Supplementary Information

A study on the chiral inversion of mandelic acid in humans

Maksims Yevglevskis, Catherine R. Bowskill, Chloe C. Y. Chan, Justin H.-J. Heng, Michael D. Threadgill, Timothy J. Woodman, and Matthew D. Lloyd*

Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U. K.

Email: M.D.Lloyd@bath.ac.uk
<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characterisation of synthetic compounds</td>
<td>3 - 8</td>
</tr>
<tr>
<td>Incubations of acyl-CoA esters with recombinant human AMACR 1A</td>
<td>9 - 16</td>
</tr>
<tr>
<td>Kinetic plots for ACOT1</td>
<td>17 - 26</td>
</tr>
<tr>
<td>Kinetic plots for ACOT2</td>
<td>27 – 36</td>
</tr>
<tr>
<td>Supplementary Figure S1: Sequence alignment of AMACR and MCR</td>
<td>37</td>
</tr>
</tbody>
</table>
S-2-Hydroxyphenylacetyl-CoA 2S ($^1$H NMR, 500.13 MHz)
R-2-Hydroxy-2-phenylacetyl-CoA \textbf{2R} (\textsuperscript{1}H NMR, 500.13 MHz)
S-2-Methyl-2-phenylacetyl-CoA \(8S\) (\(^1\)H NMR, 500.13 MHz)
R-2-Methyl-2-phenylacetyl-CoA 8R (\(^1\)H NMR, 500.13 MHz)
$S$-2-Hydroxy-2-phenylacetyl-CoA $2S$ (as Na adduct)
5-2-Phenylpropanoyl-CoA 8S
Incubation of ±-Fenoprofenoyl-CoA with human recombinant AMACR 1A

Heat-inactivated enzyme

Live enzyme
Incubation of $S$-2-hydroxy-2-phenylacetyl-CoA 2S with human recombinant AMACR 1A

Heat-inactivated enzyme

Live enzyme
$S$-2-Hydroxy-2-phenylacetyl-CoA 2$S$ incubated with heat inactivated AMACR (full spectrum)

$S$-2-Hydroxy-2-phenylacetyl-CoA 2$S$ incubated with active AMACR (full spectrum)
Incubation of $R$-2-hydroxy-2-phenylacetyl-CoA $2R$ with human recombinant AMACR 1A

Heat-inactivated enzyme

Live enzyme
$R$-2-Hydroxy-2-phenylacetyl-CoA $2R$ incubated with heat inactivated AMACR (full spectrum)

![Chemical structure](image1)

$R$-2-Hydroxy-2-phenylacetyl-CoA $2R$ incubated with active AMACR (full spectrum)

![Chemical structure](image2)
Stack plot showing incubation of $2R$ and $2S$ with live and heat-inactivated AMACR

$2R$, Live enzyme

$2R$, heat-inactivated enzyme

$2S$, Live enzyme

$2S$, heat-inactivated enzyme
Incubation of \( S-2\)-phenylpropanoyl-CoA 8S with human recombinant AMACR 1A

Heat-inactivated enzyme

Live enzyme
Incubation of $R$-2-phenylpropanoyl-CoA $8R$ with human recombinant AMACR 1A

Heat-inactivate enzyme

Live enzyme
ACOT1, Myristoyl-CoA

Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>± Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>68.1711</td>
<td>118.7410</td>
<td>-178.7692 to 315.1114</td>
</tr>
<tr>
<td>Km</td>
<td>84.3676</td>
<td>170.3888</td>
<td>-269.9825 to 438.7178</td>
</tr>
<tr>
<td>Ki</td>
<td>28.1262</td>
<td>69.3833</td>
<td>-116.1673 to 172.4196</td>
</tr>
</tbody>
</table>

Goodness of Fit

- Degrees of Freedom: 21
- AICc: 48.022
- R²: 0.832
- Sum of Squares: 116.501
- Sy.x: 2.355
- Runs Test p Value: 0.501

Data

- Number of x values: 8
- Number of replicates: 3
- Total number of values: 24
- Number of missing values: 0

---

Michaelis-Menten

![Michaelis-Menten Graph]
ACOT-1, S-2-hydroxy-2-phenylacetyl-CoA 2S

Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>±Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>75.4081</td>
<td>56.1754</td>
<td>-41.4173 to 192.2335</td>
</tr>
<tr>
<td>Km</td>
<td>156.4288</td>
<td>138.3438</td>
<td>-131.2787 to 444.1362</td>
</tr>
<tr>
<td>Ki</td>
<td>40.4438</td>
<td>38.0679</td>
<td>-38.7243 to 119.6120</td>
</tr>
</tbody>
</table>

Goodness of Fit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degrees of Freedom</td>
<td>21</td>
</tr>
<tr>
<td>AICc</td>
<td>28.657</td>
</tr>
<tr>
<td>R²</td>
<td>0.913</td>
</tr>
<tr>
<td>Sum of Squares</td>
<td>51.989</td>
</tr>
<tr>
<td>Sy.x</td>
<td>1.573</td>
</tr>
<tr>
<td>Runs Test p Value</td>
<td>0.486</td>
</tr>
</tbody>
</table>

Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of x values</td>
<td>8</td>
</tr>
<tr>
<td>Number of replicates</td>
<td>3</td>
</tr>
<tr>
<td>Total number of values</td>
<td>24</td>
</tr>
<tr>
<td>Number of missing values</td>
<td>0</td>
</tr>
</tbody>
</table>

Michaelis-Menten

![Michaelis-Menten graph](image-url)
ACOT-1, *R*-2-hydroxy-2-phenylacetyl-CoA 2R

![Chemical Structure](image)

### Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>±Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$</td>
<td>12.2541</td>
<td>1.5178</td>
<td>9.0975 to 15.4106</td>
</tr>
<tr>
<td>$K_m$</td>
<td>61.6170</td>
<td>18.1924</td>
<td>23.7830 to 99.4509</td>
</tr>
</tbody>
</table>

### Goodness of Fit

- Degrees of Freedom: 21
- AICc: 13.563
- $R^2$: 0.837
- Sum of Squares: 30.247
- Sy.x: 1.200
- Runs Test p Value: 0.334

### Data

- Number of x values: 8
- Number of replicates: 3
- Total number of values: 23
- Number of missing values: 1

### Michaelis-Menten

![Graph of Michaelis-Menten kinetics](image)
ACOT-1, S-2-Phenylpropanoyl-CoA 8S

\[
\begin{align*}
\text{Parameters} & \\
\text{Value} & \quad \pm \text{Std. Error} & \quad 95\% \text{ Conf. Interval} \\
V_{\text{max}} & 26,802.5216 & 6.645e+6 & \text{to} \quad 1.379e+7 \\
K_m & 35,967.0081 & 8.924e+6 & \text{to} \quad 1.852e+7 \\
K_i & 0.1140 & 28.2948 & \text{to} \quad 58.9574 \\
\end{align*}
\]

Goodness of Fit
- Degrees of Freedom: 21
- AICc: 62.143
- \( R^2 \): 0.892
- Sum of Squares: 209.830
- \( \text{Sy.x} \): 3.161
- Runs Test p Value: 0.126

Data
- Number of x values: 8
- Number of replicates: 3
- Total number of values: 24
- Number of missing values: 0

Michaelis-Menten
ACOT-1, R-2-phenylpropanoyl-CoA 8R

Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>± Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>4.9646</td>
<td>0.3707</td>
<td>4.1959 to 5.7334</td>
</tr>
<tr>
<td>Km</td>
<td>22.1012</td>
<td>5.7426</td>
<td>10.1914 to 34.0109</td>
</tr>
</tbody>
</table>

Goodness of Fit

- Degrees of Freedom: 22
- AICc: -18.897
- $R^2$: 0.800
- Sum of Squares: 8.090
- Sy.x: 0.606
- Runs Test p Value: 0.501

Data

- Number of x values: 8
- Number of replicates: 3
- Total number of values: 24
- Number of missing values: 0

Michaelis-Menten

![Michaelis-Menten graph](image-url)
ACOT-2, Myristoyl-CoA 11

Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>±Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>29.3472</td>
<td>11.0834</td>
<td>6.2271 to 52.4672</td>
</tr>
<tr>
<td>Km</td>
<td>15.7935</td>
<td>9.9807</td>
<td>-5.0262 to 36.6132</td>
</tr>
<tr>
<td>Ki</td>
<td>152.5699</td>
<td>205.6633</td>
<td>-276.4448 to 581.5847</td>
</tr>
</tbody>
</table>

Goodness of Fit

Degrees of Freedom: 20
AICc: 48.032
R²: 0.826
Sum of Squares: 119.032
Sy.x: 2.440
Runs Test p Value: 0.500

Data

Number of x values: 8
Number of replicates: 3
Total number of values: 23
Number of missing values: 1

Michaelis-Menten

![Michaelis-Menten graph]
ACOT-2, S-2-hydroxy-2-phenylacetyl-CoA 2S

Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>± Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>102.8182</td>
<td>103.7608</td>
<td>-112.9686 to 318.6050</td>
</tr>
<tr>
<td>Km</td>
<td>171.6409</td>
<td>198.9373</td>
<td>-242.0801 to 585.3619</td>
</tr>
<tr>
<td>Ki</td>
<td>26.8023</td>
<td>32.3655</td>
<td>-40.5068 to 94.1115</td>
</tr>
</tbody>
</table>

Goodness of Fit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degrees of Freedom</td>
<td>21</td>
</tr>
<tr>
<td>AICc</td>
<td>38.715</td>
</tr>
<tr>
<td>R²</td>
<td>0.902</td>
</tr>
<tr>
<td>Sum of Squares</td>
<td>79.053</td>
</tr>
<tr>
<td>Sy.x</td>
<td>1.940</td>
</tr>
<tr>
<td>Runs Test p Value</td>
<td>0.338</td>
</tr>
</tbody>
</table>

Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of x values</td>
<td>8</td>
</tr>
<tr>
<td>Number of replicates</td>
<td>3</td>
</tr>
<tr>
<td>Total number of values</td>
<td>24</td>
</tr>
<tr>
<td>Number of missing values</td>
<td>0</td>
</tr>
</tbody>
</table>

Michaelis-Menten

![Michaelis-Menten Graph](image-url)
Lineweaver-Burk

Residuals
ACOT-2, \(R\)-2-hydroxy-2-phenylacetyl-CoA \(2R\)

\[
\begin{align*}
\text{Parameters} & \\
V_{\text{max}} & = 20.3377 \pm 3.1959 \quad (13.6231 \text{ to } 27.0522) \\
K_m & = 130.7584 \pm 37.3894 \quad (52.2047 \text{ to } 209.3122)
\end{align*}
\]

**Goodness of Fit**

- Degrees of Freedom: 18
- AICc: 14.166
- \(R^2\): 0.893
- Sum of Squares: 27.911
- Sy.x: 1.245
- Runs Test p Value: 0.179

**Data**

- Number of x values: 7
- Number of replicates: 3
- Total number of values: 20
- Number of missing values: 1

![Michaelis-Menten Graph](attachment:image.png)
ACOT-2, S-2-Phenylpropanoyl-CoA 8S

Parameters

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>±Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>33,339.3892</td>
<td>7.917e+6</td>
<td>-1.643e+7 to 1.650e+7</td>
</tr>
<tr>
<td>Km</td>
<td>46,916.6408</td>
<td>1.115e+7</td>
<td>-2.314e+7 to 2.323e+7</td>
</tr>
<tr>
<td>Ki</td>
<td>0.1081</td>
<td>25.6837</td>
<td>-53.3051 to 53.5212</td>
</tr>
</tbody>
</table>

Goodness of Fit

- Degrees of Freedom: 21
- AICc: 54.443
- R²: 0.930
- Sum of Squares: 152.241
- Sy.x: 2.693
- Runs Test p Value: 0.501

Data

- Number of x values: 8
- Number of replicates: 3
- Total number of values: 24
- Number of missing values: 0

Michaelis-Menten

![Michaelis-Menten Graph](attachment:image.png)
**Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>±Std. Error</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vmax</td>
<td>5.3167</td>
<td>0.3275</td>
<td>4.6375 to 5.9960</td>
</tr>
<tr>
<td>Km</td>
<td>27.3880</td>
<td>5.4159</td>
<td>16.1558 to 38.6202</td>
</tr>
</tbody>
</table>

**Goodness of Fit**

- Degrees of Freedom: 22
- AICc: -31.069
- \( R^2 \): 0.882
- Sum of Squares: 4.872
- Sy.x: 0.471
- Runs Test p Value: 0.023

**Data**

- Number of x values: 8
- Number of replicates: 3
- Total number of values: 24
- Number of missing values: 0

---

Michaelis-Menten

![Michaelis-Menten Plot](image-url)
Figure S1: Sequence alignment of MCR from *M. tuberculosis* (O06543) and human AMACR 1A (Q9UHK6), showing residues involved in methyl group binding pocket (Bold).