Bioorganic & Medicinal Chemistry Letters 23 (2013) 6860–6863

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Cytidine derivatives as IspF inhibitors of *Burkolderia pseudomallei*

Zheng Zhang [a], Sriram Jakkaraju [a], Joy Blain [a], Kenneth Gogol [a], Lei Zhao [a], Robert C. Hartley [c,d], Courtney A. Karlsson [c,d], Bart L. Staker [b,c,d], Thomas E. Edwards [c,d], Lance J. Stewart [c,d], Peter J. Myler [b,c], Michael Clare [d], Darren W. Begley [c,d], James R. Horn [a], Timothy J. Hagen [a,*]

[a] Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb, IL, USA
[b] Seattle BioMed, 307 Westlake Avenue N, Seattle, WA 98109-5219, USA
[c] Seattle Structural Genomics Center for Infectious Disease (www.ssgcid.org), USA
[d] Emerald Bio, 7869 NE Day Road West, Bainbridge Island, WA 98110, USA

ARTICLE INFO

Article history:
Received 1 September 2013
Revised 30 September 2013
Accepted 30 September 2013
Available online 8 October 2013

Keywords:
Fragment screening
MEP pathway
IspF
Non-mevalonate
Anti-infective
SPR

ABSTRACT

Published biological data suggest that the methyl erythritol phosphate (MEP) pathway, a non-mevalonate isoprenoid biosynthetic pathway, is essential for certain bacteria and other infectious disease organisms. One highly conserved enzyme in the MEP pathway is 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (IspF). Fragment-bound complexes of IspF from *Burkholderia pseudomallei* were used to design and synthesize a series of molecules linking the cytidine moiety to different zinc pocket fragment binders. Testing by surface plasmon resonance (SPR) found one molecule in the series to possess binding affinity equal to that of cytidine diphosphate, despite lacking any metal-coordinating phosphate groups. Close inspection of the SPR data suggest different binding stoichiometries between IspF and test compounds. Crystallographic analysis shows important variations between the binding mode of one synthesized compound and the pose of the bound fragment from which it was designed. The binding modes of these molecules add to our structural knowledge base for IspF and suggest future refinements in this compound series.

Enzymes from the methyl erythritol phosphate (MEP) biosynthetic pathway are regarded to be promising targets for new anti-infective agents and herbicides.[1,2] This pathway is essential in *Plasmodium falciparum*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Burkholderia pseudomallei*, *Escherichia coli*, and other infectious disease causing organisms.[3–6] MEP enzymes are absent in mammalian species, which utilize the well-characterized and druggable mevalonate-dependent (MAD) pathway to synthesize isoprenoids.[7] Studies on fosmidomycin have indicated clinical utility with *P. falciparum* infections by inhibiting the MEP enzyme IspC, thus demonstrating the druggability in this alternative route for isoprenoid production.[1,8] Some cases of drug resistance have been observed for lone fosmidomycin chemotherapy, but if given in combination, fosmidomycin appears to retain its clinical activity towards *P. falciparum*.[9] Thus novel or combination chemotherapies which target multiple MEP enzymes should minimize the possibility for resistance mutations to develop and survive in pathogenic species, with minimal off-target effects in mammalian hosts lacking the MEP pathway.

The fifth enzyme in the MEP pathway is 2C-methyl-D-erythritol-2,4-cyclo-diphosphate synthase (IspF). Sequence analysis shows over 30% identity for IspF proteins across prokaryotic and eukaryotic species.[10] Structural studies show high conservation of residues essential in substrate-binding, catalysis, and other aspects of IspF enzymatic function.[8] Thus compounds which inhibit the IspF enzyme from any given pathogenic organism could possess some potential for cross-species activity. Previously, we conducted fragment-based screens with IspF from *B. pseudomallei* (*Bp*IspF) by nuclear magnetic resonance (NMR) spectroscopy, and by X-ray crystallography.[10] Screening generated a collection of hits (Fig. 1), and led to 14 fragment-bound complexes with as many as three small molecules bound in unique sites of the protein.

Three distinct binding sites for fragments emerged, two of which exist in the *Bp*IspF active site: the cytidine recognition pocket, and an adjacent pocket surrounding the catalytic zinc ion (Fig. 2). Only cytosine, cytidine and cytidine phosphate derivatives were observed crystallographically in the cytidine binding pocket, despite broad chemical diversity and metabolite variants, including other close analogs of cytidine, in our screening collection.[11] Conversely, a range of chemical entities with differing orientations bound in the zinc pocket. In each case, an aromatic nitrogen occupies the available metal coordination site in the fragment-bound structure.[10] Structural characterization of these fragment hits provide chemical starting points as well as relative distance and orientation information, suitable to a linking strategy. We therefore set

* Corresponding author. Tel.: +1 815 753 1463; fax: +1 815 753 4802.
E-mail address: thagen@niu.edu (T.J. Hagen).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.09.101

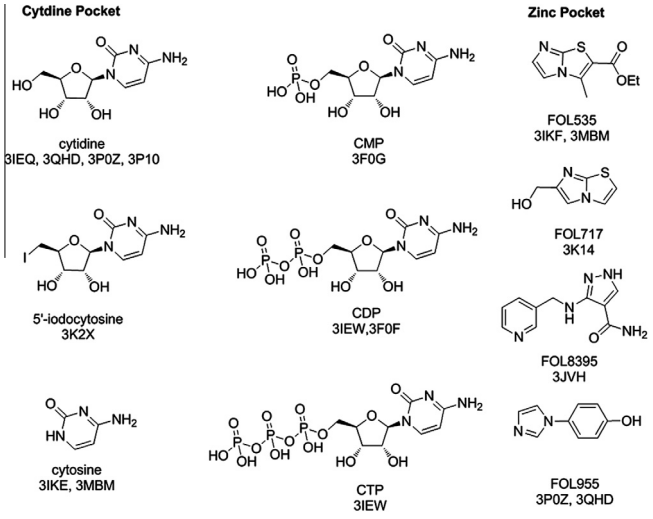


Figure 1. Fragment screening hits that bind to the cytidine and zinc pockets of *Bp*IspF.

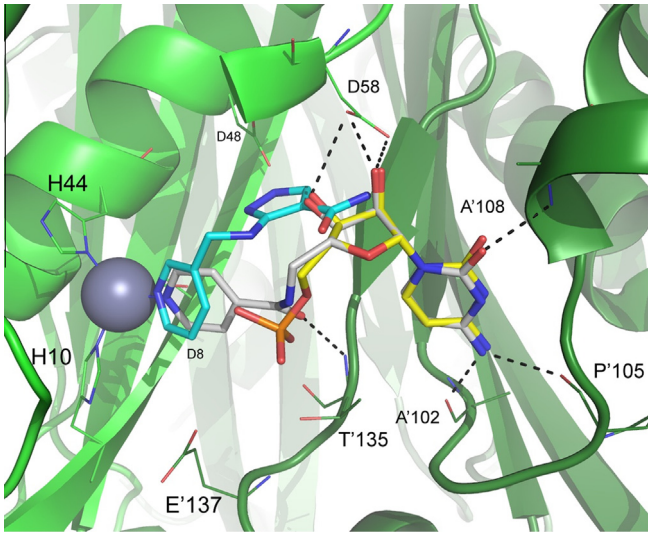
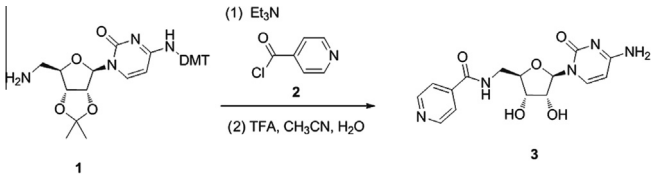


Figure 2. Crystal structure of compound **3** (carbon atoms white) bound to *Bp*IspF (PDB 3KE1) depicting the active site comprised of chains A (light green ribbon) and B (dark green ribbon). Overlaid are binding poses of fragments FOL8395 (carbon atoms cyan) and cytidine monophosphate (carbon atoms yellow) from PDB entries 3JVH and 3F0G, respectively[10] Ligand oxygen atoms are colored red, nitrogen blue, and phosphorus orange. Active site residues critical for ligand binding with high conservation across pathogenic organisms are indicated. Residues 64–72 from chain A were unmodeled due to poor electron density in the diffraction data. Residues 60–63 were removed from image for clarity. Image created using PyMol.[14]

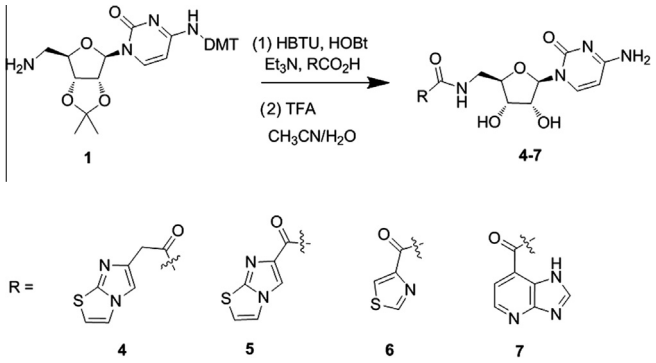
out to design and synthesize a series of molecules that connect the cytidine moiety to different zinc pocket fragment binders to form 'fusion' ligands.

Synthesis of fusion compounds began with intermediate **1**, which was synthesized according to literature procedure in six steps from commercially available (+)-cytidine.[12] Target fusion compounds were obtained by amide formation followed by deprotection. Two different amide coupling procedures were utilized. First, **3** was synthesized from **1** by coupling with the commercially available acid chloride **2** (Scheme 1).

Compounds **4**–**7** were synthesized from the amine **1** and coupled with commercially available acids using HBTU, HOBt and Et_3N followed by deprotection of acetonide and DMT with trifluoroacetic acid in acetonitrile/water (Scheme 2). The final products



Scheme 1. Synthesis of fusion compound **3**.



Scheme 2. Synthesis of fusion compounds **4**–**7**.

3–**7** were purified by reverse phase HPLC to yield the products as TFA salts.

Dissociation constants for the fusion analogs **3**–**7** binding *Bp*IspF were determined using surface plasmon resonance (SPR) similar to literature methods.[12] *Bp*IspF was immobilized using random amine, NHS/EDC coupling to a carboxylmethyl dextran SPR slide to give 3000–5000 μRIU (Refractive Index Units).

Concentration series for each compound from 2 mM to 1.7 μM (5% DMSO) were generated by serial dilution and injected over the IspF surface. Buffer-only injections were included between every compound injection at the same DMSO concentration. Data analysis was performed in scrubber (BioLogic Software), allowing double referencing (subtraction of buffer-only injection and reference flow cell). The binding responses were recorded as a function of time and at all compound concentrations, the responses reached equilibrium and dissociated rapidly, indicating that binding was reversible. Values for K_D were obtained by fitting the steady-state binding curves to a 1:1 Langmuir binding model.

Cytidine diphosphate (CDP) was found to have a K_D of 75 μM, similar to the K_D determined by Ramsden for IspF from *E. coli*.[12] Much of this binding energy appears due to polar interactions between the phosphates of CDP and the catalytic zinc of IspF, as demonstrated in *E. coli* crystal structures of IspF bound to the enzymatic product.[13] Our fusion series molecules generated a range of K_D from 70 to 200 μM (Table 1) without the diphosphate group of CDP present in the final molecule. Interestingly, each molecule in the fusion series gave exactly threefold higher molecular weight-adjusted RU_{max}, compared to CDP and several fragment hits tested by SPR. This is strongly indicative of 3:1 stoichiometric binding for the fusion series (Table 1), a result consistent with binding to each of the three active sites in the trimeric structure of *Bp*IspF. Binding to only one active site per trimer is also observed crystallographically for certain fragments.[10] However, this result does not correlate with affinity, nor explain the 1:1 binding observed for CDP. We speculate that the apparent 1:1 binding observed for CDP and certain fragments may be due to differential affinity across the three active sites in the *Bp*IspF trimer. Threefold symmetry is not preserved in any crystal structures solved for *Bp*IspF, due to slight structural differences in each protomer of the trimer in the crystal lattice.[10] Thus the 3:1 versus 1:1 binding observed by SPR