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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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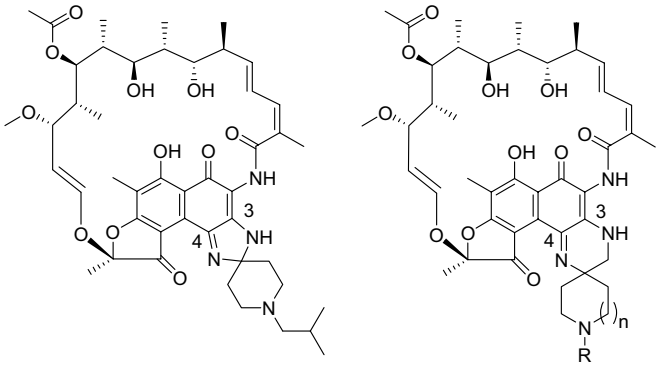

Rifabutin (**1**)

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