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Supporting information for article:

Rational modification of vanillin derivatives to stereospecifically destabilize sickle hemoglobin polymer formation

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S1. Synthesis of Compounds

All reagents used in the syntheses were purchased from Sigma-Aldrich (St. Louis, MO) and ThermoFisher Scientific (Waltham, MA) and utilized without additional purification. Melting points were determined on a Fisher-Scientific melting point apparatus (Serial# 410N0117), and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a BPLker 400 MHz spectrometer and tetramethylsilane (TMS) was used as an internal standard. Peak positions were given in parts per million (δ). Column chromatography was performed on silica gel (grade 60 mesh; Bodman Industries, Aston, PA). Routine thin-layer chromatography (TLC) was performed on silica gel GHIF plates (250 μm, 2.5 x 10 cm; Analtech Inc., Newark, DE). MS spectra were obtained from a Perkin Elmer Flexar UHPLC with AxION 2 Time of Flight (TOF) Mass Spectrometer, and the molecular weight of the compounds was within 0.05% of calculated values. Infrared spectra were obtained on a Thermo Nicolet iS10 FT-IR. Purity of the compounds was determined by HPLC using a Varian Microsorb 100-5 C18 column (250 x 4.6 mm), using Prostar 325 UV-Vis (254 nm) as the detector.

S1.1. Synthesis of 2-((6-(Hydroxymethyl)pyridin-2-yl)methoxy)-5-methoxybenzaldehyde, TD-7

Compound TD-7 was prepared according to standard procedures for similar compounds.(Nnamani *et al.*, 2008) A mixture of 2-hydroxy-5-methoxybenzaldehyde (0.20 mL, 1.5 mmol), 6-(bromomethyl)-2-pyridinemethanol (0.30 g, 1.5 mmol), and K_2CO_3 (0.25 g, 1.8 mmol) in anhydrous DMF (20 mL) was allowed to stir at room temperature for 12 h. The reaction mixture was diluted with EtOAc (20 mL), washed with H_2O (2 x 20 mL), brine (2 x 10mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield a crude product. The residue was purified by column chromatography (hexanes/EtOAc; 1:1) to yield 0.32g (78%) of TD-7 as a yellow-colored solid: mp 84-85 °C; IR (KBr, cm^{-1}): 3396 (O-H), 1678 (C=O); ^{1}H-NMR ($CDCl_3$): δ 3.51 (s, 1H, OH), 3.81 (s, 3H, OCH_3), 4.79 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 6.99 (d, *J* = 9.0 Hz, 1H, ArH), 7.09-7.13 (dd, *J* = 9.0, 3.2 Hz, 1H, ArH), 7.21 (d, *J* = 7.7 Hz, 1H, ArH), 7.37 (d, *J* = 3.2 Hz, 1H, ArH), 7.43 (d, *J* = 7.6 Hz, 1H, ArH), 7.74 (t, 1H, ArH), 10.58 (s, 1H, CHO); ^{13}C-NMR ($CDCl_3$): δ 189.24, 158.80, 155.61, 154.17, 154.17, 137.66, 125.56, 123.40, 119.84, 119.64,

114.92, 110.917, 71.65, 64.02, 55.86. MS (ESI) *m/z* found 274.15 $(M + H)^+$, 296.14 $(M + Na)^+$. The purity of the compound was checked by HPLC and was found to be 97.8% pure.

S1.2. Synthesis of 2-((6-(Hydroxymethyl)pyridin-2-yl)methoxy)-4-methoxybenzaldehyde, TD-9

Compound TD-9 was prepared according to literature procedure for a similar compound.(Nnamani *et al.*, 2008) A mixture of 2-hydroxy-4-methoxybenzaldehyde (113 mg, 0.74 mmol), 6-(bromomethyl)-2-pyridinemethanol (150 mg, 0.74 mmol), and K_2CO_3 (123 mg, 0.90 mmol) in anhydrous DMF (20 mL) was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc (20 mL), washed with H_2O (2 x 20 mL), brine (2 x 10mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield a crude product. The crude was purified by column chromatography (Hexanes/EtOAc; 1:1) to yield 190 mg (94%) of TD-9 as a yellow-colored solid: mp 104-105 °C; IR (KBr, cm^{-1}): 3153 (O-H), 1599 (C=O); ^{1}H-NMR ($CDCl_3$): δ 3.58 (s, 1H, OH), 3.85 (s, 3H, OCH_3), 4.78 (d, 2H, CH_2), 5.30 (s, 2H, CH_2), 6.53 (d, *J* = 2.2 Hz, 1H, ArH), 6.60 (d, *J* = 2.16 Hz, 1H, ArH), 7.22 (d, *J* = 7.68 Hz, 2H, ArH), 7.47 (d, *J* = 7.76 Hz, 2H, ArH), 7.76 (t, 1H, ArH), 7.85 (d, *J* = 8.64 Hz, 1H, ArH), 10.43 (s, 1H, CHO); ^{13}C-NMR ($CDCl_3$): δ 166.16, 162.27, 158.67, 155.51, 137.74, 131.04, 119.85, 119.69, 118.1, 106.68, 99.16, 70.85, 63.87, 55.66. MS (ESI) *m/z* found 274.11 $(M + H)^+$, 296.09 $(M + Na)^+$. The purity of the compound was checked by HPLC and was found to be 96.9% pure.

S1.3. Synthesis of 3-((6-(Hydroxymethyl)pyridine-2-yl)methoxy)-4-methoxybenzaldehyde, TD-8

Compound TD-8 was prepared according to literature procedure for a similar compound.(Nnamani *et al.*, 2008) A mixture of 3-hydroxy-4-methoxybenzaldehyde (200 mg, 1.0 mmol), 6-(bromomethyl)-2-pyridinemethanol (152 mg, 1.0 mmol), and potassium carbonate (165 mg, 1.2 mmol) in anhydrous DMF (20 mL) was stirred at room temperature for 12 h. The reaction mixture was diluted with EtOAc (20 mL), washed with H_2O (2 x 20 mL), brine (2 x 10mL), dried over Na_2SO_4 and evaporated under reduced pressure to yield a crude product. The crude was purified by column chromatography (Hexanes/EtOAc; 1:1) to yield 220 mg (80%) of **TD-8** as a white-colored solid: mp 120-121 °C; IR (KBr, cm^{-1}): 3176 (O-H), 1681 (C=O); ^{1}H-NMR ($CDCl_3$): δ 3.65 (s, 1H, OH), 4.00 (s, 3H, OCH_3), 4.78 (d, 2H, CH_2), 5.32 (d, 2H, CH_2), 7.03 (d, *J* = 8.72 Hz, 1H, ArH), 7.17 (d, *J* = 7.68 Hz, 1H, ArH), 7.44-7.51 (m, 3H, ArH), 7.71 (t, 1H, ArH); ^{13}C-NMR

($CDCl_3$): δ 190.66, 155.53, 154.95, 137.54, 130.11, 126.88, 119.99, 119.60, 111.92, 110.98, 158.79, 71.40, 63.90, 56.22. MS (ESI) *m/z* found 274.11 $(M + H)^+$, 296.09 $(M + Na)^+$. The purity of the compound was checked by HPLC and was found to be 99.5% pure.

S2. Hemoglobin Modification, Oxygen Equilibrium and Antisickling Studies Using Human Sickle Blood

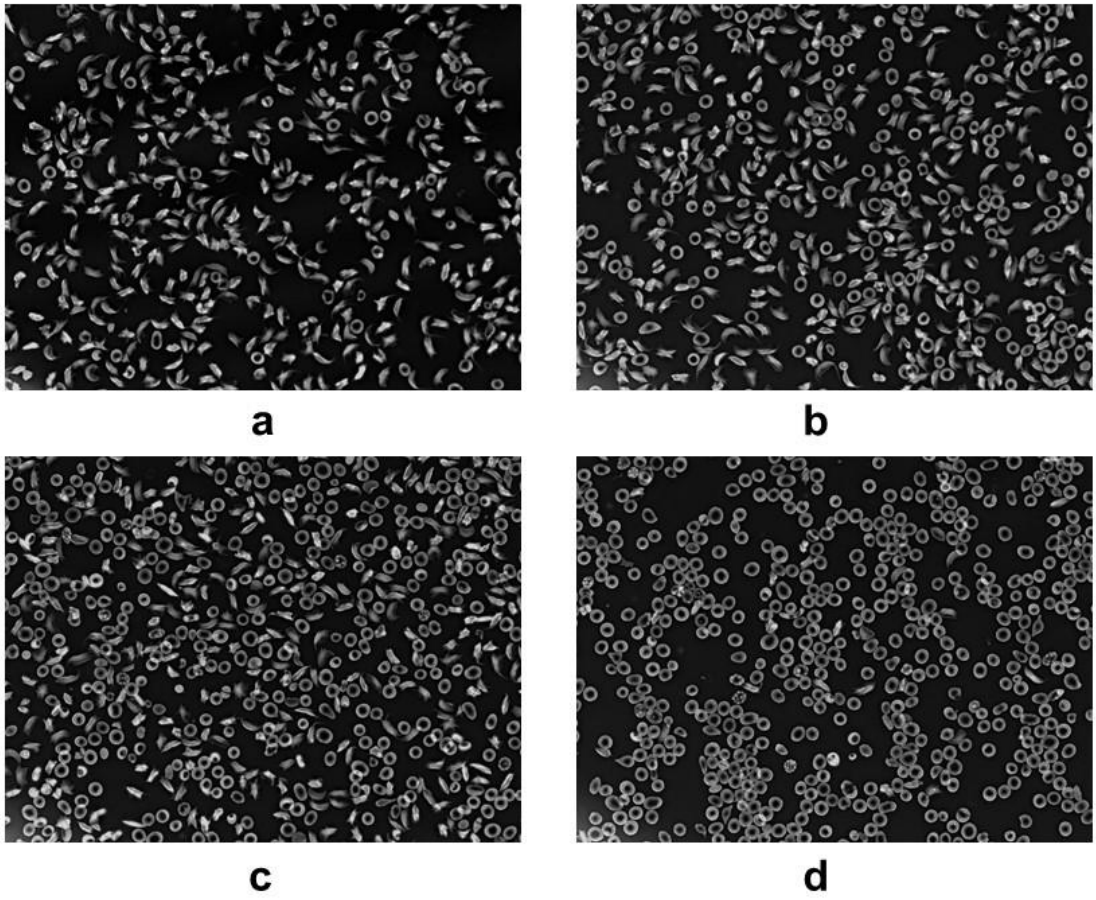

Figure S1 Morphology of SS cells with and without various concentrations of TD-7 under 2.5 % oxygen at 37°C for 2 hrs. (a) Without TD-7. (b, c, d) Upon incubation in the presence of various concentrations of TD-7 (0.5, 1.0 and 2.0 mM), SS cell sickling was prevented in a dose-dependent manner.

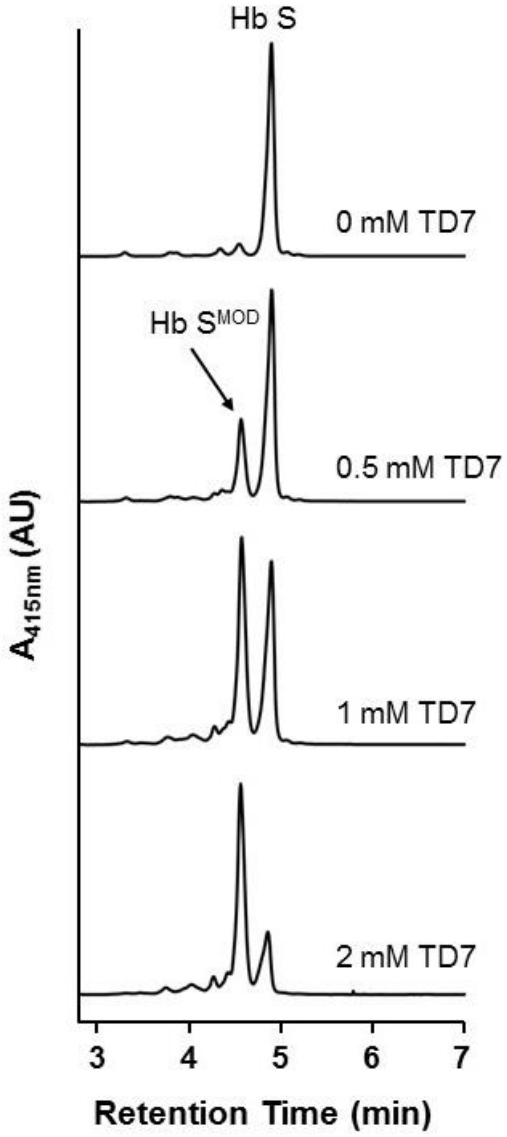

Figure S2 Cation-exchange HPLC patterns of hemoglobin prepared from SS cells that had been pre-incubated with 0 (control), 0.5, 1, and 2 mM TD-7.