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Supplementary information

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Pharmacological restriction of genomic binding sites redirects PU.1 pioneer transcription factor activity

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Supplementary Information: Methods

Synthesis of DB2373, DB2750 and DB2826

Progress of the chemical reactions were monitored by thin-layer chromatography on silica gel 60-F254 aluminum plates and detected employing UV light. Melting points were measured using a capillary melting point apparatus and are uncorrected. NMR spectra were recorded using a 400 MHz spectrometer, and chemical shifts (δ) are reported in ppm relative to TMS as the internal standard. Electrospray ionization (ESI) Q-Tof and Orbitrap were used for the mass spectra measurements. Elemental analyses are within ±0.4 of the theoretical values. Compounds reported as salts frequently analyzed for fractional moles of water or other solvents; the proton NMR results showed the presence of the indicated solvate. See Source data for detailed synthesis methods.

1, 3-Bis{4[4(5)-N-isopropylamidinobenzimidazolyl] phenoxymethyl]} benzene tetrahydrochloride (DB 2373) A well stirred solution of 1, 3-bis (4-formylphenoxymethyl)-benzene (0.173 g, 0.0005 mole), 4- (*N*-isopropylamidino)-1, 2-phenylenediamine hydrochloride-$0.2H_2O$[1] (0.232 g, 0.001 mole) and 1, 4-benzoquinone (0.108 g, 0.001 mole) in anhydrous ethanol (40 ml) (under nitrogen) was heated at reflux for 8-10 h, following workup as previously described[2] , yielded bluish grey solid 0.32 g (73%); mp > 320°C; ^{1}HNMR (DMSO-d_6/65^{0}C): 9.71 (s, 1H), 9.69 (s, 1H), 9.56 (s, 2H), 9.16 (s, 2H), 8.42 (d, 4H, J = 8.0 Hz), 8.08 (s, 2H), 7.85 (d, 2H, J = 8.4 Hz), 7.68 (d, 2H, J = 8.4 Hz), 7.64 (s, 1H), 7.49 (brm, 3H), 7.32(d, 4H, J = 8.0 Hz), 5.30 (s, 4H), 4.12 (quintet, 2H, J = 7.5 Hz), 1.32 (d, 12H, J = 7.5 Hz), 3.70-3.20 (vbr, exchanged NH); MS: HRMS-ESIPOS.: Calc. for $C_{42}H_{42}FN_8O_2$ *m/z* 691.3508(M^++1), found *m/z* 691.4123; Analysis Calc. for $C_{42}H_{42}N_8O_2.4HCl.2.5H_20$: C, 57.32; H, 5.84; N, 12.74; Found: C,57.39; H,5.91; N, 12.81.

2-(4-(4-(4-(6-Carbamimidoyl-1H-benzo[d]imidazol-2-yl)-2-(hex-5-yn-1-yloxy)phenoxy)butoxy)phenyl)-1H-benzo[d]imidazole-6-carboximidamide. (DB2750)

Sodium metabisulphite (0.25 g, 1.32 mmol) solution in water (2 ml) was added to a stirred solution of the 4-(4-(4-formylphenoxy)butoxy)-3-(hex-5-yn-1-yloxy)benzaldehyde (0.26 g, 0.66 mmol) in ethanol (20 ml) and stirring was continued for 1h, 3,4-diaminobenzimidamide hydrochloride (prepared as described in ref. 1) (0.24 g, 1.32 mmol) was added to the reaction mixture which was then heated at reflux for 24 h. The reaction mixture was concentrated under reduced pressure, filtered, the precipitate was suspended in water, neutralized with sodium hydroxide solution (2 N), filtered, and dried under vacuum at room temperature. The free base was dissolved in methanol (80 ml) and filtered. Finally, ethanolic HCl was added to the filtrate and stirred for 24 h at room temperature. The solution was concentrated to 5 ml, filtered, washed with ethanol and acetone, and dried at 100°C for 12 h. White solid, yield (0.38 g, 74%), mp > 250 °C dec., ^{1}HNMR (DMSO-d_6) δ 9.57 (br s, 4H), 9.24 (br s, 4H), 8.42 (d, *J* = 8.8 Hz, 2H), 8.26 (brs, 3H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.91 (m, 2H), 7.85 (m, 2H), 7.31 (d, *J* = 8.4, 1H), 7.26 (d, *J* = 8.8 Hz, 2H), 4.20 (m, 6H), 2.78 (s, 1H), 2.28 (m, 2H), 1.97 (brs, 4H), 1.87 (t, *J* = 7.2, 2H), 1.66 (t, *J* = 7.2, 2H); ESI-MS: m/z calculated for $C_{38}H_{40}N_8O_3$: 328.1606, found: 328.1593 (double charged amidine base M^++2); Anal. Calcd. For $C_{38}H_{38}N_8O_3$. 4HCl. $3.5H_2O$: C, 52.84; H, 5.71; N, 12.97. Found: C, 53.03; H, 5.80; N, 12.79.

1, 3-Bis{4[4(5)-N-isopropylamidinobenzimidazolyl]-3-fluorophenoxymethyl]]-2-fluorobenzene dihydrochloride (DB2826)

A mixture of 1,3-bis(bromomethyl)-2-fluorobenzene[3](1.41 g, 0.005 mole), 4-hydroxy-2-fluoro benzaldehyde (1.40 g, 0.01 mole) and anhydrous K_2CO_3 (2.07 g, 0.015 mole in 10 ml DMF was heated at 45^0C for 4 h, yielded 1.61 g (80%) of 1, 3-bis(3-fluoro-4-formylphenoxymethyl)-2-fluorobenzene; mp 133-5^0C ; ^{1}H NMR (DMSO- d_6): 10.09 (s, 2H),7.82 (t, 2H, J = 8.4 Hz),7.64 (t, 2H, J = 7.2 Hz),7.32 (t, lH, J = 7.6 Hz), 7.17 (dd, 2H, J = 2.0 Hz, J = 12.8 Hz), 7.07 (dd, 2H, J = 2.0 Hz, J = 8.4 Hz),5.32 (s,4H); MS: HRMS-ESI-POS: calc. for $C_{22}H_{15}F_3O_4Na$ m/z 423.0820 (M^++Na), found m/z 423.0835; Analysis Calc. for $C_{22}H_{15}F_3O_4$: C, 66.00; H, 3.78; Found: C, 66.12; H, 3.71.

A well stirred solution of 1, 3-bis(3-fluoro-4-formylphenoxymethyl)-2-fluorobenzene (0.20 g, 0.0005 mole), 4-(*N*-isopropylamidino)-1, 2-phenylenediamine hydrochloride-0.2H_2O (ref. 1) (0.2329, 0.001 mole) and 1,4-benzoquinone (0.108 g, 0.001 mole) in anhydrous ethanol (40 ml) (under nitrogen) was heated under reflux for 8-10 h. The reaction mixture was cooled, concentrated to 10 ml stirred in 50 ml acetone and filtered, washed with dry ether and dried to yield dihydrochloride salt. This salt was dissolved into a 1:1 mixture of warm ethanol-methanol (50 ml), filtered, the volume was reduced to 10 ml, diluted with anhydrous 50-60 ml ether, filtered, washed with ether, and dried under vacuum 70^0C (12 h) yielding purple-bluish grey solid 0.26 g (70%); mp> 320^0C dec.; ^{1}H NMR (DMSO- d_6): 9.71 (s, 1H), 9.69 (s, 1H), 9.56 (s, 2H), 9.16 (s, 2H), 8.42 (d, 4H, J = 8.4 Hz), 8.08 (s, 2H), 7.85 (d, 2H, J = 8.4 Hz),7.68 (d, 2H, J = 8.4Hz), 7.64 (s, 1H), 7.49 (brs, 2H),7.32 (d, 2H, J = 8.0 Hz), 5.31 (s, 4H), 4.12 (quintet, 2H, J = 6.4 Hz), 1.32(d, l2H, J = 6.4 Hz), 3.7-3.2 (vbr, exchanged NH); MS: HRMS-ESI-POS.: Calc. for $C_{42}H_{40}F_3N_8O_2$ m/z 745.3226 (M^++1), found m/z 745.3212; analysis calc. for $C_{42}H_{39}F_3N_8O_2$.2HCl.4.25H_20: C, 56.41; H,5.58; N, 12.53; Found: C, 56.49; H, 5.67; N, 12.61.