Design, Synthesis and Structure−Activity Relationships of Novel Taxane-Based Multidrug Resistance Reversal Agents

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A series of novel taxane-based multidrug resistance (MDR) reversal agents (TRAs) has been designed and synthesized. Structure−activity relationship (SAR) study clearly indicates that modification of the C-7 position with hydrophobic arenecarbonylcinnamoyl groups brings about high potency against drug efflux mediated by P-glycoprotein (P-gp). Six TRAs exhibit ability to modulate a wide range of ATP-binding cassette (ABC) transporters, such as P-gp, multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP), which may serve as novel broad-spectrum modulators of ABC transporters.

Introduction

Clinical resistance to drugs is a major obstacle in cancer chemotherapy today. Although there are many antineoplastic drugs in use, only a few are effective in the treatment of each specific tumor type because of intrinsic or acquired drug resistance. A broad-spectrum resistance to structurally and mechanistically diverse antitumor agents is known as multidrug resistance (MDR).[1] Classical MDR results from the overexpression of ATP-binding cassette (ABC) transporters.[2] The two best-known and extensively studied ABC transporters are P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1).[3] A large number of structurally unrelated compounds are known to be P-gp substrates, only having in common high hydrophobicity, an amphiphilic nature, and a net positive charge.[4] MRP1 also effluxes a broad range of substrates, either by a glutathione cotransport mechanism or after their conjugation to glutathione.[5,6] P-gp and MRP1 are typically coexpressed with other ABC transporters such as breast cancer resistance protein (BCRP).[7−11]

As soon as P-gp and sister proteins were recognized to be responsible for MDR, blocking the efflux of drugs by inhibiting the functions of these transporters became a realistic way to overcome MDR.[12] The use of noncytotoxic chemosensitizers (MDR-reversal agents, MDR modulators) which can block the anticancer drug binding sites, thus preventing their exclusion from the cells, has received considerable attention to date. Tsuruo and co-workers[13] were the first to demonstrate the ability of the calcium channel blocker, verapamil, to reverse MDR. Since then, 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).[14−18] However, undesirable side effects limited their use in clinical trials. Thus, potent noncytotoxic reversal agents with minimum undesirable side effects are in demand.

Kobayashi et al.[19−22] reported that natural taxanes isolated from the Japanese yew tree, *Taxus cuspidata*, could increase the cellular accumulation of vincristine in MDR tumor cells as potent as verapamil. This discovery inspired us to investigate the taxane structure for the design of new MDR reversal agents. Our research is based on the derivatization of 10-deacetylbaccatin III (DAB) (Figure 1) and 14β-hydroxybaccatin III (14-OH-DAB). Previous structure−activity relationship (SAR) studies of structurally different classes of MDR reversal agents pointed out the importance of a hydrophobic, conjugated, planar ring.[23,24] Accordingly, benzophenone, naphthalene-containing carboxylic acids and other hydrophobic groups were chosen to modify the hydroxyl groups of DAB. The SAR study of the taxane-based MDR reversal agents (TRAs) focused on two areas: (1) exploring the structural and flexibility requirements of a key hydrophobic pendant group; (2) the position of the hydrophobic group.

MDR reversal activity of these taxanes was evaluated by testing the cytotoxicity of paclitaxel (Taxol) coadministered with a TRA against drug-resistant human breast cancer cell line MCF7-R or MDA-435/LCC6-MDR. In our previous study,[25−28] the taxanes with modifications at the C-7 position of DAB proved to be very active with >95% reversal activity in most cases. The best activity reached 99.8% recovery of the original antitumor activity of paclitaxel at 1 μM level of a TRA.[25]

The majority of the previous studies in our laboratories as well as others have focused on specific MDR inhibitors, only targeting P-gp, despite the fact that additional ABC transport proteins contribute to clinical MDR. It has been shown that P-gp and BCRP are significantly coexpressed, and P-gp expressed with MRP-1 greatly increases the efflux of chemotherapeutic

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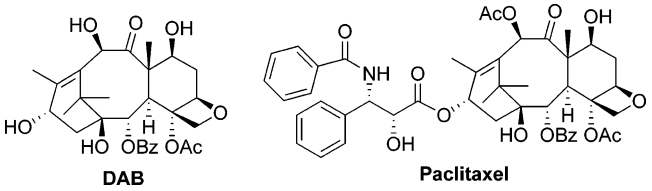


Figure 1. Structure of DAB and paclitaxel.

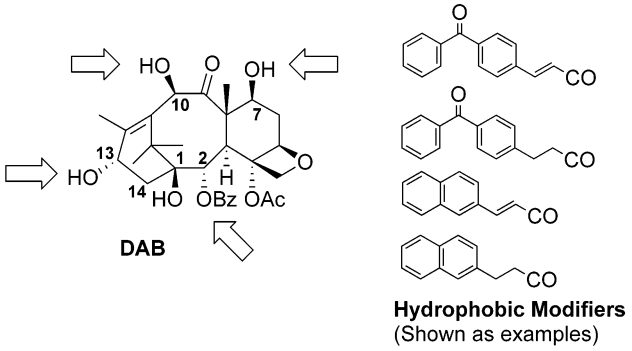


Figure 2. Design of TRAs.

agents.[7−11] Accordingly, the efficacy of broad spectrum modulation of these transporters by TRAs are worthy to explore.

A subset of the TRAs was selected for further testing to check their ability to block the efflux of mitoxantrone mediated by MRP-1, P-gp and BCRP. Then, six TRAs were found to be able to modulate all three efflux pumps, which would serve as broad-spectrum modulators of ABC transporters.

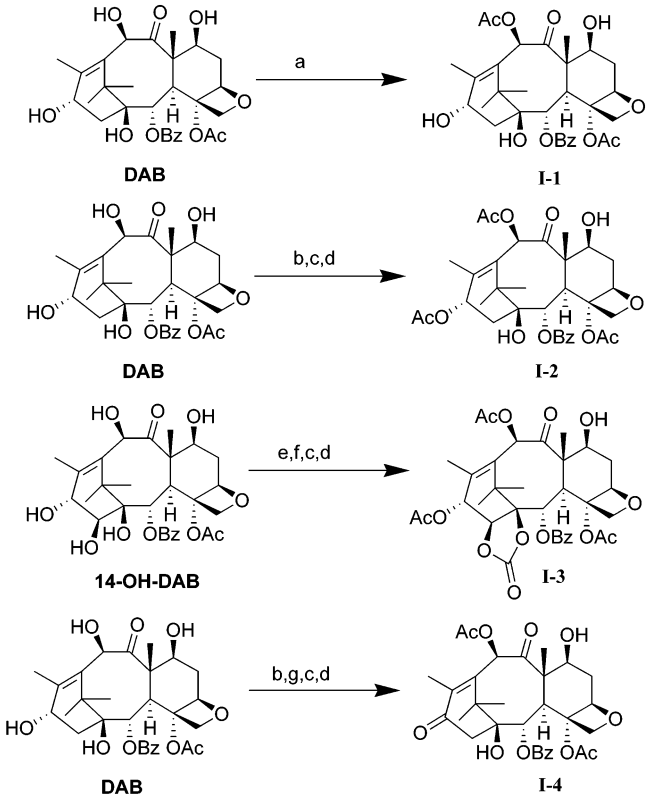
We describe here a full account of our work on the design, synthesis and SAR of TRAs.

Results and Discussion

Rational Design. Our design is based on the derivatization of DAB or 14-OH-DAB, which provides the crucial component of paclitaxel and taxoids, but is noncytotoxic by itself. These naturally occurring complex diterpenes possess several hydroxyl groups that can easily be modified with hydrophobic side chains by esterification (Figure 2). Benzophenone, naphthalene-containing carboxylic acids, and other hydrophobic groups were selected to modify C-2, C-7, C-10, and/or C-13 positions of either DAB or 14-OH-DAB. The structural requirements for the hydrophobic modifiers and the positional preference have been investigated in our SAR study.

Chemical Synthesis. Baccatin III (**I-1**), 13-Ac-baccatin III (**I-2**), 13-Ac-1,14-CO-baccatin III (**I-3**), and 13-oxo baccatin III (**I-4**) were prepared using previously established procedures (Scheme 1).[29] Baccatin III (**I-1**) was synthesized through acetylation of the C-10 hydroxyl group of DAB.[30,31] 13-Ac-baccatin III (**I-2**) was prepared by the selective TES-protection of the C-7 hydroxyl group of DAB and acetylation of the C-10 and C-13 hydroxyl groups, followed by deprotection of the C-7 silyl ether.[30] 13-Ac-1,14-CO-baccatin III (**I-3**) was prepared from 14-OH-DAB through the selective TES-protection of the C-7 hydroxyl group, carbonate formation between the C-1 and C-14 hydroxyl groups, acetylation of the C-10 and C-13 hydroxyl groups, and subsequent deprotection of the C-7 silyl ether. 13-Oxo-baccatin III (**I-4**)[32] was obtained through the selective

Scheme 1. Preparation of Modified Baccatin Cores[31] [a]



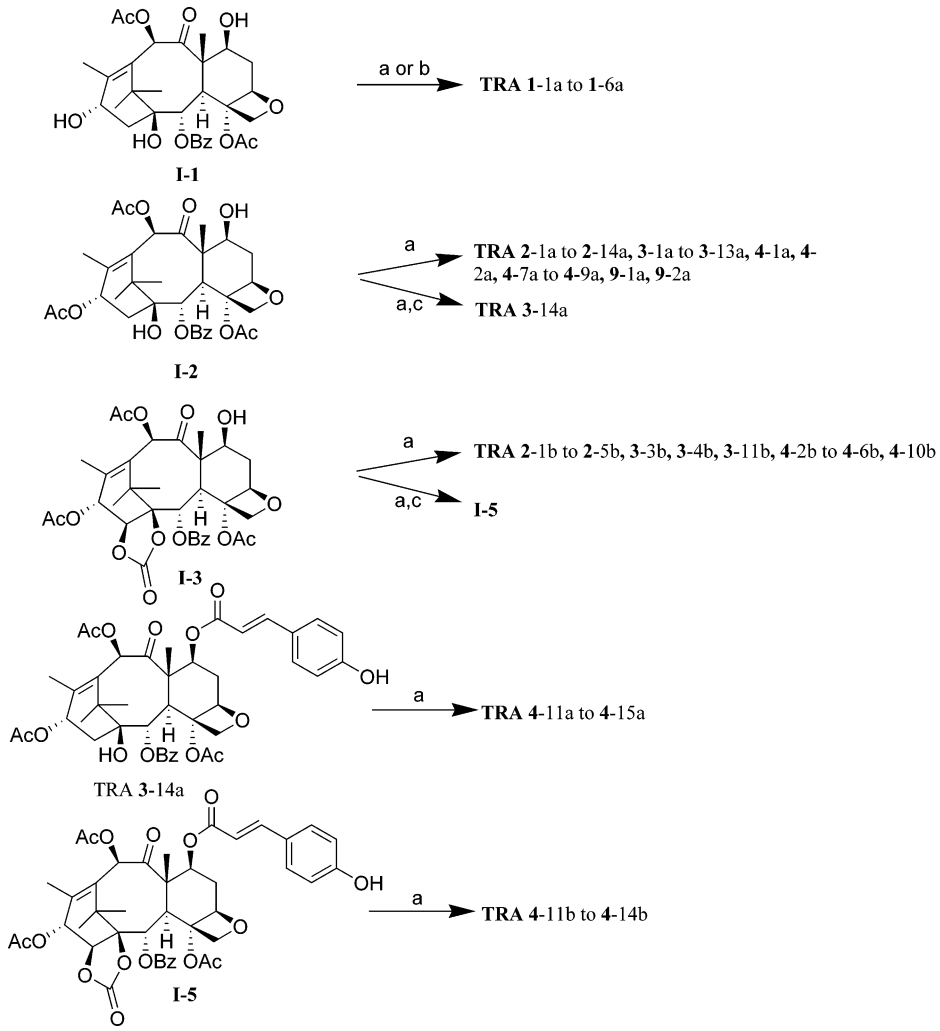
[a] Reagents and conditions: (a) Ac_2O, $CeCl_3$, THF, rt; (b) TESCl, Imidazole, DMF, 25 °C; (c) Ac_2O, DMAP, CH_2Cl_2, 25 °C; (d) HF−pyridine, pyridine/CH_3CN, 0 °C to 25 °C; (e) TESCl, pyridine/DMF, 25 °C; (f) $Cl_3COCOOCCl_3$, pyridine/CH_2Cl_2, 0 °C; (g) TPAP, NMO, CH_2Cl_2, 25 °C.

TES-protection of the C-7 hydroxyl group of DAB, oxidation of the C-13 hydroxyl group, and acetylation of the C-10 hydroxyl group, followed by deprotection.

TRA **1**-1a to TRA **1**-6a were obtained through acylations at the C-7 position of **I-1** with two coupling methods. One was the preparation of the acyl chloride of the corresponding acid and subsequent coupling with the 7-OH of a baccatin in the presence of DMAP. The other was a direct coupling of the acid with the 7-OH of a baccatin using EDC and DMAP. Acylations of the C-7 hydroxyl group of **I-2** and **I-3** were carried out with appropriate carboxylic acids in the presence of EDC and DMAP to afford the corresponding TRA **2**-1a to TRA **2**-14a, TRA **2**-1b to TRA **2**-5b, TRA **3**-1a to TRA **3**-13a, TRA **3**-3b, TRA **3**-4b, TRA **3**-11b, TRA **4**-1a, TRA **4**-2a, TRA **4**-7a to TRA **4**-9a, TRA **4**-2b to TRA **4**-6b, TRA **4**-10b, TRA **9**-1a, and TRA **9**-2a ("a" and "b" means the corresponding TRA is derived from DAB and 14-OH-DAB respectively). TRA **3**-14a and **I-5**, bearing a phenol moiety in the side chain, were obtained by treating the silyl-protected intermediates with HF/pyridine. Acylation of the phenolic hydroxyl groups of TRA **3**-14a and **I-5** with different acids led to TRA **4**-11a to TRA **4**-15a, and TRA **4**-11b to TRA **4**-14b. (Scheme 2).

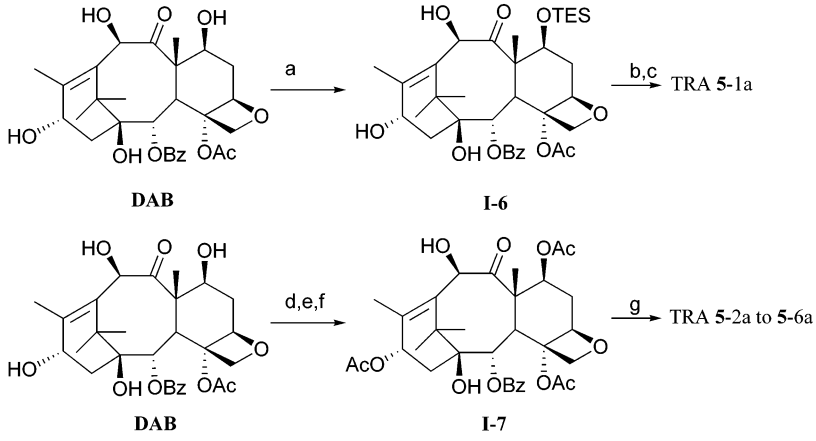
Syntheses of C-10 modified TRAs are shown in Scheme 3. Preparation of TRA **5**-1a started from 7-TES-DAB **I-6**,[33] and the C-10 modification was carried out with the use of *N*-(4-benzoylcinnamoyloxy)succinimide. Subsequent removal of the TES group with HF/pyridine afforded TRA **5**-1a. For the syntheses of other C-10 modified TRAs, 7,13-diacetyl-10-DAB (**I-7**) was used.

Scheme 2. Synthesis of 7-Modified TRAs[a]



[a] Reagents and conditions: (a) RCO_2H, EDC, DMAP, CH_2Cl_2, 25 °C; (b) RCOCl, Et_3N, DMAP, CH_2Cl_2, 25 °C; (c) HF−pyridine, pyridine/CH_3CN, 0 °C to 25 °C.

Scheme 3. Synthesis of 10-Modified TRAs[a]

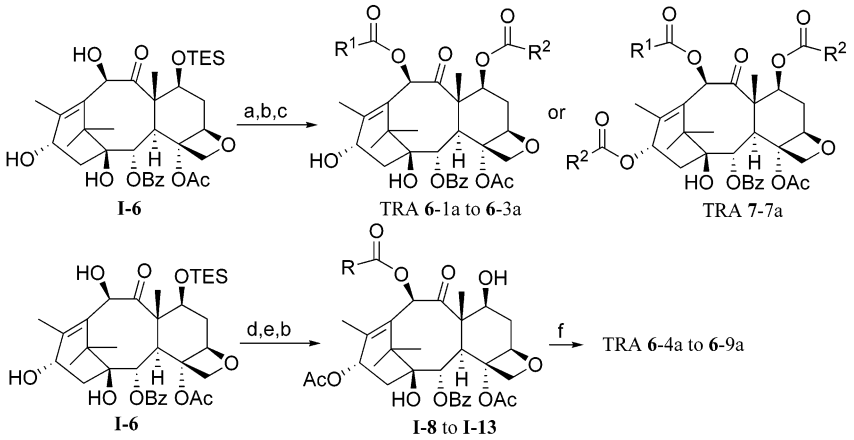


[a] Reagents and conditions: (a) TESCl, pyridine, room temperature; (b) *N*-hydroxysuccinimide ester of RCO_2H, LiHMDS, THF, −40 °C; (c) HF−pyridine, pyridine/CH_3CN, 0 °C to room temperature; (d) $CF_3C(OTMS)NTMS$, THF, 0 °C; (e) Ac_2O, DMAP, CH_2Cl_2, 25 °C; (f) 0.1N HCl, EtOH, 25 °C; (g) RCO_2H, EDC, DMAP, CH_2Cl_2, 25 °C.

Syntheses started from the selective protection of DAB at C-10, followed by acetylation at C-7 and C-13. Deprotection of C-10 and coupling with the corresponding acid afforded the desired TRA **5**-2a to TRA **5**-6a (Scheme 3).

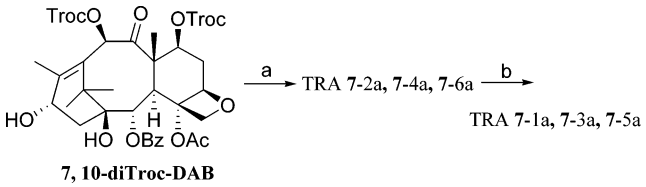
C-7,C-10 dimodified TRAs were also prepared using previously established procedures.[33,34] Starting from 7-TES-DAB **I-6**, the C-10 position was modified by reacting with the corresponding succinimide esters. Removal of the 7-TES group with HF/pyridine, followed

Scheme 4. Synthesis of 7,10-Modified and 7,10,13-Modified TRAs[a]



[a] Reagents and conditions: (a) *N*-hydroxysuccinimide ester of RCOOH, LiHMDS, THF, −40 °C; (b) HF−pyridine, pyridine/CH_3CN, 0 °C to room temperature; (c) RCOOH, DCC, DMAP, CH_2Cl_2, room temperature; (d) RCOCl, LiHMDS, THF, −40 °C; (e) Ac_2O, DMAP, CH_2Cl_2, 25 °C; (f) *p*-benzoylcinnamic acid, EDC, DMAP, CH_2Cl_2, 25 °C.

Scheme 5. Synthesis of 13-Modified TRAs[a]



[a] Reagents and conditions: (a) RCOOH, DCC, DMAP, CH_2Cl_2, room temperature; (b) Zn, AcOH/MeOH, 70 °C.

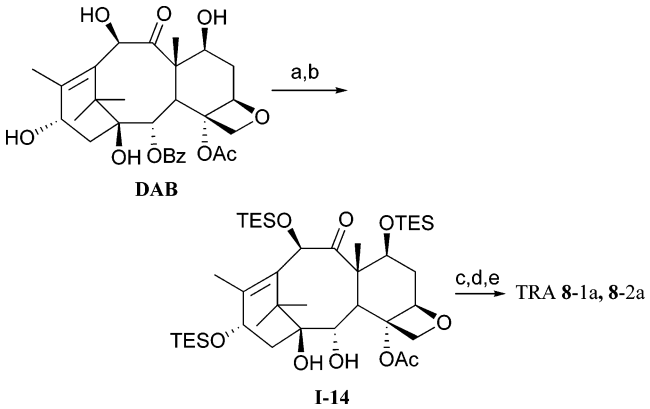
by coupling with the corresponding acids, provided TRA **6**-1a to TRA **6**-3a. TRA **7**-7a was synthesized in the same manner with the third modifier at the C-13 position. TRAs **6**-4a to TRA **6**-9a were prepared in parallel using a slightly different method. Selective protection of the C-7 hydroxyl group was followed by acylation of the C-10 hydroxyl group with the corresponding acid chloride. Acetylation of the C-13 alcohol and deprotection of the silyl ether gave alcohols **I-8** to **I-13**. Coupling of the resulting alcohols with *p*-benzoylcinnamic acid in the presence of EDC and DMAP yielded C-10 modified TRA **6**-4a to TRA **6**-9a (Scheme 4).

Coupling of the appropriate pendant groups with 7, 10-diTroc-DAB afforded 13 modified TRA **7**-2a, TRA **7**-4a, and TRA **7**-6a (Scheme 5). Further deprotection of the Troc groups was carried out using zinc in AcOH/MeOH at 70 °C to produce TRA **7**-1a, TRA **7**-3a, and TRA **7**-5a.

To prepare C-2 modified analogues, the hydroxyl groups at the C-7, C-10, and C-13 positions were suitably protected with TES groups (Scheme 6). Debenzoylation at C-2 using Red-Al proceeded smoothly to afford 2-debenzoyl-triTES-DAB (**I-14**)[35] which was then coupled with acids in the presence of DCC and DMAP. Finally, the TES groups were replaced by acetyl groups in two steps to give TRA **8**-1a and TRA **8**-2a.

Acylation of the C-2 hydroxyl group with *m*-anisic acid followed by deprotection and selective protection of the resulting C-7 alcohol yielded C-2 modified baccatin **I-15**. Acetylation of the C-10 and C-13 positions, removal of the silyl group, and acylation of the resulting C-7 alcohol with *p*-benzoylcinnamic acid in the presence of EDC and DMAP yielded the C-2,C-7 modified TRA **8**-3a (Scheme 7).

Scheme 6. Synthesis of 2-Modified TRAs[a]



[a] Reagents and conditions: (a) TESCl, imidazole, DMF, room temperature; (b) Red-Al, THF, 0 °C; (c) RCOOH, DCC, DMAP, CH_2Cl_2, room temperature; (d) HF−pyridine, pyridine/CH_3CN, 0 °C to room temperature; (e) Ac_2O, DMAP, CH_2Cl_2, room temperature.

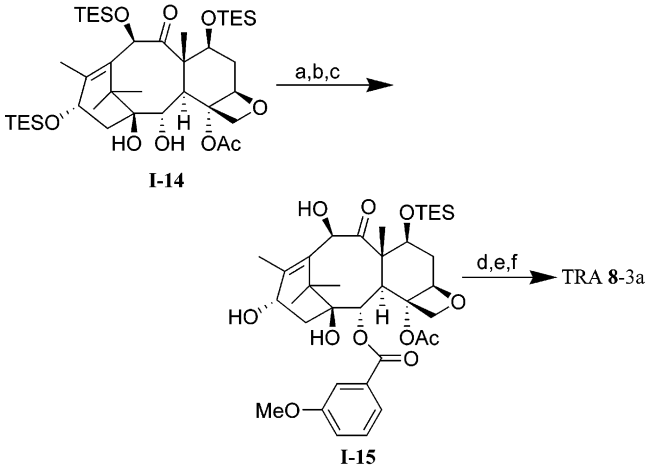
Hydrogenation of the C-2 benzoate of 13-Ac-baccatin III (**I-2**) over Pt/C catalyst followed by acylation of the C-7 alcohol with *p*-benzoylcinnamic acid afforded C-2,C-7 modified TRA **8**-4a (Scheme 8).

Acylation of the C-7 hydroxyl of 13-oxo baccatin III (**I-4**) with *p*-benzoylcinnamic acid in the presence of EDC and DMAP gave 7-modified-13-keto analogue, TRA **8**-5a (Scheme 9).

Evaluation of Biological Activities. MDR-Reversal Activity in Combination with Paclitaxel. For the assessment of MDR-reversal activity of these TRAs, the cytotoxicity of paclitaxel in combination with a TRA is evaluated against the resistant human breast tumor cell lines MCF7-R and/or MDA-435/LCC6-MDR. We have found that all TRAs, except for the ones bearing a side chain at the C-13 position, possess very weak cytotoxicities (i.e., $IC_{50} > 10\ \mu M$) or are noncytotoxic up to the solubility limit (ca. 30 μM).

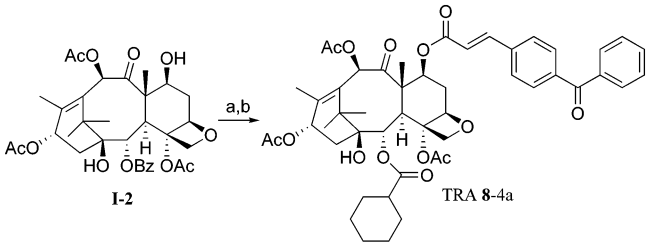
MDR-reversal activity of the six TRAs, TRA **1**-1a to TRA **1**-6a, bearing different side chains at C-7 and a free hydroxyl group at C-13, is listed in Table 1. Four of them, TRA **1**-1a to TRA **1**-4a, show high level of activity in the range of 96−99.8% at 1−3 μM concentration. These results clearly indicate that the modification

Scheme 7. Synthesis of 2,7-Modified TRA **8**-3a[a]



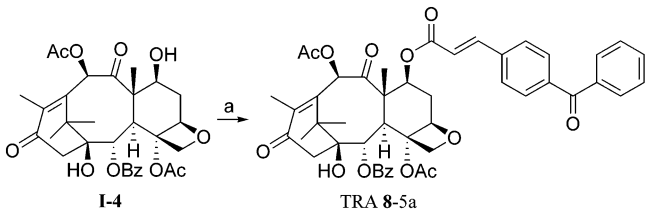
[a] Reagents and conditions: (a) *m*-anisic acid, DIC, DMAP, CH_2Cl_2, 37 °C; (b) HF−pyridine, pyridine/CH_3CN, 0 °C to 25 °C; (c) TESCl, imidazole, DMF, 25 °C; (d) Ac_2O, DMAP, CH_2Cl_2, 25 °C; (e) HF−pyridine, pyridine/CH_3CN, 0 °C to 25 °C; (f) *p*-benzoylcinnamic acid, EDC, DMAP, CH_2Cl_2, 25 °C.

Scheme 8. Synthesis of 2,7-Modified TRA **8**-4a[a]



[a] Reagents and conditions: (a) Pt/C, H_2, EtOAc, 25 °C; (b) *p*-benzoylcinnamic acid, EDC, DMAP, CH_2Cl_2, 25 °C.

Scheme 9. Synthesis of 13-Oxo-TRA **8**-5a[a]

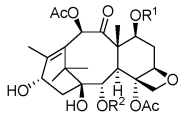


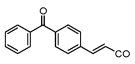
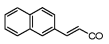
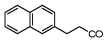
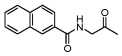
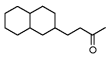
[a] Reagents and conditions: (a) *p*-benzoylcinnamic acid, EDC, DMAP, CH_2Cl_2, 25 °C.

of C-7 position is suitable for strong MDR reversal activity. Benzophenone and naphthalene side chains are appropriate pendant groups, and the former appears to be slightly better than the latter. Shortening the side chain by two carbons does not have influence on the reversal activity (TRA **1**-4a compared with TRA **1**-3a). However, increasing the hydrophilicity of the TRA by introducing a glycine linker (TRA **1**-5a, 42%), or reducing the aromatic groups to saturated cycloalkyl groups (TRA **1**-6a, 81%), results in dramatic or considerable reduction of activity. This implies the need of a conjugated planar ring structure in the pharmacophore of TRAs.

Further evaluation of the TRAs containing a benzophenone moiety in the C-7 side chain is summarized in Table 2. TRA **2**-1a gave 99.7% reversal activity, similar to TRA **1**-1a. This means that the C-13 acetylation does not affect MDR-reversal activity. The length of the linker plays an important role in reversal activity.

Table 1. MDR-reversal Activity of TRAs Bearing a Hydrophobic Moiety at C-7 and a Free Hydroxyl Group at the C-13



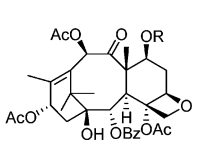
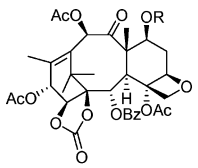
TRA	R^1	R^2	%Reduction[a] MCF7-R	%Reduction[a] MDA-435/LCC6-MDR
1-1a			**99.8**	--
1-2a			**96**[b]	50
1-3a			**96**	--
1-4a			**97.5**	--
1-5a			42	--
1-6a			81	--

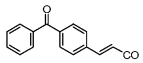
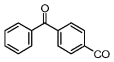
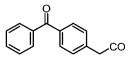
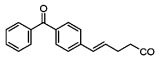
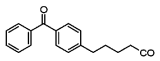
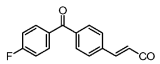
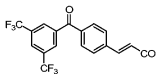
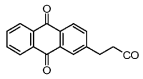
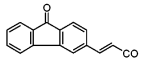
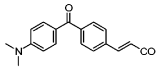
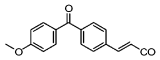
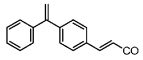
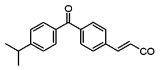
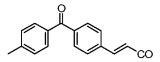
[a] % Reduction = [1 − {IC_{50}(0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100. For example, for TRA **1**-1a, in MCF7-R cell line, IC_{50}(3 μM reversal agent + paclitaxel) = 1.6 nM, IC_{50}(paclitaxel) = 860 nM, % reduction = [1-(1.6/860)] × 100 = 99.8%. [b] See ref 36.

While the removal of the ethylene linker (TRA **2**-2a and TRA **2**-2b) was well tolerated, shortening the linker by one carbon (TRA **2**-3a and TRA **2**-3b), or increasing the chain length (TRA **2**-4a, TRA **2**-5a, TRA **2**-4b, and TRA **2**-5b), resulted in severely muted reversal activity. *Para* substituents on the benzophenone moiety were generally well tolerated (TRA **2**-6a, TRA **2**-10a, TRA **2**-11a, TRA **2**-13a, and TRA **2**-14a), resulting in retained or slightly increased activities. Formation of a C−C bond between the two ortho positions of the benzophenone (TRA **2**-9a) was well tolerated, and the insertion of a carbonyl linker and reduction of the double bond (TRA **2**-8a) resulted in loss of activity. Replacement of the benzophenone carbonyl with an alkene resulted in muted activity (TRA **2**-12a). A cyclic carbonate functionality at the C-1 and C-14 positions of the baccatin does not seem to change the activity [compare (TRA **2**-1b to TRA **2**-5b) with (TRA **2**-1a to TRA **2**-5a)].

MDR-reversal activity of TRAs bearing a variety of hydrophobic side chains at the C-7 position is summarized in Table 3. TRA **3**-11a bearing a naphthalene moiety at the C-7 position exhibited very high activity against MCF7-R cell line, while 14-OH-DAB analogue (TRA **3**-11b) gave fairly good activity against MDA-435/LCC6-MDR cell line. The reduction of the ketone moiety to a racemic alcohol and saturation of the double bond (TRA **3**-1a) resulted in about a 50% loss in activity. Further reduction of the alcohol to an alkane (TRA **3**-2a) drastically reduced activity. Replacing the benzophenone moiety with a diphenyl ether (TRA **3**-3a and TRA **3**-3b) retained activity, while replacement with a thioether (TRA **3**-4a and TRA **3**-4b) reduced activity. Replacement of the benzophenone with an indole ring (TRA **3**-8a) or a naphthyl ether (TRA **3**-12a) was well tolerated, but replacement with a C-1 naphthyl ether (TRA **3**-13a) resulted in muted activity.

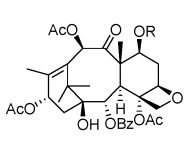
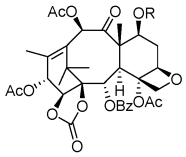
Table 2. MDR-Reversal Activity of TRAs Bearing a Benzophenone Moiety with a Variable Chain Length at the C-7 Position

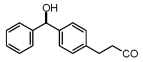
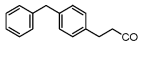
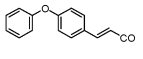
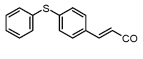
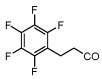
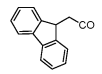
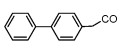
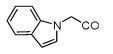
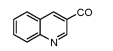
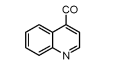
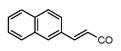
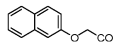
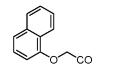
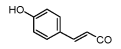



TRA	R	%Reduction[a] MDA-435/LCC6-MDR	%Reduction[a] MCF7-R
2-1a		**92**[d]	**99.7**
2-1b[c]		**94**[d]	--
2-2a		88[d]	--
2-2b[c]		**92**[d]	--
2-3a		41	--
2-3b[c]		44	--
2-4a		21	--
2-4b[c]		11	--
2-5a		6	--
2-5b[c]		0	--
2-6a		**92**[d]	--
2-7a		36	--
2-8a		29	--
2-9a		**91**[d]	--
2-10a		89[d]	--
2-11a		89[d]	--
2-12a		52	--
2-13a		**94**[d]	--
2-14a		**91**[d]	--

[a] % Reduction = [1 − {IC_{50}(0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100. [c] Taxanes derived from 14-OH DAB. [d] See ref 36.

Table 3. MDR-Reversal Activity of TRAs Bearing Various Hydrophobic Pendant Groups at the C-7 Position

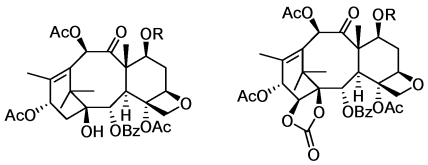
TRA	R	%Reduction[a] MDA-435/LCC6-MDR	%Reduction[a] MCF7-R
3-1a		48	--
3-2a		16	--
3-3a		**90**[d]	--
3-3b[c]		**90**[d]	--
3-4a		64	--
3-4b[c]		63	--
3-5a		59	--
3-6a		67	--
3-7a		57	--
3-8a		83	--
3-9a		41	--
3-10a		36	--
3-11a		--	**99.3**
3-11b[c]		60	--
3-12a		86	--
3-13a		60	--
3-14a		34	--

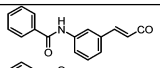
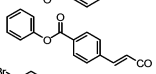
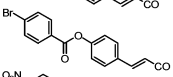
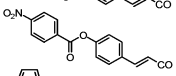
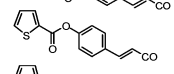
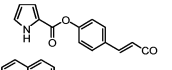
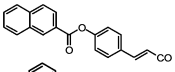
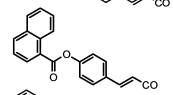
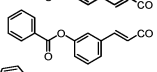
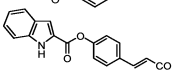
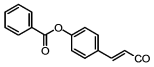
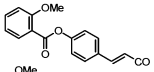
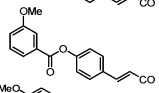
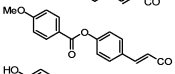
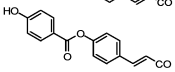
[a] % Reduction = [1 − {IC_{50}(0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100. [c] Taxanes derived from 14-OH DAB. [d] See ref 36.

As Table 4 illustrates, replacement of the benzoyl group with a benzamide (TRA **4**-1a) is well tolerated, and the corresponding ester isosteres (TRA **4**-11a, TRA **4**-11b) also retain high levels of potency. Reversing the ester orientation, however, resulted in decreased activity for the TRA derived from DAB (TRA **4**-2a), while the corresponding TRA based on 14-OH DAB (TRA **4**-2b) retained activity. Introduction of para-substituted benzoyloxy substituents (both electron donating and withdrawing) (TRA **4**-3b, TRA **4**-4b, TRA **4**-14a, TRA **4**-14b, and TRA **4**-15a) resulted in drastically reduced activities, indicating that there are definite steric requirements for good activity. Introduction of a heteroaromatic group (TRA **4**-5b, TRA **4**-6b, and TRA **4**-10b) gave significantly reduced activities, in all cases as expected. This can be abscribed to a reduction of hydrophobic nature of the group. Introduction of a hydroxyl group to the benzoyloxy moiety (TRA **4**-15a) led to a significant loss in activity, so as that of a methoxy substituent (TRA **4**-12a to TRA **4**-14a, TRA **4**-12b to TRA **4**-14b).

As Table 5 shows, modification of the C-10 hydroxyl group with a benzophenone side chain (TRA **5**-1a) results in a very good reversal activity (95%). However, in all other instances, the attachment of a hydrophobic side chain containing diphenyl ether, diphenyl thioether, benzamide, benzoate to the C-10 position (TRA **5**-2a to TRA **5**-6a) resulted in significant loss of activity.

Table 4. MDR-Reversal Activity[a] of TRAs Bearing Aromatic or Heteroaromatic Benzoate or Benzamide Moiety at the C-7 Position

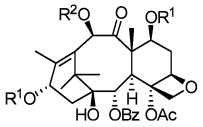


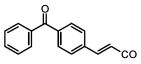
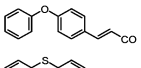
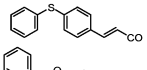
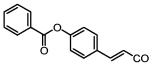
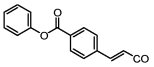
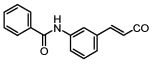
TRA	R	% Reduction[b]
4-1a		83[d]
4-1b[c]		91[d]
4-2a		27
4-2b[c]		84
4-3b[c]		34
4-4b[c]		27
4-5b[c]		55
4-6b[c]		63
4-7a		29
4-8a		89
4-9a		17
4-10b[c]		43
4-11a		**91**[d]
4-11b[c]		**95**[d]
4-12a		57
4-12b[c]		79
4-13a		34
4-13b[c]		62
4-14a		39
4-14b[c]		69
4-15a		24

[a] MDA-435/LCC6-MDR cell line was used. [b] % Reduction = [1 − {IC_{50}(0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100. [c] Taxanes derived from 14-OH DAB. [d] See ref 36.

As Table 6 indicates, the effects of introducing two hydrophobic side chains at the C-7 and C-10 positions on the reversal activity are more complicated. An activity ranging from 0% (TRA **6**-2a**,** Table 6) to 99.7% (TRA **6**-1a) is observed depending on the substitution pattern. TRA **6**-1a with modification at both C-7 and C-10 positions with benzophenone moiety proved to be very active (99.7%). Replacement of the C-10 benzophenone moiety with either a methyl formate (TRA **6**-8a) or a propanoyl group (TRA **6**-9a) was well tolerated, resulting in only minimal loss of activity. However, replacement with larger aromatic substituents (TRA **6**-4a to TRA **6**-7a), or replacement of the C-7 benzophenone side chain with a naphthalene moiety (TRA **6**-2a and TRA **6**-3a), resulted in significant loss of activity. Thus, hydrophobicity is not the only requirement for an efficient MDR reversal activity. These results strongly suggest that there is a specific binding site for TRAs on P-gp that has rather strict steric/shape requirements.

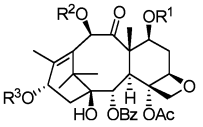
Table 5. MDR-Reversal Activity of TRAs Bearing a Hydrophobic Moiety at the C-10 Position

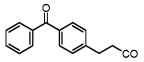
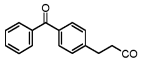
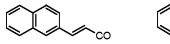
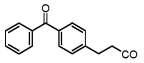
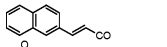
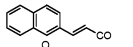
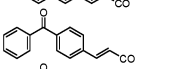
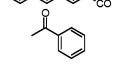
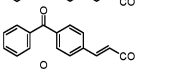
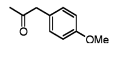
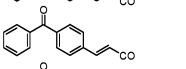
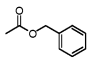
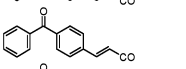
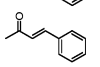
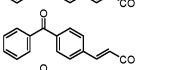
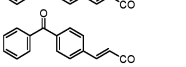


TRA	R^1	R^2	% Reduction[a] MDA-435/LCC6-MDR	% Reduction[a] MCF7-R
5-1a	H		--	**95**
5-2a	Ac		29	--
5-3a	Ac		28	--
5-4a	Ac		7	--
5-5a	Ac		24	--
5-6a	Ac		43	--

[a] % Reduction = [1 − {IC_{50}(0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100.

Table 6. MDR-reversal Activity of TRAs Bearing Various Pendant Groups at the C-7 and C-10 Positions



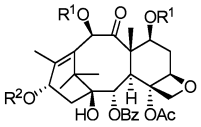
TRA	R^1	R^2	R^3	% Reduction[a] MDA-435/LCC6-MDR	% Reduction[a] MCF7-R
6-1a			H	--	**99.7**
6-2a			H	--	0
6-3a			H	--	45
6-4a			Ac	51	--
6-5a			Ac	7	--
6-6a			Ac	10	--
6-7a			Ac	11	--
6-8a		MeOCO	Ac	**91**	--
6-9a		CH_3CH_2CO	Ac	88	--

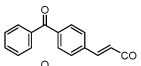
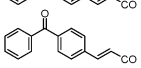
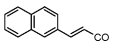
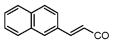
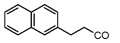
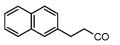
[a] % Reduction = [1 − {IC_{50}(0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100.

Table 7 shows the activity of TRAs bearing a hydrophobic moiety at the C-13 position. In general, C13-modified TRAs showed little activity except for TRA **7**-2a and TRA **7**-3a, which exhibited relatively strong reversal activity (80% and 88%, respectively). Additionally, C7,C10,C13-trisubstituted TRA **7**-7a (Figure 3) showed no activity.

Modification of the C-2 position with a benzophenone or a naphthalene side chain also resulted in very little

Table 7. MDR-reversal Activity[a] of TRAs Bearing a Hydrophobic Moiety at the C-13 Position



TRA	R^1	R^2	% Reduction[b]
7-1a	**H**		0
7-2a	**Troc**		80
7-3a	**H**		88
7-4a	**Troc**		12
7-5a	**H**		37
7-6a	**Troc**		0

[a] MCF7−R cell line was used. [b] % Reduction = [1 − {IC_{50}(0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100.

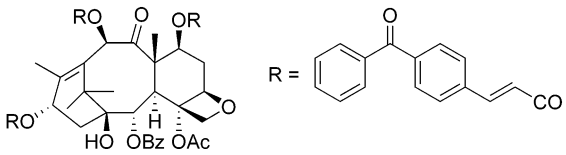
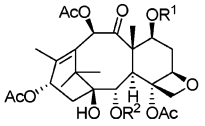
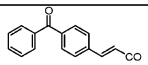
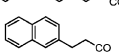
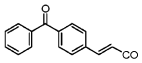
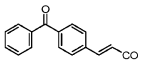


Figure 3. Structure of TRA **7**-7a.

Table 8. MDR-reversal Activity[a] of TRAs Bearing Hydrophobic Moieties at the C-2 or C-7 and C-2 Positions



TRA	R^1	R^2	% Reduction[b]
8-1a	Ac		27
8-2a	Ac		24
8-3a			95[c]
8-4a			86

[a] MDA-435/LCC6-MDR cell line was used. [b] % Reduction = [1 − {IC_{50} (0.1 μM reversal agent + paclitaxel)/IC_{50}(paclitaxel)}] × 100. [c] See ref 36.

activity (TRA **8**-1a and TRA **8**-2a) as shown in Table 8. When the C-7 hydroxyl group was substituted by an arenecarbonylcinnamoyl group, replacement of the C-2 benzoyl group with a *m*-methoxybenzoyl group (TRA **8**-3a) resulted in very good activity (Table 8). Replacement with a cyclohexanoyl group (TRA **8**-4a) instead of a benzoyl group was tolerated, causing only a minor loss of activity. Oxidation of the C-13 position to a ketone (TRA **8**-5a, Figure 4) resulted in a significant loss in activity (37% reduction at 0.1 μM against MDA-435/LCC6-MDR cell line). These results indicate that a C-2 modification with a bulky group or a C-13 modification

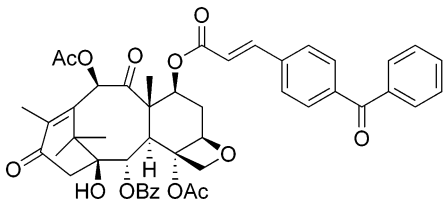


Figure 4. Structure of TRA **8**-5a.

is not appropriate for achieving good MDR reversal activity.

We should add that besides paclitaxel, the sensitization of MCF7-R cells by the TRAs was also observed with another commonly used anthracycline-class anticancer agent, doxorubicin. TRA **1**-2a exhibited 92% MDR reversal activity (at 1 μM concentration) when coadministered with doxorubicin.[25]

As mentioned above, we hypothesized that TRAs would block the efflux pump of P-gp and other ABC transporters, thereby restoring the drug sensitivity in the MDR-expressing cancer cells by allowing cytotoxic drugs to accumulate in the cancer cells. To verify this hypothetical mechanism, we investigated the effects of TRA **1**-2a on the intracellular accumulation of paclitaxel in the drug-resistant cancer cell MCF7-R. As a control experiment, we also looked at the effects of TRA **1**-2a on the concentration of paclitaxel in the parent drug sensitive cancer cells MCF7. As Figure 5 shows, TRA **1**-2a does not have any appreciable effects on the accumulation of paclitaxel in the drug sensitive cells. In sharp contrast to this, the addition of TRA **1**-2a (1 μM) dramatically increased the accumulation of paclitaxel in the drug resistant cells although only a very low level of paclitaxel is detected without TRA **1**-2a. These results strongly support that TRAs block the P-gp efflux, allowing the anticancer drug to accumulate in the drug-resistant cancer cells and exert its chemotherapeutic effect.

Broad Spectrum Modulation of ABC Transport Proteins by TRAs and Mitoxantrone.[36,37] Since the majority of previous studies on MDR-reversal agents have only focused on P-gp modulators, clinical trials of the known MDR-reversal agents have been disappointing to date.[38−42] The true measure of an effective MDR-reversal agent is its ability to modulate drug efflux mediated by a broad spectrum of ABC transporters. Recently, Okumura et al.[43] found that a newly synthesized taxoid, 5-*O*-benzoylated taxinine K (BTK), has the ability to reverse P-gp- and MRP-mediated multidrug resistance. Our group also examined the broad-spectrum modulatory activity of TRAs synthesized in our laboratories. Among the large number of TRAs synthesized, the best 20 compounds in terms of their MDR reversal activity in combination with paclitaxel against P-gp-overexpressing MDA435/LCC6-MDR cell lines were chosen for further testing to check their ability to block the efflux of mitoxantrone mediated by MRP-1, P-gp, and BCRP.[36] Cell lines used for this study were drug-resistant human myelogenous HL60-ADR (overexpresses MRP-1), drug-resistant human myeloma line 8226-Dox6 (overexpresses P-gp), and 8226-MR20 (overexpresses BCRP). The bioassay results showed that four TRAs were able to modulate all three efflux pumps (TRA **2**-10a, TRA **2**-11a, TRA **4**-1b, TRA **2**-2a). Based on these results, two more new TRAs, TRA **9**-1a and TRA **9**-2a,

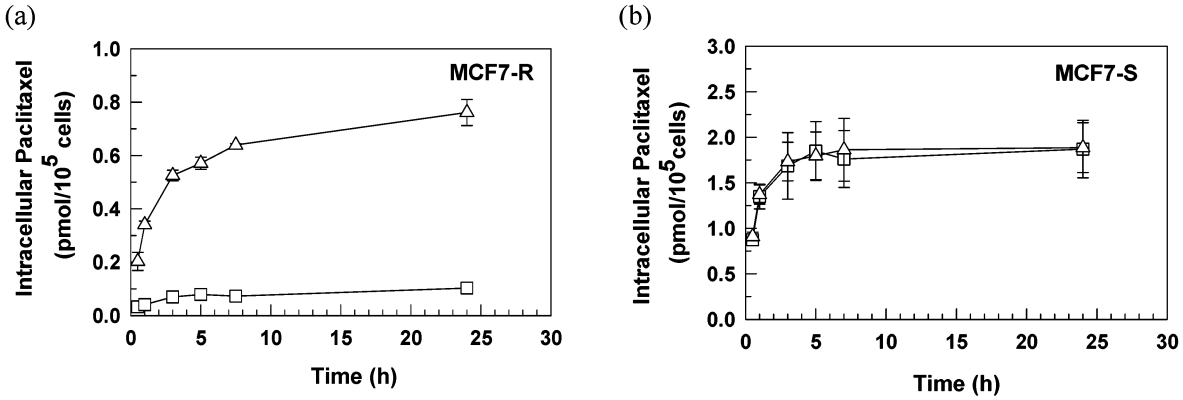


Figure 5. Modulation of tritiated paclitaxel accumulation by MDR reversal TRA **1**-2a in MCF7 cells, resistant (a) and sensitive (b). Square, 10 nM of paclitaxel; triangle, 10 nM of paclitaxel and 1 μM of TRA **1**-2a.

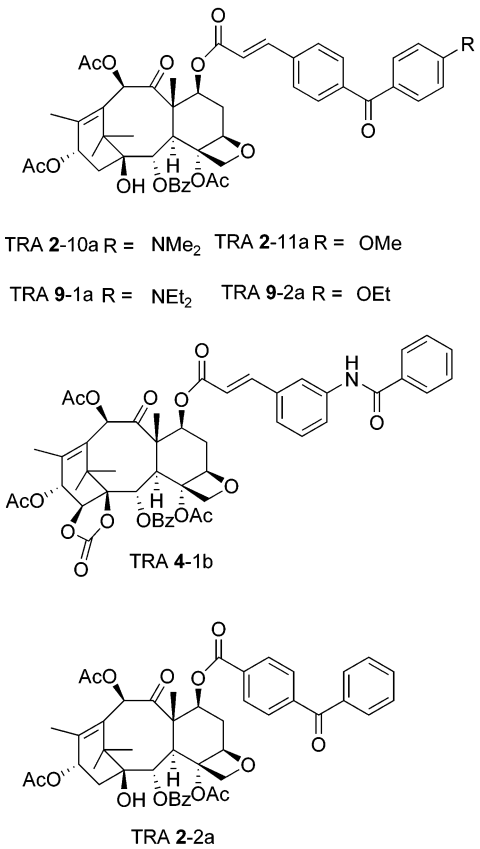


Figure 6. TRAs that modulate all three efflux pumps.

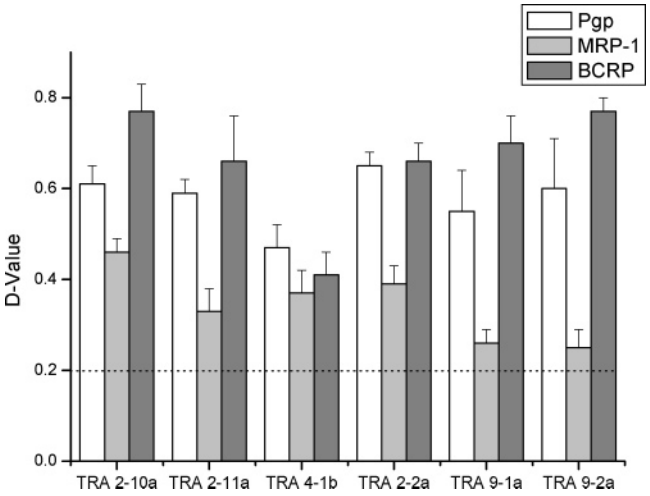


Figure 7. Effect of TRAs on drug efflux in resistant cell lines.[36] D-values represent the separation of the distribution histograms for uptake in RPMI 1640 + 3 μM mitoxantrone and uptake in RPMI 1640 + 3 μM mitoxantrone + 10 μM TRA in Kolmogorov-Smirnov (KS) statistics. Screening of TRAs for modulation of mitoxantrone efflux mediated by Pgp (8226-Dox6), MRP-1 (HL60-ADR) and BCRP (8226-MR20) is demonstrated. D-value $\geq$ 0.20 indicates modulatory activity. The graph shows the mean values from triplicate experiments, with standard errors.

were also synthesized as analogues of TRA **2**-10a and TRA **2**-11a, which were the best two TRAs so far, and evaluated for their broad-spectrum modulatory activities. The structures of these six TRAs, TRA **2**-10a, TRA **9**-1a, TRA **2**-11a, TRA **9**-2a, TRA **4**-1b and TRA **2**-2a, are shown in Figure 6, and their broad-spectrum modulatory activities are illustrated in Figure 7.

The ability of these TRAs to increase the uptake of mitoxantrone was examined. At 10 μM, all six TRAs exerted significant effects on increasing the amount of mitoxantrone taken up into the cancer cells overexpressing either P-gp or BCRP. However, MRP-1 was found to be rather difficult to modulate efficiently (Figure 7).[36]

Among the six TRAs that possess broad-spectrum reversal activity, demonstrating modulation of P-gp, MRP-1, and BCRP, TRA **2**-10a has been selected as the leading broad-spectrum modulator with the highest reversal activity in all three cell lines. TRA **9**-2a is the next active compound, which is less active than TRA **2**-10a against HL60-ADR cell line, overexpressing MRP-1, but comparable against the P-gp- and BCRP-overexpressing cell lines.

The ability of TRAs to enhance mitoxantrone cytotoxicity was also studied. Cells were treated either with mitoxantrone alone or in combination with each of the four TRAs, TRA **2**-10a, TRA **2**-11a, TRA **4**-1b, and TRA **2**-2a, at different doses (0.1 μM, 1 μM, 10 μM). Figure 8 shows the effects of TRAs on mitoxantrone cytotoxicity for 96 h continuous exposure to 8226-Dox6 (A), HL60-ADR (B), and 8226-MR20 (C) cell lines.

All four TRAs effectively enhanced cytotoxicity of mitoxantrone in the P-gp-overexpressing and BCRP-overexpressing cell lines. However, the effect on the MRP-1-overexpressing cell line was more limited. Nevertheless, a significant improvement in the IC_{50} was still observed with TRA **2**-10a and TRA **2**-11a at the con-

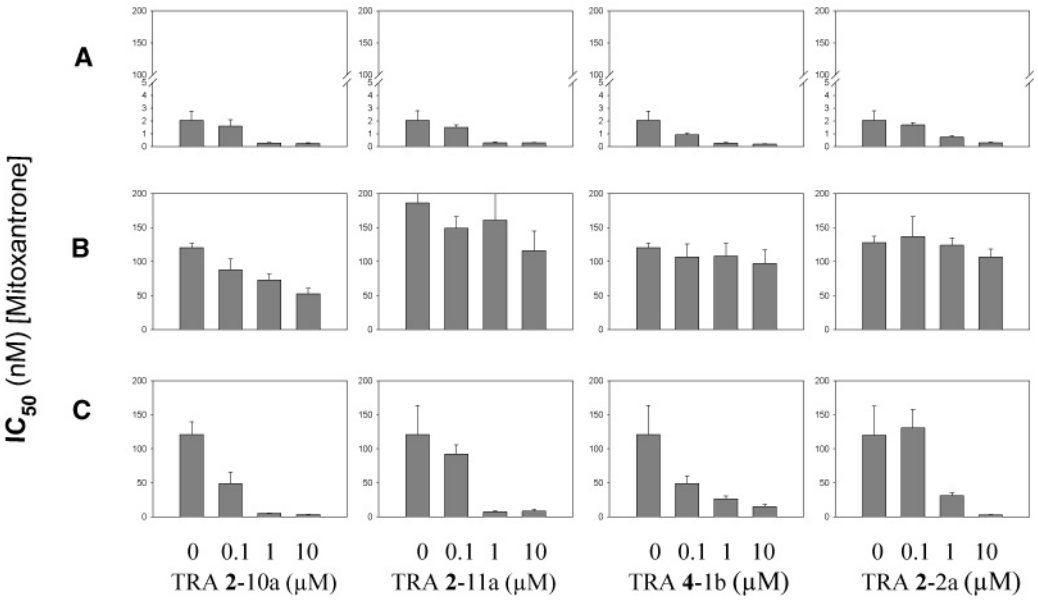


Figure 8. Effect of TRAs on mitoxantrone cytotoxicity 96 h continuous exposure to cell lines A: 8226-Dox6 (overexpresses P-gp); B: HL60-ADR (overexpresses MRP 1); C: 8226-MR20 (overexpresses BCRP).

centration of 10 μM. The best result for the reduction of the IC_{50} of mitoxantrone against HL60-ADR (MRP-1+) cell line was observed with TRA **2**-10a at 10 μM concentration (lowering the IC_{50} by 40% to 73 $\pm$ 9 nM). Comparison of activity at 1 μM concentration also shows that TRA **2**-10a is the most effective agent against 8226-Dox6 (P-gp+) cell line, lowering the IC_{50} by 87% to 0.27 $\pm$ 0.04 nM, and 8226-MR20 (BCRP+) cell line, lowering the IC_{50} by 96% to 5.02 $\pm$ 0.5 nM. TRA **2**-11a is also very effective against these three cell lines.

Conclusion

Libraries of novel taxane-based MDR reversal agents (TRAs) were designed, synthesized, and evaluated for their modulating ability against P-glycoprotein overexpressed in the drug-resistant cancer cell lines, MCF7-R and MDA-435/LCC6-MDR. These TRAs exhibited MDR-reversal activities up to 99.8% when coadministered with paclitaxel, acting as efficient sensitizing agents. These TRAs are noncytotoxic and can potentially revitalize known and well-studied anticancer agents toward the treatment of resistant tumors.

The SAR studies of TRAs indicate that the C-7 position of the baccatin skeleton is the most effective position for introducing hydrophobic pendant group as compared to the C-10, C-13, and C-2 positions. Among the hydrophobic pendant groups designed and examined, the one consisting of two aromatic rings, spaced by a 1- or 2-atom linker and bearing a carbonyl or ether group, was identified as the highly effective unit structure. For example, an arenecarbonylcinnamoyl group is found to be the best pendant group so far to date. While the P-gp transporter is known for its ability to nonselectively eliminate hydrophobic contaminants from cells, our SAR study suggests the presence of a discrete recognition site of TRAs in the P-gp system and a strict steric requirement for the TRAs to be effective.

Besides P-gp, selected TRAs were found to modulate efflux pumps mediated by the MDR-associated ABC-transporters MRP-1 and BCRP. Six TRAs have been identified as triple-modulators to date, which can effectively modulate mitoxantrone efflux from drug-resistant cancer cell lines overexpressing P-gp, MDR-1, and BCRP. Among these, TRA **2**-10a exhibited the best activity as broad-spectrum modulator and has been identified as the lead drug candidate for further pharmacological evaluations directed toward clinical applications.

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Supporting Information Available: Synthetic procedures and characterization data for all TRAs and intermediates; procedures for biological evaluations. This material is available free of charge via the Internet at http://pubs.acs.org.

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