Antimicrobial Activity of Non-Hydroxamate LpxC Inhibitors

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Abstract

Background: LpxC is a zinc-dependent deacetylase responsible for the biosynthesis of lipid A, an essential component of Gram-negative bacteria. LpxC inhibitors have been shown to beidal or key Gram-negative pathogens. Presented herein is the in vitro characterization of Forge's non-hydroxamate LpxC inhibitors including enzymatic and antimicrobial activity, time-kill studies, frequency of resistance profiling, and off-target effect screening.

Methods: LpxC inhibitors were screened in an enzymatic assay using the RapidFire/MS system and a fluorescent thermal stability assay with Sypro Orange Dye. The antimicrobial activities of Forge's LpxC inhibitors were measured by minimum inhibitory concentration (MIC) determination using CLSI methods against a broad panel of Gram-negative and Gram-positive reference strains as well as MDR strains and clinical isolates, which included extended-spectrum beta-lactamases (ESBL), carbapenem-resistant Enterobacteriaceae (CRE), and E. coli harboring mcr-1. Development of resistance by Enterobacteriaceae was studied by standard spontaneous mutation frequency method. Bacterial kinetics against Gram-negative organisms were determined by time-kill assays. Potential off-target activity was assessed using commercially available fluorescence-based enzyme assays and cytotoxicity assay in several human cell lines.

Results: LpxC Inhibitor FG-944 has nanomolar \(IC_{50}\) values for E. coli LpxC enzyme, increases thermal stability of E. coli LpxC protein by 14\(^{\circ}\)C and has MIC values less than 0.5\(\mu\)g/mL against wild type and MDR Enterobacteriaceae while having no activity against Gram-positive organisms such as S. aureus. Resistance frequencies and time-kill kinetics are comparable to historical values for hydroxamate-based LpxC inhibitors. Compounds show no cytotoxicity against human cell lines and no activity against other metabolic enzymes in enzymatic assays at levels > 50X in vivo efficacious free drug concentrations.

Antimicrobial Activity of FG-944 and comparators against a panel of MDR and KDR. Enterobacteriaceae spp. clinical isolates including ESBL and CRN strains.

Enzyme Assay

Inhibition of E. coli and K. pneumoniae LpxC activity by FG-944. \(IC_{50}\) values were determined using a mass spectrometry-based assay to measure de-acetylation of LPS substrate by LpxC protein. Shift in E. coli LpxC stabilization upon binding of FG-944 is 14\(^{\circ}\)C, as determined by StepOnePlus instrument using recombinant E. coli protein and Sypro Orange Dye.

Frequency of Resistance

Frequency of Resistance (FoR) studies in E. coli and P. mirabilis confirm FG-944 has similar resistance profile to historical LpxC inhibitors. To confirm that the reduced susceptibility to FG-944 was stable, the resistant mutants were passaged five times in the absence of selection before being subjected to further susceptibility testing with levofoxacin, polymyxin B, FG-944, CHIR-090 and PF-5081090. The results showed that reduced susceptibility to FG-944 was retained for every isolate tested, and the increase in MIC was in the range 8- to 64-fold (mode 16- fold); similar increases in MIC were also observed for CHIR-090 and PF-5081090 (cross-resistance), but no MIC change for polymyxin B and polymyxin B. Preliminary data indicates that resistant clones carry mutations in FabZ gene and have slower growth rates in vitro.

Summary and Conclusions

Forge has identified mcr-hydroxamate LpxC inhibitor, FG-944 that:
- demonstrates in vitro on-target activity
- not cross-reactive with other tested Zn(II) metalloenzymes
- shows excellent activity against a variety of Gram-negative bacteria, including clinical isolates harboring plasmids containing the resistance genes mcr-1, ESBL, KPC, and NDM
- shows rapid bacterial activity against E. coli, K. pneumoniae and P. mirabilis
- inactive against Gram-positive organisms
- has FC50 rate on par with hydroxamic acid based LpxC inhibitors
- efficacious in mouse infection models (data presented in poster 644)

Acknowledgements

Dr. Michael Barbachyn, Dr. Seth Cohen, Dr. John Rex, Dr. Karen Shaw, Dr. Lynn Silver, Dr. Andres Tomaras, and Dr. Mark Whittaker for support and helpful discussions.

Inhibition of MMP-2 and MMP-12 (red) by FG-944 (solid lines) and PF-5081090 (dotted lines). Assays were run in triplicate using commercially available fluorescence-based enzyme assay kits.

FG-944 showed no activity in assays with other human metalloproteinases such as Mac, ADAMs, TIMPs, and MMP-12.

Time-Kill Experiments

FG-944 achieved bactericidal activity against E. coli BAA-2469, K. pneumoniae ATCC 43816 and P. mirabilis HM 752 with rapid concentration-independent killing rates. MIC\(_{50}\) values determined by microdilution method. Bacterial activity refers to 23 log\(_{10}\) CFU/mL (horizontal dotted line on the graph) reduction in viability relative to the starting inoculum after 24 h exposure to FG-944. The limit of detection for horizontal dashed line on the graph for these assays was 55 CFU/mL.

E. coli BAA-2469 FG-944 MIC = 0.25 \(\mu\)g/mL

K. pneumoniae ATCC 43816 FG-944 MIC = 2 \(\mu\)g/mL

P. mirabilis HM 752 FG-944 MIC = 4 \(\mu\)g/mL

Antimicrobial Activity of FG-944 and comparators against a panel of selected Gram-positive bacterial species. LpxC enzyme is found only in Gram-negative bacteria, hence no antimicrobial activity is expected against Gram-positive bacteria.

E. coli (n=100)

K. pneumoniae (n=100)

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Off-target Activity Assays

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