Targeting FtsZ for Antituberculosis Drug Discovery: Noncytotoxic Taxanes as Novel Antituberculosis Agents

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Abstract: Screening of 120 taxanes identified a number of compounds that exhibited significant antituberculosis activity. Rational optimization of selected compounds led to the discovery that the C-seco-taxane-multidrug-resistance (MDR) reversal agents (C-seco-TRAs) are noncytotoxic at the upper limit of solubility and detection (>80 μM), while maintaining MIC_{99} values of 1.25−2.5 μM against drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* (MTB). Treatment of MTB cells with TRA **3aa** and **10a** at the MIC caused filamentation and prolongation of the cells, a phenotypic response to FtsZ inactivation.

Tuberculosis (TB) is the leading cause of death in the world from a single infectious disease, claiming over three million lives each year. The AIDS pandemic has led to an explosion of HIV/TB coinfection, as TB is the most common opportunistic infection for patients living with HIV/AIDS. Poor chemotherapeutics and inadequate local-control programs contribute to the inability to manage TB and lead to the emergence of drug resistant strains of *Mycobacterium tuberculosis* (MTB).[1] Consequently, there is a pressing need for the development of novel TB drugs for treating AIDS-related opportunistic infections that are effective against both drug-sensitive and drug-resistant MTB strains. To this end, we select FtsZ, a tubulin homologue in MTB, as a novel target for anti-TB drug discovery.

FtsZ (filamentation temperature-sensitive protein Z) is an essential cell division protein in bacteria and has been shown to be a homologue of the mammalian cytoskeletal protein tubulin. FtsZ and tubulin share extensive similarity in function. In a process strongly reminiscent of microtubule formation by tubulin, FtsZ polymerizes in a GTP-dependent manner into filaments, which assemble into a highly dynamic structure known as the Z ring on the inner membrane at the mid cell.[2] Following recruitment of the other cell division proteins, the Z-ring contracts, resulting in septation. Inactivation of FtsZ results in the absence of septum formation. Accordingly, FtsZ is a very promising target for new antimicrobial drug discovery.

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Table 1. Antimicrobial Activities of Taxanes against Drug-Sensitive and Mutidrug-Resistant *M. tuberculosis*[a]

		MIC (μM)		cytotoxicity (IC_{50}, μM)	
entry	taxane	*M. tuberculosis* H37Rv	*M. tuberculosis* IMCJ946.K2	MCF7	A549
1	paclitaxel	40	40	0.019	0.028
2	**11**	5	1.25	0.65	0.65
3	**2a**	5	2.5	4.5	15.7
4	**2b**	5	2.5	7.6	14.0
5	**4a**	5	5	5.4	80
6	**4b**	10	10	9.3	12.5
7	**4c**	2.5	2.5	3.4	4.5
8	**4d**	5	5	5.3	5.0
9	**6**	20	10	14	N.D.
10	**7a**	10	10	7.0	10.8
11	**7b**	10	5	3.9	9.6
12	**8a**	20	5	>20	4.3
13	**8b**	10	20	9.4	17.0
14	**10a**	2.5	1.25	>80	>80
15	**10b**	2.5	2.5	>80	>80
16	**10c**	2.5	1.25	>80	>80
17	**10d**	2.5	1.25	>80	>80

[a] *M. tuberculosis* (*M. tb*.) H37Rv is sensitive to all antibiotics tested. *M. tuberculosis* IMCJ946.K2 is resistant to nine drugs including isoniazid (INH), rifampicin (RFP), ethambutol (EB), streptomycin (SM), kanamycin (KM), ethionamide (ETH), *p*-aminosalicylic acid (PAS), cycloserine (CS), and enviomycin (EVM). MCF7 and A549 cells: human breast and non small cell lung cancer cell lines, respectively.

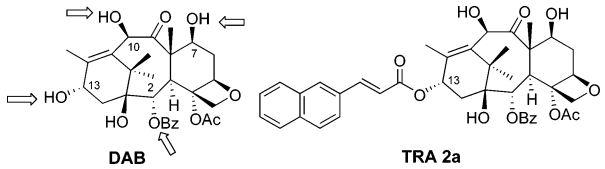

Figure 1. Structures of DAB and TRA **2a**.

A starting point for discovering inhibitors of FtsZ polymerization or depolymerization is a library of compounds that are known to affect the assembly of the FtZ homologue tubulin into microtubules, since the latter protein has been a target for anticancer chemotherapeutics for over 35 years. The fact that the sequence homology between FtsZ and tubulin is low (<20% identity) suggests that there is an excellent possibility in discovering FtsZ specific taxanes that are noncytotoxic to human host cells.

Taxanes were first screened for inhibitory activity by a real time PCR-based (RT-PCR) assay.[3] These taxanes represent two diverse activities, highly cytotoxic taxoids (i.e., "taxol-like compound") that stabilize microtubules[4−6] and noncytotoxic (or very weakly cytotoxic) taxane-multidrug-resistance (MDR) reversal agents (TRAs)[7−14] which inhibit the efflux pumps of ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp), multidrug resistant protein (MRP-1), and breast cancer resistant protein (BCRP). Screening of 120 taxanes revealed that a number of taxanes exhibited significant anti-TB activity. The antibacterial activity of each compound was confirmed by determining MIC_{99} values using the conventional microdilution broth assay. Treatment of MTB cells with two TRAs at the MIC caused filamentation and prolongation of the cells (see Supporting Information for electron microscope images), a phenotypic response to FtsZ inactivation.

In the MIC assay, it was found that TRA **2a**,[14] bearing a *(E)*-3-(naphth-2-yl)acryloyl (2-NpCH═CHCO) group at the C-13 position possessed very promising anti-TB activity against drug-

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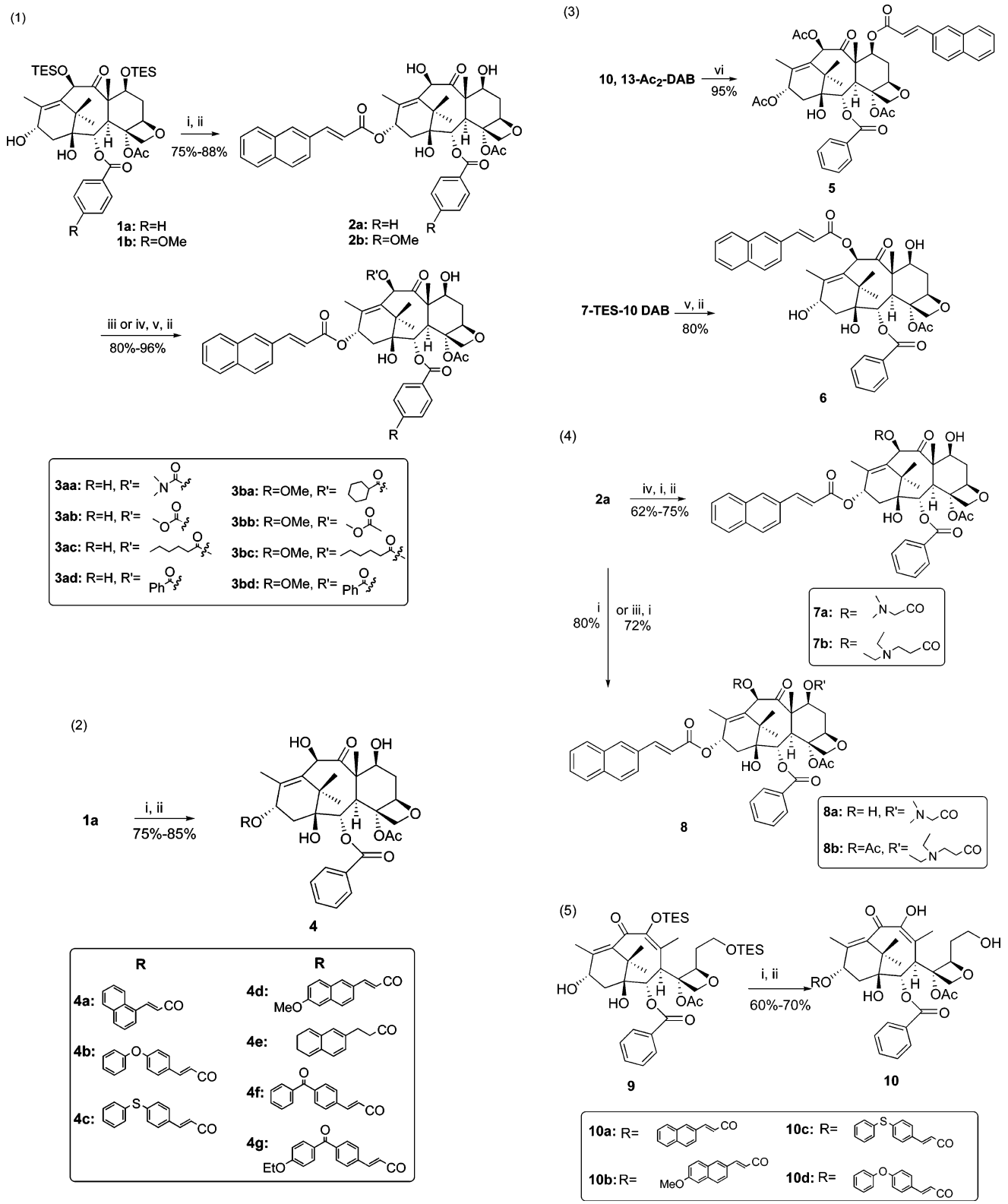
Scheme 1. Synthesis of Taxane-Based Anti-TB Agents[a]



[a] Reagents and conditions: (i) RCOOH, DIC, DMAP, CH_2Cl_2; (ii) HF/pyridine, CH_3CN/pyridine, room temperature, overnight; (iii) $CeCl_3$, acid anhydride, THF, room temperature, 4−6 h; (iv)TESCl, imidazole, room temperature; (v) acid chloride, LiHMDS, THF, −40 °C; (vi) RCOOH, EDC, DMAP, CH_2Cl_2, room temperature.

sensitive as well as drug-resistant MTB strains (MIC_{99} = 2.5−5 μM; Table 1). TRA **2a**[14] was selected as the lead compound for further optimization, and a new library of taxanes was prepared by modification of 10-deacetylbaccatin III (DAB) (Figure 1 and Scheme 1).

For the FtsZ-interacting taxane-based anti-TB agents to be useful as therapeutic drugs, these agents should not be cytotoxic at the concentration required for their antibacterial activity. Accordingly, it is necessary for the agents to distinguish human β tubulin from the MTB FtsZ. It has been shown in the SAR

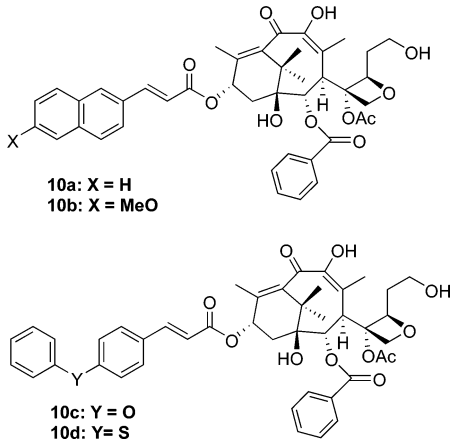


Figure 2. Structures of highly promising noncytotoxic anti-TB taxane leads derived from C-seco-baccatin.

studies of paclitaxel (Taxol, Figure 3) and taxoids that substitution at the para-position of the C-2 benzoate[6,15] substantially diminishes the binding ability of the analogues. Furthermore, the C-10 position may affect anti-TB activity. Therefore, we synthesized C-2 and C-10 modified TRA **2a** (Scheme 1, eq 1) to examine the effects of those modifications on the cytotoxcity, FtsZ binding ability, and anti-TB activity. Some C-10 modified TRA **2a** analogues show little or no anti-TB activity, while C-2 modification of TRA **2a** results in slightly decreased cytotoxicity and does not affect the anti-TB activity.

A variety of hydrophobic side chains were appended to the C-13 position of DAB in order to generate a series of TRA **2a** analogues (Scheme 1, eq 2). Screening of these compounds revealed several with activity as good as that of TRA **2a** (entries 3, 5, 7, and 8, Table 1).

We also examined whether the attachment of the 3-(2-naphthyl)acrylate side chain to the C-13 position is crucial for its anti-TB activity through binding to FtsZ or not for the TRA **2a** series. Accordingly, we attached the same side chain moiety to the C-7 and C-10 position to see the effects of these changes on the potency and profile of the resulting taxanes (Scheme 1, eq 3). In fact, the 10-modified analogue **6** showed only slightly reduced anti-TB activity (entry 9, Table 1).

In addition to the above modifications, we also introduced functionalities to improve the water solubility of these TRAs. Thus, *N*,*N*-dimethylglycine and *N*,*N*-diethyl-β-alanine esters were introduced to TRA **2a** as a pendant group at the C-7 or C-10 position (Scheme 1, eq 4). This modification caused only minor reduction in the anti-TB activity of these analogues (TRAs **7a**, **7b**, **8a**, and **8b**) as compared with TRA **2a** (entries 3, 10, 11, 12, and 13, Table 1)

Although TRA **2a** is certainly an excellent lead compound for optimization, it will be even better if a noncytotoxic lead compound, which does not bind to microtubules at all, is identified. Recently, we have been investigating a novel antiangiogenic taxoid (IDN5390),[16,17] which bears a C-seco-baccatin (i.e., C-ring-opened baccatin) skeleton and is much less cytotoxic than paclitaxel. Accordingly, we prepared the C-seco-analogue of TRA **2a**, i.e., TRA **10a** (Scheme 1, eq 5). Three analogues of TRA **10a** (Figure 2) were also prepared and assayed for their anti-TB activity and cytotoxicity. Significantly, TRA **10** series compounds (entries 14−17, Table 1) possessed potent anti-TB activity (MIC 1.25−2.5 μM) against drug-sensitive and drug-resistant MTB strains without appreciable cytotoxicity (IC_{50} > 80 μM).

As Table 1 shows, paclitaxel, 10-hexanoylpaclitaxel **11** (Figure 3), TRA **2a**, and its congeners were assayed for their

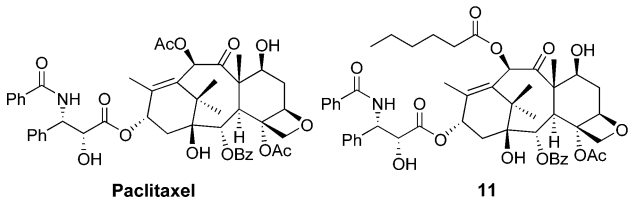


Figure 3. Structures of paclitaxel and **11**.

growth inhibitory activity against drug-sensitive MTB strain (H37Rv) and a multi-drug-resistant strain (IMCJ946K2), cultured from clinical isolates of MDR-TB. The MTB strain IMCJ946K2 is associated with nosocomial outbreaks in Japan and is resistant to all the clinically prescribed anti-TB drugs used in Japan (9 drugs; see Table 1 legend).

Paclitaxel (Figure 3), a microtubule-stabilizing anticancer agent, exhibits modest antibacterial activity against both MTB strains (MIC 40 μM), but its cytotoxicity against human cancer cell lines (a benchmark for activity against human host cells) is 3 orders of magnitude more potent (IC_{50} 0.019−0.028 μM; entry 1, Table 1). These data clearly indicate that paclitaxel is highly specific for microtubules. Taxoid **11** (Figure 3) exhibits 1 order of magnitude higher antibacterial potency and 20−30 times reduced cytotoxicity compared to paclitaxel. Since it is likely that the IC_{99} values would be at least 10 times larger than the IC_{50} values (as the former measures complete cell growth inhibition while the latter only measures 50% inhibition), it appears that **11** has comparable affinities to microtubules and FtsZ (entry 2, Table 1). TRA **2a** and its congeners derived from DAB (entries 3−13, Table 1) are clearly much less cytotoxic than paclitaxel (200−1000 times less toxic) and **11**, while keeping the same level of antibacterial activity to that of **11**. These TRAs appear to have higher specificity to FtsZ than microtubules. As entries 14−17, Table 1 clearly indicated, C-seco-TRAs **10a**−**d** are noncytotoxic so far at the upper limit of solubility and detection, while keeping the MIC values of 1.25−2.5 μM against drug-resistant and drug-sensitive MTB strains. Thus, we have now discovered noncytotoxic taxane lead compounds to develop a novel class of anti-TB agents. The specificity of these novel taxanes to microtubules as compared to FtsZ appears to have been completely reversed through systematic rational drug design. Moreover, we observed that the treatment of MTB cells with TRA **10a** at the MIC caused filamentation and prolongation of the cells (see Supporting Information), a phenotypic response to FtsZ inactivation. In addition, a preliminary study on the effect of TRA **10a** on the polymerization−depolymerization, using the standard light-scattering assay exhibited a dose-dependent stabilization of FtsZ against depolymerization. The details will be reported elsewhere in due course.

Further optimization and biological evaluation of these newly discovered lead compounds are actively underway in these laboratories.

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

References

(1) Raviglione, M. C. Issues facing TB control (7). Multiple drug-resistant tuberculosis. *Scottish Med. J.* **2000**, *45*, 52−55; discussion 56.

(2) Bi, E.; Lutkenhaus, J. FtsZ ring structure associated with division in Escherichia coli. *Nature (London)* **1991**, *354*, 161−164.
(3) Huang, Q.; Pepe, A.; Zanardi, I.; Tonge, P. J.; Slayden, R. A.; Kirikae, F.; Kirikae, T. Ojima, I. Targeting FtsZ for anti-tuberculosis drug discovery: noncytotoxic taxanes as novel anti-TB agents. *Abstracts of Papers*, 229th American Chemical Society National Meeting, Mar 13−17, 2005, San Diego, CA; ACS: Washington, DC, 2005 MEDI-386.
(4) Georg, G. I.; Chen, T. T.; Ojima, I.; Wyas, D. M.; Eds. Taxane Anticancer Agents: Basic Science and Current Status. *ACS Symp. Ser*. **1995**, *583*, 1−353.
(5) Ojima, I.; Kuduk, S. D.; Chakravarty, S. Recent Advances in the Medicinal Chemistry of Taxoid Anticancer Agents. *Adv. Med. Chem.* **1998**, *4*, 69−124.
(6) Kingston, D. G. I.; Jagtap, P. G.; Yuan, H.; Samala, L. The Chemistry of Paclitaxel (Taxol) and Related Taxoids. *Prog. Chem. Org. Nat. Prod.* **2002**, *84*, 53−225.
(7) Ojima, I.; Bounaud, P.-Y.; Takeuchi, C. S.; Pera, P.; Bernacki, R. J. New Taxanes as Highly Efficient Reversal Agents for Multi-Drug Resistance in Cancer Cells. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 189−194.
(8) Ojima, I.; Bounaud, P.-Y.; Bernacki, R. J. New Weapons in the Fight Against Cancer. *CHEMTECH* **1998**, *28*, 31−36.
(9) Ojima, I.; Bounaud, P.-Y.; Oderda, C. F. Recent Strategies for the Treatment of Multi-drug Resistance in Cancer Cells. *Expert Opin. Ther. Pat.* **1998**, *8*, 1587−1598.
(10) Ojima, I.; Bounaud, P.-Y.; Bernacki, R. J. Designing Taxanes to Treat Multidrug-Resistant Tumors. *Modern Drug Discovery* **1999**, *2* (3), 45−52.
(11) Brooks, T. A.; Minderman, H.; O'Loughlin, K. L.; Ojima, I.; Baer, M. R.; Bernacki, R. J. Taxane-Based Reversal Agent Modulation of P-glycoprotein-, Multidrug Resistance Protein- and Breast Cancer Resistance Protein-Mediated Drug Transport. *Mol. Cancer Ther*. **2003**, *2*, 1195−1205.
(12) Minderman, H.; Brooks, T. A.; O'Loughlin, K. L.; Ojima, I.; Bernacki, R. J.; Baer, M. R. Modulation of ATP−Binding-Cassette Transport Proteins by the Taxane Derivatives BAY 59−8862 and tRA96023. *Cancer Chemother. Pharm.* **2004**, *53*, 363−369.
(13) Brooks, T. A.; Kennedy, D. R.; Gruol, D. J.; Ojima, I.; Baer, M. R.; Bernacki, R. J. Structure−Activity Analysis of Taxane-Based Broad-Spectrum Multidrug Resistance Modulators. *Anticancer Res.* **2004**, *24*, 409−415.
(14) Ojima, I. B., C. P.; Wu, X.; Bounaud, P.-Y.; Fumero-Oderda, C.; Sturm, M.; Miller, M. L.; Chakravarty, S.; Chen, J.; Huang, Q.; Pera, P.; Brooks, T. A.; Baer, M. R.; Bernacki, R. J. Design, Synthesis and SAR of Novel Taxane-Based Multi-Drug Resistance Reversal Agents. *J. Med. Chem.* **2005**, *48*, 2218−2228.
(15) Georg, G. I.; Harriman, G. C. B.; van der Velde, D. G.; Boge, T. C.; Cheruvallath, Z. S.; Datta, A.; Hepperle, M.; Park, H.; Himes, R. H.; Jayashinge, L. Medicinal Chemistry of paclitaxel: Chemistry, structure-activity relationships, and conformational analysis. *Taxane Anticancer Agents: Basic Science and Current Status*, Georg, G. I., Chen, T. T., Ojima, I., Wyas, D. M., Eds. *ACS Symp. Ser.*, **1995**, *583*, 217−232.
(16) Appendino, G.; Danieli, B.; Jakupovic, J.; Belloro, E.; Scambia, G.; Bombardelli, E. Synthesis and Evaluation of C−Seco Paclitaxel Analogues. *Tetrahedron Lett.* **1997**, *38*, 4273−4276.
(17) Taraboletti, G.; Micheletti, G.; Rieppi, M., Poli; M., T., M.; Rossi, C., Borsotti, P.; Roccabianca, P.; Scanziani, E.; Nicoletti, M. I.; Bombardelli, E.; Morazzoni, P.; Riva, A.; Giavazzi, R. Antiangiogenic and Antitumor Activity of IDN 5390, a New Taxane Derivative. *Clin. Cancer Res.* **2002**, *8*, 1182−1188.

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