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Preparation and in vitro anti-staphylococcal activity of novel 11-deoxy-11-hydroxyiminorifamycins

Jing Li,[a] Zhenkun Ma,[a,†] Katrina Chapo,[b] Dalai Yan,[b] A. Simon Lynch[b] and Charles Z. Ding[a,*]

[a]*Department of Medicinal Chemistry, Cumbre Pharmaceuticals Inc., 1502 Viceroy Drive, Dallas, TX 75235, USA*
[b]*Department of Biology, Cumbre Pharmaceuticals Inc., 1502 Viceroy Drive, Dallas, TX 75235, USA*

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Abstract—We report herein the preparation and anti-staphylococcal activity of a series of novel 11-deoxy-11-hydroxyiminorifamycins. Many of the compounds synthesized exhibit potent activity against wild-type *Staphylococcus aureus* with MICs equivalent to, or better than, rifamycin reference agents. In addition, some of the compounds retain potent activity against an intermediate rifamycin-resistant strain of *Staphylococcus aureus*. For instance, compound **5k** exhibits an MIC of 0.12 μg/mL against an intermediate rifamycin-resistant strain, while the rifamycin reference agents, rifampin and rifalazil, exhibit MICs of 16 μg/mL and 2 μg/mL, respectively, against the same strain.

Rifamycins are a group of ansamycin natural product derived antibiotics 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 mechanism of action of the rifamycins is mediated via inhibition of bacterial DNA-dependent RNA polymerase and they act to efficiently terminate the synthesis of nascent RNA transcripts with secondary, indirect effects on protein synthesis. Various single-point 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] Their unique mode-of-action, potent activity against gram-positive aerobic and anaerobic bacteria, and their superior pharmacokinetic properties make the rifamycin class of antibiotics clinically indispensable for some hard-to-treat infections.[2] Antibiotics of the rifamycin class, such as, rifampin and rifapentine, 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 due to *Staphylococcus aureus*.[2,3] However, even during standard combination therapy, resistance to the rifamycin component of standard regimens still occurs.[4]

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 the known rifamycins.[5] Most rifamycin derivatives have chemical modifications at the C3 and/or C4 position of the naphthalene ring structure. To our knowledge, there has been only one published report of chemical modifications at the C-11 position of rifamycin core. Specifically, reduction of the C-11 ketone group to an alcohol yielded a derivative that is reported to retain the potent antimicrobial activity of the parent; however,

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* Corresponding author. Tel.: +1 214 631 4700x7670; fax: +1 214 631 4710; e-mail: charles.ding@cumbrepharma.com
† Present address: Zhenkun Ma, Global Alliance for TB drug development, 80 Broad Street, 3rd Floor, New York, NY 10004, USA.

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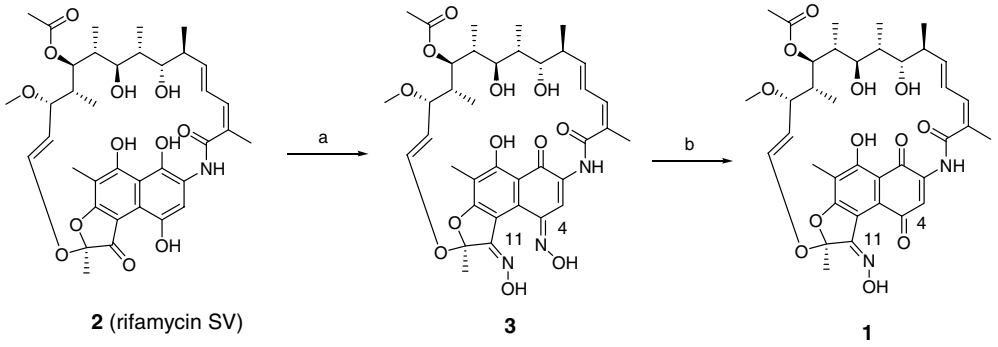
the activity of this compound against strains exhibiting rifamycin-resistance was not reported.[6] In this letter, we report the synthesis of a series of novel rifamycin derivatives bearing substitutions of the C-11 ketone group with an oxime functionality. The anti-staphylococcal activity and resistance development potential of the synthesized compounds are described and compared to those of rifamycin comparator agents, rifampin and rifalazil.

11-Deoxy-11-hydroxyiminorifamycin S (**1**) was prepared as shown in Scheme 1. Rifamycin SV (**2**) reacting with hydroxylamine hydrochloride in the presence of pyridine produced 4,11-dideoxy-4,11-dihydroxyiminorifamycin S (**3**) in 85% yield. Reduction of the 4,11-dioxime **3** with stannous chloride in the presence of ammonium chloride selectively cleaved 4-hydroxyimino group to give 11-deoxy-11-hydroxyiminorifamycin S (**1**) in excellent yield. The regioselective reduction is presumably due to difference in accessibility of the two oximes. C-4 oxime is more accessible than sterically hindered C-11 oxime functionality. The antibacterial activity and resistance profile of 11-deoxy-11-hydroxyiminorifamycin S (**1**) was evaluated by determining minimal inhibitory concentrations (MICs) against wild-type and its isogenic resistant variants of *S. aureus* CB190 (ATCC# 29213): *S. aureus* CB372 bears an Asp471Tyr substitution mutation in the *rpoB* gene encoding the β-subunit of RNA polymerase and confers intermediate levels of rifamycin-resistance, whereas *S. aureus* CB370 bears a His481Tyr substitution mutation in *rpoB* that confers high levels of rifamycin-resistance. The MIC data for 11-deoxy-11-hydroxyiminorifamycin S (**1**) and rifamycin comparators are shown in Table 1. The C-11 oxime compound **1** retains potent antibacterial activity against wild-type *S. aureus* with an MIC of 0.002 μg/mL that is more potent than rifampin or its C-11 ketone analogues, rifamycin SV (**2**) and rifamycin S (**6**). Compound **1** also exhibits more potent activity against the intermediate rifamycin-resistant strain CB372 with an MIC of 0.5 μg/mL that is 32-fold lower than that of rifampin (16 μg/mL). Against the high-level rifamycin-resistant strain CB370, compound **1** exhibits a measurable MIC of 32 μg/mL that is similar to that of rifalazil (**4**), while rifampin and rifamycin SV do not reach an MIC end-point at the maximum concentration tested (64 μg/mL). We attribute this enhanced overall activity of the 11-deoxy-11-hydroxyiminorifamycin S (**1**) compound to the presence of C-11 oxime functionality.

The encouraging results obtained for the 11-deoxy-11-hydroxyiminorifamycin S (**1**) against rifamycin-resistant strains of *S. aureus* prompted us to similarly derivatize other chemotypes of the rifamycin class. Benzoxazinorifamycins are a relatively new class of semi-synthetic rifamycins characterized by the presence of a 3,4-benzoxazine moiety fused onto the naphthalene nucleus. A representative member of this series is rifalazil (**4**, as shown in Figure 1). Rifalazil is currently undergoing clinical trials for peripheral arterial disease due to its potent anti-chlamydial activity.[7] Benzoxazinorifamycins as a class offer advantages over earlier generations of rifamycins in their chemical stability, low potential for CYP-450 induction, and improved activity against certain rifampin-resistant strains of *S. aureus*.[8] Therefore, it seemed reasonable to assume that 11-deoxy-11-hydroxyimino-benzoxino-rifamycins, that combine the C-11 oxime functionality with the fused benzoxine functionality, may exhibit additional benefits at the level of resistance development. Hence, a series of 11-deoxy-11-hydroxyimino-benzoxino-rifamycins (**5a–5l**, Figure 1) was prepared, and their activity and resistance profile was similarly evaluated.

A general synthesis of 11-deoxy-11-hydroxyimino-benzoxazino-rifamycins (**5a–5l**) is illustrated in Scheme 2. The synthesis of the benzoxazinorifamycins followed a method described in the literature.[9] Briefly, rifamycin S (**6**) was prepared from rifamycin SV sodium (**2**) by oxidation with $K_3Fe(CN)_6$ in aqueous ethyl acetate. 2-Aminoresorcinol (**7**, R′ = OH) was prepared by reaction of 2-nitroresorcinol with sodium metasulfide. Other required 2-aminophenols (**7**, R′ = H, Me) were purchased from various chemical vendors. Coupling reaction of rifamycin S (**6**) and 2-aminophenol (**7**) was accomplished in a mixture of THF and toluene to afford benzoxazinorifamyins (**8**) in 60–80% yield. Addition of various mono-substituted piperazines (**9**) to benzoxazinorifamyins (**8**) was carried out in DMSO in the presence of MnO_2. The substituted benzoxazinorifamcyin **10** was isolated in 50–90% yield. Reaction of benzoxazinorifamcyins **10** with hydroxylamine hydrochloride in methanol in the presence of pyridine at room temperature produced 11-deoxy-11-



Scheme 1. Reagents: (a) $NH_2OH{\cdot}HCl$, pyridine, MeOH, 85%; (b) $SnCl_2 \cdot 2H_2O$, NH_4OAc, 6 N HCl, THF/MeOH/H_2O, 98%.

Table 1. Antibacterial activities and resistance profile of 11-deoxy-11-hydroxyiminorifamycins as compared to those of rifamycin standards (MICs; μg/mL)

Compound	R^1	$R^{2,a}$	*S. aureus* CB190[b]	*S. aureus* CB372[c]	*S. aureus* CB370[d]
	Rifampin		0.008	16	>64
	Rifalazil **(4)**		0.004	2	32
	Rifamycin SV **(2)**		0.016	2	>64
	Rifamycin S **(6)**		0.016	4	>64
	1		0.002	0.5	32
5a	OH	*i*-Bu	0.004	2	32
5b	OH	Me	0.004	1	32
5c	OH	Et	0.008	2	32
5d	OH	*i*-Pr	0.008	1	32
5e	OH	*n*-Bu	0.004	2	16
5f	OH	ChMe	0.008	8	8
5g	OH	1-Me-4-Pip-Me	0.13	4	32
5h	CH_3	*i*-Bu	0.008	4	>64
5i	CH_3	Me	0.004	1	16
5j	H	*i*-Bu	0.004	0.5	8
5k	H	*i*-Pr	0.0005	0.12	8
5l	H	ChMe	0.008	2	16

[a] ChMe means cyclohexylmethyl, 1-Me-4-Pip-Me means 1-methyl-4-piperidinylmethyl.
[b] CB190 (ATCC# 29213) a wild-type, rifamycin-sensitive strain of *S. aureus*.
[c] Laboratory derived variant of CB190 bearing an *rpoB* Asp471Tyr mutation that confers intermediate rifamycin resistance.
[d] Laboratory derived variant of CB190 bearing an *rpoB* His481Tyr mutation that confers high level rifamycin resistance.

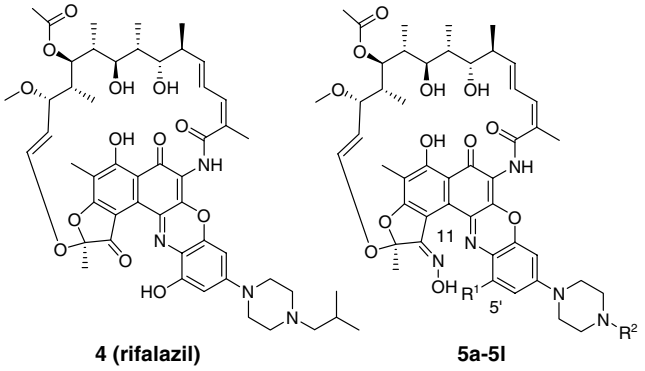
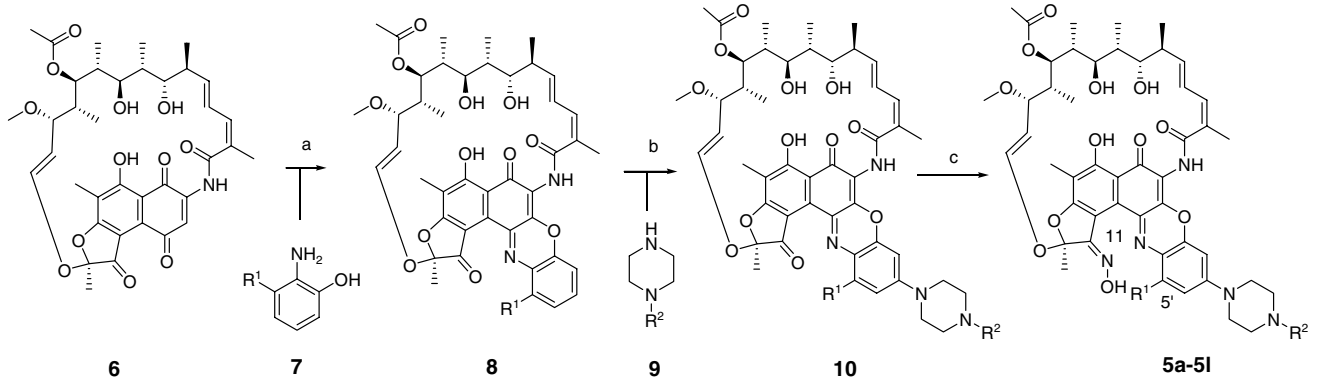


Figure 1. Structures of rifalazil (**4**) and 11-deoxy-11-hydroxyimino-benzoxazino-rifamycins (**5a–5l**).

hydroxyimino-benzoxazino-rifamycins **(5a–5l)** in good yield (75-85%).

The anti-staphylococcal activities of the 11-deoxy-11-hydroxyimino-benzoxazino-rifamycins **5a–5l** are also summarized in Table 1. In general, all the newly synthesized 11-deoxy-11-hydroxyimino-benzoxazino-rifamycins retain potent activity and compare favorably to the rifalazil reference compound. The compound **5g** is not as potent as rifalazil presumably due to the presence of a basic piperidine amine group. Otherwise, various lipophilic groups attached to the distal end of the piperazine ring nitrogen are tolerated and exhibit uniformly potent activity (compare the group of compounds from **5a** to **5f**). It was somewhat disappointing that **5a**, a direct C-11 oxime analogue of rifalazil, did not display added benefit as observed with compound **3**. Specifically, the MICs for **5a** against wild-type and resistant strains overlap with those of rifalazil indicating a similar sensitivity to mutations conferring rifamycin resistance. Interestingly, replacement of the C-5′ hydroxyl group by a methyl group has no effect on activity against wild-type *S. aureus* and has a variable impact on rifamycin-resistant strains (compare compound **5a** to **5h**; **5b** to **5i**). However, removal of C-5′ hydroxyl group produced compounds (**5j** to **5l**) that exhibit antibacterial activity



Scheme 2. Reagents and conditions: (a) **7**, toluene/THF, rt, 60–80%; (b) **9**, MnO_2, DMSO, rt, 50–90%; (c) $NH_2OH{\cdot}HCl$, pyridine, MeOH, rt, 75–85%.

and resistance profiles that are markedly improved. Compound **5j** is a direct analogue of rifalazil and exhibits an MIC versus wild-type *S. aureus* that is identical to that of rifalazil. However, MICs against the intermediate- and high-level rifamycin-resistant strains are 4-fold improved over rifalazil, possibly indicating a better resistance development profile. The compound **5k** with an isopropyl group attached to the distal nitrogen of piperazine is even more potent than the compound **5j**, with MICs 8-fold and 4-fold lower against the wild-type and CB372 strains, respectively. However, despite its improved activity against wild-type and intermediate rifamycin-resistant strains, the activity of compound **5k** against high-level resistant strain CB370 is similar to that of **5j**, indicating an apparent limitation of this series of 11-deoxy-11-hydroxyimino-benzoxazino-rifamycins.

In summary, we have prepared a novel series of 11-deoxy-11-hydroxyimino-rifamycins. 11-Deoxy-11-hydroxyimino-rifamycin S (**3**) exhibited enhanced antibacterial activity and an improved resistance development profile when compared to rifampin. This enhancement of activity may be due to an additional binding interaction(s) of the C-11 oxime functionality with the RNA polymerase enzyme. In an attempt to further improve the resistance profile of C11-oxime derivatives of rifamycin class compounds, we prepared 11-deoxy-11-hydroxyimino-benzoxino-rifamycins. Some of the latter compounds have both improved activity and resistance development profiles when compared to rifalazil. The MIC of the best compound of the series (compound **5k**) against an intermediate rifamycin-resistant strain of *S. aureus* (CB372) is more than 10-fold lower than that of rifalazil. However, the MIC of the same compound against a high-level rifamycin-resistant strain of *S. aureus* (CB370) is still as high as 8 μg/mL. This level of activity provides little comfort for the future development of a rifamycin agent that will circumvent resistance development. Indeed, suppression of the development of resistance may not be adequately addressed by exploration of novel binding interactions with the RNA polymerase enzyme as mediated through small variations or substitutions of the core rifamycin scaffold due of the existence and prevalence of single-step mutations in the *rpoB* gene that confer high level resistance. Hence other medicinal chemistry strategies, like incorporation of other antibacterial pharmacophores, 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 *Mycobacterium tuberculosis* and in hard-to-treat settings like biofilm-associated infections of indwelling medical devices mediated by *S. aureus*.[2] Therefore, development of a rifamycin agent without the resistance development liability and that could be employed in monotherapy would be expected to be highly valued as a new addition to the antimicrobial armamentarium.

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