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Targeting FtsZ for anti-tuberculosis drug discovery: non-cytotoxic taxanes as novel anti-tuberculosis agents

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Abstract

Screening of 120 taxanes identified a number of compounds that exhibited significant anti-tuberculosis activity. Rational optimization of selected compounds led to the discovery that the C-seco-taxane-multidrug-resistance (MDR) reversal agents (C-seco-TRAs) are non-cytotoxic at the upper limit of solubility and detection ($>80 \mu\text{M}$), while maintaining MIC₉₉ values of 1.25–2.5 μM against drug-resistant and drug-sensitive MTB 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 co-infection, 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 sensitive and 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 homolog 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 dependant 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.

A starting point for discovering inhibitors of FtsZ polymerization or depolymerization are compounds that are known to affect the assembly of the FtZ homolog tubulin into microtubules,

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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 non-cytotoxic 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 non-cytotoxic (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 MIC₉₉ 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-sensitive as well as drug-resistant MTB strains (MIC₉₉ = 2.5–5 μ M; 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 studies of paclitaxel (Taxol[®], Figure 2) and taxoids that substitution at the *para*-position of the C-2 benzoate^{6,15} substantially diminishes the binding ability of the analogs. Another interesting position is the C-10 position, which 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 cytotoxicity, FtsZ binding ability as well as anti-TB activity. Some C-10 modified TRA **2a** analogs 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** analogs (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, 10-modified analog **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 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 analogs (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 non-cytotoxic lead compound, which does not bind to microtubules at all, is identified. Recently, we have been investigating a novel anti-angiogenic 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-analog of TRA **2a**, i.e., TRA **10a** (Scheme 1, Eq. 5). Three analogs of TRA **10a** 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 μ M) against drug-sensitive and drug-resistant MTB strains without appreciable cytotoxicity ($IC_{50} > 80 \mu$ M).

As Table 1 shows, paclitaxel (taxol), 10-hexanoylpaclitaxel **11** (Figure 2), TRA **2a** and its congeners were assayed for their growth inhibitory activity against drug-sensitive MTB strain (H37Rv) and a multi-drug resistant strain (IMCJ946K2), originated 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 2), a microtubule-stabilizing anticancer agent, exhibits modest antibacterial activity against both MTB strains (MIC 40 μ M), but its cytotoxicity against human cancer cell lines (a benchmark for activity against human host cells) is three orders of magnitude more potent (IC_{50} 0.019–0.028 μ M; entry 1, Table 1). These data clearly indicate that paclitaxel is high specific for microtubules. Taxoid **11** (Figure 2) exhibits one 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 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 (200–1,000 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 non-cytotoxic so far at the upper limit of solubility and detection, while keeping the MIC values of 1.25–2.5 μ M against drug-resistant and drug-sensitive MTB strains. Thus, we have now discovered *non-cytotoxic* 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. In addition, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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

Figure 2.
Structures of paclitaxel and **11**

Figure 3.
Structures of highly promising non-cytotoxic anti-TB taxane leads derived from C-seco-baccatin

Scheme 1. Synthesis of taxane-based anti-TB agents^a

^aReagents and conditions: i) RCOOH, DIC, DMAP, CH₂Cl₂; (ii) HF/Pyridine, CH₃CN/Pyridine, room temperature, overnight; (iii) CeCl₃, acid anhydride, THF, room temperature, 4h–6h; (iv) TESCl, imidazole, room temperature; (v) Acid chloride, LiHMDS, THF, –40°C; (vi) RCOOH, EDC, DMAP, CH₂Cl₂, room temperature.

Table 1
Antimicrobial activities of taxanes against drug-sensitive and multidrug-resistant *M. tuberculosis*

Entry	Taxane	MIC (µM)		Cytotoxicity (IC ₅₀ , µM)	
		M. tuberculosis H37Rv	M. tuberculosis IMCJ946.K2	MCF7	A549
1	Paclitaxel	40	40	0.019	0.028
2		5	1.25	0.65	0.65
3		5	2.5	4.5	15.7
4		5	2.5	7.6	14.0
5		5	5	5.4	80
6		10	10	9.3	12.5
7		2.5	2.5	3.4	4.5
8		5	5	5.3	5.0
9		20	10	14	N.D.
10		10	10	7.0	10.8
11		10	5	3.9	9.6
12		20	5	>20	4.3
13		10	20	9.4	17.0
14		2.5	1.25	>80	>80
15		2.5	2.5	>80	>80
16		2.5	1.25	>80	>80
17		2.5	1.25	>80	>80

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.