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NEW TAXANES AS HIGHLY EFFICIENT REVERSAL AGENTS FOR MULTI-DRUG RESISTANCE IN CANCER CELLS

Iwao Ojima,*[a] Pierre-Yves Bounaud,[a] Craig Takeuchi,[a] Paula Pera,[b] and Ralph J. Bernacki[b]

[a]*Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400*
[b]*Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263*

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Abstract: New non-cytotoxic taxanes synthesized from 10-deacetylbaccatin III and special hydrophobic acylating agents show remarkable MDR reversal activity (≤99.8%) against drug-resistant human breast cancer cells when co-administered with paclitaxel or doxorubicin. This activity is ascribed to the highly efficient blocking of P-glycoprotein efflux by these new taxanes.

Clinical resistance to drugs has been a major obstacle in cancer chemotherapy since most metastatic cancers have an intrinsic or acquired resistance to the treatment. A broad-spectrum resistance to structurally and mechanistically diverse antitumor agents, such as *Vinca* alkaloids, anthracyclines, epipodophyllotoxins, antibiotics, colchicine or Taxol® (paclitaxel), constitutes the multi-drug resistance (MDR) phenotype. Tumor cells carrying this phenotype are characterized by the overexpression of an energy-dependent drug transport protein, P-glycoprotein (P-gp), resulting in a decreased accumulation of the drug within the cell because of its efficient efflux system against hydrophobic anticancer drug molecules.[1] One approach to overcome this problem is the inhibition of P-gp with a noncytotoxic compound, thus restoring the sensitivity towards the anticancer agent. Since Tsuruo and coworkers discovered that verapamil, a calcium channel blocker, was able to reverse the multidrug resistance,[2] many other reversal agents were brought to light, such as calmodulin antagonists (trifluoperazine), antiarrhythmics (amiodarone), antihypertensive agents (reserpine), antipsychotics (phenothiazines), and immunosuppressants (cyclosporine A, FK506).[3] Undesirable side-effects limited their use in clinical trials, however. New potent noncytotoxic reversal agents are therefore needed to overcome MDR in cancer.

Recently Kobayashi reported the isolation of several noncytotoxic natural taxanes from the Japanese yew tree *Taxus cuspidata*.[4] Taxanes that were binding to P-gp were shown to increase cellular accumulation of the antitumor drug vincristine in MDR tumor cells as potent as verapamil. This led us to investigate the taxane structure for the design of new MDR reversal agents.

The pharmacophore for MDR reversal agents is not fully defined yet. Nevertheless, a reported systematic structure–activity relationship (SAR) study of different classes of chemosensitizing agents pointed out the importance of a hydrophobic, conjugated planar ring, and a tertiary (preferably cyclic) amino group.[1] The last item, however, does not seem to be a requisite since highly potent MDR reversal agents reported here do not have this functional group at all. Our design of new MDR reversal agents is based on the derivatization of 10-deacetylbaccatin III (DAB) that is a crucial constituent of paclitaxel, but noncytotoxic by itself. This complex

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diterpene possesses several hydroxyl groups that can easily be modified with hydrophobic side chains byesterification (Figure 1). Another baccatin, 14-hydroxyl 10-deacetylbaccatin III (14-OH DAB), was also used as starting material.

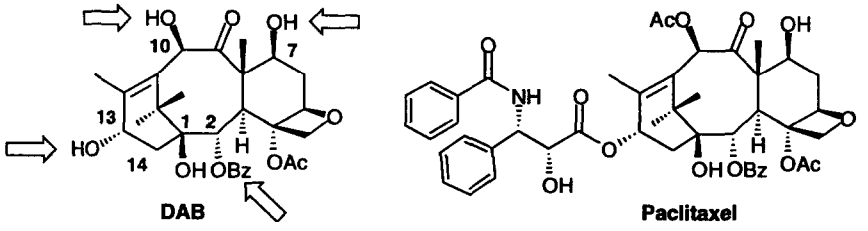
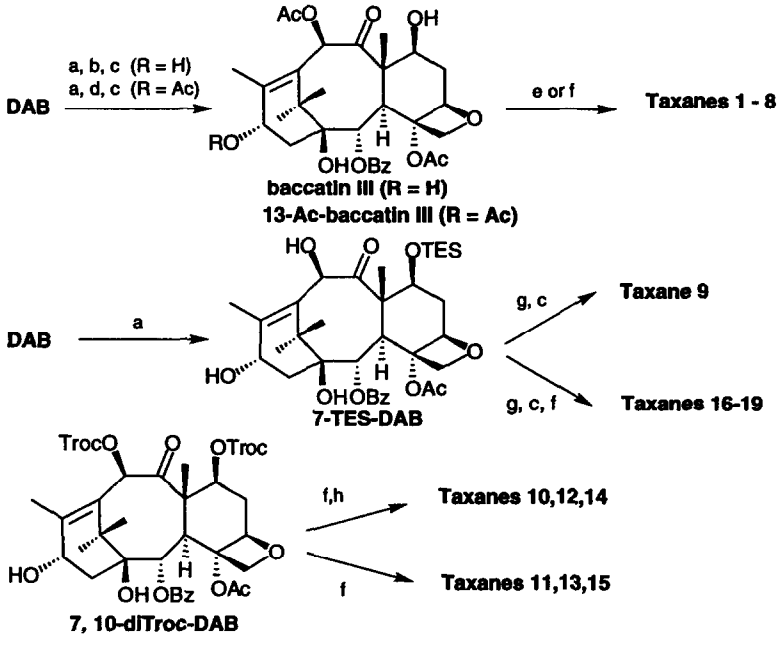
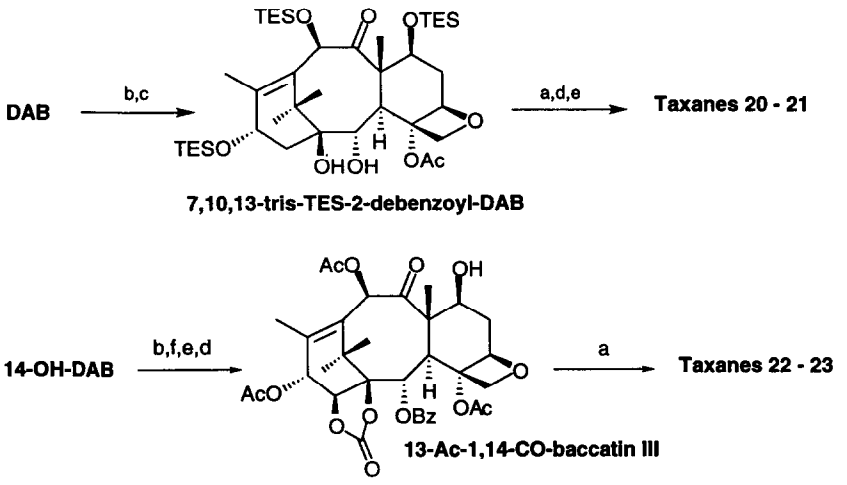


Figure 1. Structures of DAB and paclitaxel.

A benzophenone- and a naphthalene-containing carboxylic acids were chosen first as hydrophobic modifiers. For the selective protection and deprotection of the hydroxyl groups of DAB, well-established procedures developed for taxoid syntheses[5] were used to introduce the hydrophobic side chain(s)[6] at the desired position(s) in the taxane skeleton (Schemes 1 and 2).



Scheme 1. (a) TESCl, pyridine, room temperature; (b) LiHMDS, AcCl, THF, –40 °C; (c) HF-pyridine, pyridine/CH_3CN, 0 °C to room temperature; (d) Ac_2O, DMAP, CH_2Cl_2, room temperature; (e) RCOCl, Et_3N, DMAP, CH_2Cl_2, room temperature; (f) RCO_2H, DCC, DMAP, CH_2Cl_2, room temperature; (g) *N*-hydroxysuccinimide ester of RCO_2H, LiHMDS, THF, –40 °C; (h) Zn, AcOH/MeOH, 70 °C.
TES = triethylsilyl.

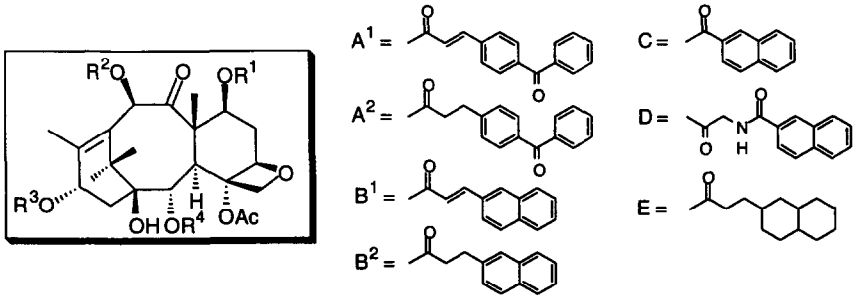


Scheme 2. (a) RCO_2H, DCC, DMAP, CH_2Cl_2, room temperature; (b) TESCl, imidazole, DMF, room temperature; (c) Red-Al®, THF, 0 °C; (d) HF-pyridine, pyridine/CH_3CN, 0 °C to room temperature; (e) Ac_2O, DMAP, CH_2Cl_2, room temperature; (f) $COCl_2$, pyridine, CH_2Cl_2, 0 °C.
TES = triethylsilyl; Troc = 2,2,2-trichloroethyoxy carbonyl

Most of the new taxanes thus synthesized possess only weak cytotoxicities (IC_{50} >10 μM). For the assessment of MDR reversal activity of these new taxanes, the cytotoxicity of Taxol® (paclitaxel) in combination with a new taxane is evaluated against the resistant human breast tumor cell lines MCF7-R and MDA-435/LCC6-MDR. Table 1 summarizes new synthetic taxanes that have been screened for their MDR reversal activity. Two series of new taxanes with modifications at the C-7 (taxanes **1**–**8**) and C-10 positions (taxane **9**) of DAB prove to be very active with >95% reversal activity in most cases while the taxanes modified at the C-13 (taxanes **10**–**15**) or C-2 positions (taxanes **20** and **21**) of DAB show a little or no activity in general. Taxanes **1** and **7** can restore the sensitivity of MCF7-R cells toward paclitaxel almost completely. When the hydrophilicity of the taxane is increased by introducing a glycine linker (taxane **5**), the activity drops dramatically. A considerable loss of activity is also observed when the aromatic rings are totally reduced (taxane **6**), implying the need of conjugated planar rings in the MDR pharmacophore. A carbonate functionality at the 1,14 position on the baccatin does not seem to change much the activity (taxanes **22** and **23**, as opposed to **7** and **8**). The effects on the MDR reversal activity of introducing two or three hydrophobic side chains at the C-7, C-10 and C-13 positions of DAB (taxanes **16**–**19**) appear to be complicated, that is an activity ranging from 0% (taxanes **17** and **19**) to 99.7% (taxane **16**) is observed depending on the substitution pattern. The results strongly suggest that there is a specific binding site for taxanes on P-gp which has rather strict steric/shape requirements. This is consistent with our recent observation of specific photoaffinity labeling of P-gp with a paclitaxel analog bearing [$^3H_2$]-3-(4-benzoylphenyl)propanoyl group at the 3′-N position in place of benzoyl group.[7]

The MDR reversal activity exhibited by this series of new taxanes is not limited to the cases using paclitaxel, the sensitization of MCF7-R cells has also been observed with another commonly used anthracycline class anticancer drug, doxorubicin, that is taxane **2** exhibited 92% MDR reversing activity when co-administered with doxorubicin against MCF7-R.

Table 1. Modulation of the sensitivity of the resistant human breast tumor cell line against paclitaxel by taxane reversal agents **1–23**.



Taxanes	R^1	R^2	R^3	R^4	Conc. (μM)[a]	IC_{50} (nM)[b,c] [% reduction]	IC_{50} (nM)[b,d] [% reduction]
paclitaxel	H	Ac	side-chain	Bz	--	860 [0]	860 [0]
1	A^1	Ac	H	Bz	3	1.6 **[99.8]**	--
2	B^1	Ac	H	Bz	1	36 **[96]**	430 [50]
3	B^2	Ac	H	Bz	1	33 **[96]**	--
4	C	Ac	H	Bz	1	21 **[97.5]**	--
5	D	Ac	H	Bz	1	500 [42]	--
6	E	Ac	H	c-HexCO	1	160 [81]	--
7	A^1	Ac	Ac	Bz	3	2.6 **[99.7]**	69 **[92]**
8	B^1	Ac	Ac	Bz	3	5.8 **[99.3]**	--
9	H	A^1	H	Bz	1	42 **[95]**	--
10	H	H	A^1	Bz	1	860 [0]	--
11	Troc	Troc	A^1	Bz	3	170 [80]	--
12	H	H	B^1	Bz	1	100 [88]	--
13	Troc	Troc	B^1	Bz	3	750 12]	--
14	H	H	B^2	Bz	1	540 [37]	--
15	Troc	Troc	B^2	Bz	3	860 [0]	--
16	A^2	A^2	H	Bz	1	2.6 **[99.7]**	--
17	B^1	A^2	H	Bz	1	860 [0]	--
18	B^1	B^1	H	Bz	1	470 [45]	--
19	A^2	A^2	A^2	Bz	0.1	--	860 [0]
20	Ac	Ac	Ac	A^1	0.1	--	630 [27]
21	Ac	Ac	Ac	B^2	0.1	--	650 [24]
22[e]	A^1	Ac	Ac	Bz	0.1	--	52 **[94]**
23[e]	B^1	Ac	Ac	Bz	0.1	--	340 [60]

[a]Concentration of a taxane administered in combination with paclitaxel; [b]Cytotoxicity of paclitaxel (10 nM) when co-administered with a taxane; [c]MCF7-R cell line; [d]MDA-435/LCC6-MDR cell line; [e]Taxanes derived from 14-OH DAB. % reduction = [1–{IC_{50}(reversal agent + paclitaxel)/IC_{50} (paclitaxel)}] × 100.

The dose–response for MDR reversal activity was examined for two analogs, **2** and **7**, against the human breast cancer cell line MDA-435/LCC6-MDR, at three different taxane concentrations (Figure 2). As Figure 2 shows, both taxanes display excellent MDR reversal activity at 1 μM. Taxane **7** exhibits strong MDR reversal activity even at 0.1 μM (Figure 3b). However, no appreciable activity was observed at 0.01 μM.

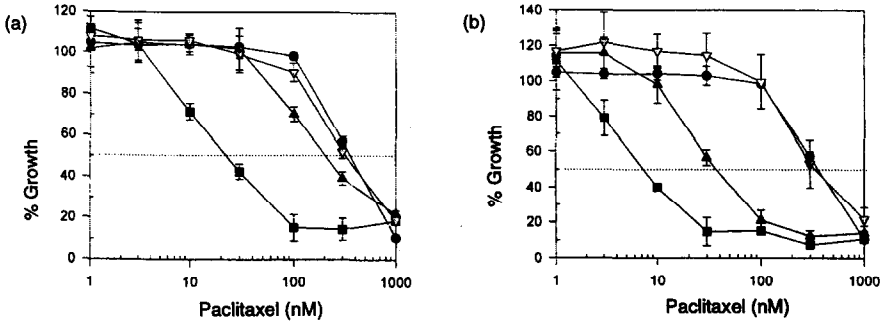


Figure 2. Modulation of MDA-435/LCC6-MDR sensitivity to paclitaxel by MDR reversal taxane **2** (a) and **7** (b) at three different concentrations (circle, 0 μM; square, 1 μM ; triangle, 0.1 μM; inversed triangle, 0.01 μM).

In order to verify our hypothetical mechanism of action for these new taxane MDR reversal agents, we investigated the effects of taxane **2**, as a representative, on the intracellular concentration of paclitaxel in MCF7-R with and without taxane **2**. As a control experiment, we also looked at the effects of taxane **2** on the paclitaxel concentration in the parent MCF7 cancer cells that are drug sensitive. As Figure 3a shows, taxane **2** does not have any appreciable effects on the accumulation of paclitaxel in MCF7 as expected. In sharp contrast with this, taxane **2** indeed enables paclitaxel to increase its accumulation in MCF7-R cells dramatically although only a very low level of paclitaxel is detected without taxane **2** (Figure 3b). These results strongly indicate that the new taxane MDR reversal agents represented by taxane **2** indeed block the efflux mechanism of P-gp to allow paclitaxel (and doxorubicin) to penetrate into drug-resistant cancer cells and accumulate.

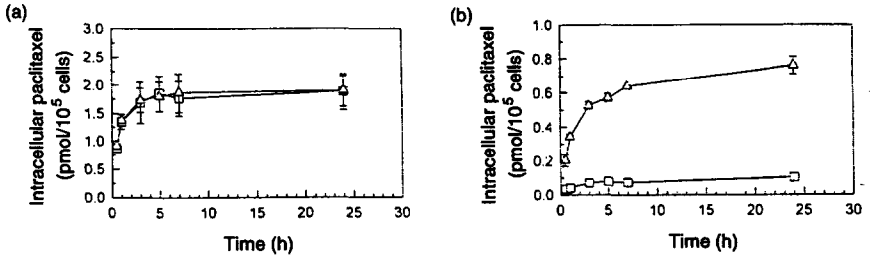


Figure 3. Modulation of tritiated paclitaxel accumulation by MDR reversal taxane **2** in MCF7 cells, sensitive (a) and resistant (b). Square, 10 nM of paclitaxel; triangle, 10 nM of paclitaxel and 1 μM of taxane **2**.

Further MDR reversal activity assay in animal models is in progress for taxanes **2**, **7** and **16**. Also, further SAR study for design and synthesis of more potent taxane MDR reversal agents is actively underway in these laboratories.

In summary, a series of new taxanes were synthesized through the modifications of 10-deacetylbaccatin III (DAB) and 14-hydroxyl 10-deacetylbaccatin III (14-OH DAB), and their MDR reversal activities were evaluated mainly against drug-resistant human breast cancer cell lines. Several of these new taxanes exhibited >99% MDR reversal activity at 1–3 μM level when co-administered with paclitaxel, and a strong activity was still maintained at 0.1 μM for taxanes **7** and **22**. The structure–activity relationships (SAR) of these new taxanes revealed clear positional and steric/shape requirements for strong activity. The mechanism of action for the new taxane MDR reversal agents is shown to be blocking the efflux system of P-glycoprotein (P-gp) to allow paclitaxel to penetrate and accumulate into MDR cancer cells.

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6. Side-chain carboxylic acids, A^1-OH and A^2-OH were readily prepared in a few steps from 4-bromobenzophenone through the modified Heck reaction with methyl acrylate (A^1-OH), followed by hydrogenation catalyzed by $RhCl(PPh_3)_3$ (A^2-OH). Similarly, B^1-OH and B^2-OH were obtained through the Wittig reaction of 2-naphthalenecarboxaldehyde (B^1-OH), followed by hydrogenation (B^2-OH). Taxane **6** was obtained through the hydrogenation (500 psi) of taxane **2** or **3** over 5% Rh/C.
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