Targeting Key Biosynthetic Pathways as an Antichlamydial Strategy

McCafferty Lab
Duke University
Department of Chemistry
Chlamydia trachomatis is a public health threat

C. trachomatis

Infection

Often Asymptomatic

Increases chance of contracting HIV

Linked to cervical cancer

Pelvic Inflammatory Disease

Ectopic Pregnancies

Infertility

*C. trachomatis* is internalized through inclusions that increase in size over infection progression.

*Chlamydia trachomatis* is an obligate intracellular pathogen that relies on menaquinone for survival.

- Limited Genome → Limited Biosynthetic Activity → Heavily Reliant on Host for Metabolites → Obligate Intracellular Pathogen
- Obligate Intracellular Pathogen → Retained Bacterial Electron Transport Pathway → Requires Means to Transport Electrons → Menaquinone


Menaquinone biosynthesis relies on two biosynthetic pathways:

- **Shikimate pathway**
- **Futalosine pathway**

Aromatic Amino Acids

Chorismate

Folates

Menaquinone-7
Shikimate Pathway

1. + 2. \[ \rightarrow 3. \]

3. \[ \rightarrow 4. \]

4. \[ \rightarrow 5. \]

5. \[ \rightarrow 6. \]

6. \[ \rightarrow 7. \]

7. \[ \rightarrow 8. \]

8. \[ \rightarrow 9. \]

Shikimate Kinase

Menaquinone Biosynthesis

Aromatic Amino Acids Biosynthesis

Folates

chorismate
Futalosine Pathway

\[ \text{chorismate} \rightarrow \text{10} \rightarrow \text{11} \rightarrow \text{12} \]

\[ \text{13} \rightarrow \text{14} \rightarrow \text{15} \]

\[ \text{16} \rightarrow \text{17} \text{ menaquinone} \]
Known futalosine pathway inhibitors reduce chlamydia inclusion size and number

Dudiak, B. M.; Nguyen, T. M.; McCafferty, D. G. In Preparation.
Targeting enzymes in both shikimate and futalosine pathways

- **Shikimate pathway**
- **Futalosine pathway**
- Aromatic Amino Acids
- Folates

Starting from chorismate, the shikimate pathway leads to aromatic amino acids, while the futalosine pathway leads to folates. Both pathways ultimately contribute to the production of menaquinone-7.
CT367 is a shikimate kinase from the shikimate pathway.

\[ \text{CT367} \] is a shikimate kinase from the shikimate pathway.
Growing interest in kinase inhibitors for pathogens

Fluorescence Linked Chemoproteomic Strategy Screen (FLECS) for kinase inhibitors

ATP resin loaded with crude CT367-GFP E. coli lysate

Loaded ATP resin aliquoted into 96-well filter plate placed atop a 96-well catch plate

Each well treated with a known kinase inhibitor and incubated for 15 min

Wells showing fluorescence contain a kinase inhibitor that bound CT367

Compounds that do not bind CT367 will leave CT367 attached to the ATP resin

Compounds that bind CT367 result in elution of GFP tagged CT367

Validate CT367 activity *in vitro* through enzyme assays
Targeting enzymes in both shikimate and futalosine pathways

Shikimate pathway

Futalosine pathway

Aromatic Amino Acids

Folates

chorismate

menaquinone-7
CT263 is an MTAN-like enzyme from the futalosine pathway.

The diagram illustrates the biochemical pathway, starting with chorismate (9) and ending with menaquinone (17). The reactions involve the enzymes CT427, CT426, CT767, CT262, CT263, CT085/CT220, and CT219.
Synthesis of Potential CT263 inhibitor

Synthesis of CT263 Substrate

Enzymatic and Cell Based Assays

CT263 + Adenine -> 2,8-dihydroxyadenine

Absorbs at 305 nm

DAPI  Chlamydia trachomatis