Chemoenzymatic synthesis of prodigiosin analogues – exploring the substrate specificity of PigC

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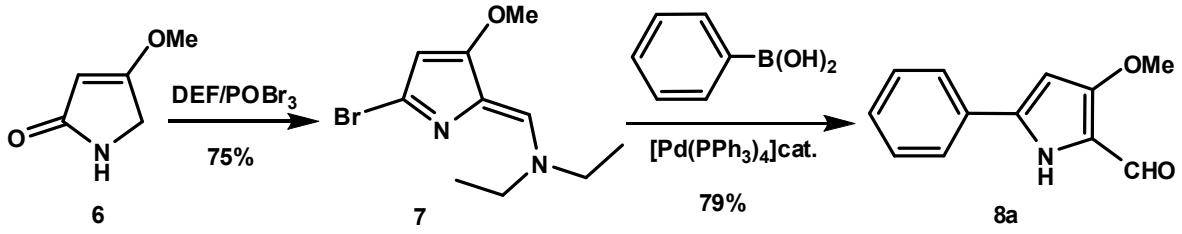
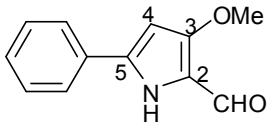
Supplementary Material

Representative Experimental Details



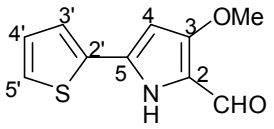
***N*-((5-Bromo-3-methoxy-2*H*-pyrrol-2-ylidene)methyl)-*N*-ethylethanamine**[13] **7**

To a mixture of diethylformamide (3 equiv, 0.3 mL) and DCM (0.5 mL) at 0 ^{o}C was added dropwise a solution of phosphorus oxybromide (2.5 equiv, 0.634 g) in DCM (1.5 mL). The mixture was stirred at 0 ^{o}C for 30 min and then evaporated to dryness in vacuo. DCM (1.0 mL) was added to the solid and cooled to 0 ^{o}C. A solution of 4-methoxy-3-pyrrolin-2-one (0.1 g, 0.884 mmol) in DCM (2.0 mL) was added dropwise. The mixture was warmed to room temperature, then heated at 40 ^{o}C for 30 min, then stirred at room temperature for 4 h and finally poured onto aqueous sodium bicarbonate-ice-EtOAc mixture and stirred for 10 min. The resulting mixture was filtered through Celite and the two layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel neutralized with 0.75% TEA; eluent, EtOAc:petroleum ether, 1.5:10) to obtain the enamine **7** as an oil (170 mg, 75%), which turned into to a crystalline solid when kept in a refrigerator, m.p. 38–40 ^{o}C (Found: C, 46.25; H, 5.8; N, 10.6. Calc. for $C_{10}H_{15}BrN_2O$: C, 46.35; H, 5.8; N, 10.8%); R_f 0.70 (MeOH:DCM; 0.7:9.3); ν_{max} (neat) cm^{-1} 1630s (C=C); δ_H (400 MHz, $CDCl_3$) 1.27 and 1.28 (each 3H, t, J 7.2, NCH_2CH_3), 3.37 and 4.10 (each 2H, q, *J* 7.1, NCH_2CH_3), 3.74 (3H, s, OCH_3), 5.57 (1H, s, C*H*=C-OCH_3), 6.98 (1H, s, C=C*H*-N); δ_C (100 MHz, $CDCl_3$) 12.71 and 14.82 (CH_3CH_2), 44.76 and 51.31 (CH_3CH_2), 58.20 (OCH_3), 96.70 (*C*H=C-O), 121.08 (NCH=*C*), 133.96 (Br*C*), 138.78 (N*C*H=C), 165.56 (*C*-OCH_3); HRMS, *m/z* found 259.0444, $C_{10}H_{15}BrN_2O$ requires M+H$^+$ 259.0446.



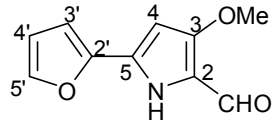
3-Methoxy-5-phenyl-1*H*-pyrrole-2-carbaldehyde 8a

To a degassed solution of toluene (0.5 mL) were added $Pd(OAc)_2$ (0.009 g, 0.038 mmol) and PPh_3 (0.046 g, 0.174 mmol). The mixture immediately became dark yellow and was stirred at 70 ^oC for 20 min under argon. A solution of bromoenamine **7** (0.1 g, 0.387 mmol) and benzeneboronic acid (0.071 g, 0.581 mmol) in 5% water/dioxane (1.5 mL) was degassed and purged with argon. The suspension of $Pd(PPh_3)_4$ in toluene was added to this solution and solid Na_2CO_3 (0.124 g, 1.163 mmol) was added. The mixture was stirred at 98 ^oC for 90 min and then poured into water. The pH of the mixture was lowered to pH 7 with 3 M hydrochloric acid and it was filtered through Celite. The Celite was washed several times with acetone and the washings were concentrated under reduced pressure. The residue was purified by column chromatography on silica gel neutralized with 0.75% Et_3N (eluent, EtOAc:petroleum ether, 2:10) to give the phenylpyrrole **8a** as pale yellow solid (95 mg, 79%), m.p: 150–153 ^oC (Found: C, 71.3; H, 5.5; N, 6.9. Calc. for $C_{12}H_{11}NO_2$: C, 71.6; H, 5.5; N, 6.95%). R_f 0.51 (MeOH:DCM; 0.7:9.3); ν_{max} (neat) cm^{-1} 3248s (N-H), 2822m (aldehyde C-H), 1616s (C=O); δ_H (400 MHz, $CDCl_3$) 3.92 (3H, s, OC*H*$_3$), 6.19 (1H, d, *J* 3, 3-*H*), 7.36 (1H, tt, *J* 7 and 2, *p*-*H*), 7.43 (2H, t, *J* 7, *m*-*H*), 7.55 (2H, dd, *J* 7 and 2, *o*-*H*), 9.0 (1H, br s, N*H*), 9.55 (1H, s, C*H*O); δ_C (100 MHz, $CDCl_3$) 58.2 (O*C*H$_3$), 93.5 (C-4), 119.8 (C-2), 125.4 (2 x *o*-*C*H), 129.09 (*p*-*C*H), 129.29 (2 x *m*-*C*H), 130.74 (phenyl-*C*), 139.03 (C-5), 159.00 (C-3), 174.99 (*C*HO); HRMS, *m/z* 202.0870, $C_{12}H_{11}NO_2$ requires *M*+H^+ 202.0868.



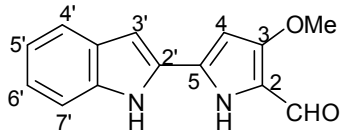
3-Methoxy-5-thien-2-yl-1*H*-pyrrole-2-carbaldehyde 8b

The thienylpyrrole **8b** was obtained as a brick-red coloured solid (71 mg, 88%), m.p. 154-159 ^oC (dec.); R_f 0.52 (MeOH:DCM; 0.7:9.0); ν_{max} (neat) cm^{-1} 3207s (N-H), 2826m (aldehyde C-H), 1615s (C=O); δ_H (400 MHz, $CDCl_3$) 3.90 (3H, s, OC*H*$_3$), 6.08 (1H, d, *J* 2, 4-H), 7.07 (1H, dd, *J* 5 and 4, 4'-H), 7.30 (1H, dd, *J* 4 and 1, 5'-H), 7.32 (1H, dd, *J* 5 and 1, 3'-H), 9.07 (1H, bs, N*H*), 9.52 (1H, s, CHO); δ_C (100 MHz, $CDCl_3$) 58.2 (OCH_3), 93.9 (C-4), 119.27 (C-2), 124.95, 126.26 and 128.45 (3 x thienyl-*C*H), 133.86 and 133.97 (C-5 and C-2'), 159.01 (C-3), 174.68 (*C*HO); (Found: C, 54.9; H, 4.4; N, 5.9. Calc. for $C_{10}H_9O_2S$: C, 57.95; H, 4.4; N, 6.8%); HRMS, m/z 208.0427, $C_{10}H_9NO_2S$ requires *M*+H^+ 208.0432.



3-Methoxy-5-fur-2-yl-1*H*-pyrrole-2-carbaldehyde 8c

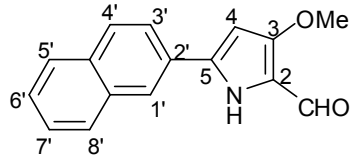
The furylpyrrole **8c** was obtained was obtained as golden coloured solid (35 mg, 95%), m.p.: 143-146 ^{o}C. R_f 0.52 (MeOH:DCM; 0.7:9.0); ν_{max} (neat) cm^{-1} 3207s (N-H), 2826m (aldehyde C-H), 1616s (C=O); δ_H (400 MHz, $CDCl_3$) 3.84 (3H, s, OC*H*$_3$), 6.04 (1H, s, 4-H), 6.42 (1H, dd, *J* 4 and 2, 4'-H), 6.64 (1H, dd, *J* 4 and 1, 3'-H), 7.39 (1H, dd, *J* 2 and 1, 5'-H), 9.30 (1H, br s, N*H*), 9.47 (1H, s, C*H*O); δ_C (100 MHz, $CDCl_3$; due to low solubility only carbons with protons attached gave visible peaks) 58.2 (O*C*H$_3$), 92.4 (C-4), 107.9 (C-4'), 112.2 (C-3'), 143.1 (C-5'), 174.8 (*C*HO). LCMS, m/z 192, $C_{10}H_9NO_3$ requires *M*+H$^+$ 192.



3-Methoxy-5-indol-2-yl-1*H*-pyrrole-2-carbaldehyde 8d

In this case the starting boronic acid was the *N*-Boc derivative of indole-2-boronic acid and two products were obtained, the indolylpyrrole **8d** and its *indolyl-N*-Boc derivative in a combined yield of 96%. Indolylpyrrole **8d** was obtained as a colourless solid (13 mg, 28%), m.p. 245-248 ^{o}C (dec.). R_f 0.26 (EtOAc:Hexane; 1:1); ν_{max} (neat) cm^{-1} 3204s (N-H), 2835m (aldehyde C-H), 1584s (C=O); δ_H (400 MHz, CD_3OD; COSY used for assignments) 3.97 (3H, s, OC*H*$_3$), 6.44 (1H, s, 4-H), 6.97 (1H, s, 3'-H), 7.05 and 7.17 (each 1H, td, *J* 8 and 1, 5'- and 6'-H), 7.39 and 7.57 (each 1H, dd, *J* 8 and 1, 4'- and 7'-H), 9.38 (1H, s, C*H*O); LCMS, m/z 241, $C_{14}H_{12}N_2O_2$ requires *M*+H$^+$ 241.

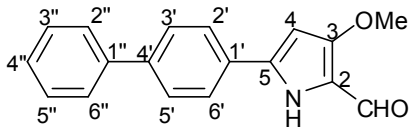
Chromatography also gave the *indolyl-N*-Boc derivative (45 mg, 68%). R_f 0.45 (EtOAc:Hexane; 1:1); ν_{max} (neat) cm^{-1} 3204s (N-H), 2835m (aldehyde C-H), 1584s (C=O); δ_H (400 MHz, CD_3OD) 3.96 (3H, s, OC*H*$_3$), 6.20 (1H, s, 4-H), 6.88 (1H, s, 3'-H), 7.27 and 7.37 (each 1H, td, *J* 8 and 1, 5'- and 6'-H), 7.61 and 8.22 (each 1H, dd, *J* 8 and 1, 4'- and 7'-H), 9.45 (1H, s, C*H*O); δ_C (100 MHz, CD_3OD) 27.0 (C*Me*$_3$), 57.7 (OMe), 84.2 (*C*Me$_3$), 96.4 (C-4), 112.4 (C-3'), 115.4 (C-7'), 119.4 (C-5), 120.9 (C-5'), 123.3 (C-4'), 125.3 (C-6'), 129.1 and 130.8 (C-2' and -3a'), 138.0 (C-2), 149.9 (N-C=O), 158.8 (C-3), 174.8 (*C*HO); LCMS, m/z 341, $C_{19}H_{20}N_2O_4$ requires *M*+H$^+$ 341.



3-Methoxy-5-naphth-2-yl-1*H*-pyrrole-2-carbaldehyde 8e

The naphthylpyrrole **8e** was obtained as colourless solid (79 mg, 81%), m.p.: 216-218 ^{o}C. R_f 0.55 (MeOH:DCM; 0.7:9.0); ν_{max} (neat) cm^{-1} 3254s (N-H), 2818m (aldehyde C-H), 1620s (C=O); δ_H (400 MHz, CD_3OD) 4.00 (3H, s, OC*H*$_3$), 6.62 (1H, s, 4-H), 7.56 (2H, m, 6'- and 7'-H), 7.90-7.98 (4H, m, 3'-, 4'-, 5'- and 8'-H), 8.34 (1H, s, 1'-H), 9.47 (1H, s, C*H*O); δ_C (100 MHz, CD_3OD; due to

low solubility only carbons with protons attached gave visible peaks) 57.8 (OMe), 93.8 (C-4), 123.5 (C-3'), 124.8 (C-1'), 126.9 and 127.1 (C-6' and -7'), 127.9, 128.5 and 128.8 (C-4', -5' and -8'), 174.7 (*C*HO); LCMS, m/z 252, $C_{16}H_{13}NO_2$ requires M+H^+ 252.



3-Methoxy-5-*p*-biphenyl-1*H*-pyrrole-2-carbaldehyde 8f

The biphenylpyrrole **8f** was obtained as colourless solid (88 mg, 82%), m.p.: 234-237 ^oC (dec.). R_f 0.57 (MeOH:DCM; 0.7:9.0); ν_{max} (neat) cm^{-1} 3239s (N-H), 2826m (aldehyde C-H), 1624s (C=O); δ_H (400 MHz, DMSO) 3.90 (3H, s, OCH_3), 6.64 (1H, d, *J* 2, 4-H), 7.39 (1H, tt, *J* 1 and 8, 4"-H), 7.49 (2H, t, *J* 8, 3" and 5"-H), 7.72-7.77 (4H, m, 3', 5', 2" and 6"-H), 8.00 (2H, d, *J* 8, 2' and 6'-H), 9.47 (1H, s, C*H*O), 11.91 (1H, br s, N*H*); δ_C (100 MHz, DMSO; HMQC used for assignments) 58.3 (O*C*H_3), 94.2 (C-4), 119.6 (C-2), 126.5 (C-2' and 6'), 126.9 and 127.3 (C-3', 5', 2" and 6"), 128.1 (C-4"), 129.4 (C-3" and 5"), 130.1 (C-1'), 138.1 (C-5), 139.7 and 140.1 (C-4' and 1"), 158.5 (br, C-3), 173.8 (*C*HO); LCMS, m/z 278, $C_{18}H_{15}NO_2$ requires M+H^+ 278.

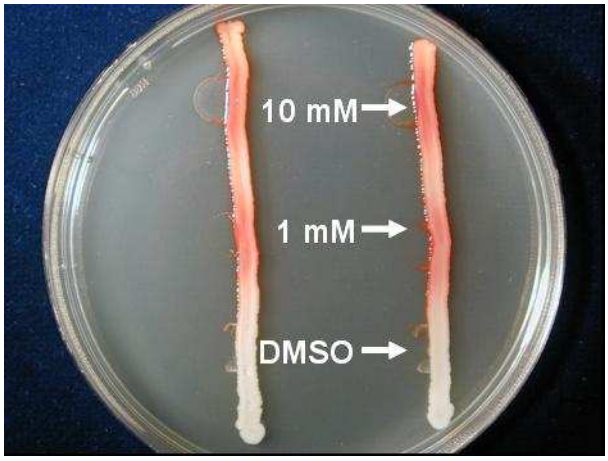
Feeding Experiments

(a) On agar plates

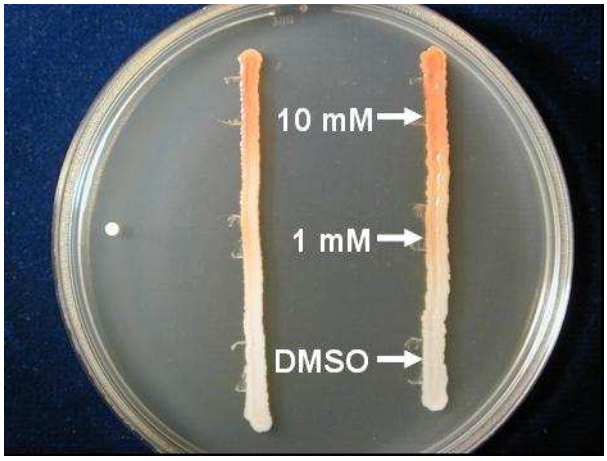
Streaks of the following *Serratia* mutants were grown for two days on PGM-agar plates: *pigH*57Δ, *pigM*Δ, *pigB*Δ, *pigC*Δ, and *pigD*Δ. Beside each streak was spotted on the agar 10 μL of a DMSO solution (0, 1, or 10 mM) of MBC **1** or one of its analogues **8a-f**. The plate was then incubated at 30 ^oC for 48 h before being photographed, as shown below. The photographs shown are of the *pigH*57Δ strain. The *pigM*Δ strain gave very similar results. No pigmentation was observed when **1** or **8a-f** were fed to either of the mutants, *pigB*Δ or *pigD*Δ, blocked in the biosynthesis of the monopyrrole MAP **2** or to the *pigC*Δ mutant, which is defective in the production of the condensing enzyme.

Fig. S1. Feeding of MBC **1** and its analogues to streaks of the *Serratia pigH*57Δ strain grown on agar plates.

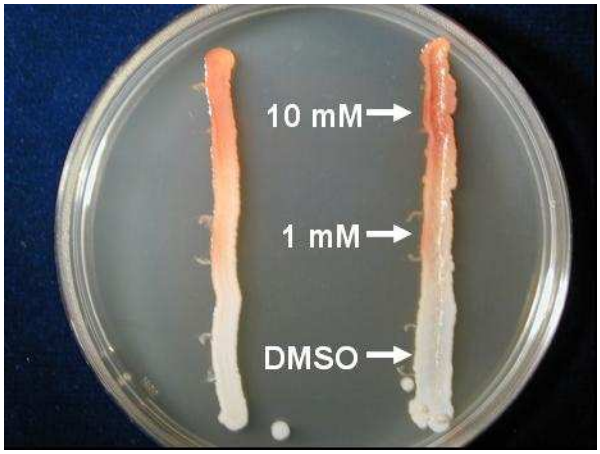
(i) MBC **1**



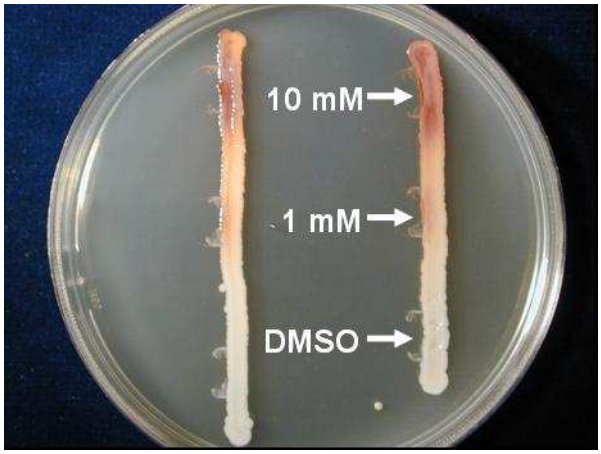
(ii) Phenyl analogue **8a**



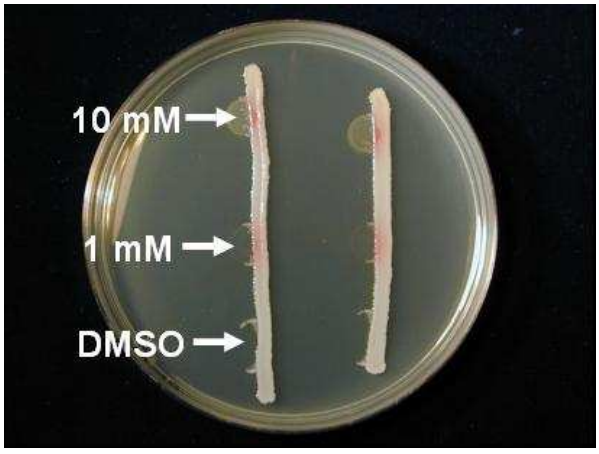
(iii) Thienyl analogue **8b**



(iv) Furyl analogue **8c**



(v) Indolyl analogue **8d**



(vi) Naphthyl analogue **8e**

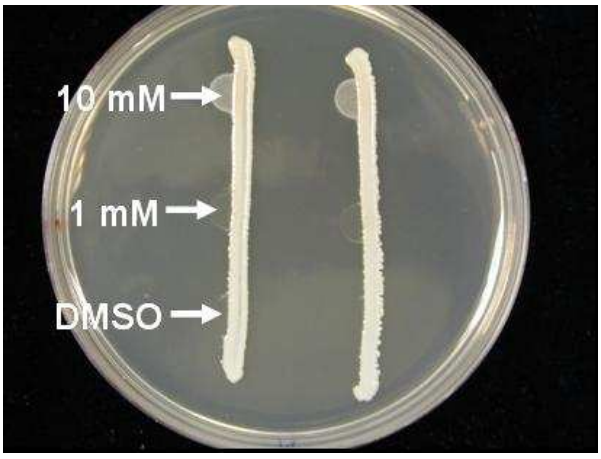
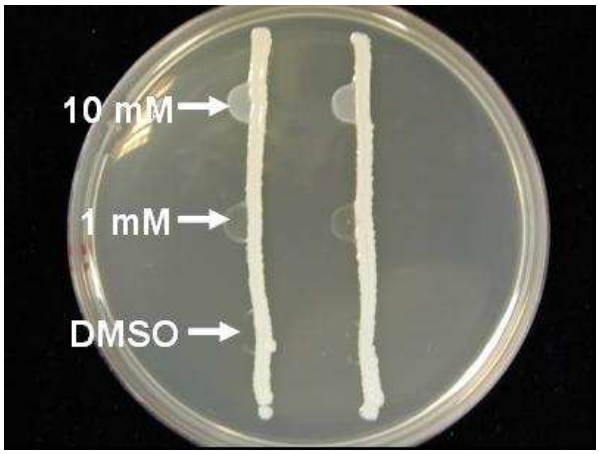


Fig. S1 (continued).

(vii) Biphenyl analogue **8f**



(b) In liquid culture

Cells were grown in LB broth (Tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L) supplemented with sorbitol (0.25 M) for *Serratia* strains or ampicillin (100 μg/mL) for *E. coli* strains. Flasks were inoculated with an appropriate starter culture to get an OD_{600} of 0.04 and then grown in the shaker for 11-12 h at 30 ^{o}C for *Serratia* strains or at 37 ^{o}C for *E. coli* strains. Sufficient solution of MBC **1** or one of its analogues **8a-f** (10 mM in DMSO) was added to give the final concentrations shown in Table 1 of the paper. The cultures were then shaken for a further 16-17 h, to an OD_{600} of about 40 for the *Serratia* strains or about 3-4 for the *E. coli* strains. Each culture was centrifuged and the cell pellet was extracted with ethanol acidified with 4% of 1 M hydrochloric acid (1 mL) and then centrifuged again. A UV/Vis spectrum was recorded for the ethanolic supernatant (with appropriate dilution when the absorbance exceeded 1) and it was then evaporated to dryness and analysed by LC-MS.

Cuvettes from the *Serratia pig*H57Δ strain incubated with 100 μM MBC or analogue are shown in the paper, Fig. 1.

Fig. S2. Cuvettes from the *Serratia pig*H57Δ strain incubated with 10 μM MBC **1** or analogues **8a-f**.

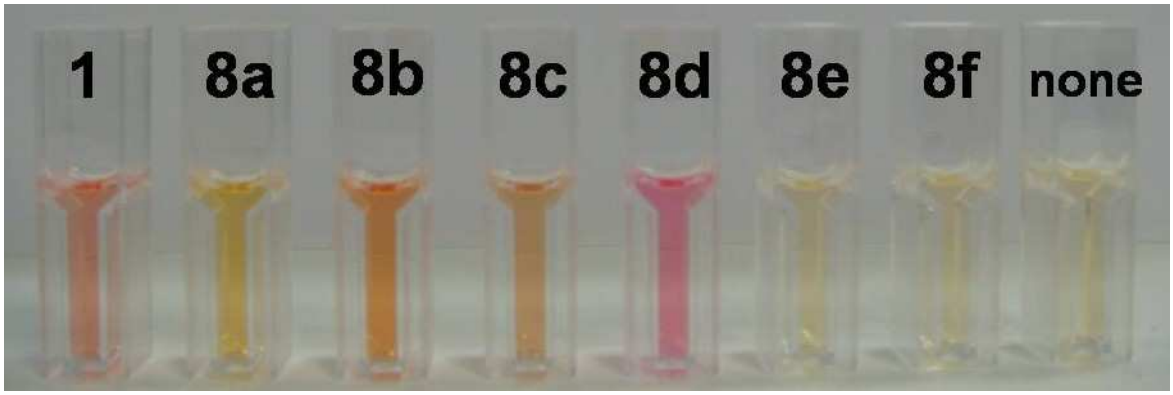
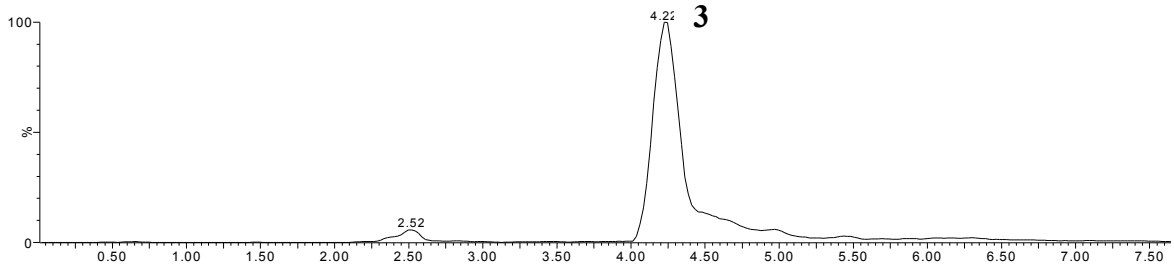
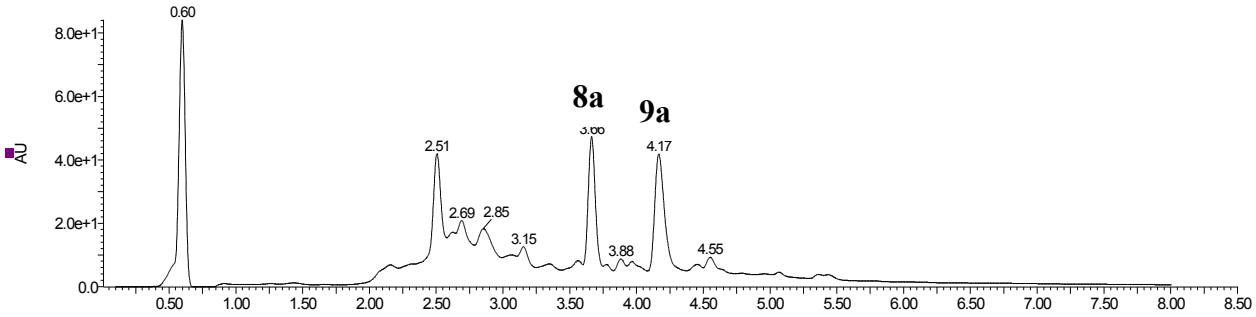


Fig. S3. Selected chromatograms from the LC-MS analysis of the extracts

(a) Extract of medium from culture of *E. coli* strain DH5α-pNRW73 supplemented with MBC **1**; selective ion chromatogram for *m/z* = 323 (M+H$^+$ for prodigiosin **3**).



(b) Extract of medium from culture of *E. coli* strain DH5α-pNRW73 supplemented with phenyl analogue **8a**; total ion chromatogram; the peaks labelled **8a** and **9a** can be identified by their mass spectrum as corresponding to those compounds.



(c) Extract of medium from culture of *E. coli* strain DH5α-pNRW73 supplemented with thienyl analogue **8b**; total ion chromatogram; the peaks labelled **8b** and **9b** correspond to those compounds.

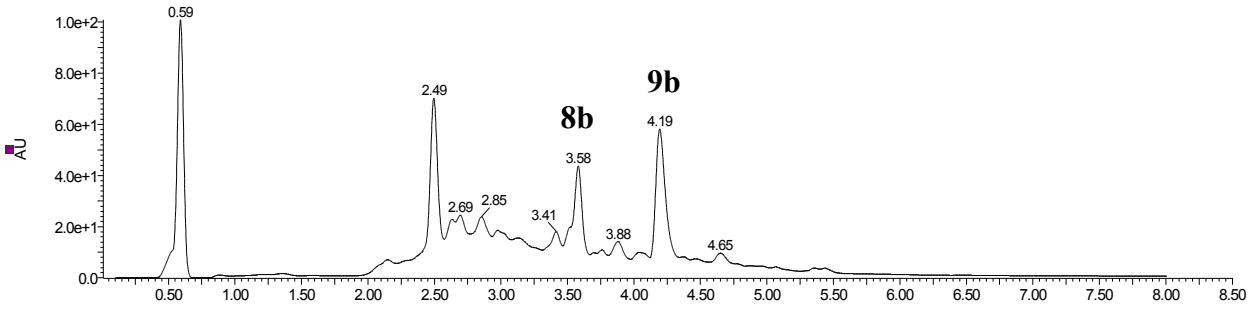
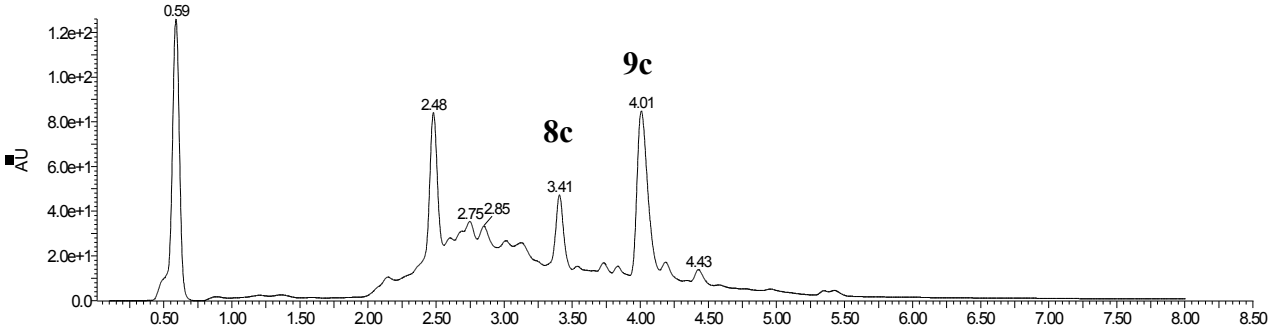
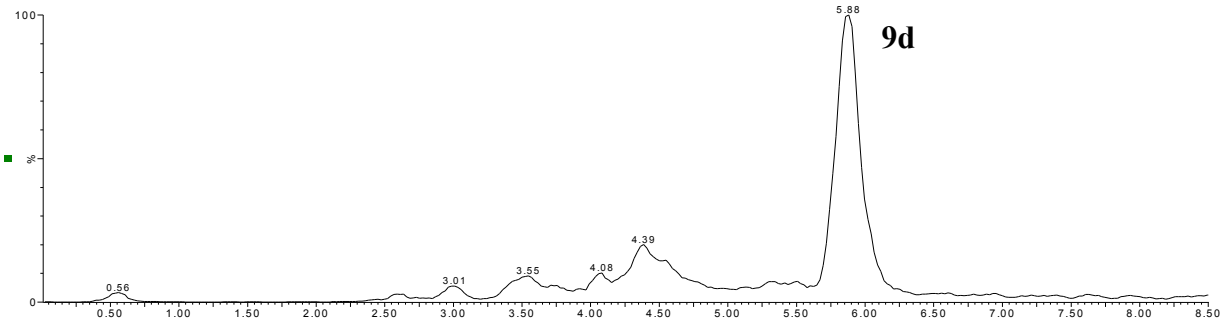


Fig. S3 (continued).

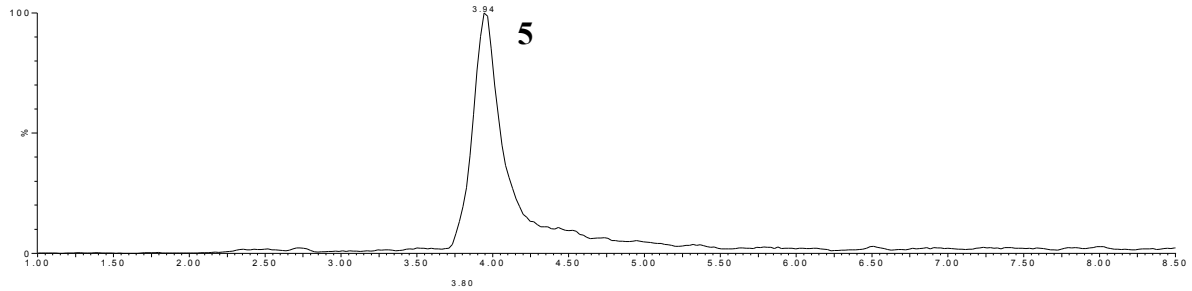
(d) Extract of medium from culture of *E. coli* strain DH5α-pNRW73 supplemented with furyl analogue **8c**; total ion chromatogram; the peaks labelled **8c** and **9c** correspond to those compounds.



(e) Extract of medium from culture of *Serratia* mutant *pigH*57Δ supplemented with indolyl analogue **8d**; selective ion chromatogram for *m/z* = 373 (M+H$^+$ for prodigiosin analogue **9d**).



(f) Extract of medium from culture of *Serratia* mutant *pigH*57Δ supplemented with indolyl analogue **8d** and 2,4-dimethylpyrrole **10**; selective ion chromatogram for *m/z* = 317 (M+H$^+$ for obatoclax **5**).



(g) Extract of medium from culture of *E. coli* strain DH5α-pNRW73 supplemented with naphthyl analogue **8e**; total ion chromatogram; the peak labelled **8e** corresponds to that compound; no peak was found for **9e**.

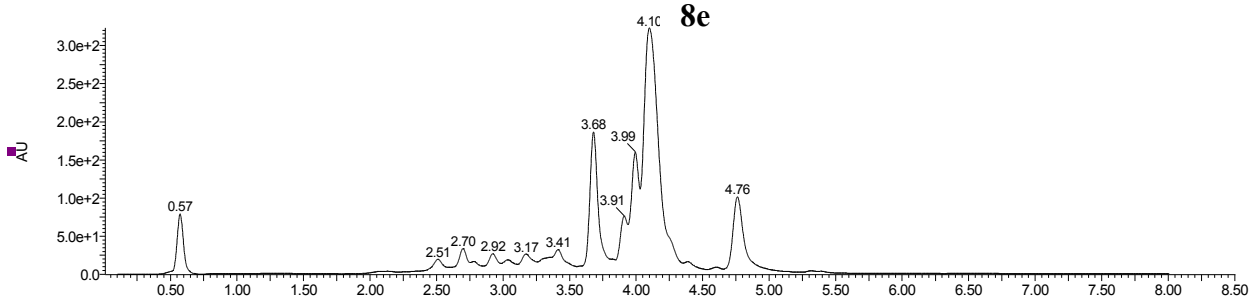
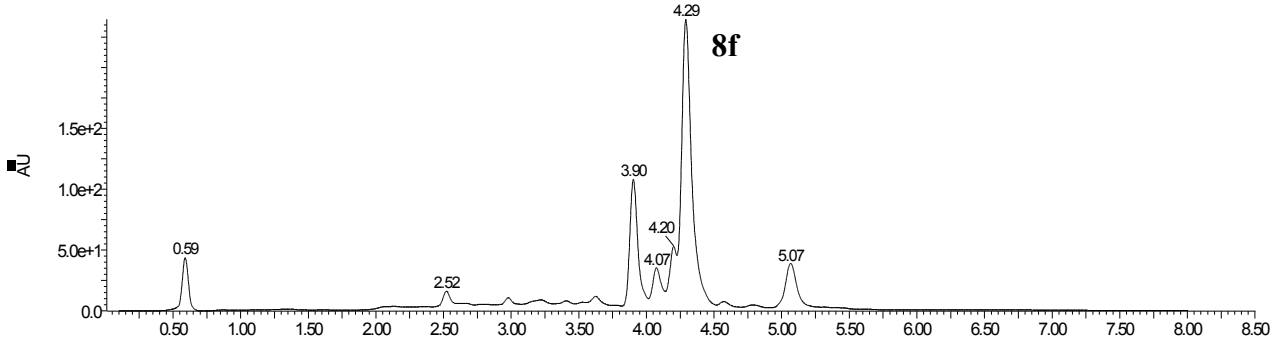


Fig. S3 (continued).

(g) Extract of medium from culture of *E. coli* strain DH5α-pNRW73 supplemented with biphenyl analogue **8f**; total ion spectrum; the peak labelled **8f** corresponds to that compound; no peak was found for **9f**.



(h) Extract of medium from culture of *E. coli* strain DH5α-pNRW73; total ion spectrum; the peak labelled **2** corresponds to MAP.