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New rifabutin analogs: Synthesis and biological activity against *Mycobacterium tuberculosis*

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Abstract—The synthesis, structure, and biological evaluation of a series of novel rifamycin derivatives, Rifastures **(RFA)** with potent anti-tuberculosis activity are presented. Some of these derivatives showed higher in vitro activity than rifabutin and rifampicin against not only *Mycobacterium tuberculosis* strains but also against MAC and *Mycobacterium kansasii*.

The rifamycins are a family of naphthalenic ansamycin antibiotics of remarkable interest because of their structure, biogenesis, mechanism of action, and therapeutic efficacy.[1] They were isolated for the first time in 1959 from *Amycolatopsis mediterranei* as a mixture of at least five active substances, designated rifamycin A–E. Only rifamycin B was isolated as a pure crystalline substance, and it has the unusual property that in oxygenated aqueous solutions, it tends to change spontaneously into rifamycin S (Fig. 1), a compound with higher antibacterial activity.

Ansamycins are macrolide antibiotics characterized as having a 17-membered aliphatic bridge connecting two nonadjacent positions on a chromophoric naphthahydroquinone nucleus via an amide linkage,[2] and are generally active against Gram-positive bacteria and mycobacteria, especially *Mycobacterium tuberculosis*. Some rifamycins have interesting levels of activity against viral RNA-dependent DNA polymerase[3] but they are mostly known as potent inhibitors of all bacterial DNA-dependent RNA polymerases (DDRP).[4] It is believed that the mechanism of action of rifamycins involves primarily formation of a stable complex with bacterial RNA polymerase, binding with the β subunit of the enzyme, and effectively inhibiting RNA synthesis.[5] Most rifamycins are not effective on the mammalian RNA polymerase; therefore, they possess the necessary requisite of low toxicity.

Studies of structure–antibacterial activity relationships have shown the minimal requirements for activity to be the presence of oxygenated functions at the C-1, C-8, C-21, and C-23 positions, that are thought to be directly involved in the attachment to the enzyme.[6] Therefore, modifications at C-3 and/or C-4 positions, which are on the opposite side of the aromatic ring, have been mostly exploited for the preparation of new active derivatives. It is believed that these structural changes do not affect the antibiotic mode of action, but they are able to enhance activity by improving membrane permeability and pharmacokinetic properties.[7] Among all new products, Rifampicin (Fig. 1) (U.S. generic name is rifampin)[8] has been introduced into therapy for the last 30 years as a first-line agent against tuberculosis.[9]

Spiropiperidyl-substituted rifamycin derivatives[10] are a class of semisynthetic rifamycin antibiotics[11] in which the C-3 and C-4 positions have been incorporated into an imidazolyl ring bearing a spiropiperidyl group. The nitrogen of the piperidyl ring can be substituted with linear and branched aliphatic chains.[12] Among them,

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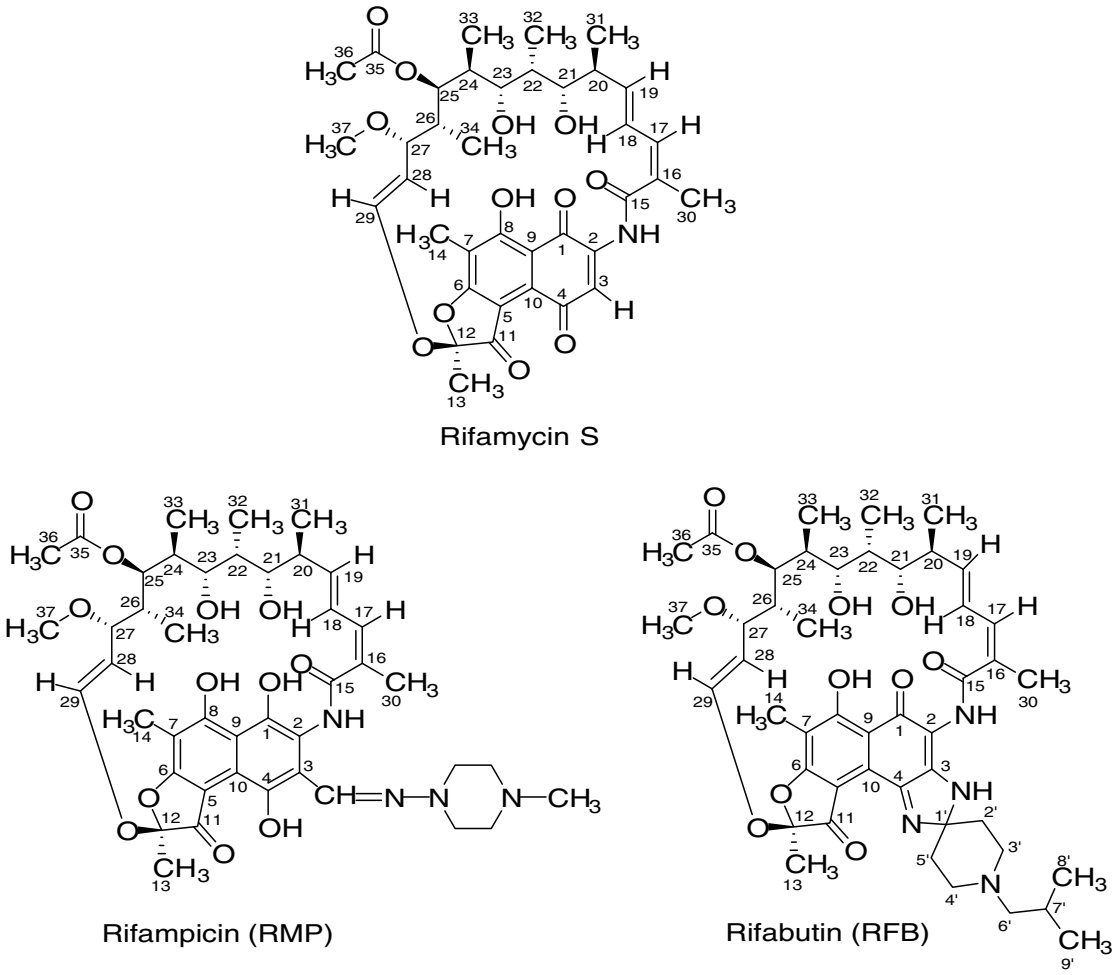

Figure 1. Chemical structure of some rifamycins.

rifabutin[13] (Fig. 1) is an alternative agent[14] for the treatment of several mycobacterial infections, including both prophylaxis or therapy against disseminated MAC (*Mycobacterium avium-intracellulare* complex) infections in AIDS patients, and multidrug-resistant tuberculosis (MDR-TB) strains.[15] Unfortunately, rifabutin presents a high level of toxicity in vivo.

Recently, organizations such as the World Health Organization (WHO)[16] and the International Union against Tuberculosis and Lung Disease[17] have promulgated efforts to develop new drugs that will shorten the treatment duration and improve the therapeutic response of MDR-TB. In this paper, we report the synthesis, structure, and bioactivity of seven new rifabutin analogs, called Rifastures (**RFA-3**),[18] which show a high level of activity against *M. tuberculosis*.

For the preparation of the new spiropiperidylrifamycin derivatives we used a modification of the method previously published for rifabutin and its analogs[11b] involving the condensation reaction between the common intermediate 3-amino-4-iminorifamycin S[19] and several *N*-substituted-4-piperidones (Scheme 1). As far as we are aware, there have not been any examples using 4-piperidones substituted in other positions on the piperidyl moiety. For this reason the first objective of this study was to synthesize a variety of di- and trisubstituted 4-piperidones (**2a**–**f**) to evaluate the importance of the substituents in the biological activity of the new derivatives.

For several years, we have been engaged in the study of the stereoselective synthesis of 4-piperidones by the imino-Diels–Alder reaction of 2-amino-1,3-butadienes with imines.[20] We decided to utilize this methodology toward the preparation of new rifabutin analogs for further biological evaluation. For our studies we chose a variety of *meso*-2,6-disubstituted-4-piperidones (**2a**–**f**)[21] (Scheme 2). The selection of this particular system was motivated by various reasons: symmetrical 4-piperidones would afford two diastereoisomers as reaction products, due to the formation of a new stereocenter at the spiranic carbon atom C-1′, and also would facilitate the structural characterization of these new analogs. Moreover, the new analogs prepared in this way will constitute the first examples in which *meso*-2,6-disubstituted-4-piperidones are used for the condensation reaction with 3-amino-4-iminorifamycin S (**1**).

The condensation reaction of **1** was carried out with the *meso*-2,6-disubstituted 4-piperidones (**2a**–**f**), as shown in