Electronic Supporting Information

for

An iron(III) complex selectively mediated cancer cell death: crystal structure, DNA targeting and *in vitro* antitumor activities

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temperature in Tris-HCl/NaCl buffer (pH = 7.2). Inset: Plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ vs. [DNA] for the absorption titration of CT-DNA with complexes.

Figure S14. Absorption spectra of complex **3** (12.5 μ M) in the absence (black line) and presence (other lines) of increasing amounts of CT-DNA (3.65, 7.22, 10.73, 14.17, 17.55, 20.86 and 24.11 μ M) at room temperature in Tris-HCl/NaCl buffer (pH = 7.2). Inset: Plot of ($\varepsilon_a - \varepsilon_f$)/($\varepsilon_b - \varepsilon_f$) vs. [DNA] for the absorption titration of CT-DNA with complexes.

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Figure S16. Emission spectra of EB-CT-DNA in the absence (black line) and in the presence (other lines) of **3** with increasing amounts (0.99-7.41 \times 10⁻⁵ M). The arrow shows the intensity changes on increasing the complex concentration. Inset: plot of I₀/I vs. [complex].

Figure S17. Cleavage of pUC19 DNA (0.1 μ g μ L⁻¹) in the varied concentrations of NH-Tone, MH-Tone, ME-Tone, FeCl₃ and cisplatin after 3 h incubation at 37 °C in Tris-HCl/NaCl buffer (pH 7.2).

Figure S18. Cell viability of iron complexes and cisplatin for 48 h against HUVEC.

Table S1. The Fe/Pt (ng) content in per million cells after treatments by varied complexes at 50 μ M for 8 h.

Experimental details

Materials and Instrumentations. All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. 2-Acetylpyridine and 2-Cyanopyridine were obtained from Shanghai Aladdin Bio-Chem Technology Co., LTD Introduction. Thiosemicarbazide and 4-ethyl-3-thiosemicarbazide were purchased from J&K Scientific Ltd. (Beijing, China). Soybean Lecithin and cholesterol were acquired from Solarbio. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Sigma-Aldrich. FBS (Fetal bovine serum) was purchased from Hyclone. DMEM medium was obtained from Gibco. Cells were counted using Countstar cell automatic counter acquired from Advanced Lab Instrument & Technology Co., Ltd. ¹H and ¹³C spectra were recorded on 400 MHz Varian Bruker NMR spectrometer. Infrared spectra (IR) were recorded on a Nicolet 380 spectrometer.

Synthesis of NH-TSC. 2-Cyanopyridine (2.40 g, 23.0 mmol) was dissolved in a solution of sodium (0.16 g, 7.0 mmol) in MeOH (70 mL), which was dried over CaSO₄. Thiosemicarbazide (2.10 g, 23.0 mmol) was added in small portions to the resulting solution, then the mixture was refluxed for 4 h and the resulting yellowish solid was filtered off, recrystallized from ethanol. Yield 59.78% (2.68 g, 13.7 mmol). Elemental analysis (%): calc. for C₇H₉N₅S: C, 43.06; H, 4.65; N, 35.87. Found: C, 43.35; H, 4.58; N, 35.63. IR (KBr, cm⁻¹): 3420.6, 3360, 3233.9, 2988.7, 1661.6, 1610.1, 1588.7, 1542.2, 1480.5, 1464.4, 1435.7, 1381.1, 1298.3, 1251.6, 1151.6, 1130.2, 1049.1, 998.5, 852.8, 792.2, 744.1, 701.7, 619.1, 526.2, 460.5. ¹H NMR (400 MHz, DMSO-*d*₆, ppm) 10.06 (s, 1H), 8.55 (d, 1H), 8.44 (d, 1H), 7.95 (s, 1H), 7.86–7.75 (m, 2H), 7.43 (dd, 1H), 6.92 (s, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆, ppm): 176.64(s), 150.15 (s), 147.85 (s), 141.86–140.20 (m), 136.64 (s), 124.48 (s), 121.10 (s).

Synthesis of MH-TSC. 2-Acetylpyridine (2.50 g, 0.02 mol) dissolved in ethanol (15 mL), was mixed with the aqueous solution (20 mL) of thiosemicarbazide (1.88 g, 0.02 mol) and the acetic acid (2–4 drops) was added under stirring. After gently refluxing for 6 h, the resulting solution was cooled to room temperature and filtered. The bright yellow product was filtered off, washed with ethanol (15 mL), and dried by diethyl ether. Yield 93.6% (3.75 g, 0.02 mol). Elemental analysis (%): calc. for

C₈H₁₀N₄S: C, 49.46; H, 5.19; N, 28.84. Found: C, 49.33; H, 5.41; N, 28.63. IR (KBr, cm⁻¹): 3420, 3372.2, 3185.5, 1601.2, 1501.8, 1466.1, 1428.2, 1367.6, 1314.1, 1244.1, 1150.1, 1107.1, 1083.0, 1049.4, 995.0, 965.6, 845.9, 780.9, 760.1, 740.1, 649.7, 619.4, 560.2, 485.5, 464.5, 418.6. ¹H NMR (400 MHz, DMSO-*d*₆, ppm) δ 10.30 (s, 1H), 8.57 (d, J = 4.7 Hz, 1H), 8.41 (d, J = 8.1 Hz, 2H), 8.17 – 8.06 (m, 1H), 7.78 (td, J = 8.1, 1.5 Hz, 1H), 7.37 (dd, J = 6.8, 5.4 Hz, 1H), 2.38 (d, J = 6.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆, ppm): δ 178.68 (s), 154.64 (s), 148.37 (s), 148.22 (s), 136.34 (s), 123.90 (s), 120.84 (s), 12.08 (s).

Synthesis of ME-TSC. The synthesis was carried out as described for MH-TSC, with 4-ethyl-3-thiosemicarbazide (2.40 g, 0.02 mol) and 2-Acetylpyridine (2.44 g, 0.02 mol) to give a pale yellow solid. Yield 81.27% (3.64 g, 0.016 mol). Elemental analysis (%): calc. for C₁₀H₁₄N₄S: C, 54.03; H, 6.35; N, 25.20. Found: C, 54.22; H, 6.61; N, 25.01. IR (KBr, cm⁻¹): 3348.6, 3210.8, 2977.8, 2930.9, 1580.6, 1533.1, 1503.7, 1469.7, 1434.5, 1388.5, 1343.5, 1311.5, 1297.4, 1221.2, 1154.3, 1102.2, 1083.2, 1065.3, 990.7, 965.8, 931.3, 900.8, 823.8, 781.6, 747.8, 666.7, 640.2, 618.0, 586.4, 549.4, 485.7, 430.8. ¹H NMR (400 MHz, DMSO-*d*₆, ppm) 10.27 (s, 1H), 8.70 (t, *J* = 5.7 Hz, 1H), 8.59 (ddd, *J* = 4.8, 1.6, 0.9 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 7.83 (tt, *J* = 7.5, 3.7 Hz, 1H), 7.40 (ddd, *J* = 7.4, 4.8, 1.1 Hz, 1H), 3.72 – 3.52 (m, 2H), 2.40 (s, 3H), 1.17 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆, ppm): 177.60 (s), 154.68(s), 148.43 (s), 147.83 (s), 136.28 (s), 123.85 (s), 120.75 (s), 38.61 (s), 14.43 (s), 12.09 (s).

Synthesis of NH-Tone (HL1). NH-TSC (2.72 g, 14.0 mmol), Chloroacetic acid (1.42 g, 15.0 mmol) and triethylamine (1.52 g, 15.0 mmol) were dissolved in toluene (30 mL). The reaction mixture was refluxing at 120 °C for 4 h, and the resulting solution was reduced under high vacuum. Finally the product was recrystallization from ethanol to get a white flocculent solid. Yield 68.65% (2.26 g, 9.61 mmol). Elemental analysis (%): calc. for C₉H₉N₅OS: C, 45.95; H, 3.86; N, 29.77. Found: C, 45.81; H, 3.59; N, 29.98. IR (KBr, cm⁻¹): 3436.6, 3307.3, 3069, 2926.6, 2761.9, 1708.6, 1641.8, 1616, 1589.2, 1564, 1476.7, 1435.6, 1391.7 1333.8, 1241.3, 1201.5, 1134.5, 1047.8, 989.6, 884.4, 796.6, 742.4, 717.1, 621.2, 580.7, 508, 462.2. ¹H NMR (400 MHz, DMSO-*d*₆, ppm) δ 11.65 (s, 1H), 8.73–8.47 (m, 1H), 8.10 (d, 1H), 7.89 (tt, 1H), 7.59–7.38 (m, 1H), 6.33 (d, 2H), 3.85 (s, 2H). ¹³C NMR (101 MHz, DMSO-*d*₆, ppm): δ 173.84 (s), 157.38 (s), 152.70 (s), 150.20 (s), 148.37 (s), 136.83 (s), 124.97 (s), 120.59 (s), 32.76 (s)

Synthesis of MH-Tone (HL2). The synthesis was carried out as described for NH-Tone, with MH-TSC (0.80 g, 4.12 mmol) and Chloroacetic acid (0.44 g, 4.74 mmol) to give a a pale red solid. Yield 52.76% (0.51 g, 2.17 mmol). Elemental analysis (%): calc. for C₁₀H₁₀N₄OS: C, 51.27; H, 4.30; N, 23.91. Found: C, 51.49; H, 4.51; N, 23.82. IR (KBr, cm⁻¹): 2936, 2735, 1715.7, 1630.3, 1570.2, 1478.5, 1430.6, 1345.3, 1307.3, 1242.3, 1210.2, 1039.4, 1145.3, 1112.6, 1092.4, 1039.4, 1000.5, 880.5, 812.5, 773.5, 737.5, 718, 631.5, 608.3, 592.9, 560, 537, 507.2, 453, 434.3. ¹H NMR (400 MHz, DMSO-*d*₆, ppm) δ 12.06 (s, 1H), 8.63 (d, J = 4.3 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.86 (td, J = 7.9, 1.6 Hz, 1H), 7.43 (dd, J = 6.8, 5.3 Hz, 1H), 3.89 (s, 2H), 2.42 (s, 3H). ¹³C NMR (101 MHz, DMSO-*d*₆, ppm): δ 173.93 (s), 165.48 (s), 161.39 (s), 155.12 (s), 148.77 (s), 136.60 (s), 124.40 (s), 120.36 (s), 32.86 (s), 13.51 (s).

Synthesis of ME-Tone (HL3). The synthesis was carried out as described for NH-Tone, with ME-TSC (0.80 g, 3.60 mmol) and Chloroacetic acid (0.39 g, 4.14 mmol) to give a yellow needle-shaped crystal. Yield 48.14% (0.46 g, 1.73 mmol). Elemental analysis (%): calc. for C₁₂H₁₄N₄OS: C, 54.94; H, 5.38; N, 21.36. Found: C, 54.75; H, 5.56; N, 21.22. IR (KBr, cm⁻¹): 3052.0, 2980.2, 2938.8, 2739.5, 2677, 2603.5, 2529.9, 2494.2, 1715.5, 1622.4, 1592.5, 1566.1, 1466.8, 1433.2, 1391.9, 1347.2, 1309, 1283.8, 1243, 1172.3, 1129.1, 1108.2, 1086.4, 1036, 991.8, 960.9, 941, 898.6, 851.2, 805.5, 781.8, 737.7, 715.5, 638.2, 604.8, 564.6, 538.7, 505.1, 459.7. ¹H NMR (400 MHz, CDCl₃, ppm) δ 8.63 (d, *J* = 4.7 Hz, 1H), 8.25–8.14 (m, 1H), 7.72 (dd, *J* = 10.9, 4.5 Hz, 1H), 7.37–7.18 (m, 1H), 3.95 (q, *J* = 7.1 Hz, 2H), 3.79 (s, 2H), 2.56 (d, *J* = 0.8 Hz, 3H), 1.32 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃, ppm): δ 171.81 (s), 164.06 (s), 162.64 (s), 155.76 (s), 148.66 (s), 136.12 (s), 124.16 (s), 121.18 (s), 38.75 (s), 32.66 (s), 12.40 (s), 8.62 (s).



























Figure S7. ¹H NMR spectrum of NH-Tone



Figure S8. ¹³C NMR spectrum of NH-Tone







Figure S10. ¹³C NMR spectrum of MH-Tone











Figure S13. Absorption spectra of complex **2** (12.5 μ M) in the absence (black line) and presence (other lines) of increasing amounts of CT-DNA (3.65, 7.22, 10.73, 14.17, 17.55, 20.86 and 24.11 μ M) at room temperature in Tris-HCl/NaCl buffer (pH = 7.2). Inset: Plot of ($\varepsilon_a - \varepsilon_f$)/($\varepsilon_b - \varepsilon_f$) vs. [DNA] for the absorption titration of CT-DNA with complexes.



Figure S14. Absorption spectra of complex **3** (12.5 μ M) in the absence (black line) and presence (other lines) of increasing amounts of CT-DNA (3.65, 7.22, 10.73, 14.17, 17.55, 20.86 and 24.11 μ M) at room temperature in Tris-HCl/NaCl buffer (pH = 7.2).

Inset: Plot of $(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)$ vs. [DNA] for the absorption titration of CT-DNA with complexes.



Figure S15. Emission spectra of EB-CT-DNA in the absence (black line) and in the presence (other lines) of **2** with increasing amounts (0.99-7.41 × 10⁻⁵ M). The arrow shows the intensity changes on increasing the complex concentration. Inset: plot of I_0/I vs. [complex].



Figure S16. Emission spectra of EB-CT-DNA in the absence (black line) and in the presence (other lines) of **3** with increasing amounts (0.99-7.41 × 10^{-5} M). The arrow

shows the intensity changes on increasing the complex concentration. Inset: plot of I_0/I vs. [complex].



Figure S17. Cleavage of pUC19 DNA (0.1 μ g μ L⁻¹) in the varied concentrations of NH-Tone, MH-Tone, ME-Tone, FeCl₃ and cisplatin after 3 h incubation at 37 °C in Tris-HCl/NaCl buffer (pH 7.2).



Figure S18. Cell viability of iron complexes and cisplatin for 48 h against HUVEC.

Table S1. The Fe/Pt (ng) content in per million cells after treatments by varied complexes at 50 μ M for 8 h.

| Cell lines | HeLa | LO2 | FI | SI |
|---|----------------|---------------|------|------|
| 1 | 54.69 ± 1.11 | 91.28 ± 0.55 | 0.60 | 1.2 |
| 2 | 94.54 ± 1.52 | 178.48 ± 4.35 | 0.53 | 1.06 |
| 3 | 218.85 ± 10.53 | 98.72 ± 3.72 | 2.22 | 4.44 |
| Cisplatin | 81.71 ± 7.13 | 163.91 ± 9.65 | 0.50 | |
| FI = the net increase of metal in HeLa / that of LO2. SI = FI (complex) / FI (cisplatin). | | | | |