Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 809–810

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Metabolically Blocked Paclitaxel Analogues

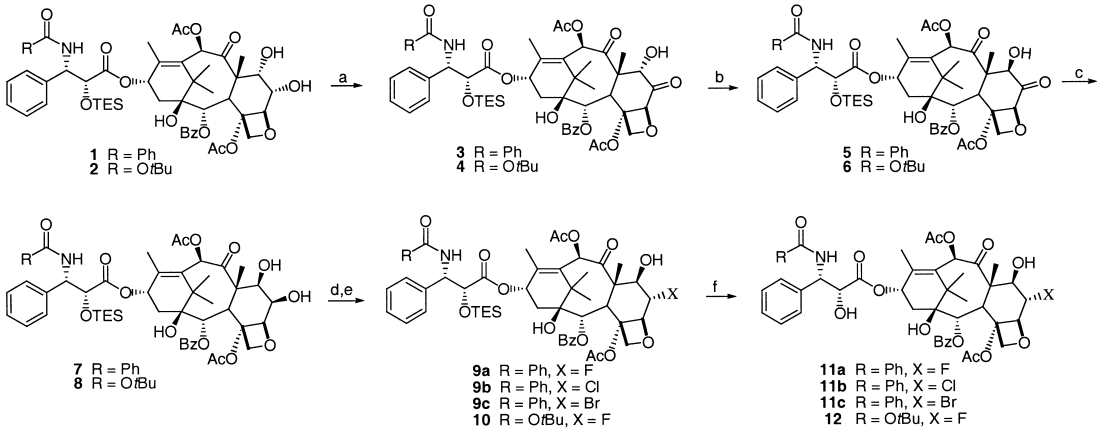
Mark D. Wittman,[a,*] Thomas J. Altstadt,[a] Craig Fairchild,[b] Stephen Hansel,[c] Kathy Johnston,[b] John F. Kadow,[a] Byron H. Long,[b] William C. Rose,[b] Dolatrai M. Vyas,[a] Mu-Jen Wu[a] and Mary E. Zoeckler[c]

[a]*Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 5100, Wallingford, CT 06492-7660, USA*
[b]*Oncology Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, USA*
[c]*Department of Metabolism and Pharmacokinetics, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 5100, Wallingford, CT 06492-7660, USA*

Received 1 November 2000; accepted 25 January 2001

Abstract—The stereospecific syntheses of the metabolically blocked 6-α-F, Cl, Br paclitaxel, and 6-α-F-10-acetyldocetaxel are described and in vitro and in vivo activity is presented.

Several studies have elucidated the metabolic fate of paclitaxel in humans, rats, and mice. The major human metabolite of paclitaxel has been identified as 6-α-hydroxypaclitaxel and is 30-fold less active than paclitaxel itself.[1,2] Studies in humans have revealed a significant portion of the dose of paclitaxel is excreted through the bile as 6-α-hydroxypaclitaxel.[3] Thus, 6-hydroxylation of paclitaxel represents a route for the detoxification and elimination of paclitaxel in humans. Blockade of this metabolic pathway should therefore provide taxanes with improved therapeutic efficacy and reduced clearance. Metabolic stabilization could also reduce the toxicity associated with taxane treatment by reducing the dose needed to achieve antitumor efficacy. For this reason, we became interested in preparing taxane analogues that maintained antitumor efficacy but would be less prone to metabolic deactivation via 6-α-hydroxylation. We felt that introduction of an α-fluorine would



Scheme 1. Reagents and conditions: (a) 4-BzO-TEMPO (2%), KBr, Chlorox®; (b) silica gel, CH_2Cl_2; (c) $Na(OAc)_3BH$, HOAc, CH_3CN (75% overall yield for a–c); (d) $SOCl_2$, Et_3N; $NaIO_4$, $RuCl_3$, $CCl_4/CH_3CN/H_2O$ (77%); (e) Bu_4NX (F, Cl, Br), THF (76%); (f) 1 N HCl, acetonitrile (85%).

*Corresponding author. Tel.: +1-203-677-6318; fax: +1-203-677-7702; e-mail: mark.wittman@bms.com

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00066-X

Table 1. Biological activity of C-6α halogenated taxanes

Compound	Tubulin[a]	IC_{50} HCT-116 (nM)	M109[b]
Paclitaxel	1.0	2–4	197–215 (40, 60)
11a	1.0	1.4	[c]
11b	0.8	1.2	203 (200)
11c	1.1	2.6	214 (200)
12	0.6	0.2	269 (100)
10-Acetyldocetaxel	0.7	1.8	177 (32)

[a]Tubulin assay measures the initial rate of polymerization and is expressed as a ratio to that obtained for paclitaxel. Ratios less than 1 reflect analogues that show a more rapid polymerization rate than paclitaxel (see ref 6).
[b]%T/C analogue at the maximum tolerated dose (dose mg/kg). %T/C values of greater than 125 % are considered active.
[c]Not determined in this model system.

not significantly alter the biological activity of paclitaxel but would effectively block 6-α hydroxylation. With this goal in mind, we turned to chemistry we had developed for the synthesis of 6-α-hydroxy paclitaxel.[4]

Previously, we demonstrated a convenient procedure for conversion of the readily available C-6,7-α-diol to the corresponding β-diol.[4] The β-diol, **7**, served as a useful intermediate for introducing functionality at the 6-α-position via the cyclic sulfate since nucleophiles regioselectively add to the 6 position. The regioselectivity observed for the addition of nucleophiles to the cyclic sulfate results from the steric compression between the 7 position and the B ring acetate bearing methine. The cyclic sulfate obtained from diol **7** was treated with fluoride,[5] chloride, and bromide ion to provide 6-α halo analogues **9a**–**c**. Deprotection under acidic conditions afforded the targeted taxane analogues **11a**–**c**. This sequence was repeated with the docetaxel side chain to provide 6-α-fluoro-10-acetyldocetaxel, **12** (Scheme 1).

Analogues **11a**–**c** and **12** were evaluated for inhibition of tubulin polymerization[6] and cytotoxicity against the human colon tumor cell line, HCT-116.[7] Analogues **11a**–**c** and **12** had in vitro activity indistinguishable from paclitaxel or C-10-acetyldocetaxel. Analogues **11b**,**c** and **12** were evaluated in vivo using the M109 Madison murine lung carcinoma tumor model.[8] The in vivo activity of **11b**,**c** was equivalent to that observed for a reference dose of paclitaxel[9] (known to be quite effective but not necessarily optimized). The 6-α-fluoro-10-acetyldocetaxel, **12**, did produce an increase in lifespan greater than that observed for 10-acetyldocetaxel and paclitaxel in this model (Table 1). These results suggest that halogenation of the 6-α position does not alter the in vitro or in vivo efficacy of paclitaxel analogues. The potential advantages of these analogues in the clinic could not be adequately tested in murine model systems since both 6-α hydroxylation and 3′-*p*-phenyl hydroxylation pathways are observed in mice[10] as opposed to humans in which 6-hydroxylation predominates. To address the potential for improved metabolic stability in humans the analogues were evaluated in a human liver S9 fraction capable of hydroxylating paclitaxel.[11,4] There was no detectable metabolite formation from the halogenated taxanes **11a**–**c** in comparison with paclitaxel which generated detectable levels of the 6-α hydroxylated metabolite. These results show that 6-α halogenation does block to some degree the major metabolic pathway for paclitaxel.

References and Notes

1. Harris, J. W.; Katki, A.; Anderson, L. W.; Chmurny, G. N.; Paukstelis, J. V.; Collins, J. M. *J. Med. Chem.* **1994**, *37*, 706.
2. Sparreboom, A.; Huizing, M. T.; Boesen, J. J. B.; Nooijen, W. J.; van Tellingen, O.; Beijnen, J. H. *Cancer Chemother. Pharmacol.* **1995**, *36*, 299.
3. Walle, T.; Walle, U. K.; Kumar, G. N.; Bhalla, K. N. *Drug Metab. Dispos.* **1995**, *23*, 506.
4. Wittman, M. D.; Kadow, J. K.; Vyas, D. M. *Tetrahedron Lett.* **2000**, *41*, 4729.
5. Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.
6. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665 . An $E_{0.01}$was determined for each analogue and expressed as a ratio relative to paclitaxel.
7. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tieney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827. The IC_{50} for each compound was determined against HCT-116, a human colon tumor cell line sensitive to paclitaxel.
8. %T/C values of greater than 125 are consider active: Rose, W. C. *Cancer Treat. Rep.* **1981**, *65*, 299.
9. Rose, W. C. *Anti-Cancer Drugs* **1992**, *3*, 311.
10. Sparreboom, A.; van Tellingen, O.; Nooijen, W. J.; Beijnen, J. H.; Jos, H. *Anti-Cancer Drugs* **1996**, *7*, 78.
11. A pooled lot of P450 characterized human liver S-9 fractions were obtained from The International Institute for the Advancement of Medicine. All incubations were performed in duplicate at concn of 12.5 μg/mL (solubilized in 1.25% ethanol final volume) in S-9 preparations containing approximately 5 mg microsomal protein/mL, phosphate buffer (0.1 M pH 7.4) and an NADPH regenerating system. Incubations were performed at 37 °C for 120 min in a shaking water bath. Sample aliquots were analyzed by HPLC/UV after acetonitrile deproteinization. Paclitaxel was used as a control and generated detectable amounts of 6-α-hydroxy paclitaxel. Due to the limited extent of paclitaxel metabolism in this system it is difficult to make definitive conclusions about the extent to which analogues **11a**–**c** block this metabolic pathway.