Mycobacterium tuberculosis NAD kinase: druggability analysis for lead generation

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Abstract

The NAD kinase (NadF) enzyme is an attractive target for the discovery of novel antibiotics against many pathogenic bacteria, including M. tuberculosis. SiteMap analyses using the apo and NAD bound 1Y3I structures suggested that hydrophilic contributions to binding dominated the NAD binding site, leading to a poor drug score. This binding was further probed by biochemical screening campaigns using diverse and focused libraries. These campaigns resulted in low hit rates as compared to similar targets. The NadF hits were confirmed to genuine leads using protein-thermal stability (Tm shift) and enzyme kinetic assays. The hits appeared to be highly hydrophilic and were attributed to hydrophilic interactions, suggesting hypotheses that this could potentially be due to interactions with the predominantly hydrophilic active site. The dearth of adequate hydrophobic surface area based binding studies as well as ligand-observed 1D-NMR. Interestingly, the most potent compounds were found to have higher polar surface area (PSA). We hypothesize that this could potentially be due to interactions with the predominantly hydrophilic active site. The studies of druggability led to the conclusion that NadF is a challenging target to find drug like leads.

Introduction & Objective

• This co-factor NAD is essential for the growth of many microorganisms, including M. tuberculosis. SiteMap analyses using the apo and NAD bound 1Y3I structures suggested that hydrophilic contributions to binding dominated the NAD binding site, leading to a poor drug score. This binding was further probed by biochemical screening campaigns using diverse and focused libraries. These campaigns resulted in low hit rates as compared to similar targets. The NadF hits were confirmed to genuine leads using protein-thermal stability (Tm shift) and enzyme kinetic assays. The hits appeared to be highly hydrophilic and were attributed to hydrophilic interactions, suggesting hypotheses that this could potentially be due to interactions with the predominantly hydrophilic active site. The dearth of adequate hydrophobic surface area based binding studies as well as ligand-observed 1D-NMR. Interestingly, the most potent compounds were found to have higher polar surface area (PSA). We hypothesize that this could potentially be due to interactions with the predominantly hydrophilic active site. The studies of druggability led to the conclusion that NadF is a challenging target to find drug like leads.

Mtb NadF – Site map analysis of the NAD binding site

• A 1D WaterLOGSY NMR experiment was carried out and the data were processed to determine the NAD binding site.

Mtb NadF – Experimental Assessment & Screening Strategy

• Thermal melt analysis of compound 1
• Thermal melt analysis of compound 2
• Thermal melt analysis of compound 3
• Thermal melt analysis of compound 4

Thermal melt analysis of compound 1

Thermal melt analysis of compound 2

Thermal melt analysis of compound 3

Thermal melt analysis of compound 4

Mtb NadF – Experimental Assessment - Results

• Hit rates lower compared to screens against other targets using the same set of libraries (Typical hit rates for a focused screen 1-4 % using a cutoff >50% @ 20uM). For high throughput parallel screening 0.4-0.8% using a cutoff >50% @ 20uM).

Representative screening hits – confirmation of binding to Mtb NadF by ligand observed 1D NMR (WaterLOGSY) and thermal melt studies

• As anticipated from the SiteMap analysis, screening for Mtb NadF inhibitors resulted in hit rates that are appreciably lower as compared to screens against other targets.

• Many potent inhibitors have high polar surface area (PSA), which can be attributed to the hydrophilic nature of the NAD binding pocket.

• Improving inhibitor potency could be a challenge.

• Highly polar inhibitors may also pose permeation challenges for Mtb.

• The results from biochemical screening and computational analyses suggest that NadF is a challenging target to find drug like leads.

Methods

• All data are shown as mean ± SEM (n = 3) and obtained from experiments carried out in 50 mM Hepes pH 7.5, 50 mM NaCl, 5 mM MgCl2, 4% DMSO. 0.15 mg/ml of enzyme, 50 ìM compound, 6.25X Sypro Orange (Invitrogen) in 25 ul 96 well PCR plates in Bio-Rad IQ5 Thermocycler. Ex: 470nM Em: 540nM

• Assays were carried out in 50 mM Hepes pH 7.5, 50 mM NaCl, 5 mM MgCl2, 4% DMSO. 0.15 mg/ml of enzyme, 50 ìM compound, 6.25X Sypro Orange (Invitrogen) in 25 ul 96 well PCR plates in Bio-Rad IQ5 Thermocycler. Ex: 470nM Em: 540nM

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• Tm shift experiments

• Detection mix containing 0.25 mM XTT, 0.1 mM PES, 0.008 % Sodium dodecyl sulfate and 10 mM N-ethylmaleimide and the plate read at 490 nm

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