

Supporting Information

Chemical Intervention Suggests a Critical Role for Lysyl Oxidase in Zebrafish Notochord Morphogenesis

Morphogenesis

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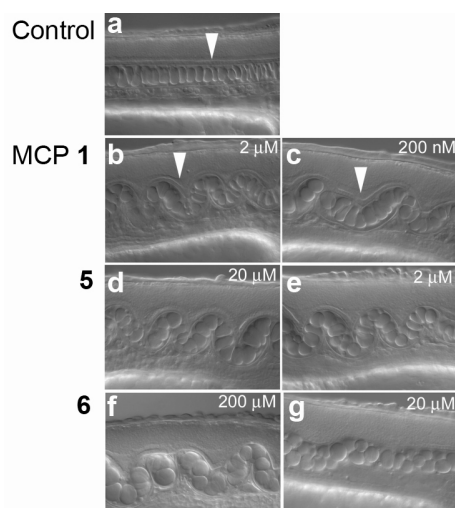


Figure S1: MCP derivatives 5 and 6 show similar effects on notochord morphology

Lateral views of posterior trunk of 25 hpf embryos after treatment with MCP 1, 5, or 6 at each specified concentration. Note that all treated embryos (B-I) show severely kinked notochord morphology, in contrast to control embryos (embryo medium with 1% DMSO, A), but that MCP 1 shows higher potency than the derivative compounds. Note too the larger, more spherical shape of the vacuolated notochord cells in treated embryos, compared with smaller, disc-shaped cells in controls. The distorted notochord results in distortions of the neural tube, as seen by comparing appearance of neurocoel (arrowhead) in untreated (A) and treated embryos (B,C).

Cyclohexanone (5.0 mmol, 515 μ l) was added to a suspension of thiosemicarbazide (5.0 mmol, 456 mg) and acetic acid (100 μ l) in methanol (10 ml). The suspension was heated at reflux, under nitrogen, for 24 h. On cooling the reaction mixture to room temperature a white precipitate formed, this was collected and washed with methanol to yield the *2-cyclohexylidene-hydrazinecarbothioamide* (650 mg, 3.8 mmol, 76%) as a colourless solid δ_{H} (400 MHz; CDCl_3) 8.77 (1H, br. s, C=NNH), 7.30 (1H, brs, C=SNH), 6.39 (1H, brs, C=SNH), 2.36-2.28 (4H, m, CH_2) and 1.79-1.64 (6H, m, CH_2); δ_{C} (100 MHz; CDCl_3) 178.8 (C=S), 156.9 (C=N), 35.4, 27.1, 26.8, 25.9 and 25.5; m/z (FAB^+) 171 (100%, M^+); found MH^+ 172.0914; $\text{C}_7\text{H}_{14}\text{N}_3\text{S}$ requires M^+ 172.0908.

2-butylidene-hydrazinecarbothioamide 3

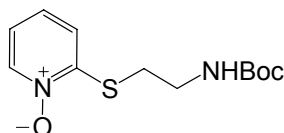
Butyraldehyde (25 mmol, 2.24 ml) was added to a solution of thiosemicarbazide (25 mmol, 2.28 g) in triethylorthoformate (50 ml) and stirred under nitrogen at ambient temperature for 16 h. The reaction mixture was partitioned between EtOAc and water, the organics were separated and washed with water (2×100 ml), brine (100 ml), dried (MgSO_4) and evaporated under reduced pressure. The crude product was purified by column chromatography eluting with 0–40% EtOAc in petrol to yield the *2-butylidene-hydrazinecarbothioamide* (260 mg, 2.6 mmol, 11%) as a colourless solid R_{F} 0.50 (40% EtOAc in petrol); δ_{H} (400 MHz; CDCl_3) 10.10 (1H, br. s, NH) 7.38 (1H, t, J 5.4, $\text{CH}=\text{N}$), 7.12 (1H, brs, $\text{NHC}=\text{S}$), 6.52 (1H, brs, $\text{NHC}=\text{S}$), 2.27 (2H, dt, J 7.5 and 5.4, CHCH_2), 1.59 (2H, AX_5 , J 7.5, CH_2CH_3) and 0.97 (3H, t, J 7.5, CH_3CH_2); δ_{C} (100 MHz; CDCl_3) 177.9 (C=S), 148.7 (C=N), 34.2 (CHCH_2), 19.6 (CH_2CH_3) and 13.7 (CH_2CH_3); m/z (FAB^+) 146 (100%, MH^+).

2-cyclopentylidene-hydrazinecarbothioamide 4

Cyclopentanone (5.0 mmol, 442 μ l) was added to a suspension of thiosemicarbazide (5.0 mmol, 456 mg) and acetic acid (100 μ l) in methanol (10 ml). The suspension was heated at reflux, under nitrogen, for 16 h. The reaction mixture was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2 (40 ml) and washed with water (2×40 ml), saturated aqueous NaHCO_3 solution (1×40 ml) and brine (1×40 ml). The organics were dried (MgSO_4) and evaporated to yield the *2-cyclopentylidene-hydrazinecarbothioamide* (643 mg, 4.2 mmol, 82%) as a colourless solid δ_{H} (400 MHz; CDCl_3) 8.19 (1H, br. s, C=NNH), 6.97 (1H, br. s, C=SNH), 6.18 (1H, brs,

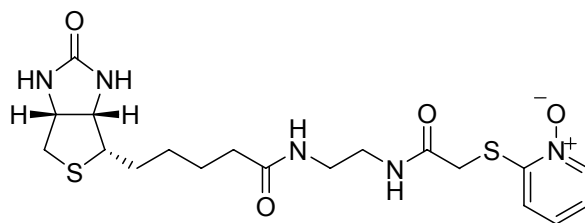
C=S_{NH}), 2.21-2.08 (4H, m, CH₂) and 1.70-1.56 (4H, m, CH₂); δ_C (100 MHz; CDCl₃) 178.4 (C=S), 163.3 (C=N), 33.1, 27.6, 24.6 and 24.4; *m/z* (FAB⁺) 157 (100%, M⁺); found MH⁺ 158.0750; C₆H₁₂N₃S requires M⁺ 158.0752.

N-tert-Butoxycarbonylamino-N'-2-[(1-oxido-2-pyridinyl)thio] ethylenediamine 5



1-(N-tert-Butoxycarbonylamino)-2-bromoethane (3.0 mmol, 775 μl) was added to a stirring solution of 2-mercaptopyridine *N*-oxide (3.0 mmol, 381 mg) and triethylamine (6.0 mmol, 842 μl) in acetonitrile (10 ml) and the mixture stirred under nitrogen, at ambient temperature for 15 hours. The solvent was removed under reduced pressure and the crude product purified by column chromatography eluting with 5% MeOH in CH₂Cl₂, followed by lyophilization to yield the *N*-tert-Butoxycarbonylamino-N'-2-[(1-oxido-2-pyridinyl)thio] ethylenediamine (89 mg, 0.20 mmol, 91%) as a colourless powder δ_H (270 MHz; CDCl₃) 8.23 (1H, d, *J* 6.4, Ar-6H), 7.40 (1H, d, *J* 6.4, Ar-3H), 7.25 (1H, t, *J* 6.4, Ar-5H), 7.03 (1H, dt, *J* 6.4 and 1.8) 5.03 (1H, br. s, NH), 3.38 (2H, q, *J* 6.7, CH₂NH), 3.05 (2H, t, *J* 6.7, SCH₂) and 1.40 (9H, s, C(CH₃)₃); δ_C (100 MHz; CD₃OD) 158.6 (C), 154.3 (C), 140.5 (CH), 130.6 (CH), 123.7 (CH), 122.5 (CH), 80.5 (C), 40.3 (CH₂), 30.9 (CH₂), 28.9 (CH₃); *m/z* (ES-HRES) C₁₂H₁₈N₂O₃SNa requires 293.0936 found 293.0939 (20%, MNa⁺) and C₁₂H₁₉N₂O₃S requires 271.1116 found 271.1111 (10%, MH⁺).

N-(Biotinoyl)-N'-(acetyl 2-[(1-oxido-2-pyridinyl)thio] ethylenediamine 6



N-(Biotinoyl)-N'-(iodoacetyl] ethylenediamine (50 mg, 0.11 mmol) was added to a stirring solution of 2-mercaptopyridine *N*-oxide (0.11 mmol, 14 mg) and triethylamine (0.22 mmol 30 μl) in acetonitrile (10 ml) and the mixture stirred under nitrogen, at ambient temperature for 72 h. The solvent was removed under reduced pressure and the crude product purified silica chromatography

Supplementary Material (ESI) for Molecular BioSystems

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eluting with 10% MeOH in CH₂Cl₂, followed by lyophilization to yield the biotinylated compound as a colourless powder (89 mg, 0.20 mmol, 91%). *R_F* 0.10 (10 % MeOH in CH₂Cl₂); δ_H (400 MHz; DMSO) 8.32-8.26 (2H, m, NH and ArH₃), 7.81 (1H, brs, NH), 7.42-7.32 (2H, m, ArH₅ and ArH₆), 7.22 (1H, dt, *J* 6.8 and 2.4, ArH₄), 6.45 (1H, brs, biotin NH), 6.38 (1H, brs, biotin NH), 4.32-4.27 (1H, m, CHCH₂S), 4.15-4.10 (1H, m, CHCHS), 3.69 (2H, s, SCH₂CO), 3.16-3.04 (5H, m, NHCH₂CH₂NH and SCHR), 2.81 (1H, dd, *J* 12.6 and 5.2, CHCH_{a/b}S), 2.57 (1H, d, *J* 12.6, CHCH_{a/b}S), 2.03 (2H, t, *J* 7.5, NHCOCH₂CH₂) and 1.65-1.21 (6H, m, CH₂); δ_C (100 MHz; DMSO) 172.3 (C=O), 167.2 (C=O), 162.7 (C=O), 150.8 (ArC₂), 138.1 (ArC₆), 125.5 (ArC₃), 122.1 (ArC₄), 121.3 (ArC₅), 61.0 (NHCHCHS), 59.3 (NHCHCH₂S), 55.4 (biotin SCHCH₂), 39.5 (SCH₂ - under solvent peak), 38.7 (NHCH₂CH₂NH), 38.1 (NHCH₂CH₂NH), 35.3 (CH₂CH₂CONH), 33.6 (SCH₂CO), 28.3 (CH₂), 28.1 (CH₂) and 25.2 (CH₂); *m/z* (ES) 476 (100%, MNa⁺) and 454 (10%, MH⁺); *m/z* (ES-HRES) C₁₉H₂₇N₅O₄S₂Na requires 476.1402 found 476.1406.