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Synthesis and antibacterial evaluation of a novel series of rifabutin-like spirorifamycins

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Abstract—A novel series of spirorifamycins was synthesized and their antibacterial activity evaluated both in vitro and in vivo. This new series of rifamycins shows excellent activity against *Staphylococcus aureus* that is equivalent to rifabutin. However, some compounds of the series exhibit lower MICs than rifabutin against rifampin-resistant strains of *S. aureus*. Further, compound **2e** exhibits comparable efficacy in vivo in a murine model of *S. aureus* septicemia model following administration by either oral or parenteral dosing routes.

Rifamycins are a group of ansamycins characterized by an aliphatic bridge spanning a naphthalene nucleus and the structures of several rifamycins have been elucidated spectroscopically, chemically, and by X-ray crystallography.[1] The antibacterial activity of the rifamycins is due to inhibition of the initiation of transcription by bacterial DNA-dependent RNA polymerase, thus effectively terminating further RNA synthesis and hence protein synthesis. Multiple different single-step mutations in the *rpoB* gene, encoding the β-subunit of RNA polymerase, can confer high levels of resistance to rifamycins and their clinical use is therefore restricted to combination therapy with the addition of antibiotics from other classes serving to minimize the ease of development of clinical resistance.[2,3] Antibiotics of the rifamycin class, such as rifampin, rifapentine, or rifabutin (**1**), have been employed on a global basis in a number of well-established combination regimens for the treatment of *Mycobacterium tuberculosis* (TB) infections and are also similarly used for the treatment of a number of other life-threatening or persistent infections.[2,3] However, even during standard combination therapy, resistance to the rifamycin component of standard regimens still occurs.[4]

Rifabutin is clinically used as a standard component of a combination regimen for tuberculosis treatment in HIV-infected patients where rifampin therapy is contraindicated, and relatively rapid resistance development to rifabutin in these patients has been reported.[2,5] In a medicinal chemistry program aimed at the development of novel rifamycin antibiotics that exhibit significantly reduced resistance development characteristics, we sought to prepare novel rifamycin derivatives that either bind RNA polymerase tighter and/or exploit new binding interactions with the enzyme compared to rifabutin. A similar medicinal chemistry approach was elegantly used to optimize macrolides to defeat both efflux and ribosome- (target) binding associated resistance mechanisms in the discovery of telithromycin.[6] To this end, we sought to prepare spirorifamycins (**2**, **3**) where the fused-imidazoline ring of rifabutin (**1**) is replaced by a fused-piperazine ring at the 3,4-positions of rifamycin as shown in Figure 1. The distinct conformation of the

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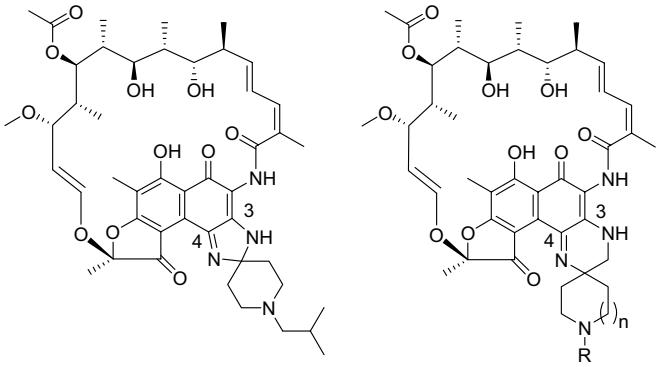


Rifabutin (**1**)

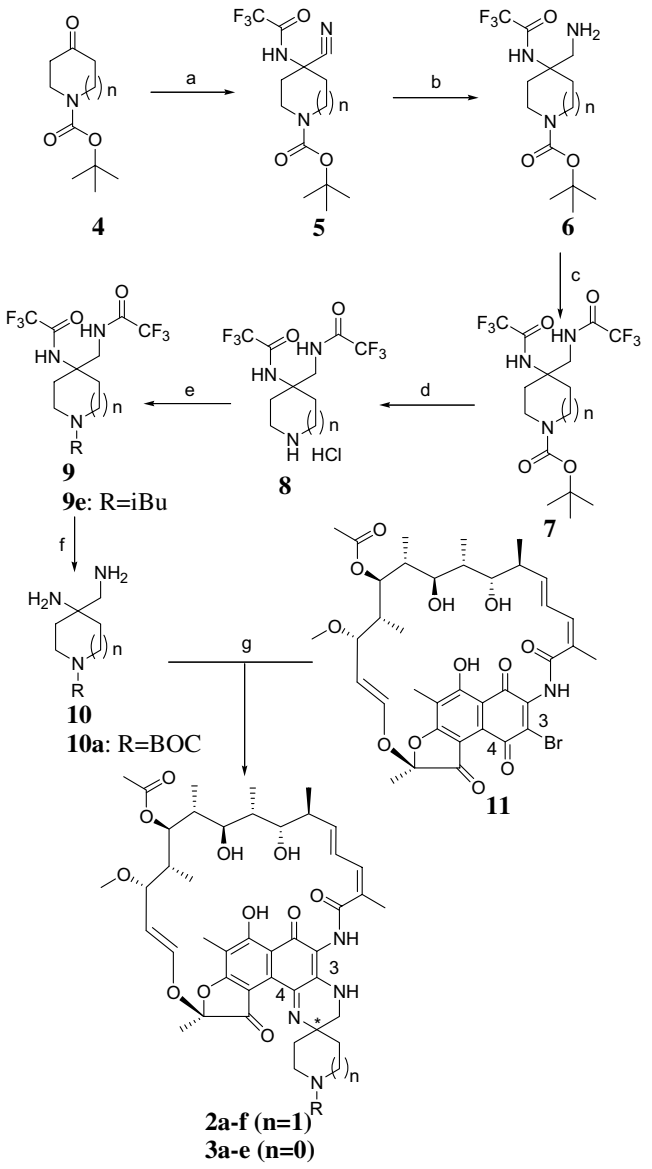
Spirorifamycins
(**2**) n = 1
(**3**) n = 0

Figure 1. The structure of rifabutin (**1**) and novel spirorifamycins (**2**, **3**).

fused-piperazine ring in combination with different spirocylic ring structures is predicted to project groups at the 3,4-positions of the resultant spirorifamycin derivatives into spatial orientations that are distinct from that of rifabutin.[7] Such differences may confer unique RNA polymerase interactions and potentially improved resistance development properties. This communication discloses preliminary results of our medicinal chemistry effort in optimization of this novel series of rifamycins.

The synthesis of spirorifamycins is shown in Scheme 1. Spirorifamycins containing a piperidine ring structure (**2a**–**f**) were prepared from 1-Boc-4-piperidone (**4**, $n = 1$) and those (**3a**–**e**) containing a pyrrolidine ring were from 1-Boc-3-pyrrolidinone (**4**, $n = 0$). Both sets of compounds were prepared via the same synthetic route with similar yields for each step. Direct hydrogenation of the Strecker aminonitrile product with Raney-Ni, in an attempt to prepare ethylene diamines, was not successful. Instead the Strecker product aminonitrile from **4**, which was not isolated but directly treated with trifluoroacetic anhydride in pyridine to generate compound **5** in 56% overall yield.[8] The Raney-Ni promoted hydrogenation of **5** went smoothly to give primary amine **6**. Compound **6** slowly decomposed upon isolation; presumably the trifluoroacetyl group underwent slow acetyl migration from a more hindered amine to the primary amine (see compound structure **6**). So compound **6** was converted to bis-trifluoroacetamide **7** immediately upon formation by treatment with trifluoroacetic anhydride in pyridine. The choice of two trifluoroacetyls as protecting groups facilitates deprotection later in the synthesis. Compound **7** is stable to purification and was isolated in 88% yield. Removal of the BOC group was accomplished using HCl in ethyl acetate to give piperidine **8** as its HCl salt in 84% yield.[9] N-substitution of piperidine **8** was completed by either reductive amination or amine substitution with alkyl halides.[10] Compound **9e** was prepared in 85% yield by reaction of **8** with isobutylbromide in the presence of K_2CO_3 in DMF. Compound **9** was prepared by reductive amination of piperidine **8** with appropriate aldehydes using sodium triacetoxyborohydride. The two trifluoroacetyl groups of **9** were removed in a single transformation to give diamine **10** in excellent yield (77–85%), the product is ready for coupling with 3-bromorifamycin S (**11**).[11] In our initial experiments, reaction of diamine **10** with 3-bromorifamycin resulted in a complex mixture with none of the desired spirorifamycin product detected by LCMS. Reaction of 3-bromorifamycin S with phenylenediamine or 2,3-diaminopyridine to form a fused-pyrazine structure is well documented in the literature.[11] However, reaction of an ethylene diamine such as compound **10** with 3-bromorifamycin S or other rifamycin derivatives has not been reported to our knowledge. The failure of the coupling reaction between ethylene diamine **10** and 3-bromorifamycin S was attributed to the basic nature of ethylene diamine in general. The copper (II) salts were used to mediate fused-piperazine ring formation between 1,4-dihydroxyanthraquinone and ethylene diamine.[12] Indeed, it was found that reaction of 3-bromorifamycin with ethylene diamine **10** in the presence of copper (II) bromide in THF afforded desired spirorifamycin, albeit in low yield (<5%). Further investigation of the reaction revealed that addition of $K_3Fe(CN)_6$ to the reaction mixture in aqueous dioxane without copper (II) bromide gave us the best results and sufficient quantities for antibacterial evaluation of spirorifamycins were consistently achieved from each reaction run using a variety of substituted ethylene diamines. In some cases, the isolated yield was as high as 31%. $K_3Fe(CN)_6$ is an oxidant, but other roles, like chelating with ethylene diamine, in the reaction cannot be ruled out. Further optimization of this reaction is ongoing. Unlike homochiral spirorifamycins **2a**–**f**, the pyrrolidinone-derived spirorifamycins **3a**–**e** should exist in two possible diastereomeric forms due to the presence of a prochiral spirocarbon (denoted by '*').[13] The two presumed diastereomers were not separable by normal purification methods (column chromatography or preparative HPLC) and the materials tested in antimicrobial assays were therefore a mixture of the two diastereomers.[13]

The antibacterial activity of the spirorifamycins **2a**–**f** and **3a**–**e** against three strains of *Staphylococcus aureus* is shown in Table 1. Rifamycins are very potent antistaphylococcal agents with the minimum inhibitory



Scheme 1. Reagents and conditions: (a) 1—NaCN, NH_3, NH_4Cl, MeOH, reflux 3 h; 2—TFAA, pyridine, 0 °C, 18 h (56% for two steps); (b) Raney-Ni, H_2, ethanol, room temperature, 18 h (>95%); (c) TFAA, pyridine, 0 °C, 18 h (88%); (d) HCl, EtOAc, room temperature, 18 h (84%); (e) aldehyde, $Na(OAc)_3BH$, THF, 0 °C, 4 h for **9** or isobutylbromide, K_2CO_3 (85%) for **9a**; (f) NH_3, MeOH, reflux 18 h (77–85%), **10a** was prepared directly from **7** by the same conditions; (g) $K_3Fe(CN)_6$, dioxane/water (4:1), room temperature, 18 h (1.6–31%). '*n*' in structures denotes 0 or 1.

concentration (MIC) for rifabutin against the wild-type strain (CB190) corresponding to 31 ng/mL. The MICs of the newly synthesized spirorifamycins are within 2- to 4-fold dilutions of that of rifabutin and therein retain excellent antibacterial activity despite the structural changes introduced. Interestingly, the size of the substituents (*R*) within each set, or between the two sets, of compounds ($n = 0, 1$) does not impact antibacterial activity significantly, for instance, compare MICs for **2b** (methyl) with **2e** (isobutyl). The same can be said for the polarity and basicity nature of the spirocycles; for instance, compare MICs for the *N*-BOC compound **2a** with the basic *N*-*i*-Bu derivative **2e**.

To evaluate the activity of the novel spirorifamycins in terms of their potential to alter resistance properties, we employed two mutant strains that exhibit intermediate and high-level resistance to rifamycins. CB372 bears a Asp471Tyr substitution mutation in *rpoB* that confers intermediate levels of rifamycin resistance. Against CB372, rifabutin has an MIC of 15.6 μg/mL, whereas the newly synthesized spirorifamycins exhibit MICs in the range 0.98 to >62.5 μg/mL. The MICs are compound specific and no clear structure-activity relationship trends are apparent. The *N*-quinolin-3-ylmethyl analog **2f** and the two *N*-methyl derivatives **2b** and **3b** have the lowest MICs. CB370 bears a His481Tyr substitution mutation in *rpoB* that confers high levels of rifamycin resistance and against which rifabutin has an MIC of >62.5 μg/mL. With the exception of compound **2f** (MIC of 15.6 μg/mL), all of the newly synthesized spirorifamycins did not show any measurable activity (MICs >62.5 μg/mL). Although the origin of this activity is still the subject of ongoing investigations, one cannot rule out the possibility that the *N*-quinolin-3-ylmethyl group binds to RNA polymerase in a fashion that is distinct from rifabutin and the other synthesized analogs.

Selected spirorifamycins were also assayed in vivo in an acute lethal mouse model of septicemia mediated by wild-type *S. aureus*;[14] see Table 2 for a summary of the results. Rifabutin is a potent anti-staphylococcal agent in this model with protection ED_{50}s determined to be 0.6 mg/kg when given orally (po) and <0.1 mg/kg given subcutaneously (sc). Compounds **2b**, **2e**, and **2f** exhibited good protection ED_{50}s when given subcutaneously, but only **2e** exhibited good oral efficacy that is comparable to rifabutin.

In conclusion, a novel series of spirorifamycins was synthesized by coupling substituted ethylene diamines **10** with 3-bromorifamycin S in the presence of $K_3Fe(CN)_6$. The reaction seems to be general for preparation of this series of spirorifamycins. The spirorifamycins are potent anti-staphylococcal agents with in vitro activity similar to rifabutin, and some exhibit lower MICs against rifamycin-resistant mutant strains. The mechanistic basis of this latter activity has yet to be elucidated. However, the best compound in this series (**2f**) exhibited only marginal in vitro activity against a high-level rifamycin-resistant strain of *S. aureus* (MIC of 15.6 μg/mL). This provides little comfort for the development of a rifamycin agent by this approach that will circumvent resistance development. Indeed, unlike the case of the macrolides, suppression of the development of resistance may not be adequately addressed by exploration of novel-binding interactions with the RNA polymerase enzyme in the 3,4-positions of the rifamycin structure. Hence other medicinal chemistry strategies may be necessary to develop novel rifamycin agents that circumvent the rapid resistance development liability. However, clinical agents of the rifamycin class of antibiotics exhibit unique efficacy in the treatment of persistent infections mediated by *M. tuberculosis* and in hard-to-treat settings like biofilm-associated infections of indwelling medical devices mediated by *S. aureus*.[2] Therefore, development

Table 1. Antibacterial activities of spirorifamycins **2a–f** and **3a–e** as compared to rifabutin (MIC: μg/mL)

Compound	R	*S. aureus* CB190[a]	*S. aureus* CB372[b]	*S. aureus* CB370[c]
Rifabutin		0.031	15.6	>62.5
2a	Boc	0.026	7.81	>62.5
2b	Me	0.016	1.95	>62.5
2c	Bn	0.003	1.95	32
2d	Allyl	0.006	15.6	>62.5
2e	*i*-Bu	0.063	7.81	>62.5
2f	Quinolin-3-yl methyl	0.063	0.98	15.6
3a	Boc	0.016	7.81	>62.5
3b	Me	0.013	0.98	>62.5
3c	Bn	0.031	3.91	>62.5
3d	i-Bu	0.063	>62.5	31.3
3e	Quinolin-3-yl methyl	0.063	7.81	>62.5

[a] ATCC # 29213 a wild-type, rifamycin-sensitive strain of *S. aureus*.
[b] Laboratory-derived variant of ATCC # 29213 (CB190) bearing an *rpoB* Asp471Tyr mutation that confers intermediate rifamycin resistance.
[c] Laboratory-derived variant of ATCC # 29213 (CB190) bearing an *rpoB* His481Tyr mutation that confers high-level rifamycin resistance.

Table 2. In vivo activity of selected spirorifamycins tested against a wild-type *S. aureus* strain ATCC # 6538 in an acute lethal mouse model of septicemia (ED_{50}: mg/kg)

Compound	ED_{50} (po)	ED_{50} (sc)
Rifabutin	0.6	<0.1
2b	>10	0.3
2e	<0.1	0.6
2f	5.0	0.6

of a rifamycin agent without the resistance development liability and that could be employed in monotherapy would be expected to be highly prized as a new addition to the antimicrobial armamentarium.

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13. *Analytical characterization of* **3d**. ESI-MS *m/z* 847 ($M+H^+$); HPLC: single peak at retention time 3.37 min (Xterra® MS C18 3.5 μm; 2.1 × 30 mm column, 8 min gradient elution, 0 to 100% solvent B in solvent A; solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in methanol); ^1H NMR (400 MHz, $CDCl_3$) δ 13.73 (s, 1H), 8.14 (s, 1H), 7.31 (br s, 1H), 6.44 (dd, *J* = 10.8 and 16.0 Hz, 1H), 6.25 (d, *J* = 10.4 Hz, 1H), 6.10 (dd, *J* = 16.0 and 6.4 Hz, 1H), 6.01 (dd, *J* = 12.8 and 0.8 Hz, 1H), 5.09 (dd, *J* = 6.0 and 12.4 Hz, 1H), 4.97 (d, *J* = 9.6 Hz, 1H), 3.83 (d, *J* = 5.6 Hz, 2H), 3.76 (d, *J* = 10.0 Hz, 2H), 3.56 (br s, 2H), 3.39 (br d, *J* = 6.0 Hz, 2H), 3.20 (br d, *J* = 12.0 Hz, 2H), 3.05 (s, 3H), 3.05–3.00 (m, 1H), 2.82 (m, 2H), 2.40–2.34 (m, 1H), 2.25 (s, 3H), 2.07 (s, 3H), 2.06–1.62 (m, 6H), 2.03 (d, *J* = 3.6 Hz, 6H), 2.02 (s, 3H), 1.71 (s, 3H), 1.03 (d, *J* = 6.8 Hz, 3H), 1.03–1.02 (m, 1H), 0.85 (d, *J* = 7.2 Hz, 3H), 0.65 (d, *J* = 7.2 Hz, 3H), 0.01 (d, *J* = 7.2 Hz, 3H).
14. *Staphylococcus aureus* in 5% porcine gastric mucin in PBS was given at a dose of 50 times the LD_{50} intraperitoneally (ip) to mice. Survival was monitored for the next 4 days for drug-treated and control groups. ED_{50}s were calculated based on three doses with five mice in each dose group.