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Synthesis and Antitumor Activity of Novel Paclitaxel–Chlorambucil Hybrids

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Abstract—The syntheses and antitumor activity of three paclitaxel–chlorambucil hybrids are presented. Hybrid **3** showed significant in vivo efficacy.

The synergistic combination of Taxol® with other antitumor agents has been well recognized to provide clinically effective treatment regimes. Our own preclinical evidence suggested that the combination of paclitaxel and an alkylating agent could yield supraadditive antitumor effects.[1] M109taxlR,[2] a cell line that was made resistant to paclitaxel, was found to be 2- to 5-fold hypersensitive in vitro to cisplatin, Mitomycin C, Melphalan and BCNU versus the parental cell line (M109). This hypersensitivity to alkylating agents was also demonstrated in vivo. This suggested that a hybrid molecule combining an alkylating agent with paclitaxel might extend the therapeutic utility of paclitaxel to resistant or paclitaxel insensitive tumors.

Two reports describing paclitaxel hybrid molecules have appeared[3,4] in which the individual molecules are connected via a chemically stable linker. In this study, we envisioned using hybrids with a range of stability to esterase cleavage capable of releasing both paclitaxel and the alkylating agent simultaneously. In general, hybrid molecules are not as effective as combination therapy since optimization of individual drug concentration and schedule is not possible. Nevertheless, there may be potential advantages if both drugs can be deactivated as a result of the linking function and then released together. To test this hypothesis three paclitaxel–chlorambucil hybrid molecules were prepared (Fig. 1). Hybrid **1** is a simple ester of chlorambucil and

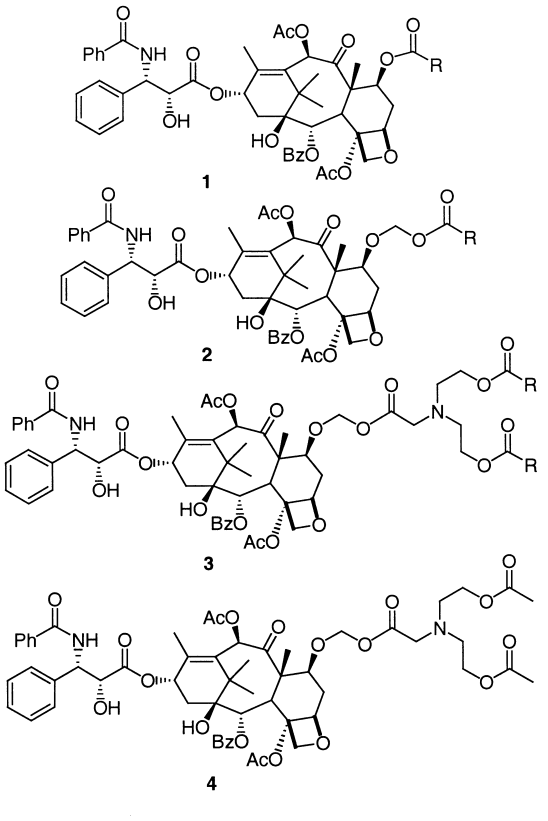

Figure 1.

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paclitaxel and as such is expected to be quite stable to esterase cleavage.[5] Extending the ester by means of an acetal linkage, as in **2**, renders the ester more susceptible to esterase cleavage. Hybrid **3** takes advantage of the acetal extension to provide two molecules of chlorambucil and one molecule of paclitaxel upon esterase cleavage. Preliminary experiments on the bis silyl protected diethanolamine prodrug **5**, showed paclitaxel was generated rapidly upon silyl ether cleavage (buffered Bu_4NF) presumably from self-immolative cyclization of the hydroxyl group on the extended ester (Fig. 2). In comparison, the directly linked prodrug **6** generated paclitaxel much more slowly, and the desilylated intermediate was observed. The rapid degradation of **5** suggested this would be an ideal platform on which to introduce an alkylating agent. Prodrug **4**, with two acetates rather than two molecules of chlorambucil, was also prepared as a control.

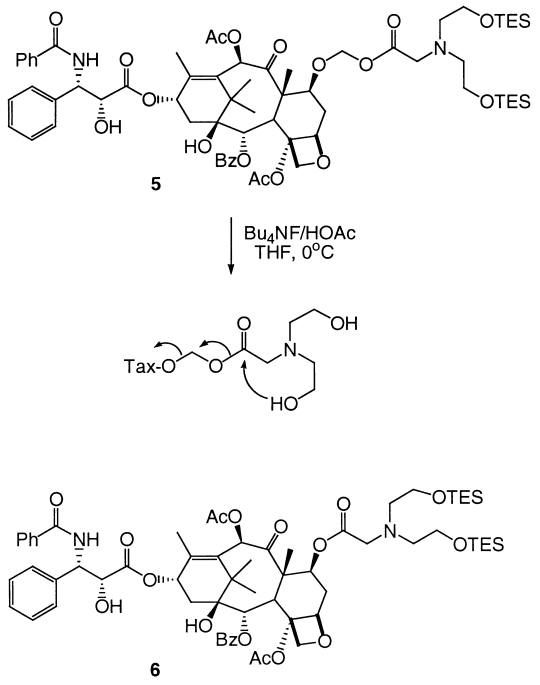
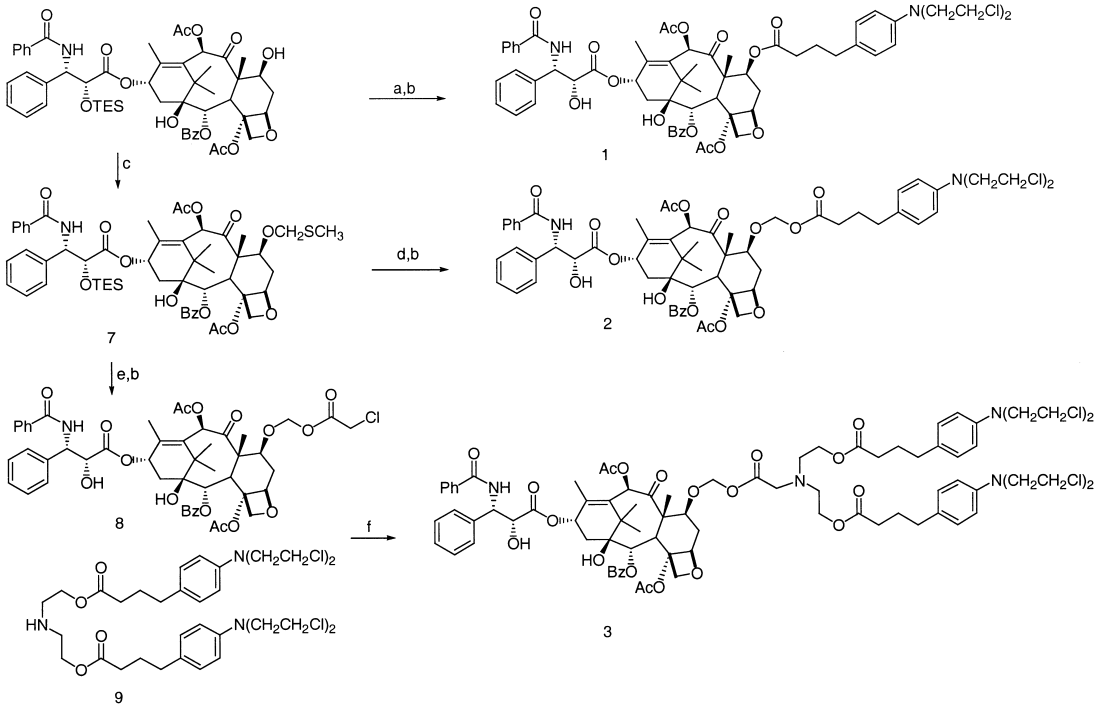


Figure 2.

The hybrid molecules **1**–**4** were prepared following Scheme 1. Starting with 2′-*O*-TES paclitaxel, hybrid **1** was prepared by simple acylation of the 7-hydroxyl group followed by 2′ desilylation. The extended ester **2** was prepared using the MTM intermediate **7**. Activation of the MTM ether using NIS with AgOTf as a catalyst provided the extended ester **2**. Treatment of the MTM ether with chloroacetic acid under similar activation conditions provided the extended chloroacetyl ester **8** which could be reacted with the amine **9** to give hybrid **3**. The diacetoxy prodrug **4** was prepared using the sequence used to prepare **3**. Amine **9** was prepared in straightforward fashion from diethanolamine. Silylation of the hydroxyls (TBSCl, imidazole, DMF) followed by protection of amine as a Cbz carbamate (CbzCl, iPr$_2$NEt, CH_2Cl_2) and deprotection of the silyl ethers (Dowex 50W-X8, MeOH) provided the *N*-protected diethanolamine. Esterification of the hydroxyls with chlorambucil using DCC and DMAP followed by Cbz removal provided the amine **9** in 70% overall yield. The *N,N* diacetoxyethyl amine was prepared similarly.

The hybrid molecules **1**–**4** were evaluated in vitro for inhibition of tubulin polymerization[6] and cytotoxicity against the HCT-116 human colon tumor cell line.[7]



Scheme 1. Reagents and conditions: (a) chlorambucil, DCC, DMAP, toluene (79%); (b) 1 N HCl, CH_3CN, O°C; (c) DMS, Bz_2O_2, CH_3CN (93%); (d) chlorambucil, NIS, AgOTf, 4 Å sieves, THF (26%); (e) $ClCH_2CO_2H$, NIS, AgOTf, 4 Å sieves, THF (51%); (f) NaI, acetone, **9** (47%).

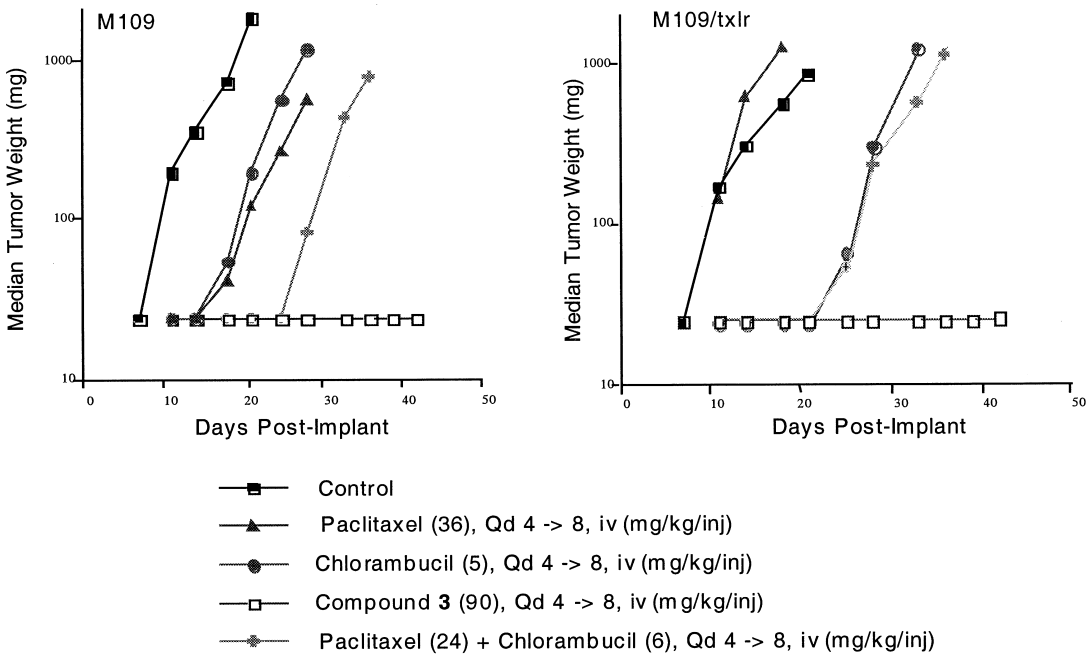


Figure 3. Activity of hybrid **3** versus im bilaterally implanted M109 and M109/txlr.

Table 1. Biological activity of compounds **1**–**4**

Compound	Tubulin[a]	IC_{50} HCT-166 (nM)	Ip/ip M109[b]
Paclitaxel	1	2–4	194–246
1	53	161	127
2	62	4.6	155
3	70	24	224
4	6.5	3.9	17

[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.[6]
[b]Values are %T/C; lifespan of treatment group divided by the lifespan of the the control group multiplied by 100.

Compounds were tested in vivo using the M109 Madison murine lung carcinoma M109 tumor model[8] (Table 1). The lack of activity in the cell-free tubulin polymerization assay suggested that compounds **1**–**3** are inactive until cleavage of the ester takes place. The cytotoxicity of compounds **2** and **4** suggests that these analogues may undergo cleavage to generate paclitaxel. All four molecules showed in vivo activity in the paclitaxel-sensitive M109 model.

Compound **3** was tested in a bilateral[9] distal site model against M109 (paclitaxel and chlorambucil-sensitive) and M109/taxlR (paclitaxel-resistant, chlorambucil-sensitive). Compound **3** was curative in both the paclitaxel-sensitive M109 arm and paclitaxel-resistant M109/taxlR arm (Fig. 3). The clear superiority of hybrid **3** relative to the optimal combination treatment demonstrates that a paclitaxel–alkylating agent hybrid can yield super-additive antitumor activity in paclitaxel-resistant tumors that are responsive to alkylating agents such as M109/taxlR.

To better understand what was driving the antitumor activity of **3**, it was tested in the A2780 ovarian carcinoma xenograft tumor model[10] (paclitaxel-sensitive, chlorambucil-resistant), HCT/pk human ovarian carcinoma xenograft model[11] (paclitaxel-resistant, chlorambucil-resistant), and the M5076 murine sarcoma model[12] (paclitaxel-insensitive, chlorambucil-sensitive). In the paclitaxel-sensitive A2780, hybrid **3** was equally active to paclitaxel but not as efficacious as the optimal combination treatment. In the HCT/pk model (paclitaxel and chlorambucil-resistant) compound **3** did not achieve an active result. In the M5076 model (paclitaxel-insensitive, chloroambucil-sensitive) hybrid **3** showed significant antitumor activity that was equivalent to chlorambucil or the optimal combination treatment. These results show that hybrid **3** shows significant antitumor efficacy in tumor models that are responsive to either paclitaxel or chlorambucil, suggesting that it retains both paclitaxel and chloroambucil-like activity. However, in the HCT/pk model which is resistant to both agents no activity was observed for compound **3**. The HCT/pK results show that not all paclitaxel resistant cell lines are hypersensitive to alkylating agents but, in cell lines that are (such as M109/taxlR), hybrid molecules such as **3** can yield supra-additive antitumor effects superior to combination treatment.

In summary, a series of three paclitaxel–chlorambucil hybrids was prepared and their antitumor activity evaluated in vitro and in vivo. All hybrids showed in vivo antitumor activity with compound **3** being the most active. Compound **3** showed excellent in vivo activity in M109 and the paclitaxel resistant M109/taxlR models that was superior to single agent or combination treatment. Compound **3** showed in vivo efficacy in the paciltaxel-sensitive, A2780 ovarian carcinoma model and the chlorambucil-sensitive, M5076 murine sarcoma model.

References and Notes

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8. %T/C values of greater than 125 are considered active. Rose, W. C. *Cancer Treat. Rep.* **1981**, *65*, 299.
9. In this experiment the tumor was implanted intra-muscularly (hind leg) and the drug given intra-venously. The same animal had both an M109 tumor and a M109/taxlR tumor.
10. A2780 human ovarian carcinoma xenograft model is highly sensitive to paclitaxel. Rose, W. C. *Anti-Cancer Drugs* **1992**, *3*, 311.
11. HCT/pk human ovarian carcinoma xenograft model (multidrug resistant tumor model). HCT/pk was established by exposing parental HCT-116 cells to concentrations of paclitaxel that mimic clinically achievable plasma levels. This resulted in a multidrug resistant tumor model that expressed elevated levels of P-glycoprotein, was cross resistant to a number of natural product anticancer agents, and was resistant to paclitaxel in vivo.
12. M5076 murine sarcoma model is insensitive to paclitaxel. Rose, W. C. *Anti-Cancer Drugs* **1992**, *3*, 311.