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Structure-activity relationships of macrolides against *Mycobacterium tuberculosis*

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KEYWORDS
Mycobacterium tuberculosis;
Macrolides;
Ketolides;
SAR;
Synthesis

Summary
Existing 14, 15 and 16-membered macrolide antibiotics, while effective for other bacterial infections, including some mycobacteria, have not demonstrated significant efficacy in tuberculosis. Therefore an attempt was made to optimize this class for activity against *Mycobacterium tuberculosis* through semisyntheses and bioassay. Approximately 300 macrolides were synthesized and screened for anti-TB activity. Structural modifications on erythromycin were carried out at positions 3, 6, 9, 11, and 12 of the 14-membered lactone ring; as well as at position 4" of cladinose and position 2' of desosamine. In general, the synthesized macrolides belong to four subclasses: 9-oxime, 11,12-carbamate, 11,12-carbazate, and 6-*O*-substituted derivatives. Selected compounds were assessed for mammalian cell toxicity and in some cases were further assessed for CYP3A4 inhibition, microsome stability, in vivo tolerance and efficacy. The activity of 11,12-carbamates and carbazates as well as 9-oximes is highly influenced by the nature of the substitution at these positions. For hydrophilic macrolides, lipophilic substitution may result in enhanced potency, presumably by enhanced passive permeation through the cell envelope. This strategy, however, has limitations. Removal of the C-3 cladinose generally reduces the activity. Acetylation at C-2' or 4" maintains potency of C-9 oximes but dramatically decreases that of 11,12-substituted compounds. Further significant increases in the potency of macrolides for *M. tuberculosis* may require a strategy for the concurrent reduction of ribosome methylation.

Introduction

One practical approach for expeditious TB drug discovery is to consider current antibiotic classes that already possess acceptable pharmacological and toxicological profiles, and to then optimize for potency against *M. tuberculosis*. Erythromycin (EM, Fig. 1), the first generation prototypical macrolide, is a natural product produced by *Streptomyces erythreus*. The compound inhibits protein synthesis by binding to the 50S subunit of 70S ribosomes near the peptidyl transferase center, thus blocking the movement of nascent peptides through the exit tunnel. Erythromycin has a short serum half-life (thus necessitating tid or qid dosing) as well as acid lability, the acid degradation product of which results in

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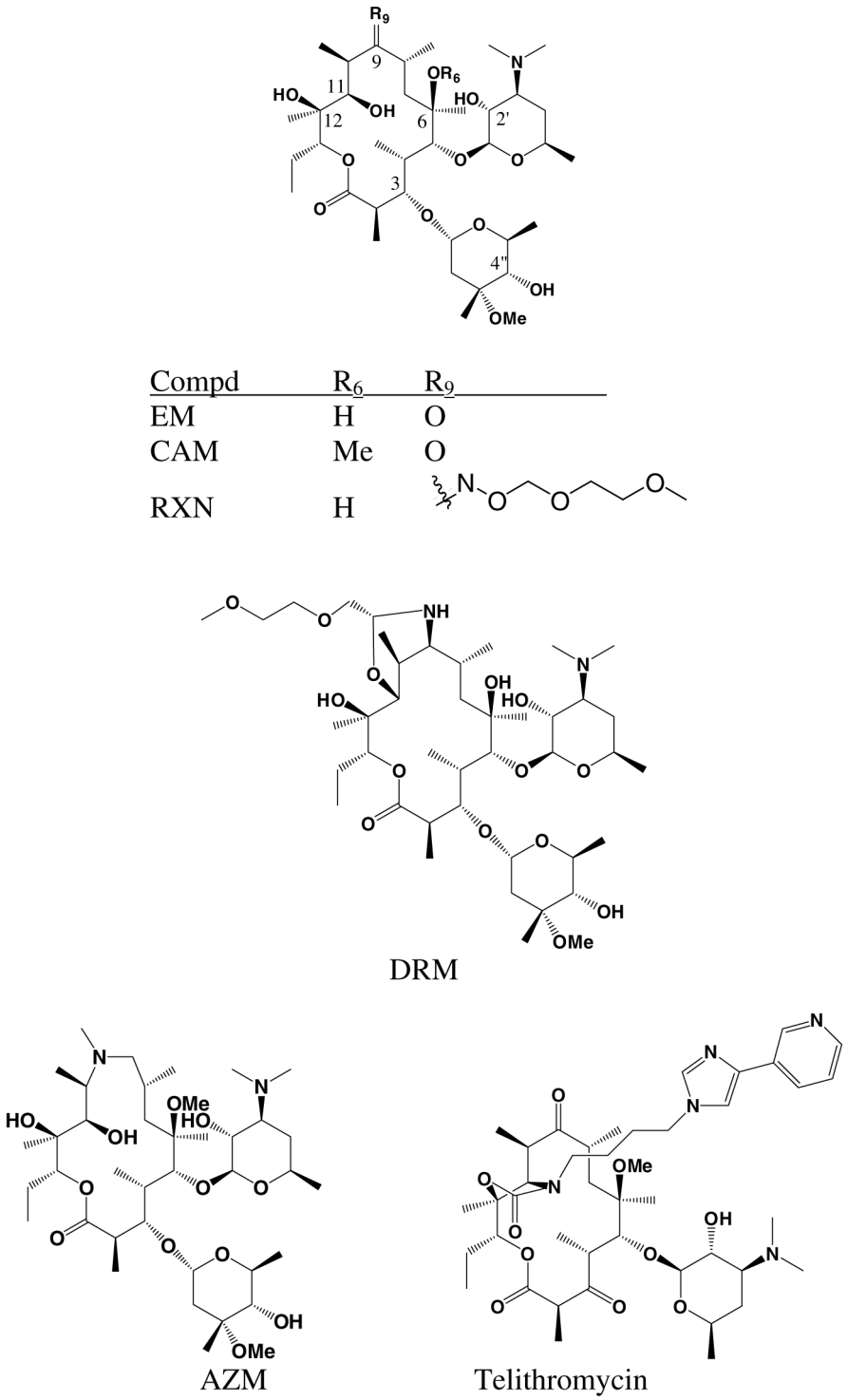


Figure 1 Macrolide antibiotics.

gastric motility-based discomfort. In addition, activity was restricted to Gram-positive bacteria and no activity was observed against *M. tuberculosis*. The second generation macrolides clarithromycin (CAM), roxithromycin (RXM), dirithromycin (DRM) and azithromycin (AZM)[1] (Fig. 1) were therefore developed to have superior acid stability and serum half-life. CAM and AZM were, along with rifabutin, the most active clinical agents against *M. avium*.[2,3] With the exception of AZM (an azalide which possesses a different spectrum of activity than other macrolides), these compounds were also found to possess potent activity against *M. leprae* in axenic media[4,5] in macrophages,[6] mice[7,8] and ultimately in man.[9–11] CAM is currently recommended by the WHO for treatment of leprosy in cases of rifampin resistance or intolerance.[12] Other studies demonstrated low MICs and/or clinical

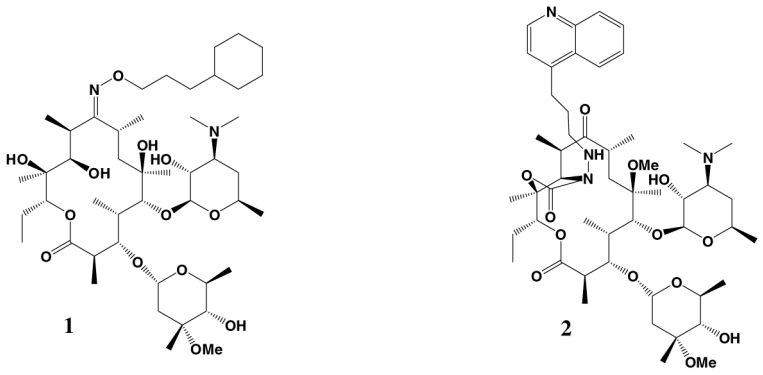


Figure 2 GI-448 (**1**) and RU-66252 (**2**).

utility of second generation macrolides for *M. kansasii*,[13] *M. marinum*,[14–17] *M. xenopi*[18] and other mycobacterial opportunistic pathogens.[15,19–21]

The impressive activity of second generation macrolides unfortunately also did not include *M. tuberculosis*. Reported MICs of CAM or RXM against the tubercle bacillus range from 4->128 μg/mL[22–25] and activity of CAM in mouse models was marginal[23] to non-existent.[22] The weak in vitro and in vivo activity of CAM against *M. tuberculosis* and the demonstrated activity in leprosy and *M. avium* infection has formed the basis of its use in MDR-TB when therapeutic options are extremely limited.[26] However, the in vitro and in vivo results would strongly suggest that CAM cannot be expected to offer significant antimicrobial clinical benefits in tuberculosis.

Ribosome modification leading to macrolide resistance can occur either through mutation in 23S ribosomal RNA or ribosomal proteins or through specific mono or di-methylation at A2058 (*E. coli* numbering system) by ribosome methylases. Monomethylation of A2058 typically results in variable level resistance to macrolides, lincosamides and streptogramin B (MLS_B) but no resistance to the newer ketolides (type I resistance) wherease dimethylation of A2058 results in high level resistance to MLS_B and ketolides (type II resistance).[27] Although it is possible that efflux and/or macrolide modification might play a role in macrolide resistance in *M. tuberculosis*, there is more definitive evidence that ribosome methylation plays a major role in the innate resistance of this organism to this class of antibiotics.[28]

The third generation of macrolides were developed to overcome the primary macrolide resistance mechanisms of common respiratory pathogens: ribosome methylation and to a lesser degree, drug efflux. Major areas of focus were the removal of the cladinose (ketolides) and the addition of aryl groups at positions 6, 9 and 11,12. The first such ketolide to be approved for clinical use, telithromycin (Fig 1), confers significant activity against a broad range of respiratory pathogens, including macrolide-resistant strains[4] but is not active against *M. tuberculosis*.[24,29]

Reports of the activity of newer experimental macrolides against *M. tuberculosis* are very limited. Hokuriku Seiyaku Co., Ltd. patented 9-oxime ether derivatives of EM with representative compound GI-448 (**1** in Fig. 2) demonstrating a MIC of 3.13 μg/mL against *M. tuberculosis*.[30]

We recently reported the activities of approximately 30 macrolides and ketolides from Abbott and Aventis against *M. tuberculosis*.[29] Sub-micromolar MICs were observed for at least one representative from series substituted at positions 6, 9 or 11,12. One of the latter macrolides, RU-66252 (**2** in Fig. 2), a 4-quinolinylpropyl 11,12-carbazate of CAM, demonstrated dose-dependent inhibition of *M. tuberculosis* growth in mice. This compound did not, however, demonstrate consistent bactericidal activity in vivo and appeared unstable in the presence of microsome (author's unpublished data). Nonetheless, based on the encouraging in vitro activity described above, an extensive lead identification/optimization project on anti-TB macrolides and ketolides ensued with a focus on positions 6, 9 and 11,12.

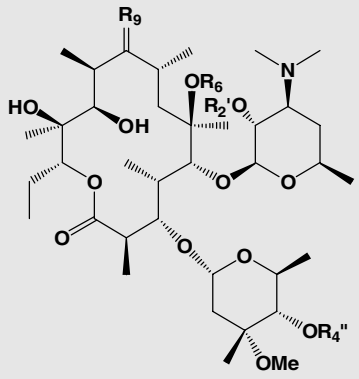
Chemistry

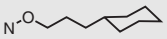
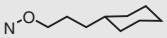
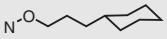
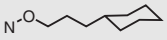
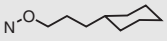
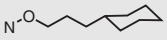
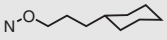
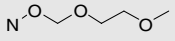
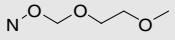
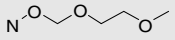
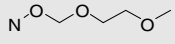
EM (Fig. 1) constituents include a 14-membered lactone ring, cladinose at the 3-position, and desosamine at the 5-position. On the lactone ring, positions 6, 9, 11, and 12 are relatively accessible for modifications; besides 3-cladinose can be hydrolyzed under acidic conditions. The cladinose 4"- and desosamine 2'-hydroxyls are accessible to chemical modification.

The synthesis of the 9-oxime ether of macrolide ITR051 (Table 1) was carried out by converting EM to 9-oxime with hydroxylamine and followed by a nucleophilic substitution (Scheme 1).[31] The 4"-acylated ITR051 (GI-448) and its precursor ITR150 were prepared according to a literature procedure[32] as was 4"-acylated RXN (ITR056). Because of the catalytic function of the amino group in desosamine, position 2' is preferentially acylated and deacylated over the hydroxyl group at the position 4" of cladinose. ITR052, the 6-methyl analog of ITR051, was synthesized by a similar procedure using CAM instead of EM as the starting material. The 4"-benzoyl ester of RXN (ITR057), the 4"-acetyl ester of CAM (ITR054), and 4"-benzoyl ester of CAM (ITR036) were prepared similarly with corresponding starting materials.

9-Azine derivatives of EM (Table 1) were synthesized as shown in Scheme 2 based on previously described

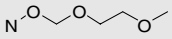
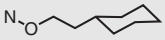
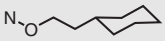
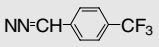
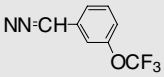
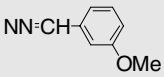
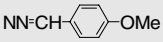
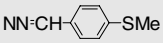
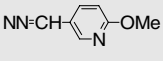
Table 1 In vitro inhibition activity of 4",2'- and 9-modified EM and CAM derivatives.



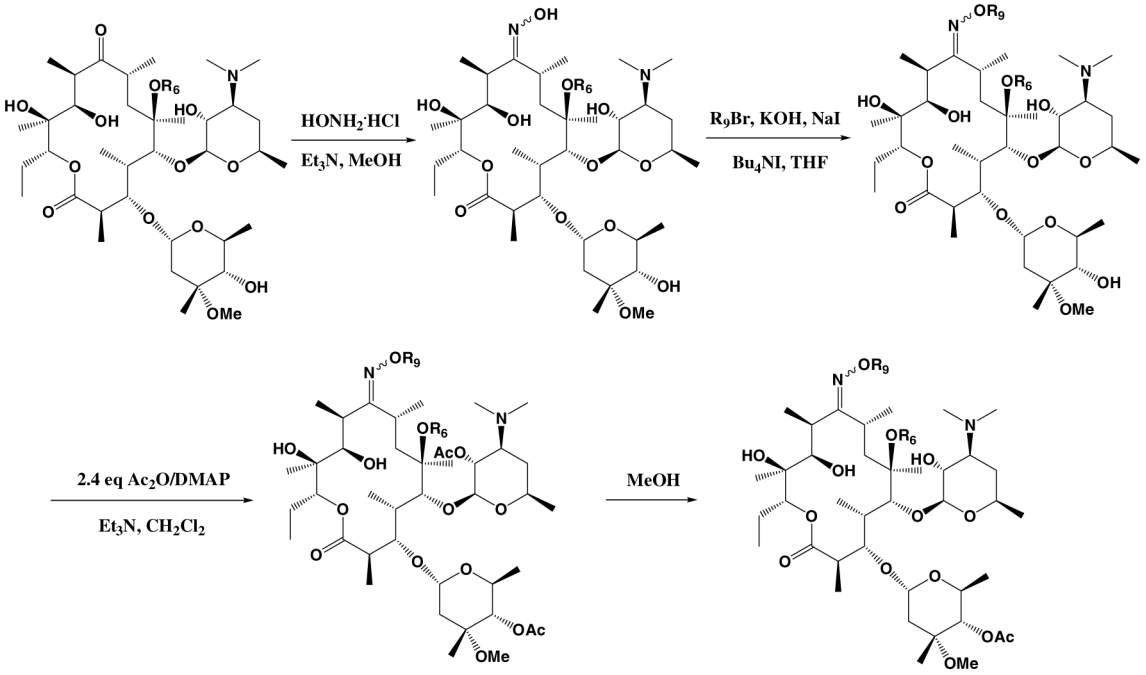
Compound	$R_{4"}$	$R_{2'}$	R_6	R_9	MIC μM	IC_{50} μM	CYP3A4 IC_{50} μM	LogP
EM	H	H	H	O	128	N/A	1.3	2.8
ITR051	H	H	H		12 (±4)[a]	9.03	N/A	7.6
GI-448	Ac	H	H		3.5	13.6	5.6	8.0
ITR150	Ac	Ac	H		3.4	12.0	9.6	8.8
ITR052	H	H	Me		1.1(±0.1)	12.4	N/A	8.0
ITR161	Ac	H	Me		0.97	14	7.8	8.5
ITR159	H	Ac	Me		0.69	45	4.7	8.7
ITR160	Ac	Ac	Me		0.96	39	6.7	9.2
RXN	H	H	H		117 (±1)	>128	2.9	3.7
ITR056	Ac	H	H		128	N/A	25.3	4.2
ITR057	Bz	H	H		64	N/A	8.6	6.2
CAM	H	H	Me	O	15 (±0.3)	40.9	9.8	3.2
ITR054	Ac	H	Me	O	117 (±1.5)	35.6	N/A	3.7
ITR036	Bz	H	Me	O	>128	N/A	N/A	5.7
ITR073	H	C(O)NHPh	H		9.5 (±2.9)	14.2	N/A	6.0

Continued overleaf

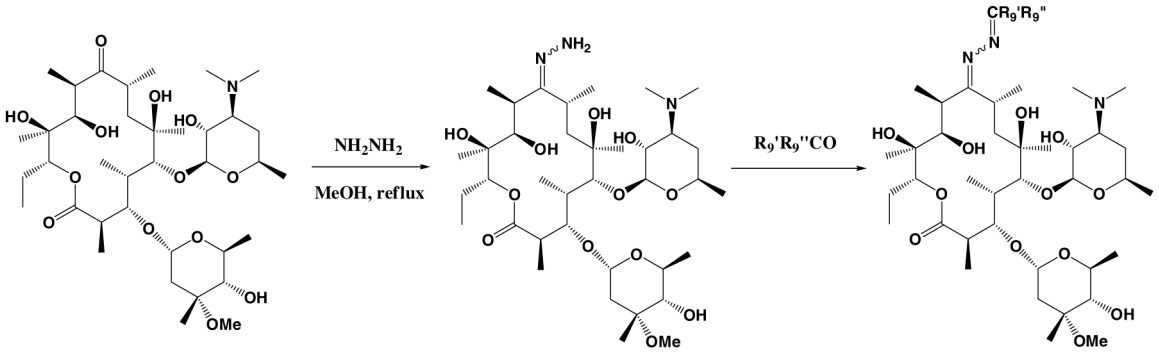
Table 1 *Continued.*

Compound	$R_{4''}$	$R_{2'}$	R_6	R_9	MIC μM	IC_{50} μM	CYP3A4 IC_{50} μM	LogP
ITR076	C(O)NHPh	C(O)NHPh	H		3.5 (±0.2)	24.8	3.8	8.1
ITR077	H	H	Me		0.96 (±0.01)	16.3	3.0	7.5
ITR120	C(O)NHPh	C(O)NHPh	Me		103 (±1)	115	N/A	11.9
ITR086	H	H	Me	NOC_2H_4Ph	2.0 (±0.03)	15.4	1.3	6.5
ITR126	C(O)NHPh	C(O)NHPh	Me	NOC_2H_4Ph	17	48.0	43.0	10.9
ITR103	H	H	Me	NOC_3H_6Ph	0.5	16.6	3.5	7.0
ITR138	C(O)NHPh	C(O)NHPh	Me	NOC_3H_6Ph	>128	107	31	11.4
ITR145	C(O)NHEt	H	Me	O	92	>128	N/A	3.9
ITR122	C(O)NHEt	C(O)NHEt	Me	O	>128	N/A	N/A	4.9
ITR074	C(O)NHPh	H	Me	O	55 (±1.6)	7.9	N/A	5.2
ITR121	C(O)NHPh	C(O)NHPh	Me	O	58	6.8	N/A	7.6
ITR083	C(O)NHBn	H	Me	O	91 (±3)	39	N/A	5.1
ITR091	$C(O)NHC_2H_4Ph$	H	Me	O	57 (±0.4)	16	N/A	5.6
ITR105	H	H	H	NNH_2	>128	4.70	N/A	2.3
ITR116	H	H	H		122	N/A	5.4	3.4
ITR125	H	H	H		27.2	8.6	N/A	5.9
ITR128	H	H	H		11.4	N/A	14	6.0
ITR129	H	H	H	$NN{=}CH{-}(CH_2)_2CF_3$	56	N/A	10	4.3
ITR132	H	H	H		59	4.8	N/A	5.0
ITR133	H	H	H		58	18.0	N/A	5.0
ITR148	H	H	H		43	12.0	N/A	5.8
ITR140	H	H	H		56	35.8	29	4.6

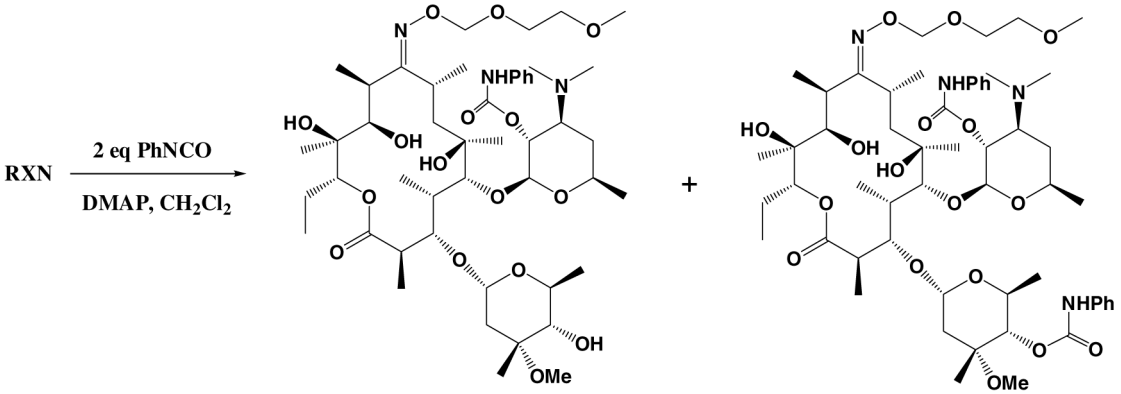
[a]Values are means of three experiments. Standard deviation is given in parentheses.



Scheme 1 Synthesis of C-9 oxime derivatives of EM and CAM (R_6 = H, Me; for R_9, see Table 1).



Scheme 2 Synthesis of 9-azine derivatives of EM.

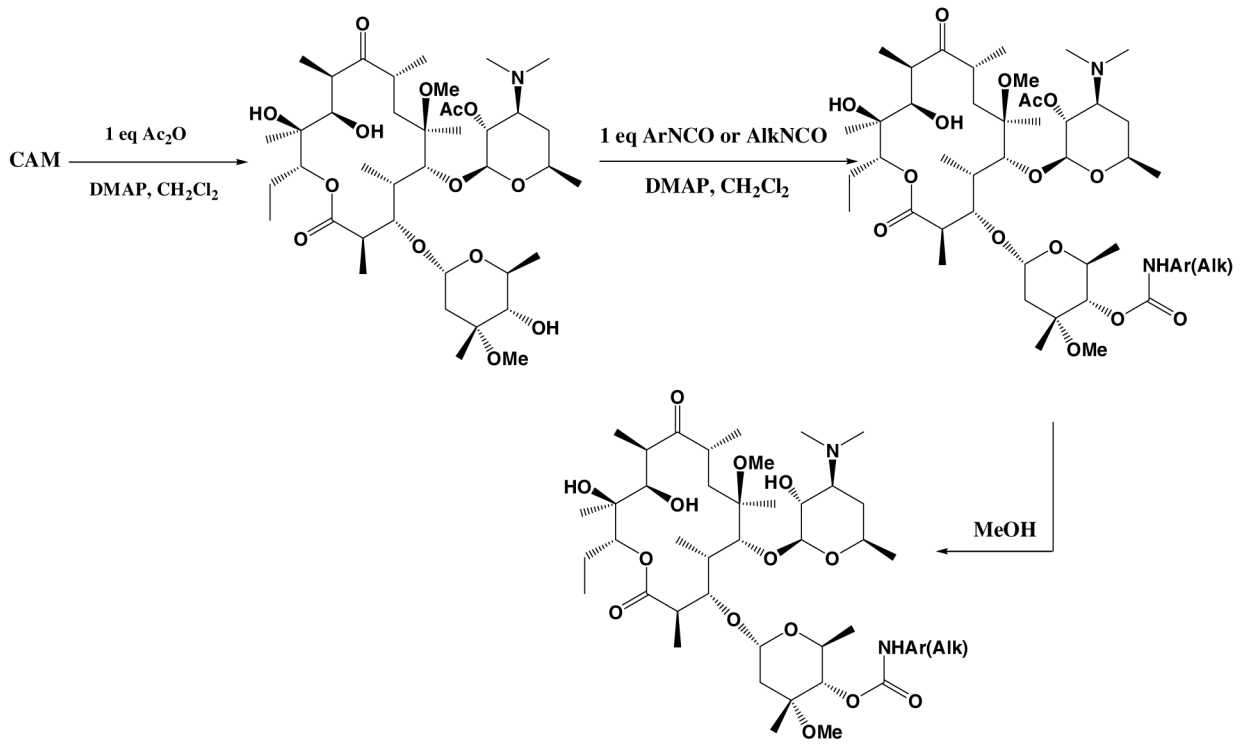


Scheme 3 Synthesis of 2'-carbamate and 2',4"-dicarbamate of RXN.

procedures[33,34] beginning with the synthesis of erythromycin 9-hydrazone, followed by reaction with aldehydes and ketones (Scheme 2).

Reacting two-equivalents of phenyl isocyanate with RXN provided both 2',4"-dicarbamate (ITR076) and 2'-carbamate (ITR073) as shown in Scheme 3.[35] The two products were isolated by flash chromatography.

Dicarbamates of CAM (ITR121 and ITR122) were prepared similarly using CAM as the starting material. Reacting one-equivalent of isocyanates with 2'-acetyl



Scheme 4 Synthesis of 4"-carbamates of CAM. Ar=Ph; Alk= Et, $PhCH_2$ and $PhCH_2CH_2$.

CAM followed by deacylation provided the corresponding 4"-carbamates (ITR145, ITR074, ITR083, and ITR091 in Table 1) as shown in Scheme 4.

The synthesis of 11,12-carbamates ITR004, ITR020, and ITR021 (Table 3) was carried out with a similar procedure as described in the literature (Scheme 5).[36] The 11,12-carbamates ITR048 and ITR049 in Table 3 were prepared according to a literature procedure.[37]

The syntheses of 11,12-carbazates ITR033, ITR005, ITR053, ITR099, ITR023 and ITR022 (Table 3) were carried out as depicted in Scheme 6.[38] Derivatization at positions 3 and 6 was based upon the procedures published by Ma et al (Scheme 7).[36]

Biological activity

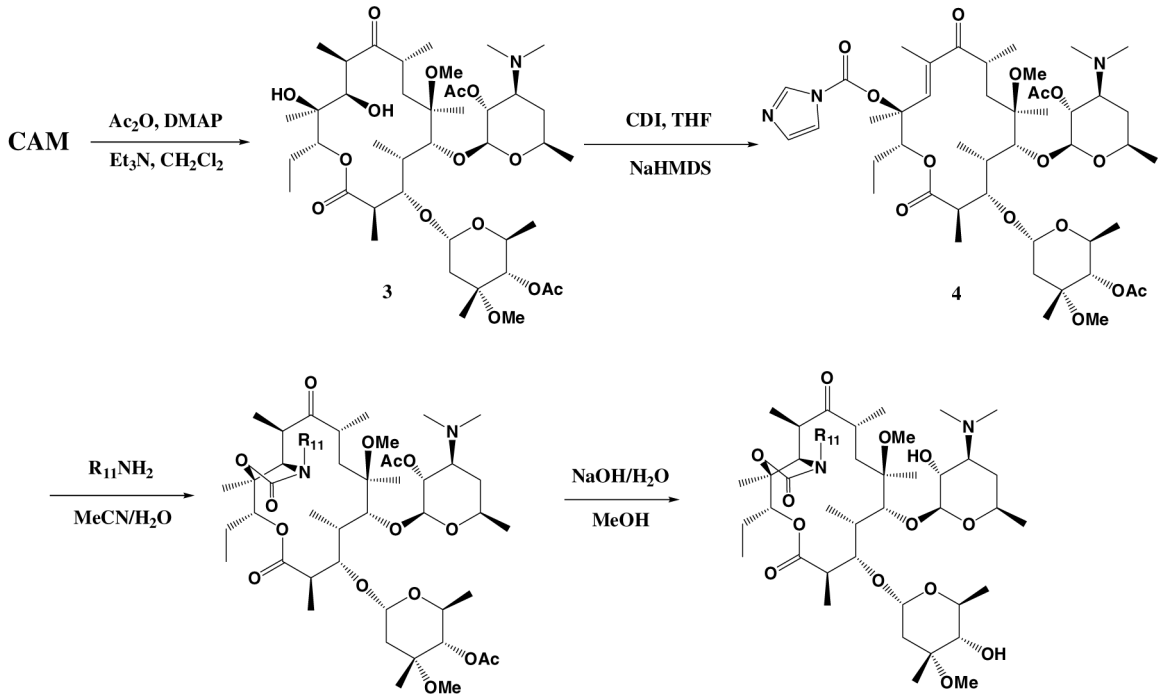
MICs against *M. tuberculosis* H37Rv, a strain that is susceptible to the clinical tuberculosis drugs, and toxicity for VERO (green monkey kidney) cells were determined as previously described.[29] The MIC was defined as the lowest concentration resulting in 90% reduction in fluorescence. Inhibition of CYP3A4 was determined using the flourescent Invitrogen Vivid™ assay. LogP values were calculated with ACD/LogP freeware (acdlabs.com).

With respect to activity of macrolides against *M. tuberculosis* there are two additional major aspects that need to be considered: innate resistance and the unique structure and composition of the highly hydrophobic cell wall that must be traversed. It is generally accepted that increasing lipophilicity of compound leads to improved cell wall permeability.[39,40] It is tempting to attribute the difference in anti-TB activity of CAM and EM to the difference in lipophilicity.

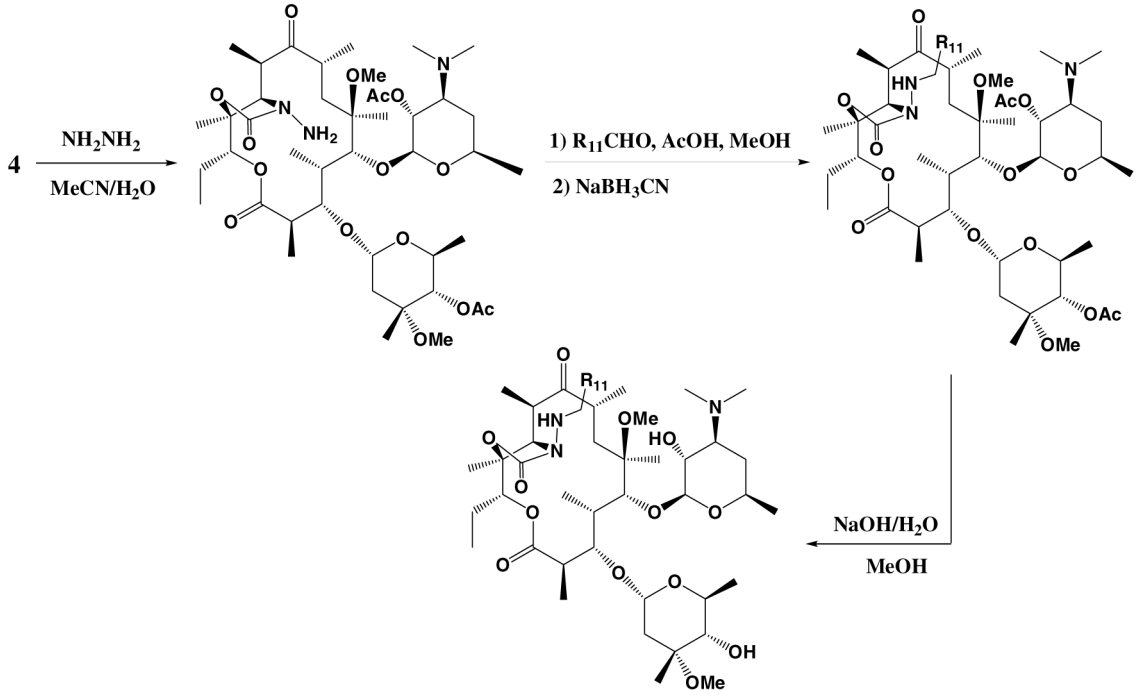
Within each of two series — EM and CAM 9-oxime derivatives (Table 1) — there is a correlation between lipophilicity of the substituent on the 9-position (defined as calculated logP) and both anti-TB activity and toxicity; both activities increase with increasing lipophilicity. A similar type of MIC-clogP relationship was previously reported for 9-oxime derivatives of ketolides.[29] In pairs of corresponding 9-oximes, the CAM derivatives were more active than the EM counterparts (for example compounds ITR051 and ITR052, respectively).

For CAM and 9-oxime derivatives of both CAM and EM, further enhancement of lipophilicity was undertaken via modification of 2' and 4"-positions on desosamine and cladinose rings, respectively. Although esterification of macrolides has been observed to decrease activity for Gram-positive and Gram-negative bacteria[41] such modifications might be expected to promote penetration through the mycobacterial cell envelope[40] with subsequent removal via bacterial esterase.

4"-Acetyl- and 4"-benzoyl-CAM (ITR054 and ITR036 respectively; Table 1) are significantly less potent compared to CAM while moderate improvement in the activity can be seen for some acetylated EM derivatives (ITR051 vs. GI448 and ITR150). However, in general 4"-acylation or 2',4"-diacylation of both EM and CAM 9-oximes does not result in significant changes in potency. Interestingly, however, 2'-acetylation of CAM 9-oximes may decrease mammalian cell toxicity, resulting in a higher selectivity index (VERO IC_{50}/MIC) as observed for ITR052 vs. ITR159.



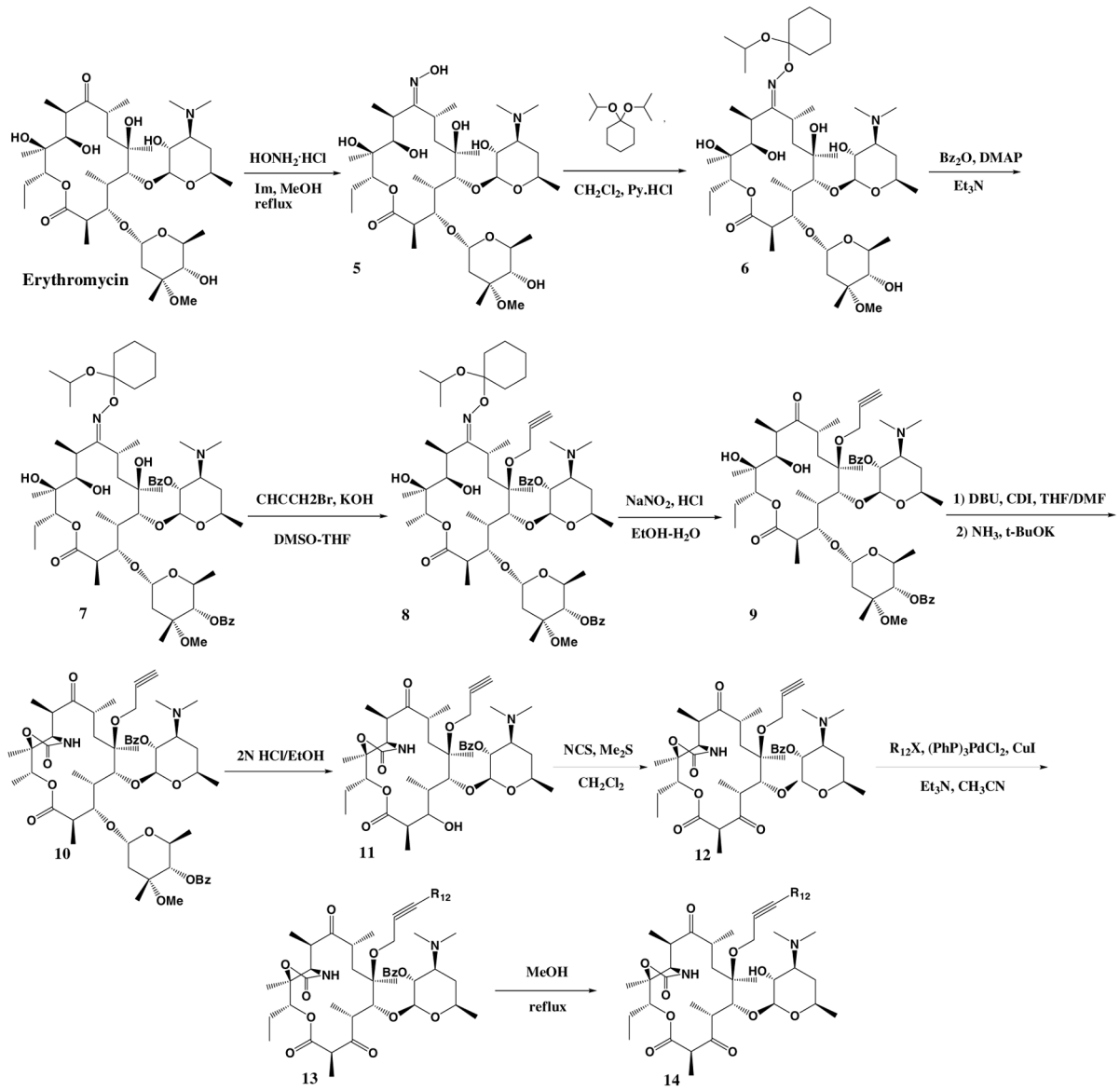
Scheme 5 Synthesis of 11,12-carbamates of CAM.



Scheme 6 Synthesis of 11,12-carbazates of CAM.

4”-Carbamates of macrolides have good in vitro and in vivo activities against macrolide-susceptible and -resistant organisms.[42] However, modification of CAM and its 9-oxime derivatives at 4”- and 2’-hydroxyl groups to carbamates all resulted in dramatically decreased potency. For 2’,4”-diphenylcarbamoyl derivatives of CAM we observed an unusual trend: increasing lipophilicity of the substituent on the 9-position of oxime leads to decreasing toxicity. Interestingly, the roxithromycin derivatives 2’,4”-diphenylcarbamate (ITR076) and 2’-phenylcarbamate (ITR073) are significantly more active than the parent compound.

Overall for anti-TB activity of 9-oxime derivatives of both EM and CAM, the substituent on the 9-position is



Scheme 7 Modifications at positions 3 and 6 of EM.

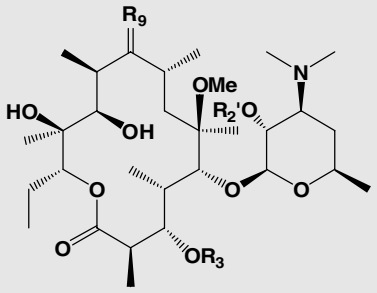
generally more important than modifications on 2' and 4" positions. For example, replacing 9-carbonyl in EM with cyclohexyl ether of 9-oxime in ITR051 enhanced potency by 10 fold (128 to 12 μM, Table 1). In contrast, further modification at 4" (GI-448), or 4" and 2' positions enhanced potency to a lesser extent - 3.5 fold (from 12 to 3.4 μM). Another example is that ITR052 enhanced potency of CAM from 15 to 1.1 μM (more than 10 fold); however, further modification at 4" and/or 2' positions (ITR159, ITR160, and ITR161; Table 1) only enhanced potency marginally. 9-Azines of EM are toxic and poorly active; however, the trend is the same: the more lipophilic compounds yielding lower MIC and IC_{50} values (e.g.. ITR125 vs. ITR133).

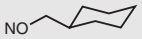
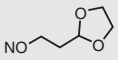
It has been reported that the cladinose ring at C-3 contributes to the metabolic instability of macrolides as well as to drug efflux and inducible macrolide resistance; removing the cladinose and consequently oxidizing the resultant hydroxyl group to a ketone group yields more stable and desirable compounds (ketolides).[4] Similar approaches adopted in this study by hydrolyzing the cladinose followed by derivatizing the 3-OH resulted in a reduction of activity (Table 2).

As we reported previously, two 11,12-substituted macrolides, the carbazate RU66252 and the carbamate RU69874 demonstrated potent MIC values.[29] Therefore, several new C-11,12 substituted analogs were synthesized (Table 3). It appears that the substituent at 11,12 position affects the potency significantly. For example, replacing the 4-quinolyl substituent of RU66252 with a 3-quinolyl resulted in a 20-fold decrease in activity for ITR023.

Several of the most active C-11,12 substituted macrolides were esterified at C2' and/or C4" in an attempt to increase activity. Unlike the 9-oxime derivatives, activity of 11,12-carbamate and 11,12-

Table 2 In vitro activity of 3-0-modified compounds.



Compound	R_3	$R_{2'}$	R_9	MIC μM	IC_{50} μM	CYP3A4 IC_{50} μM	LogP
ITR156	C(O)NHEt	H	O	>128	N/A	2.7	2.9
ITR163	C(O)NHHexyl	H	O	62	73	0.06	5.0
ITR151	C(O)NHPh	H	O	>128	N/A	6.1	4.3
ITR157	H	H		4.0	45	0.4	5.1
ITR173	H	H		>128	N/A	N/A	2.7
ITR175	H	H	NOC3H6Ph	6.3	N/A	0.02	5.7
ITR174	Bn	Bn	NOBn	7.1	N/A	N/A	10.3

carbazate derivatives dramatically decreases after acylation on C4" position and even more after C2', C4"-diacylation, except ITR020, that retains its activity after C4"-acylation (ITR021); MICs 6 μM and 4 μM respectively (Table 3).

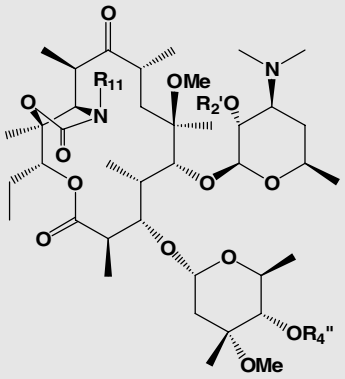
Retaining the 11, 12-carbamate moiety that is favorable for activity, further combinations of modifications were made on the 3- and 6- as well as 2'- and 4"-positions (Table 4). In most cases less lipophilic terminal heterocycles attached to an isoxazole or thiophene ring in the 6-substituent yielded higher MICs. Compounds containing 3-quinolyl are more active than the corresponding 3-pyridil- or the tetrazolyl-containing derivatives (A323348 vs. ITR273, ITR275 vs. ITR257, ITR255 vs. ITR261, ITR285 vs. ITR 270 etc). Interesting counterparts are A323348 vs. ITR266 and I10 vs. ITR276, demonstrating that replacing the nitrogen atom in the quinolyl substituent of a terminal ring (e.g. 3-quinolyl- vs. 6-quinolyl-) leads to a decrease in the activity and an increase in the cytoxicity. For all substituents on the 6-position, ketolides are less potent than the corresponding cladinose-containing compounds (ITR250 vs. ITR286, ITR248 vs. ITR285, ITR263 vs. ITR270, ITR264 vs. ITR 271).

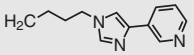
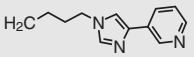
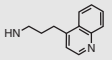
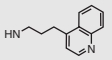
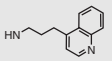
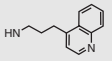
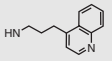
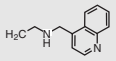
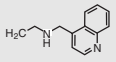
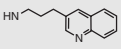
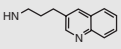
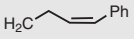
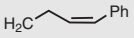
2'-Benzoylation of ketolides and 4"-benzoylation of cladinose containing derivatives slightly/moderately improves activity in most cases. The 2', 4"-dibenzoylated macrolides are not active. The 2-fluorinated ketolides are more potent than their C2-H des-fluoro counterparts (A323348 vs. ITR250, ITR278 vs. ITR248). The 6-allyl linker confers the same or slightly more potency and moderately less cytoxicity than the propargyl linker.

Because the studies reported here exclusively used whole cell inhibition as the biological endpoint, it was not possible to try to separate SAR related to ribosome binding from other factors such as rate of passage through the cell wall/membrane and/or drug metabolism. Indeed, it is certainly possible that some of the inhibitory activity noted here, especially in the case of the more hydrophobic compounds with significant mammalian cell toxicity, may have been due in whole or in part to off-target activity[43] rather than ribosome binding.

Because tuberculosis must be treated with multiple drugs and because of the frequent need for concurrent treatment of both HIV and TB, drug-drug interactions are of particular concern. Macrolides are well known inhibitors of CYP3A4, therefore it was of interest to measure this activity among these derivatives. In cladinose-containing compounds, with one exception (ITR048 vs. ITR049, Table 3) inhibition is decreased in 2'

Table 3 In vitro activity of 11,12-carbamate and 11,12-carbazate derivatives of CAM.

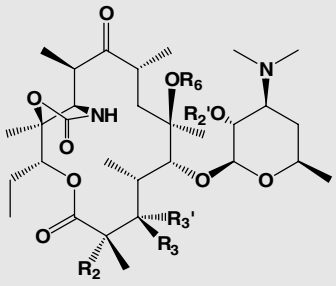


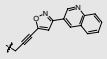
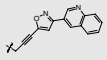
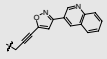
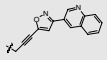
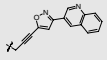
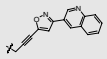
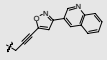
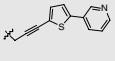
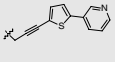
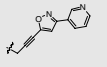
Compound	$R_{4"}$	$R_{2'}$	R_{11}	MIC μM	IC_{50} μM	CYP3A4 IC_{50} μM	LogP
RU69874[29]	H	H		0.38	27.0[29]	0.2	5.5
ITR004	Ac	Ac		32	N/A	N/A	6.7
RU66252[29]	H	H		0.25	24.9[29]	0.4	6.6
ITR033	Ac	H		64	N/A	N/A	7.1
ITR005	Ac	Ac		6	N/A	3.6	7.9
ITR053	Bz	H		1.7	5.5	19	9.2
ITR099	Bz	Bz		11	N/A	N/A	11.9
ITR049	H	H		2.5	38.8	0.07	5.3
ITR048	Ac	H		10	13.5	0.01	5.8
ITR023	H	H		6	N/A	0.01	5.2
ITR022	Ac	Ac		16	N/A	5.0	6.4
ITR020	H	H		6	>128	N/A	6.5
ITR021	Ac	H		4	N/A	N/A	6.9

and/or 4"-substituted compounds including ITR086 vs. ITR126, RXN vs. ITR056 and ITR057 (Table 1), ITR023 vs. ITR 022, (Table 3), ITR286 vs. ITR258, ITR281 vs. ITR 280, ITR271 vs. ITR268 and ITR270 vs. ITR267 (Table 4) . Among 2'-benzoylated ketolides there were both examples of decreased CYP3A4 inhibition (A323348 vs. I10, ITR266 vs. ITR276) or modestly increased inhibition (ITR255 vs. ITR248, ITR277 vs. ITR278 and ITR259 vs. ITR275) (Table 4).

It should be noted that a number of macrolides described here (as well as several related compounds not shown here including cethromycin[29]) were orally administered to TB-infected mice[29] at a highest dosage of 200 mg/kg, including ITR161, ITR77, ITR103 (Table 1), ITR157 (Table 2) and ITR258 and ITR255 (Table 4) or at 100 mg/kg (the maximum tolerated dose) for A323348, (Table 4) and cethromycin. All failed to significantly reduce cfu in the lungs of mice treated once daily for 3

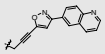
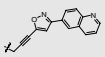
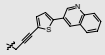
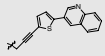
Table 4 MICs of 6-*O*-Substituted Macrolides



Compound	R_2	R_3,R_3	$R_{2'}$	R_6	MIC μM	IC_{50} μM	CYP3A4 IC_{50} μM	LogP
I10	F	=O	Bz		1.3	>128	85.45	8.3
A323348	F	=O	H		0.99	33.9	21.47	5.5
ITR250	H	=O	H		3.7	35.0	55.40	5.2
ITR283	H	H,OH	H		3.37	>128	0.11	5.2
ITR286	H	H,Clad	H		0.5	12	0.13	6.3
ITR258	H	H,BzClad	H		0.68	37.0	35.37	8.8
ITR272	H	H.BzClad	Bz		>128	N/A	N/A	11.5
ITR274	H	H,BzClad	H		1.8	2.99	21.43	9.5
ITR282	H	H,BzClad	Bz		>128	>128	N/A	12.5
ITR284	H	H,BzClad	H		2.27	24.9	34.3	9.8
ITR285	H	H,Clad	H		3.31	27.7	0.12	7.3
ITR255	H	=O	Bz		1.3	>128	13.83	9.0
ITR248	H	=O	H		12	11.9	38.6	6.2
ITR278	F	=O	H		3.8	44	15	6.5
ITR277	F	=O	Bz		3.7	>128	6.0	9.3
ITR256	H	=O	Bz		3.2	>128	5.0	9.0
ITR257	H	=O	H		16	15.0	0.72	6.2
ITR273	F	=O	H		>13	35.4	5.7	4.5

Continued overleaf

Table 4 *Continued*

Compound	R_2	R_3,R_3	$R_{2'}$	R_6	MIC μM	IC_{50} μM	CYP3A4 IC_{50} μM	LogP
ITR266	F	=O	H		3.3	51.3	17.9	5.7
ITR276	F	=O	Bz		2.4	42.4	>128	8.4
ITR275	H	=O	H		5.0	14.07	10.19	7.3
ITR259	H	=O	Bz		3.4	N/A	5.68	10.1
ITR261	H	=O	Bz		8.0	>128	N/A	7.5
ITR263	H	=O	H		31	51.1	N/A	4.8
ITR262	H	=O	Bz		53	>128	1.8	7.5
ITR264	H	=O	H		>128	>128	N/A	4.8
ITR265	H	H,BzClad	Bz		>128	>128	N/A	11.3
ITR267	H	H,BzClad	H		1.7	6.52	37.3	8.6
ITR270	H	H,Clad	H		7.2	18.9	2.6	6.1
ITR269	H	H,BzClad	Bz		>128	>128	N/A	10.8
ITR280	H	H,BzClad	H		1.9	30.2	47.0	8.1
ITR281	H	H,Clad	H		3.3	41.9	0.08	5.6
ITR279	H	H,BzClad	Bz		>128	12.7	N/A	11.3
ITR268	H	H,BzClad	H		2.0	7.3	11.8	8.6
ITR271	H	H,Clad	H		4.0	48.2	0.36	6.1

weeks, possibly due to poor pharmacokinetics. Therefore although compounds with improved MICs relative to clarithromycin have been identified, none with the exception of RU66252[29] have demonstrated superior in vivo activity. The latter demonstrated a dose response and at the upper dosage of 200 mg/kg, reduced lung cfu by 1–2+ $\log_{10}$ relative to untreated mice. The failure, however, of RU66252 to achieve more potent bactericidal activity in vivo may have been due, at least in part, to metabolic instability as indicated by a relatively rapid loss of parent compound in a microsome incubation experiment (author's unpublished data).

The combination of high in vitro and in vivo potency and favorable pharmacokinetic properties in a single macrolide against *M. tuberculosis* has yet to be attained. After completion of the studies described above, two important publications appeared that shed light on the mechanism of resistance to macrolides in *M. tuberculosis* which may in turn at least partially explain its unique macrolide resistance pattern. It now appears that the *M. tuberculosis* ribosome (and that of other *M. tuberculosis* complex species[27]) is not only methylated at A2058, a key residue implicated in macrolide resistance, but is in fact monomethylated at three consecutive residues: A2057, 2058 and 2059.[27] This is apparently due to a truncated C-terminal region of the chromosomally-encoded ribosome methylase erm (37) (aka ermMT, encoded by Rv1988), presumably resulting in

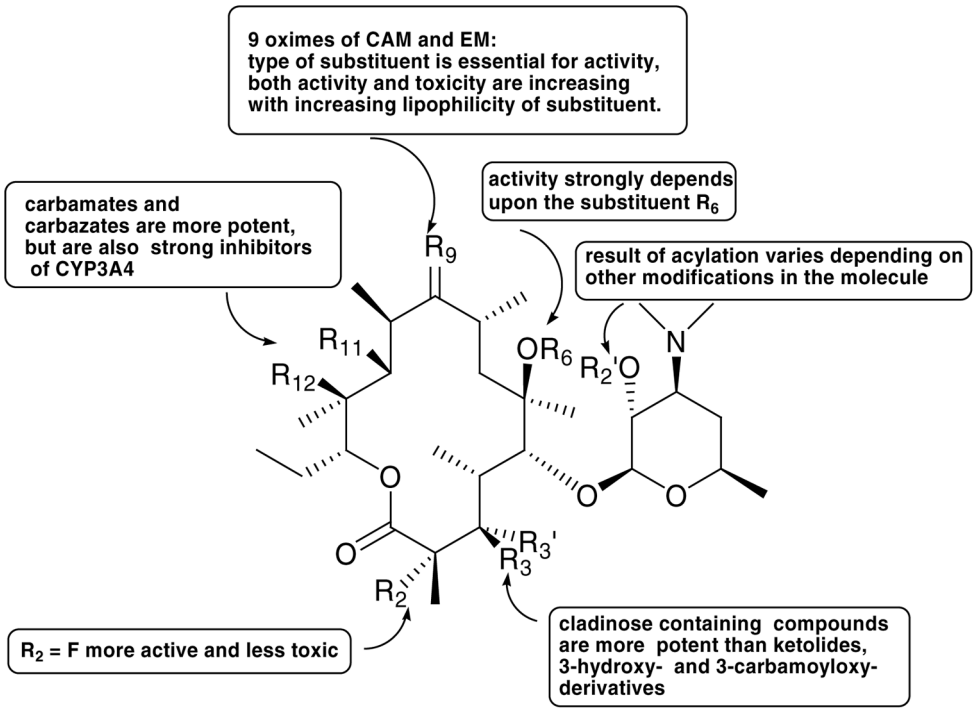


Figure 3 Effect of modifications on the anti-tuberculosis activity of macrolides.

lower stability with regard to positioning at the A2058 locus. The second report confirmed that ribosome methylation in *M. tuberculosis* is indeed an inducible process[44] even by ketolides (which do not induce methylation in some other bacteria with type I resistance).

It is possible that the unique ribosome methylation pattern in *M. tuberculosis* may make it very difficult to considerably enhance potency through new macrolide design alone. Of major interest is the recent observation that, besides exposure to MLS_B antibiotics, erm (37) may also be induced following exposure to other antibiotics and fatty acids as well as residence of *M. tuberculosis* inside of a macrophage, all mediated through the transcriptional regulator whiB7 [45]. Therefore, in addition to the continued pursuit of an optimal macrolide for *M. tuberculosis*, it may be valuable to gain a better understanding of the full range of factors that may influence the expression and activity of erm (37). Reducing ribosome methylation would presumably make *M. tuberculosis* highly sensitive to an optimized macrolide.

In summary, appropriate substitution on the C-9, C-11,12 or C6 positions in the macrolactone ring results in better in vitro activity against *M. tuberculosis*. However, there appears to be little similarity in the direction and magnitude of effect of specific modifications on these positions (Fig. 3). Moreover, it appears that C-3 cladinose is important for anti-TB potency of macrolides. Despite improvements in in vitro activity, a macrolide with optimal in vivo anti-TB activity has yet to be developed. Recent elucidation of the unique ribosome methylation pattern and regulation in *M. tuberculosis* suggests that inhibition of this process may be required for the full potential of an optimized macrolide to be realized in treating tuberculosis.

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