Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 5, pp. 545-548, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00058-9

DESIGN AND SYNTHESIS OF NEW TAXOL-CONTAINING AMINOPHOSPHATES AS PROTAXOLS

Jih Ru Hwu,*[a,b] Gholam H. Hakimelahi,[b] Thota Sambaiah,[a] Himatkumar V. Patel,[a] Shwu-Chen Tsay,[a] Yiu-Kay Lai,[c] and Chien-Hui Lieu[c]

[a]*Organosilicon and Synthesis Laboratory, Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan 30043, Republic of China;*

[b]*Institute of Chemistry, Academia Sinica, Nankang, Taipei, Taiwan 11529, Republic of China; and*

[c]*Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan 30043, Republic of China*

Abstract: Taxol-containing aminophosphates **5–7** were synthesized as protaxols by a "one-flask" method; the intrinsic zwitterionic character increases their solubility to about six times higher than that of taxol in a phosphate buffer solution.

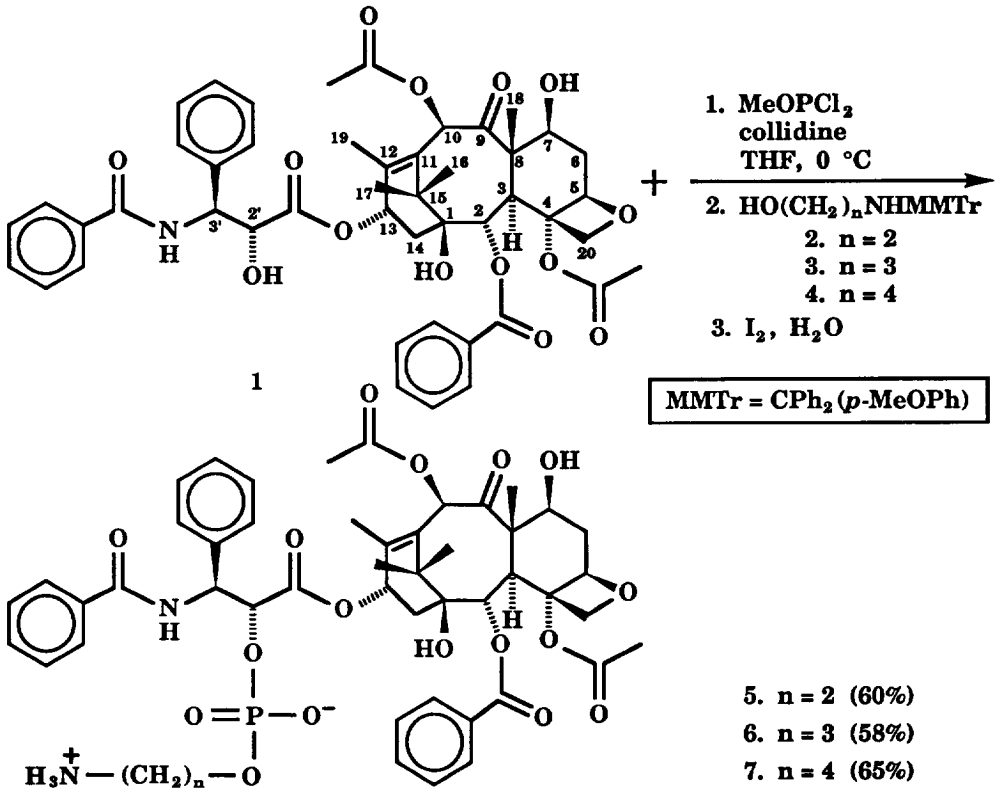
Taxol (**1**) exhibits excellent antitumor activity in a wide variety of tumor models, such as B16 melanoma, L1210 and P388 leukemias, MX-1 mammary tumor, and CX-1 colon tumor xenografts.[1–5] The mechanism of its action has been studied extensively;[6] the antitumor activity is due to its ability to promote tubulin assembly into micro-tubules.[6,7] In the presence of taxol, micro-tubules resist depolymerization; taxol thus stops the cell cycle.[8] In spite of its excellent antitumor activity, considerable difficulties exist in developing taxol as a chemotherapeutic agent. For example, the low aqueous solubility precludes its formulation and delivery into biological systems.

Structure–activity relationships and variations in solubility of taxol have been investigated through substitution at the C(2') hydroxyl group.[9–11] Esterification at the C(2') position results in loss of tubulin assembly activity in vitro but not cytotoxicity in vivo.[12,13] Therefore the problem of low solubility of taxol could be circumvented by development of a water soluble taxol prodrug that is chemically stable yet labile in vivo.[14,15]

In general, dephosphorylation occurs easier in cancer cells than in normal cells. Thus chemotherapeutic agents possessing a phosphate unit would preferentially interact with the cancer cells.[16,17] As the C(2') hydroxyl group in taxol is the most suitable site to attach the designed unit, we decided to synthesize aminophosphates **5–7**, which may exhibit specificity towards the tumor cells. Upon action of phosphodiesterases,[18] taxol will then be liberated in vivo as drug against tumor cells. Herein, we report the synthesis of taxol-containing aminophosphates **5–7** as protaxols.

We successfully developed a new "one-flask" method for the syntheses of aminophosphates **5–7**. Treatment of taxol (**1**) with $MeOPCl_2$ and collidine in THF and then with (monomethoxy)tritylated amino alcohol **2**, I_2, and water produced the desired zwitterionic compound **5** in 60% yield (Scheme 1).[19] Under the same conditions, we converted **1** to **6** in 58% yield and to **7** in 65% yield by using amino alcohols **3** and **4**, respectively. The solubility (μM/mL) in a phosphate buffer (0.10 M, pH 6.5) was 359 for **5**, 263 for **6**, 299 for **7**, and 58.6 for taxol (**1**). Taxol-containing aminophosphates **5–7** in their zwitterionic form showed ~6 times higher solubility than the parent taxol (**1**).

Scheme 1



The standard procedure for the preparation of taxol-2'-(aminoalkyl) phosphates **5–7** is as follows. Taxol (1.0 equiv) in dry THF (1.0 mL) was added dropwise to a solution of dry THF (1.0 mL) containing methyldichlorophosphite (1.5 equiv) and collidine (20.0 equiv) at 0 °C with stirring. After 20 min, *N*-(4-methoxytrityl)aminoalkanol (**2–4**, 1.0 equiv) in THF (0.50 mL) was added at 0 °C and stirring was continued for 30 min. To the solution at room temperature was added iodine (2.0 equiv) in a mixture of THF and H_2O (2:1, 3.0 mL). After 30 min, the solvent was removed under reduced pressure and the resultant solid was

dissolved in ethyl acetate (20 mL). The solution was washed with water (3 × 20 mL), 5% aqueous $Na_2S_2O_3$ (5.0 mL), and brine (1 × 20 mL), and then dried over $MgSO_4$(s). It was then filtered and concentrated under reduced pressure. The residue was chromatographed through a column packed with silica gel (5% MeOH in ethyl acetate as eluent) to give the desired products.

Taxol-containing aminophosphates **5–7** and taxol (**1**) were tested in vitro against HL-60 leukemia cells.[20,21] The IC_{50} values (μM/mL) were 0.80 for **5**, 4.0 for **6**, 0.30 for **7**, and 1.0×10^{-3} for taxol (**1**).

The free C(2')-OH group is essential for anticancer activity of taxol in vitro. We designed the zwitterionic aminophosphates **5–7** to be the substrates of phosphodiesterases in vivo. These new compounds were found to be stable in the culture medium and exhibited lower anti-leukemic activity in vitro relative to taxol. We believe that aminophosphates **5–7** have greater affinity towards the tumor cells than the normal tissue. Removal of the aminophosphate component in **5–7** by the aid of phosphodiesterases is expected to be responsible for the anticancer activity of these new protaxols in vivo.

Acknowledgment: For financial support, we thank the National Health Research Institutes of Republic of China.

References

1. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665–667.
2. McLaughlin, J. L.; Miller, R. W.; Powell, R. G.; Smith, C. R. *J. Nat. Prod.* **1981**, *44*, 312–319.
3. Zee-Cheng, R. K. Y.; Cheng, C. C. *Drugs Future* **1986**, *11*, 45–46.
4. Kingston, D. G. I.; Hawkins, D. R.; Ovington, L. *J. Nat. Prod.* **1982**, *45*, 466–470.
5. Wiernik, P. H.; Schwartz, E. L.; Strauman, J. J.; Dutcher, J. P.; Lipton, R. B.; Paietta, E. *Cancer Res.* **1987**, *47*, 2486–2493.
6. Manfredi, J. J.; Horwitz, S. B. *Pharmacol. & Ther.* **1984**, *25*, 83–125.
7. Ringel, I.; Horwitz, S. B. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 692–698.
8. Schiff, P. B.; Horwitz, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1561–1565.
9. Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1991**, *34*, 1176–1184.
10. Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. J. *J. Med. Chem.* **1992**, *35*, 145–151.
11. Greenwald, R. B.; Gilbert, C. W.; Pendri, A.; Conover, C. D.; Xia, J.; Martinez, A. *J. Med. Chem.* **1996**, *39*, 424–431.
12. Parness, J.; Kingston, D. G. I.; Powell, R. G.; Harracksingh, C.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1082–1089.

13. Lataste, H.; Senilh, V.; Wright, M.; Guénard, D.; Potier, P. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4090–4094.
14. Stella, V. J.; Himmelstein, K. J. *Design of Prodrugs*; Bundgaard, H. Ed.; Elsevier: New York, 1985; pp. 446–472.
15. Stella, V. J. *Prodrugs as Novel Drug Delivery Systems*; Higuchi, T.; Stella, V. J. Eds.; American Chemical Society: Washington, DC, 1975; pp. 1–115.
16. Dugas, H; Penney, C. *Bioorganic Chemistry, A Chemical Approach to Enzyme Action*; Cantor, C. R. Ed.; Springer–Verlag: Berlin, 1981; p. 36.
17. Zakerinia, M.; Davary, H.; Hakimelahi, G. H. *Helv. Chim. Acta* **1990**, *73*, 912–915.
18. Hakimelahi, G. H.; Moosavi–Movahedi, A. A.; Sadeghi, M. M.; Tsay, S.-C.; Hwu, J. R. *J. Med. Chem.* **1995**, *38*, 4648–4659.
19. For removal of (monomethoxy)trityl group under acidic conditions, see Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L. *Can. J. Chem.* **1978**, *56*, 2768–2780.
20. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro–Wolff, A.; Gray–Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
21. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.

(*Received in Japan* 11 *December* 1996; *accepted* 21 *January* 1997)