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Synthesis and bioactivities of paclitaxel analogs with a cyclopropanated side-chain

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Abstract—Four paclitaxel analogs with a cyclopropanated side-chain were synthesized by coupling of the spirocyclopropanated oxazoline-5-carboxylic acid **4** with 7-TES-baccatin III, followed by hydrolytic ring opening and rearrangement. The absolute configuration of the 2′-position was determined by NMR analysis of the corresponding Mosher esters. The two new paclitaxel analogs with the *R* configuration at C-2′ were both active in the A2780 mammalian cell line cytotoxicity assay, but much less than paclitaxel itself.

Paclitaxel (Taxol®) **1**, originally isolated from the Pacific yew (*Taxus brevifolia*),[1] has become an important anticancer drug, especially for the treatment of breast and ovarian cancer.[2] Many analogs of paclitaxel have been prepared in studies directed at finding compounds with improved activity, and several new analogs are in clinical trials.[3] Cyclopropyl groups have proved to be highly effective in improving the activity of many biologically active compounds,[4,5] and several cyclopropane-bearing analogs of paclitaxel[6] and epothilone[7] have previously been synthesized and shown to have improved or retained anticancer activity. The synthesis of cyclopropanated paclitaxel analogs such as **3** was thus of interest as a possible route to analogs with enhanced bioactivity (Fig. 1). In particular, the 1,1-disubstituted cyclopropyl group in the side-chain would restrain the conformational mobility of the side-chain and potentially bring it closer to the biologically active binding conformation of paclitaxel. Thus a paclitaxel analog with a methyl group at the 2′-position was prepared by two groups,[8,9] and was found to be slightly more cytotoxic than paclitaxel; restricted conformational mobility was offered as one explanation for this enhanced activity.[8] On the other hand, a paclitaxel analog with a deleted 3′-phenyl substituent was found to be orders of magnitude less active than paclitaxel,[10] but analogs with alkyl substituents in place of the 3′-phenyl ring and some additional modifications have been shown to be more active than paclitaxel.[11] Placing the 1,1-disubstituted cyclopropyl group in the 3′-position would also mimic *gem*-dimethyl substitution there, the effect of which has not been tested yet.

The synthesis of **3** requires the attachment of a cyclopropanated side-chain to a protected baccatin III. The preferred method of attaching paclitaxel side chains to baccatin III is by ring-opening condensation with an appropriately substituted β-lactam,[12] but the β-lactam required to apply this method to the synthesis of **3** cannot be prepared by the established [2+2] cycloaddi-

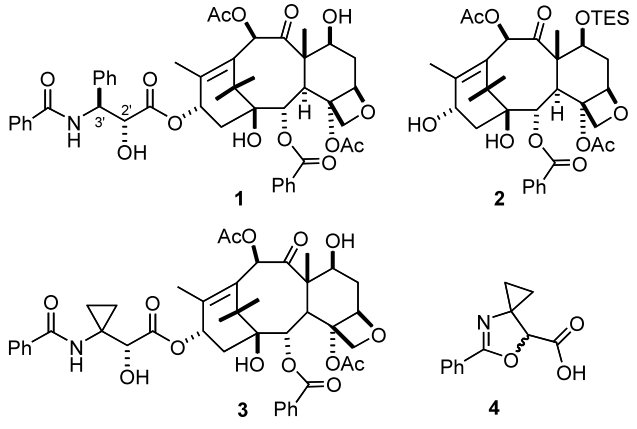

Figure 1. Structures of paclitaxel (**1**), 7-TES-baccatin III (**2**), synthetic target (**3**), and (±)-2-phenylspiro(cyclopropane-1′,4-oxazoline)-5-carboxylic acid (**4**).

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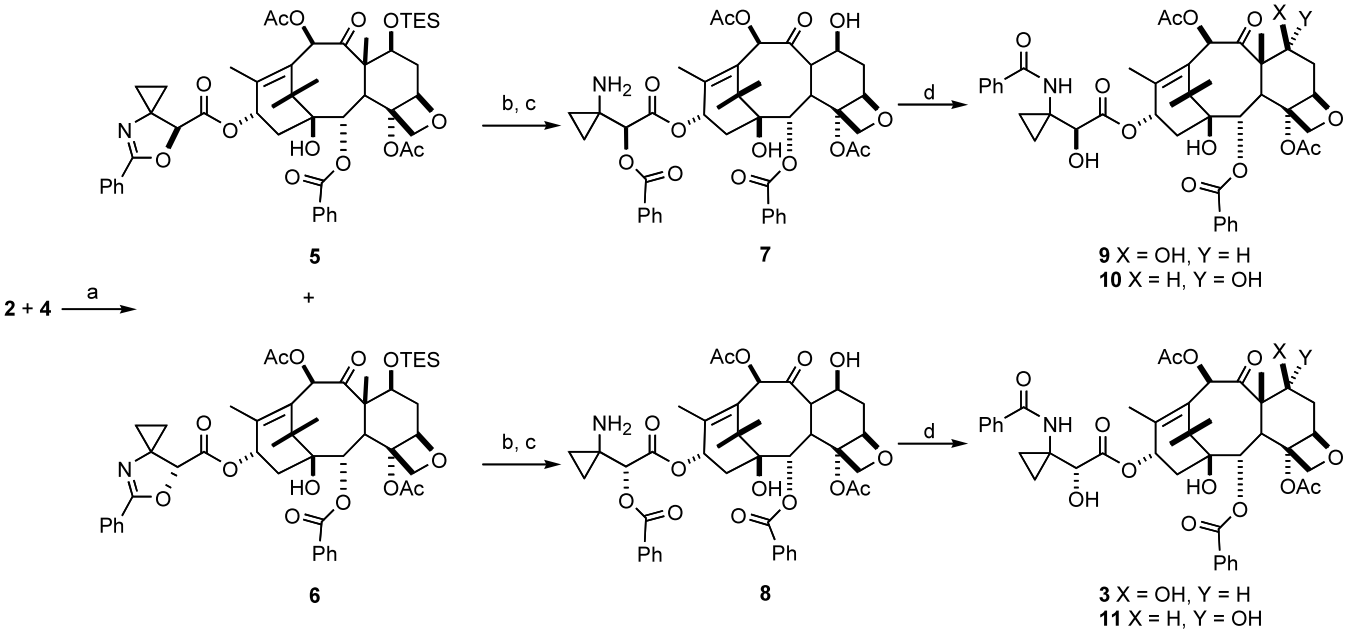
tion of an ester enolate to an *N*-silylimine. Recently, a new simple access to the spirocyclopropanated oxazoline-5-carboxylic acid **4** was disclosed.[13] Compound **4** is a potential precursor to **3**, since oxazolines have been shown to be suitable precursors of the paclitaxel side-chain.[14] It can also be noted that the synthetic route used to prepare **4** probably could not be used to prepare its *gem*-dimethyl analog because of the steric problems associated with doing a Michael reaction on a 3,3-dimethylacrylate.

The coupling of 7-TES-baccatin III **2** with (±)-2-phenylspiro(cyclopropane-1′,4-oxazoline)-5-carboxylic acid (**4**) using DCC/4-PP occurred in good yield (85%),[15] and the two diastereomers **5** and **6** were obtained in a ratio of 2:3 and were easily separated by thin-layer chromatography. Unfortunately, **5** and **6** proved resistant to hydrolysis under the conditions previously used [0.1N HCl/dioxane (1:1)].[14] Only starting material and various decomposition products could be detected under the literature conditions, while prolonged reaction times led to cleavage of the side-chain. Surprisingly, however, when the triethylsilyl groups in **5** and **6** were first removed by treatment with HF–pyridine, and the product subsequently hydrolyzed with 0.1N HCl/dioxane (1:1) at 50°C, the *O*-benzoyl derivatives **7** and **8** with free amino groups were obtained (Scheme 1).[16] These compounds were less prone to rearrange by benzoyl migration from the 2′-oxy group to the 3′-amino group than other taxol analogs.[17] Thus, under neutral aqueous conditions and basic non-aqueous conditions, migration of the benzoyl group did not take place, but treatment with 0.1N $NaHCO_3$/dioxane (1:1) led to rearrangement accompanied by epimerization at C-7,[18] and the four cyclopropane-containing paclitaxel analogs (**9**, **10**, **3**, and **11**)[19] were obtained. High resolution FAB mass spectra (HRFABMS) showed that all four of them had the same mass and elemental composition. The chemical shift differences between the 7-H and 10-H protons in the ^{1}H NMR spectra of compounds **9** and **10** (or **3** and **11**) were quite large. By comparison with literature values,[20] it could be concluded that **10** and **11** had the 7-(*R*) (7-*epi*) configuration rather than the normal 7-(*S*) configuration.

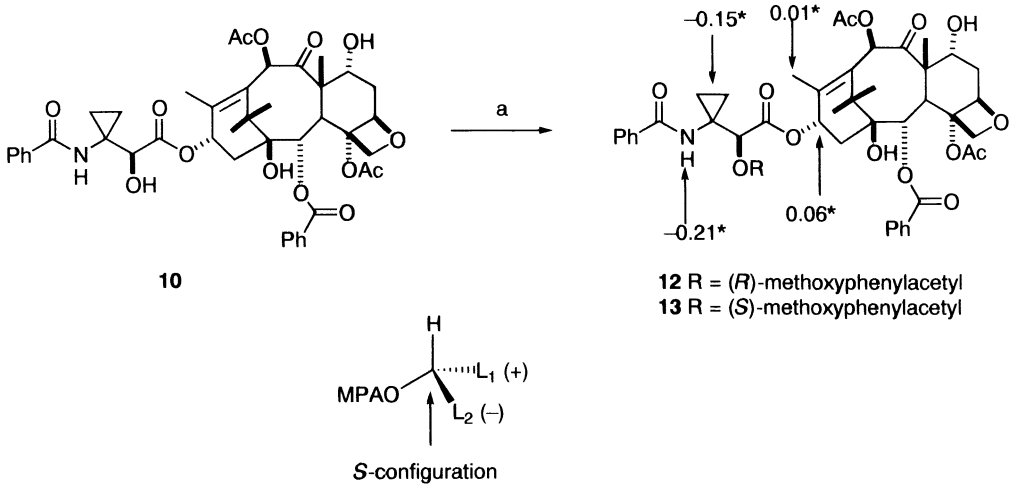
In order to determine the absolute configuration at the 2′-position, compound **10** was converted to two esters with (*R*)- or (*S*)-methoxyphenylacetic acid (MPA), using the EDC/DMAP coupling conditions. Since the 7-hydroxy group in this compound is highly hindered,[21] reaction occurred only at the 2′-OH position to yield the Mosher esters **12** and **13**. This observation also supported the assigned configuration being (*R*) at C-7 for **10** and **11**, since 7-*epi*-paclitaxel is much less reactive at this position than paclitaxel itself. The resulting 2′-MPA esters **12** and **13** were subjected to NMR analysis.[22] Fortunately, the chemical shift differences $\Delta\delta^{RS}$ were significant (Scheme 2). From these data it was concluded that the taxoid **10** must have the (*S*)-configuration at C-2′, which is the configuration of 2′-*epi*-paclitaxel. Thus, the diastereomers **3** and **11** must be 2′-(*R*), and **9** must be 2′-(*S*).

The cytotoxic activity of these novel taxoids was evaluated in vitro using the A2780 ovarian cancer cell line. This assay showed that compounds **9** and **10**, with the unnatural configuration at 2′, are essentially inactive, while the isomers **3** and **11** with the natural configuration at 2′ are active, but much less so than paclitaxel itself (Table 1).

This finding could be due to a conformational restriction such that the cyclopropyl analogs do not bind well



Scheme 1. Keys: (a) DCC, 4-PP, toluene, rt, 24 h, 85%; (b) HF–pyridine, THF, 0°C to rt, 24 h, 90%; (c) 0.1N HCl, 1,4-dioxane (1:1), 50°C, 1 h, 85%; (d) 0.1N $NaHCO_3$, 1,4-dioxane (1:1), rt, 6 h, 80%.



Scheme 2. Key: (a) (*R*)-Methoxyphenylacetic acid or (*S*)-methoxyphenylacetic acid, EDC, DMAP, CH_2Cl_2, rt, 24 h, 85%. $*\Delta\delta^{RS}=\delta^{R}$ (**7**) $-\delta^{S}$ (**8**).

Table 1. Cytotoxicities (IC_{50} values) of compounds **9**, **10**, **3**, and **11**, in the A2780 mammalian cell assay[a]

Compound	Cytotoxicity (IC_{50}, μg/mL)
1	0.02
9	18
10	>20
3	7
11	8

[a] Data are the mean of at least three determinations, and are rounded to the nearest whole number.

to the receptor, but it is perhaps more likely that the cyclopropyl group is too small to have good van der Waals or aromatic interactions at the binding site.[23] In the closest published analogy to this work, a 9-dihydrodocetaxel analog with a methyl group in place of the 3′-phenyl group was an average of 30-fold less cytotoxic than the corresponding 3′-phenyl derivative, while the 3′-isobutyl derivative was significantly more active than the 3′-phenyl derivative.[24] The synthesis of substituted cyclopropyl derivatives of **3** might thus yield some compounds with interesting bioactivities.

The observation that **9** and **10** are even less cytotoxic than **3** and **11** is consistent with the above-mentioned configurational assignments, as taxoids with a 2′-(*S*)-configuration are usually less active than those with a 2′-(*R*)-configuration.[17,25]

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15. Preparation of compounds **5** and **6**: To a solution of **4** (70 mg, 0.323 mmol) in 5 ml toluene was added DCC (68 mg, 0.323 mmol). After 15 min stirring, 4-PP (2 mg, cat.) was added and keep stirring for 5 min before **2** (70 mg, 0.100 mmol) was added. The reaction mixture was allowed to stir at room temperature for 24 h, followed by washing through a short silica gel column using EtOAc as solvent. The organic phase was concentrated in vacuum. The residue was applied to PTLC (30% EtOAc/hexane) to give **5** (33 mg, 36%) and **6** (44 mg, 49%).
16. Preparation of compounds **7** and **8**. To a solution of **5** (32 mg, 0.0355 mmol) in 2.5 ml THF was added pyridine (0.5 ml) and HF–pyridine (0.5 ml) at 0°C. The reaction mixture was then allowed to warm up to room temperature and stirred overnight. The reaction mixture was diluted with EtOAc (30 ml), and then washed with sodium bicarbonate, water, and brine. The organic phase was concentrated in vacuum. The residue was applied to PTLC (50% EtOAc/hexane) to give the desired deprotection product (25.5 mg, 90%). To a solution of the deprotection product (25 mg, 0.032 mmol) in 5 ml 1,4-dioxane was added HCl (0.1N, 5 ml) and stirring was continued at 50°C for 1 h. The reaction mixture was diluted with EtOAc (30 ml), and then washed with sodium bicarbonate, water, and brine. The organic phase was dried with sodium sulfate, concentrated in vacuum, and applied to PTLC (60% EtOAc/hexane) to give **7** (21.3 mg, 85%). Compound **8** was prepared in similar yield using the same procedure.
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18. Preparation of compounds **9**, **10**, **3**, and **11**: To a solution of **7** (16 mg, 0.02 mmol) in 1,4-dioxane (5 ml) was added $NaHCO_3$ solution (0.1N, 5 ml) at room temperature. The reaction was allowed to stir for 6 h and TLC showed no starting material was left. The reaction mixture was diluted with EtOAc (20 ml), and then washed with water, brine, and dried with sodium sulfate. The organic phase was concentrated in vacuum, and the residue was applied to PTLC (50% EtOAc/hexane) to give **9** (7.7 mg, 48%) and **10** (5.1 mg, 32%). Compounds **3** and **11** were prepared in similar yield by this procedure.
19. Compound **9**: $[\alpha]_D^{20}=-60°$ (*c* 0.23); ^{1}H NMR δ (ppm) 1.14 (3H, s), 1.24 (3H, s), 1.05–1.40 (4H, m), 1.68 (3H, s), 1.88 (1H, m), 2.12 (3H, d, $J=1.1$ Hz), 2.15–2.35 (2H, m), 2.24 (3H, s), 2.28 (3H, s), 2.51–2.61 (1H, m), 3.68 (1H, s), 3.84 (1H, d, $J=7.1$ Hz), 4.16 (1H, d, $J=8.3$ Hz), 4.29 (1H, d, $J=8.3$ Hz), 4.45 (1H, dd, $J=10.7$, 6.6 Hz), 4.96 (1H, dd, $J=7.7$, 1.8 Hz), 5.66 (1H, d, $J=7.1$ Hz), 6.15 (1H, td, $J=8.9$, 1.3 Hz), 6.34 (1H, s), 6.66 (1H, s), 7.33–7.52 (5H, m), 7.61 (1H, m), 7.67 (2H, m), 8.05 (2H, m); ^{13}C NMR δ (ppm) 9.7, 13.7, 14.7, 15.9, 21.1, 21.9, 22.8, 26.9, 35.8, 36.3, 36.8, 43.3, 46.0, 58.8, 72.0, 72.5, 75.1, 75.9, 76.6, 77.3, 79.6, 81.2, 84.6, 127.2, 128.93, 128.94, 129.3, 130.3, 132.5, 132.8, 133.4, 134.0, 143.0, 167.2, 170.3, 170.4, 171.5, 172.3, 204.0; HRFABMS, MH^+ calcd 804.3231, found 804.3197. **10**: $[\alpha]_D^{20}=-56°$ (*c* 0.41); ^{1}H NMR δ (ppm) 1.14 (3H, s), 1.17 (3H, s), 1.07–1.31 (4H, m), 1.65 (3H, s), 1.65–1.75 (1H, m), 2.00 (3H, d, $J=1.3$ Hz), 2.19 (3H, s), 2.20–2.39 (3H, m), 2.39 (3H, s), 3.66–3.73 (1H, m), 3.72 (1H, s), 3.94 (1H, d, $J=7.4$ Hz), 4.33 (1H, d, $J=8.6$ Hz), 4.38 (1H, d, $J=8.6$ Hz), 4.70 (1H, d, $J=10.3$ Hz), 4.92 (1H, dd, $J=5.7$, 3.3 Hz), 5.73 (1H, d, $J=7.4$ Hz), 6.11 (1H, td, $J=8.9$, 1.5 Hz), 6.71 (1H, s), 6.83 (1H, s), 7.32–7.52 (5H, m), 7.62 (1H, m), 7.68 (2H, m), 8.06 (2H, m); ^{13}C NMR δ (ppm) 14.0, 14.6, 15.9, 16.4, 21.1, 21.3, 22.7, 26.2, 35.6, 36.6, 37.0, 40.6, 42.8, 57.8, 71.5, 75.4, 75.9, 77.8, 78.4, 79.5, 82.2, 82.9, 127.3, 128.96, 129.00, 129.4, 130.2, 132.6, 133.1, 133.4, 134.0, 140.3, 167.2, 169.7, 170.9, 172.1, 172.3, 207.5; HRFABMS, MNa^+ calcd 826.3050, found 826.3050. **3**: $[\alpha]_D^{20}=-68°$ (*c* 0.30); ^{1}H NMR δ (ppm) 1.15 (3H, s), 1.27 (3H, s), 1.07–1.35 (4H, m), 1.69 (3H, s), 1.79 (3H, d, $J=1.3$ Hz), 1.88 (1H, m), 2.23 (3H, s), 2.25–2.38 (2H, m), 2.46 (3H, s), 2.57 (1H, m), 3.82 (1H, d, $J=7.1$ Hz), 3.91 (1H, s), 4.18 (1H, d, $J=8.5$ Hz) 4.33 (1H, d, $J=8.5$ Hz), 4.44 (1H, dd, $J=10.8$, 6.7 Hz), 4.99 (1H, dd, $J=7.5$, 2.1 Hz), 5.68 (1H, d, $J=7.1$ Hz), 6.24 (1H, td, $J=9.2$, 1.5 Hz), 6.25 (1H, s 6.83), 1H (s), 7.44–7.60 (5H, m), 7.64 (1H, m), 7.74 (2H, m), 8.10 (2H, m); ^{13}C NMR δ (ppm) 9.6, 13.6, 14.3, 14.8, 20.9, 22.01, 22.04, 26.7, 35.5, 35.9, 37.2, 43.2, 45.6, 58.5, 71.0, 72.1, 75.1, 75.6, 76.4, 77.9, 79.5, 80.7, 84.5, 127.2,128.7, 128.9, 129.2, 129.3, 130.1, 132.59, 132.60, 132.7, 132.8, 133.8, 142.8, 167.0, 170.6, 170.8, 171.2, 172.3, 203.8, 207.4; HRFABMS, MH^+ calcd 804.3231, found 804.3239. **11**: $[\alpha]_D^{20}=-70°$ (*c* 0.24); ^{1}H NMR δ (ppm) 1.16 (3H, s), 1.22 (3H, s), 1.08–1.37 (4H, m) 1.66 (3H, s), 1.71 (3H, d, $J=1.4$ Hz), 2.18 (3H, s), 2.23–2.41 (3H, m), 2.57 (3H, s), 3.69 (1H, m), 3.89 (1H, s), 3.92 (1H, d, $J=7.5$ Hz), 4.35 (1H, d, $J=8.5$ Hz), 4.41 (1H, d, $J=8.5$ Hz), 4.78 (1H, d, $J=11.7$ Hz), 4.96 (1H, dd, $J=5.7$, 3.4 Hz), 5.76 (1H, d, $J=7.5$ Hz), 6.21 (1H, td, $J=9.0$, 1.4 Hz), 6.74 (1H, s) 6.80 (1H, s) 7.44–7.60 (5H, m) 7.65 (1H, m), 7.73 (2H, m), 8.12 (2H, m); ^{13}C NMR δ (ppm) 13.8, 14.6, 14.8, 16.2, 20.9, 21.3, 22.1, 25.9, 35.3, 36.2, 37.5, 40.4, 42.7, 57.6, 70.9, 75.4, 75.7, 77.5, 78.2, 78.6, 79.5, 81.6, 82.8, 127.2, 128.7, 128.9, 129.3, 130.1, 132.5, 132.7, 133.8, 140.3, 167.1, 169.3, 171.2, 172.3, 172.6, 207.4; HRFABMS, MH^+ calcd 804.3231, found 804.3226.
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