultimately, such research can be expected to lead to the identification of structurally simpler and clinically more effective analogs that could be made in a practical fashion through total synthesis.[8] Toward this end, we now report the synthesis and assay of the novel taxol analogs **6** and **10**. The former is a representative member of a new analog class, the first to incorporate selenium and is readily derived from baccatin III (**1**). The latter is a novel C-ring contracted analog which has also been independently obtained from taxol (**7a**) by the Kingston group.[9]

The synthesis of **6** started with baccatin III (**1**) (Scheme I). Reductive cleavage of the 10-acetoxy group was achieved with samarium diiodide and acetic acid in THF.[10] Treatment of the resultant product (**2**) with an excess amount (10–15 equiv) of selenium dioxide in pyridine at 85 °C yielded the selenophene-containing compound **3**[11] in 45% yield together with 10-deacetoxy-13-oxo-baccatin III (**4**) (25%). Coupling of **3** with β-lactam **5**[12] in the presence of sodium hexamethyldisilazide (2.5 equiv) in THF followed by deprotection with hydrogen fluoride in pyridine led to the selenophene-containing taxol analog **6** in 85% yield. The structure of **6** was assigned on the basis of ^{1}H, ^{13}C, ^{77}Se NMR spectra, and FAB-HRMS[13] and by comparison with the 7β-epimer, which was independently obtained through the coupling procedure by using only one equivalent of sodium hexamethyldisilazide.

For the preparation of the 5-membered C-ring taxol analog (**10**), taxol (**7a**) was treated with *t*-butyldimethylsilyl chloride and imidazole in dimethyl formamide[14] to give 2'-(*t*-butyldimethylsilyl)taxol (**7b**)

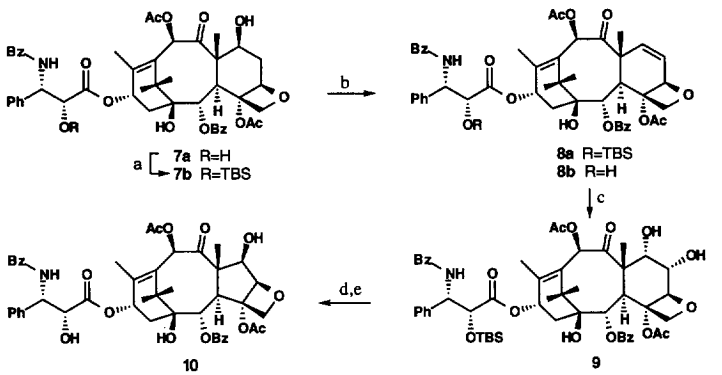
Scheme I



(a) SmI_2, HOAc, THF; (b) SeO_2, Pyr; (c) NaHMDS, Ojima's β-lactam (5), THF; (d) HF, Pyr

in 99% yield (Scheme II). Treatment of **7b** with trifluoromethanesulfonic anhydride and pyridine in dichloromethane at 25 °C and subsequently with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at the same temperature for 20 h afforded 7-deoxy-2'-(*t*-butyldimethylsilyl)-$\Delta^{6,7}$-taxol (**8a**) in 70% yield together with 24% of the deprotected compound **8b**. Dihydroxylation of **8a** with osmium tetroxide in pyridine-THF (1:1) at 25 °C yielded 2'-(*t*-butyldimethylsilyl)-6α-hydroxy-7-epitaxol (**9**) in 85% yield.[15] Cleavage of diol **9** with lead tetraacetate in the presence of sodium bicarbonate in benzene at 25 °C yielded three compounds.

Scheme II



(a) TBSCl, Imidazole, DMF; (b) Tf_2O, Pyr then DBU; (c) OsO_4, Pyr-THF
(d) $Pb(OAc)_4$, $NaHCO_3$, PhH; (e) HF, Pyr

The major component was isolated in 40% yield after deprotection of the *t*-butyldimethylsilyl group. Extensive spectroscopic characterization of this compound[16] led to the structural assignment as the C ring-contracted taxol **10**.

Both structurally modified taxol analogs **6** and **10** were directly compared to taxol for their biological activities. At identical concentrations, both analogs were found to promote polymerization of tubulin and stabilize the microtubules, although neither was as active as taxol. Both analogs are also less cytotoxic than taxol for J774.2 cells. ED_{50} values for taxol and analogs **6** and **10** are shown in Table I. These results show that activity is retained in A- and C-ring modified taxol analogs **6** and **10**. Both analogs can mimic the active

binding conformation of taxol. Conformational analyses of **6** and **10** by NMR spectroscopy and molecular modeling indicated that both assume conformations similar to taxol in chloroform.[17]

Table I: Cytotoxicity of taxol analogs **6** and **10** versus taxol

compound	$ED_{50}(\mu M)$
taxol	0.016
6	0.90
10	0.25

Exponentially growing J774.2 cells (2 x 10^4 / mL) were placed in multi-well plates. Either taxol, **6**, or **10** was added at various concentrations and incubated at 37°C. After 72h the cell number was determined.

Previous work has shown that taxol-like activity is retained in taxol analogs with partially modified or deleted functionality. The current and related studies suggest that activity can also be retained through modifications of the taxol carbotricyclic core. Further studies are in progress.

Acknowledgment: This research was supported by a grant from the National Institutes of Health (CA31845). A Fellowship Award to D.L. from Syntex is also gratefully acknowledged.

References and Notes

1. Taxol is a registered trademark of Bristol-Myers Squibb Inc. which offers the generic name paclitaxel.
2. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggen, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325.
3. (a) Rowinsky, E. K.; Cazenave, L. A.; Donehower, R. C. *J. Natl. Cancer Inst.* **1990**, *82*, 1247; (b) Slichenmyer, W. J.; Von Hoff, D. D. *J. Clin. Pharmacol.* **1990**, *30*, 770; (c) Slichenmyer, W. J.; Von Hoff, D. D. *Anti Cancer Drugs* **1991**, *2*, 519.
4. Suffness, M. *Ann. Rep. Med. Chem.* **1993**, *28*, 305.
5. (a) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665; (b) Horwitz, S. B. *Trends Pharmacol. Sci.* **1992**, *13*, 134; (c) Andreu, J. M.; Diaz, J. F.; Garcia de Ancos, J.; Gil, R.; Mendrano, F. J.; Nogales, E.; Pantos, E.; Towns-Andrews, E. *J. Mol. Biol.* **1992**, *226*, 169; (d) Diaz, J. F.; Andreu, J. M. *Biochemistry* **1993**, *32*, 2747; (e) Jordan, M. A.; Toso, R. J.; Thrower, D.; Wilson, L. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 9552; (f) Arnal, I.; Wade, R. H. *Current Biol.* **1995**, *5*, 900; (g) ter Har, 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; (h) Hung, D. T.; Chen, J.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 287.
6. (a) Suffness, M.; Cordell, D. A. *The Alkaloids: Chemistry and Pharmacology*; Academic: New York, 1985, Vol. 25, pp 3–355; (b) Blechert, S.; Guénard, D. *The Alkaloids: Chemistry and Pharmacology*; Academic: San Diego, 1990; Vol. 39, pp 195–238.
7. (a) Rao, S.; Horwitz, S. B.; Ringel, I. *J. Natl. Cancer Inst.* **1992**, *84*, 785; (b) Rao, S.; Krauss, N. E.; Heerding, J. M.; Swindell, C. S.; Ringel, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1994**, *269*, 3132; (c) Rao, S.; Orr, G. A.; Chaudhary, A. G.; Kingston D. G. I.; Horwitz, S. B. *J. Biol. Chem.* **1995**, *270*, 20235; (d) Dasgupta, D.; Park, H.; Harriman, G. C. B.; Georg, G. I.; Himes, R. *J. Med. Chem.* **1994**, *37*, 2976.
8. (a) Wender, P. A.; Mucciaro, T. P. *J. Am. Chem. Soc.* **1992**, *114*, 5878; (b) Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; Gränicher, C.; Houze, J. B.; Jänichen, J.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Mucciaro, T. P.; Mühlebach, M.; Natchus, M. G.; Paulsen, P.; Rawlins, D. B.; Satkofsky, J.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E.; Tomooka, K. *J. Am. Chem. Soc.* **1997**, *119*, 2755; (c) Wender, P. A.; Badham, N. F.; Conway, S. P.; Floreancig, P. E.; Glass, T. E.; Houze, J. B.; Krauss, N. E.; Lee, D.; Marquess, D. G.; McGrane, P. L.; Meng, W.; Natchus, M. G.; Shuker, A. J.; Sutton, J. C.; Taylor, R. E. *J. Am. Chem. Soc.* **1997**, *119*, 2757; (d) Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. *Taxane Anticancer Agent: Basic Science and Current Status*; American Chemical Society: Washington, DC, 1994; (d) Suffness, M. *Taxol®: Science and Applications*; CRC: Boca Raton, FL, 1995.
9. During the preparation of this manuscript, the synthesis of **10** was published. (a) Liang, X.; Kingston, D. G. I.; Long, B. H.; Fairchild, C. A.; Johnston, K. A. *Tetrahedron Lett.* **1995**, *36*, 7795; (b) Liang, X.; Kingston, D. G. I.; Long, B. H.; Fairchild, C. A.; Johnston, K. A. *Tetrahedron* **1997**, *53*, 3441.
10. Georg, G. I.; Cheruvallath, Z. S. *J. Org. Chem.* **1994**, *59*, 4015.

11. A similar skeletal rearrangement was observed on a related systems. (a) Samaranayake, G.; Magri, N. F.; Jitrangsri, C.; Kingston, D. G. I. *J. Org. Chem.* **1991**, *56*, 5114; (b) Chen, S.-H.; Huang, S.; Wei, J.; Farina, V. *J. Org. Chem.* **1993**, *58*, 4520; (c) Zamir, L.; Zheng, Y. F.; Caron, G.; Sauriol, F.; Mamer, O. *Tetrahedron Lett.* **1996**, *37*, 6435. Spectroscopic data for **3**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.19 (t, J = 46 Hz, 1H, 18-H), 8.08 (d, J = 7.2 Hz, 2H), 7.64 (t, J = 7.5 Hz, 1H), 7.49 (t, J = 7.6 Hz, 2H), 6.25 (d, J = 8.9 Hz, 2H), 5.18 (q, J = 6.2 Hz, 1H, 13-H), 4.92 (dd, J = 9.2, 2.8 Hz, 1H, 5-H), 4.55 (d, J = 8.2 Hz, 1H, 20-H), 4.18 (d, J = 8.2 Hz, 1H, 20-H), 3.76 (ddd, J = 10.1, 6.9, 1.5 Hz, 1H, 7-H), 3.18 (d, J = 9.5 Hz, 1H, 3-H), 3.08 (d, J = 1.5 Hz, 1H, 7-OH), 3.00 (dd, J = 14.3, 6.7 Hz, 1H, 14-H), 2.58 (ddd, J = 14.4, 9.2, 6.9 Hz, 1H, 6-H), 2.46 (dd, J = 14.3, 7.3 Hz, 1H, 14-H), 2.18 (s, 3H, 4-OAc), 2.06 (d, J = 6.5 Hz, 1H, 13-OH), 1.94 (ddd, J = 14.4, 10.1, 2.8 Hz, 1H, 6-H), 1.84 (s, 3H, 19-Me), 1.11 (s, 3H, 17-Me), 1.02 (s, 3H, 16-Me). The selenium NMR spectra was recorded using neat PhSeH as external standard. ^{77}Se NMR ($CDCl_3$, 95.3 MHz) δ 1959.68 (d, J = 46 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.9, 169.8, 165.9, 154.5, 153.8, 133.8, 129.8, 129.5, 129.3, 128.7, 83.7, 79.9, 75.5, 75.2, 69.9, 69.7, 69.2, 62.8, 57.4, 46.9, 45.6, 35.5, 27.0, 26.0, 21.8, 9.6. HRMS (FAB) calcd for $C_{29}H_{33}O_9{}^{80}Se$ (MH^+) 605.1289, found 605.1286. The structure of **3** was further secured by x-ray crystallography. Crystallographic data (collected at CHEXRAY of UC Berkeley): A colorless orthorhombic crystal with dimensions of 0.14 x 0.25 x 0.30 mm^3 was mounted on a glass fiber in paratone oil at -80 °C. The space group is $C222_1$ (#20) and cell constants are a = 10.0936(2) Å, b = 15.3218(2) Å, c = 35.5927(4) Å, V = 5504.5(1) Å^3, and Z = 8. All measurements were made on a Siemens SMART diffractometer with graphite-monochromated Mo-Kα radiation. The data were collected at a temperature of -129 ± 1°C using the ω scan technique to a maximum 2θ value of 46.6°. The structure was solved by direct method (SHELXS-86) and expanded using Fourier techniques. The final cycle of full-matrix least-square refinement was based on 3422 observed reflections ($I > 3\sigma(I)$) and 361 variable parameters and converged with R = 0.030, R_w = 0.035.
12. (a) Ojima, I.; Habus, I.; Zhao, M.; Georg, G. I.; Jayasinghe, L. R. *J. Org. Chem.* **1991**, *56*, 1681; (b) Holton, R. A. *Chem. Abstr.* **1990**, *114*, 164568q.
13. Spectroscopic data for **6**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.20 (d, J = 7.8 Hz, 2H), 8.01 (s, 1H, 18-H), 7.70 (d, J = 7.8 Hz, 2H), 7.57 (t, J = 7.5 Hz, 1H), 7.34-7.52 (m, 10H), 6.93 (d, J = 9.2 Hz, 3'-NH), 6.28 (d, J = 9.5 Hz, 1H, 2-H), 6.11 (t, J = 7.0 Hz, 1H, 13-H), 5.79 (dd, J = 9.2, 1.8 Hz, 1H, 3'-H), 4.93 (t, J = 6.4 Hz, 1H, 5-H), 4.75 (dt, J = 3.6, 1.8 Hz, 1H, 2'-H), 4.68 (d, J = 8.5 Hz, 1H, 20-H), 4.25 (d, J = 8.5 Hz, 1H, 20-H), 4.15 (dt, J = 11.8, 3.5 Hz, 1H, 7-H), 4.02 (d, J = 11.8 Hz, 1H, 7-OH), 3.44 (d, J = 3.6 Hz, 1H, 2'-OH), 3.40 (d, J = 9.5 Hz, 1H, 3-H), 2.91 (d, J = 7.0 Hz, 2H, 14-H), 2.38 (s, 3H, Me), 2.36 (dd, J = 6.4, 3.5 Hz, 2H, 6-H), 1.78 (s, 3H, Me), 1.11 (s, 3H, Me), 1.01 (s, 3H, Me). ^{77}Se NMR ($CDCl_3$, 95.3 MHz) δ 1948.92 (d, J = 46 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 202.6, 172.6, 172.0, 167.0, 165.9, 151.6, 148.0, 138.3, 138.1, 133.9, 133.7, 131.8, 130.5, 130.2, 129.3, 129.0, 128.8, 128.6, 128.1, 127.0, 126.9, 126.8, 83.0, 81.1, 76.1, 75.7, 73.6, 73.3, 68.9, 63.0, 56.6, 54.7, 42.2, 41.5, 35.5, 29.7, 26.9, 25.9, 21.8, 15.8. HRMS (FAB) calcd for $C_{45}H_{46}NO_{12}{}^{80}Se$ (MH^+) 872.2185, found 872.2235.
14. Magri, N. F.; Kingston, D. G. I. *J. Nat. Prod.* **1988**, *51*, 298.
15. (a) Johnson, R. A.; Nidy, E. G.; Dobrowolski, P. J.; Gebhard, I.; Qualls, S. J.; Wicnienski, N. A.; Kelly, R. C. *Tetrahedron Lett.* **1994**, *35*, 7893; (b) Liang, X.; Kingston, D. G. I.; Lin, C. M.; Hamel, E. *Tetrahedron Lett.* **1995**, *36*, 2901.
16. Spectroscopic data for **10**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.20 (d, J = 8.8 Hz 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.61 (t, J = 7.6 Hz, 1H), 7.31-7.53 (m, 10H), 6.95 (d, J = 9.1 Hz, 3'-NH), 6.45 (s, 1H, 10-H), 6.27 (br t, J = 8.5 Hz, 1H, 13-H), 5.81 (m, J = 9.1 Hz, J = 7.6, 1.5 Hz, 2H, 2-H, 3'-H), 5.13 (d, J = 4.4 Hz, 1H, 5-H), 4.79 (d, J = 1.5 Hz, 1H, 2'-H), 4.45 (d, J = 8.2 Hz, 1H, 20-H), 4.35 (d, J = 7.6 Hz, 1H, 3-H), 4.33 (dd, J = 7.6, 4.4 Hz, 1H, 7-H), 4.28 (d, J = 8.2 Hz, 1H, 20-H), 3.48 (br s, 1H, 2'-OH), 2.53 (d, J = 7.6 Hz, 1H, 7-OH), 2.43 (s, 3H, Me), 2.32 (dd, J = 15.6, 9.5 Hz, 1H, 14-H), 2.21 (s, 3H, Me), 2.20 (dd, J = 15.6, 8.5 Hz, 1H, 14-H), 1.85 (s, 3H, Me), 1.84 (s, 3H, Me), 1.23 (s, 3H, Me), 1.14 (s, Me, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.3, 172.6, 169.7, 167.0, 166.9, 140.4, 138.0, 135.9, 133.7, 133.5, 131.9, 130.3, 129.2, 128.8, 128.7, 128.6, 128.3, 127.0, 126.8, 87.7, 81.7, 78.6, 75.6, 74.2, 73.5, 73.2, 72.3, 59.8, 54.7, 44.5, 42.7, 36.9, 26.5, 22.0, 21.0, 20.7, 14.6, 12.9. HRMS (FAB) calcd for $C_{46}H_{49}NNaO_{14}$ (MNa^+) 862.3051, found 862.3063.
17. (a) Williams, H. J.; Scott, A. I.; Dieden, R. A.; Swindell, C. S.; Chirlian, L. E.; Francl, M. M.; Heerding, J. M.; Krauss, N. E. *Can. J. Chem.* **1994**, *72*, 252; (b) Dubois, J.; Guénard, D.; Guéritte-Voegelein, F.; Guedira, N.; Poiter, P.; Gillet, B.; Beloeil, J-C. *Tetrahedron* **1993**, *49*, 6533; (c) Paloma, L. G.; Guy, R. K.; Wrasidlo, W.; Nicolaou, K. C. *Chem. Biol.* **1994**, *1*, 107.

(*Received in USA* 21 *April* 1997; *accepted* 24 *June* 1997)