Drug Targeting of Novel Antimicrobials in the MEP Pathway:

Utilizing an *in silico* and *in vitro* based screening approach

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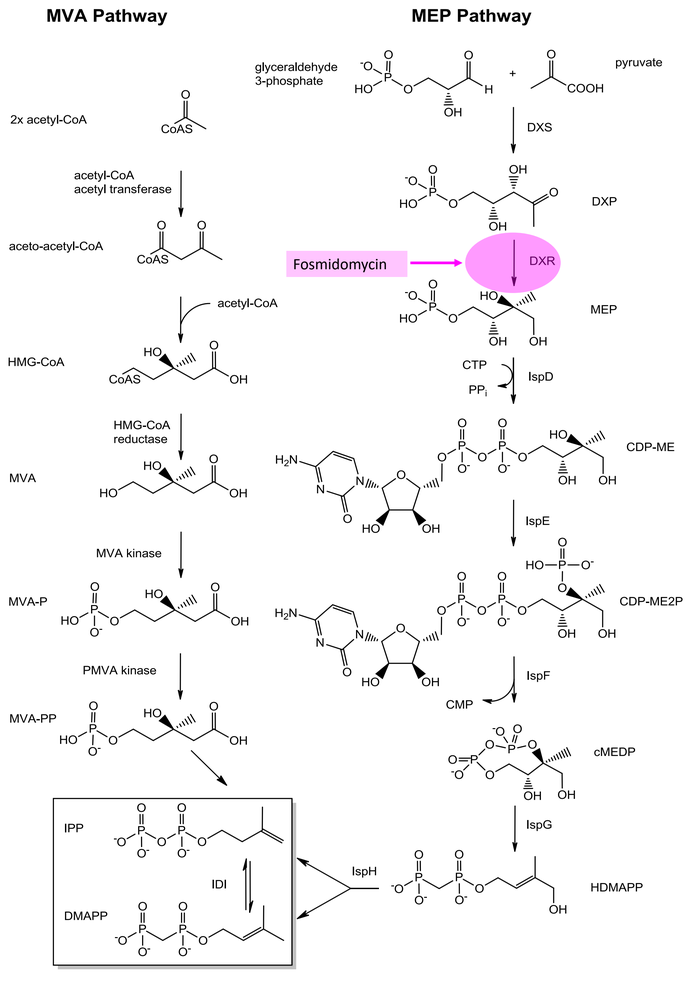
OVERVIEW

- Antibiotic resistance is a global problem, and the search for new antibiotics or bacterial targets continues to be a fruitful area of research, making the methylerythritol phosphate (MEP) pathway an attractive underexplored target.
- Utilizing computational methods we virtually screened a set of > 150,000 compounds and identified 200 potential deoxyxylulose reductoisomerase (DXR) inhibitors.
- A medium throughput enzyme inhibitor screen, which kinetically monitors inhibition of the DXR enzyme, was developed to evaluate these potential inhibitors.

INTRODUCTION

- Two unrelated essential pathways exist in nature for the biosynthesis of isoprenoid metabolites, which include the methylerythritol phosphate (MEP) pathway, unique to bacteria, and the mevalonate (MVA) pathway used by humans.
- The individual enzymatic steps of the MEP pathway are attractive for the development of new antibiotics targeted against them, as few inhibitors of this pathway have been described.
- The third committed step in the MEP Pathway, involving the conversion of deoxyxylulose phosphate (DXP) to MEP, was chosen as the target based on the known inhibitor fosmidomycin.



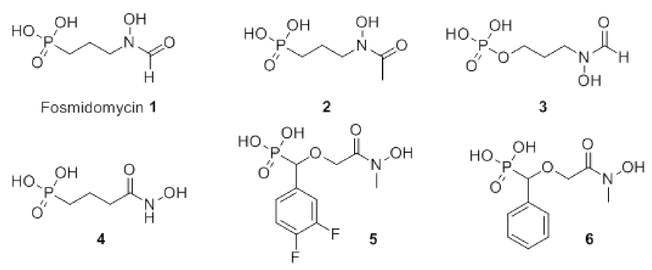
Screening Strategies for the Identification of Potential Antimicrobials

- ❖ *In Silico* Virtual Screen of the DXR enzyme and compound library
- ❖ *In Vitro* Inhibitor Screen developed to validate enzymatic target
- ❖ Whole-Cell Based Screening approach to confirm hits and further analyze bacterial permeability, cell uptake, and efflux pump interactions.

Methods

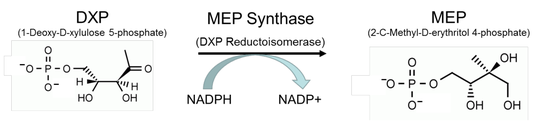
In Silico Screening: Computational Background

- The published crystal structure (PDB 2v2z) was used to model the binding site.
- The antibiotic fosmidomycin and a series of known inhibitors (2-6) were used as a training set to determine plausible conformations of the binding site.
- A database of 63,808 molecules was obtained in SDF format and converted into a 3D database with 5 conformations selected for each structure for further evaluation in docking experiments.

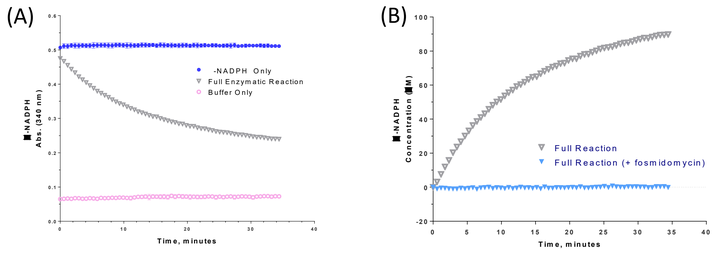


In Vitro Screening: MEP Synthase (DXR) Inhibitor Screen

DXR Inhibitor Screen



- 189 compounds were selected, weighed, and "blindly" plated in a 96 well plate format for screening. *(Performed by Frontier Scientific, Newark, DE)*
- Compound plates were transferred to Echelon Biosciences for testing
- Plated compounds were pre-incubated with the DXR enzyme with shaking for 10 minutes.
- Deoxyxylulose Phosphate (DXP) substrate was then added to start the reactions.
- The absorbance was recorded in kinetic mode at 340 nm.
- Data was analyzed blindly at Echelon Biosciences before compounds were decoded by Frontier Scientific

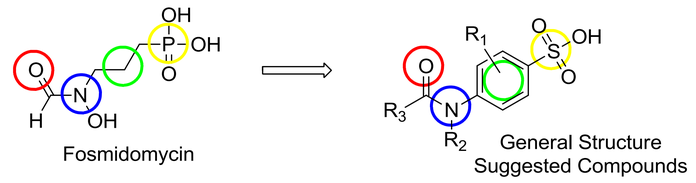


The DXR Inhibitor Screen monitors a decrease in β-NADPH levels which directly corresponds with the conversion of the DXP substrate to MEP product (A). The screen will monitor compounds for inhibition of DXR activity, as demonstrated by the fosmidomycin control inhibitor, shown at 100 µM (B).

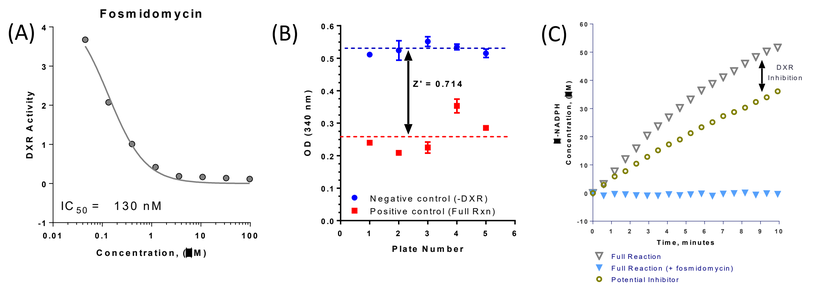
RESULTS

Results

- The library was docked into the binding site using ROCS (OpenEye Scientific) that identified 200 suggested compounds for *in vitro* screening. *(Performed by Hudson Robotics, Springfield, NJ)*
- Many of the top suggestions contained similar pharmacophore traits as fosmidomycin. For example, many of the hits suggested a sulfate group (yellow) in substitution for the phosphate group (yellow) and a substituted aryl or alkyl chain for the middle linker group (green) in fosmidomycin.
- A wide range of variations was suggested for combinations of the carbonyl (red) and hydroxyl amine moiety (blue), but in general a combination of hydrogen accepting groups were presented.



Results



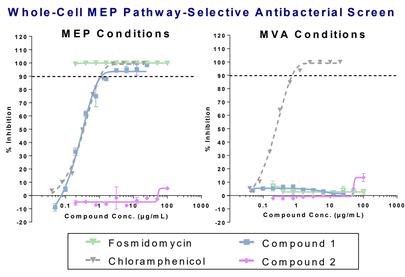
- Following assay optimization; the fosmidomycin positive control was further evaluated and demonstrated an IC_{50} value of 130 nM, similar to inhibitory values described previously (A).
- The inhibitor screen when averaged demonstrated a Z' factor of 0.714 (B).
- Two of the 200 test compounds showed modest inhibition of 5 - 10% at 100 µM with a third compound inhibiting 32% of DXR activity at this same concentration (C).

Next

Follow up hit to lead identification in a cell based screening assay.

We developed an innovative genetically-engineered whole-cell (Salmonella typhimurium) phenotypic screen to identify compounds that selectively inhibit the MEP pathway. Hits from this screen produce three significant results:

1. The compound is antibacterial.
2. The compound hits the MEP pathway.
3. The compound does not affect the MVA pathway- presumably with low potential for human toxicity.



CONCLUSIONS & ACKNOWLEDGEMENTS

- ❑ Our work highlights the potential of multiple discovery platforms to target the bacterial MEP pathway for identifying future novel antibiotics.
- ❑ The DXR Inhibitor Screen is quick, convenient, and an effective assay for measuring DXR inhibition.
- ❑ *In Silico* screening coordinated with optimized compound libraries remains a promising method for early identification of potential inhibitors.
- ❑ We thank Dr. Colin Ferguson, Dr. Charles Testa, and Dr. Mark Brown for the early development of MEP associated reagents.

DXR Inhibitor Screen is available from Echelon Biosciences, Inc.
For more information, please visit our website (www.echelon-inc.com)

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