Natural Antibiotics

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Lost in Transcription—Inhibition of RNA Polymerase**

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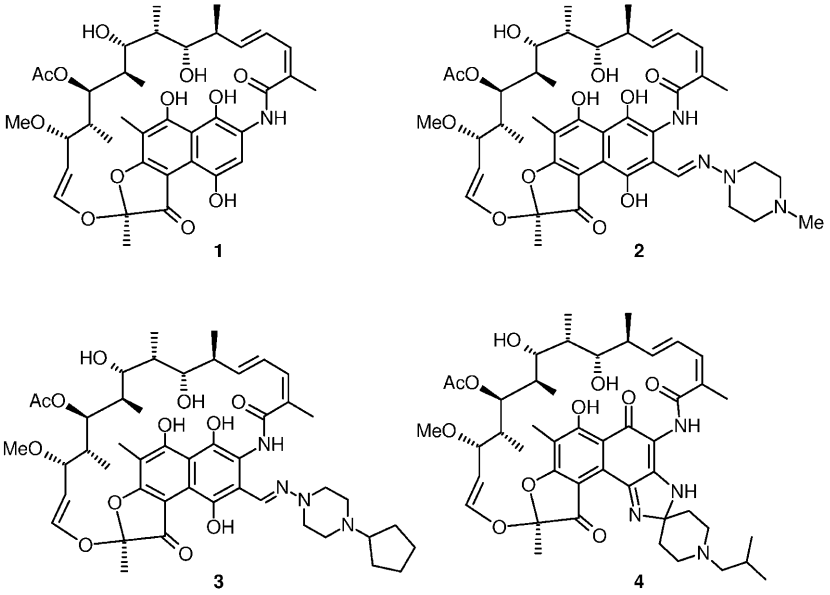
antibiotics · drug discovery · drug resistance · natural products · RNA

A Dormant Beast

Tuberculosis (TB) is the most widespread and persistent bacterial infection.[1] About 2 billion people carry its causative agent, *Mycobacterium tuberculosis*, a refractory, slow-growing pathogen that kills about 1.7 million persons each year, mostly in developing countries.[2] And more and more people in the developed world are contracting tuberculosis because their immune system is compromised (immunosuppressive drugs, substance abuse, AIDS).[3] Together, HIV and TB are a deadly duo; each disease speeds up the progress of the other. In particular, multiresistant variants of *M. tuberculosis* (MDR-TB and XDR-TB)[4] require new and more effective drugs.[5] The evolution of antibacterial drug resistance in life-threatening human pathogens has been meticulously documented, and experts have warned time and again of a nascent public health crisis.[6] Infections by methicillin-resistant *Staphylococcus aureus* (MRSA)[7] are on the rise, and related mortality has exceeded the number of HIV-associated deaths in the U.S.[8]

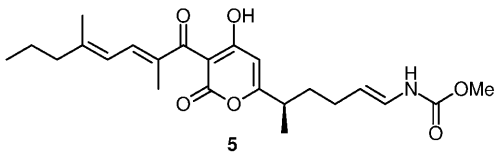


Scheme 1. Natural rifamycin SV (**1**) as well as semisynthetic rifampicin (**2**), rifapentine (**3**), and rifabutin (**4**) are clinically approved inhibitors of DNA-dependent bacterial RNA polymerase.

The rifamycin antibiotics (Scheme 1), in particular rifampicin (**2**), rifapentine (**3**), or rifabutin (**4**), are essential components of the internationally recommended first-line TB treatment regimen.[9] The rifamycins selectively bind to bacterial—not to mammalian—RNA polymerase (RNAP) and block its essential function: transcription. They bind to an allosteric site close to the active center and avert extension of nascent microbial RNA.[10] Apart from the rifamycins, no other inhibitors of bacterial RNAP are clinically approved. Yet, the emergence of mycobacterial strains resistant to rifamycins, caused in part by improper antibiotic use, has considerably hampered TB treatment.

Learning from Nature

In the October 17th, 2008 issue of *Cell*, the research teams led by Eddy Arnold and Richard H. Ebright described the mechanism by which the natural α-pyrone antibiotic myxopyronin A (**5**) blocks bacterial RNAP.[11] Grasping the fine



mechanics of antibiotic action is of far more than purely academic interest. A vital insight into these processes should spark new ideas for the chemical design of future antibiotics.

DNA-dependent RNAP is responsible for transcription. As a result of our common evolutionary origin, RNAP is

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conserved in all living organisms. Bacterial RNAP is essential for microbial growth, conserved among Gram-positive and Gram-negative pathogens, and sufficiently different from its mammalian counterparts (tolerability). Despite structural and functional similarities, prokaryotic RNAP and eukaryotic RNAP do not share broad sequence homology and possess properties that distinguish them from each other.[12] Because of the clinical experience with the rifamycins, RNAP qualifies as one of the rare validated—but underexploited—targets for broad-spectrum antibacterial therapy.[13]

Form Follows Function

Transcription proceeds in three distinct stages: initiation, elongation, and termination. The process defines the form of the RNAP transcriptional machinery—a crab-claw-like structure (Figure 1). The bacterial RNAP enzyme consists of five protein subunits, $\alpha_2\beta\beta'\omega$, and an additional dissociable σ factor. The β and β′ domains form the pincers of the claw and the active center, a Mg^{2+}-containing cleft. The pincers open and close by a 30° rotation of the larger β′ subunit (clamp) around the switch region (hinge). Clamp opening allows double-stranded DNA to bind in the catalytic cleft (diameter ≈20 Å), and clamp closure around the bound DNA permits transcription initiation.[14] RNAP shifts along the DNA template and synthesizes a complementary strand of messenger RNA. First, RNAP recognizes the nucleoside triphosphate by complementary base pairing with the DNA template, and then it incorporates the new nucleotide into the growing RNA releasing diphosphate. Such a two-step mechanism ensures the high fidelity required for the vital transcription process. During elongation, nascent RNA leaves the enzyme through the exit channel. Many bacteria contain several σ factors, which fine-tune gene expression by directing the core polymerase to transcribe particular genes (promoter-specific transcription initiation).[15]

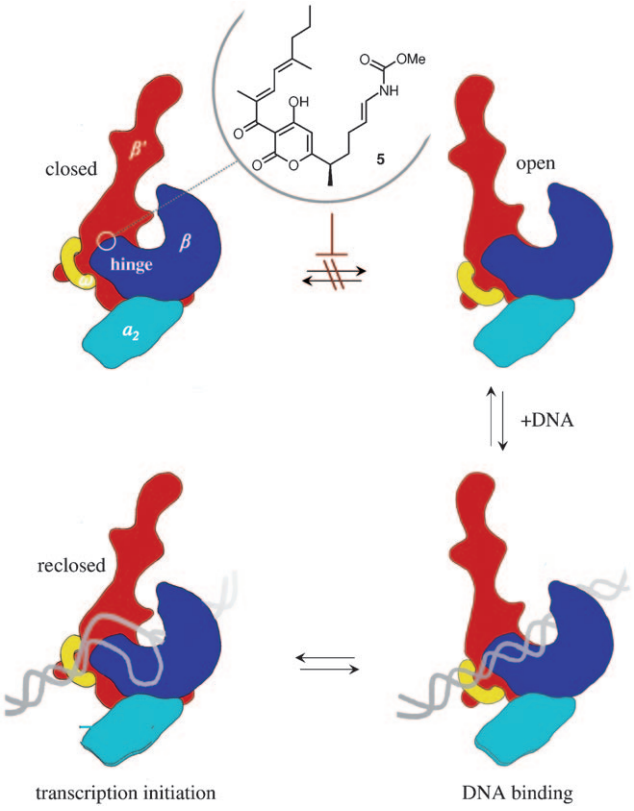


Figure 1. Structure and function of bacterial RNA polymerase. The crab-claw-like RNAP can open and close by a 30° rotation of the larger β′ subunit (clamp) around the switch region (hinge). Myxopyronin A (**5**) blocks transcription initiation by jamming the hinge.

Jamming the Hinge

In a sequence of well-conceived biochemical, genetic, and structural studies, E. Arnold, R. H. Ebright, et al.[11] characterize the mechanism of RNAP inhibition by the α-pyrone antibiotic myxopyronin A (**5**), a secondary metabolite produced by the myxobacterium *Myxococcus fulvus*.[16] Their proficient study has helped to better understand the fundamentals of RNAP structure and action. The complex function and the large structure of the bacterial RNAP enzyme offer diverse binding sites for small-molecule inhibitors. In particular, naturally occurring antibiotics have been reported to specifically inhibit bacterial RNAP.[13b,17] From an evolutionary point of view, this is no surprise. Natural scaffolds[18] contain the key to bacterial vulnerability, and it appears wise not to ignore them in antibacterial drug discovery. Myxopyronin A (**5**) has been known to specifically inhibit bacterial RNAP ($IC_{50} \approx 1$ μM).[16a] Now, E. Arnold and R. H. Ebright, et al. have discovered its binding site and mechanism of action.[11] Exploring myxopyronin resistance in *Escherichia coli* by mutagenesis and biochemical studies, the authors proved that **5** binds to the RNAP switch region at the base of the β′ pincer, preventing the interaction of RNAP with double-stranded DNA. An X-ray crystal structure of RNAP from *Thermus thermophilus* in complex with **5** (Figure 2) revealed the binding contacts and explained the mode of

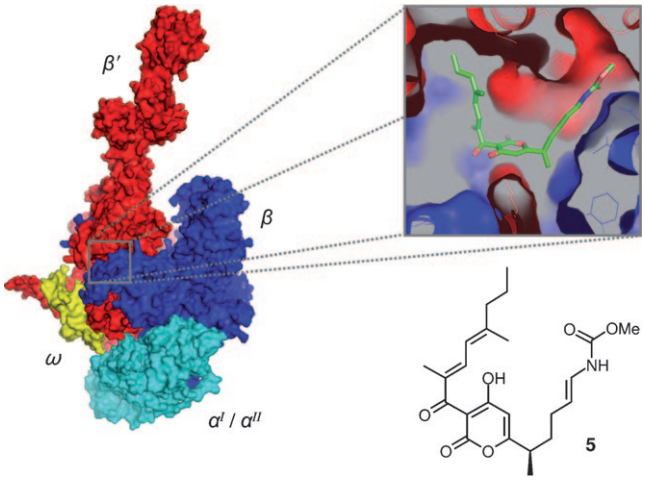
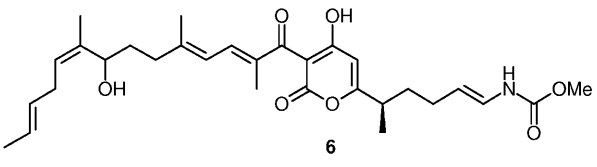


Figure 2. X-ray crystal structure of myxopyronin A (**5**) buried in the switch region of bacterial RNAP from *T. thermophilus*; σ factors omitted for clarity.

action: myxopyronin is locking the clamp in a partly to fully closed conformation, thereby preventing entry of promoter DNA into the active-center cleft during transcription initiation. Myxopyronin's allosteric binding site is a crescent-shaped hydrophobic pocket distant from the RNAP catalytic center and away from the binding site of the rifamycins.

Three Antibiotics—One Mode of Action

The myxopyronin study is not a singular episode. The authors provide experimental evidence that two other myxobacterial metabolites share myxopyronin's mode of action: the α-pyrone antibiotic corallopyronin A (**6**),[19] produced by the myxobacterium *Corallococcus coralloides*, and the 14-membered macrolactone ripostatin A (**7**)[20] from *Sorangium cellulosum*. Corallopyronin A (**6**) differs from **5**



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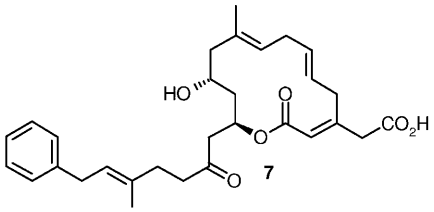

7

by a longer hydroxylated dienone side chain. The structurally unrelated macrolactone **7** also fits into the spacious myxopyronin binding site. Both antibiotics, discovered and characterized by G. Höfle and H. Reichenbach and their research groups, specifically block bacterial RNAP ($IC_{50} \approx 4$ μM for **6** and ≈0.8 μM for **7**) but not eukaryotic RNAP II.

Valid Lead Structures?

Myxopyronin (**5**), corallopyronin (**6**), and ripostatin (**7**) suffer from various biological, structural, and physicochemical deficiencies that will need amending by medicinal chemistry. This is typical for many natural product leads. In evolutionary processes,[21] distinct parameters such as target affinity, potency, antibacterial spectrum, cytotoxicity, and biosynthetic effort contribute to the "fitness" of a specific molecule. Yet, a pharmaceutical drug has to match additional physicochemical, pharmacological, toxicological, and technical requirements that have not been selectors in the evolution of antibacterial secondary metabolites.

Antibacterial in vitro activity (MIC) of compounds **5**, **6**, and **7** is species dependent, overall. Whereas the growth of *S. aureus* and *S. epidermidis* is inhibited at low concentrations, MIC is only moderate against many other bacterial species such as streptococci and enterococci because of limited membrane permeability. The antibacterial spectrum is mostly restricted to Gram-positive pathogens (efflux) and does not cover *Pseudomonas aeruginosa*. The tremendous lipophilicity (high lg*P*; Table 1) of **5**–**7** reflects an inadequate physicochemical and pharmacokinetic profile which does not yet meet the requirements of a drug. The antibiotics are photosensitive polyenes, chemical stability is low in the case of the macrolactone, and solubility (low polarity) is insufficient except for the acid **7**. All three antibiotics show high serum protein binding (high lg(HSA)) which reduces their biologically active free fraction in blood plasma. As only the free, unbound drug can act on the target in vivo, this is a key parameter for therapeutic efficacy. Consequently, **6** did not exhibit in vivo efficacy after parenteral administration of up to 25 mg kg^{-1} in a murine *S. aureus* sepsis model.[22] Nevertheless, the scaffolds of **5**, **6**, and **7** present seminal opportunities for medicinal chemistry. An acceptable safety profile—a frequent issue with antibiotics—seems feasible (low acute toxicity of **5** and **6**: $LD_{50} > 100$ mg kg^{-1}).[16a] Apart from its lipophilicity, **5** is the most attractive oral lead, since it offers a starting profile of modest molecular weight (M_{rel}) and potentially tractable appendages.

Table 1: Physicochemical profiles of the antibiotic lead structures **5**–**7**; calculated data.

Antibiotic	M_{rel}	lg *P*[a]	lg(HSA)[b]	Polar surface area [Å^2][c]
myxopyronin A (**5**)	432	6.7	4.6	102
corallopyronin A (**6**)	528	6.7	5.1	122
ripostatin A (**7**)	495	4.7	5.4	101

[a] Descriptor for lipophilicity. [b] Descriptor for the binding to human serum albumin. [c] Descriptor for oral absorption.

Outlook

In the evolutionary contest between humans and microbes, it is crucial to learn how antibiotics act on the molecular level. E. Arnold, R. H. Ebright, and their groups[11] show precisely how the natural α-pyrone antibiotics inhibit bacterial RNA polymerase. This new mechanistic understanding is an excellent starting point for the design of new therapies based on RNAP inhibition. As often the case, the unmodified natural products **5**, **6**, and **7** cannot be used directly as clinical drugs for various reasons (physicochemical profile);[23] yet, these antibiotics represent hopeful lead structures for medicinal chemistry. Total syntheses[24,25] of the α-pyrone antibiotics have provided a solid chemical basis but only a few active congeners so far (7-demethylmyxopyronin B and 11,12-*cis*-myxopyronin B). A complete de novo synthesis of ripostatin is in progress.[26] By structural modification, improvements in potency and antibacterial spectrum should be approachable. To achieve in vivo efficacy, serum protein binding must be reduced and solubility should be increased by incorporating polar functionality, while retaining potency. The spontaneous rate of resistance should be lower than that observed with the rifamycins. Simple structural

modifications will not suffice to achieve the optimal balance of the manifold parameters that mark a good drug. Success will ultimately depend on daring structural redesign and the courage to explore beyond the obvious bioisosters. In the patients' interest, chemistry should not miss this unique chance.

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