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ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ
ORIGINAL RESEARCH

A new inhibitor binding site in bacterial RNA polymerase

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Abstract. *Relevance.* Growing resistance of bacterial pathogens to antibiotics is a fundamental threat to healthcare worldwide. Many antibiotics currently used in clinical practice were developed decades ago, and their efficiency has been declining over time. Research efforts focusing on identification and optimization of new promising antibacterial compounds and new molecular targets in bacterial cells establish the foundation for developing new potent antibiotics relevant to modern day clinical needs. The bacterial RNA polymerase is a validated target for a number of natural, semisynthetic, and synthetic antibacterial compounds. This essential enzyme maintains a high potential for developing a new generation of antibiotics. *Materials and methods.* We prepared a complex of recombinant *Escherichia coli* RNA polymerase with a new small-molecule transcription inhibitor, and studied it using cryoelectron microscopy to determine the mechanism of inhibition. *Results and discussion.* The current work establishes the molecular basis of RNA polymerase inhibition by the compound N-(9H-fluoren-9-yl)-3-(2-methylpropyl)-[1,2,4] triazolo[4,3-a]pyridine-8-carboxamide (compound A) that blocks transcription through binding to a previously unknown binding site. We describe interactions of the compound A with RNA polymerase and reveal its mechanism of action that involves steric restriction of template DNA binding in the enzyme active site. We demonstrate that the compound A targets only certain types of transcription complexes since its binding site is formed in part by a dissociable subunit of RNA polymerase. *Conclusion.* The results of the study may be used for development of conjugates of rifamycins with the compound A to obtain a new class of bacterial RNA polymerase inhibitors with a new mechanism of action.

Keywords: bacterial transcription, RNA polymerase, inhibitor, mechanism of action

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Introduction

Antibiotics are a pillar of modern medicine; however, existing antibacterial drugs are increasingly proving ineffective in treating infectious diseases due to the growing prevalence of drug-resistant bacterial pathogens [1]. Revolutionary changes in structural biology, particularly the increasing availability and efficiency of cryo-electron microscopy, are leading to the rapid accumulation of solved structures of biological macromolecules, facilitating the dissemination of methods for the targeted design of inhibitors of essential target molecules in bacterial cells for the development of new antibacterial drugs [2–4]. The structure of a complex of a biological macromolecule with an inhibitor allows for a detailed study of the molecular mechanism of inhibition, thereby creating the opportunity for sequential structural modification and optimization of the original inhibitor for the rational design of a new inhibitor with increased affinity and efficacy.

Bacterial RNA polymerase is an enzyme essential for gene expression, cell growth, and division [5]. During transcription, the bacterial RNA polymerase core enzyme (with the subunit formula α2ββ′ω) binds the σ factor for specific recognition and binding to the gene promoter and initiates *de novo* synthesis of RNA transcript [6]. Bacterial RNA polymerase has a shape commonly described as a "crab claw", with the active site at its base located deep within the main binding channel for DNA and RNA substrates [7]. The catalytic center of bacterial RNA polymerases is formed by a rather conserved amino acid sequence that has low homology with the catalytic centers of eukaryotic RNA polymerases, which allows bacterial RNA to be used as a target for the development of broad-spectrum antibiotics with high selectivity [8–14].

Currently, antibiotics that inhibit bacterial RNA polymerase and are approved for clinical use belong to two classes: rifamycins and fidaxomicin. Rifamycins, widely used to treat tuberculosis and other infectious diseases [15], bind RNA polymerase in a shallow pocket within the active site and sterically block elongation of the RNA transcript after it reaches a length of 2–3 nucleotides [16]. Fidaxomicin, used to treat *Clostridium difficile* infections [17], binds to RNA polymerase at switches, blocking conformational changes required for the formation of an open promoter complex [12]. A recent study analyzing components of a library of small molecules that bind to bacterial RNA polymerase revealed a novel component, N-(9H-fluoren-9-yl)-3-(2-methylpropyl)-[1,2,4]triazolo[4,3-a] pyridine-8-carboxamide (compound A), with antibacterial activity [18]. This molecule was found to inhibit transcription in rifamycin-resistant *E. coli* strains, and mutations in RNA polymerase associated with acquired resistance to compound A were located outside the known binding sites of bacterial RNA polymerase inhibitors. **The aim of this work** was to investigate the

binding site of the inhibitor compound A in *E. coli* RNA polymerase and to establish the molecular mechanism of transcription inhibition.

Materials and methods

E. coli RNA polymerase

E. coli RNA polymerase σ70 holoenzyme was prepared as described previously [9].

Sample preparation for cryo-electron microscopy

An aliquot of *E. coli* RNA polymerase σ70 holoenzyme was thawed on ice, and 10 μL of 20 μM enzyme were mixed with 1 μL of 1 mM N-(9H-fluoren-9-yl)-3-(2-methylpropyl)-[1,2,4]triazolo[4,3-a] pyridine-8-carboxamide (Merck; CAS No. 2095432-38-3) diluted in DMSO (Sigma-Aldrich), 2 μL of buffer (10 mM Tris-HCl, pH 7.6, 100 mM NaCl, 0.1 mM EDTA, 5 mM dithiothreitol), and 7 μL of distilled water. After 20 min of incubation at 25 °C, the complex was immediately applied to cryo-electron microscopy substrates. Substrates with cryo-electron microscopy samples were prepared on a Vitrobot Mark IV autoplunger (FEI/ThermoFisher) at 22 °C and 100% humidity in the blotting chamber. Samples (3 μL) were applied to 2/1 Quantifoil Cu 300 holey-carbon grids (Quantifoil), treated for 60 s in a PELCO surface charge remover (Ted Pella) immediately before application. Grids with samples were blotted with #595 filter paper disks for 7 s at 22 °C, after which they were flash-frozen by plunging to liquid ethane cooled with liquid nitrogen. The prepared grids with the sample were stored frozen in liquid nitrogen until the beginning of experimental data collection.

Cryo-electron microscopy: data acquisition and processing

Experimental data were collected at the Rutgers University Cryo-Electron Microscopy and Nanoimaging Facility (USA) on a Talos Arctica 200 keV electron microscope (FEI/ThermoFisher) equipped with a GIF Quantum K2 direct electron detector (Gatan). Data were collected automatically using the EPU software (FEI/ThermoFisher) at a nominal resolution of 130,000x, a calibrated pixel size of 1.038 Å/pixel, and a irradiance of 4.8 electrons/pixel/sec. Video images were recorded at 200 milliseconds/frame for 6 sec (30 frames), resulting in a total cumulative irradiance dose of 26.7 electrons/Å2. The defocus distance ranged from –1.25 μm to –2 μm. 2000 micrographs were recorded from one sample substrate over 2 days.

Micrographs were normalized and corrected for defects. Data processing was performed on a Tensor TS4 Linux GPU workstation equipped with four GTX 1080 Ti video cards (NVIDIA). Dose-dependent correction of particle motion (3 × 3 tiles; b-factor = 150) was performed using Motioncor2 [19]. Contrast transfer function (CTF) estimation was performed using CTFFIND-4.1[20]. Post-processing of images was performed using Relion 3.0 [21]. Automatic particle extraction using a Laplace-Gaussian filter yielded a set of 364,630 particles. Particles were extracted using a 250 × 250 pixel extraction square and subjected to reference-free 2D classification, removing sparsely populated and misshapen classes. This yielded a set of 107,112 selected particles. This set was then subjected to 3D classification using C1 symmetry and a *de novo* 3D model obtained using 3D_initial_model in Relion 3.0. Classes with clear density corresponding to RNA polymerase were combined and subjected to automatic 3D refinement. The resulting particle set was further refined using the "solvent flattening" setting and postprocessed, with a final reconstruction of the complex at a resolution of 3.9 Å, determined according to the Fourier distribution correlation (FSC) standard. The initial atomic model was constructed based on the σ70 structure of the *E. coli* RNA polymerase holoenzyme obtained by X-ray crystallography (PDB 4KN7) [9]. The structure of compound A was drawn in an online graphics editor, converted into a SMILES formula in an online converter, and processed in the eLBOW program [22] in PHENIX 1.20.1–4487 [23]. The resulting structure of compound A was manually fitted into the additional electron density present in the active site of the initial RNA polymerase model, after which the model was subjected to several rounds of manual refinement in Coot [24] and automated refinement using real-space refinement in PHENIX 1.20.1–4487 [23]. Visualization of the resulting structure was performed using Coot and PyMOL (Schrödinger).

Results and discussion

Binding site and mechanism of transcription inhibition of compound A

In the study identifying compound A as a bacterial transcription inhibitor [18], mutations leading to resistance to compound A were clustered in a small segment of RNA polymerase comprising the β' and ω subunits (Figure 1*a*). For this reason, initial analysis of the electron density map of the resulting RNA polymerase complex with compound A expected to see additional electron density at this site, corresponding to the bound inhibitor. However, additional electron density, with a size and geometry consistent with compound A, was found away from the putative binding site, within the main RNA polymerase channel near the catalytic center and the rifamycin-binding site (Figure 1*a*). compound A was inserted into this additional electron density, and its interactions with RNA polymerase were analyzed (Figure 1 *b, c*). The binding site of compound A is a shallow pocket formed by amino acid residues of the β' and σ70 subunits. The β' subunit interacts with compound A via β' amino acid residues L255, D256, and R259, which are part of the conserved structural element, the β' lid, which contacts the active site RNA during transcription and promotes the separation of RNA from DNA in the RNA-DNA hybrid [25]. The σ70 subunit interacts with the inhibitor via σ70 amino acid residues I505, I511, and F522, which are part of the conserved domain 3.2 of the basic σ-factor (Figure 1 *b, c*). Domain 3.2 of the σ-factor interacts with RNA at the early stages of transcription initiation, preventing its elongation after reaching a length of 4–6 nucleotides [26] and blocking the RNA exit channel from the transcription complex, which triggers σ-factor dissociation during the transition from transcription initiation to elongation [27]. Thus, since the binding site of compound A is present only in the free RNA polymerase holoenzyme, the inhibitor presumably blocks transcription at the stage of promoter complex formation and is unable to affect transcription at other stages. Modeling of the DNA open promoter complex (PDB 4YLP) [28] into the derived structure of the RNA polymerase complex with compound A shows that binding of the template DNA strand leads to a steric clash with one end of compound A (Figure 2), prevents the formation of contacts between the template DNA strand and the amino acid residues of the β' cap, and does not allow the template DNA strand to assume a position in the catalytic center that promotes optimal placement of the first two ribonucleotides during the formation of the first phosphodiester bond during transcription initiation. The combination of these factors explains the mechanism of bacterial transcription inhibition by compound A. However, the obtained structure leaves open the question of the allosteric mechanism of resistance to the inhibitor of RNA polymerase variants with mutations in the β' and ω subunits, discovered in a previous study [18].

Potential of a new binding site for the development of antibiotics

Given the low affinity of compound A for bacterial RNA polymerase (Kd ~ 3 μM) [18], the potential of the inhibitor as a platform for the development of a new stand-alone antibiotic seems low. However, in recent years, studies of the activity of drug conjugates based on drugs used in clinical practice have become increasingly common in the field of medicinal chemistry. A recent study provides an example of the successful development of a new type of antibiotic by chemical conjugation of rifabutin with metronidazole, which made it possible to achieve a synergistic effect and high efficacy in the treatment of infectious diseases caused by *H. pylori*, including drug-resistant strains [14]. We note that in RNA polymerase, compound A and rifamycin antibiotics such as 25-O-benzoyl-rifabutin [13] and TNP-2198 [14] are separated by a distance of 9–12.9 Å (Figure 3 *a, b*). This observation suggests that compound A and rifamycin antibiotics can be conjugated using a linker to create novel antibacterial compounds with enhanced affinity for bacterial RNA polymerase. Such conjugates may have a different mechanism of transcription inhibition than both parent molecules, based on complete steric blocking of the template-strand DNA loading to the active site of the RNA polymerase holoenzyme by a linker connecting the two pharmacophores. The synthesis and testing of such compounds will be the subject of future research.

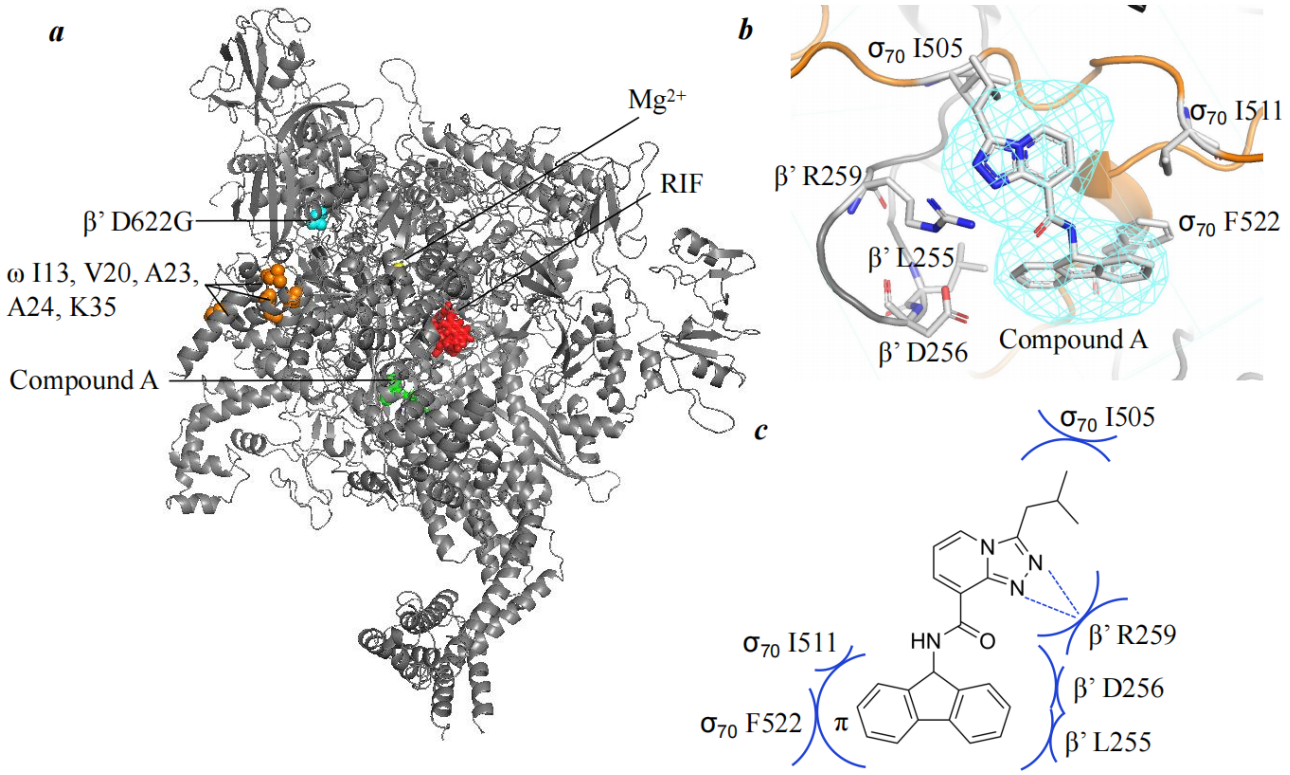

Figure 1. Structural basis of bacterial RNA polymerase inhibition. (*a*) Model of the transcription initiation complex of E. coli RNA polymerase. RNA polymerase, rifampin, and compound A are shown in gray, red, and green, respectively. The Mg^{2+} ion in the active site is shown as a yellow sphere. Amino acid residues in the β′ and ω subunits, mutations in which lead to resistance to the inhibitory action of compound A, are shown in the enzyme structure as blue and orange spheres. (*b*) The binding site of compound A. Some structural elements of the binding site that obscure the view have been omitted for clarity. The experimental three-dimensional electron density of compound A is shown as a blue grid with an embedded atomic model of compound A colored according to atomic composition. The β′ and σ70 subunits are shown in gray and orange, respectively. The side chains of the amino acid residues of the β′ and σ70 subunits interacting with compound A are colored according to their atomic composition. (c) Schematic representation of the interactions of compound A with the amino acid residues in the binding site. Van der Waals interactions are shown as blue arcs, hydrogen bonds are indicated by blue dashed lines, and the π-stacking interaction is marked with the letter π.

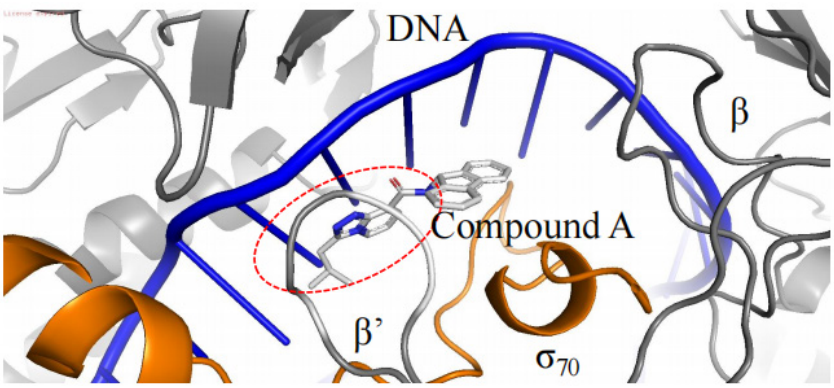

Figure 2. Mechanism of bacterial transcription inhibition by compound A. Some structural elements of RNA polymerase that obscure the overview have been omitted for clarity. compound A, β, β', and σ70 subunits are shown as in Figure 1*a-b*. The DNA model is shown in blue. The collision of the template-strand DNA with compound A in the open promoter complex of *E. coli* RNA polymerase is shown by the red dotted oval

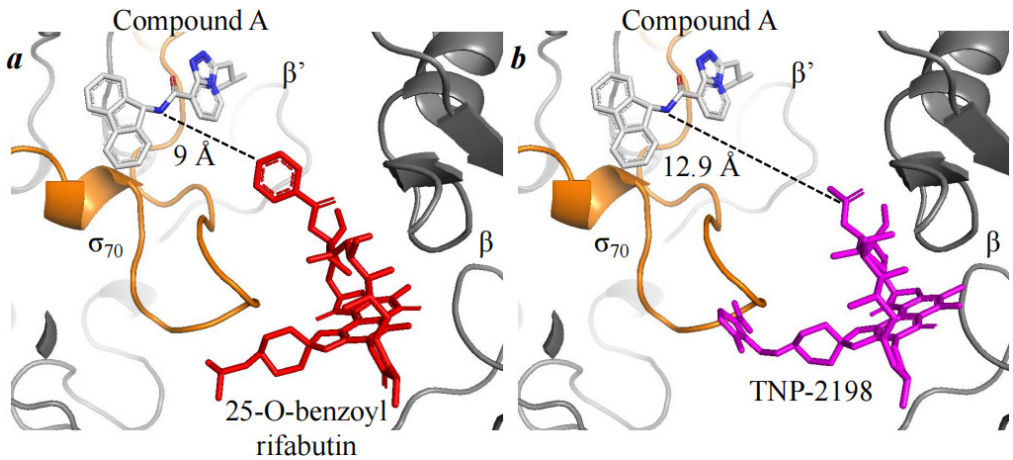

Figure 3. Relative positioning of compound A and rifamycin antibiotics in the binding sites of bacterial RNA polymerase. Some structural elements of RNA polymerase that obscure the overview have been omitted for clarity. Compound A, β, β', and σ70 subunits are shown as in Figure 1*b*. (*a*) Modeling of compound A and 25-O-benzoyl-ribofabutin in the active site of *E. coli* RNA polymerase. The atomic model of 25-O-benzoyl-ribofabutin is shown in red. The minimum distance between compound A and 25-O-benzoyl-ribofabutin is shown as a dotted line. (*b*) Modeling of compound A and TNP-2198 in the active site of *E. coli* RNA polymerase. The atomic model of TNP-2198 is shown in magenta. The minimum distance between compound A and TNP-2198 is shown as a dotted line.

Conclusion

The obtained results expand the existing list of inhibitor binding sites in bacterial RNA polymerase and provide an opportunity for the rational design of new antibacterial drugs that inhibit bacterial transcription by binding to this site. Further research in this direction can be aimed at creating dual inhibitors based on rifamycins and compound A-like conjugates that bind to bacterial RNA polymerase at both binding sites simultaneously.

References / Список литературы

1. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem*. 2014;6:25–64. doi: 10.4137/PMC.S14459
2. Bagchi A. Latest trends in structure based drug design with protein targets. *Adv Protein Chem Struct Biol*. 2020;121:1–23. doi: 10.1016/bs.apcsb.2019.11.008
3. Varela MF, Kumar S. Strategies for discovery of new molecular targets for anti-infective drugs. *Curr Opin Pharmacol*. 2019;48:57–68. doi: 10.1016/j.coph.2019.04.015
4. Neville N, Jia Z. Approaches to the structure-based design of antivirulence drugs: therapeutics for the post-antibiotic era. *Molecules*. 2019;24(3):378. doi: 10.3390/molecules24030378
5. Villain-Guillot P, Bastide L, Gualtieri M, Leonetti JP. Progress in targeting bacterial transcription. *Drug Discov Today*. 2007;12(5–6):200–8. doi: 10.1016/j.drudis.2007.01.005
6. Basu RS, Warner BA, Molodtsov V, Pupov D, Esyunina D, Fernández-Tornero C, Kulbachinskiy A, Murakami KS. Structural basis of transcription initiation by bacterial RNA polymerase holoenzyme. *J Biol Chem*. 2014;289(35):24549–59. doi: 10.1074/jbc.M114.584037
7. Murakami KS, Darst SA. Bacterial RNA polymerases: the wholo story. *Curr Opin Struct Biol*. 2003;13(1):31–9. doi: 10.1016/s0959-440x(02)00005-2
8. Ho MX, Hudson BP, Das K, Arnold E, Ebright RH. Structures of RNA polymerase-antibiotic complexes. *Curr Opin Struct Biol*. 2009 Dec;19(6):715–23. doi: 10.1016/j.sbi.2009.10.010
9. Molodtsov V, Nawarathne IN, Scharf NT, Kirchhoff PD, Showalter HD, Garcia GA, Murakami KS. X-ray crystal structures of the Escherichia coli RNA polymerase in complex with benzoxazinorifamycins. *J Med Chem*. 2013;56(11):4758–63. doi: 10.1021/jm4004889
10. Molodtsov V, Fleming PR, Eyermann CJ, Ferguson AD, Foulk MA, McKinney DC, Masse CE, Buurman ET, Murakami KS. X-ray crystal structures of Escherichia coli RNA polymerase with switch region binding inhibitors enable rational design of squaramides with an improved fraction unbound to human plasma protein. *J Med Chem*. 2015;58(7):3156–71. doi: 10.1021/acs.jmedchem.5b00050
11. Zhang Y, Degen D, Ho MX, Sineva E, Ebright KY, Ebright YW, Mekler V, Vahedian-Movahed H, Feng Y, Yin R, Tuske S, Irschik H, Jansen R, Maffioli S, Donadio S, Arnold E, Ebright RH. GE23077 binds to the RNA polymerase 'i' and 'i+1' sites and prevents the binding of initiating nucleotides. *Elife*. 2014;3: e02450. doi: 10.7554/eLife.02450
12. Lin W, Das K, Degen D, Mazumder A, Duchi D, Wang D, Ebright YW, Ebright RY, Sineva E, Gigliotti M, Srivastava A, Mandal S, Jiang Y, Liu Y, Yin R, Zhang Z, Eng ET, Thomas D, Donadio S, Zhang H, Zhang C, Kapanidis AN, Ebright RH. Structural Basis of Transcription Inhibition by Fidaxomicin (Lipiarmycin A3). *Mol Cell*. 2018;70(1):60–71.e15. doi: 10.1016/j.molcel.2018.02.026
13. Lan T, Ganapathy US, Sharma S, Ahn YM, Zimmerman M, Molodtsov V, Hegde P, Gengenbacher M, Ebright RH, Dartois V, Freundlich JS, Dick T, Aldrich CC. Redesign of rifamycin antibiotics to overcome ADP-ribosylation-mediated resistance. *Angew Chem Int Ed Engl*. 2022;61(45): e202211498. doi: 10.1002/anie.202211498
14. Ma Z, He S, Yuan Y, Zhuang Z, Liu Y, Wang H, Chen J, Xu X, Ding C, Molodtsov V, Lin W, Robertson GT, Weiss WJ, Pulse M, Nguyen P, Duncan L, Doyle T, Ebright RH, Lynch AS. Design, synthesis, and characterization of TNP-2198, a dual-targeted rifamycin-nitroimidazole conjugate with potent

Activity against Microaerophilic and Anaerobic Bacterial Pathogens. *J Med Chem*. 2022;65(6):4481–4495. doi: 10.1021/acs.jmedchem.1c02045

15. Aristoff PA, Garcia GA, Kirchhoff PD, Showalter HD. Rifamycins — obstacles and opportunities. *Tuberculosis (Edinb)*. 2010;90(2):94–118. doi: 10.1016/j.tube.2010.02.001

16. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural mechanism for rifampicin inhibition of bacterial rna polymerase. *Cell*. 2001;104(6):901–12. doi:

17. Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol*. 2009;7(7):526–36. doi: 10.1038/nrmicro2164

18. Walker SS, Degen D, Nickbarg E, Carr D, Soriano A, Mandal M, Painter RE, Sheth P, Xiao L, Sher X, Murgolo N, Su J, Olsen DB, Ebright RH, Young K. Affinity Selection-Mass Spectrometry Identifies a Novel Antibacterial RNA Polymerase Inhibitor. *ACS Chem Biol*. 2017;12(5):1346–1352. doi: 10.1021/acschembio.6b01133

19. Zheng SQ, Palovcak E, Armache JP, Verba KA, Cheng Y, Agard DA. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat Methods*. 2017;14(4):331–332. doi: 10.1038/nmeth.4193

20. Rohou A, Grigorieff N. CTFFIND4: Fast and accurate defocus estimation from electron micrographs. *J Struct Biol*. 2015;192(2):216–21. doi: 10.1016/j.jsb.2015.08.008

21. Zivanov J, Nakane T, Forsberg BO, Kimanius D, Hagen WJ, Lindahl E, Scheres SH. New tools for automated high-resolution cryo-EM structure determination in RELION-3. *Elife*. 2018;7: e42166. doi: 10.7554/eLife.42166

22. Moriarty NW, Grosse-Kunstleve RW, Adams PD. electronic Ligand Builder and Optimization Workbench (eLBOW): a tool for ligand coordinate and restraint generation. *Acta Crystallogr D Biol Crystallogr*. 2009;65(Pt 10):1074–80. doi: 10.1107/S0907444909029436

23. Liebschner D, Afonine PV, Baker ML, Bunkóczi G, Chen VB, Croll TI, Hintze B, Hung LW, Jain S, McCoy AJ, Moriarty NW, Oeffner RD, Poon BK, Prisant MG, Read RJ, Richardson JS, Richardson DC, Sammito MD, Sobolev OV, Stockwell DH, Terwilliger TC, Urzhumtsev AG, Videau LL, Williams CJ, Adams PD. Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta Crystallogr D Struct Biol*. 2019;75(Pt 10):861–877. doi: 10.1107/S2059798319011471

24. Emsley P, Lohkamp B, Scott WG, Cowtan K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr*. 2010;66(Pt 4):486–501. doi: 10.1107/S0907444910007493.

25. Naryshkina T, Kuznedelov K, Severinov K. The role of the largest RNA polymerase subunit lid element in preventing the formation of extended RNA-DNA hybrid. *J Mol Biol*. 2006;361(4):634–43. doi: 10.1016/j.jmb.2006.05.034

26. Pupov D, Petushkov I, Esyunina D, Murakami KS, Kulbachinskiy A. Region 3.2 of the σ factor controls the stability of rRNA promoter complexes and potentiates their repression by Dks A. *Nucleic Acids Res*. 2018;46(21):11477–11487. doi: 10.1093/nar/gky919

27. Li L, Molodtsov V, Lin W, Ebright RH, Zhang Y. RNA extension drives a stepwise displacement of an initiation-factor structural module in initial transcription. *Proc Natl Acad Sci U S A*. 2020;117(11):5801–5809. doi: 10.1073/pnas.1920747117

28. Zuo Y, Steitz TA. Crystal structures of the E. coli transcription initiation complexes with a complete bubble. *Mol Cell*. 2015;58(3):534–40. doi: 10.1016/j.molcel.2015.03.010

Новый сайт связывания ингибитора в бактериальной РНК полимеразе

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Аннотация. *Актуальность.* Растущая устойчивость бактериальных патогенов к антибиотикам представляет собой фундаментальную угрозу для здравоохранения во всем мире. Многие антибиотики, используемые в настоящее время в клинической практике, были разработаны десятилетия назад, и их эффективность со временем снижается. Исследования, направленные на идентификацию и оптимизацию новых перспективных антибактериальных соединений и новых молекулярных мишеней в бактериальных клетках, закладывают основу для разработки новых сильнодействующих антибиотиков, отвечающих современным клиническим потребностям. Бактериальная РНК-полимераза является подтвержденной мишенью для ряда природных, полусинтетических и синтетических антибактериальных соединений. Этот важный фермент обладает высоким потенциалом для разработки нового поколения антибиотиков. *Материалы и методы.* Мы получили комплекс рекомбинантной РНК-полимеразы *Escherichia coli* с новым низкомолекулярным ингибитором транскрипции и изучили его с помощью криоэлектронной микроскопии для определения механизма ингибирования. *Результаты и обсуждение.* В данной работе установлена молекулярная основа ингибирования РНК-полимеразы соединением N-(9H-флуорен-9-ил)-3-(2-метилпропил)-[1,2,4]триазоло[4,3-a]пиридин-8-карбоксамидом (соединение А), которое блокирует транскрипцию путем связывания с ранее неизвестным сайтом связывания. Мы описываем взаимодействие соединения А с РНК-полимеразой и раскрываем его механизм действия, который

включает стерическое ограничение связывания матричной ДНК в активном центре фермента. Мы демонстрируем, что соединение А нацелено только на определенные типы транскрипционных комплексов, поскольку его сайт связывания частично образован диссоциируемой субъединицей РНК-полимеразы. *Выводы*. Результаты исследования могут быть использованы для разработки конъюгатов рифамицинов с соединением А с целью получения нового класса ингибиторов бактериальной РНК-полимеразы с новым механизмом действия.

Ключевые слова: Бактериальная транскрипция, РНК полимераза, ингибитор, механизм действия

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