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A New Class of Small Molecule RNA Polymerase Inhibitors with Activity Against *S. aureus*

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Abstract

Introduction: The RNA polymerase holoenzyme is a particularly attractive target for the development of new antibacterial agents, yet there are few current RNA polymerase inhibitors used clinically. Herein is described the development of a new class of compounds, with antibacterial activity, acting via the RNA polymerase holoenzyme.

Methods: The compounds were tested against the *S. aureus* RNA polymerase holoenzyme in a functional assay measuring the incorporation of α-[^{32}P]-UTP in acid-precipitate. All other assays were performed using standard procedures.

Results: A high-throughput screen of 250,000 synthetic small molecules allowed the identification of a 2-ureidothiophene-3-carboxylate submicromolar inhibitor of the RNA polymerase holoenzyme with some antibacterial activity. The preparation of synthetic analogs revealed the structure-activity relationships within this class of compounds as RNA polymerase holoenzyme inhibitors. Among these analogs are nanomolar inhibitors with antibacterial activity against *Staphylococci*, including antibiotic resistant strains. A key analog was further assessed and behaved properly in all counter-screens. It however lacked antibacterial activity in the presence of serum, and displayed rapid development of resistance. Protection was obtained in the peritonitis murine infection model when administered i.p. but not i.v. nor p.o. Conclusion: A class of 2-ureidothiophene-3-carboxylate esters inhibitors of the RNA polymerase holoenzyme with selective antibacterial mode of action was developed. However the rapid development of resistance and the effect of serum on activity remain significant obstacles to the therapeutic use of these compounds.

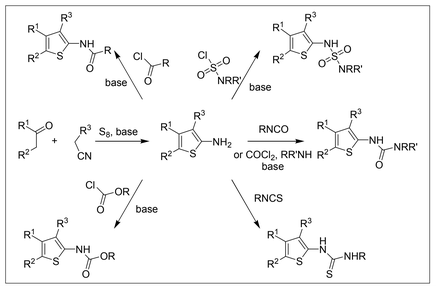
Introduction

Transcription is an essential event for cell growth and survival, catalyzed by the DNA dependent RNA polymerase (RNAP) core enzyme. This protein associates with a variety of sigma factors to form the RNA polymerase holoenzyme. The essential role of this holoenzyme has been established through the intracellular production, under regulated expression, of bacteriophage inhibitory peptides targeting the sigma factor of *S.aureus*. In addition, the enzyme is a validated drug target, through its inhibition by the Rifamycin class of antibacterials. There have been few reports of other classes of RNA polymerase inhibitors as antibacterials, but in nearly all cases, these were complex natural products. Small molecule RNAP inhibitors have been recently reported for E.coli, but their inability to eradicate the host bacterium was also noted. Herein is described the development of a new class of compounds, with antibacterial activity, acting via the *S.aureus* RNAP holoenzyme.

Biological methods

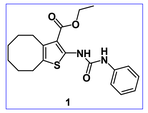
MIC's were determined by a microdilution method following NCCLS guidelines using cation-adjusted Mueller-Hinton broth (CAMHB) as the test medium. Resistance was measured at 8xMIC.
In vitro transcription assays were conducted using pB6 template DNA, 12.5nM σ^{SA}, 12.5nM RNA polymerase core enzyme, with α-[^{32}P]-UTP as marker. The progress of the reaction was measured in terms of counts after TCA precipitation.
Macromolecular synthesis studies were conducted using exponentially growing *S.aureus* in the presence of test compound and methyl-[^{3}H]- thymidine, 5-[^{3}H]-uridine and [^{35}S]-methionine to determine the extent of DNA, RNA and protein syntheses respectively.
In vivo assays involved the i.p. infection of female CD-1 mice followed by i.p. treatment with compound.

Synthetic scheme



Results

The screening of a library of 250,000 compounds (Array Biopharma, Chembridge, Maybridge, Talon) resulted in the identification of **1** as a hit.



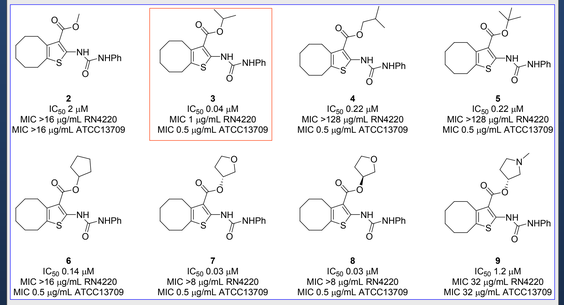
1

profile

IC50 1 μM
MIC 1-2 μg/mL in *S.aureus* ATCC13709
MIC 0.25 mg/mL in *S.epidermidis*
MIC > 128 μg/mL in 7 other *S.aureus* strains including RN4220
MIC > 128 μg/mL in other G+/G- strains
No Cytotoxicity
No promiscuity or effect on E.coli and mammalian *in vitro* transcription
Serum binding issue (MIC >128μg/mL in 50% serum)

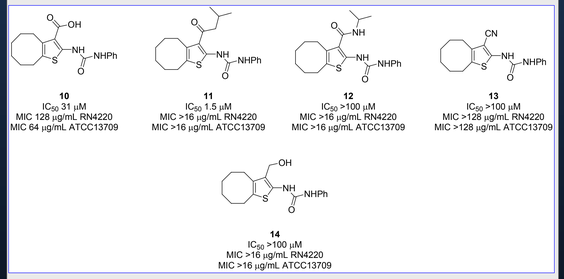
Structure activity relationships

Alterations of the ester functional group
• Ester substituents ►Isopropyl is optimum◄



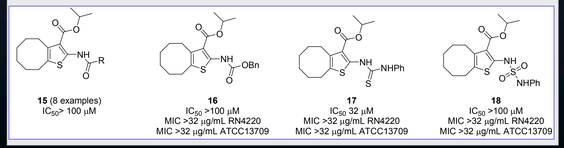
2 IC_{50} 2 μM; MIC >16 μg/mL RN4220; MIC >16 μg/mL ATCC13709
3 IC_{50} 0.04 μM; MIC 1 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
4 IC_{50} 0.22 μM; MIC >128 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
5 IC_{50} 0.22 μM; MIC >128 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
6 IC_{50} 0.14 μM; MIC >16 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
7 IC_{50} 0.03 μM; MIC >8 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
8 IC_{50} 0.03 μM; MIC >8 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
9 IC_{50} 1.2 μM; MIC 32 μg/mL RN4220; MIC 32 μg/mL ATCC13709

• Ester replacements ►ester is optimum◄



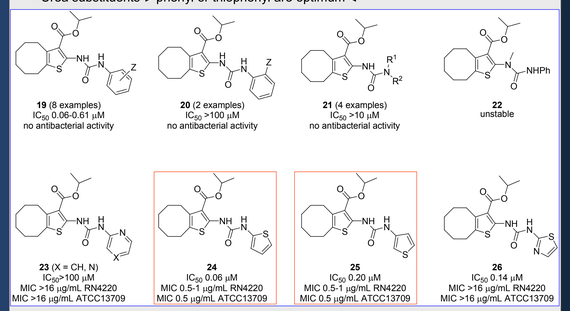
10 IC_{50} 31 μM; MIC 128 μg/mL RN4220; MIC 64 μg/mL ATCC13709
11 IC_{50} 1.5 μM; MIC >16 μg/mL RN4220; MIC >16 μg/mL ATCC13709
12 IC_{50} >100 μM; MIC >16 μg/mL RN4220; MIC >16 μg/mL ATCC13709
13 IC_{50} >100 μM; MIC >128 μg/mL RN4220; MIC >128 μg/mL ATCC13709
14 IC_{50} >100 μM; MIC >16 μg/mL RN4220; MIC >16 μg/mL ATCC13709

Alterations of the urea functional group
• Urea replacements ►urea is optimum◄



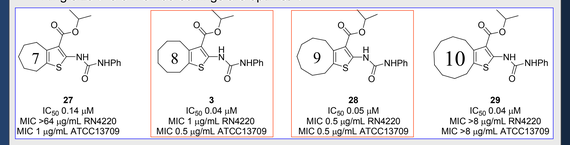
15 (8 examples) IC_{50}> 100 μM
16 IC_{50} >100 μM; MIC >32 μg/mL RN4220; MIC >32 μg/mL ATCC13709
17 IC_{50} 32 μM; MIC >32 μg/mL RN4220; MIC >32 μg/mL ATCC13709
18 IC_{50} >100 μM; MIC >32 μg/mL RN4220; MIC >32 μg/mL ATCC13709

• Urea substituents ►phenyl or thiophenyl are optimum◄



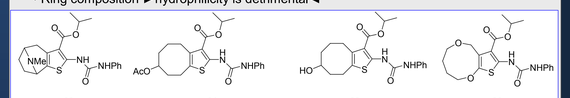
19 (8 examples) IC_{50} 0.06-0.61 μM; no antibacterial activity
20 (2 examples) IC_{50} >100 μM; no antibacterial activity
21 (4 examples) IC_{50} >10 μM; no antibacterial activity
22 unstable
23 (X = CH, N) IC_{50}>100 μM; MIC >16 μg/mL RN4220; MIC >16 μg/mL ATCC13709
24 IC_{50} 0.06 μM; MIC 0.5-1 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
25 IC_{50} 0.20 μM; MIC 0.5-1 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
26 IC_{50} 0.14 μM; MIC >16 μg/mL RN4220; MIC >16 μg/mL ATCC13709

Alterations of the hydrophobic ring
• Ring size ►8-9 membered rings are optimal◄



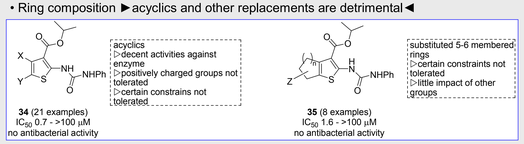
27 IC_{50} 0.14 μM; MIC >64 μg/mL RN4220; MIC 1 μg/mL ATCC13709
3 IC_{50} 0.04 μM; MIC 1 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
28 IC_{50} 0.05 μM; MIC 0.5 μg/mL RN4220; MIC 0.5 μg/mL ATCC13709
29 IC_{50} 0.04 μM; MIC >8 μg/mL RN4220; MIC >8 μg/mL ATCC13709

• Ring composition ►hydrophilicity is detrimental◄



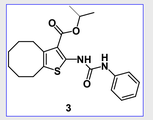
30 IC_{50} 2.5 μM; MIC 128 μg/mL RN4220; MIC 128 μg/mL ATCC13709
31 IC_{50} 0.9 μM; MIC >16 μg/mL RN4220; MIC >16 μg/mL ATCC13709
28 IC_{50} 1.5 μM; MIC >128 μg/mL RN4220; MIC >128 μg/mL ATCC13709
29 IC_{50} 0.8 μM; MIC >128 μg/mL RN4220; MIC >128 μg/mL ATCC13709

• Ring composition ►acyclics and other replacements are detrimental◄



34 (21 examples) IC_{50} 0.7 - >100 μM; no antibacterial activity
35 (8 examples) IC_{50} 1.6 - >100 μM; no antibacterial activity

Biological profiling of antibacterial activity



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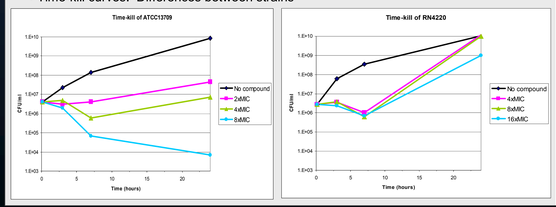
Antibacterial and cellular activity
• Antistaphylococcal spectrum
MIC 0.25 - 2 μg/mL observed in 47/48 Staph strains tested including *S.epidermidis*, *S.hyicus*, *S.carnosus* and 35 resistant *S.aureus* strains(rif-r, mup-r, MRSA,van-i MRSA)
• MIC>128 μg/mL with 50% serum and 16-32 μg/mL with 4% serum albumin
• Time-kill curves. Differences between strains



• No activity against other G+ organisms (*S.pneumoniae*, *E.faecalis*, *B.subtillis*, *S.pyogenes*)

• No activity against other G- organisms (*E.coli*, *P.aeruginosa*, *H.influenzae*, *S.tymphimurium*)

• No antifungal activity (*C.albicans*, *C.parapsilosis*)

• No cytotoxicity with HeLa cells (MTS assay) or human primary hepatocytes (ATP assay)

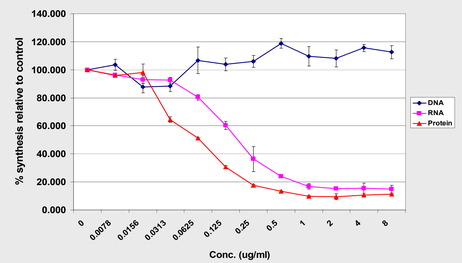
Resistance
• Strain dependent resistance
• Frequency of resistance at 4xMIC
3.2x10^{-5} against RN4220
6.8x10^{-8} against ATCC13709

Counterscreens
• No activity against *E.coli* and mammalian *in vitro* transcription

• No activity against reconstituted *S.aureus* DNA replication machinery

• No DNA-binding (dye displacement assay)

Inhibition of macromolecular synthesis



In vivo activity
• No acute toxicity at up to 2x100 mg/Kg *i.p.* or 2x25 mg/Kg *i.v.*
• Demonstrated efficacy on mouse peritonitis model when used *i.p.* at 2x100 mg/Kg
• No effect on mouse peritonitis model when used *i.v.* or *p.o.* (bioavailability of ~8%)
• No effect in thigh infection model
• No effect in wound infection model

Conclusion

❶ A new class of selective *S.aureus* RNA polymerase inhibitors with selective activity against *Staphylococci* has been developed. The structural requirements for activity have been fairly stringently defined.

❷ A key member of this class has been shown to selectively inhibit transcription and translation over replication, and to demonstrate efficacy in the mouse peritonitis model when used *i.p.*

❸ The strain dependence of the time-kill curves and frequency of resistance would require further investigation and the detrimental effect of serum on antibacterial activity remains a significant hurdle to the further development of this class of compounds into useful therapeutics.

Further readings

1) Salgado, C.D. et al *Clin. Infect. Dis.* **2003**, *36*, 131-139
2) Liu, J. et al *Nat Biotechnol.* **2004**, *22*,185-191
3) Dehbi, M. et al *45th ICAAC* **2005**, poster and abstract F-1845