Leiden University
Medical Center

Novel antibiotics inhibiting DNA polymerases from Gram-positive bacteria

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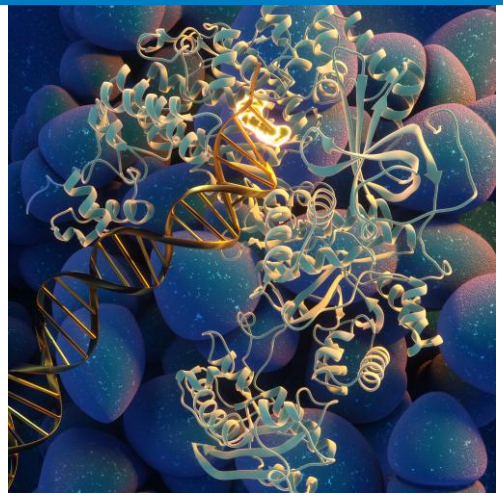
MACHINES ON GENES 2025



Art by Ella Maru Studio

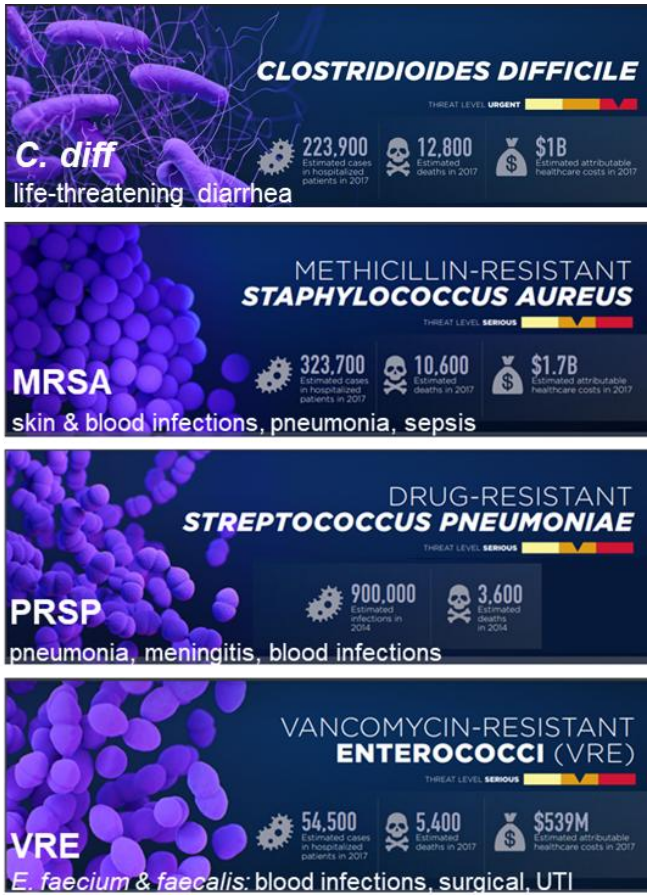
The urgent need for novel antibiotics

- Antibiotic resistance is a silent pandemic
- Predicted to cause 10 million deaths annually by 2050 (WHO)
- In the last 30 years only 1 novel antibiotic with new mode-of-action

We need:

- **new chemotypes**
- **new targets**

DNA polymerase PolC: novel antibiotic target



New chemotype

- class of guanine analogues
- specifically inhibit PolC

New Target

- PolC is <u>the</u> replicative DNA pol in Gram^{+}
- Clinically unexploited
- Distinct structure from human DNA polymerases

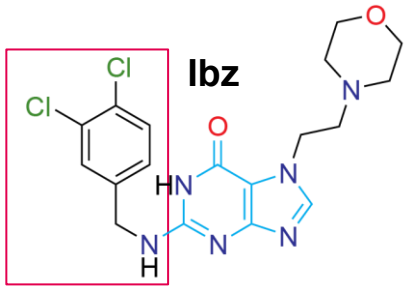
Xu (2019) Bioorg Med Chem
CDC: https://www.cdc.gov/antimicrobial-resistance/data-research/threats/index.html

First-in-class: Ibezapolstat (Ibz) entering Phase 3 trials

Phase 2 clinical trials **Acurx Pharmaceuticals**

- eradicates *C. difficile* infection (gut)
- favourable safety profile
- narrow spectrum → microbiome-sparing

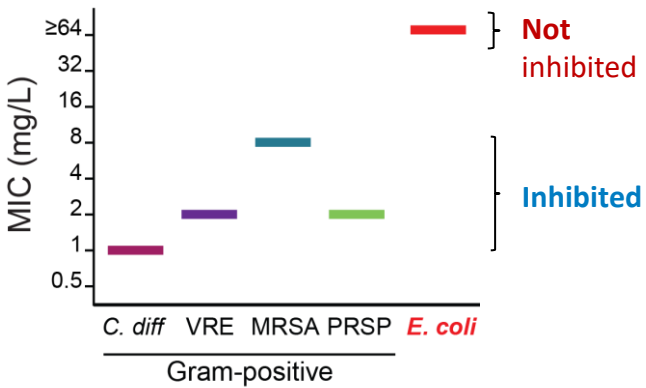
Garey (2022) Clin Infect Dis / Garey (2020) J Antimicrob Chemother



PolC-binding guanine core

Acurx Pharmaceuticals

Ibz specifically inhibits Gram⁺ growth

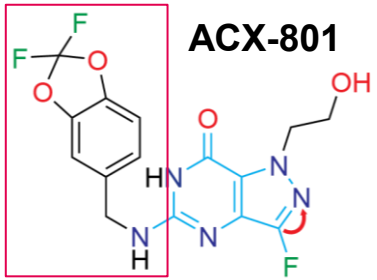


- Specific via N2-aromatic group
- Hydrophobic: only suitable in gastro-intestinal tract (and not for other infections)

Modified core improves absorption & pharmacokinetics

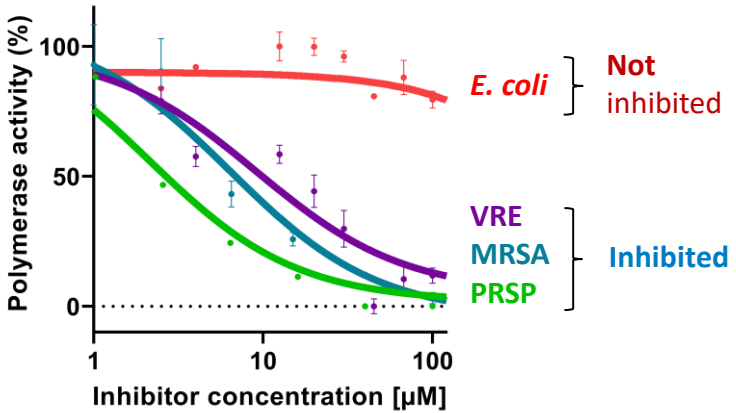
Basis for next generation inhibitors

- Screening: inhibition of bacterial growth → Gram$^+$ specific
- Assess candidates: inhibition of polymerase activity → PolC specific
- ACX-801 was identified as a potent inhibitor

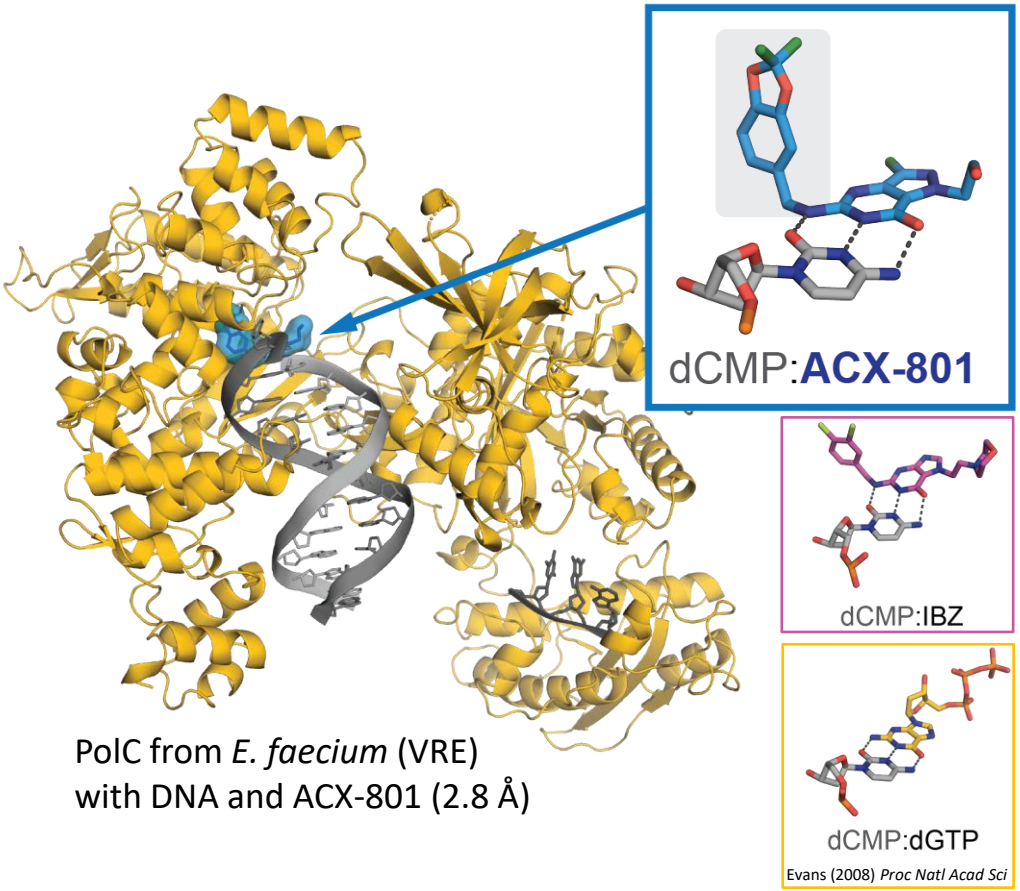


Acurx Pharmaceuticals

Only PolC activity inhibited *in vitro*



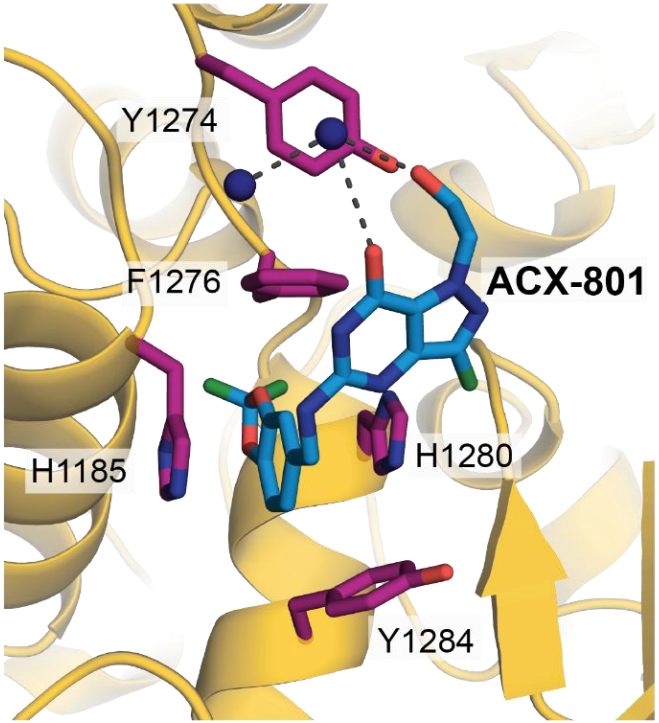
PolC-bound inhibitors adopt non-planar conformation



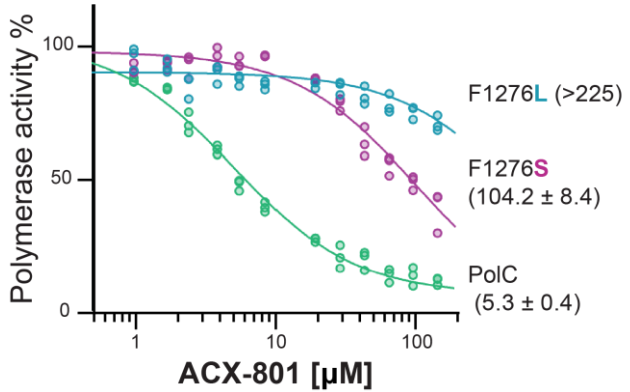
PolC from *E. faecium* (VRE)
with DNA and ACX-801 (2.8 Å)

- Cryo-EM structure to elucidate mode-of-action of Ibz and ACX-801
- Nucleobases: base-pair with dCMP in template strand
- The N2-linked aromatic groups
  - perpendicular to the nucleobase
  - not observed in solution (NMR)
  - no binding pocket in the apo structure (DNA- and inhibitor-free)

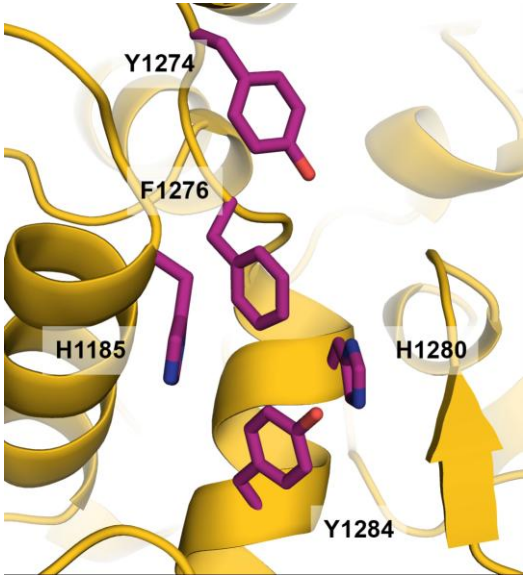
Highly conserved aromatic residues form the binding pocket



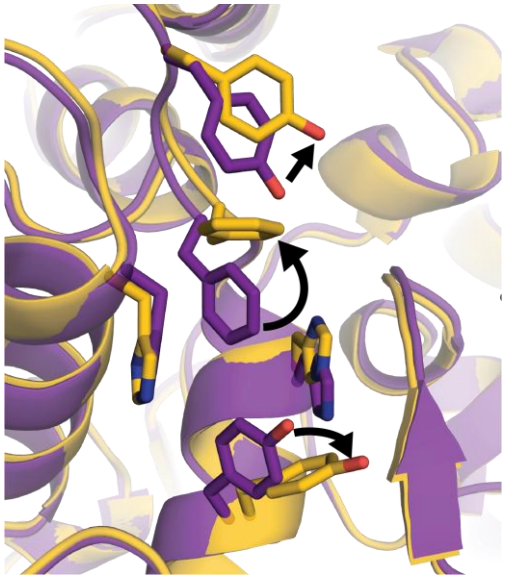
- π-π stacking interactions with N2 aromatic group
  - F1276 with N2 group **and** nucleobase
  - F1276 is a hotspot for lab-evolved mutations
  - Mutants retain active but decrease susceptibility



The inhibitors induce pocket formation by displacing the aromatic residues



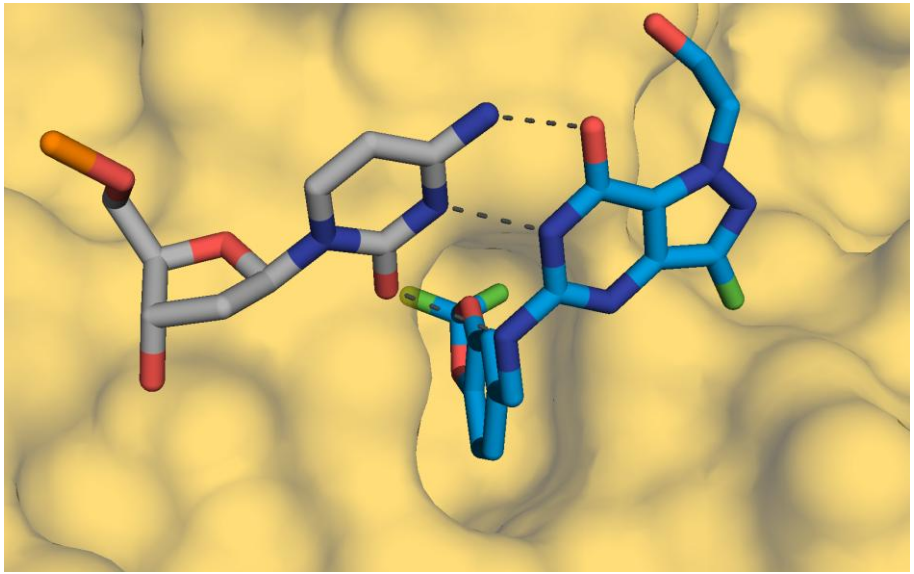
Movement required to create a full inhibitor binding pocket



ACX-801 vs Apo

- Pocket not observed in absence of inhibitor (apo)
- H1185: not conserved in Gram-
- F1276: hotspot for lab-evolved resistance mutations

Key to inhibitor mode-of-action:



- Pocket formation through induced conformational change
- Dependent on non-planar inhibitor conformation

Guide rational design of new compounds with improved inhibitory activity

Leiden University Medical Center

Acknowledgments

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NeCEN Netherlands Centre for Electron Nanoscopy

Health~Holland

Acurx Pharmaceuticals