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SYNTHESIS AND EVALUATION OF PACLITAXEL C7 DERIVATIVES: SOLUTION PHASE SYNTHESIS OF COMBINATORIAL LIBRARIES

Laxminarayan Bhat, Yanbin Liu, Sam F. Victory, Richard H. Himes,# and Gunda I. Georg*

Department of Medicinal Chemistry, Drug Discovery Program, Higuchi Bioscience Center, and Department of Molecular Biosciences,# University of Kansas, Lawrence, Kansas 66045, USA

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Abstract: A novel and efficient two-step, automated solution phase synthesis of a 26-membered combinatorial chemistry library of paclitaxel C7 esters was accomplished using the HP 7686 Solution Phase Synthesizer. Results of combinatorial synthesis, purification, analysis, and biological evaluation are described.

The antitumor agent paclitaxel (Taxol®), isolated from the bark of the Pacific yew (*Taxus brevifolia*), is an important chemotherapeutic agent that is particularly effective against ovarian and breast cancer and Kaposi's sarcoma.[1] However, difficulties related to formulation and multiple drug resistance (MDR) limit the application of Taxol® and its close analog Taxotere® in cancer treatments.[1b] The combinatorial chemistry technique[2] is an attractive tool for use in the discovery of new and better pharmacological agents. In recent years, a variety of methodologies and techniques[3] have been developed or redefined for use in solid phase[4] as well as solution phase[5] synthesis of small molecule libraries. Although extensive analog studies in the taxoid field have been done, the application of this new technique to the taxol template has the potential of uncovering new taxoids with improved pharmacological properties. Recently, Xiao *et al.*[6] have reported a solid phase synthesis of a taxoid library using a radio frequency encoding technique.

Previous studies on the structure-activity relationships of paclitaxel derivatives have shown that the C13 side chain including the C2′ hydroxyl group is critical for maintaining the biological properties of paclitaxel,[7] whereas the C7 and C10 hydroxyl groups are less sensitive to alteration.[8] A number of derivatives carrying groups at C7, C10, or both hydroxyl groups have been synthesized, and many of them have been found to possess high cytotoxicity.[8] Some of these derivatives were prepared as prodrugs[8,9] and a few as photoaffinity analogs[10] in order to study the paclitaxel binding site on microtubules. However, little work has been done to prepare paclitaxel analogs with activity against MDR cancer cells. P-glycoprotein over-expression and consequent multidrug resistance causes a major problem in cancer chemotherapy.[11] Paclitaxel is known to induce P-glycoprotein over-expression.[11a] Ojima and coworkers[12] have prepared a variety of C3′ and C10 modified taxol analogs and have found that proper modifications at the C10 position of 3′-(2-methyl-1-propenyl) and 3′-(2-methylpropyl) taxoids result in significant increase in their cytotoxicity, particularly against the drug-resistant human breast cancer cell line MCF7-R.

We intended to synthesize various paclitaxel analogs using combinatorial techniques in order to find compounds with good activity against MDR cell lines without lowering the cytotoxic profile. In search of such taxanes, we decided to synthesize paclitaxel C7 derivatives utilizing solution phase combinatorial chemistry techniques. In an effort to automate the synthesis of libraries, we used the HP 7686 Solution Phase Synthesizer (HP PrepStation SPE Module). Herein, we describe an automated two-step combinatorial synthesis, purification, and analysis of paclitaxel C7 esters as well as results of their biological evaluation.

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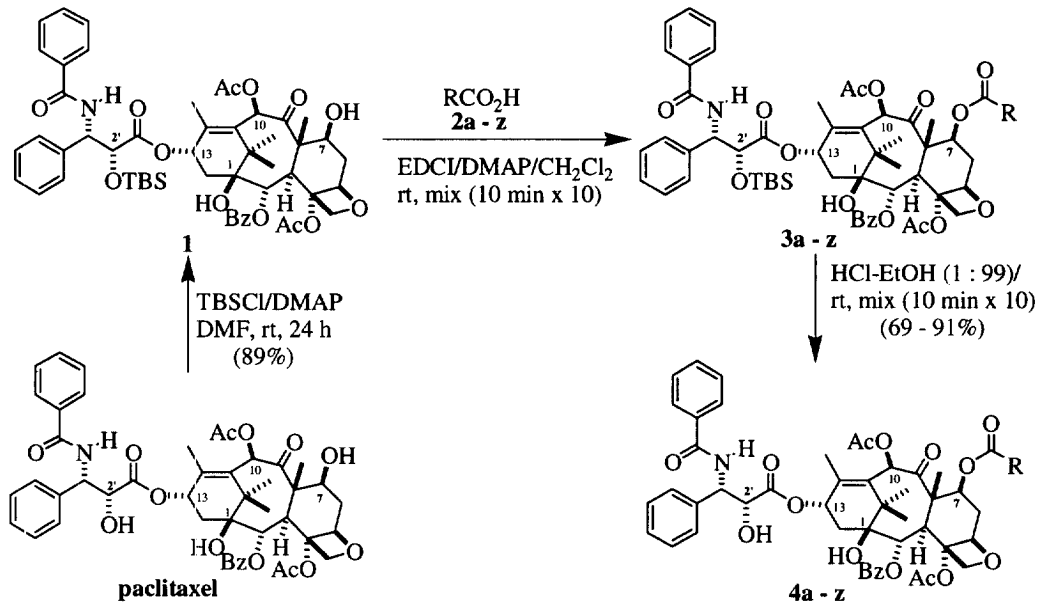
The common intermediate 2′-*O*-(*tert*-butyldimethylsilyl)paclitaxel (**1**) was prepared from paclitaxel by its treatment with *tert*-butyldimethylsilyl chloride (TBSCl) in the presence of dimethylaminopyridine (DMAP) in 89% yield (Scheme).[13] Condensation of **1** with commercially available acids **2** in the presence of the dehydrating agent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and DMAP afforded the corresponding C7 esters **3** in good yields. An efficient and selective deprotection of the TBS group was achieved with ethanolic-conc. HCl (99:1 mL) to furnish the desired paclitaxel C7 esters **4** in excellent yields.[14]

Scheme



The HP Synthesizer is a stand-alone instrument that allows for automation of many traditional chemistries by combining flexibility in reaction capabilities with cleanup options. The synthesizer does this by automating the various sample preparation processes critical to successful microscale solution phase synthesis, workup, and liquid/liquid or solid phase cleanup. The Microsoft® Windows® based operating software is used to create automated methods to run single and multiple reaction sequences.

One of our objectives in this investigation was to have a good diversity in the library. Thus, we chose a variety of carboxylic acids such as aromatic, heteroaromatic, cyclic, aliphatic, α,β-unsaturated, and *N*-Boc protected aminoacids (Table 1) to synthesize paclitaxel C7 esters. The first step of the synthesis involves the reaction of the starting 2′-*O*-TBS-paclitaxel (**1**) with excess of carboxylic acids **2a-z**. The weighed amount of acids **2a-z** (0.1 mmol each) were manually placed into 26 reaction vials, which was the only operation carried out manually. All other subsequent steps were performed by the HP Synthesizer. Methylene chloride solutions of 2′-*O*-TBS-paclitaxel (0.1 mL, 0.01 mmol), EDCI (0.2 mL, 0.2 mmol), and DMAP (0.1 mL, 0.1 mmol) were dispensed into each reaction vial from stock solution bottles. The reaction mixtures were mixed at high speed sequentially 10 min each for 10 times (10 min × 10), for each reaction mixture a total of 100 min mixing at room temperature. The synthesizer has only one mixer, and the contents of reaction vials are mixed with vortex-like rotary action. While mixing one reaction at high speed, the remaining ones were stirred using magnetic stir bars in order to reduce the reaction time. The progress of all reactions was monitored by tlc. The reactions with acids **2h-i**, **2s** and **2y** were not complete at room temperature; hence, these reaction mixtures were further stirred at 40 °C for 6 h. After completion of the reaction, the automated workup procedures, *i.e.* liquid/liquid extraction, drying, evaporation, and purification (cleanup) were performed by the instrument. In the liquid/liquid extraction step, the

Table 1. Yield, Purity, and Mass Spectral Data of Paclitaxel C7 Esters 4a - z

Entry	Acid 2	Product[a] 4	Yield (purity)[b] (%)	Mass[d] m/z (calc. mass)
1	**a** benzoic acid	**a**	72 (84)	959 (959)
2	**b** *p*-toluic acid	**b**	69 (90)	972 (972)
3	**c** *p*-chlorobenzoic acid	**c**	82 (61)	992 (992)
4	**d** *p*-anisic acid	**d**	82 (82)	988 (988)
5	**e** 2-naphthoic acid	**e**	86 (65)	1008 (1008)
6	**f** 3,4-dichlorobenzoic acid	**f**	83 (70)	1027 (1026)
7	**g** dihydrocinnamic acid	**g**	70 (40)	986 (986)
8	**h** 2-methylnicotinic acid[c]	**h**	68 (91)	973 (973)
9	**i** 5,6-dichloronicotinic acid[c]	**i**	58 (30)	1027 (1027)
10	**j** 2-furoic acid	**j**	80 (95)	948 (948)
11	**k** 5-nitro-2-furoic acid	**k**	84 (70)	993 (993)
12	**l** *N*-methyl-2-pyrrolecarboxylic acid	**l**	79 (63)	960 (961)
13	**m** 3-thiophenecarboxylic acid	**m**	76 (58)	963 (964)
14	**n** cyclopentanecarboxylic acid	**n**	83 (45)	950 (950)
15	**o** cyclopropanecarboxylic acid	**o**	81 (55)	922 (922)
16	**p** acetic acid	**p**	87 (>99)	896 (896)
17	**q** valeric acid	**q**	94 (98)	938 (938)
18	**r** tridecanoic acid	**r**	82 (83)	811[e] (1050)
19	**s** trimethylacetic acid[c]	**s**	81 (>99)	938 (938)
20	**t** cyanoacetic acid	**t**	76 (>99)	921 (921)
21	**u** acetoxyacetic acid	**u**	85 (53)	952 (953)
22	**v** methoxyacetic acid	**v**	91 (85)	926 (926)
23	**w** methylthioacetic acid	**w**	80 (68)	942 (942)
24	**x** crotonic acid	**x**	92 (77)	924 (923)
25	**y** *trans*-3-chloroacrylic acid[c]	**y**	84 (>99)	907, 909[f] (942)
26	**z** *N*-(*tert*-butoxycarbonyl)-4-aminobutanoic acid (*N*-Boc-GABA)	**z**	89 (>99)	1040 (1039)

[a]All the derivatives were characterized by ^{1}H NMR (300 MHz). [b] Yield for the two-step synthesis from **1** after purification (solid phase cleanup). The product purity was estimated by HPLC analysis and is given in parenthesis. HPLC conditions: Phenomenex ODS Hypersil C-18 column (5μ, 150 × 3.2 mm); eluents: acetonitrile/water (85:15); UV detection at 245 nm. [c]The reaction mixture was stirred for an additional 6 h at 40 °C. [d]FABMS: all the compounds gave satisfactory M^+, M+1 or M−1 peaks except compounds **4r** and **4y**. Calculated values are given in parenthesis. [e][M - $C_{15}H_{14}NO_2$ of side chain)] peak. [f][M - Cl] peak.

reaction mixtures were washed successively with saturated $NaHCO_3$ and water. The organic extracts were dried over granular Na_2SO_4, which previously had been placed manually into the vials. The solvents were evaporated on the instrument at 50 °C under a stream of nitrogen. The resulting residues, esters **3a-z**, were cleaned up in an automated step by passing them through silica cartridges using ethyl acetate as eluent. The 2′-*O*-TBS-paclitaxel esters **3a-z** thus obtained were carried to the next step. In the subsequent TBS deprotection step, an ethanol-conc. HCl (99:1 mL) solution was dispensed (0.5 mL each) into the reaction vials containing 2′-*O*-TBS-paclitaxel C7 esters **3a-z** and mixed sequentially at high speed (10 min × 10) at room temperature, for a total of 100 min for each vial. Again, while mixing the reagents in one reaction vial, the remaining ones were stirred using magnetic stir bars in order to reduce the reaction time. After completion of the reaction (monitored by tlc), the reaction mixtures were diluted with ethyl acetate and subjected to automated workup and cleanup procedures on the instrument, as described above, to afford fair to high purities of the paclitaxel C7 esters **4a-z** in good yields.[15] The purities of the final products **4a-z** were determined by HPLC analysis (Table 1). The structures were

Table 2. Biological Evaluation of Paclitaxel C7 Esters 4a - z

Paclitaxel C7 Ester	Microtubule Assembly[a] [$ED_{50}/ED_{50(Paclitaxel)}$][b]	MCF7-R Cell Assay[a] [$ED_{50}/ED_{50(Paclitaxel)}$][c]	MCF7 Cell Assay[a] [$ED_{50}/ED_{50(Paclitaxel)}$][d]
4a	8.3	1.1	14
4b	25	0.90	31
4c	13	1.6	ED_{50}>80 nM[f]
4d	22	2.2	ED_{50}>80 nM[f]
4e	60	ED_{50}>5000 nM[e]	ED_{50}>80 nM[f]
4f	24	ED_{50}>5000 nM[e]	ED_{50}>80 nM[f]
4g	inactive	inactive	inactive
4h	32	ED_{50}>5000 nM[e]	ED_{50}>80 nM[f]
4i	inactive	inactive	inactive
4j	22	1.0	27
4k	8.7	ED_{50}>5000 nM[e]	12
4l	8.3	1.2	ED_{50}>80 nM[f]
4m	1.7	1.3	ED_{50}>80 nM[f]
4n	1.0	1.3	37
4o	22	ED_{50}>5000 nM[e]	35
4p	0.70	0.70	8.0
4q	0.70	0.40	10
4r	inactive	inactive	inactive
4s	8.3	1.5	3.0
4t	1.3	ED_{50}>5000 nM[e]	8.0
4u	1.7	1.8	17
4v	1.0	1.3	14
4w	0.70	0.50	10
4x	6.7	ED_{50}>5000 nM[e]	ED_{50}>80 nM[f]
4y	5.3	ED_{50}>5000 nM[e]	ED_{50}>80 nM[f]
4z	0.70	ED_{50}>5000 nM[e]	ED_{50}>80 nM[f]

[a]Data reported relative to paclitaxel = 1. [b]ED_{50} is the concentration which causes polymerization of 50% of the tubulin present in 15 min. at 37 °C. ED_{50} for paclitaxel = 0.3 μM. [c]ED_{50} refers to the concentration which produces 50% inhibition of proliferation after 72 h incubation at 37 °C. ED_{50} for paclitaxel = 2220-3700 nM. [d]ED_{50} refers to the concentration which produces 50% inhibition of proliferation after 72 h incubation at 37 °C. ED_{50} for paclitaxel = 2 nM. [e]Did not inhibit 50% proliferation at the maximum concentration tested (MCF7-R = 5000 nM). [f]Did not inhibit 50% proliferation at the maximum concentration tested (MCF7 = 80 nM).

confirmed by ^{1}H NMR (300 MHz) and FAB mass spectral data (Table 1). The major by-product of the reaction was paclitaxel along with small amounts of the starting acid. The purity of analogues **4a-z** was nevertheless, judged to be sufficient for biological evaluation since we were trying to identify analogues with significantly increased cytotoxicity (100-fold to 1000 fold) against the MCF7-R cell line in comparison to paclitaxel. A 100-fold to 1000-fold increase of cytotoxicity against the MCF7-R cell line ($ED_{50}/ED_{50paclitaxel}$ = 0.01-0.001 for an analytically pure compound) would provide an approximate $ED_{50}/ED_{50paclitaxel}$ = 0.02-0.002 for a 1:1 mixture of the analog and paclitaxel. Thus, we should be able to identify analog with promising activities against this cell line, even though compounds **4a-z** contain paclitaxel as an impurity.

The paclitaxel C7 esters **4a-z** were evaluated for their ability to initiate the polymerization of tubulin in the tubulin assembly assay (Table 2). In addition, they were screened for cytotoxicity against the human breast cancer cell line MCF7 and the resistant human breast cancer cell line MCF7-R (Table 2).

The aromatic acid derived analogs **4a-f** demonstrated very weak activity in the tubulin assembly assay (8-fold to 60-fold decreased activity compared to paclitaxel). Similar results were obtained for the heteroaromatic analogues **4h-l**. Only the 3-thiophenecarboxylic acid **4m** displayed good ability to stimulate the assembly of tubulin ($ED_{50}/ED_{50paclitaxel}$ = 1.7). The majority of the aliphatic acid analogs **4n-w** and **4z** displayed paclitaxel-like activity in our assay. One of the exceptions is the cyclopropanecarboxylic acid analogue **4o**, which was 22 times less active than paclitaxel. This is a surprising result, since the related cyclopentanecarboxylic acid analog **4n** showed paclitaxel-like activity. It is possible that this result is due to the relatively low purity (55%) of the sample. The acetic acid analogue **4p**, valeric acid analogue **4q**, and methylthioacetic analog **4w** are slightly more potent ($ED_{50}/ED_{50paclitaxel}$ = 0.70) than paclitaxel in the tubulin assembly assay. Increasing the length of the aliphatic acid moiety to a 13C acid (tridecanoic acid derivative **4r**) led to an analog that did not possess any activity in our assays. The attachment of the sterically demanding trimethylacetic acid moiety (**4s**) also produced an analog with greatly reduced activity in the tubulin assembly assay ($ED_{50}/ED_{50paclitaxel}$ = 8.3). Introduction of unsaturated acids such as crotonic acid (**4x**) and *trans*-3-chloroacrylic acid (**4y**) provided analogs that showed weak activity in the tubulin assembly assay.

Our study also demonstrated that none of the derivatives **4a-z** exhibited significant activity against the non-drug resistant human cancer cell line MCF7, suggesting that modifications at the 7-position of paclitaxel are detrimental to activity against this cell line. The cytotoxicity of analogs **4a-z** against the drug resistant MCF7-R cell line was also weak although some aliphatic acid derivatives were identified to display better activity than paclitaxel against this cell line.

In most cases, the activity of a compound in the tubulin assembly assay is a good predictor for its cytotoxicity against cancer cells. Thus, we expected good cytotoxicity for the aliphatic acid derived analogs. However, the acetic acid analog **4p**, the valeric acid analog **4q**, and methylthioacetic acid analog **4w** showed an approximately 10-fold reduced cytotoxicity against MCF7 cells suggesting a difference in uptake or metabolism for these analogs in comparision to paclitaxel. These three analogs (**4p**, **4q**, and **4w**) were the most potent analogues in the MCF7-R cell cytotoxicity assay ($ED_{50}/ED_{50paclitaxel}$ ranged from 0.40 to 0.70). The introduction of more sterically demanding aliphatic acids such as trimethylacetic acid, cyclopropanecarboxylic acid, and cyclopentanecarboxylic acid did not lead to an improvement of cytotoxicity against the MCF7-R cell line. Introduction of a phenyl group (dihydrocinnamic acid analog **4g**) provided an analog that was inactive in all three assays. Replacement of the *S*-methyl group of methylthioacetic acid analog (**4w**) with a cyano (**4t**), an acetoxy, and a methoxy (**4v**) moiety did not improve activity against MCF7-R cells in comparison to the methylthioacetic acid analog (**4w**).

A decrease in activity was also seen for the cytotoxicity of aromatic acid derived compounds **4a-f** against the MCF7 cell line. The cytotoxicities of **4a-f** against the MCF7-R cell line are also weak comparable to the cytotoxicity displayed by paclitaxel. Similar results were observed for the heteroaromatic acid analogs **4h-m.**

It is of interest to note that analog **4z**, possessing the *N*-Boc-GABA moiety, was more active than paclitaxel in the tubulin assembly assay but was not active against the MCF7-R and MCF7 cell lines. This result suggests that this compound is either not taken up into the cancer cells or is metabolized *in vivo* to an inactive analog.

In summary, we have accomplished an automated two-step solution phase synthesis of a 26-membered paclitaxel C7 ester library. The conversion of the traditional esterification procedure to the HP 7686 Synthesizer was straightforward. Fair to high purity esters in milligram quantities were synthesized in good yields in a very short start-up time. Evaluation in the microtubule assembly assay showed that esters **4p-q**, **4w**, and **4z** are more active than paclitaxel. Esters **4b**, **4p-q**, and **4w** were found to be slightly more active than paclitaxel against the drug-resistant human breast cancer cell line MCF7-R. We are currently pursuing additional automated syntheses of combinatorial libraries of paclitaxel analogs using the HP 7686 Solution Phase Synthesizer.

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