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Dual mode of Action Broad-spectrum Bacterial Gyrase Inhibition by the Peptide Antibiotic Albicidin

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Albicidin[1] is the lead structure of a new class of topoisomerase IIA bacterial topoisomerase 4/IV (parE and parC protein domains) - gyrase (GyrA and GyrB protein domains) inhibitors with a dual mode of action that overcomes resistance to fluoroquinolones and other agents that inhibit this target. It exhibits broad spectrum antibacterial activity against key human Gram-positive and Gram-negative pathogens including *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. The observed resistance rate of <10-10 was mainly related to mutations in a nucleoside channel (Tsx) that facilitates entry of albicidin and analogs into the bacterial cytosol. In-vivo proof-of-concept has been demonstrated with a pharmacological optimised analog in a mouse infection study challenged with a ciprofloxacin resistant clinical E. coli isolate[2].

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[1] Grätz, S.et al. *ChemMedChem* **2016**, *11* (14), 1499–1502. https://doi.org/10.1002/cmdc.201600163.

[2] Zborovsky, L. et al. *Chem. Sci.* **2021**, *2021* (12), 14606–14617. https://doi.org/10.1039/D1SC04019G.

Its unique structure consists of four substituted and unsubstituted paraaminobenzoic acids (PABA), a central β-cyano-L-alanine (Cya) and at the N-terminus a methyl coumaric acid (MCA)[3].

The exact mechanism of action remained elusive[4]. To shed light on the binding mode, the structure of the complex of albicidin and DNA Gyrase was determined using cryoelectron microscopy[5]. As a result, a ternary complex consisting of E. coli DNA gyrase, a 217 bp double-stranded DNA fragment and albicidin was elucidated. The C-terminal part of the peptide obstructs the gyrase dimer interface, while the N-terminal part intercalates into the gyrase bound DNA indicating a unique dual binding mechanism. The most relevant interactions involve hydrophobic interactions of the methoxy groups on the substituted PABA units with a hydrophobic pocket on the gyrase dimer interface, hydrogen bonding of Cya with Glu744 and the amide backbone with a conserved Mg2+-ion and π-π-stacking interactions of the MCA with the gyrase bound DNA.

Consequently, albicidin effectively blocks DNA gyrase, preventing it from reconnecting the DNA strand and completing its catalytic cycle This results in irreversible DNA damage, which triggers an SOS response of the bacterial cell, which leads to cell apoptosis.

The elucidated inhibition complex enables rational design of novel albicidin derivatives to further improved the potency and spectrum of activity of our albicidin analogs, and to further optimise the solubility, permeability, ADMET, PK and PD towards IND-enabling candidates.

350 words

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[3] Cociancich, S. et al. *Nature Chemical Biology* **2015**, *11* (3), 195–197. https://doi.org/10.1038/nchembio.1734.

[4] Hashimi, S. M. et al. *Antimicrob. Agents Chemother.* **2007**, *51* (1), 181. https://doi.org/10.1128/AAC.00918-06.

[5] Michalczyk, E et al. *Nature Catalysis* **2023**. https://doi.org/10.1038/s41929-022-00904-1.