interface. This latter structural event may lead to a tightening down of the FtsZ polymer spiral, much like the compression of a spring, resulting in Z-ring contraction.

Remodeling of cytoskeletal polymers is induced by transitions between nucleotide hydrolysis intermediates. The energy from hydrolysis can be used to destabilize a previously stable structure and to produce mechanical work [15, 19, 23, 43]. In organisms without a cell wall, FtsZ is the only conserved protein of the cell division machinery, suggesting that FtsZ might use GTP hydrolysis to direct cytokinesis [20, 23, 44]. Three mechanisms have been proposed for how FtsZ may transmit energy from nucleotide hydrolysis into mechanical force for constriction of the Z ring [15, 19, 23, 43]. The release of FtsZ subunits from the Z ring through depolymerization will cause the Z ring to become smaller [22], or, alternatively, FtsZ filaments may move relative to each other to reduce the circumference of the ring without depolymerization occurring, as observed for the actomyosin ring in eukaryotes [44]. In addition, the FtsZ filaments may bend upon GTP hydrolysis [43].

Since the rate-limiting step in the turnover of FtsZ polymers is GTP hydrolysis, and protofilaments consist mostly of FtsZ-GTP, GTP hydrolysis will release energy in small quanta, and will not generate a large force [23]. This situation is different from that observed for microtubules, in which almost every subunit is bound with GDP and the energy from hydrolysis is stored as strain in the polymer [33]. Perhaps microtubules need to generate a larger force because they operate on a greater geometric scale than does the Z ring [23].

Structural and Functional Homology Between FtsZ and Tubulin

The possibility that FtsZ might be a homologue of tubulin was first suggested by a short segment of its amino acid sequence, GGGTGTG, which is virtually identical to the tubulin signature motif, (G/A)GGTGSG, found in all α, β and γ tubulins [45-47]. It was not until the crystal structure of FtsZ from *Methanococcus jannaschii* was determined in 1998 that the similarity between tubulin and FtsZ was fully appreciated [15, 48]. Despite limited (10-18%) sequence similarity [49], FtsZ and tubulin share a common fold, comprised of two domains linked by an α-helix [50]. Conserved residues between the two proteins map to the nucleotide-binding domain and a region involved in protofilament formation in tubulin. Consistent with these observations, like tubulin, FtsZ GTP hydrolysis is self-activated, with the active site being formed by interaction of two monomers [38]. FtsZ subunits polymerize in the presence of GTP into straight, 5 nm-wide protofilaments, while the subsequent hydrolysis of GTP results in the filaments adopting a curved conformation. Just as microtubule stability is controlled by microtubule associated proteins (MAPs), assembly of FtsZ is influenced *in vivo* by proteins such as FtsA, ZipA and ZapA [51-54]. Taken together, the similarities between both protein families suggest that FtsZ may be a prokaryotic cytoskeletal protein homologue of tubulin [49, 55, 56] and support the hypothesis that ancient FtsZ might have evolved into tubulin [49, 57, 58]. While tubulin and FtsZ share some common features, the two proteins also differ in important ways. While FtsZ subunits are all identical, microtubules are formed from non-identical paired subunits (α- and β-tubulin). As shown in Fig. **4**, the two Mtb FtsZ subunits (A and B) in the crystallographic asymmetric unit associate laterally, rather than longitudinally like tubulin protofilaments (Fig. **4C**), to form an arc-shaped dimer [16]. A model for a Mtb FtsZ spiral filament is shown in Fig. **4D**.

Given the importance of FtsZ assembly in cell division, compounds that interfere with FtsZ function have good potential as novel antibacterial agents. Because of structural and functional homology between FtsZ and tubulin, compounds that are known to affect the assembly of tubulin into microtubules, provide a starting point for targeting FtsZ assembly. While the structures of tubulin and FtsZ are similar, the fact that FtsZ and tubulin have limited sequence homology (<20% identity) at the protein level, affords the opportunity to discover FtsZ-specific compounds with limited cytotoxicity to eukaryotic cells. Therefore, FtsZ can be considered as an attractive target for the development of agents with selective inhibitory activity against bacterial pathogens. In addition, investigating the effects of tubulin inhibitors on FtsZ assembly should also provide important information for FtsZ structure and function.

COMPOUNDS TARGETING *ESCHERICHIA COLI* FTSZ

Viriditoxin

Using a high-throughput FtsZT65C-fluorescein polymerization assay [60], Wang *et al.* [61] screened more than 100,000 microbial fermentation broths and plant extracts and discovered a small molecule, viriditoxin (Fig. **5**), which blocked *E. coli* FtsZ polymerization (IC_{50} 8.2 μg/ml) and inhibited GTP hydrolysis (IC_{50} 7.0 μg/ml). Morphological assays with a specific *E. coli* strain (SOS-, SulA-) showed that viriditoxin caused filamentation, and that the filaments were not formed as a result of DNA damage. The increased MIC resulting from the induction of FtsZ expression provided solid evidence that viriditoxin interacts with FtsZ. Furthermore, viriditoxin exhibited broad-spectrum antibacterial activity against many clinically relevant Gram-positive pathogens, which indicated a high functional conservation of FtsZ in these clinically important species. Presumably, the structure of the FtsZ molecule is highly conserved in the viriditoxin-binding site, a fact that may limit the ability of FtsZ to develop resistance to this drug.

Zantrins

After a high throughput protein based chemical screening of 18,320 compounds, Margalit *et al.* [11] reported the identification of five structurally diverse molecules, named Zantrins (Fig. **6**), that inhibit GTPase activity of *E. coli* FtsZ at IC_{50} values below 50 μM. Results from electron microscopy and quantification of effects of Zantrins on steady state FtsZ polymer mass and structure, indicated that Zantrins inhibit FtsZ GTPase either by destabilizing the FtsZ protofilaments (Z1 and Z4) or by inducing filament hyperstability (Z2, Z3 and Z5). Margalit *et al.* [11] proposed that Zantrins that destabilize the FtsZ polymer may bind at a site between the FtsZ subunits such that the T7 synergy loop in one FtsZ monomer fails to make optimum contact with the GTP bound to loops T1–T6 in the neighboring monomer, an

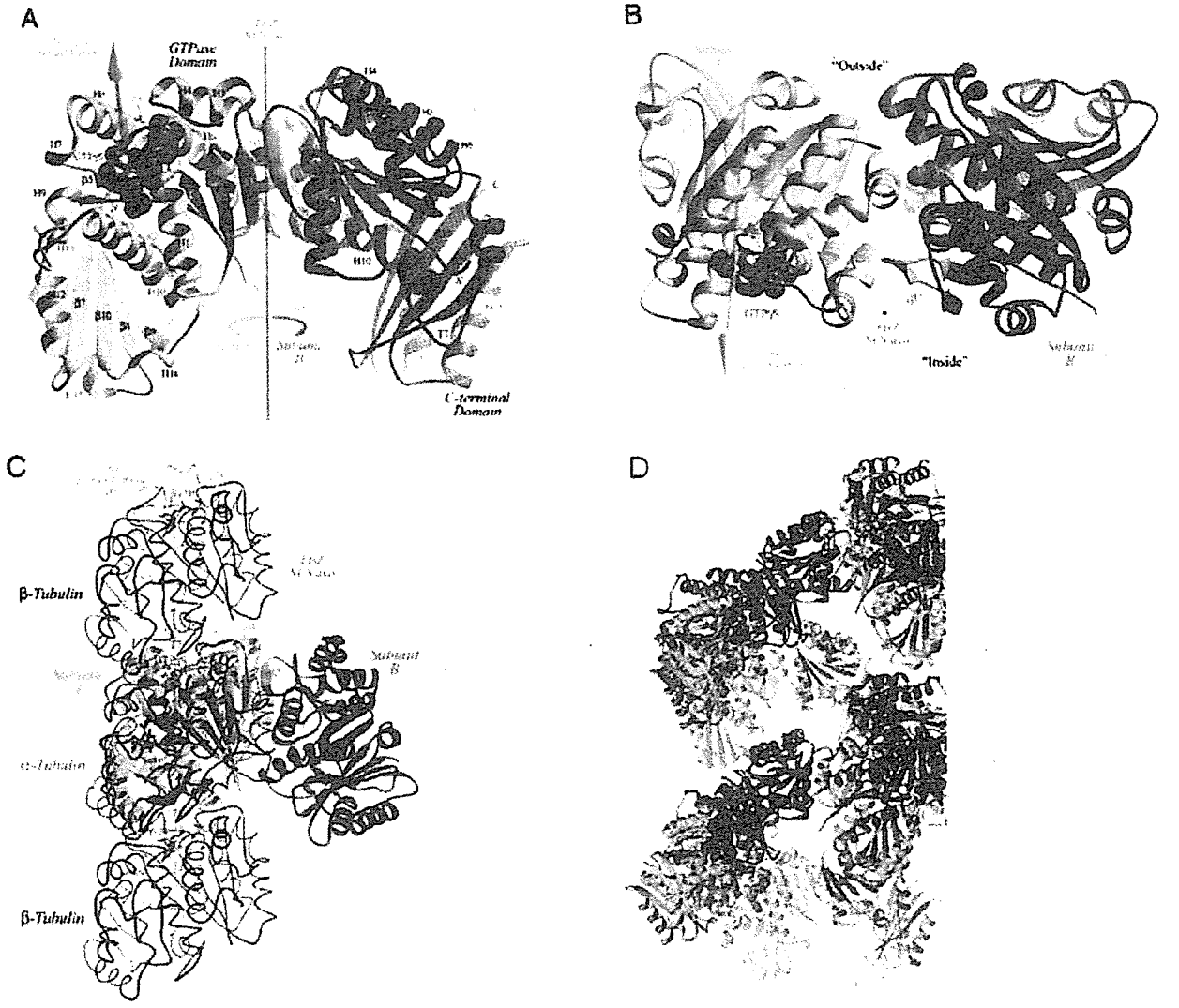
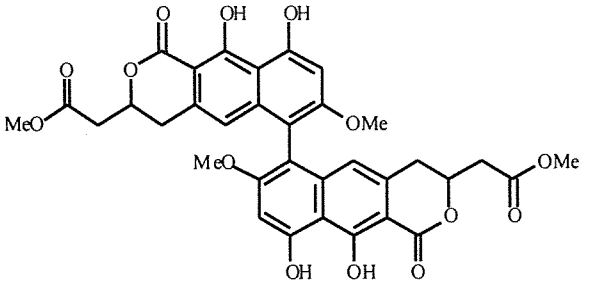


Fig. (4). (A) The GTPγS complex (Mtb FtsZ, subunit A, yellow; subunit B, brown) is viewed from the "inside" of a corresponding microtubule. GTPγS bound to the subunit A active site is shown as a space-filling model (nitrogen, blue; oxygen, red; phosphorous, yellow-green; carbon, grey; sulfur, purple). The switch elements at the subunit interface within the dimer are highlighted in light blue. The active sites are lavender. The two Mtb FtsZ subunits are related by a ~92° rotation about the vertical axis (grey); this axis is canted by ~50° from the αβ-tubulin protofilament axis (light green), drawn with the arrowhead pointing in the (+)-direction. **(B)** Rotated by 90° about a horizontal axis (from the top in A), to better illustrate the dimmer interface. **(C)** Comparison of the Mtb FtsZ dimer with the αβ-tubulin protofilament (PDB entry 1jff) [59]. Subunit A was aligned with the exchangeable (E) α-tubulin subunit (blue), which contains GDP (green) in its active site and a bound Taxol (purple) molecule. The two adjacent non-exchangeable (N) β-tubulin subunits (black) in the protofilament contain GTP (red). The protofilament axis is vertical. **(D)** A model for an FtsZ spiral filament. A, Twenty-four Mtb FtsZ subunits forming a right-handed spiral are shown in this stereoview. The B (brown) and A (yellow) subunits alternate.

Reprinted from [16] *J. Mol. Biol, 342*, (3), Leung, A. K. W.; White, E. L.; Ross, L. J.; Reynolds, R. C.; DeVito, J. A.; Borhani, D. W., Structure of *Mycobacterium tuberculosis* FtsZ Reveals Unexpected, G Protein-like Conformational Switches, 953-970., Copyright (2004), with permission from Elsevier.

interaction essential for polymerization and for stimulating nucleotide hydrolysis [62, 63]. They also proposed that the stabilizing Zantrins could inhibit FtsZ depolymerization by opposing the movement of the T3 switch loop that has been proposed to cause a bend in the filament upon GTP hydrolysis. In support of this suggestion, results from immunofluorescence microscopy demonstrate that Zantrins perturb FtsZ ring assembly in *E. coli* cells. Interestingly, Zantrins Z3 and Z5, which stabilize FtsZ polymers *in vitro*, caused a significant reduction in Z ring assembly in *E. coli*. Z3 and Z5 may disrupt FtsZ's recruitment of other stabilizing factors, such as ZipA and FtsA, to the septum. Zantrins have also been tested against FtsZ from Mtb. The majority of Zantrins inhibited Mtb FtsZ GTPase with IC_{50} values up to one order of magnitude higher than the corresponding values against *E. coli* FtsZ. Zantrins have also been observed to cause lethality to a variety of bacteria in broth cultures, including antibiotic-resistant and virulent pathogens, further supporting the hypothesis that FtsZ is a good target for the development of new broad-spectrum antibacterial agents.

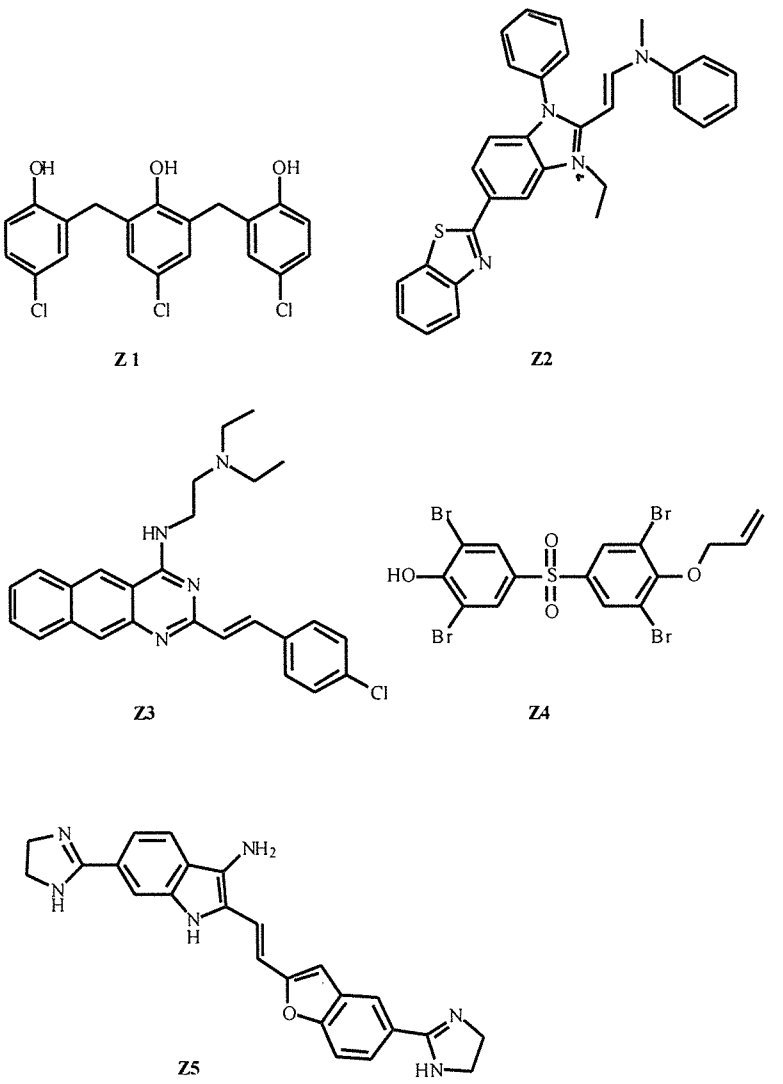


viriditoxin

Fig. (5). Chemical structure of viriditoxin.

GTP Analogue BrGTP

Läppchen *et al.* [42] designed a selective *E. coli* FtsZ inhibitor BrGTP (Fig. 7) based on the structure of the natural substrate GTP. Presumably, BrGTP competes with GTP for the binding site on soluble FtsZ. The inhibitory activity of BrGTP was first demonstrated by electron microscopy which showed that addition of BrGTP resulted in shorter and thinner FtsZ filaments. The inhibitory activity of BrGTP was further characterized by a coupled assay, which allowed simultaneous detection of the extent of polymerization and GTPase activity. The results demonstrated the reversible competitive inhibition of FtsZ by BrGTP. In the GTP concentration range studied, the IC_{50} values depend on the ratio of BrGTP to GTP, which is approximately 1/2 for assembly and 1/1 for GTPase activity, suggesting that both nucleotides bind with equal affinity and that BrGTP-FtsZ is inactive. Interestingly, the addition of a 2-fold excess of BrGTP when FtsZ had fully polymerized resulted in complete FtsZ depolymerization and inhibition of GTPase activity within 5 seconds, indicating that BrGTP could also lead to polymer destabilization by directly replacing GTP/GDP in the polymers. The observation that BrGTP does not inhibit tubulin assembly indicates that there are subtle differences between the GTP binding sites in FtsZ and tubulin.



Z1 Z2 Z3 Z4 Z5

Fig. (6). Chemical structures of Zantrins.

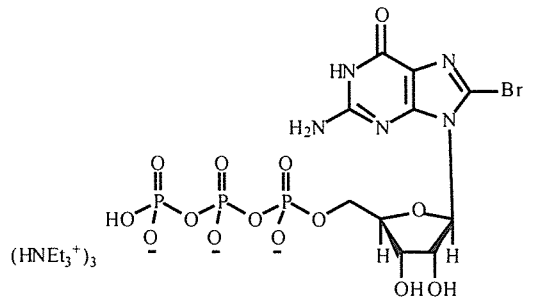


Fig. (7). Chemical structures of BrGTP.

Sanguinarine

Sanguinarine (Fig. **8**) a benzophenanthridine alkaloid derived from the rhizomes of *Sanguinaria canadensis*, has a wide range of antimicrobial activity [64]. It is also known to inhibit proliferation of various types of cancer cells [65, 66] and has been shown to depolymerize microtubules both *in vitro* and in cancer cells [67, 68]. Recently, Beuria *et al.* [69] reported that sanguinarine inhibited cytokinesis in both Gram-positive and Gram-negative bacteria by perturbing Z-ring assembly through FtsZ binding. In both *E. coli* and *B. subtilis* cells, sanguinarine not only reduced the frequency of Z-ring occurrence, but also perturbed the Z-ring morphology, resulting in increased cell length in bacteria. Further *in vitro* experiments demonstrated that sanguinarine was found to bind to FtsZ with a dissociation constant of 18-30 μM. Sanguinarine was shown to reduce the light-scattering caused by FtsZ assembly, to decrease the sedimentable polymeric mass, and to perturb the bundling of FtsZ protofilaments.

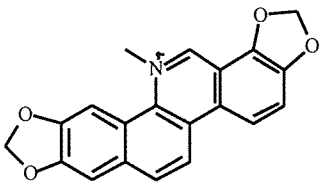


Fig. (8). Chemical structures of sanguinarine.

FtsZ is essential for bacterial cell division and represents an excellent novel target for anti-bacterial drug discovery. Although FtsZ shows a high degree of similarity among bacterial species, there are some important differences between the *E. coli.* and Mtb enzymes. Mtb FtsZ shares ~46% amino acid identity with *E. coli.* FtsZ, and has been shown to be a markedly slower GTPase *in vitro* [11, 70]. In addition, Mtb FtsZ has some characteristics more reminiscent of its homologue tubulin than the *E. coli* protein [70]. Therefore, compounds targeting *E. coli* FtsZ may not inhibit the Mtb FtsZ or have anti-TB activity.

COMPOUNDS TARGETING MTB FTSZ AS POTENTIAL ANTI-TB AGENTS

Bis-ANS

Bis-ANS (Fig. **9**), a well known fluorescent probe for hydrophobic surfaces on proteins, was shown to inhibit tubulin polymerization [71]. In 1998, Yu and Margolin [72] reported the inhibition of *E. Coli* FtsZ assembly by bis-ANS and proposed that bis-ANS inhibited FtsZ polymerization by blocking FtsZ intermolecular hydrophobic interactions. The titration of FtsZ with bis-ANS and *vice versa*, using the same methods that were previously applied to tubulin, suggested that FtsZ has a high affinity bis-ANS binding site as well as multiple low affinity binding sites, with K_d values similar to those of tubulin. The inhibition of bis-ANS binding by GTP binding, and *vice versa*, suggested that the GTP and bis-ANS binding sites overlapped. Subsequently, Nair *et al.* [73] demonstrated that 50 μM bis-ANS significantly reduced the GTPase activity of Mtb FtsZ as well as completely abolished FtsZ polymerization in a light scattering assay. Interestingly, in support of the observed *in vitro* inhibition, Slayden *et al.* [74] demonstrated that bis-ANS inhibited Mtb cell growth($H37_{Rv}$) with a MIC_{99} value of 1 μM, and also that sub-MIC concentrations of bis-ANS caused filamentation in Mtb. ANS (Fig. **9**), a hydrophobic probe similar to bis-ANS, had no inhibitory effect on FtsZ assembly or tubulin assembly, suggesting that FtsZ and tubulin share similar conformational properties and may interact similarly with bis-ANS and ANS [72].

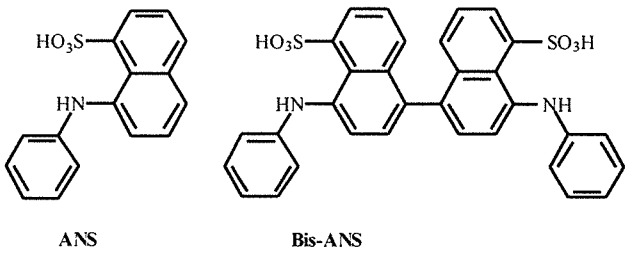
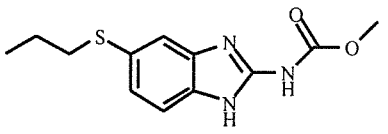
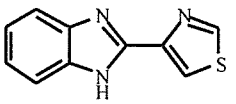


Fig. (9). Chemical structures of ANS and Bis-ANS.

Thiabendazole and Albendazole

Albendazole and thiabendazole (Table **1**) are known inhibitors of tubulin polymerization via competitive binding at the same site as colchicine. Importantly, Sarcina and Mullineaux [75] demonstrated that thiabendazole caused cell elongation in *E. coli* and cyanobacteria, a phenotypic response identical to that elicited by disruption of the FtsZ gene in these organisms, suggesting that these tubulin inhibitors may act in a similar manner on the FtsZ gene product as they do on tubulin. They also observed unaffected DNA replication and mobility of thylakoid membrane components accompanied by cell elongation, which indicated that thiabendazole had a specific effect on cell division. Later, Slayden *et al.* [76] determined the MIC_{99} values of thiabendazole and albendazole against Mtb cell growth (Table **1**), and studied their effects on bacterial

Table 1. Data for Tubulin Polymerization Inhibitors: Albendazole and Thiabendazole

Compound	MIC_{99} (H37Rv)
 Albendazole	16 μg/mL (61 μM)
 Thiabendazole	16 μg/mL (80 μM)

ultrastructure (filamentation) and transcriptional response. The results indicated that thiabendazole and albendazole interfere and delay Mtb cell division processes at inhibitory concentrations. In addition, the fact that these drugs have inhibitory activity provides compelling evidence that the inhibition of FtsZ polymerization is a novel drug target that warrants further research focus.

2-Alkoxycarbonylaminopyridines

Investigators at Southern Research Institute [77-79] screened their compound library of 200 2-alkoxy-carbonylaminopyridines, developed to inhibit tubulin polymerization, for the ability to inhibit FtsZ polymerization and for the ability to inhibit growth of Mtb. Using this approach, they identified several compounds with the desired properties. Colchicine, a known tubulin inhibitor, was also included in the study as a reference compound. SRI-3072, SRI-7614, and colchicine inhibited Mtb FtsZ polymerization and GTP hydrolysis in a dose dependant manner (Table **2**). SRI-3072 and SRI-7614 were equipotent against susceptible and single-drug-resistant strains of Mtb (Table **3**). Importantly, there is a clear correlation between the antibacterial activity of selected compounds (as illustrated by SRI-3072 and SRI-7614) and inhibition of FtsZ polymerization and GTP hydrolysis. Like colchicine, SRI-7614 inhibited polymerization of both FtsZ and tubulin, while SRI-3072 was specific for FtsZ and did not affect the polymerization of tubulin. Furthermore, SRI-3072 reduced the growth of Mtb in mouse-derived macrophages.

Table 2. Results for Inhibition of *M. tuberculosis* H37Rv Growth

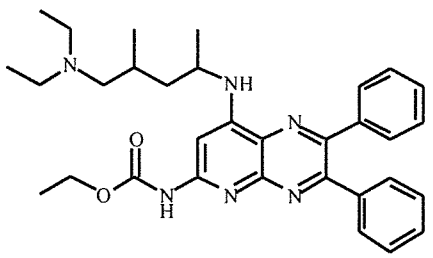
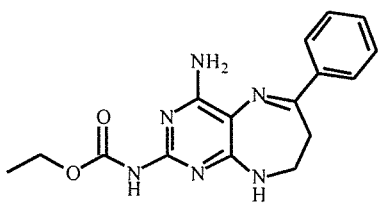
Compound	Structure	MIC_{99} (mg/L)	IC_{50} (mg/L)	ST (IC_{50}: MIC)
SRI-3072		0.15	6.3	42.0
SRI-7614		6.25	>200	>32

Table 3. Inhibitors of FtsZ and Tubulin Polymerization and GTP Hydrolysis

	M. tuberculosis FtsZ		Bovine brain tubulin
Compounds	Polymerization ID_{50} (μM)	GTP hydrolysis % inhibition (100 μM)	Polymerization ID_{50} (μM)
Colchicine	104 ± 2	35	6.5
SRI-3072	52 ± 12	20	100 (no inhibition)
SRI-7614	60 ± 0	25	4

Taxanes

Taxanes [80, 81], synthesized in our lab, were first screened for inhibitory activity by a real time PCR-based (RT-PCR) assay [81]. These taxanes represent two diverse activities; highly cytotoxic taxoids (i.e., "taxol-like compounds") that stabilize microtubules [82-84] and noncytotoxic (or very weakly cytotoxic) taxane-multidrug-resistance (MDR) reversal agents (TRAs) [85-92] 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 [81].

In the MIC assay, it was found that SB-RA-2001 [92], 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-resistant as well as drug sensitive Mtb strains (MIC_{99} = 2.5-5 μM; Table **4**). SB-RA-2001 [92] was selected as the lead compound for further optimization, and a new library of taxanes was prepared by modification of 10-deacetylbaccatin III (DAB) (Fig. **10** and Scheme **1**).

For the FtsZ-binding 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 Mtb FtsZ. It has been shown in the SAR studies of paclitaxel (Taxol, Fig. **11**) and taxoids that substitution at the *para*-position of the C-2 benzoate [84, 93] 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 SB-RA-2001 (Scheme **1**, eq 1) to examine the effects of those modifications on cytotoxicity, FtsZ binding ability, and anti-TB activity. Some C-10 modified SB-RA-2001 analogues show little or no anti-TB activity, while C-2 modification of SB-RA-2001 results in slightly decreased cytotoxicity and does not affect the anti-TB activity.

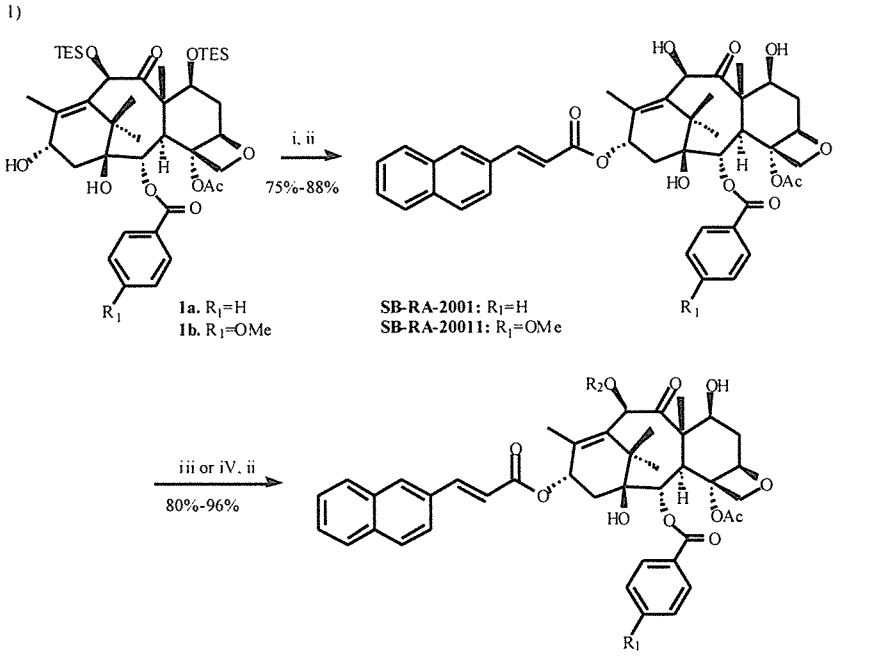
A variety of hydrophobic side chains were appended to the C-13 position of DAB in order to generate a series of SB-RA-2001 analogues (Scheme **1**, eq 2). Screening of these compounds revealed several taxanes with activity as good as that of SB-RA-2001 (entries 3, 5, 7, and 8, Table **4**).

We also examined whether the attachment of the 3-(2-naphthyl)acrylate side chain to the C-13 position is crucial

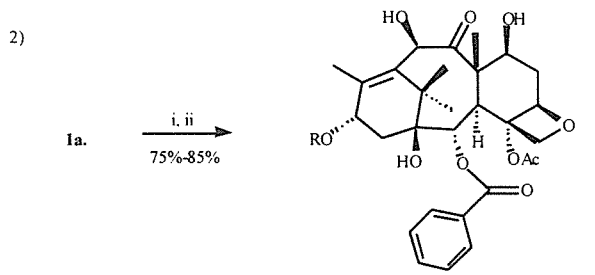
Table 4. Antimicrobial Activities of Taxanes Against Drug-Sensitive and Mutidrug-Resistant *M. tuberculosis*[a]

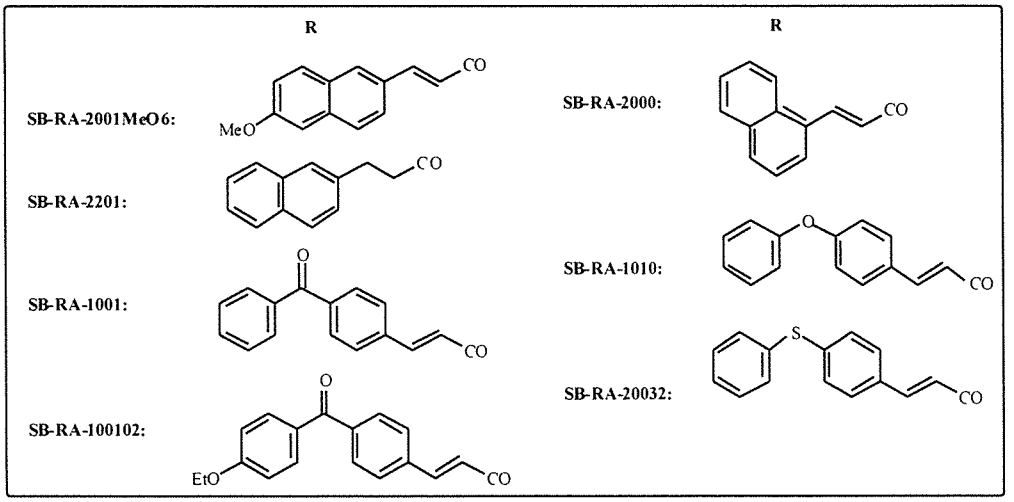
Entry	Taxane	MIC (μM)		Cytotoxicity (IC_{50}, μM)	
		M. tuberculosis H37Rv	*M. tuberculosis* IMCJ946.K2	MCF7	A549
1	**Paclitaxel**	40	40	0.019	0.028
2	**SB-T-0032**	5	1.25	0.65	0.65
3	**SB-RA-2001**	5	2.5	4.5	15.7
4	**SB-RA-20011**	5	2.5	7.6	14.0
5	**SB-RA-2000**	5	5	5.4	80
6	**SB-RA-1010**	10	10	9.3	12.5
7	**SB-RA-20032**	2.5	2.5	3.4	4.5
8	**SB-RA-2001MeO6**	5	5	5.3	5.0
9	**SB-RA-4010**	20	10	14	N.D.
10	**SB-RA-200101**	10	10	7.0	10.8
11	**SB-RA-200102**	10	5	3.9	9.6
12	**SB-RA-200200**	20	5	>20	4.3
13	**SB-RA-2002002**	10	20	9.4	17.0
14	**SB-RA-5001**	2.5	1.25	>80	>80
15	**SB-RA-5001MeO6**	2.5	2.5	>80	>80
16	**SB-RA-5011**	2.5	1.25	>80	>80
17	**SB-RA-5012**	2.5	1.25	>80	>80

[a]*M. tuberculosis* (*Mtb*) H37Rv is sensitive to all antibiotics tested. *M. tuberculosis* IMCJ946.K2 is resistant to nine drugs including INH, REF, EB, streptomycin (SM), kanamycin (KM), ethionamid (ETH), *p*-aminosalicilic acid (PAS), cycloserine (CS) and enviomycin (EVM). MCF7 and A549 cells: human breast and non-small cell lung cancer cell lines, respectively. Reprinted with permission from [80] *J. Med. Chem.* **2006**, *49*, (2), 463-466. Copyright 2006 American Chemical Society.



SB-RA-20012: R_1=H, R_2= Ac
SB-RA-20013: R_1=H, R_2=
SB-RA-20014: R_1=H, R_2=
SB-RA-20015: R_1=H, R_2=
SB-RA-20021: R_1=OMe, R_2= Ac
SB-RA-20031: R_1=OMe, R_2=
SB-RA-20041: R_1=OMe, R_2=
SB-RA-20051: R_1=OMe, R_2=





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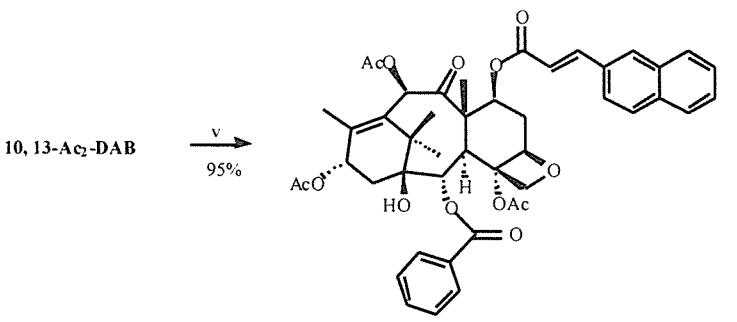
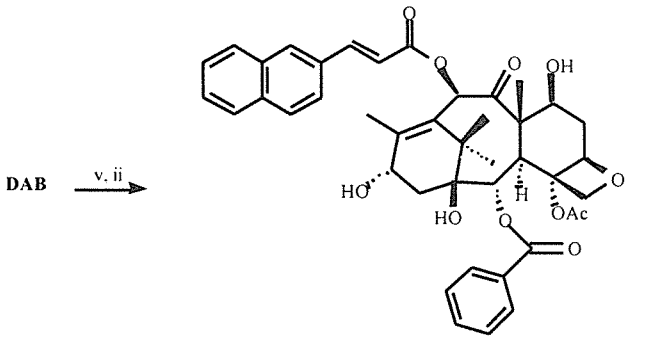
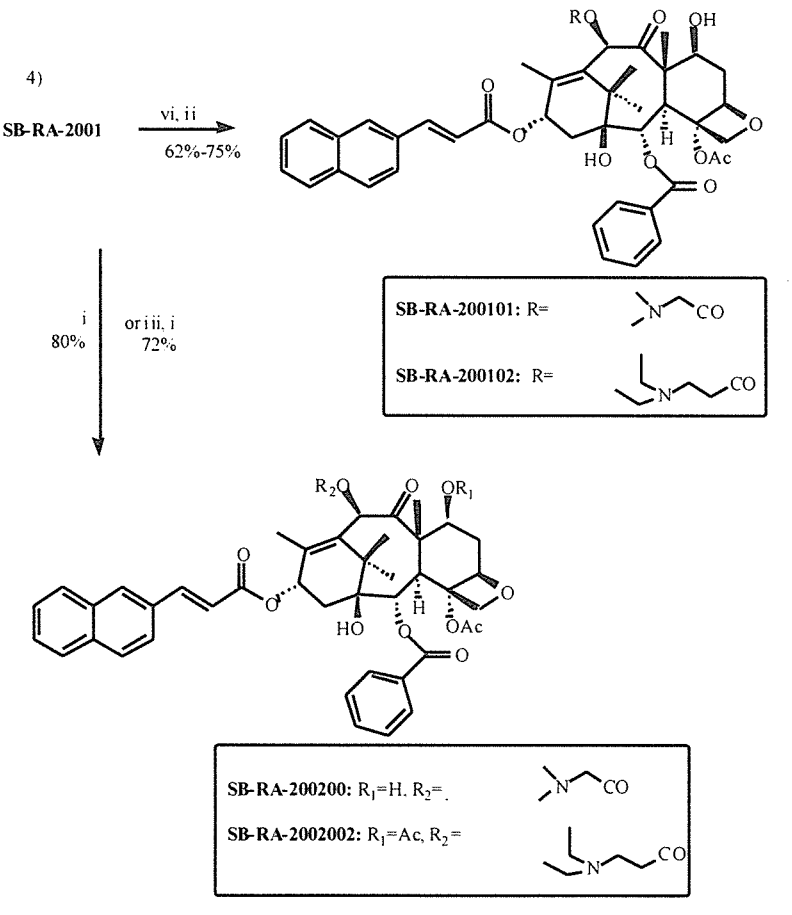


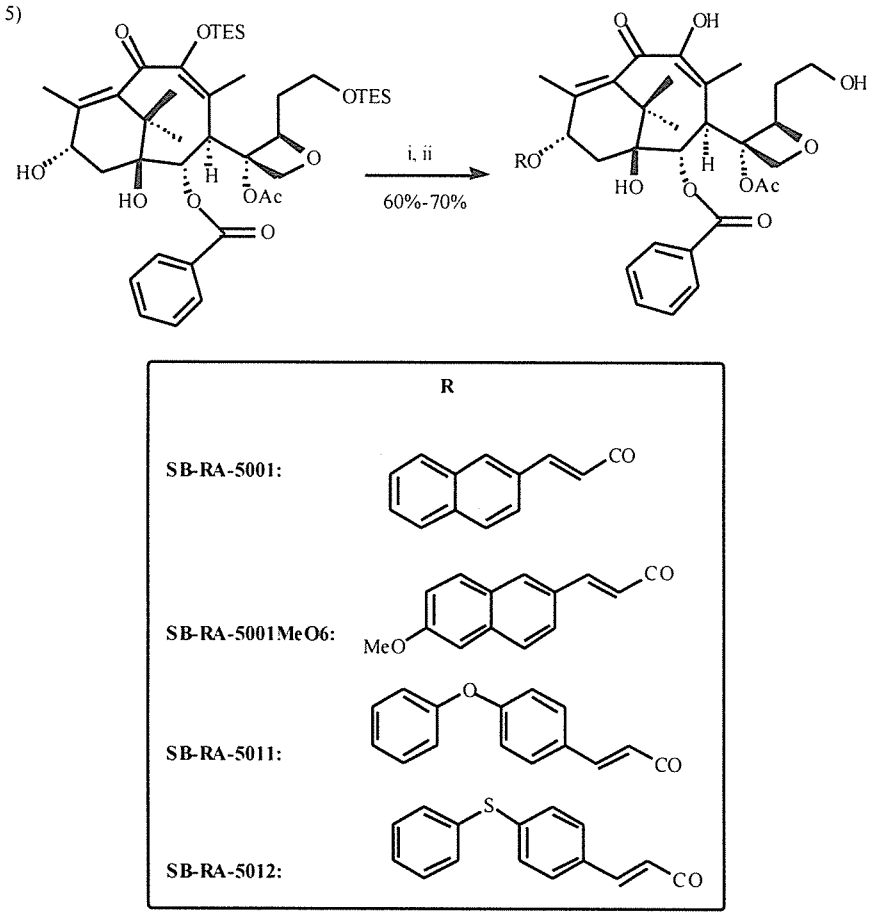
SB-RA-30012



SB-RA-4010

4)





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, 4h-6h; (iv) TESCl, imidazole, room temperature; Acid chloride, LiHMDS, THF, -40°C; (v) RCOOH, EDC, DMAP, CH_2Cl_2, room temperature. Reprinted with permission from [80] *J. Med. Chem.* **2006**, *49*, (2), 463-466. Copyright 2006 American Chemical Society

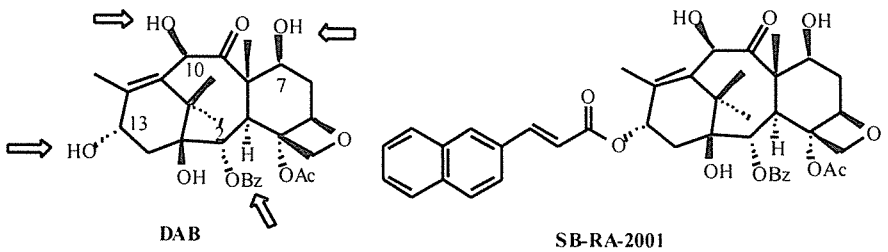


Fig. (10). Chemical structures of DAB and SB-RA-2001. Reprinted with permission from [80] *J. Med. Chem.* **2006**, *49*, (2), 463-466. Copyright 2006 American Chemical Society.

for the anti-TB activity of the SB-RA-2001 series via interaction with FtsZ. 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 SB-RA-4010 showed only slightly reduced anti-TB activity (entry 9, Table **4**).

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 SB-RA-2001 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 (SB-RA-200101, SB-RA-200102, SB-RA-

200200, and SB-RA-2002002) as compared with SB-RA-2001 (entries 3, 10, 11, 12, and 13, Table **4**).

Although SB-RA-2001 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) [94, 95], which bears a C-secobaccatin (i.e., C-ring-opened baccatin) skeleton and is much less cytotoxic than paclitaxel. Accordingly, we prepared the C-seco analogue of SB-RA-2001, i.e., SB-RA-5001 (Scheme **1**, eq 5). Three analogues of SB-RA-5001 (Fig. **12**) were also prepared and assayed for their anti-TB activity and cytotoxicity. Significantly, SB-RA-5001 series compounds (entries 14-17, Table **4**) 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 **4** shows, paclitaxel, SB-T-0032 (Fig. **11**), SB-RA-2001 and its congeners were assayed for their 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 IMCJ 946K2 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 **4** legend).

Paclitaxel (Fig. **11**), 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 **4**). These data clearly indicate that paclitaxel is highly specific for microtubules. SB-T-0032 (Fig. **11**) 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 inhibition while the latter only measures 50% inhibition), it appears that SB-T-0032 has comparable affinities to microtubules and FtsZ (entry 2, Table **4**). SB-RA-2001 and its congeners derived from DAB (entries 3-13, Table **4**) are clearly much less cytotoxic than paclitaxel (200-1000 times less toxic) and SB-T-0032, while keeping the same level of antibacterial activity to that of SB-T-0032. These TRAs appear to have higher specificity to FtsZ than microtubules. As entries 14-17, Table **4** clearly indicated, C-seco-TRAs 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 SB-RA-5001 at the MIC caused filamentation and prolongation of the cells (Fig. **13**), a phenotypic response to FtsZ inactivation. In addition, a preliminary study on the effect of TRA SB-RA-5001 on FtsZ polymerization and depolymerization using the standard light scattering assay

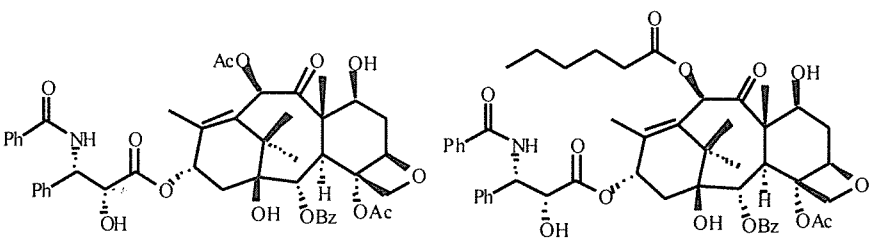


Fig. (11). Chemical structures of paclitaxel and SB-T-0032.

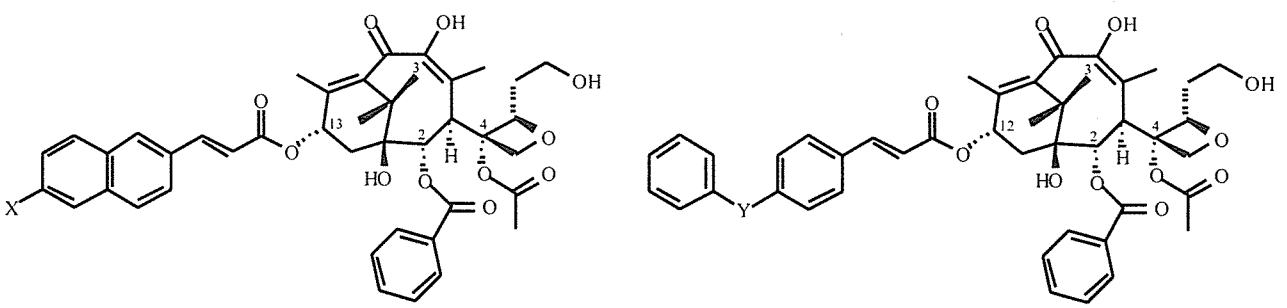


Fig. (12). Chemical structures of highly promising noncytotoxic anti-TB taxane leads derived from C-seco-baccatin.

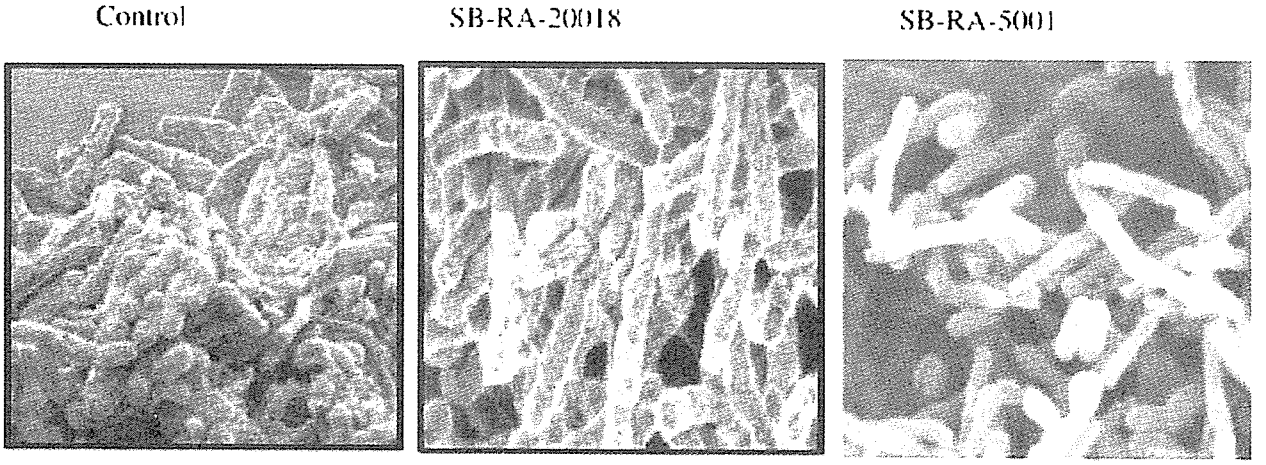


Fig. (13). Electron micrographs of Mtb cells before (Control) and after treatment with SB-RA-20018 and SB-RA-5001. Reprinted with permission from [80] *J. Med. Chem.* **2006**, *49*, (2), 463-466. Copyright 2006 American Chemical Society.

demonstrated a dose-dependent stabilization of FtsZ against depolymerization.

CONCLUSION

Multi-drug resistant Mtb is a major worldwide health problem. Therefore, it is critical to develop new antibiotics with novel modes of action to overcome this emerging resistance problem. FtsZ, a tubulin-like GTPase, plays an essential role in bacterial cell division, and is present in almost all eubacteria and archaea. Inhibitors of the GTP-dependent polymerization of FtsZ are expected to result in a new class of antibacterial agents.

The strong structural homology between FtsZ and tubulin raises the possibility that some of tubulin inhibitors could affect bacterial cell division. The search for a suitable lead compound was greatly facilitated by screening libraries of tubulin inhibitors. The fact that the protein sequence homology between FtsZ and tubulin is low (<20% identity) strongly indicates an excellent possibility in discovering FtsZ specific agents that are non-cytotoxic to eukaryotic cells. Recently, a number of FtsZ inhibitors have been reported. Some of them have been observed to perturb Z ring assembly, and cause bacterial lethality, confirming the hypothesis that FtsZ is a sensitive target for anti-TB drug discovery. However, much remains to be done to exploit FtsZ as a new target. Structural and kinetic analysis of compounds binding to FtsZ, determining the mechanism of drug action and evaluating structure relationships of active compounds are all critical aspects of the rational drug discovery process, which will facilitate the further optimization of chemical leads into specific FtsZ inhibitors with high affinity.

ACKNOWLEDGMENT

The authors gratefully acknowledge the grant support from the National Institutes of Health and the Japan Health Science Foundation. Generous support from Indena, SpA, Italy is also appreciated.

ABBREVIATIONS

ABC	=	ATP-binding cassette
AIDS	=	Acquired Immune Deficiency Syndrome
ANS	=	1-Anilinonaphthalene-8-sulfonate
BCRP	=	Breast cancer resistant protein
Bis-ANS	=	4,4'-Dianilino-1,1'-binaphthyl-5,5'-sulfonate
BrGTP	=	8-bromoguanosine 5'-triphosphate
DAB	=	10-deacetylbaccatin III
DNA	=	Deoxyribonucleic acid
E. Coli	=	*Escherichia coli*
HIV	=	Human immunodeficiency virus
IC_{50}	=	Concentration of compound that inhibits the growth of cells by 50%
IC_{99}	=	Concentration of compound that inhibits the growth of cells by 99%
MAPs	=	Microtubule associated proteins
MDR	=	Multi-drug resistant
MIC	=	Minimum inhibitory concentration
MRP	=	Multidrug resistant protein
Mtb	=	*Mycobacterium tuberculosis*
P-gp	=	P-glycoprotein
FRAP	=	Fluorescence recovery after photobleaching
Fts	=	Filament-forming temperature-sensitive genes
GDP	=	Guanosine-5'-diphosphate
GFP	=	Green fluorescent protein
GTP	=	Guanosine-5'-triphosphate
RT-PCR	=	Real-time polymerase chain reaction
SAR	=	Structure activity relationship
TRAs	=	Taxane-multidrug-resistance reversal agents

WHO = World Health Organization

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Received: February 18, 2006 Accepted: March 15, 2006

抗酸菌感染症

結　　核

Tuberculosis

森　亨

Key words：結核，結核対策，感染症

はじめに

世界をみると，結核はいまだ年々900万人の患者，170万人の死亡者を作り出し，わずかながらとはいえ増加を続けている．特にHIV蔓延のひどいサハラ砂漠以南のアフリカではかなりの勢いで増えている．旧体制崩壊の後，多剤耐性結核という悪性の結核が増えてしまった旧共産主義諸国の状態も痛々しい．しかし，これを迎え撃つ対策の側の勢いはこの15年間目覚ましいものがある．2000年には，WHOや世界銀行の肝煎りで結核関連の政府・非政府機関がStop TB Partnershipの下に，世界の結核対策に一致団結してあたる運動を発足させた．これまでの努力の勢いがこのまま続けば，2015年までには世界の結核を減少傾向に転ずることも夢ではないと予測されている[1]．

一方，日本でも1999年に'結核緊急事態宣言'を発表し，対策への気の引き締めと見直しをし，2005年から制度大改定に踏み切った．しかし，日本の罹患率は今なお米国の5倍のレベルにあり，改善も遅い．その間質的に厄介な問題が浮上している．重症例の増加による治療成績の悪化，集団感染や院内感染の増加などである．この問題は根本的には結核発生の特定階層（ハイリスク集団）への集中によるものである．すなわち，①高齢者：現在発生する患者の60％が60歳以上で，この年齢層は戦前・終戦直後，日本の結核大流行時代に生まれ育ったことで結核感染に濃厚に曝露しているからである．②医学的結核リスク集団：日本で最も重要なのが糖尿病（結核患者の15–20％にみられる）であり[2]，また最も手強いのがHIV感染・エイズである．その一部に'医原病'のそしりを免れ得ないものもある．③社会経済弱者：大都会のホームレスの結核は周知のとおりだが，これほどまでの生活困窮者でなくとも結核は'健康管理の機会に恵まれない人々'（外国人労働者，無職，零細企業労働者などが含まれる）の問題になりつつある．

以下，新しい対策とそれを更に強化させるための技術，特に近未来の技術開発への展望を含めて述べてみたい．

1. 予防接種

BCG接種は0歳児1回に限定し，ツベルクリン反応（ツ反）検査なしの'直接接種'で行うことになった．更に，より安全な直接接種のために原則として6カ月に達するまでに接種することとした．新制度の導入にあたり接種の始期をめぐって，法律的には出生直後の接種も可との解釈が行われたが，専門家の間では先天性免疫障

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0047-1852/07/¥40/頁/JCLS

害に対する従来の配慮が必要，ということから‘生後3カ月過ぎの接種’が勧奨されている．従来よりも接種機会が非常に短期間に限定されて，接種率・技術の確保が更に重い課題となった．接種の技術評価については，1歳半健診のときなどに接種瘢痕の体系的調査を保健所・市町村が行い，その結果を接種医師に還元することが望ましい．

また‘直接接種法’に伴い，頻度は低い(1万人中3-5人程度)が結核既感染の児に接種をしてしまう可能性が出てきた．そのようなことの未然の防止に向けて，接種前の予診における感染の可能性に関する慎重な問診と，接種後のコッホ現象の発見とそれに対する適切な対処(精密検査と予防投薬)が重要である．

現在のBCGワクチンによる予防接種に代わり，より効果の確実で強力な(予防効果率および持続期間の点で)ワクチンを開発する努力が行われている(文献[3]参照)．

2. 化学予防

国民の15%が結核に感染し，また大半の結核患者はこれまでに感染を受けた人の中から発生することを考えれば，真の結核根絶は予防接種よりも化学予防によってより早期に達成され得るはずである．短期的に考えても，結核患者の90%が30歳以上なので，この年齢層からの患者発生防止が日本の結核を減らすことにつながる．その意味で従来29歳以下に限定してきた化学予防をより実効あるものとするためには，日本結核病学会などが勧奨するように，年齢枠を取り払った‘発病リスクの高い者すべて’を対象とすべきである[4]．このような考えは世界的標準であり，早急に制度化されるべきである．

a. 対象枠の拡大

上記学会が勧告している化学予防の対象は以下のとおりである．

(1) 結核患者接触者で結核感染を受けたと判定された者．

(2) 胸部X線上，明らかな陳旧性結核の所見(胸膜癒着像や石灰化のみの者を除く)があり，ツ反強陽性で，結核治療歴のない者．

(3) 医学的な結核発病リスク要因をもった者：①HIV感染者，②免疫抑制剤治療中の者，③健康上，結核発病リスクの高い者(糖尿病，塵肺，白血病，Hodgkin病，頭頸部癌，重症の胃疾患(透析中を含む)，低栄養(標準体重より10%以上の低体重)，胃切除後，空腸回腸バイパスなど)．

b. 新しい治療方式の必要性

化学予防に用いる薬剤はこれまで①イソニアジド(INH)6カ月であったが，米国ではこのほかに，②リファンピシン(RFP)で4カ月，③RFPとピラジナミド(PZA)で2カ月といった方式も認められ，患者の規則的治療継続の難易などによって柔軟に選択される．特にHIV陽性者などでは③が推奨されている．更に国によってはRFP+INH 4カ月も認められている．日本でもそれらの適用も検討すべきである．

化学予防対象者の感染源がINH耐性患者ということがある(日本の最近の調査[5]では，初回治療患者のINH耐性頻度は2.8%，再治療患者では18.9%)．その場合は化学予防にはRFPを用いる(日本ではこの場合も6カ月間とされている)．多剤耐性(INHとRFP両剤に耐性)の場合には方針は未確立である．ニューキノロン剤を推奨する専門家もいる[6]．ただし，小児には副作用の点から推奨できない．

c. ‘静止菌’の研究

感染後，体の中で発病の時期を待っている冬眠状態の菌，いわゆる静止菌の代謝の仕組みが解明されれば，より効果的な化学予防の方式が開発される可能性もある．その点で今注目されているものの一つがisocitrate lyase(ICL)である．ICLは結核菌の脂肪酸代謝に必須の酵素で，これを発現する遺伝子を欠失した菌は脂肪酸を含んだ培地の上でも，マウス骨髄由来のマクロファージ内でも生育せず，逆に遺伝子を担ったプラスミドを投与すると生育するようになるという[7]．

d. 望まれる宿主要因の研究

‘はじめに’および2-aで述べたように，糖尿病はじめ多くの医学的な結核リスク要因の中には宿主の遺伝素因との関連(例：連鎖)をうかが

表1 これまでに知られている仮想的抗酸菌症感受性遺伝子/座位
(文献[8]より引用)

遺伝子/座位	機能・直接的障害など
NRAMP1(*Slc11a1*)	マクロファージ鉄代謝に関連
	(IFNγ-IL12信号系障害)
IFNγR1	NTM感染に関連，IFNγレセプターリガンド結合鎖を発現
IFNγR2	IFNγレセプター信号誘導鎖の欠如
STAT1	転写-1の信号誘導・活性化蛋白の欠如
IL12Rβ1	IL12レセプターβ1鎖欠如
IL12B	IL12p40サブユニット欠如
IFNγ	遺伝子多型として感受性の変異を起こす(?)
Mbl2	mannan-binding lectin，食菌作用を活性化する
VDR	ビタミンDレセプター．ビタミンDは免疫調整ホルモン作用あり．遺伝子多型として発現．
HLA	DR2，DQB1*0503など．
X染色体	Xq26にミニサテライトマーカー
染色体15	15q11-13にミニサテライトマーカー

わせるものが少なくない．昔から双生児研究など現象論的な研究は幾つか行われてきたが，近年ようやく分子レベルの解明が進められ，**表1**に掲げるようなものが結核感受性遺伝子としてクローズアップされてきた[8]．これらの中には明確な免疫障害の原因となる(まれな)遺伝子異常も含まれるが，多要因疾患としての結核の宿主要因の全体像(骨子だけでも)が把握できれば，発病予防のみならず治療や再発予防に関してより効果的な対策，更には個別的な対応も可能になろう．坂谷らは多剤耐性結核に陥った患者について調査した結果，多剤耐性結核患者では*Nramp1*のSNPs解析でAsn543Aspに異常があるのではないかと報告した[9]．

3. 健康診断，特に接触者対策

結核の早期診断の代名詞であった定期健康診断は，高校以上の学校の入学時と65歳以上(市町村により多少異なる)を除いては原則として廃止され，代わって患者の周囲にいる者(接触者)への検診は強化された．これは初発患者への感染源の発見，初発患者からの被感染者の早期の発見と対応(化学予防の実施)のため今後は一層重要である．特に対象を家族以外の社会的対人関係に拡大しての検診の計画が必要である．この活動は新たな技術の導入によって一段と強化されることとなった．更に前章2でみた化学予防の強化もこれを支えるべきものである．

a. 結核感染診断の進歩

近年，DNAが99%以上の相同性があるといわれる結核菌，BCGについてその綿密な比較検討からBCGには結核菌ゲノムの一部分が欠落した領域があることが発見され，これから結核菌に特異に存在するESAT-6およびCFP-10がクローニングされ，更にはそれらのポリペプチドが合成されるに至った．これらを抗原として被験者の全血を刺激し，感作リンパ球の免疫応答をインターフェロンγのELISA定量で測定するキットが開発された．商品名を'クォンティフェロン®第二世代'といい，日本では2006年1月に健康保険適用となった．著者らの治験成績によれば，未治療の結核患者における感度が89%，またBCG既接種の健常者でみた特異度は98%であった[10]．その後，接触者での検査の経験の積み重ねから，この検査は新しい感染の検出に優れたパフォーマンスを示すと考えられている．これを用いることで，従来の便宜的なツ反に比して格段に過不足の少ない結核

表2 クォンティフェロン(QFT)の適用方法
（文献[11]より引用）

1. 接触者検診
   ・個別的には全員QFTを
   ・集団ではまずツ反，発赤10-20mm以上にQFT
2. 医療職員の結核管理
   ・採用時：二段階ツベルクリン反応検査を廃止，QFTを
   ・患者接触時：全員にQFTを
3. 臨床応用
   ・鑑別診断：結核/非結核性抗酸菌感染症/腫瘍
   ・ハイリスク者の化学予防適応決定

感染の診断が可能になった．日本結核病学会[11]はこれの応用について声明を出した(表2)．従来のツ反検査はかなりの部分がこの検査に置き換えられるようになるであろう．

ただし，問題も残っている．感染を受けてから年余の長期間の経過後ではこの試験はかなり陰性化する．陳旧性の結核所見のある者でもこの検査で陰性のことがある．陰転した人と陽性のままの人とで結核発病のリスクが同じなのかは不明である．これは成人の化学予防，特に免疫抑制剤治療のような発病リスクが大きい者の感染有無の評価には重大な問題である．また5歳未満の乳幼児では今のところかなり信頼性に問題がある．なお，同工異曲の技術でインターフェロンγ産生細胞をマーカーで検出する方法(T-SPOT®)も実用化されており[12]，原理的にはより感度が高いことも期待されるが，日本では十分な評価はまだ行われていない．

b. 結核菌遺伝子タイピング

感染経路の証明の方法としてのこの技術は，これまでのRFLPから，迅速に結果がデジタルに得られるVNTRに取って代わられつつある．同時に疫学的リンクが疑われる菌株だけの調査から，地域で発生する全部の株について分析を積極的に行う方法が普及拡大されつつある(詳しくは文献[13]を参照)．

4. 治　　療

患者支援を通した治療の向上はStop TB Partnershipによる世界の結核対策への協調の基本であり，同時に今回の法改正の大きな眼目の一つである．具体的には主治医と保健所による患者の規則的な治療の確保のための連携であり，これは'日本版DOTS'として定式化されており[14]，保健所および医療機関での積極的な取り組みが期待される．

なお，治療に関しては日本では，菌検査の外部精度保証の必要性，未承認薬剤(例えばニューキノロン剤など)の早期承認など解決の急がれる問題，更に難治症例に関する専門施設紹介制度，そして専門治療施設の確保など将来の重大な問題が残されている．技術的な話題として以下の2件を採り上げる．

a. 新抗結核薬の開発

RFPが開発された1960年代以降，新しい抗結核薬の登場は皆無である．1990年代の先進国での結核'再興'以来，官産学の連携でこの状況を打破しようとする運動がGlobal Alliance on New TB Drug Development[15]として始まり，米国政府やビル・ゲイツ財団などの援助で薬剤開発・治験促進が進められてきた．その目標は，①化学療法の更なる短期化(2-3カ月に；また1-2週1回投与など)，②化学予防の強化・短期化，③多剤耐性結核治療の強化，④HIV合併結核の治療の強化である．目下moxifloxacinの抗結核作用の治験が進行中で，これにより2010年には3カ月程度の治療が実現する可能性がある．全く新しい物質であるPA824(ニトロイミダゾピラン体)も治験に入り，有力視されている．Allianceとは別に日本の大塚製薬が独自に開発したニトロイミダゾール体も有力視されており，臨床治験が進行中と聞く[16]．いずれもINH，RFPに勝るとも劣らない力価が期待されている．

b. 新たな薬剤感受性検査

従来の固形培地上の培養による薬剤感受性検査は，時間がかかることと接種菌量の調整の困難，薬剤の吸着などの点で結果のばらつき，解釈の困難さが大きな悩みであった．これらを解消すべく，新しい技術の開発も盛んに行われている．その一方が遺伝子タイピング技術を用いるもので[17]，既にその幾つかは実用化されてい

る．いずれも薬剤耐性の基になる遺伝子変異を同定するもので，RFPのように耐性遺伝子の種類が限定的な場合には単純であるが，INHのように多彩な場合には感度に問題がある．これに対して表現型をみる，つまり薬剤存在下で微量の菌の生育を敏感に検知する方法は，原理的には既に液体培地での結核菌の検出と同じ方法で実用化されているが，更に精緻化し，薬剤感受性検査に応用しようというのがファージを用いる技術である．これにはFASTPlaque-TB, luciferase reporter phageを用いる方法などがあり，前者は既に実用化されており，臨床材料にも応用できる[18]．このような新しい技術が上手に使われるようになれば，薬剤耐性への対応も迅速・正確にできるようになり，多剤耐性の予防と治療が向上しよう．

おわりに

以上，新しい結核対策の体制の下での技術的な可能性を記述した．これらが期待される成果を発揮するためには，対策に対する医療と行政の明確な関与が欠かせない．1960-70年代に対策をおろそかにした米国では，その後多剤耐性結核が急増し，1990年にはニューヨークでは患者の19%，全米でも3%以上が多剤耐性結核という状況になった[19]（日本は0.7%，2002年）．そして1980年代の後半から結核の逆転上昇を招いた．日本の結核をめぐる社会経済的状況，そしてときに垣間見られる医療・行政の姿勢は1970年代の米国のそれに通じるものがある．米国の経験を他山の石としなければならない．

追記 本稿は多くの点で厚生労働科学研究費新興・再興感染症研究（小児結核及び多剤耐性結核の予防，診断，治療における技術開発に関する研究（主任研究者森 亨，平成15-17年度））の業績に依拠した．

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