A unique inhibitor conformation selectively targets the DNA polymerase PolC of Gram-positive priority pathogens

Machines on Genes, FASEB, May 2025

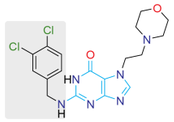
Mia Urem[1,2], Annemieke H. Friggen[1], Nina Musch[1], Michael H. Silverman[3], Christopher J. Swain[3], Michael R. Barbachyn[3], Lawrence I. Mortin[3], Xiang Yu[3], Robert J. DeLuccia[3], Meindert H. Lamers[1,4], Wiep Klaas Smits[1,2]

1 Leiden University Medical Center (LUMC), The Netherlands **2** Centre for Microbial Cell Biology, The Netherlands **3** Acurx Pharmaceuticals Inc., Staten Island, NY, USA **4** Netherlands Centre For Electron Nanoscopy (NeCEN), The Netherlands

Introduction

Bacterial replicative DNA polymerases, which are structurally distinct from their eukaryotic counterparts, are attractive yet unexploited targets for antibiotics development [1]. The DNA polymerase in Gram-positive bacteria, PolC, is specifically inhibited by guanine nucleobase analogues with aromatic modifications at the N^2-position like Ibezapolstat [2].

Ibezapolstat (IBZ)

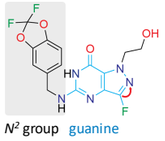


N^2 group guanine core

IBZ is currently in preparation for Phase 3 clinical trials against *Clostridioides difficile* infections (CDI) [3-4]. However, due to its poor absorbability, it is not a suitable treatment against other pathogens.

The next-generation of compounds, such as ACX-801, are being designed with distinct pharmacokinetic properties [5]. This development requires insight into the structural determinants for inhibition and resistance. However, mechanistic insight into the mode of action of PolC inhibitors was lacking.

ACX-801



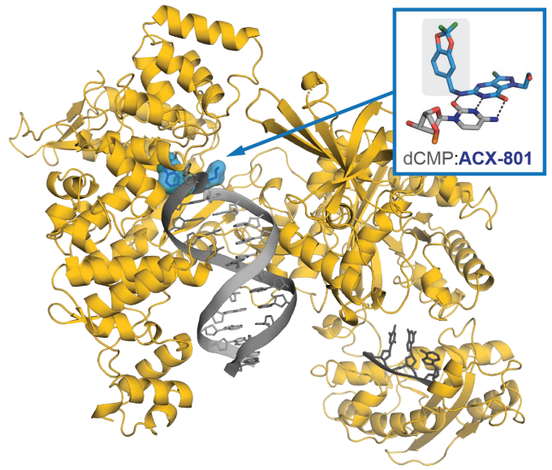
N^2 group guanine core

Redesigned to treat other Gram-positive bacteria, e.g. infections by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE).

References

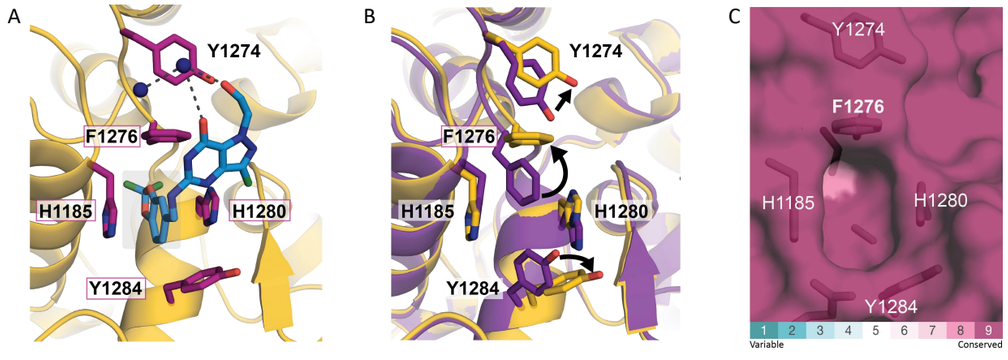
[1] M. S. Butler *et al.* (2024) https://doi.org/10.1021/acsinfecdis.4c00218
[2] W.-C. Xu *et al.* (2019) https://doi.org:10.1016/j.bmc.2019.06.017
[3] K. W. Garey *et al.* (2022) https://doi.org:10.1093/cid/ciac096
[4] https://www.acurxpharma.com/news-media/press-releases/detail/94/acurx-updates-phase-3-readiness-for-ibezapolstat-in-c
[5] https://www.acurxpharma.com/pipeline/gpss
[6] M. Landau *et al.* (2005) https://doi.org:10.1093/nar/gki370

The inhibitors adopt a non-planar conformation when bound to PolC



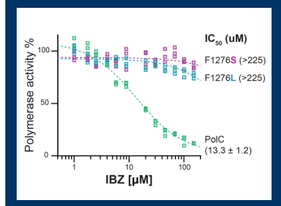
The cryo-EM structure of *E. faecium* PolC (yellow) was resolved in complex with DNA and ACX-801 (2.8 Å) or and IBZ (3.1 Å). This revealed that the nucleobase core base-pairs with DNA while the N^2 aromatic groups are positioned perpendicularly at an angle of ~90° within the polymerase domain of PolC (shown for ACX-801 in blue).

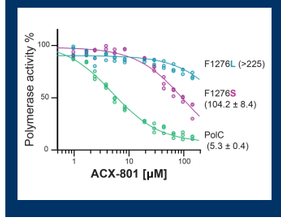
The aromatic N^2 group induces the formation of a binding pocket not observed in inhibitor-free PolC



Four aromatic residues [A, pink] are central to the formation of the induced pocket. This pocket is observed in the ACX-801 [A and B, in yellow] and Ibz structures, but not the inhibitor-free PolC structure [B, in purple]. Conformational shifts, most drastically of F1276, are required to accommodate the inhibitors [B, highlighted with arrows from inhibitor-free (purple) to ACX-801-bound state (yellow)]. Sequence conservation, plotted on the surface of PolC [C, 6], shows that these aromatic residues are strictly conserved among >220 Gram+ bacteria.

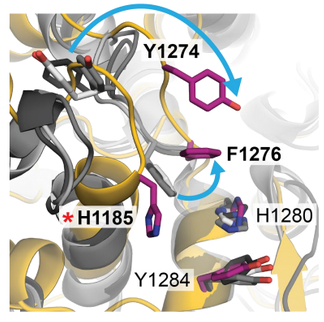
F1276 is a hotspot for lab-evolved resistance





Mutations in *polC* were observed at ≥4x MIC and predominantly at F1276, likely leading to loss of interactions with the N^2 moiety and the nucleobase. F1276S (pink) and F1276L (blue) mutations increased the resistance to Ibz and ACX801; the IC_{50} was increased 20x in polymerase activity assays and the MIC was at least 8x higher in microbial assays.

Inhibition specifically targets PolC



Screens with various inhibitor analogues show that bacterial growth and polymerase activity is inhibited in Gram+ bacteria but not in *E. coli*. Comparison of PolC to the replicate DNA polymerases of Gram-negative bacteria (in shades of grey) indicates that significant movements (arrows) would be required to create a full binding pocket and that there is no structural equivalent of the absent H1185 (*) residue.

Conclusion

The distinctive non-planar conformation of ACX-801 and IBZ, together with high conservation of the induced binding pocket in PolC, suggests that this is a general mechanism for this class of inhibitor and is conserved in Gram-positive bacteria. The data presented here pave the way for the rational design of the next generation of targeted antimicrobial therapies against Gram-positive pathogens.

NeCEN

Health~Holland

This collaboration is co-funded by the PPP allowance for POLSTOP2 made available by Health~Holland, Top Sector Life Sciences & Health, to stimulate public-private partnerships.

Acurx Pharmaceuticals