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Original article

# Pharmacophore insights into rpoB gene mutations in *Mycobacterium tuberculosis* rifampicin resistant isolates

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ABSTRACT

This paper reports the susceptibility profile to rifabutin (RFB) **1** and six recently synthesized RFB analogs **3**–**8**, of either rifampicin (RFP) susceptible *Mycobacterium tuberculosis* and resistant clinical isolates from two sources: Mexico and Brazil. Taking into account that about 95% of *M. tuberculosis* strains resistant to RFP present mutations in the *rpoB* gene, with some of these mutations being determinant also to RFB resistance, the RFB analogs were screened for activity against a set of known RFP susceptible and resistant strains. *N*′-Acetyl-RFB **5** and *N*′-(undec-10″-enoyl)-RFB **8** showed the best results, in particular with mutations in the codon 516, 522 and 531 of the *rpo*B gene, and were therefore selected for *in vivo* assessment of their efficacy. Studies conducted with tuberculous Balb/C mice previously infected with Ser531Leu mutated clinical isolate, evidenced both **5** and **8** as promoters of a significant decrease on tubercle bacilli burden in lungs associated with lower tissue damage, thus confirming them as good leads for drug discovery. The SAR of the acylated compounds **5** and **8** envisaging the identification of pharmacophore features, highlights the importance of profiling more clearly the chemistry within the molecular aspects for elucidation of the mode of action of RFB and analogs, in relation to mutations in Multidrug-Resistant (MDR) strains.



## 1. Introduction

Effective weapons against a wave of new drug-resistant strains are urgent. If we wonder where we do stand on tuberculosis (TB), most researchers would state that we are basically back to where we were before drugs. Novel approaches linking drug candidates to gene studies are needed to overcome the threat posed by the emergence of drug-resistant *Mycobacterium tuberculosis*. More effective agents against multidrug-resistant tuberculosis (MDR-TB) able to shorten the duration of treatment are needed, particularly those targeting the eradication of the latent form of TB.

For the ancient disease TB as with other "neglected diseases" for which the Pharmaceutical Industry has low expectance in terms of revenues, since these are mostly widespread in underdeveloped countries, an exciting drug development pathway is open by targeting genetic make-up in susceptibility. Forty years have passed with no introduction into the market of new anti-TB drug classes, in spite of a huge diversity of attempts, providing identification of new targets, and the discovery of novel agents with novel mode of action (MoA) [1].

In current Directly Observed Therapy (DOT) programs, Rifabutin (RFB) plays a fundamental role in the treatment of patients with active TB. Although rifampicin (RFP) and isoniazid (INH) are by far the most effective anti-TB agents, RFP and RFB are crucial for ensuring success of short-course (6-month) chemotherapy [2]. Namely, patients with INH-resistant TB can respond well to 6-month treatment without INH (on a regimen of RFP, pyrazinamide, and ethambutol), but patients with RFP-resistant TB do

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not respond well to short-course chemotherapy without RFP or RFB [3]. Without RFP or RFB, most anti-TB regimens require at least 18 months of treatment [4], and every attempt should be made to use RFP or RFB in initial anti-TB treatment. In addition, anti-TB drug regimens for patients infected with the human immunodeficiency virus (HIV) pose a special challenge when certain antiretroviral medications are also indicated. RFP should not be taken together with most protease inhibitors or non-nucleoside reverse transcriptase inhibitors. RFB, however, often has to be used in these patients [5]. Nevertheless, a 2010 Cochrane Review [6] concluded that the replacement of RFP by RFB for first-line treatment of TB is not supported by the current evidence, and also that HIV positive people with TB, the group most likely to benefit from the RFB use, are still under-represented in trials to date, and further trials in this group would be useful.

In general, the major problem in the clinical use of RFB and other rifamycins (Rifs), is that their versatility and efficiency are limited by the rapid emergence of resistant strains, mostly as a consequence of mutations that occur on the active side of its molecular target: the ribonucleic acid polymerase enzyme (RNAP). To overcome this problem, it is necessary to understand the detailed MoA and the bacterial resistance to these drugs at the molecular level. A key breakthrough was the recent determination of the crystal structures of several inhibitors with the RNAP [7].

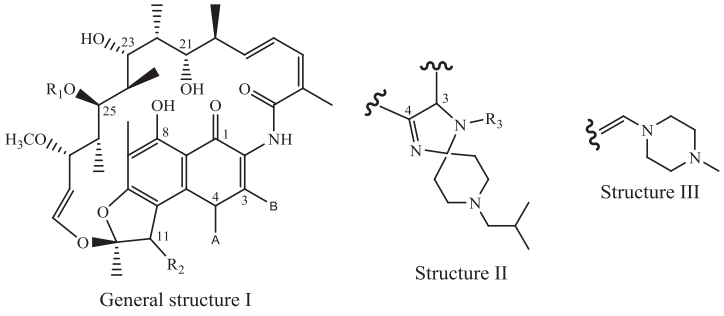
Rifs specifically inhibit bacterial transcription, by binding to RNAP. Also known as DNA-dependent RNA polymerase (DDRP), it is an intricate enzyme with a $\alpha_2\beta\beta'\omega$ subunit structure [8]. The exact mechanism by which Rifs interfere with the process had long remained elusive until 2001, when Campbell et al. [9], showed that Rifs bind to the β-subunit encoded by the *rpo*B, 12 Å away from the $Mg^{2+}$ ion at the RNAP active site, in the DNA channel, physically blocking the RNA elongation when the transcript is 2–3 nucleotide long [9]. These findings supported the steric-occlusion model for Rifs action, which basically indicates that the stronger the interactions between the Rifs and a restricted number of specific amino acid (aa) residues, encoded by the *rpo*B of the RNAP, the better it would halt transcription.

Specific parameters and constrains on the chemical transformation of anti-TB leads/current drugs have evolved from a few SAR studies [10,11], and are evolving still scarcely through *in silico* studies [12]. Drug development with an existing drug such as RFB as the lead compound, with efficacy improvement through structure-based successful manipulation, can still be considered an attractive strategy from the economic, pharmaceutical and clinical points of view [13,14]. Successful results have been reported with ongoing RFB analogs [14,15].

RFB **1** is a semi-synthetic spiropiperidyl derivative of the ansamycin family of antibiotics [16–18]. For Rifs it is well known that modifications of the ansa bridge conformation, or at O1 and O8 of the central chromophore, and O21 and O23 hydroxyl groups, reduce substantially the *in vitro* inhibitory activity. But where do we stand exactly with what we have learned about the MoA, and where do we go using improved tools from what we achieved with RFP and RFB? For decades the pharmacophore concept has always been the driver for SAR studies on ansamycins [17–19] and is defined by IUPAC as "the ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response". As pointed out by Leach et al. [20], central to the pharmacophore concept is the notion that the molecular recognition can be ascribed to a set of common features such as hydrogen-bond donors, hydrogen-bond acceptors, positively and negatively charged groups, and hydrophobic regions. As such, there is a link with contemporary pharmacophore models through the principles of bioisosterism and 3D spatial molecular and conformational relationships, leading to sophisticated computer algorithms. We focus here the challenges and constraints within the key problem of proposing the ansamycin pharmacophore mapping for a set of active molecules and their biological activities on both RFP susceptible *M. tuberculosis* and resistant clinical isolates with specific mutations.

Positions 3 and 4 of the naphthoquinone cromophore (cf. Fig. 1) are considered the centre of the pharmacophore and have been extensively modified by semi-synthesis to improve the pharmacological properties of Rifs. Differences between 3-tailed Rifs as RFP and rifapentine (RPN), and both 3,4-tailed Rifs (e.g. RFB), such as the inhibition by the former of only both the second and third phosphodiester bond formation, while the later inhibit also the first phosphodiester bond formation, fail to be explained by the proposed simple steric hindrance mechanism [21]. Also difficult to explain has been the existence of *rpo*B mutants which are resistant to RFP but yet susceptible to RFB **1** [22], regardless of the presence of point mutations in residues of the RNAP, that are known not to interact with Rifs tail (either at positions 3 or 4 or even both substitutions), where most of the differences between Rifs are located [9].

Based on these observations which were difficult to explain in the light of the steric-occlusion model, Artsimovitch et al. [21], proposed a different mechanism based on a 2.5 Å structural biology




1: Rifabutin ($R_1$: $COCH_3$; $R_2$: =O; A and B together are part of spiro structure II where $R_3$: H)
2: Rifampicin ($R_1$: $COCH_3$; $R_2$: =O; A: OH; B: structure III)
3: 25-Deacetyl-rifabutin ($R_1$: H; $R_2$: =O; A and B together are part of spiro structure II where $R_3$: H)
4: 25-Deacetyl-rifabutinol ($R_1$: H; $R_2$: OH; A and B together are part of spiro structure II where $R_3$: H)
5: N'-Acetyl-rifabutin ($R_1$: $COCH_3$; $R_2$: =O; A and B together are part of spiro structure II where $R_3$: $COCH_3$)
6: N'-Acetyl-rifabutinol ($R_1$: $COCH_3$; $R_2$: OH; A and B together are part of spiro structure II where $R_3$: $COCH_3$)
7: N'-Palmitoyl-rifabutin ($R_1$: $COCH_3$; $R_2$: =O; A and B together are part of spiro structure II where $R_3$: $COC_{15}H_{31}$)
8: N'-(Undec-10"-enoyl)-rifabutin ($R_1$: $COCH_3$; $R_2$: =O; A and B together are part of spiro structure II where $R_3$: $COC_{10}H_{19}$)

**Fig. 1.** RFB **1** and some of its previously synthesized derivatives **3**–**8** (to access the full list please see Figueiredo et al.[15], doi:10.1016/j.bmc.2008.12.006).