Supplementary Material for Organic & Biomolecular Chemistry
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Suplementary Material

Molecular Modelling Methods

Ligand and Receptor preparation

All ligands and receptors were prepared using SYBYL6.5 and used as MOL2 files. The ligands were designed with Gasteiger-Huckel charges and their structures were energy-minimised using the Tripos force-field (Clark *et al*, 1989). The receptors were prepared by manually removing all ligands and water molecules (except when relevant) from the structures. The hydrogen atoms were added automatically and the ionisation state of the pH sensitive residues in the active-site was checked manually.

GOLD docking

Each ligand was docked using the latest available version of GOLD (Jones *at al*, 1995) (version 2.1) in 25 independent runs, and for each of these a maximum number of 100000 operations were performed on a single population of 50 individuals. Operator weights for crossover, mutation and migration were used as default parameters (95, 95 and 10, respectively), as well as the hydrogen bonding (4.0 Å) and Van der Waals (2.5 Å) parameters. The α point in the centre of the active-site was introduced and the radius was set to 15 Å, with the automatic active-site detection on. Flexibility of the rings was allowed with the "flip ring corners" option.

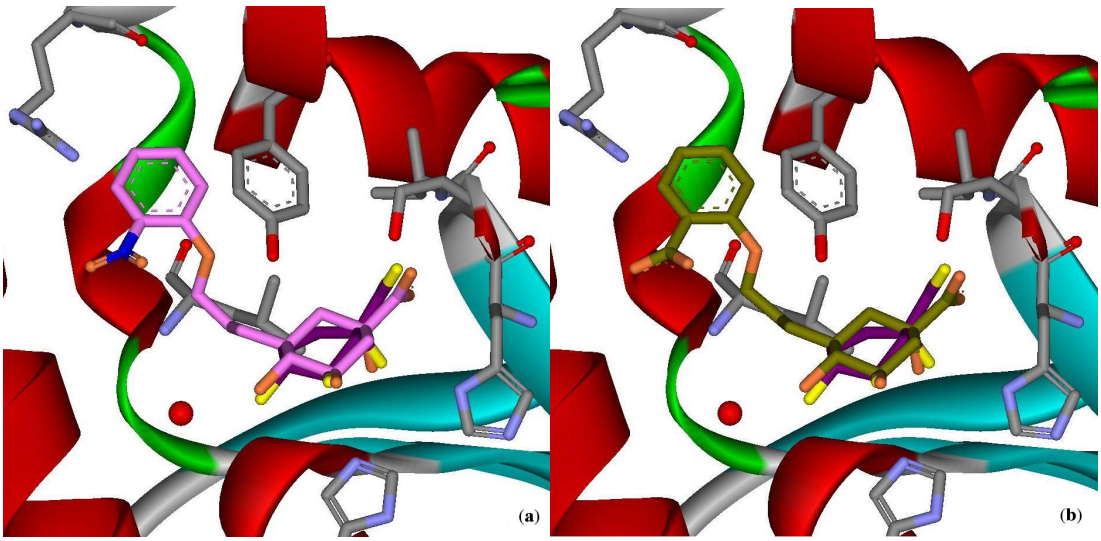

Figure 2 – docking results of **8** (a) and **9** (b) at the active-site of *S. coelicolor* type II dehydroquinase compared with the position of **3** and glycerol (purple) (PDB code: 1GU1)

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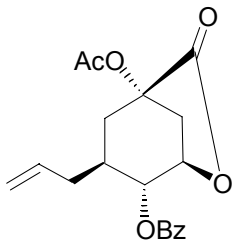
Synthetic Experimental

(1*S*, 3*R*, 4*R*, 5*S*)-1-Acetyl -5-allyl- 4-benzoylcyclohexane-1,3-carbolactone 12



To a solution of lactone **11** (1.0 g, 3.31 mmol, 1.0 eqv) in anhydrous pyridine (30 ml) was added acetic anhydride (626 µl, 6.62 mmol, 2.0 eqv) dropwise. The solution was stirred for 12 h, under N_2, at room-temperature. The solution was taken in Et_2O (150 ml) and washed with 1 M HCl (5 x 100 ml) and H_2O (100 ml). The organic layer was dried under Na_2SO_4, filtered and the solvent evaporated. The product was further purified by column chromatography on silica gel, eluting with Et_2O:Hexane (2:1) to give the *acetate* **12** as a colourless oil (1.1 g, 96%).

R_F 0.60 [Et_2O:Hex; 2:1];

υ_{max}/cm^{-1} 1802 (CO), 1745 (CO), 1719 (CO), 1642 (C=C) and 1601 (Ar);

δ_H (ppm) (400 MHz; $CDCl_3$) 8.00 (2 H, d, *J* 8.0 Hz, 2-ArH), 7.60 (1 H, t, *J* 8.0 Hz, 4-ArH), 7.47 (2 H, t, *J* 8.0 Hz, 3-ArH), 5.69 (1 H, m, =CH), 5.18 (1 H, d, *J* 2.5 Hz, =C*H*H), 5.12 (1 H, s, =CH*H*), 5.09 (1 H, dd, *J* 5.2 and 1.4 Hz, 4-H), 4.95 (1 H, dd, *J* 6.3 and 3.7 Hz, 3-H), 3.15 (1 H, ddd, *J* 11.3, 6.3 and 2.5 Hz, 2_{eq}-H), 2.59 (1 H, d, *J* 11.3 Hz, 6_{ax}-H), 2.48-2.18 (4 H, m), 2.13 (3 H, s, $OCCH_3$), 1.99 (1 H, dt, *J* 11.0 and 2.2 Hz, 1'-CH*H*);

δ_C (100 MHz, $CDCl_3$, DEPT) 176.0 (C), 172.0 (C), 167.9 (C), 137.9 (CH), 136.4 (CH), 132.4 (CH), 132.0 (C), 131.3 (CH), 121.2 (CH_2), 79.6 (C), 78.0 (CH), 73.3 (CH), 41.3 (CH_2), 39.0 (CH), 36.5 (CH_2), 35.3 (CH_2), 23.9 (CH_3);

LC/MS (ret. time/min.) 4.2 (ESI+) m/z 345 (MH^+);

HRMS calcd for $C_{19}H_{20}O_6$: *M*Na^+, 367.1158. Found: MNa^+, 367.1168.

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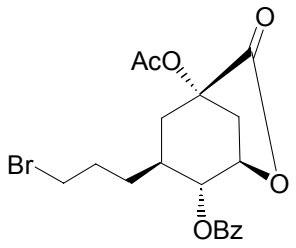
(1*S*, 3*R*, 4*R*, 5*S*) - 4 - Acetyl - 5 - (3 - bromopropyl) - 1 - benzoylcyclohexane -1,3-carbolactone 13



A solution of **12** (1.0 g, 2.90 mmol, 1.0 eqv.) and AIBN (95 mg, 0.58 mmol, 0.2 eqv.) in CCl_4 (10 ml) was bubbled slowly with HBr gas for 15 minutes at room temperature. The solution was stirred for a further 30 minutes. It was then taken up in Et_2O (100 ml) and washed with sat. $NaHCO_3$ (3 x 50 ml). The organic layer was dried with Na_2SO_4, filtered and the solvent evaporated to give the primary bromide **13** as a colourless oil (1.2 g, 97%).

R_F 0.54 [Et_2O:Hexane; 2:1];

u_{max} (NaCl)/cm^{-1} 1801s (CO), 1746s (CO), 1719s (CO) and 1602s (Ar);

δ_H (400 MHz, $CDCl_3$): 7.97 (2 H, d, J 8.0 Hz, 2-ArH), 7.56 (1 H, t, J 8.0 Hz, 4-ArH), 7.43 (2 H, t, J 8.0 Hz, 3-ArH), 5.08 (1 H, d, J 2.5 Hz, 4-H), 4.88 (1 H, t, J 2.5 Hz, 3-H), 3.30 (2 H, td, J 6.6 and 1.5 Hz, 3'-CH_2), 3.12 (1 H, ddd, J 11.5, 6.0 and 2.3 Hz, 2_{eq}-H), 2.54 (1 H, d, J 11.5 Hz, 2_{ax}-H), 2.48 (1 H, m, 2'-CHH), 2.22 (1 H, m, 5-H), 2.10 (3 H, s, $OCCH_3$), 1.95-1.85 (3 H, m), 1.78 (1 H, m, 1'-CHH), 1.55 (1 H, m, 1'-CHH);

δ_C (100 MHz, DEPT, $CDCl_3$): 173.5 (C), 169.6 (C), 165.5 (C), 134.2 (CH), 130.1 (CH), 129.6 (C), 129.0 (CH), 77.1 (C), 75.7 (CH), 71.1 (CH), 36.7 (CH), 34.3 (CH_2), 34.1 (CH_2), 33.4 (CH_2), 33.0 (CH_2), 31.9 (CH_2), 21.6 (CH_3);

LC/MS (ret. time/min.) 4.3 (ESI+) m/z 424/426 (MH^+);

HRMS calcd for $C_{19}H_{21}BrO_6$: MNa^+, 447.0414. Found: MNa^+, 447.0418.

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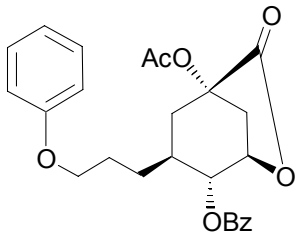
(1*S*, 3*R*, 4*R*, 5*S*) - 4 - Acetyl - 5 - [(3 - phenyloxy)-propyl] - 1 -benzoylcyclohexane-1,3-carbolactone 14



A mixture of **13** (35 mg, 0.082 mmol, 1.0 eqv.), phenol (12 mg, 0.123 mmol, 1.5 eqv.), NaH (60% in mineral oil) (4 mg, 0.098 mmol, 1.2 eqv.) and KI (1.5 mg, 0.008 mmol, 0.1 eqv.) in MeCN (2 ml) was heated at reflux under N_2 for 1 h. The mixture was allowed to cool down to room temperature, taken up in Et_2O (20 ml) and washed with sat. $NaHCO_3$ (3 x 20 ml) and H_2O (20 ml). The organic layer was dried over Na_2SO_4, filtered and the solvent evaporated. The residue was purified by column chromatography on silica gel (Et_2O:Hex; 2:1) to give the *product* **14** as a colourless oil (25mg, 69%).

R_F 0.55 [Et_2O:Hexane; 2:1];

υ_{max} (NaCl)/cm^{-1} 1800s (CO), 1745s (CO), 1719s (CO), 1600s (Ar) and 1586s (Ar);

δ_H (400 MHz, $CDCl_3$): 8.02 (2 H, d, *J* 7.9 Hz, 2-ArH), 7.59 (1 H, t, *J* 7.9 Hz, 4-ArH), 7.47 (2 H, t, *J* 7.9 Hz, 3-ArH), 7.25 (2 H, dd, *J* 7.4 and 1.0 Hz, 3''-ArH), 6.91 (1 H, t, *J* 7.4 Hz, 4''-ArH), 6.85 (2 H, dd, *J* 7.4 and 1.0 Hz, 2''-ArH), 5.21 (1 H, t, *J* 2.7 Hz, 4-H), 4.94 (1 H, dd, *J* 6.2 and 3.8 Hz, 3-H), 3.94 (2 H, t, *J* 6.0 Hz, 3'-CH_2), 3.15 (1 H, m, 2_{eq}-H), 2.61 (1 H, d, *J* 11.4 Hz, 2_{ax}-H), 2.53 (1 H, m, 2'-C*H*H), 2.31 (1 H, m, 5-H), 2.14 (3 H, s, $OCCH_3$), 1.95-1.50 (5 H, m);

δ_C (100 MHz, DEPT, $CDCl_3$): 172.1 (C), 168.1 (C), 164.0 (C), 157.7 (C), 132.5 (CH), 128.5 (CH), 128.2 (CH), 127.5 (C), 127.4 (CH), 119.5 (CH), 113.3 (CH), 75.6 (C), 74.2 (CH), 69.8 (CH), 65.9 (CH_2), 35.6 (CH), 32.7 (CH_2), 32.6 (CH_2), 29.9 (CH_2), 26.4 (CH_2), 20.0 (CH_3);

LC/MS (ret. time/min.) 4.5 (ESI+) m/z 439 (MH^+)

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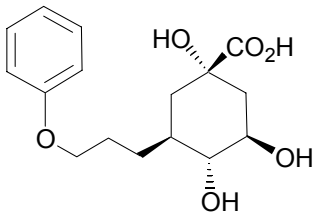
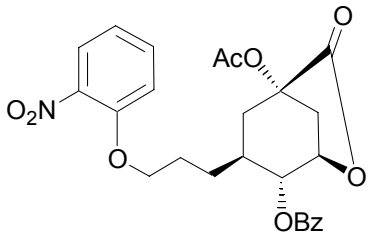
(1*S*, 3*R*, 4*R*, 5*S*) - 5 - [(3 - Phenyloxy)-propyl] - 1,3,4 - cyclohexane - 1 -carboxylic acid 7



A solution of carbolactone **14** (35 mg, 0.08 mmol, 1.0 eqv.) in H_2O/MeCN (1:1) (2 ml) was treated with NaOH (100 mg/ml solution) (128 μl, 0.32 mmol, 4.0 eqv.) and stirred for 3 h at room temperature. Amberlite IR-120 (H) (50 mg) was added, and the mixture stirred for a further 30 min. The solution was filtered and lyophilised to give the *acid* **7** as a colourless glass (34 mg, quantitative).

υ_{max} (NaCl)/cm^{-1} 3060b (OH), 1662s (CO), 1600s (Ar);
δ_H (400 MHz, $CDCl_3$): 7.28 (2 H, t, *J* 7.5 Hz, 3''-ArH), 6.95 (3 H, m, 4''-ArH), 3.97 (2 H, t, *J* 6.3 Hz, 3'-CH_2), 5.21 (1 H, m, 3-H), 3.08 (1 H, t, *J* 9.2 Hz, 4-H), 1.90-1.10 (9 H, m);
δ_C (100 MHz, DEPT, D_2O): 176.1 (C), 158.5 (C), 130.3 (CH), 121.9 (CH), 115.4 (CH), 78.5 (CH), 76.0 (C), 71.8 (CH), 69.4 (CH_2), 41.1 (CH_2), 38.5 (CH_2), 37.8 (CH), 27.6 (CH_2), 25.7 (CH_2);
MS (ESI-) m/z 309 $[(M-H]^-$
HRMS calcd for $C_{16}H_{21}O_6$: $[(M-H]^-$, 309.1344. Found: $[(M-H]^-$, 309.1339.

(1*S*, 3*R*, 4*R*, 5*S*) - 4 - Acetyl - 5 - [3 - (2 - nitrophenyloxy)-propyl)] - 1 - benzoylcyclohexane-1,3-carbolactone 15



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A mixture of **13** (160 mg, 0.38 mmol, 1.0 eqv.), *o*-nitrophenol (79 mg, 0.57 mmol, 1.5 eqv.), NaH (60% in mineral oil) (23 mg, 0.57 mmol, 1.5 eqv.) and KI (13 mg, 0.08 mmol, 0.2 eqv.) in MeCN (2 ml) was heated at reflux under N_2 for 18 h,. The mixture was allowed to cool down to room temperature, taken up in Et_2O (20 ml) and washed with 1 M HCl (3 x 20 ml) and H_2O (20 ml). The organic layer was dried over Na_2SO_4, filtered and the solvent evaporated. The residue was purified by column chromatography on silica gel (Et_2O:Hex; 2:1) to give the *product* **15** as a yellow oil (20 mg, 11%).

R_F 0.28 [Et_2O:Hexane; 2:1];

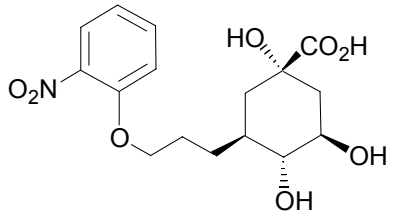
υ_{max} (NaCl)/cm^{-1} 1800s (CO), 1745s (CO), 1719s (CO), 1608s (Ar) and 1583s (Ar);

δ_H (400 MHz, $CDCl_3$): 8.01 (2 H, dd, *J* 8.2 and 1.2 Hz, 2-ArH), 7.79 (1 H, dd, *J* 8.1 and 1.7 Hz, 3''-ArH), 7.60 (1 H, t, *J* 8.2 Hz, 4-ArH), 7.48 (3 H, m), 7.04 (2 H, m), 5.19 (1 H, t, *J* 3.4 Hz, 4-H), 4.95 (1 H, dd, *J* 6.2 and 3.4 Hz, 3-H), 4.09 (2 H, t, *J* 5.7 Hz, 3'-CH_2), 3.18 (1 H, ddd, *J* 11.1, 6.2 and 2.4 Hz, 2_{eq}-H), 2.60 (1 H, d, *J* 11.1 Hz, 2_{ax}-H), 2.57 (1 H, dd, *J* 12.4 and 9.0 Hz, 2'-C*H*H), 2.43 (1 H, m, 5-H), 2.14 (3 H, s, $OCCH_3$), 2.00-1.80 (3 H, m), 1.71 (1 H, m, 1'-CH*H*);

δ_C (100 MHz, DEPT, $CDCl_3$): 174.7 (C), 170.7 (C), 166.6 (C), 153.7 (C), 141.0 (C), 135.5 (CH), 135.0 (CH), 131.1 (CH), 130.7 (C), 130.0 (CH), 127.0 (CH), 121.7 (CH), 115.9 (CH), 78.4 (C), 76.8 (CH), 72.2 (CH), 70.6 (CH_2), 37.9 (CH), 35.4 (CH_2), 35.1 (CH_2), 32.5 (CH_2), 28.5 (CH_2), 22.6 (CH_3);

LC/MS (ret. time/min.) 4.4 (ESI+) m/z 484 (MH^+).

(1*S*, 3*R*, 4*R*, 5*S*) - 5 - [3 - (2 - Nitrophenyloxy)-propyl)] - 1,3,4 - cyclohexane - 1 - carboxylic acid 8

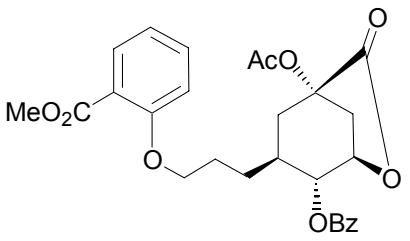


A solution of carbolactone **15** (40 mg, 0.08 mmol, 1.0 eqv.) in H_2O/MeCN (1:1) (2 ml) is treated with NaOH (100 mg/ml solution) (130 µl, 0.33 mmol, 4.0 eqv.) and stirred for 3 h at room temperature Amberlite IR-120 (H) (50 mg) was added, and the mixture stirred for a further 30

min. The solution was filtered and lyophilised to give the *acid* **8** was a yellow glass (39 mg, quantitative).

δ_H (400 MHz; D_2O): 7.78 (1 H, d, *J* 7.7 Hz, 3''-ArH), 7.51 (1 H, t, *J* 7.7 Hz, 5''-ArH), 7.13 (1 H, d, *J* 7.7 Hz, 6''-ArH), 6.96 (1 H, t, *J* 7.7 Hz, 4''-ArH), 4.06 (2 H, m, 3'-CH_2), 3.53 (1 H, m, 3-H), 3.03 (1 H, t, *J* 9.6 Hz, 4-H), 2.31 (1 H, dt, *J* 13.7, 4.3 Hz, 2_{eq}-H), 2.31 (1 H, m), 2.00-1.48 (6 H, m), 1.20 (1 H, m);
δ_C (100 MHz, DEPT, D_2O): 177.4 (C), 152.3 (C), 138.4 (C), 135.0 (CH), 125.4 (CH), 120.3 (CH), 115.0 (CH), 76.8 (CH), 74.6 (C), 70.1 (CH), 69.8 (CH_2), 36.3 (CH_2), 36.2 (CH_2), 34.6 (CH), 26.3 (CH_2), 24.5 (CH_2);
LC/MS (ret. time/min.) 3.4 (ESI+) m/z 356 (MH^+);
HRMS calcd for $C_{16}H_{21}NO_8$: MNa$^+$, 378.1165. Found: MNa$^+$, 378.1160.

(1*S*, 3*R*, 4*R*, 5*S*) - 4 - Acetyl - 5 - [3 - (2-methoxycarbonylphenyloxy) - propyl] - 1 - benzoylcyclohexane-1,3-carbolactone 16



A mixture of **13** (170 mg, 0.40 mmol, 1.0 eqv.), methyl salicylate (78 µl, 0.60 mmol, 1.5 eqv.), NaH (60% in mineral oil) (24 mg, 0.60 mmol, 1.5 eqv.) and KI (13 mg, 0.08 mmol, 0.2 eqv.) in MeCN (2 ml) was heated to reflux under N_2 for 24 h. The mixture was allowed to cool down to room temperature, taken up in Et_2O (20 ml) and washed with 1 M HCl (3 x 20 ml) and H_2O (20

ml). The organic layer was dried over Na_2SO_4, filtered and the solvent evaporated. The residue was purified by column chromatography on silica gel (Et_2O:Hex; 2:1) to give the *product* **16** as a colourless oil (45mg, 23%).

R_F 0.36 [Et_2O:Hexane; 2:1];

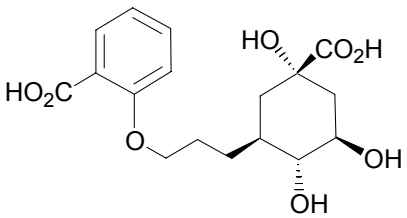
υ_{max} (NaCl)/cm^{-1} 1801s (CO), 1745s (CO), 1718s (CO), 1601s (Ar) and 1583s (Ar);

δ_H (400 MHz, $CDCl_3$): 8.01 (2 H, dd, *J* 8.2 and 1.2 Hz, 2-ArH), 7.75 (1 H, dd, *J* 7.7 and 1.8 Hz, 3''-ArH), 7.60 (1 H, t, *J* 8.2 Hz, 4-ArH), 7.45 (3 H, m, 3-ArH and 5''-ArH), 6.93 (2 H, m, 4''-ArH and 6''-ArH), 5.19 (1 H, t, *J* 2.8 Hz, 4-H), 4.94 (1 H, dd, *J* 6.2 and 2.8 Hz, 3-H), 4.02 (2 H, t, *J* 5.8 Hz, 3''-CH_2), 3.83 (3 H, s, OCH_3), 3.16 (1 H, ddd, *J* 11.2, 6.2 and 2.3 Hz, 2_{eq}-H), 2.61 (1 H, d, *J* 11.2 Hz, 2_{ax}-H), 2.55 (1 H, dd, *J* 13.6 and 9.0 Hz, 2''-C*H*H), 2.40 (1 H, m), 2.13 (3 H, s, $OCCH_3$), 2.00-1.80 (3 H, m), 1.70 (1 H, m);

δ_C (100 MHz, DEPT, $CDCl_3$): 172.0 (C), 168.0 (C), 165.6 (C), 163.9 (C), 157.1 (C), 132.4 (CH), 132.1 (CH), 130.4 (CH), 128.5 (CH), 128.1 (C), 127.4 (CH), 119.3 (C),119.0 (CH), 112.0 (CH), 77.2 (C), 75.8 (CH), 71.3 (CH), 44.0 (CH_2), 37.2 (CH), 34.3 (CH_2), 34.2 (CH_2), 32.4 (CH_2), 28.0 (CH_2), 21.6 (CH_3);

LC/MS (ret. time/min.) 4.4 (ESI+) m/z 498 (MH^+).

(1*S*, 3*R*, 4*R*, 5*S*) - 5 - [3 - (2-carboxyphenyloxy) - propyl] - 1,3,4 - cyclohexane - 1 - carboxylic acid 9



A solution of carbolactone **16** (25 mg, 0.05 mmol, 1.0 eqv.) in H_2O/MeCN (1:1) (2 ml) is treated with NaOH (100 mg/ml solution) (100 µl, 0.25 mmol, 5.0 eqv.) and stirred for 5 h at room temperature. The solution was neutralised with Amberlite IR-120 (H^+), filtered and lyophilised to give the *diacid* **9** as a colourless glass in quantitative yield (24 mg).

HPLC retention time (organic acids column): 34 minutes;

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υ_{max}/cm^{-1} 3201b (OH), 2500b (CO_2H), 1712s (CO), 1583s (Ar);

δ_H (500 MHz; D_2O): 7.63 (1 H, d, *J* 7.6 Hz, 3''-ArH), 7.48 (1 H, t, *J* 7.6 Hz, 5''-ArH), 7.11 (1 H, d, *J* 7.6 Hz, 6''-ArH), 7.03 (1 H, t, *J* 7.6 Hz, 4''-ArH), 4.12 (2 H, m), 3.65 (1 H, m), 3.13 (1 H, t, *J* 9.9 Hz), 1.95 (1 H, d, *J* 13.2 Hz), 1.90-1.60 (5 H, m), 1.55 (1 H, t, *J* 13.0 Hz), 1.32 (1 H, dd, *J* 9.2 and Hz), 1.22 (1 H, t, *J* 7.3 Hz);

δ_C (100 MHz, DEPT, D_2O): 177.7 (C), 171.0 (C), 158.0 (C), 135.4 (CH), 132.0 (CH), 121.4 (CH), 118.8 (C), 114.1 (CH), 77.9 (CH), 74.5 (C), 70.9 (CH), 69.9 (CH_2), 39.9 (CH_2), 37.6 (CH_2), 37.1 (CH), 27.2 (CH_2), 25.4 (CH_2);

HRMS calcd for $C_{16}H_{21}NO_8$: MNa$^+$, 378.1165. Found: MNa$^+$, 378.1160.

Biochemical Experimental

Assay for type I and type II dehydroquinases

Both type I and type II dehydroquinase enzymes were assayed by monitoring product formation. The initial rate of increase in absorbance at 234 nm, due to the enone-carboxylate chromophore of 3-dehydroshikimate (ε = 1.2 x 10^4 M^{-1}cm^{-1}), was measured. The assays were performed at 25 °C in potassium phosphate (0.05 M, pH 7.0) buffer (type I dehydroquinase) or Tris-HCl (0.05 M, pH 7.0) buffer (type II dehydroquinase). A standard assay of dehydroquinase includes:

100 μl of buffer (0.5 M, pH 7)

10 μl of enzyme solution (in buffer 0.05 M, pH 7)

x μl of substrate (3-dehydroquinate, ammonium salt) solution (in water)

y μl of inhibitor solution (in water)

(890 – x – y) μl of water

The assay mixture was prepared *in situ* on the cuvette, and the assay was initiated by addition of the enzyme solution to the mixture. The enzyme solutions were diluted from the concentrated stocks to 6.0 μg/ml (*S. typhi* type I dehydroquinase) ane 5.1 μg/ml (*S. coelicolor* type II dehydroquinase).

Enzyme kinetics

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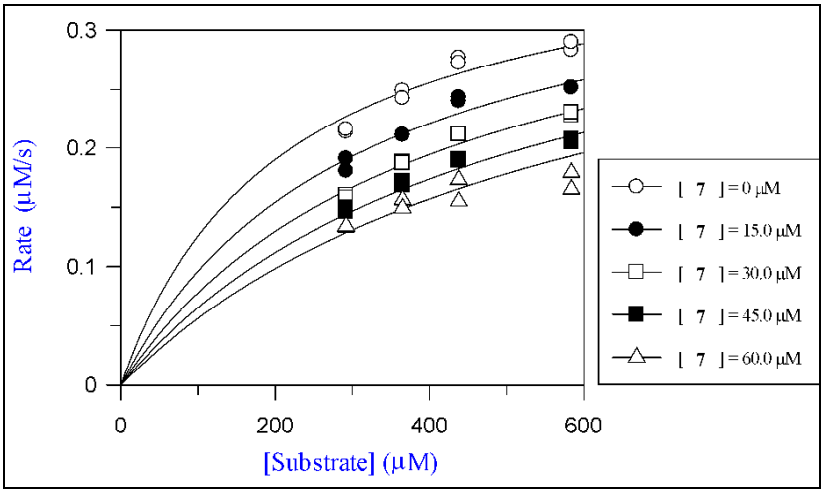
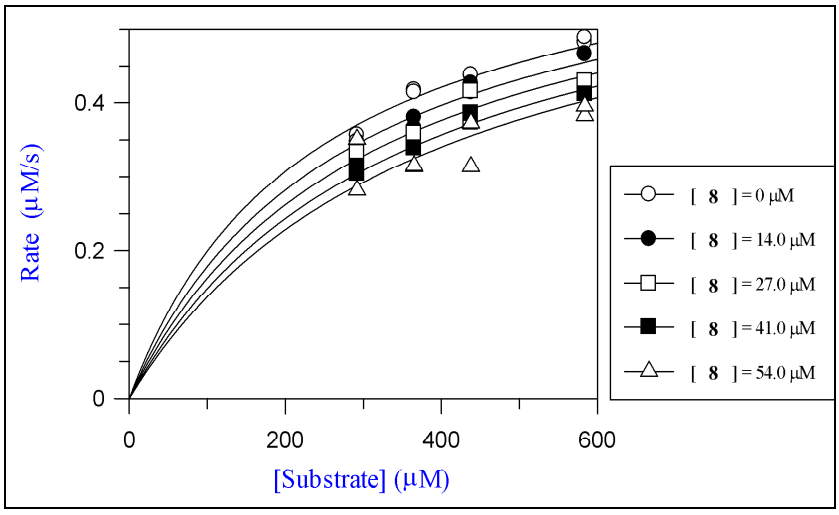
The kinetic parameters for type I and type II dehydroquinases were obtained by measuring the initial rates of reaction over a range of substrate concentrations (0.1 K_M -10 K_M). The data was fitted to Michaelis-Menten plots using the software *Lines&Kinetics* by least-squares fit, and the values for K_M and v_{max} were calculate using the Direct Linear method with the same software.

Enzyme inhibition

The inhibition kinetic data was obtained by measuring the initial rates of reaction over a range of inhibitor concentrations (typically 4 different concentrations) at 4 different substrate concentrartions (between K_M and 3 K_M). The inhibition constants K_I and standard deviation values were obtained by least-squares fitting using the software *GraFit* (Erithacus).





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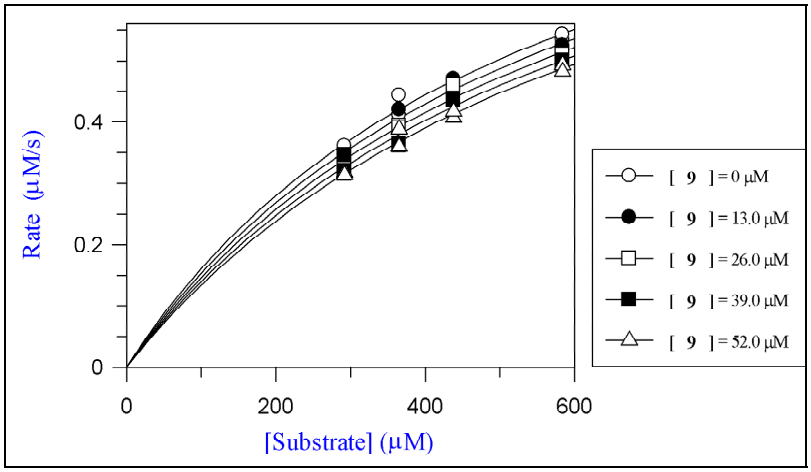

Figure 3 – Inhibition curves for *S. coelicolor* type II dehydroquinase with: **7**, **8** and **9**.

Crystallographic Methods

Crystallisation and X-ray Data Collection

Recombinant *Streptomyces coelicolor* DHQase purified from *Escherichia coli* as described previously (White *et al.*, 1990) and dialyzed into 20 mM Tris/HCl, pH 7.5, 0.5 mM DTT. The protein was concentrated using Centricon-10 centrifugal concentrators (Amicon) to 6 mg/ml. The inhibitor (1*S*, 3*R*, 4*R*, 5*S*) - 5 - [(3 - Phenyloxy)-propyl] - 1,3,4 - cyclohexane - 1 -carboxylic acid (**7**) was suspended in dialysis buffer and added to the protein at a final concentration of 4mM and incubated at 20°C for 30 minutes. Crystals were grown using the sitting-drop vapour-diffusion method using commercial and in house PEG Ions screens. The best crystals were grown by equilibrating a mixture of 1μl protein solution and 1μl precipitant solution (15%PEG8K, 0.2M NaKPhosphate, 0.1M MOPS pH 6.5) against 0.8ml of the precipitant solution.

X-ray diffraction data were collected at SRS Daresbury on beamline 14.1 using the CCD Quantum-4 detector (ADSC). Crystals were flash frozen at 100K in a stream of gaseous nitrogen using an Oxford Cryosystems cryostream, with artificial mother liquor with 20% glycerol used as a cryoprotectant. The crystals diffracted to 1.7Å and appeared to be I centered tetragonal with unit cell dimension a=198.42Å and c=396.6Å, however the data only merged successfully in P1 unit cell a=196.61 b=196.48 c=240.63 α= 65.91 β=65.91 γ=90.01. A summary of the data collection statistics is shown in table 1. The data were integrated with DENZO and scaled using

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SCALEPACK (Otwinowski and Minor, 1997). Merged intensity data were converted to structure factor amplitudes using Truncate from the CCP4 suite of programs (CCP4, 1994) and 5% of reflections flagged for use in calculation of the free-R factor.

Structure solution & Refinement

The structure was solved by molecular replacement using the program AMoRe (Navaza, 1994). The *S. coelicolor* DHQase dodecamer (PDB accession code 1GU1) was used as the search model against X-ray data between 12Å and 5.2Å (90735 reflections). Sixteen dodecamers were correctly located in the structure with a resulting correlation coefficient of 73.1% and R-factor of 38.2% after rigid body refinement in AMoRe. Refinement was performed with REFMAC5 (Murshudov *et al.*, 1997). Weighted difference Fourier maps calculated and averaged 16fold using CCP4 programs (CCP4, 1994), these clearly indicated the presence of a ligand within the active site. The ligand **7** was built and minimised using INSIGHT II (Accelrys) and fitted into the averaged structure (dodecamer A) using QUANTA (Accelrys). The unit cell contents was regenerated using the non crystallographic symmetry (NCS) before refinement.

Rounds of model refinement were performed using NCS restraints as implemented in REFMAC (Murshudov *et al.*, 1997). Model building and manual correction of models was performed using QUANTA (Accelrys), solvent molecules were added automatically using ARP (Perrakis *et al.*, 1997). Final model building using the entire unit cell contents was performed using COOT (Emsley *et al*., 2004). This resulted in a model with a final R_{work} of 19.7% and R_{free} of 24.7 %. The geometry of the model was either inside or better than expected values determined using PROCHECK (Laskowski *et al.*, 1993). The final model statistics are shown in Table 1.

Data Collection Details

Data Set	**7**
Space Group	P1
Unit Cell Dimensions (Å)	a=196.616 b=196.487 c=240.626 α=65.91 β=65.91 γ=90.01
Resolution Range (Å)	30.0 – 1.7
Observations	23,150,474
Unique Reflections	3,041,559
Completeness (%)[a]	93.7 (89.6)
Wilson B ($Å^2$)	17.4
R_{merge}[b] (%)	17.1

Refinement Statistics

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Resolution Range (Å)	27.0 – 1.7
R-factor[c] (R_{work}/R_{free})	19.7/24.7
Number of Atoms[d]	216,516 / 6,308 / 28,398
Rms Bond Length Deviation (Å)	0.021
Rms Bond Angle Deviation (°)	1.85
Mean B-factor (Å^2)[e]	15 / 25 / 33
Rms Backbone Deviation (Å)	0.12
Coordinate Error (Å)[f]	0.164

[a] values for highest resolution shell shown in brackets
[b] $R_{merge} = \sum | I - \langle I \rangle | / \sum \langle I \rangle$
[c] R factor = $\sum | F_o - F_c | / \sum F_o$
[d] number of atoms of protein, heteroatoms and water molecules respectively
[e] mean B factor for protein, inhibitor and water atoms respectively
[f] calculated using the method of Cruickshank (Cruickshank, 1999)

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