Supporting Information

Fluorescence Quenching Studies of γ -Butyrolactone Binding Protein (CprB) from *Streptomyces coelicolor* A3(2)

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Details of docking studies in terms of percentage occupancy of pocket by ligand have been provided. The scheme and synthetic details for ligand preparation and NMR of respective products have also been included. Additionally, Stern-Volmer plots for individual experiments showing slope of the initial linear stretch of the plot and their individual fluorescence quenching (inset) have been provided. Stern-Volmer plots of the main paper have also been shown here along with the error bars.

Results from docking studies of CprB and GBLs, with varying aliphatic chain length

Table S1. Table showing the percentage of ligand occupancy within the proposed ligand binding pocket for the respective GBL with the specified length of aliphatic chain at C2, from docking studies.

Carbon chain length	Percentage occupancy outside ligand binding pocket
5	10.8
6	10
7	0
8	21.6
9	21.2
10	37.2
11	46.2
12	62.8

Preparation of ligands Cp1 and Cp2

The preparation of ligands Cp1 and Cp2 has been achieved using reported procedures¹⁻³ as shown in Scheme S1. Diethyl succinate was subjected to formylation with ethyl formate and NaH in diethyl ether to yield aldehyde product **1** in 51.3% yield.^{1,2} NaBH₄ reduction of aldehydic group in **1** followed by condensation and hydrolysis resulted in **Cp1** in 13.9% yield.^{1,2} TMS protection of the hydroxyl group in **Cp1** followed by acylation with heptanoyl chloride gave the corresponding keto compound.^{1,2} TMS deprotection followed by NaBH₄ reduction of keto group gave **Cp2** in 21.4% yield.^{2,3}

Scheme S1. Preparation of ligands **Cp1** and **Cp2**. Reaction conditions: (i) NaH, HCOOEt, Et₂O, RT, 15 h; (ii) **a.** NaBH₄ with ice cooling, EtOH, RT, 3 h. **b.** K₂CO₃, MeOH and water, reflux, 3 h; (iii) **a.** TMSCl, HMDS, pyridine, RT, 2 h. **b.** LDA, THF, -78°C, heptanoyl chloride, 3 h. **c.** EtOH-H₂O, reflux. **d.** NaBH₄, MeOH, 0°C, 2h.

General notes

All chemicals and dry solvents (THF) were commercially obtained and used without any further purification. Pyridine was dried over calcium hydride. ¹H NMR and ¹³C NMR were recorded in Bruker Avance III 400 spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to CDCl₃ 7.26 ppm and 77.23 ppm for ¹H NMR and ¹³C NMR spectra respectively. Multiplicities of ¹H NMR spin couplings are reported as singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublet (dd), quartet of doublet (qd) and multiplet (m). The coupling constants (*J*) are reported in Hz. Silica gel plates precoated with fluorescent indicator were used for performing thin layer chromatography (TLC). Silica gel (100-200 mesh) was used for carrying out purification by column chromatography. MS-ESI(+) was performed with Q-TOF analyzer.

Experimental details

Diethyl formyl succinate (**1**)

To a stirred solution of NaH (2.75 g, 114 mmol) in 75 mL of diethyl ether, a mixture of diethyl succinate (20 g, 114 mmol), ethyl formate (10.2 g, 138 mmol) and 20 mL ethanol was added at room temperature and stirring was continued for 5 h. The resulting reaction mixture was left overnight. Then, 50 mL water was added to the reaction mixture and the aqueous phase was acidified using 2 M HCl followed by washing with brine solution and subsequent extraction with diethyl ether (3 × 150 mL). The ether extract was then dried over Na₂SO₄, filtered and concentrated. The remaining liquid was distilled under vacuum (bp 76 °C/6 mbar), resulting in a colorless oil (12 g, 51.3%) to yield compound **1**. R_f = 0.3 (33% ethyl acetate in hexane); ¹H NMR (400 MHz, CDCl₃), δ : 11.52 (d, J = 12.6 Hz, 1H), 7.08 (d, J = 12.7 Hz, 1H), 4.29-4.20 (m, 4H), 2.89 (qd, J = 14.7, 6.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 6H); MS-ESI(+): Calculated for C₉H₁₅O₅ [M+H]⁺ 203.09; found 203.09.

3-hydroxymethylbutanolide (Cp1)

NaBH₄ (1.01 g, 26 mmol) was added stepwise to an ice cold solution of **1** (5 g, 24 mmol) in 20 mL ethanol under stirring. Then the reaction mixture was stirred for 3 h at room temperature. 4 M aqueous HCl solution was added and the solution was stirred further for 1 h. The precipitate formed was filtered off. The filtrate was concentrated and the residue was extracted with ethyl acetate. The organic solution was washed with brine, dried over Na₂SO₄ and concentrated to give 2 g of yellowish oil. The oil was dissolved in a mixture of 12 mL methanol and 4 mL water and then K₂CO₃ (1.5 g) was added to it in small portions. The mixture was then refluxed for 3 h followed by acidification with 4 M HCl and concentrated. The residue was extracted with ethyl acetate (3 × 125 mL) and the organic solution was washed with brine, dried over Na₂SO₄ and concentrated. The product was purified by column chromatography (10% DCM in methanol) resulting in the pure lactone **Cp2** (0.4 g, 21%). R_f = 0.53 (5% ethanol in ethyl acetate); ¹H NMR (400 MHz, CDCl₃), δ : 4.41 (dd, J = 9.3, 7.5 Hz, 1H), 4.22 (dd, J = 9.3, 5.3 Hz, 1H), 3.71-3.62 (m, 2H), 2.80-2.73 (m, 1H), 2.61 (dd, J = 17.7, 8.9 Hz, 1H), 2.39 (dd, J = 17.7, 5.9 Hz, 1H); ¹³C-NMR (100 MHz; CDCl₃), δ : 177.8, 70.9, 63.1, 37.2, 31.1; MS-ESI(+): Calculated for C₅H₉O₃ [M+H]⁺ 117.0547; found 117.0552.

2-(1'-hydroxyoctyl)-3-hydroxymethylbutanolide (Cp2)

Hexamethyldisilazane (0.09 mL, 0.40 mmol) and trimethylsilyl chloride (0.08 mL, 0.62 mmol) were added to an ice-cooled solution of **Cp1** (60 mg, 0.52 mmol) in 2 mL pyridine, and the reaction mixture was stirred for 2 h at room temperature under a nitrogen atmosphere resulting in the formation of white precipitate. Then, 5 mL of benzene/hexane (1:1 v/v) was added to the reaction mixture and the precipitate was removed by filtration. The filtrate was concentrated and the crude material subjected to column chromatography (33% ethyl acetate in hexane) to produce a yellowish oily product (78 mg).

The oily compound was dissolved in 3 mL of THF and added to LDA solution [prepared by using N,N-diisopropylamine (0.145 mL, 1.06 mmol) and n-butyl lithium (0.65 mL, 1.06 mmol, 1.6 M in hexane) at -78°C followed by stirring for 15 min]. To this mixture heptanoyl chloride (60 mg, 0.41 mmol), was added and the reaction was stirred for another 1.5 h, at -78°C . The reaction mixture was allowed to warm up to 0°C followed by the addition of 10 mL ice-cold water with 1.5 mL acetic acid. The product formed was subsequently extracted with dichloromethane (3×100 mL) and concentrated. The residue was dissolved in 200 mL dichloromethane, washed with 100 mL saturated aq. NaHCO_3 and brine. After removal of the solvents, the silyl protected compound was obtained as yellowish oil. The trimethylsilyl group was removed by refluxing with 10 mL of ethanol and 4 mL of water for 30 min. After concentrating, the resulting mixture was purified by column chromatography (20-33% ethyl acetate in hexane) to yield 2-(octanoyl)-3-hydroxymethylbutanolide (50 mg, 0.2 mmol) as an oily liquid.

The compound was dissolved in 8 mL of ethanol and NaBH_4 (10.5 mg, 0.3 mol) was added at 0°C with stirring for 3 h followed by the addition of 3 M aqueous HCl solution. The precipitate formed was removed by filtration and the aqueous solution was extracted with ethyl acetate (3×50 mL) and the organic extract was dried over Na_2SO_4 . The compound was purified by HPLC to obtain **Cp2** in 21.4% yield (27.73 mg). $R_f = 0.32$ (33% ethyl acetate in hexane); ^1H NMR (400 MHz, CDCl_3), δ : 4.23-4.13 (m, 2H), 3.96-3.91 (m, 1H), 3.70 (dd, $J = 11.4, 4.0$ Hz, 1H), 3.60 (dd, $J = 11.5, 5.7$ Hz, 1H), 3.19-3.11 (m, 1H), 2.35 (t, $J = 7.6$ Hz, 1H), 1.67 (m), 1.25 (m), 0.88 (t, $J = 6.8$ Hz, 3H); ^{13}C -NMR (100 MHz; CDCl_3), δ : 70.5, 65.4, 63.6, 34.4, 32.2, 29.67, 29.58, 29.47, 29.35, 25.1, 22.9, 14.3; MS-ESI(+): Calculated for $\text{C}_{13}\text{H}_{25}\text{O}_4$ $[\text{M}+\text{H}]^+$ 245.1753; found 245.1754.

Stern-Volmer plots of individual experiments with fluorescence intensity quenching spectra provided inset.

Figure S1. (a), (b) and (c) represent the fluorescence quenching and Stern-Volmer plots for CprB protein at concentrations 1.50 μM , 3.75 μM and 7.50 μM respectively, with the quencher Cp1.

Figure S2. (a), (b) and (c) represent the fluorescence quenching and Stern-Volmer plots for CprB protein at concentrations $1.50 \mu M$, $3.75 \mu M$ and $7.50 \mu M$ respectively, with the quencher Cp2.

Figure S3. (a) and (b) represent the fluorescence quenching and Stern-Volmer plots for mutant W185L at concentrations 3.75 μM and 7.50 μM respectively, with the quencher Cp1.

Figure S4. (a) and (b) represent the fluorescence quenching and Stern-Volmer plots for mutant W185L at concentrations 3.75 μM and 7.50 μM respectively, with the quencher Cp2.

Figure S5. The Stern-Volmer plots for the native CprB at concentrations 1.50 μM , 3.75 μM and 7.50 μM with compounds Cp1 (a) and Cp2 (b). (Figure 7 of main paper with error bars included).

NMR Spectra for compounds Cp1 and Cp2

For all NMR spectra, peaks for grease have been marked as G.

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