Design, Synthesis and Characterization of Novel Inhibitors Against
Mycobacterial β-Ketoacyl CoA Reductase FabG4

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Supporting Information

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S1. NMR spectra of selected compounds

Compound 6: $^1$H NMR (CDCl$_3$, 400 MHz) spectrum
Compound 8: $^1$H NMR (CDCl$_3$, 400 MHz) spectrum

Compound 10: $^1$H NMR (CDCl$_3$, 200 MHz) spectrum
Compound 10: $^{13}$C NMR (CDCl$_3$, 50 MHz) spectrum

Compound 11: $^1$H NMR (CDCl$_3$, 400 MHz) spectrum
**Compound 11:** $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum

![Compound 11: $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum](image)

**Compound 12:** $^1$H NMR (CDCl$_3$, 200 MHz) spectrum

![Compound 12: $^1$H NMR (CDCl$_3$, 200 MHz) spectrum](image)
Compound 12: $^{13}$C NMR (CDCl$_3$, 50 MHz) spectrum

Compound 13: $^1$H NMR (CDCl$_3$, 200 MHz) spectrum

Compound 13: $^1$H NMR (CDCl$_3$, 50 MHz) spectrum
Compound 16: $^1$H NMR (CDCl$_3$, 200 MHz) spectrum
**Compound 16: $^{13}$C NMR (CDCl$_3$, 50 MHz) spectrum**

**Compound 14: $^1$H NMR (CDCl$_3$, 400 MHz) spectrum**
Compound 14: $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum

Compound 15: $^1$H NMR (CDCl$_3$, 400 MHz) spectrum
Compound 15: $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum

Compound 17: $^1$H NMR (CDCl$_3$, 400 MHz) spectrum
Compound 17: $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum

Compound 19: $^1$H NMR (CDCl$_3$, 400 MHz) spectrum

Compound 19: $^{13}$C NMR (CDCl$_3$, 100 MHz) spectrum
Compound 1: $^1$H NMR (Acetone-$d_6$, 400 MHz) spectrum
**Compound 1:** $^{13}$C NMR (Acetone-$d_6$, 100 MHz) spectrum

**Compound 1:** DEPT-135 NMR (Acetone-$d_6$, 100 MHz) spectrum
Compound 2: $^1$H NMR (Acetone-$d_6$, 400 MHz) spectrum

Compound 2: $^{13}$C NMR (Acetone-$d_6$, 100 MHz) spectrum
Compound 2: DEPT-135 NMR (Acetone-d$_6$, 100 MHz) spectrum

Compound 3: $^1$H NMR (Acetone-d$_6$, 400 MHz) spectrum
**Compound 3:** $^{13}$C NMR (Acetone-$d_6$, 100 MHz) spectrum

**Compound 4:** $^1$H NMR (Acetone-$d_6$, 400 MHz) spectrum
**Compound 4:** $^{13}$C NMR (Acetone-d$_6$, 100 MHz) spectrum

**S2. Mass spectra of final compounds**

![Mass spectrum of Compound 1](image1)

**Compound 1:** LCMS mass spectrum

![Mass spectrum of Compound 2](image2)
**Compound 2: LCMS mass spectrum**

![LCMS mass spectrum of Compound 2](image)

**Compound 3: HRMS mass spectrum**

![HRMS mass spectrum of Compound 3](image)
Compound 4: HRMS mass spectrum

S3. HPLC traces of the synthesized final compounds

HPLC trace of compound 1, Eluent: 100% methanol, Flow rate: 1 ml/min, Ret. Time: 5.49 min

HPLC trace of compound 2, Eluent: 100% methanol, Flow rate: 0.5 ml/min, Ret. Time: 9.06 min

HPLC trace of compound 3, Eluent: 100% methanol, Flow rate: 1 ml/min, Ret. Time: 10.26 min
HPLC trace of 4

HPLC trace of compound 4, Eluent: 100% methanol, Flow rate: 1 ml/min, Ret. Time: 9.66 min

S5. Secondary plots of inhibition kinetics

Compound 1 (Competitive Inhibitor)

Compound 2 (Mixed Inhibitor)

S6. Images of REMA assay

Resazurin (Blue) → Resorufin (Pink)

Reduction by viable cell
Resazurin assay plate. Pink colour indicates growth and blue indicates inhibition. Row A = only media, negative control; Row B = only culture, growth control; Row C = culture + INH, positive control; Row D = culture + compound 2; Row E = culture + compound 1.

**S7. Details interaction from docking studies with distances**

Compound 1:

<table>
<thead>
<tr>
<th>A-subsite of NADH binding region</th>
<th>P-subsite of NADH binding region</th>
<th>N-subsite of NADH binding region</th>
<th>Catalytic tetrad</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val268 (H-bond, 3.7 Å)</td>
<td>Gly297 (H-bond, 2.5 Å)</td>
<td>Ser346 (H-bond, 2.8 Å)</td>
<td>Lys364 (H-bond, 2.9 Å)</td>
</tr>
<tr>
<td>Leu26 (H-bond, 3.7 Å)</td>
<td>Thr299 (H-bond, 3.3 Å)</td>
<td>Ser346 (H-bond, 2.6 Å)</td>
<td></td>
</tr>
<tr>
<td>Gly220 (H-bond, 2.7 Å)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Compound 1 totally competes at all three binding subsites of NADH binding region; supports that compound 1 is a competitive inhibitor.
Compound 2:

Compound 2 with interacting residues (distances in Å)

<table>
<thead>
<tr>
<th>A-subsite of NADH binding region</th>
<th>P-subsite of NADH binding region</th>
<th>N-subsite of NADH binding region</th>
<th>Loop I</th>
<th>Catalytic tetrad</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td>Ser346 (H-bond, 2.0 Å)</td>
<td>Arg300 (H-bond, 3.3 Å)</td>
<td>Ser347 (H-bond, 3.4 Å)</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>Gly391 (H-bond, 3.0 Å)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Compound 2 mainly interacts with catalytic tetrad and loop I; It can bind with free enzyme as well as enzyme-NADH complex resulting mixed inhibition.
Compound 3:

Compound 4:
A typical kinetic plot of absorbance vs time with increasing concentration of inhibitor (compound 4) in presence of substrate acetoacetyl CoA (positive control).