Supplementary information

Chiral inversion of 2-arylpropionoyl-CoA esters by human α-methylacyl-CoA racemase 1A (P504S) – a potential mechanism for the anti-cancer effects of ibuprofen

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Synthesis of substrates

The required acyl-CoA esters were synthesized by reaction of the 2-APA with carbonyl diimidazole,[1,2] with the formation of the acyl-imidazole intermediates confirmed by ^{1}H NMR. These intermediates were immediately reacted with the reduced thiol form of coenzyme A, to afford the desired esters on ~10 mg scales.

Scheme S1: Synthesis of acyl-CoA esters from their corresponding acids. Reagents and conditions: i) N,N'-carbonyldiimidazole, CH_2Cl_2, N_2, ambient temperature, 1 hr; CoA-SH $(Li^+)_3$, 0.1 M aq. $NaHCO_3$, ambient temperature, 16 h.

Experimental details

Sources of materials:

All chemicals were obtained from the Sigma-Aldrich Chemical Co. or Fisher Ltd and were used without further purification unless otherwise stated. Experiments were conducted at ambient temperature, unless noted otherwise. Organic extracts were dried over $MgSO_4$ and filtered. Solvents were evaporated under reduced pressure. NMR spectra were obtained on Varian Mercury (400 MHz), Varian Inova (600 MHz), or Bruker Avance III (400 and 500 MHz) spectrometers, in $CDCl_3$ solution, except where noted. Spectra were referenced to the residual solvent peak, or externally via the solvent lock signal. Coupling constants (*J*) are reported in Hz to the nearest 0.1 Hz. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block. Coenzyme A tri-lithium salt was purchased from Calbiochem. Rosetta2 (DE3) cells and benzonase were from Novagen. *S*-2-Methyldecanoic acid was synthesized as previously described.[3] Recombinant human AMACR 1A was expressed in *E. coli* Rosetta2 (DE3) cells (Novagen). Cells (in 30 mL buffer) were pretreated with 250 U of benzonase before lysis, and the crude extract was treated with *N*-lauroyl-sarcosine and purified as previously described.[3]

±-Ibuprofenoyl-imidazole (±-1-(2-(4-(2-methylpropyl)phenyl)propanoyl)imidazole) (14).

±-Ibuprofen **1** (20 mg, 97 μmol) was stirred with N,N'-carbonyldiimidazole (25 mg, 154 μmol) in CH_2Cl_2 (2.0 mL) under N_2 for 1 h. The solution was washed repeatedly with water (2 mL portions) until the organic layer was no longer cloudy. Drying and evaporation gave **14** (24 mg, 94.1 μmol, 97%) as a colourless solid: ^{1}H NMR (500.13 MHz) δ 8.13 (1 H, t, *J* = 1.1 Hz, imidazole-*H*), 7.44 (1 H, t, *J* = 1.5 Hz, imidazole-*H*), 7.16 (2 H, d, *J* = 8.2 Hz, arom-*H*), 7.11 (2 H, d, *J* = 8.2 Hz, arom-*H*), 6.99 (1 H, dd, *J* = 1.1, 1.5 Hz, imidazole-*H*), 4.26 (1 H, q, *J* = 6.6 Hz, C*H*Me), 2.42 (2 H, d, *J* = 8.2 Hz, CH_2), 1.82 (1 H, nonet, *J* = 7.0 Hz, C*H*Me$_2$), 1.60 (3 H, d, *J* = 7.0 Hz, CHCH_3), 0.87 (6 H, d, *J* = 6.6 Hz, CH(CH_3)$_2$); ^{13}C NMR (125.77 MHz) δ 170.56, 141.48, 136.53, 136.32, 130.41, 130.11, 126.73, 116.43, 46.00, 44.83, 29.99, 22.23, 19.59.

±-Ibuprofenoyl-CoA (2).

Compound **14** (21 mg, 82 μmol) in dry THF (1.5 mL) was treated with CoA-SH tri-lithium salt (11 mg, 14 μmol) in aq. $NaHCO_3$ (0.1 M, 1.0 mL). The mixture was then stirred for 16 h at room temperature. The mixture was washed with EtOAc (2 × 2 mL). The aqueous layer was acidified with Dowex X50 (H^+ form) to *ca.* pH 4-5, then repeatedly extracted with EtOAc (2 mL portions) until no

more free acid remained (as shown by NMR). The aqueous layer was then lyophilized overnight to give **2** (9.1 mg) as a colourless solid: ^{1}H NMR (400.04 MHz, D_2O) δ 8.46 (1 H, s, adenosine C*H*), 8.13 (1 H, s, adenosine C*H*), 7.13 (2 H, d, *J* = 7.8 Hz, arom-*H*), 7.06 (2 H, d, *J* = 7.8 Hz, arom-*H*), 6.06 (1 H, d, *J* = 7.0 Hz, adenosine C*H*), 4.47 (1 H, m, adenosine C*H*), 4.13 (2 H, m, adenosine CH_2), 3.90 (1 H, s, adenosine C*H*), 3.88 (1 H, q, *J* = 7.4 Hz, C*H*Me), 3.72 (1 H, m, CoA-C(CH_3)CH*H*O), 3.44 (1 H, m, CoA-C(CH_3)C*H*HO), 3.33 (1 H, m, CoA-C(OH)*H*), 3.19 (4 H, m, 2 x CoA-CH_2CH_2N), 2.94 (1 H, m, SCH), 2.83 (1 H, m, SCH), 2.31 (1 H, d, *J* = 7.0 Hz, CH_2CHMe$_2$), 2.13 (2 H, m, CoA NHC(=O)CH_2CH_2), 1.66 (1 H, nonet, *J* = 7.1 Hz, CH_2C*H*Me$_2$), 1.36 (3 H, d, *J* = 7.0 Hz, CHCH_3), 0.76 (3 H, s, CoA Me), 0.72 (6 H, d, *J* = 6.3 Hz, CH_2CH(CH_3)$_2$), 0.60 (3 H, s, CoA Me). ESI-MS *m/z* calcd. for $[M-H]^-$ $C_{34}H_{51}N_7O_{17}P_3S$: 954.2280, found: 954.2316.

±-Fenoprofenoyl-imidazole (±-1-(2-(3-phenoxyphenyl)propanoyl)imidazole) (15).

Fenoprofen **3** (19.4 mg, 75 μmol) was treated with carbonyldiimidazole, as for the synthesis of **14**, to give **15** (22 mg, 71.4 μmol, 95%) as a colourless solid: ^{1}H NMR (400.04 MHz) δ 8.13 (1 H, br s, imidazole-*H*), 7.44 (1 H, t, *J* = 1.5 Hz, imidazole-*H*), 7.34 (1 H, t, *J* = 7.4 Hz, arom-*H*), 7.28 (1 H, t, *J* = 7.4 Hz, arom-*H*), 7.12 (1 H, t, *J* = 7.1 Hz, arom-*H*), 7.03-6.93 (5 H, m, 4 × arom-*H*, 1 × imidazole-*H*), 6.87 (1 H, dd, *J* = 8.2, 2.3 Hz, arom-*H*), 4.26 (1 H, q, *J* = 7.0 Hz, C*H*Me), 1.60 (3 H, d, *J* = 7.0 Hz, CHCH_3); ^{13}C (125.77 MHz, $CDCl_3$) δ 170.04, 158.21, 156.44, 141.06, 136.54, 130.77, 130.73, 129.84, 123.72, 121.55, 119.09, 117.70, 117.41, 116.36, 46.20, 19.52.

±-Fenoprofenoyl-CoA (7).

Compound **15** (22 mg) was treated with CoA-SH tri-lithium salt (10 mg), as for the synthesis of **2,** to give **7** (9.0 mg) as a colourless solid: ^{1}H NMR (400.04 MHz, D_2O) δ 8.44 (1 H, s, adenosine C*H*), 8.10 (1 H, s, adenosine C*H*), 7.29 (2 H, t, *J* = 7.8 Hz, arom-*H*), 7.25 (1 H, t, *J* = 8.3 Hz, arom-*H*), 7.08 (2 H, t, *J* = 7.5 Hz, arom-*H*), 7.04 (1 H, s, arom-*H*), 6.99 (1 H, d, *J* = 7.8 Hz, arom-*H*), 6.91 (2 H, d, *J* = 8.2 Hz, arom-*H*), 6.84 (1 H, d, *J* = 7.8 Hz, arom-*H*), 6.04 (1 H, d, *J* = 7.0 Hz, adenosine C*H*), 4.46 (1 H, m, adenosine C*H*), 4.13 (2 H, m, adenosine CH_2), 3.90 (1 H, s, adenosine C*H*), 3.89 (1 H, m, C*H*CH_3), 3.72, 3.42 (2 H, both m, CoA-C(CH_3)CH_2O), 3.33 (1 H, m, CoA-C(OH)*H*), 3.26-3.13 (4 H, m, CoA-CH_2CH_2N), 2.92, 2.83 (2 H, both m, CoA-S CH_2CH_2), 2.15 (2 H, m, CoA-NHC(=O)CH_2CH_2), 1.35 (3 H, d, *J* = 7.0 Hz, CHCH_3), 0.75 (3 H, s, CoA-CH_3), 0.59 (3 H, s, CoA-CH_3). ESI-MS *m/z* calcd. for $[M-H]^-$ $C_{36}H_{47}N_7O_{18}P_3S$: 990.1917, found: 990.1907.

±-Flurbiprofenoyl-imidazole (*RS*-1-(2-(2-fluoro-4-phenyl)phenyl)propanoyl)imidazole) (16).

Flurbiprofen **4** (21.6 mg, 88 μmol) was treated with carbonyldiimidazole, as for the synthesis of **14**, to give **16** (24 mg, 81.6 μmol, 92%) as a colourless solid: ^{1}H NMR (400.04 MHz) δ 8.17 (1 H, br s, imidazole-*H*), 7.53-7.35 (7 H, m, arom-*H*, 1 × imidazole-*H*), 7.16-7.08 (2 H, m, arom-*H*), 7.05 (1 H, dd, *J* = 0.9, 1.5 Hz, imidazole-*H*), 4.34 (1 H, q, *J* = 6.7 Hz, C*H*Me), 1.67 (3 H, d, *J* = 6.6 Hz, CHC*H*$_3$); ^{13}C NMR (125.77 MHz$_3$) δ 169.99 (J_{CF} = 248.4 Hz), 169.86, 140.19 (d, J_{CF} = 7.3 Hz), 136.47, 134.87, 131,77 (d, J_{CF} = 4.0 Hz), 131.00, 128.87 (d, J_{CF} = 2.8 Hz), 128.79, 128.48, 127.92, 123.14 (d, J_{CF} = 3.5 Hz), 116.36, 114.95 (d, J_{CF} = 24.1 Hz), 47.76, 19.55.

±-Flurbiprofenoyl-CoA (8).

Compound **16** (24 mg) was treated with CoA-SH tri-lithium salt (11 mg), as for the synthesis of **2,** to give **8** (8.7 mg) as a colourless solid: ^{1}H NMR (400.04 MHz, D_2O) δ 8.34 (1 H, s, adenosine C*H*), 7.98 (1 H, s, adenosine C*H*), 7.38-7.22 (5 H, m, arom-*H*), 7.06-6.97 (3 H, m, arom-*H*), 5.94 (1 H, d, *J* = 5.8 Hz, adenosine C*H*), 4.40 (1 H, m, adenosine C*H*), 4.08 (2 H, m, adenosine C*H*$_2$), 3.92 (1 H, m, C*H*CH$_3$), 3.80 (1 H, m, adenosine C*H*), 3.64, 3.35 (2 H, both m, CoA-C(CH$_3$)C*H*$_2$O), 3.14 (4 H, m, 2 x CoA-CH$_2$C*H*$_2$N), 2.92, 2.80 (2 H, both m, CoA-SC*H*$_2$CH$_2$), 2.07 (2 H, m, CoA-NHC(=O)C*H*$_2$CH$_2$), 1.35 (3 H, d, *J* = 7.0 Hz, CHC*H*$_3$), 0.65 (3 H, s, CoA-CH$_3$), 0.50 (3 H, s, CoA-CH$_3$). ESI-MS *m/z* calcd. for [M-H]$^-$ $C_{36}H_{46}N_7O_{17}FP_3S$: 992.1873, found: 992.1840.

***S*-Ketoprofenoyl-imidazole (*S*-1-(2-(3-benzoylphenyl)propanoyl)imidazole) (17).**

Ketoprofen **5** (17 mg, 66.5 mmol) was treated with carbonyldiimidazole, as for the synthesis of **14**, to give **17** (19 mg, 62.5 μmol, 94%) as a colourless solid: ^{1}H NMR (400.04 MHz) δ 8.15 (1 H, br s, imidazole-*H*), 7.73 (1 H, br s, imidazole-*H*), 7.68 (2 H, d, *J* = 8.0 Hz, arom-*H*), 7.63 (1 H, t, *J* = 7.6 Hz, arom-*H*), 7.54 (1 H, t, *J* = 7.6 Hz, arom-*H*), 7.49 (1 H, d, *J* = 7.6 Hz, arom-*H*), 7.46-7.39 (4 H, m, arom-*H*), 6.98 (1 H, br d, *J* = 1.5 Hz, imidazole-*H*), 4.40 (1 H, q, *J* = 7.2 Hz, C*H*Me), 1.61 (3 H, d, *J* = 6.8 Hz, CHC*H*$_3$); ^{13}C NMR (125.77 MHz) δ 195.91, 170.00, 139.54, 138.67, 137.10, 136.46, 132.72, 131.01, 139.87, 129.97, 129.73, 129.40, 128.73, 128.38, 116.35, 46.12, 19.64.

***S*-Ketoprofenoyl-CoA (9).**

Compound **17** (19 mg) was treated with CoA-SH tri-lithium salt (11 mg), as for the synthesis of **2,** to give **9** (9.2 mg) as a colourless solid: ^{1}H NMR (400.04 MHz, D_2O) δ 8.37 (1 H, s, adenosine C*H*), 8.03 (1 H, s, adenosine C*H*), 7.58-7.46 (6 H, m, arom-*H*), 7.42-7.30 (3 H, m, arom-*H*), 5.95 (1 H, d, *J* = 5.8 Hz, adenosine C*H*), 4.42 (1 H, m, adenosine C*H*), 4.09 (2 H, m, adenosine CH_2), 3.96 (1 H, q, *J* = 7.0 Hz, C*H*CH$_3$), 3.86, (1 H, s, adenosine C*H*), 3.69, 3.40 (2 H, both m, CoA-C(CH$_3$)CH_2O), 3.30 (1 H, m, CoA-C(OH)*H*), 3.15 (4 H, m, 2 x CoA-CH$_2$CH_2N), 2.90, 2.81 (2 H, both m, CoA-S CH_2CH$_2$), 2.10 (2 H, m, CoA-NHC(=O)CH_2CH$_2$), 1.35 (3 H, d, *J* = 7.4 Hz, CHCH_3), 0.72 (3 H, s, CoA-CH$_3$), 0.57 (3 H, s, CoA-CH$_3$). ESI-MS *m/z* calcd. for [M-H]$^-$ $C_{37}H_{47}N_7O_{18}P_3S$: 1002.1917, found: 1002.1896.

***S*-Naproxenoyl-imidazole (1-(2-(6-methoxynaphthalen-2-yl))imidazole) (18).**

Naproxen **6** (18 mg, 78 μmol) was treated with carbonyldiimidazole, as for the synthesis of **14**, to give **18** (19 mg, 67.8 μmol, 87%) as a colourless solid: ^{1}H NMR (400.04 MHz) δ 8.18 (1 H, t, *J* = 0.9 Hz, imidazole-*H*), 7.73 (1 H, d, *J* = 8.6 Hz, arom-*H*), 7.68 (1 H, d, *J* = 9.0 Hz, arom-*H*), 7.65 (1 H, s, arom-*H*), 7.46 (1 H, t, *J* = 1.6 Hz, imidazole-*H*), 7.34 (1 H, dd, *J* = 8.6, 2.0 Hz, arom-*H*), 7.15 (1 H, dd, *J* = 9.0, 2.3 Hz, arom-*H*), 6.97 (1 H, dd, *J* = 0.9, 1.6 Hz, imidazole-*H*), 4.43 (1 H, q, *J* = 7.0 Hz, C*H*Me), 1.68 (3 H, d, *J* = 6.6 Hz, CHCH_3); ^{13}C NMR (125.77 MHz) δ 170.55, 158.07, 136.61, 134.96, 134.27, 130.69, 129.20, 129.06, 128.31, 125.93, 125.20, 119.61, 116.44, 105.62, 55.33, 46.42, 19.71.

***S*-Naproxenoyl-CoA (10)**

Compound **18** (19 mg) was treated with CoA-SH tri-lithium salt (10 mg), as for the synthesis of **2,** to give **10** (9.3 mg) as a colourless solid: ^{1}H NMR (400.04 MHz, D_2O) δ 8.37 (1 H, s, adenosine C*H*), 8.03 (1 H, s, adenosine C*H*), 7.58-7.46 (6 H, m, arom-*H*), 7.42-7.30 (3 H, m, arom-*H*), 5.95 (1 H, d, *J* = 5.8 Hz, adenosine C*H*), 4.47 (1 H, m, adenosine C*H*), 4.14 (2 H, m, adenosine CH_2), 4.05 (1 H, m, C*H*CH$_3$), 3.91 (1 H, s, adenosine C*H*), 3.72, 3.45 (2 H, both m, CoA-C(CH$_3$)CH_2O), 3.40-3.30 (5 H, m, CoA-C(OH)*H* and 2 x CoA-CH$_2$CH_2N), 2.96, 2.82 (2 H, both m, CoA-SCH_2CH$_2$), 2.34 (2 H, m, CoA-NHC(=O)CH_2CH$_2$), 1.46 (3 H, d, *J* = 7.2 Hz, CHCH_3), 0.66 (3 H, s, CoA-CH$_3$), 0.52 (3 H, s, CoA-CH$_3$). ESI-MS *m/z* calcd. for [M-H]$^-$ $C_{35}H_{47}N_7O_{18}P_3S$: 978.1917, found: 9978.1956.

***S*-1-(2-Methyldecanoyl)imidazole (19).**

S-2-Methyldecanoic acid **20** (15.4 mg, 82.9 μmol) was treated with carbonyldiimidazole, as for the synthesis of **14**, to give **19** (18 mg, 76.3 μmol 92%) as a low melting, colourless solid: ^{1}H NMR

(500.13 MHz) δ 8.16 (1 H, br s, imidazole-*H*), 7.47 (1 H, t, *J* = 1.5 Hz, imidazole-*H*), 7.09 (1 H, t, *J* = 1.3 Hz, imidazole-*H*), 3.04 (1 H, sextet, *J* = 7.0 Hz, decanoyl 2-H), 1.82 (1 H, m, decanoyl 3-H), 1.54 (1 H, m, decanoyl 3-H), 1.35-1.18 (15 H, m, decanoyl 4,5,6,7,8,9-H_{12} and 2-Me), 0.87 (3 H, t, *J* = 7.0 Hz, decanoyl 10-H_3). ^{13}C NMR (125.77 MHz) δ 173.43, 136.08, 130.94, 116.11, 39.45, 35.18, 33.67, 29.40, 29.26, 29.09, 27.04, 22.54, 17.25, 13.99.

_S_-Methyldecanoyl-CoA (11).

Compound **19** (18 mg) was treated with CoA-SH tri-lithium salt (11 mg), as for the synthesis of **2,** to give **11** (8.7 mg) as a colourless solid. Data were consistent with those previously reported.[3]

_S,S_-2-(3-Benzoylphenyl)-N-(1-phenylethyl)propanamide (12).

S-Ketoprofen-imidazole **17** (derived from *S*-ketoprofen **5**, 32 mg, 106 μmol) in $CDCl_3$ was treated with *S*-1-phenylethylamine (25 mg, 208 μmol). The reaction was followed at 47 °C using ^{1}H NMR (500.13 MHz). Over *ca.* 16 h, the peaks for the starting material disappeared and new peaks, corresponding to **12** arose. When **17** had been consumed, the solution was diluted with $CHCl_3$ (2 mL) and washed with aq. HCl (1 M, 3 × 2 mL). Drying and evaporation gave **12** (34 mg, 95 μmol, 89.7 %) as a colourless oil: ^{1}H NMR (500.13 MHz) δ 7.75 (2 H, m, arom-*H*), 7.71 (1 H, t, *J* = 1.5 Hz, arom-*H*), 7.66 (1 H, dt, *J* = 7.5, 1.5 Hz, arom-*H*), 7.59 (1 H, tt, *J* = 7.5,1.5 Hz, arom-*H*), 7.51 (1 H, dt, *J* = 7.5, 1.5 Hz, arom-*H*), 7.48-7.41 (3 H, m, arom-*H*), 7.25-7.17 (3 H, m, arom-*H*), 7.11 (2 H, m, arom-*H*), 5.65 (1 H, d, *J* = 7.5 Hz, NH), 5.09 (1 H, qn, *J* = 7.5 Hz, PhC*H*MeNH), 3.63 (1 H, q, *J* = 7.0 Hz, C*H*MeC=O), 1.53 (3 H, d, *J* = 7.0 Hz, CH(CH_3)C=O), 1.43 (3 H, d, *J* = 7.0 Hz, PhCH(CH_3)NH); ^{13}C NMR (125.77 MHz) δ 196.48 (C=O), 172.45 (NHC=O), 143.01, 141.79, 138.04, 137.37, 132.53, 131.45, 130.00, 129.06, 129.02, 128.74, 128.55, 128.30, 127.22, 125.81, 48.82 (*C*H(Me)NH), 46.99 (*C*H(Me)C=O), 21.77 (CH(*C*H_3)NH), 18.60 (CH(*C*H_3)C=O). ESI-MS *m/z* calcd. for $[M+Na]^+$ $C_{24}H_{23}NO_2Na$: 380.1621, found: 380.1608. $[\alpha]_D^{20}$ +32.3 ° (*c* = 1.11, dichloromethane).

_S_,_S_-2-(6-Methoxynaphthalen-2-yl)-N-(1-phenylethyl)propanamide (13).

_S_-Naproxenoyl-imidazole **18** (35 mg, 125 μmol) was treated with _S_-1-phenylethylamine (29 mg, 239 μmol), as for the synthesis of **12**, to give **13** (31 mg, 93 μmol, 74.4 %) as a colourless solid: ^{1}H NMR (500.13 MHz) δ 7.71 (1 H, d, _J_ = 8.5 Hz, arom-_H_), 7.67 (1 H, d, _J_ = 9.0 Hz, arom-_H_), 7.61 (1 H, s, arom-_H_), 7.33 (1 H, dd, _J_ = 8.5, 2.0 Hz, arom-_H_), 7.23-7.05 (7 H, m, 5 × arom-_H_ and 2 × arom-_H_), 5.58 (1 H, d, _J_ = 7.5 Hz, NH), 5.11 (1 H, qn, _J_ = 7.5 Hz, PhC_H_MeNH), 3.92 (3 H, s, OMe), 3.71 (1 H, q, _J_ = 7.5 Hz, C_H_(Me)C=O), 1.58 (3 H, d, _J_ = 7.0 Hz, CH(C_H_$_3$)C=O), 1.37 (3 H, d, _J_ = 7.0 Hz, PhCH(C_H_$_3$)NH); ^{13}C NMR (125.77 MHz) δ 173.30 (_C_=O), 157.72, 143.24, 136.47, 133.70, 129.20, 128.95, 128.46, 127.50, 127.06, 126.28, 126.12, 125.80, 55.31 (O_Me_), 48.69 (_C_H(Me)NH), 47.03 (_C_H(Me)C=O), 21.87 (CH(_Me_)NH), 18.53 (CH(_Me_)C=O). ESI-MS _m/z_ calcd. for $[M+H]^+$ $C_{22}H_{24}NO_2$: 334.1802, found: 334.1805. Melting point 176.0-176.5 °C. $[\alpha]_D^{20}$ +118.7 º (_c_ = 0.79, dichloromethane).

Kinetic assays

Assays were conducted in aq. 50 mM NaH_2PO_4-NaOH buffer, pH 7.4, and _ca._ 90% (v/v) $^{2}H_2O$ in a final volume of 550 μL for 1 h at 30ºC.[3,4] The reaction was terminated by heating at 50ºC for 10 min before ^{1}H NMR analysis. Concentrations of substrates were determined using ^{1}H NMR; at least six substrate concentrations in duplicate were used. Extents of conversion were calculated based on the integrals for both sides of the asymmetrical doublet for the α-methyl group of the ^{2}H-product at 1.45 p.p.m. (See Figure 1 of communication); The left hand side of the doublet arises from ^{1}H-containing substrate only, while the right hand side contains the signals for the right-hand side of the methyl group doublets for the ^{1}H-containing substrate and the unresolved triplet of the ^{2}H-containing product. Rates were calculated based on extents of conversion during the incubation period and corrected for non-enzymatic exchange using negative controls containing heat-inactivated enzyme. Recombinant human AMACR 1A was quantified by absorbance at 280 nm [http://www.expasy.ch/tools/protparam.html; ε = 35785 M^{-1} cm^{-1} for the His-tag enzyme[3]] giving a stock solution of 68.4 μM (3.22 μg μL^{-1}). Between 2 and 10 μL of enzyme solution were used in kinetic assays. Kinetic parameters were derived using the

Direct Linear Plot[5,6] in SigmaPlot 11 and the enzyme kinetics module 1.3 (Systat). V_{max} values in nmol min^{-1} mg^{-1} protein were calculated assuming a molecular mass of 47146.8 Da.[3]

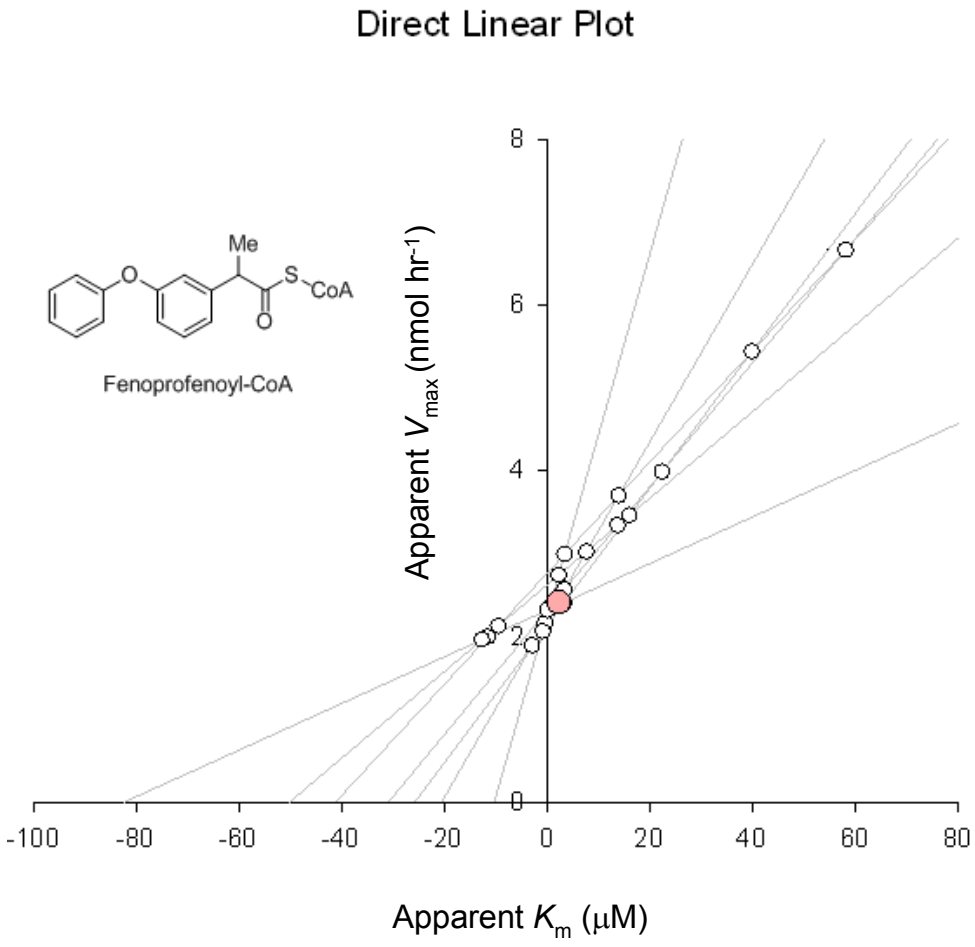
Determination of chiral inversion of 2-APA-CoA substrates

Ketoprofenoyl-CoA **9** or naproxenoyl-CoA **10** (*ca.* 10 mg; ~500 μM final concentration) were incubated with AMACR (*ca.* 3.8 mg) in 50 mM NaH_2PO_4-NaOH buffer, pH 7.4 and ^1H_2O in a total volume of *ca.* 20 mL at 30ºC for >16 h. Reactions were quenched with aq. NaOH (10 M, 1 mL) for 30 min and acidified to *ca.* pH 4-5 with aq. HCl. The acid products were extracted with $CHCl_3$, dried and the solvent evaporated. The residue was taken up in $CDCl_3$ for ^1H NMR analysis and quantification. The acids were converted into their N-(*S*-1-phenylethyl)amides **12** or **13** by addition of *S*-1-phenylethylamine (1.5 equiv.), followed of N,N'-dicyclohexylcarbodiimide (DCC, 1.5 equiv.). Reactions were typically completed in <30 min. In both cases, two diastereoisomers (*R*,*S* and *S*,*S*) were observed, as shown by the peaks for the α-proton, the methyl protons and, in the case of naproxen **6**, the methoxy protons in the ^1H NMR spectrum.

Models of substrate binding

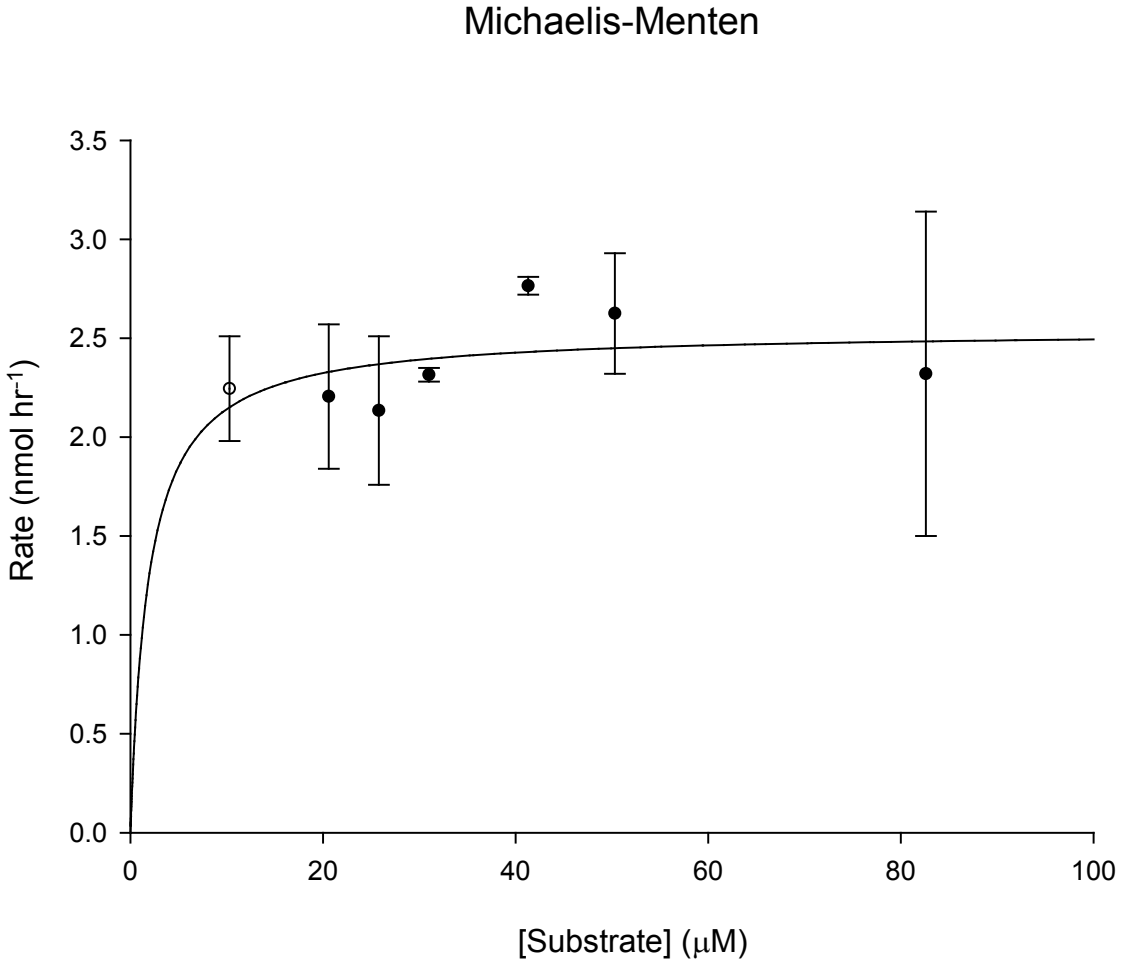
Models were produced based on the X-ray crystal structure of MCR complexed with ±-ibuprofenoyl-CoA **2**.[7] Once docked, the 2-APA-CoAs were subjected to molecular mechanics and dynamics calculations to establish optimal docking conformations; during these calculations, the enzyme and coenzyme A moiety were restrained to original conformations. The 2-APA-CoA and binding pocket were subjected to molecular dynamics and finally molecular mechanics calculations to give the final structures. Calculations were performed using the Tripos Associates force fields within the SYBYL-X software suite on an Intel dual core workstation (SUSE LINUX). Gasteiger-Hückel charges were calculated for the complexes.

±-Fenoprofenoyl-CoA 7



K_m = 2.292 μM
V_{max} = 2.415 nmol h^{-1}
Enzyme = 0.2052 nmol assay^{-1} (9.67 μg assay^{-1})

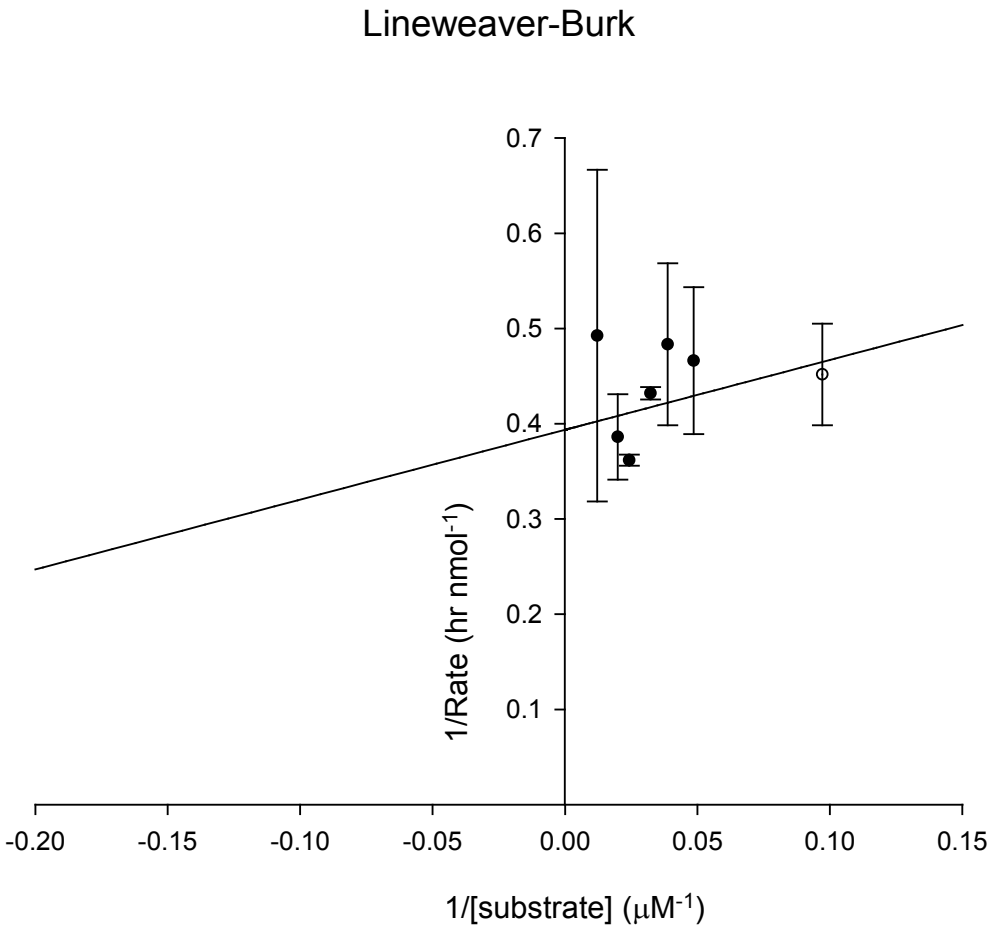
V_{max} = 4.16 nmol min^{-1} mg^{-1}
K_{cat} = 0.0033 s^{-1}
K_{cat}/K_m = 1426.33 M^{-1} s^{-1}

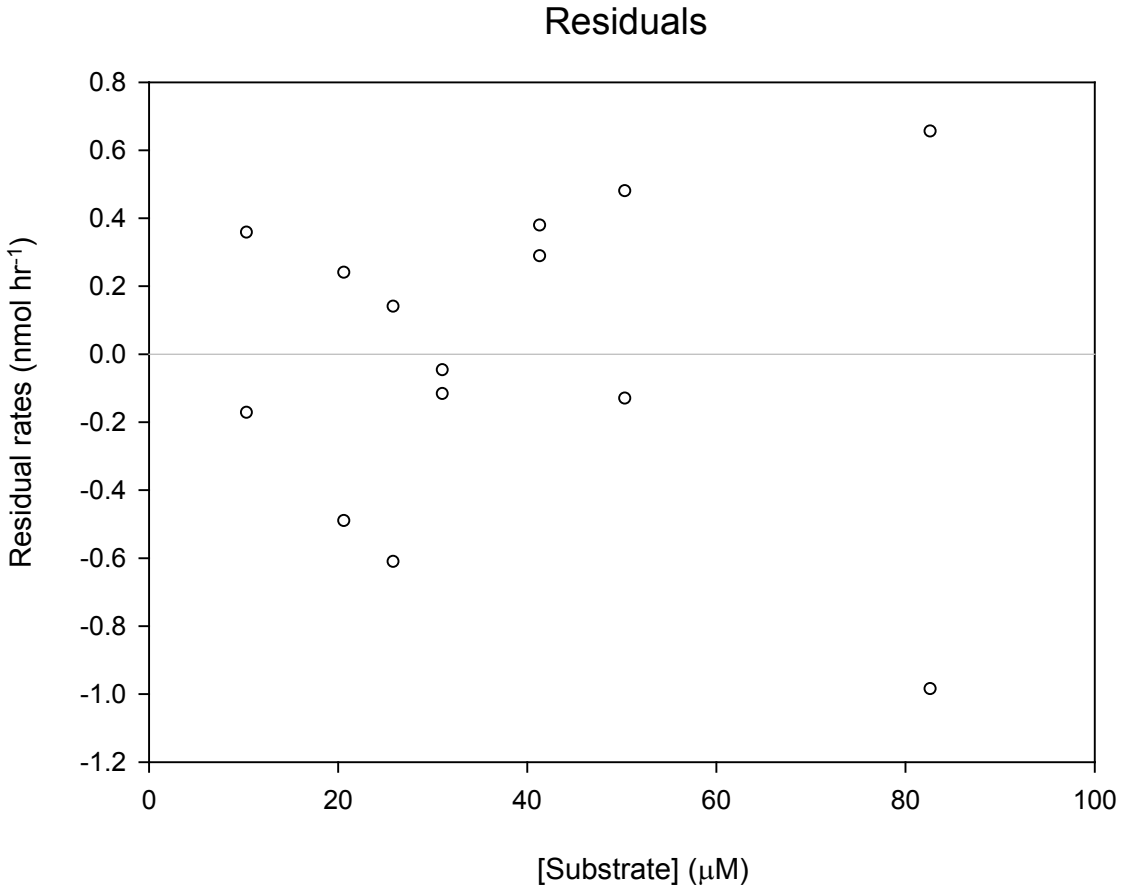


Value	±Std. Error		95% Conf. Interval		
Vmax	2.5400	0.2624	1.9682	to	3.1117
Km	1.8627	2.6299	-3.8676	to	7.5929

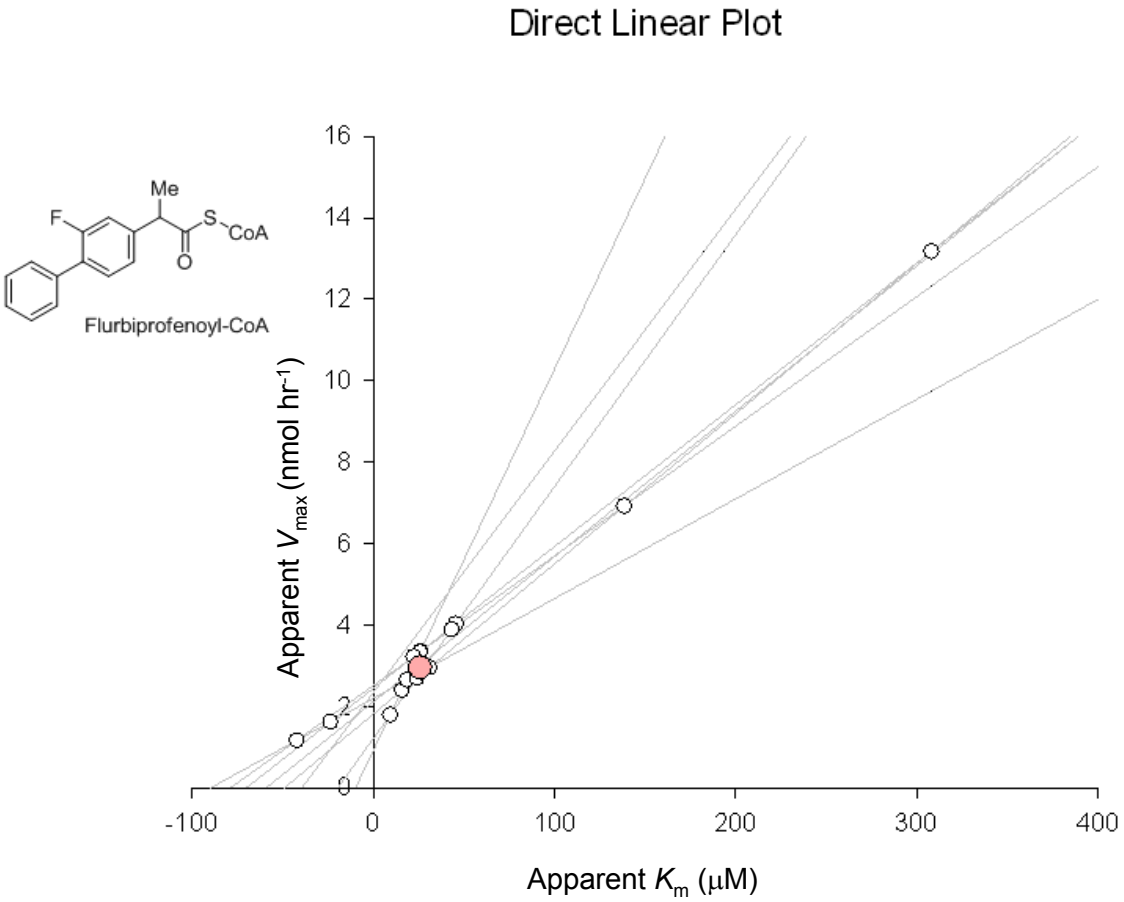
Goodness of Fit

Degrees of Freedom	12
AICc	-14.449
R^2	4.816e-2
Sum of Squares	2.737
Sy.x	0.478
Runs Test p Value	0.500



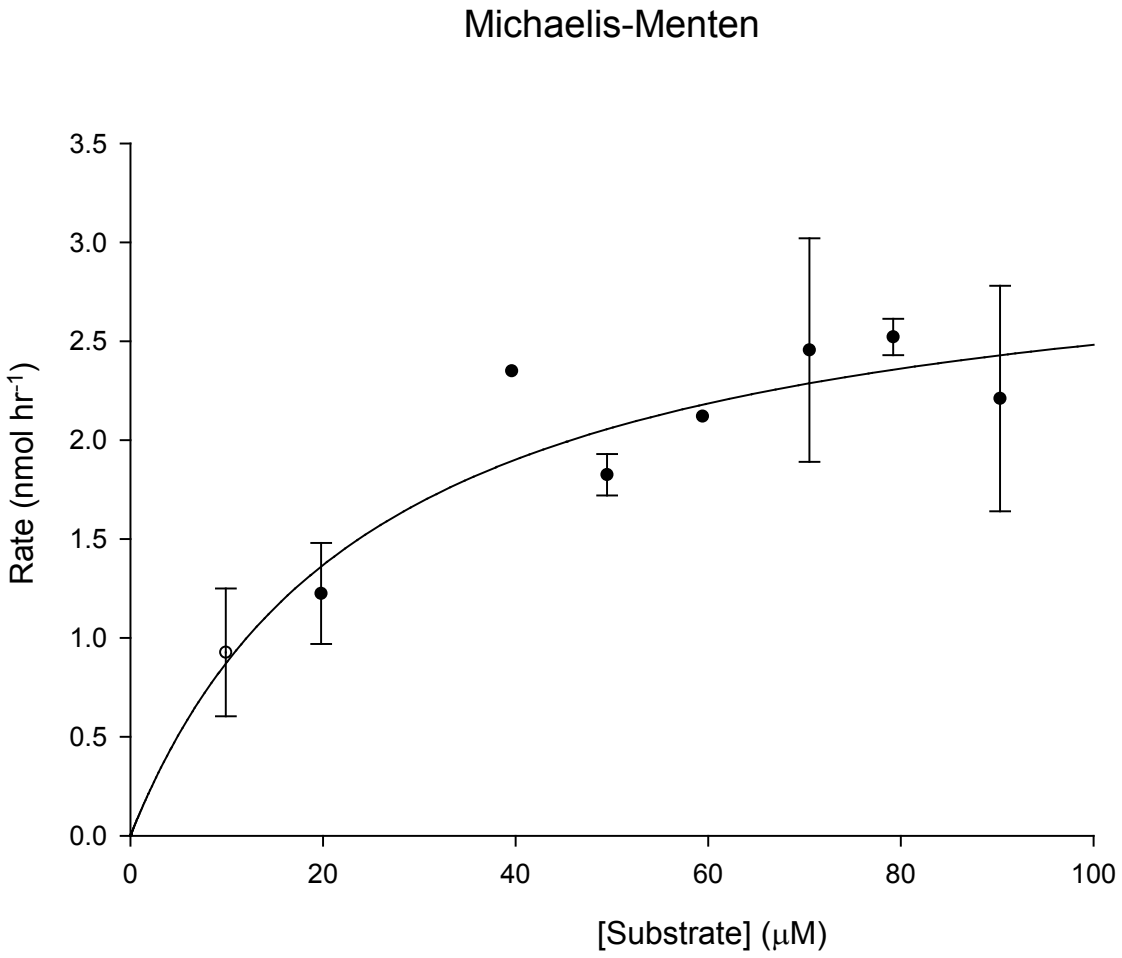


±-Flurbiprofenoyl-CoA 8



K_m = 25.8 μM
V_{max} = 2.97 nmol h^{-1}
Enzyme = 0.2052 nmol assay^{-1} (9.66 μg assay^{-1})

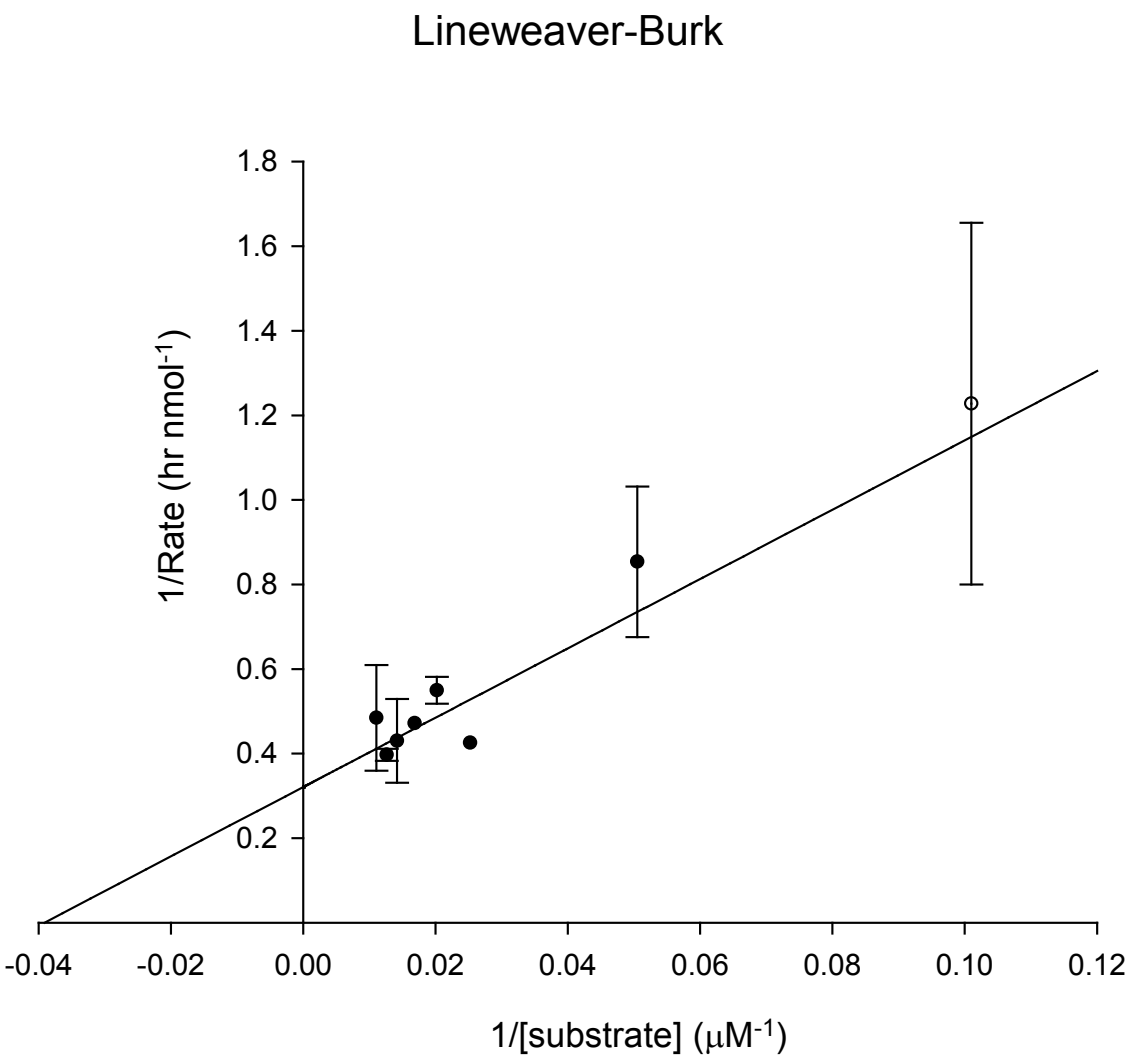
V_{max} = 5.12 nmol min^{-1} mg^{-1}
K_{cat} = 0.0040 s^{-1}
K_{cat}/K_m = 155.83 M^{-1} s^{-1}

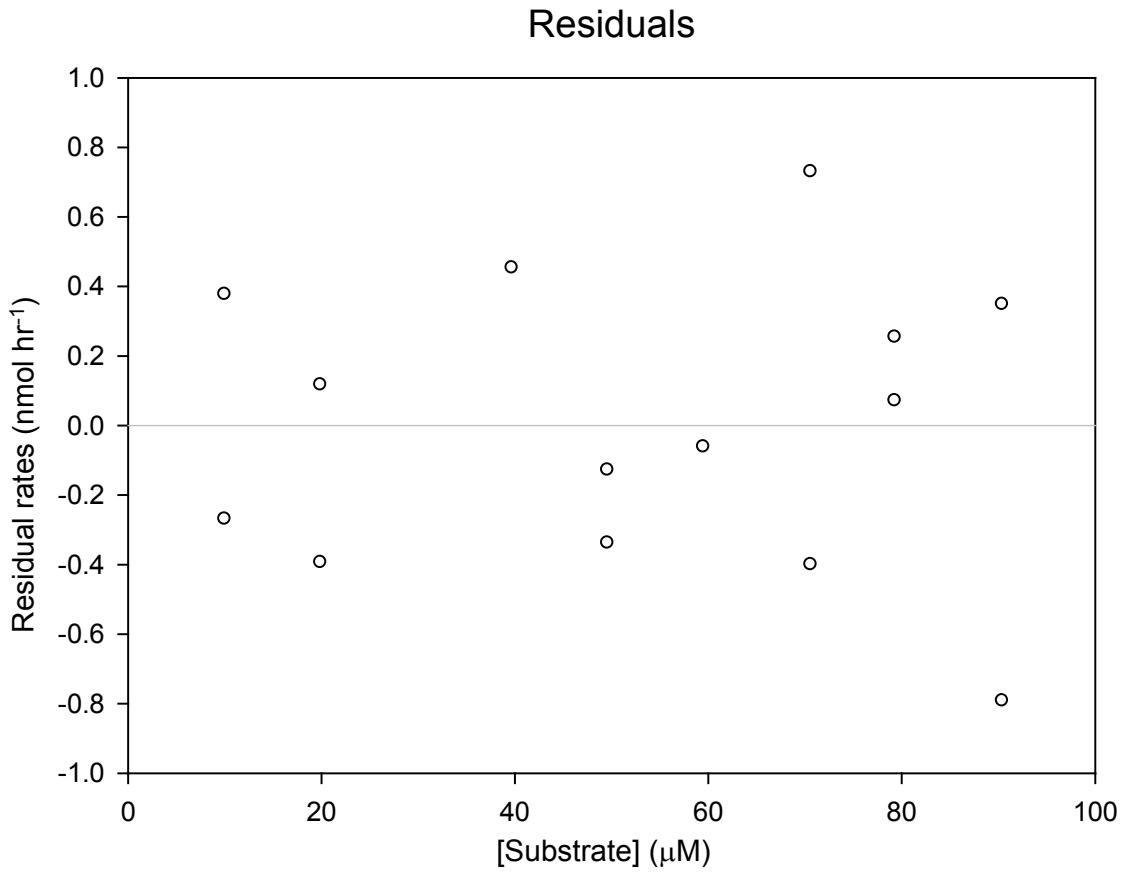


Value	±Std. Error	95% Conf. Interval			
Vmax	3.1162	0.5289	1.9638	to	4.2685
Km	25.5486	13.2867	-3.4012	to	54.4985

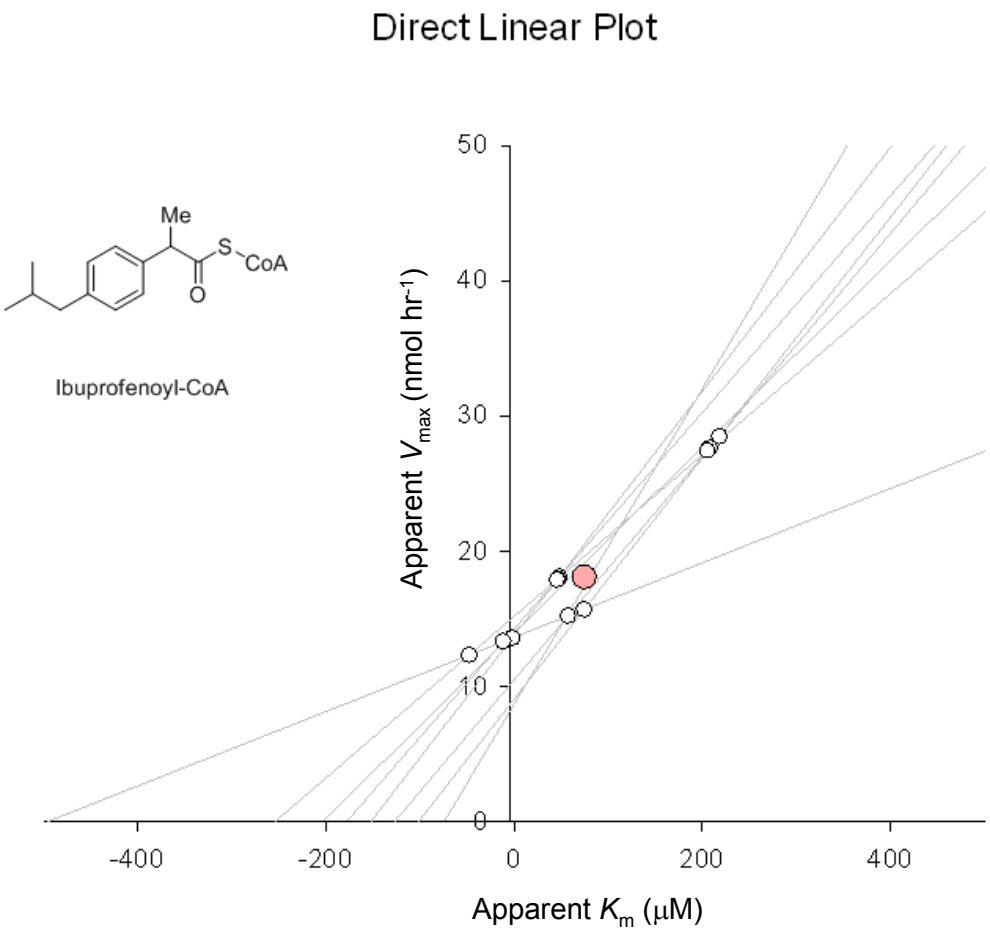
Goodness of Fit

Degrees of Freedom	12
AICc	-17.295
R^2	0.646
Sum of Squares	2.234
Sy.x	0.431
Runs Test p Value	0.133



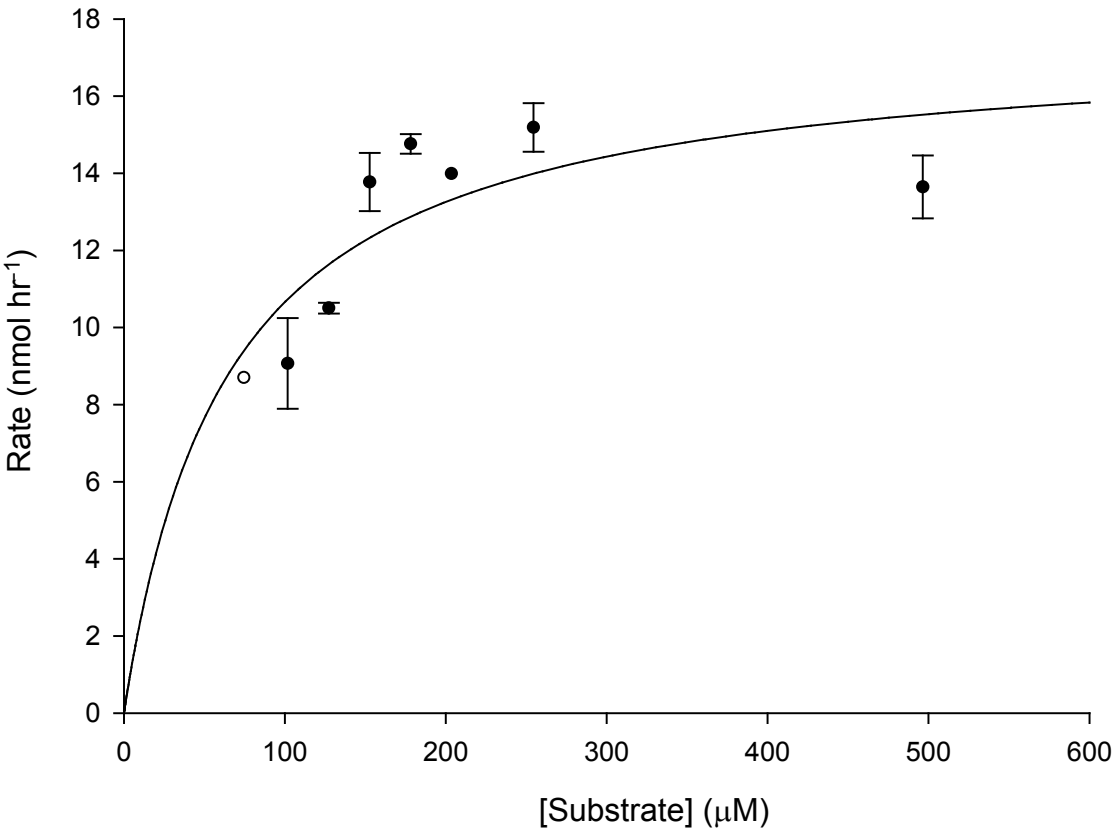


±-Ibuprofenoyl-CoA 2



K_m = 74.3 μM
V_{max} = 18.09 nmol h^{-1}
Enzyme = 0.684 nmol assay^{-1} (32.2 μg assay^{-1})

V_{max} = 9.36 nmol min^{-1} mg^{-1}
K_{cat} = 0.0073 s^{-1}
K_{cat}/K_m = 98.87 M^{-1} s^{-1}

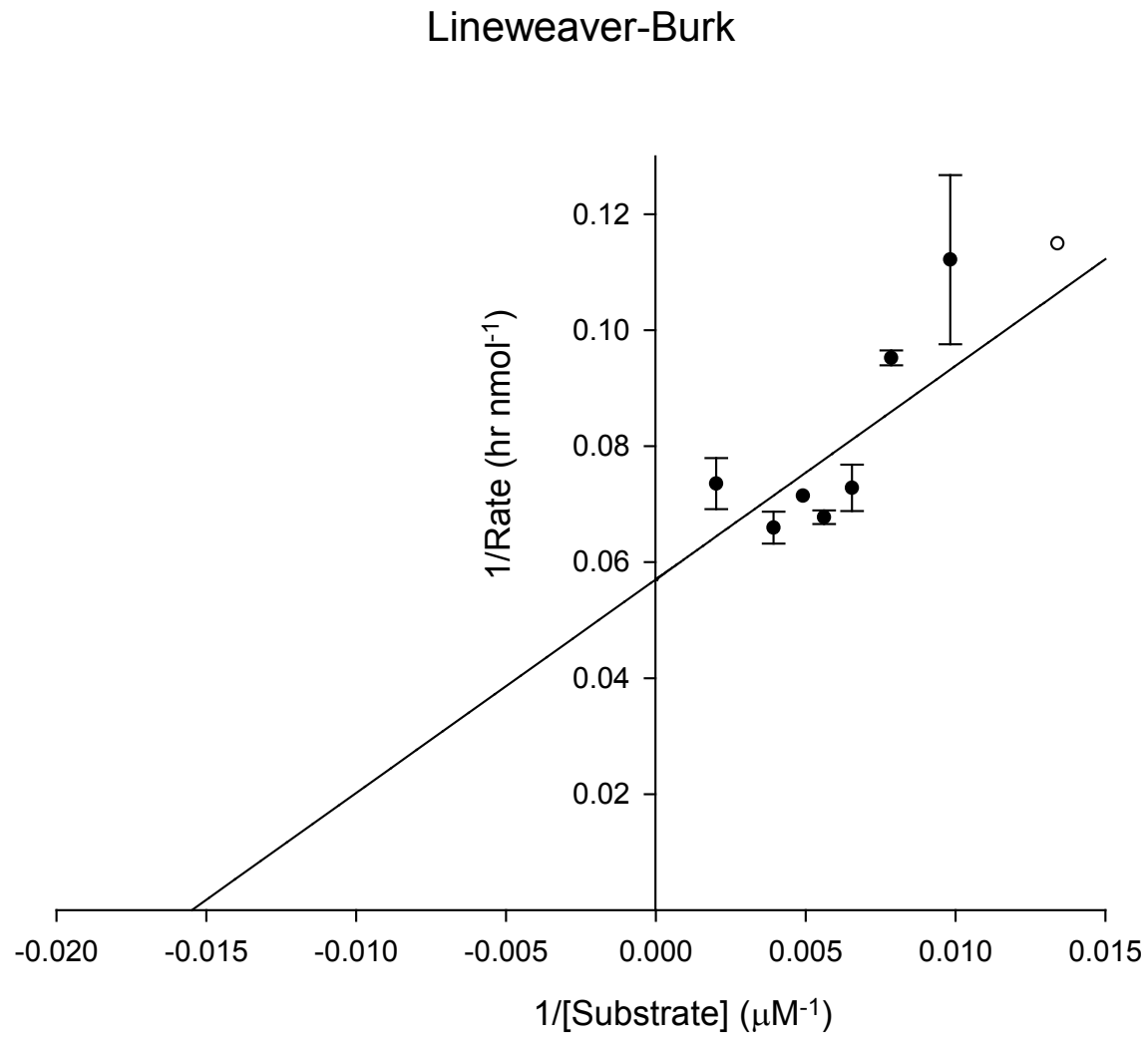


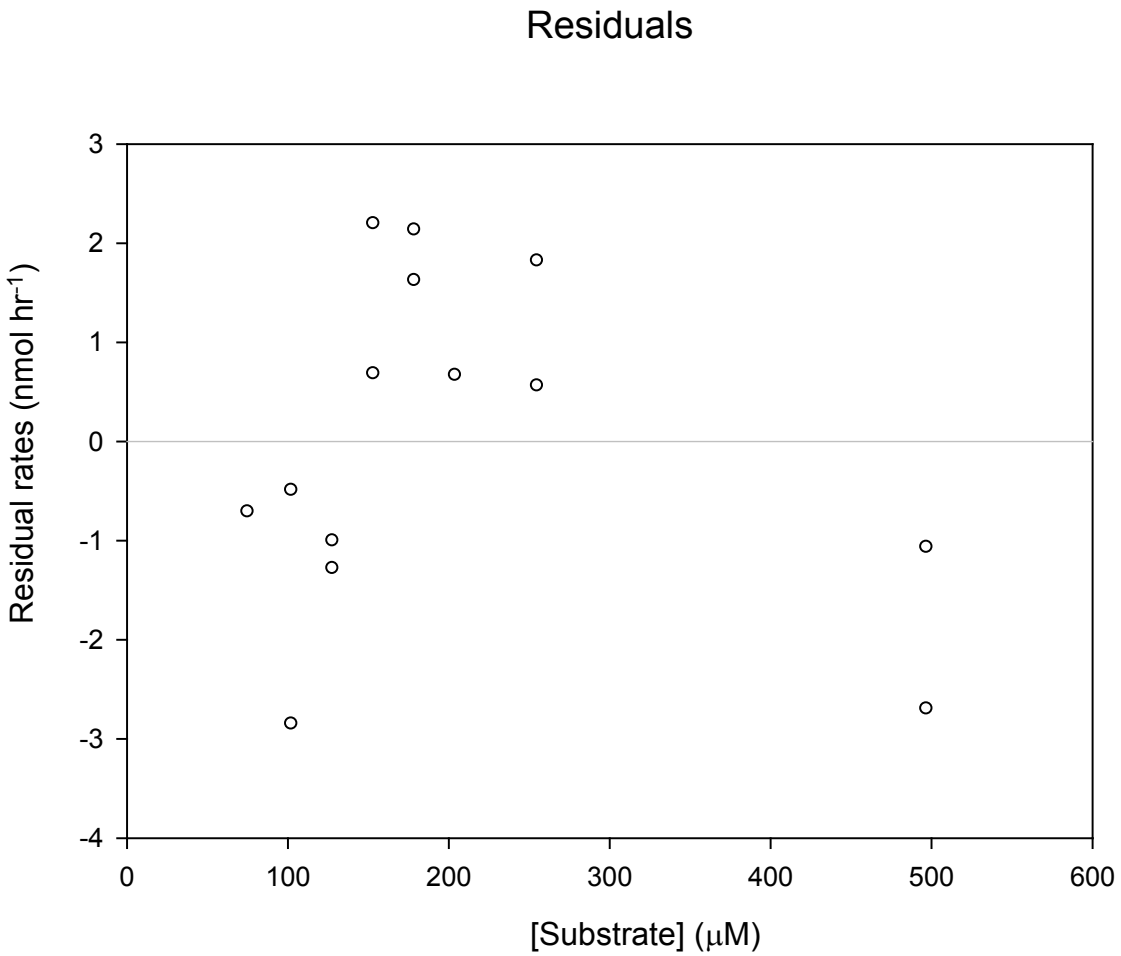
Parameters

	Value	±Std. Error	95% Conf. Interval		
Vmax	17.5378	1.8155	13.5821	to	21.4935
Km	64.5805	24.6465	10.8793	to	118.2817

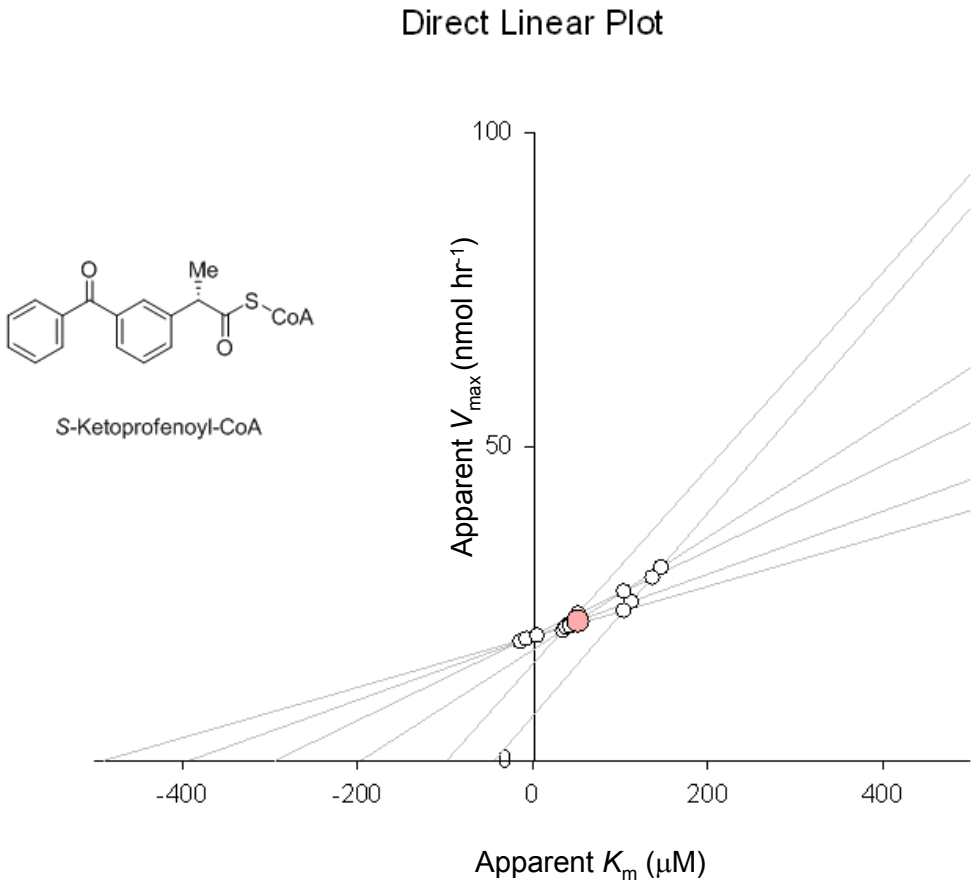
Goodness of Fit

Degrees of Freedom	12
AICc	21.803
R^2	0.570
Sum of Squares	36.468
Sy.x	1.743
Runs Test p Value	0.048



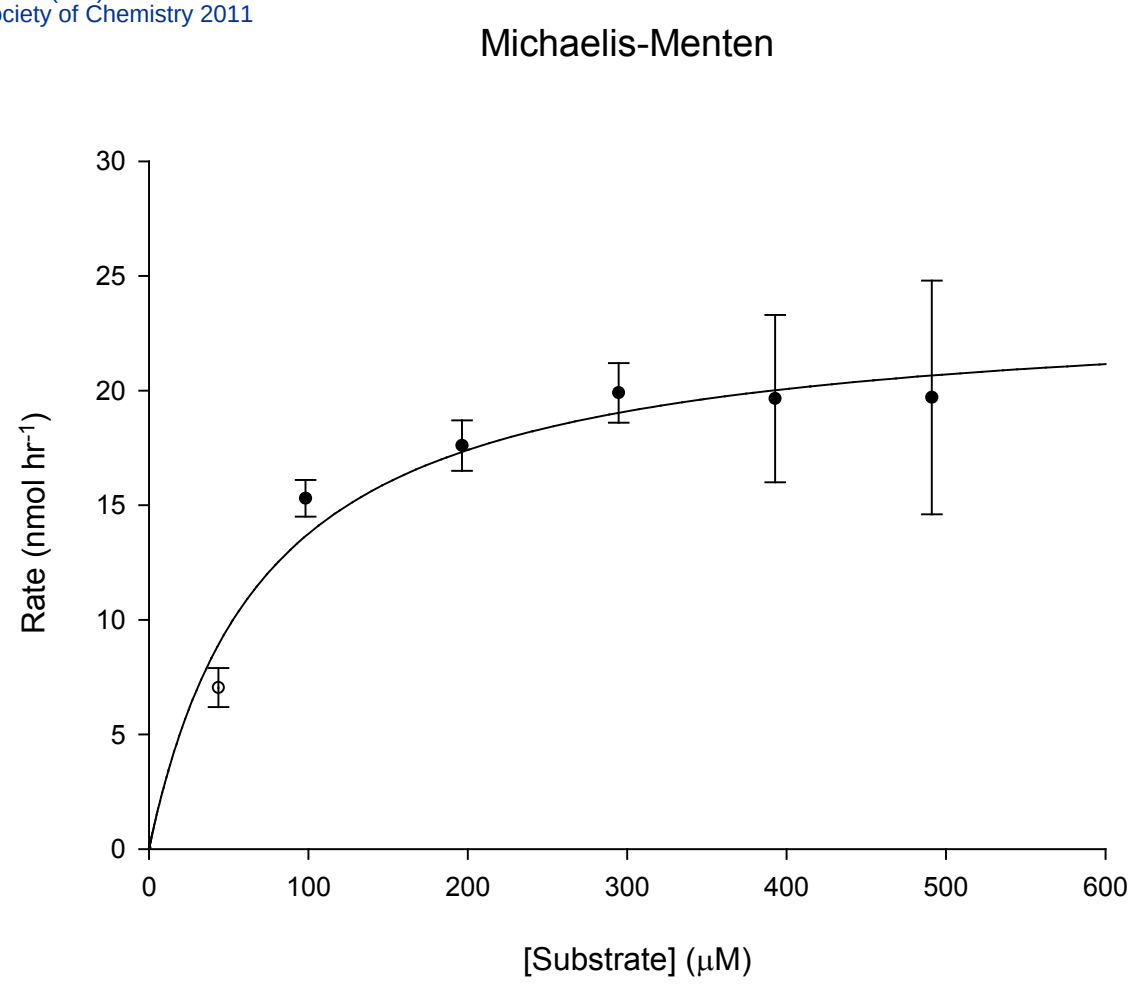


S-Ketoprofenoyl-CoA 9



K_m = 51.78 μM
V_{max} = 22.24 nmol h^{-1}
Enzyme = 0.4104 nmoles assay^{-1} (19.32 μg assay^{-1})

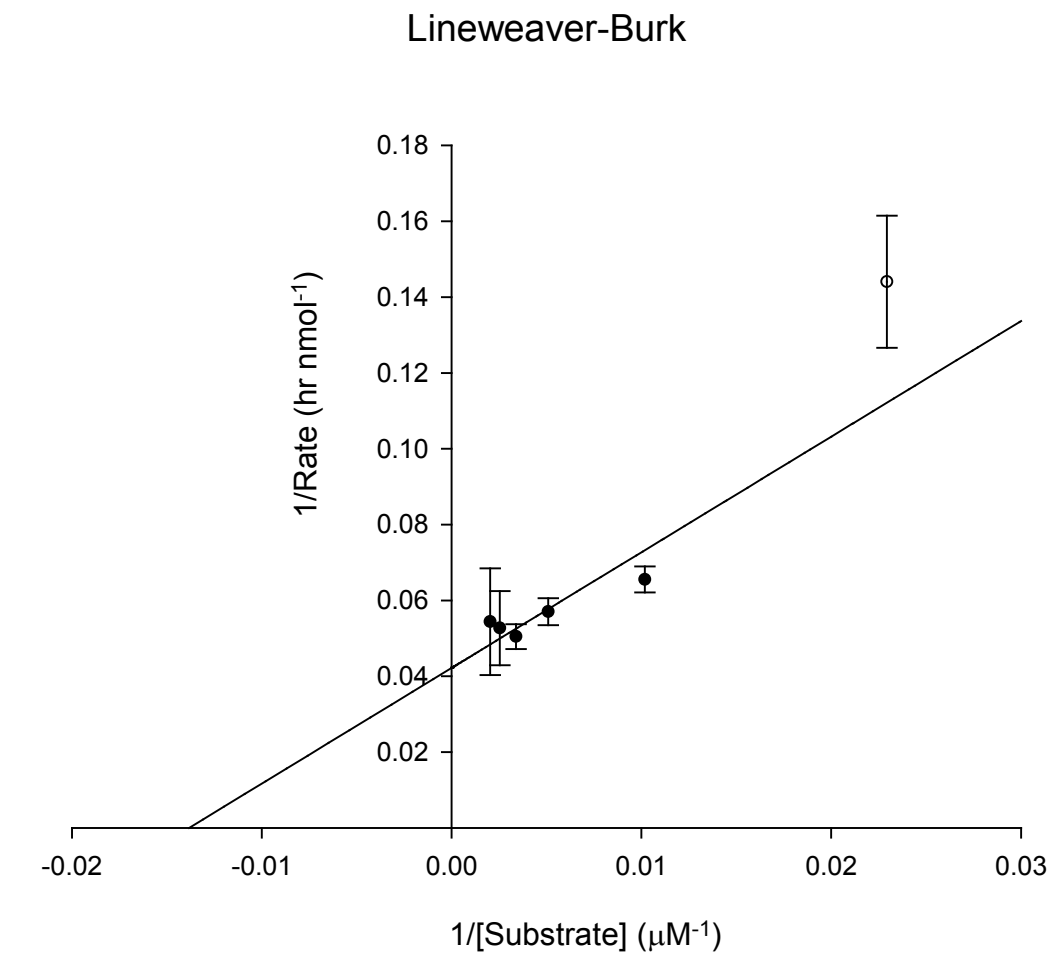
V_{max} = 19.18 nmol min^{-1} mg^{-1}
K_{cat} = 0.0150 s^{-1}
K_{cat}/K_m = 289.68 M^{-1} s^{-1}

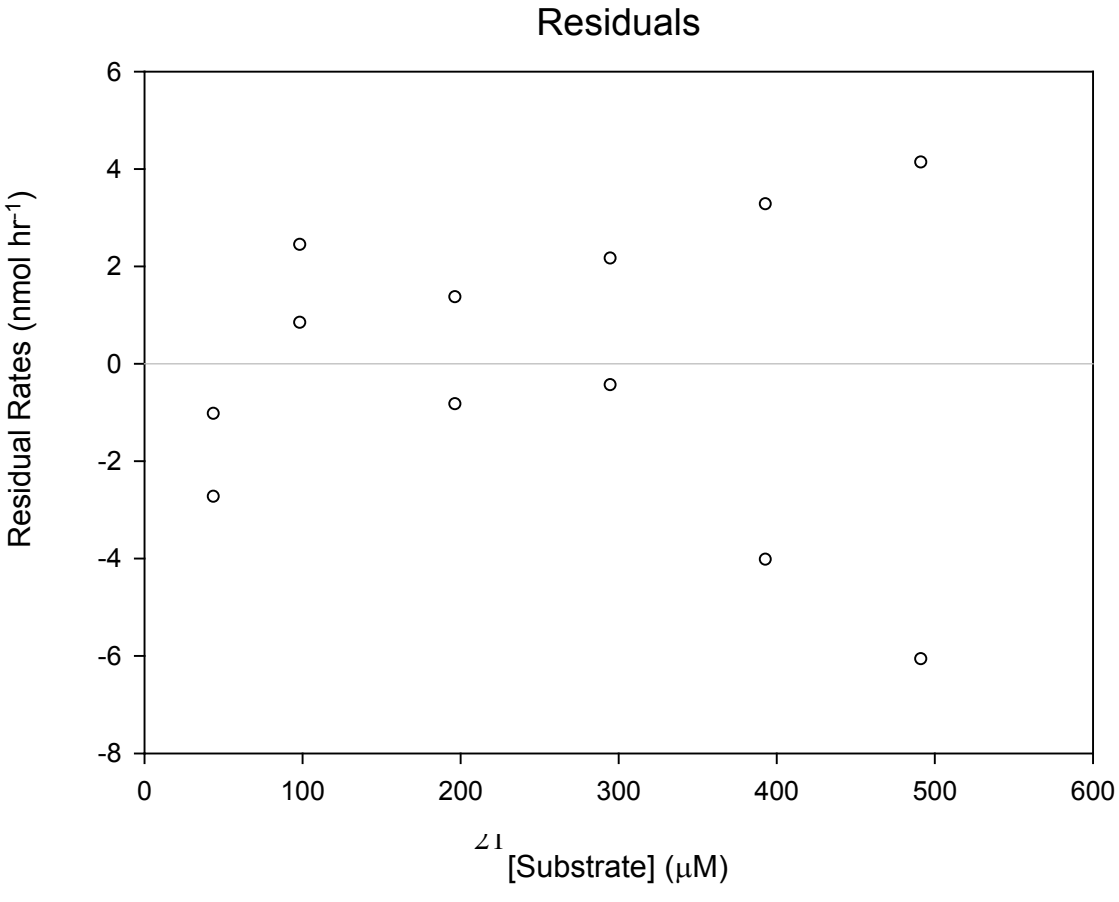


Value	±Std. Error	95% Conf. Interval			
Vmax	23.7011	2.6864	17.7153	to	29.6868
Km	72.2996	31.7504	1.5538	to	143.0453

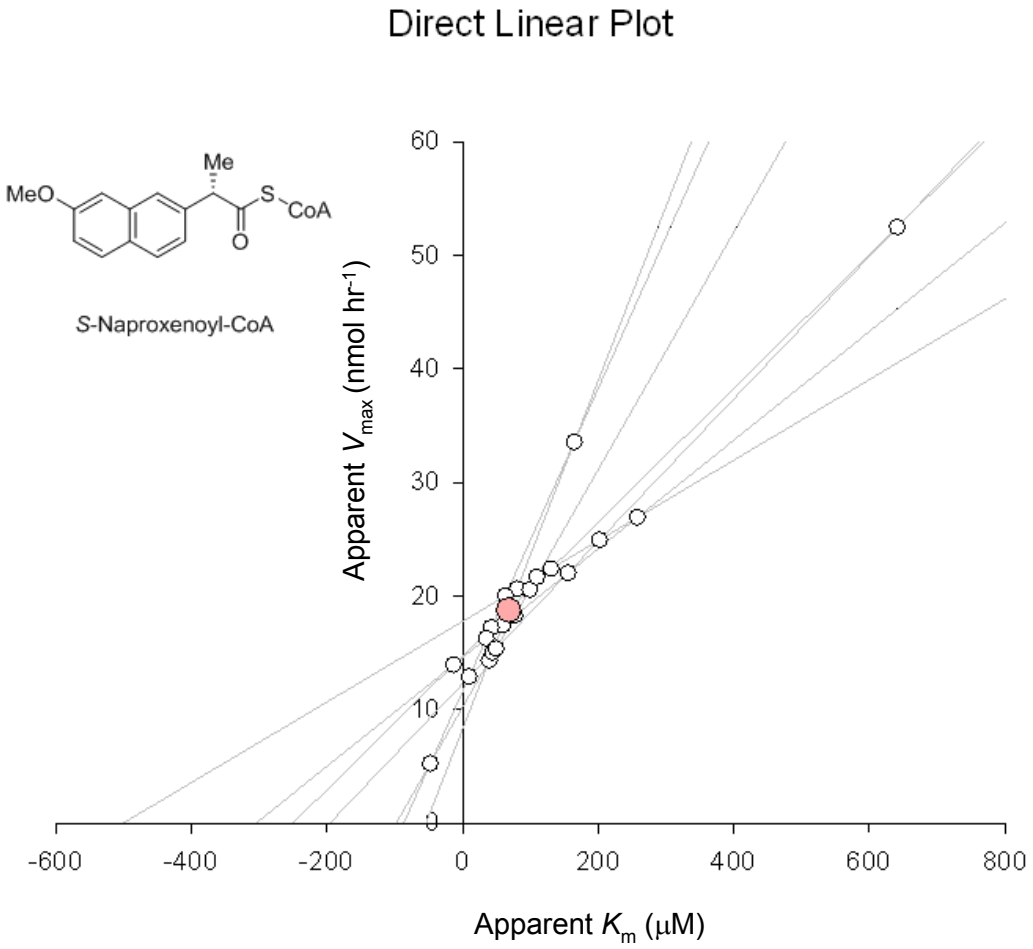
Goodness of Fit

Degrees of Freedom	10
AICc	34.847
R^2	0.691
Sum of Squares	103.419
Sy.x	3.216
Runs Test p Value	0.272



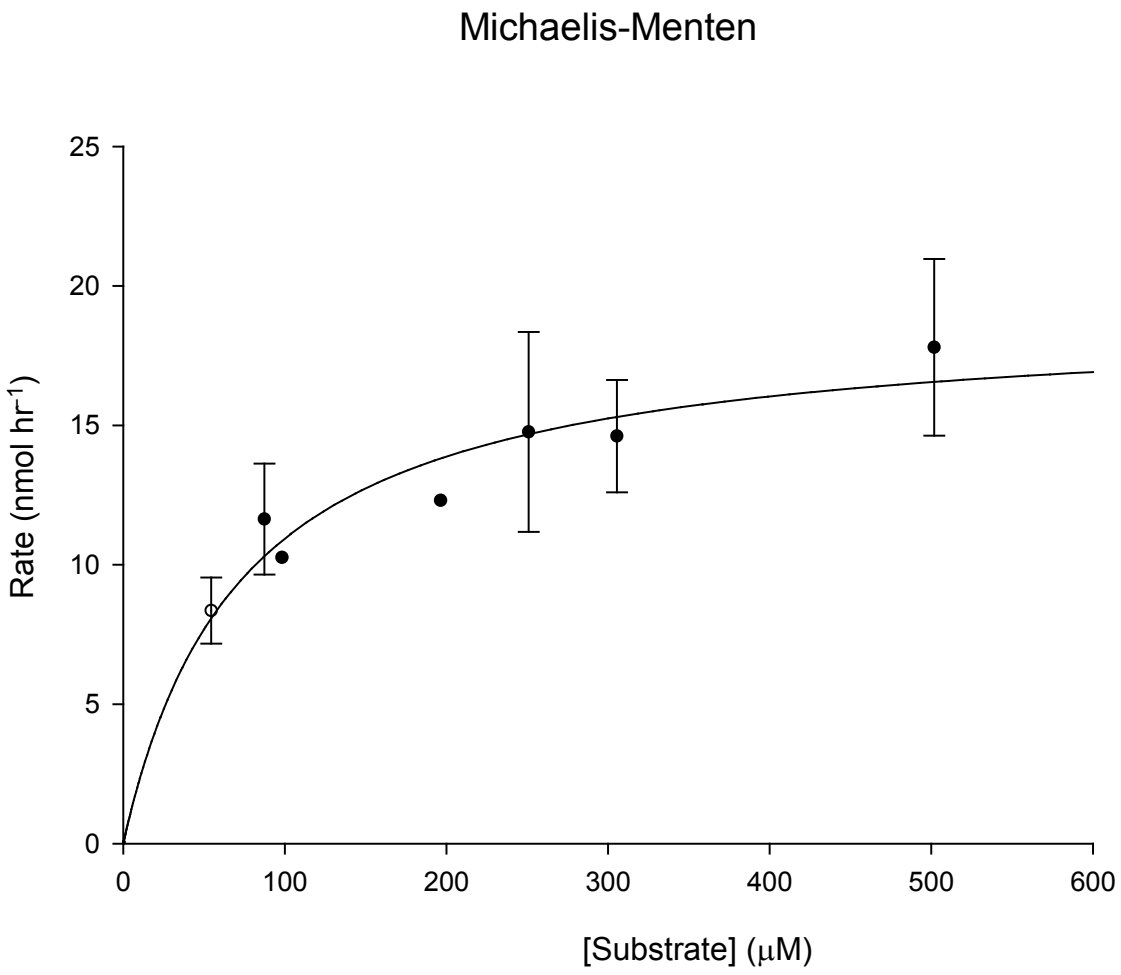


S-Naproxenoyl-CoA 10



K_m = 67.86 μM
V_{max} = 18.76 nmoles h^{-1}
Enzyme = 0.5472 nmoles assay^{-1} (25.76 μg assay^{-1})

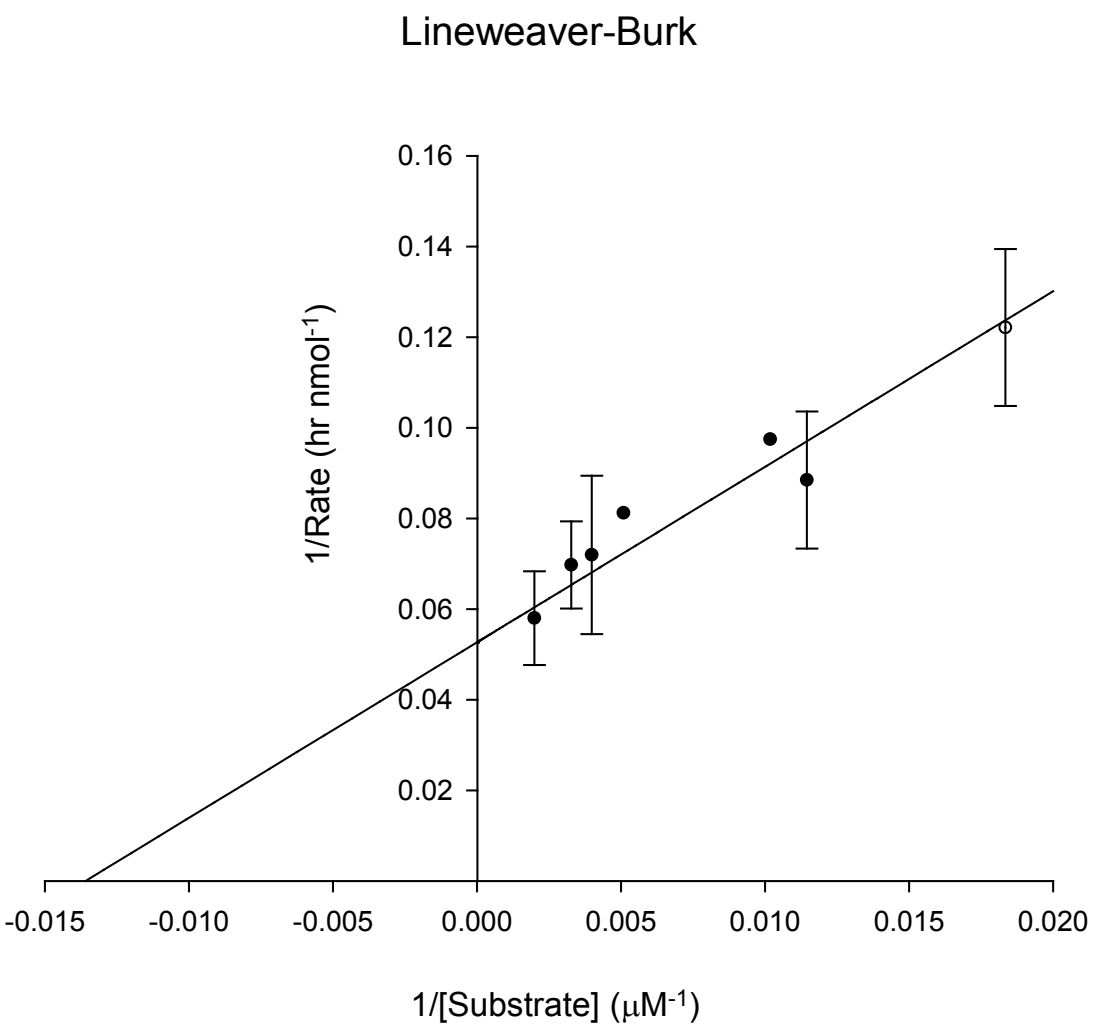
V_{max} = 12.14 nmol min^{-1} mg^{-1}
K_{cat} = 0.0095 s^{-1}
K_{cat}/K_m = 140.33 M^{-1} s^{-1}

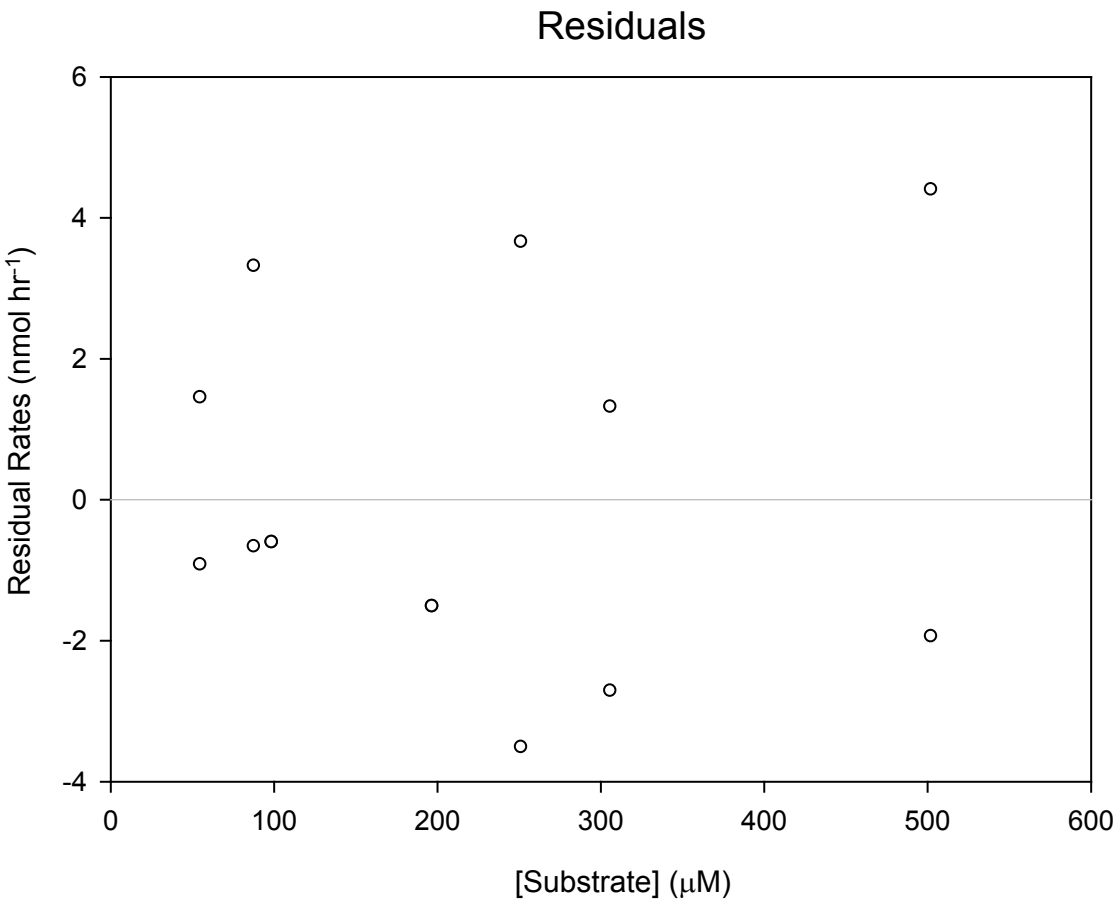


	Value	±Std. Error	95% Conf. Interval		
Vmax	18.9869	2.2443	14.0969	to	23.8768
Km	73.5604	29.5982	9.0702	to	138.0507

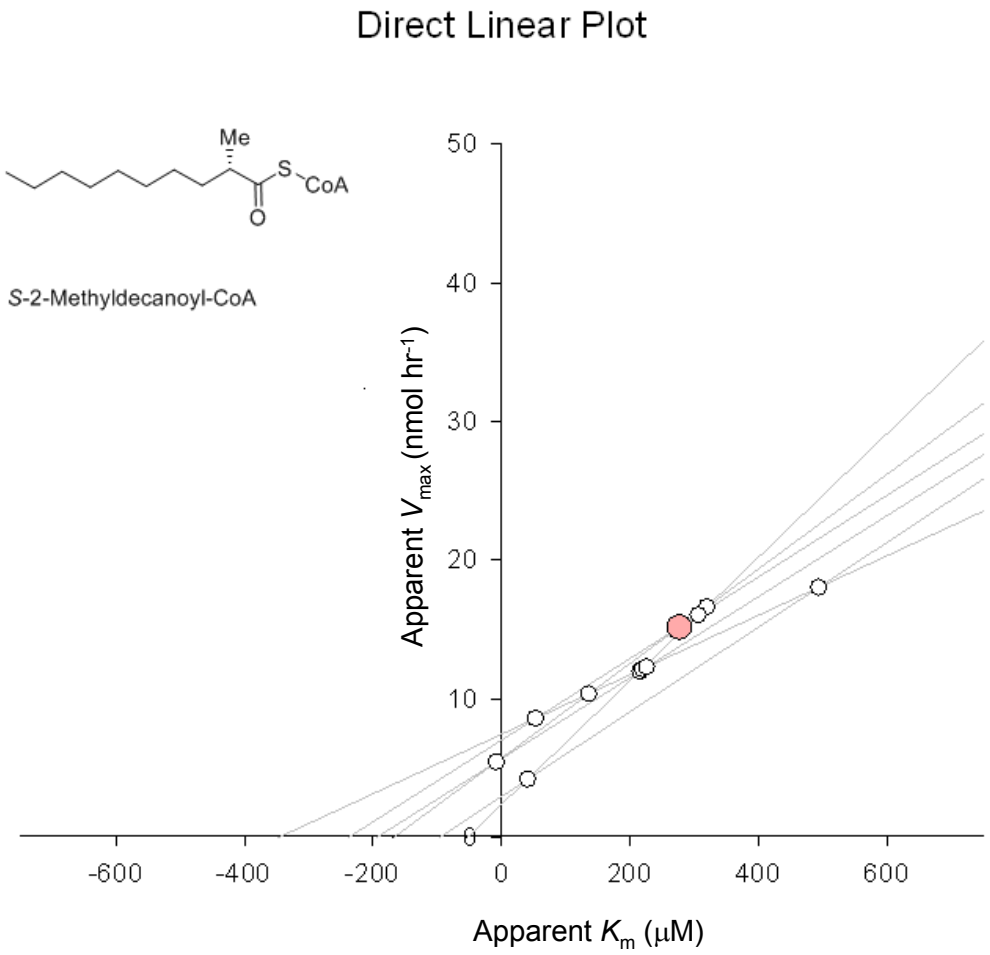
Goodness of Fit

Degrees of Freedom	12
AICc	32.381
R^2	0.579
Sum of Squares	77.635
Sy.x	2.544
Runs Test p Value	0.364



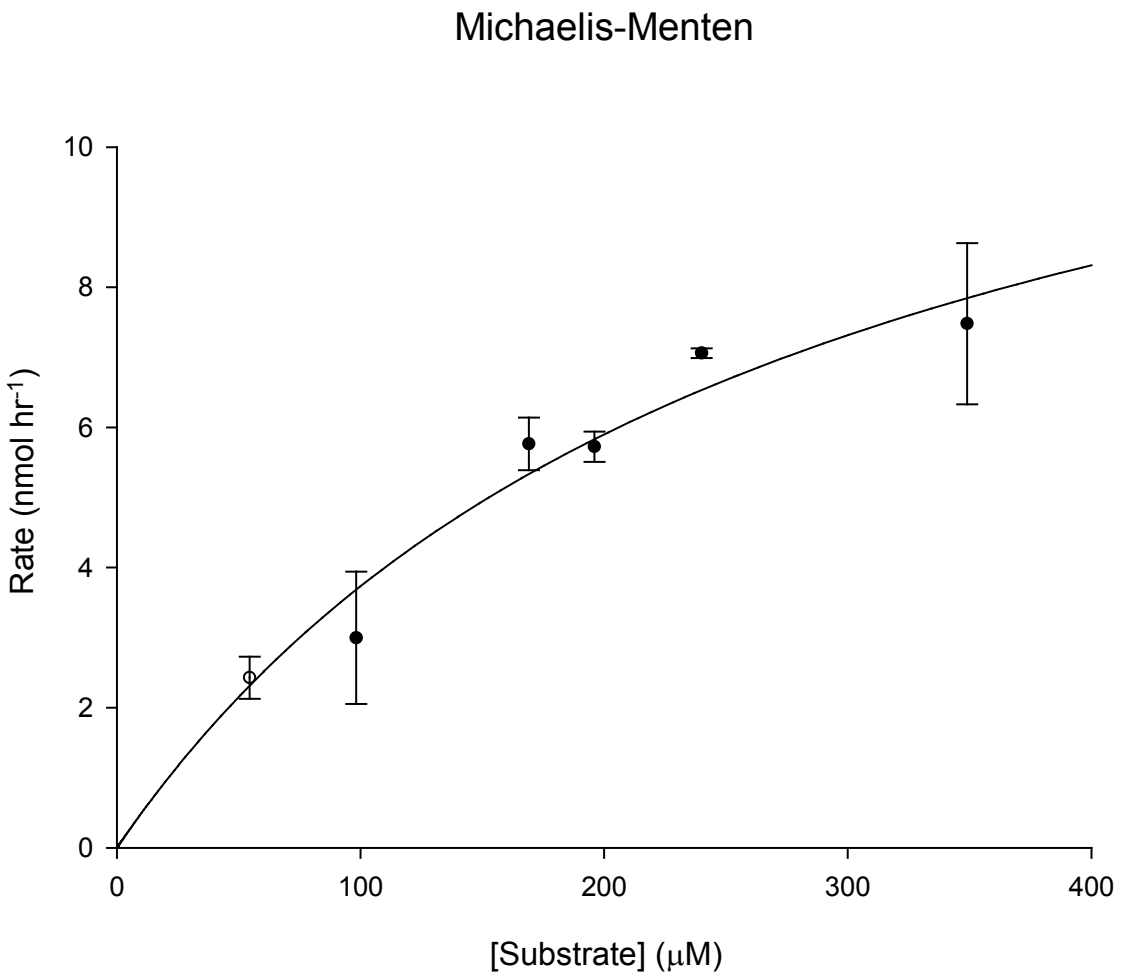


S-2-Methyldecanoyl-CoA 11



K_m = 276.5 μM
V_{max} = 15.19 nmol h^{-1}
Enzyme = 0.1368 nmoles assay^{-1} (6.45 μg assay^{-1})

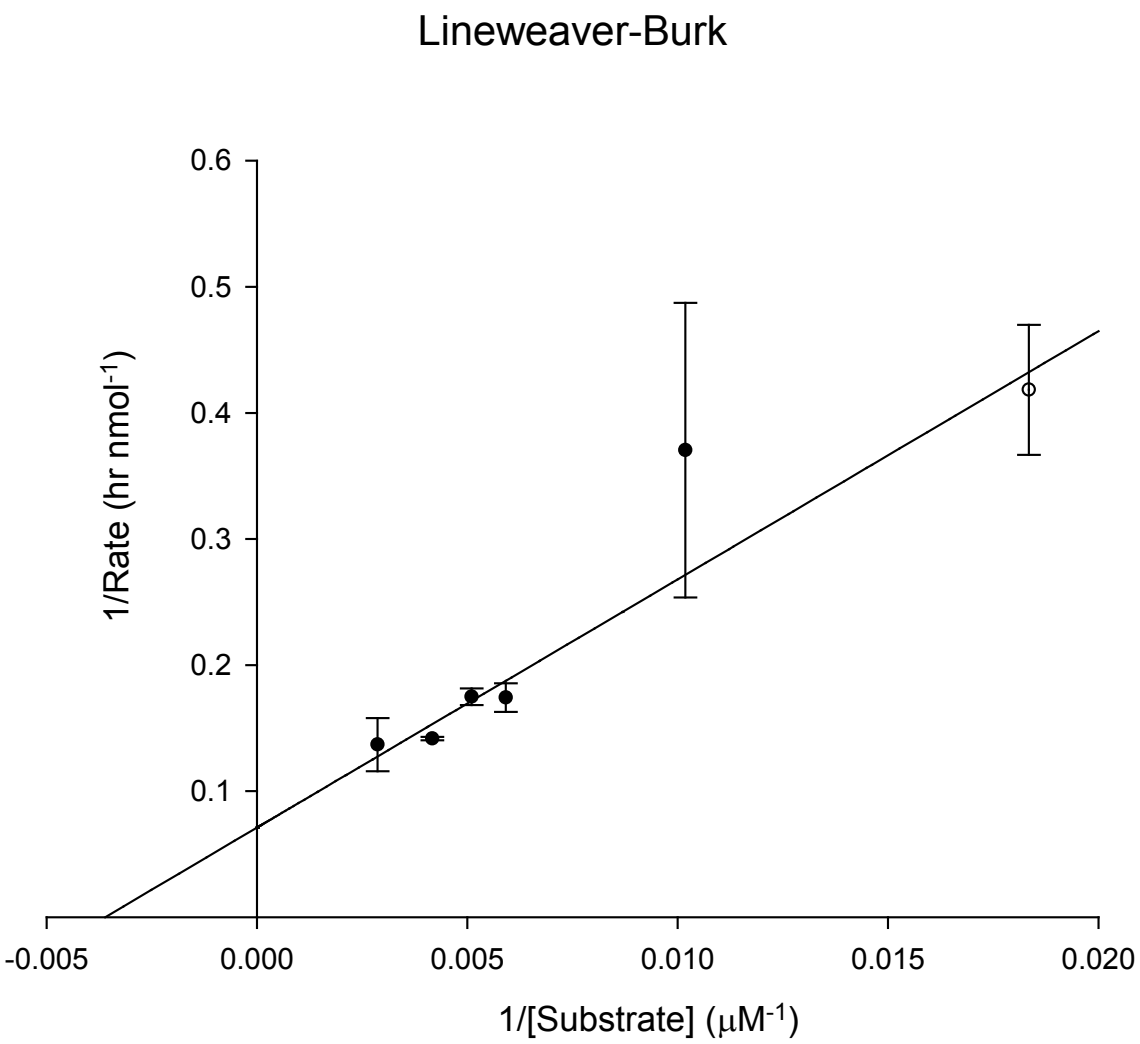
V_{max} = 39.25 nmol min^{-1} mg^{-1}
K_{cat} = 0.031 s^{-1}
K_{cat}/K_m = 111.5 M^{-1} s^{-1}

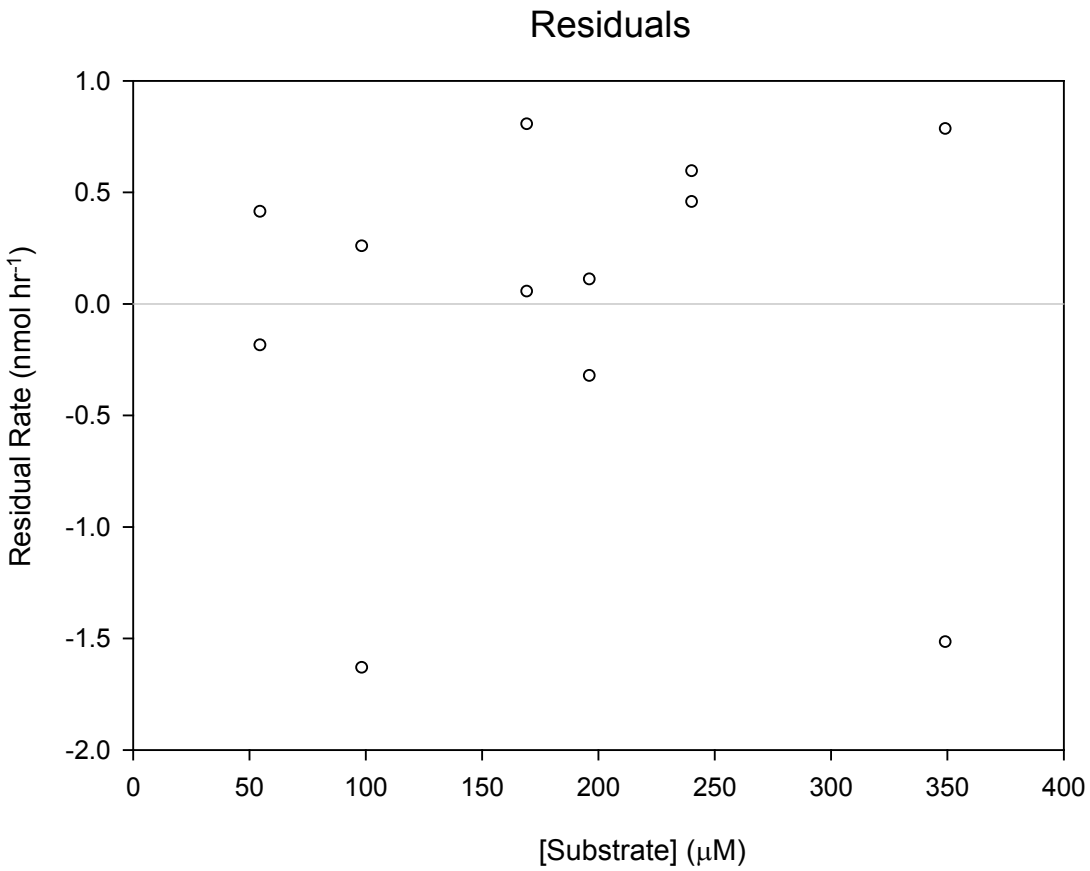


Value	±Std. Error	95% Conf. Interval			
Vmax	14.0720	3.4918	6.2915	to	21.8525
Km	277.0270	122.8954	3.1930	to	550.8609

Goodness of Fit

Degrees of Freedom	10
AICc	2.831
R^2	0.852
Sum of Squares	7.177
Sy.x	0.847
Runs Test p Value	0.125





```
AMACR 1A        --MALQGISVVELSGLAPGPFCAMVLADFGARVVRVDRPGSRYDVSR--LGRGKRSLVLD
MCR             MAGPLSGLRVVELAGIGPGPHAAMILGDLGADVVRIDRPSSVDGISRDAMLRNRRIVTAD
                   .*.*: ****:*:.***..**:*.*:** ***:***.*  .:**  : *.:* :. *

AMACR 1A        LKQPRGAAVLRRLCKRSDVLLEPFRRGVMEKLQLGPEILQRENPRLIYARLSGFGQSGSF
MCR             LKSDQGLELALKLIAKADVLIEGYRPGVTERLGLGPEECAKVNDRLIYARMTGWGQTGPR
                **. :*  :  :*  ::***:* :* ** *:* ****   : * ******::*:**:*.

AMACR 1A        CRLAGHDINYLALSGVLSKIGRSGENPYAPLNLLADFAGGGLMCALGIIMALFDRTRTGK
MCR             SQQAGHDINYISLNGILHAIGRGDERPVPPLNLVGDFGGGSMFLLVGILAALWERQSSGK
                .: *******::*.*:*  ***..*.* .****:.**.**.::  :**: **::*  :**

AMACR 1A        GQVIDANMVEGTAYLSSFLWKTQKSSLWEAPRGQNMLDGGAPFYTTYRTADGEFMAVGAI
MCR             GQVVDAAMVDGSSVLIQMMWAMRATGMWTDTRGANMLDGGAPYYDTYECADGRYVAVGAI
                ***:** **:*:: * .::*  : :.:*  .** ********:* **. ***.::*****

AMACR 1A        EPQFYELLIKGLGLKSDELPNQMSMDDWPEMKKKFADVFAKKTKAEWCQIFDGTDACVTP
MCR             EPQFYAAMLAGLGLDAAELPPQNDRARWPELRALLTEAFASHDRDHWGAVFANSDACVTP
                *****  :: ****.: *** * .   ***::  :::.**.: : .*  :* .:******

AMACR 1A        VLTFEEVVHHDHNKERGSFITSEEQDVSPRPAPLLLNTPAIPSFKRDPFIGEHTEEILEE
MCR             VLAFGEVHNEPHIIERNTFYEANG---GWQPMPAPRFSRTASSQPRPPAATIDIEAVLTD
                **:* ** :. *  **.:*  ::    . :* *    : : .*  * *    . * :* :

AMACR 1A        FGFSREEIYQLNSDKIIESNKVKASL
MCR             WDG-----------------------
                :.
```

Figure S1: Alignment of AMACR 1A (UniProtKB accession number Q9UHK6) with 2-methylacyl-CoA racemase from *M. tuberculosis* (UniProtKB accession number tr|O06543|O06543). Residues are colour coded: binding to side-chain of *S*-ibuprofenoyl-CoA; binding to side-chain of *R*-ibuprofenoyl-CoA; Methionine rich surface binding acyl side-chain during chiral inversion reaction (Leu-46 and Phe-194 from *S*-isomer binding site also involved); Catalytic residues. Residues are identified based on structure of MCR,[7] and sequences were aligned using ClusterW (http://www.ch.embnet.org/software/ClustalW.html) with default parameters. Residue numbers refer to AMACR 1A sequence.

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