Supporting Information

An iridium(III)-based irreversible protein-protein interaction inhibitor of BRD4 as a potent anticancer agent

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Experimental section

General synthesis of $[M_2(C^N)_4Cl_2]$complexes. Cyclometalated dichloro-bridged dimers of the general formula $[M_2(C^N)_4Cl_2]$,where M = Ir(III)/Rh(III), were synthesized according to a literature method.[1] In brief, $MCl_3 \cdot 3H_2O$ was heated to 150 °C with 2.2 equivalents of cyclometallated C^N ligands in 3:1 methoxymethanol and deionized water under a nitrogen atmosphere for 12 h. The reaction was cooled to room temperature, and the product was filtered and washed with three portions of deionized water and then three portions of ether (3 × 50 mL) to yield the corresponding dimer.

General synthesis of $[M(C^N)_2(ACN)_2]$OTf complexes. These complexes were synthesized according to a literature method.[2, 3] In brief, $[M_2(C^N)_4Cl_2]$ was mixed with 2.0 equivalents of silver triflate in 25 mL acetonitrile and stirred at room temperature under a nitrogen atmosphere for 15 h. The mixture was filtered and washed with two portions of ether (2 × 30 mL) to yield titled product.

The synthesis of[Rh(ppy)$_2$(N≡C-R)$_2$]OTf complex. The complex was synthesized according to a literature method.[13] In brief, the solution of [Rh(ppy)$_2$(ACN)$_2$]OTf (0.08 mmol) and naphthylisocyanide (0.18 mmol) was stirred in acetonitrile (6 mL) overnight under a nitrogen atmosphere. The solvent was removed in vacuo and the residues were washed with diethyl ether (2 × 50 mL) to yield the titled compound.

General synthesis of [M(C^N)$_2$(N^N)]PF$_6$ complexes. These complexes were synthesized using a modified literature method.[1] Briefly, a suspension of [M$_2$(C^N)$_4$Cl$_2$] (0.2 mmol) and corresponding N^N (0.44 mmol) ligands in a mixture of dichloromethane:methanol (1:1, 20 mL) was refluxed overnight under a nitrogen atmosphere. The resulting solution was allowed to cool to room temperature, and was filtered to remove unreacted cyclometallated dimer. To the filtrate, an aqueous solution of ammonium hexafluorophosphate (excess) was added and the filtrate was reduced in volume by rotary evaporation until precipitation of the crude product occurred. The precipitate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound.

Complex 1. **(**Reported)[4]

Complex 2. Yield: 56%.^{1}H NMR (400 MHz, Acetone-d_6) δ 8.68 (d, J = 8.0 Hz, 2H), 8.37 (d, J = 8.0 Hz, 2H), 8.16-8.11 (m, 4H), 8.03 (s, 2H), 7.86 (d, J = 8.0 Hz, 2H), 7.28 (t, J = 8.0 Hz, 2H), 6.80 (t, J = 8.0 Hz, 2H), 5.81 (d, J = 8.0 Hz, 2H), 2.29 (s, 6H);^{13}C NMR (100 MHz, Acetone-d_6) δ 172.8, 172.76, 172.5, 172.4, 164.8, 164.6, 162.8, 162.7, 162.4, 162.3, 162.2, 162.1, 162.06, 160.2, 160.1, 153.1, 151.2, 150.5, 141.7, 140.7, 139.8, 128.3, 128.24, 128.23, 128.20, 125.1, 124.9, 124.7, 124.5, 115.7, 115.6, 115.5, 115.4, 101.0, 100.8, 100.5, 18.5; MALDI-TOF-HRMS: Calcd. for $C_{34}H_{24}F_4RhN_4[M–PF_6]^+$: 667.0986 Found: 667.0983; Anal. ($C_{34}H_{24}N_4RhPF_{10}$+2H_2O) C, H, N: calcd 48.13, 3.33, 6.60; found 48.16, 3.29, 6.57.

Complex 3. Yield: 58%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.79 (d, J = 4.0 Hz, 2H), 8.70 (d, J = 8.0 Hz, 2H), 8.19 (s, 2H), 7.81 (d, J = 8.0 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.17 (s, 2H), 7.05 (t, J = 8.0 Hz, 2H), 6.80 (t, J = 8.0 Hz, 2H), 6.62 (t, J = 4.0 Hz, 2H), 6.11 (d, J = 8.0 Hz, 2H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 165.3, 148.9, 148.5, 147.5, 143.0, 141.0, 140.0, 135.3, 130.0, 128.7, 128.1, 127.6, 127.0, 124.7, 113.5, 109.8, 27.1; MALDI-TOF-HRMS: Calcd. for $C_{32}H_{26}RhN_6[M–PF_6]^+$: 597.1274 Found: 597.6012; Anal. ($C_{32}H_{26}N_6RhPF_6$) C, H, N: calcd 51.77, 3.66, 11.32; found 52.09, 3.48, 10.94.

Complex 4. Yield 71%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.52 (s, 2H), 8.19 (d, J = 8.0 Hz, 4H), 7.94 (t, J = 8.0 Hz, 2H), 7.89 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 4.0 Hz, 2H), 6.97 (t, J = 6.0 Hz, 4H), 6.30 (s, 2H), 2.42 (s, 6H), 2.18 (s, 6H), 2.14 (s, 6H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 169.2, 168.9, 166.1, 151.7, 150.0, 147.2, 145.2, 142.4, 140.7, 139.2, 135.7, 134.4, 130.2, 125.4, 125.2, 125.0, 123.7, 120.4, 22.0, 18.0, 15.1; MALDI-TOF-HRMS: Calcd. for $C_{40}H_{36}RhN_4[M–PF_6]^+$: 675.6461 Found: 675.2026; Anal. ($C_{40}H_{36}RhN_4PF_6$) C, H, N: calcd 58.55, 4.42, 6.83; found 58.35, 4.81, 6.58.

Complex 5. Yield: 57%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.93 (d, J = 8.0 Hz, 2H), 8.76 (d, J = 4.0

Hz, 2H), 8.71 (d, *J* = 8.0 Hz, 2H), 8.57-8.54 (m, 2H), 8.28-8.22 (m, *J* = 8.0 Hz, 4H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.32 (s, 2H), 7.15 (t, *J* = 8.0 Hz, 2H), 6.96 (t, *J* = 8.0 Hz, 2H), 6.66 (t, *J* = 4.0 Hz, 2H), 6.49 (d, *J* = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 153.8, 151.5, 144.3, 143.6, 141.0, 140.0, 136.4, 134.1, 133.4, 132.4, 132.0, 130.6, 129.0, 128.9, 127.5, 124.3, 112.9, 109.1; MALDI-TOF-HRMS: Calcd. for $C_{36}H_{24}IrN_8$[M–PF_6]$^+$: 761.1753 Found: 761.2121; Anal. ($C_{36}H_{24}N_8IrPF_6$) C, H, N: calcd 47.73, 2.67, 12.37; found 47.66, 2.62, 12.32.

Complex 6. (Reported)[5]

Complex 7. Yield 76%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.13 (s, 2H), 8.10 (d, *J* = 6.4 Hz, 2H), 7.81-7.80 (m, 4H), 7.79-7.76 (m, 4H), 7.65 (d, *J* = 6.8 Hz, 2H), 6.96-6.92 (m, 2H), 6.86-6.81 (m, 4H), 6.29 (d, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) 152.3, 150.6, 150.1, 148.8, 145.8, 145.1, 138.6, 132.6, 131.3, 131.1, 128.8, 126.4, 125.9, 124.4, 123.7, 120.8; MALDI-TOF-HRMS: Calcd. for $C_{34}H_{22}Cl_2IrN_4$ [M–PF_6]$^+$: 749.0851 Found: 749.0822; Anal. ($C_{34}H_{22}Cl_2IrN_4PF_6$) C, H, N: calcd. 45.64, 2.48, 6.26; found 45.48, 2.48, 6.18.

Complex 8. Yield: 57%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.15 (d, *J* = 8.0 Hz, 1H), 8.95 (d, *J* = 8.0 Hz, 1H), 8.72-8.70 (m, 2H), 8.64 (d, *J* = 8.0 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 2H), 8.26 (q, *J* = 8.0 Hz, 1H), 8.15 (q, *J* = 8.0 Hz, 1H), 8.03 (t, *J* = 8.0 Hz, 2H), 7.82-7.79 (m, 2H), 7.10-7.06 (m, 2H), 6.84 (t, *J* = 8.0 Hz, 2H), 5.93-5.89 (m, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 153.5, 153.0, 151.0, 148.5, 146.9, 140.7, 139.6, 137.0, 132.7, 132.1, 130.9, 129.0, 128.8, 128.5, 124.9, 124.5, 124.4, 114.9, 114.7, 100.1, 99.8, 99.6, 73.4, 62.1; MALDI-TOF-HRMS: Calcd. for $C_{34}H_{19}ClIrN_4$[M–PF_6]$^+$: 787.0864 Found: 787.0844; Anal. ($C_{34}H_{19}ClIrN_4PF_6$+2H_2O) C, H, N: calcd. 42.18, 2.39, 5.79; found 42.25, 2.44, 5.73.

Complex 9. Yield: 57%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.04-8.02 (m, 4H), 7.84-7.78 (m, 6H), 7.65-7.57 (m, 10H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.01 (t, *J* = 8.0 Hz, 2H), 6.82 (t, *J* = 8.0 Hz, 2H), 6.49 (d, *J* = 8.0 Hz,2H), 2.22 (s, 6H), 1.91 (s, 6H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 169.8, 165.2, 163.0, 151.3, 149.9, 147.0, 146.7, 139.6, 136.8, 133.2, 130.5, 130.4, 129.9, 128.7, 128.2, 126.1, 125.6, 124.6, 123.4, 117.9, 25.7, 25.2; MALDI-TOF-HRMS: Calcd. for $C_{50}H_{40}IrN_4$[M–PF_6]$^+$: 889.2882 Found: 889.2887; Anal. ($C_{50}H_{40}N_4IrPF_6$+0.5H_2O) C, H, N: calcd 57.57, 3.96, 5.37; found 57.55, 3.81, 5.31.

Complex 10. Yield: 57%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.80 (s, 2H), 8.13 (d, *J* = 8.0 Hz, 2H), 8.01 (d, *J* = 4.0 Hz, 2H), 7.95 (d, *J* = 8.0 Hz, 2H), 7.82 (t, *J* = 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 2H), 7.08 (t, *J* = 8.0 Hz, 4H), 6.87 (t, *J* = 8.0 Hz, 2H), 6.44 (d, *J* = 8.0 Hz, 2H), 1.86 (s, 6H), 1.37 (s, 18H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 169.6, 164.8, 162.5, 156.9, 149.8, 149.3, 146.5, 140.0, 133.8, 130.5, 126.3, 126.0, 124.8, 123.6, 122.5, 118.5, 36.3, 26.3; MALDI-TOF-HRMS: Calcd. for $C_{42}H_{44}IrN_4$[M–PF_6]$^+$: 797.3195 Found: 797.3144; Anal. ($C_{42}H_{44}N_4IrPF_6$) C, H, N: calcd 53.55, 4.71, 5.95; found 53.65, 4.78, 5.96.

Complex 11. (Reported)[6]

Complex 12. (Reported)[7]

Complex 13. Yield: 68%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.04 (d, J = 8.8 Hz, 2H), 8.88 (d, J = 8.8 Hz, 2H), 8.51 (d, J = 8.0 Hz, 2H), 8.43 (d, J = 5.6 Hz, 2H), 8.10 (d, J = 9.2 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H), 7.91 (d, J = 9.2Hz, 2H), 7.82 (d, J = 9.2 Hz, 2H), 7.54-7.47 (m, 6H), 7.07 (t, J = 7.6 Hz, 2H), 7.01-6.97 (m, 2H), 6.12 (d, J = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 161.3, 157.9, 151.0, 149.2, 146.0, 142.3, 140.8, 138.4, 135.4, 132.0, 130.7, 130.6, 130.3, 129.9, 129.6, 129.4, 128.9, 128.2, 124.9, 123.3, 123.0, 121.4; MALDI-TOF-HRMS: Calcd. for $C_{44}H_{28}IrN_4$[M–PF_6]$^+$: 805.1943 Found: 805.1893; Anal.: ($C_{44}H_{28}IrN_4PF_6$+3H_2O) C, H, N: calcd. 56.64, 3.41, 5.58; found 56.58, 3.67, 5.50.

Complex 14. Yield: 51%. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 8.38-8.30 (m, 4H), 8.12-8.10 (d, J = 8.0 Hz, 2H), 7.93-7.92 (d, J = 4.0 Hz, 2H), 7.86-7.83 (d, J = 8.0 Hz, 2H), 7.55 (s, 2H), 7.43-7.38 (m, 4H), 7.17-7.08 (m, 4H), 6.97 (t, J = 4.0 Hz, 2H), 6.80-6.76 (t, J = 8.0 Hz, 2H), 6.51-6.49 (d, J = 8.0Hz, 2H), 3.87 (s, 6H); ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ 171.6, 169.0, 158.3, 152.9, 150.2, 148.9, 147.5, 141.3, 135.7, 132.3, 131.8, 130.5, 129.3, 128.7, 128.1, 126.2, 124.0, 119.2, 115.4, 116.6, 57.9; MALDI-TOF-HRMS: Calcd. for $C_{42}H_{32}IrN_4O_2$[M–PF_6]$^+$: 817.2162 Found: 817.2135; Anal. ($C_{42}H_{32}IrN_4O_2\,PF_6$+2$H_2O$) C, H, N: calcd 50.55, 3.64, 5.61; found 50.24, 3.42, 5.69.

Complex 15. (Reported)[8]

Complex 16. (Reported)[9]

Complex 17. (Reported)[9]

Complex 18. (Reported)[10]

Complex 19. (Reported)[11]

Complex 20. (Reported)[5]

Complex 21. (Reported)[5]

Complex 22. (Reported)[5]

Complex 23. Yield: 55%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.29 (d, J = 8.0 Hz, 2H), 8.08-8.03 (m, 4H), 7.92 (t, J = 8.0 Hz, 4H), 7.78 (s, 2H), 7.65-7.60 (m, 10H), 7.13 (t, J = 8.0 Hz, 2H), 7.04 (t, J = 8.0 Hz, 2H), 6.88 (t, J = 8.0 Hz, 2H), 6.33 (d, J = 8.0 Hz, 2H), 2.31 (s, 6H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.5, 167.1, 164.2, 163.2, 150.0, 149.7, 146.4, 143.4, 139.0, 135.7, 132.4, 129.6, 128.9, 127.8, 126.4, 124.8, 124.2, 123.5, 123.0, 120.3, 26.3; MALDI-TOF-HRMS: Calcd. for $C_{48}H_{36}RhN_4$[M–PF_6]$^+$: 771.1995 Found: 771.0847; Anal. ($C_{48}H_{36}N_4RhPF_6$) C, H, N: calcd 62.89, 3.96, 6.11; found 62.83, 3.97, 5.91.

Complex 24. Yield: 63%. ^{1}H NMR (400 MHz; Acetonitrile-d_3): δ 8.98 (d, J = 8.4 Hz, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.49 (d, J = 8.0 Hz, 2H), 8.43 (s, 1H), 8.40-8.38 (m, 1H), 8.32 (d, J = 4.8 Hz, 1H), 7.99 (d, J = 8.8Hz, 2H), 7.89-7.83 (m, 5H), 7.77 (d, J = 8.4 Hz, 1H), 7.66 (d, J = 8.0Hz, 2H), 7.38 (d, J = 8.0, Hz, 2H), 7.29 (d, J =8.0 Hz, 2H), 6.44 (d, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz; Acetonitrile-d_3): δ

163.91, 163.75, 163.4, 154.3, 151.8, 151.4, 149.0, 146.5, 145.0, 139.8, 137.9, 137.2, 135.6, 134.0, 131.3, 130.41, 130.27, 129.38, 129.24, 127.4, 127.13, 126.93, 124.0, 122.5, 121.6; MALDI-TOF-HRMS: Calcd. for $C_{38}H_{26}ClRhN_4$[M–PF_6]$^+$: 673.0666 Found: 673.2234; Anal. ($C_{38}H_{23}ClN_4RhPF_6$+2H_2O) C, H, N: calcd 53.38, 3.18, 6.55; found 53.27, 3.27, 6.55.

Complex 25. Yield: 58%. ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.63 (d, J = 8.0 Hz, 2H), 8.53 (d, J = 4.0 Hz, 2H), 8.28 (s, 2H), 8.23 (d, J = 4.0 Hz, 2H), 8.04 (d, J = 8.0 Hz, 2H), 7.93 (t, J = 8.0 Hz, 4H), 7.68-7.62 (m, 12H), 7.52 (t, J = 8.0 Hz, 2H), 7.30 (t, J = 8.0 Hz, 2H), 6.51 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 164.6, 164.3, 153.7, 150.1, 148.9, 145.8, 139.3, 137.4, 135.5, 133.4, 129.8, 129.6, 129.4, 129.0, 128.1, 126.9, 126.8, 125.7, 124.1, 123.0, 121.4; MALDI-TOF-HRMS: Calcd. for $C_{50}H_{32}RhN_4$[M–PF_6]$^+$: 791.1682 Found: 790.0988; Anal. ($C_{50}H_{32}N_4RhPF_6$) C, H, N: calcd 64.11, 3.44, 5.98; found 64.14, 3.58, 6.01.

Complex 26. (Reported)[12]

Complex 27. (Reported)[5]

Complex 1a. Yield: 57%. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 7.94-7.92 (m, 4H), 7.62 (d, J = 8.0 Hz, 2H), 7.47 (t, J = 4.0 Hz, 2H), 6.92 (t, J = 4.0 Hz, 2H), 6.76 (t, J = 4.0 Hz, 2H), 6.12 (d, J = 4.0 Hz, 2H), 2.96 (s, 6H), 2.00 (s, 6H); ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ 167.7, 162.0, 152.4, 145.6, 141.1, 139.0, 131.2, 129.0, 124.0, 123.8, 122.4, 116.6, 27.6; MALDI-TOF-HRMS: Calcd. for $C_{28}H_{26}IrN_4$[M–2ACN–CF_3SO_3]$^+$: 529.1256 Found: 529.0762.

Complex 1b. Yield: 59%. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 9.12 (d, J = 8.0 Hz, 2H), 8.38 (d, J = 8.0 Hz, 2H), 8.16 (t, J = 8.0 Hz, 2H), 7.54 (t, J = 8.0 Hz, 2H), 6.62 (t, J = 8.0 Hz, 2H), 5.56 (t, J = 8.0 Hz, 2H), 2.00 (s, 6H); ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ 151.3, 147.5, 139.9, 128.5, 123.9, 123.6, 122.7, 120.2, 119.6, 113.6, 99.1, 98.8, 98.6. MALDI-TOF-HRMS: Calcd. for $C_{26}H_{18}IrN_4$[M–2ACN–CF_3SO_3]$^+$: 573.0566 Found: 573.0403.

Complex 1c. Yield: 67%. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 9.63 (s, 2H), 9.18 (d, J = 8.0 Hz, 2H), 8.25 (d, J = 8.0 Hz, 2H), 8.19 (t, J = 8.0 Hz, 2H), 7.88 (d, J = 8.0 Hz, 2H), 7.62 (t, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 6.43 (s, 2H), 2.07 (s, 6H); ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ 164.9, 151.2, 150.3, 142.9, 139.2, 135.9, 130.5, 124.9, 124.8, 124.3, 121.0; MALDI-TOF-HRMS: Calcd. for $C_{28}H_{22}IrN_4$ [M–2ACN–CF_3SO_3]$^+$: 557.0841 Found: 557.0948.

Complex 1d. Yield: 61%. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 8.81 (d, J = 8.0 Hz, 2H), 8.57 (d, J = 8.0 Hz, 2H), 8.25 (d, J = 8.0 Hz, 2H), 8.10 (d, J = 8.0 Hz, 2H), 7.91-7.83 (m, 4H), 7.75 (t, J = 8.0 Hz, 2H), 6.97 (t, J = 4.0 Hz, 2H), 6.75 (t, J = 4.0 Hz, 2H), 6.11 (d, J = 4.0 Hz, 2H), 1.97 (s, 6H); ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ 169.9, 147.5, 146.3, 144.0, 140.2, 132.3, 131.4, 129.7, 128.8, 128.0, 126.8, 126.7, 126.0, 122.6, 117.3. MALDI-TOF-HRMS: Calcd. for $C_{34}H_{26}IrN_4$[M–2ACN–CF_3SO_3]$^+$: 601.1256 Found: 601.4527.

Complex 1e. (Reported)[4]

Complex 1f. Yield: 58%. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 9.06-9.03 (m, 4H), 8.29 (d, J = 7.6 Hz, 2H), 8.23 (d, J = 7.6 Hz, 2H), 7.98-7.88 (m, 6H), 7.02 (t, J = 7.6 Hz, 2H), 6.74 (t, J = 7.2 Hz, 2H), 6.14 (d, J = 8.0 Hz, 2H), 2.07 (s, 6H); ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ 167.5, 146.7, 145.6, 142.4, 137.1, 131.6, 131.2, 129.7, 129.2, 128.5, 127.3, 126.3, 125.8, 121.9, 121.4, 119.2.MALDI-TOF-HRMS: Calcd. for $C_{34}H_{26}IrN_4[M–2ACN–CF_3SO_3]^+$: 601.1256 Found: 601.2065.

Complex 1g. Yield: 67%. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 8.45 (s, 2H), 8.17 (s, 2H), 7.39 (d, J = 8.0 Hz, 2H), 6.97 (t, J = 8.0 Hz, 2H), 6.90 (d, J = 8.0 Hz, 2H), 6.73 (t, J = 8.0 Hz, 2H), 6.05 (d, J = 8.0 Hz, 2H), 2.00 (s, 6H); ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ 142.9, 139.5, 132.5, 127.6, 125.3, 123.0, 111.2, 108.0; MALDI-TOF-HRMS: Calcd. for $C_{22}H_{20}IrN_4[M–2ACN–CF_3SO_3]^+$: 479.0848 Found: 479.0417.

Complex 1h. (Reported)[5]

Complex1i. (Reported)[13]

Complex 1j. (Reported)[5]

Stability analysis of complexes. Complex **1a** was dissolved in DMSO (10 μM) at 298K for 24 h, and was monitored by UV/vis absorbance at 298K for 24 h. Absorption spectra were recorded on a UV-Visible Spectrophotometer (Cary UV-100). For the stability of complex **1a** in plasma, 20 μM of complex **1a** was incubated in 2% (v/v) plasma aqueous solution then monitored by UV-Visible Spectrophotometer.

Alphascreen assay. Assays were performed as described previously with minor modifications from the manufacturer's protocol (PerkinElmer, USA). All reagents were diluted in 50 mM HEPES, 100 mM NaCl, 0.1 % BSA, pH 7.4 supplemented with 0.05 % CHAPS and allowed to equilibrate to room temperature prior to addition to plates. A 24-point 1:2 serial dilution of the ligands was prepared over the range of 0–150 μM and 4 μL transferred to low-volume 384-well plates (ProxiPlateTM-384 Plus, PerkinElmer, USA), followed by 4 μL of His-tagged protein (BRD4(1), 250 nM, BRD4(2) and CREBBP, 2000 nM, Cayman Chemical, Ann Arbor, MI, USA). Plates were sealed and incubated at room temperature for 30 min, before the addition of 4 μL of biotinylated peptide at equimolar concentration to the protein [peptide for BRD4(1) & BRD4(2): HSGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK(Biotin)-OH peptide for CREBBP: Biotin-KSAPATGGVK(Ac)KPHRYRPGT-OH (China peptide, Shanghai, China)]. 384-well plates were sealed and incubated for a further 30 min, before the addition of 4 μL of streptavidin-coated donor beads (25 μg/mL) and 4 μL nickel chelate acceptor beads (25 μg/mL) under low light conditions. Plates were foil-sealed to protect from light, incubated at room temperature for 60 min and read on an EnVision Multilabel Reader (PerkinElmer) using an AlphaScreen 680 excitation/570 emission filter set. IC_{50} values were calculated in Prism 5 (GraphPad Software, USA) after normalization against corresponding dimethyl sulfoxide (DMSO) controls and are given as the final concentration of compound in the 20 μL reaction volume.

Fluorescence polarization assay. All components were dissolved in buffer composition of 50 mM

HEPES pH 7.4, 150 mM NaCl and 0.5 mM CHAPS with final concentrations of BRD4(1) or BRD4(2), fluorescent ligand H4K5acK8acK12acK16ac, HSGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RHRK(FAM) 100 nM. 10 μL of this reaction mixture was added to wells containing 10 μL of various concentrations of test compound or DMSO vehicle (1% final) in 384-well black plate (PerkinElmer) and equilibrated in the dark for 60 min at room temperature. Fluorescence anisotropy was measured by SpectraMax M5 microplate reader (Molecular Devices) using FP module with excitation and emission wavelengths are 485 nm and 520 nm respectively.

Measurement of caspase-6 activity *in vitro.* Caspase-6 activity assay was performed using a fluorometric method (Abnova, Taiwan) according to the manufacturer's instructions. Serial dilution of **1a** were mixed with reaction buffer and 50 μM VEID-AFC (AFC, 7-amino-4-trifluoromethyl coumarin) substrate. The mixture was incubated at 37 °C for 1 h. Measured the fluorescence at an excitation and emission wavelength of 400 nm and 505 nm using SpectraMax M5 microplate reader (Molecular Devices).

STAT3 DNA-binding ELISA. The STAT3 DNA-binding assay was performed using the TransAM® Transcription Factor ELISA (Active Motif, Carlsbad, CA) according to the manufacturer's instructions. Briefly, HepG2 cells nuclear extract (2 μg) containing activated STAT3 was added with compound (20 μL) and complete binding buffer (30 μL) to microtitre wells coated with the STAT3 DNA consensus sequence. The mixture was incubated at room temperature for 1 h. The wells were washed three times with 1 × wash buffer, and incubated with STAT3 antibody for 1 h. The wells were washed as before and incubated with horseradish peroxide-conjugated secondary antibody at room temperature for 1 h. The wells were washed as before, incubated with 100 μL of developing solution, quenched with 100 μL stop solution, and the absorbance was measured at λ = 450 nm.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Cells were seeded in 96-well plates in triplicate at a density of 5,000 cells per well and given 24 h to adhere. Cells were then treated with varying concentrations of the tested compounds in the presence of 10% FBS. The cells were incubated for 72 h at 37 °C. 25 μL of MTT dye was added to each sample and incubated for 3.5 h. After this, 100 μL of DMSO was added to each well. The absorbance at 450 nm was recorded and the Half-Maximal inhibitory concentrations (IC_{50}) were determined using Prism 5.0 (Graph-Pad Software Inc., San Diego, CA, USA)

Colony formation assay. Cells were seeded at 250 cells per well in 6 cm well plates (n = 3). After 7 to 10 days of treatment, cells were stained with crystal violet, photographed, and scored.

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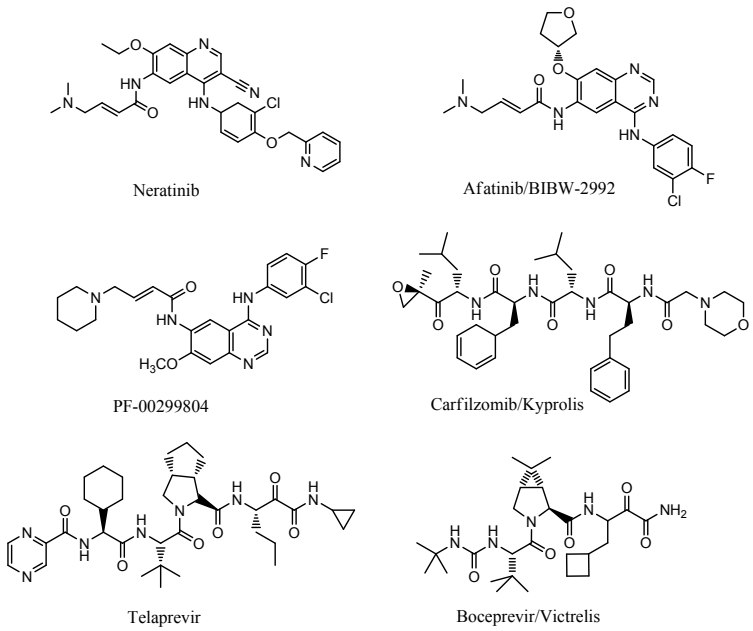

Fig. S1 The structures of Neratinib, Afatinib/BIBW-2992, PF-00299804, Carfilzomib/Kyprolis, Telaprevir and Boceprevir/Victrelis

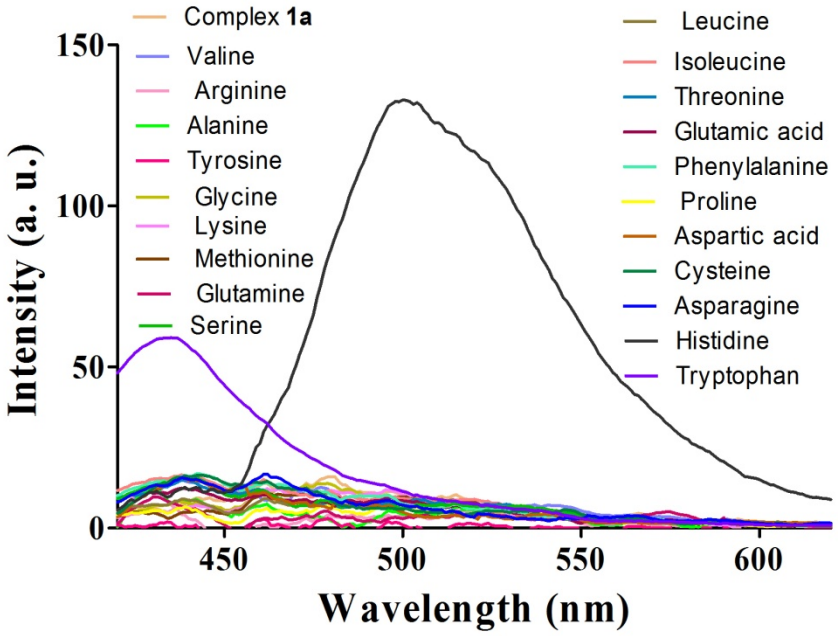

Fig. S2 Emission spectra of complex **1a** (20 μM) in HEPES buffer with various natural amino acids (100 μM) at 20 ºC.

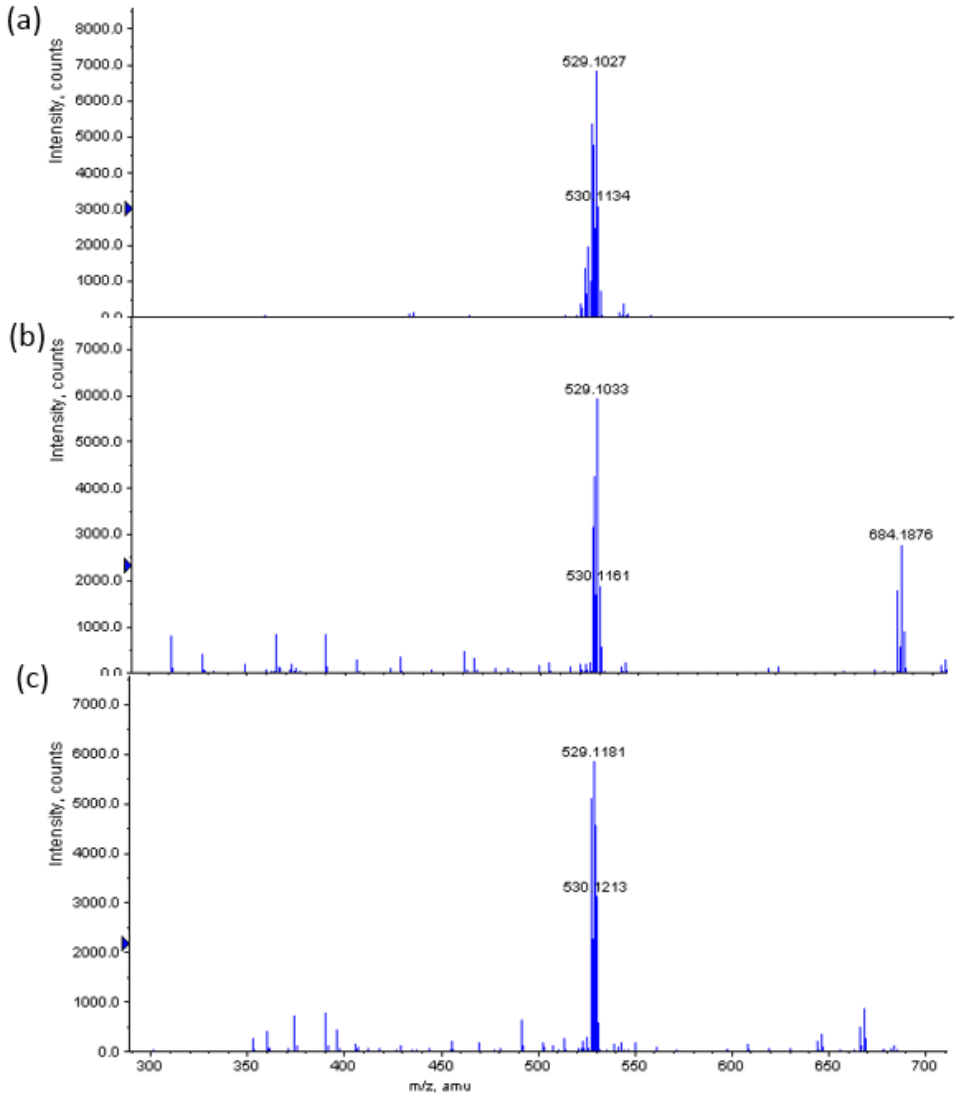

Fig. S3 Electrospray ionization time-of-flight mass spectra of (a) complex **1a**, (b) reaction mixture of complex **1a** and histidine, and (c) reaction mixture of complex **1a** and valine.

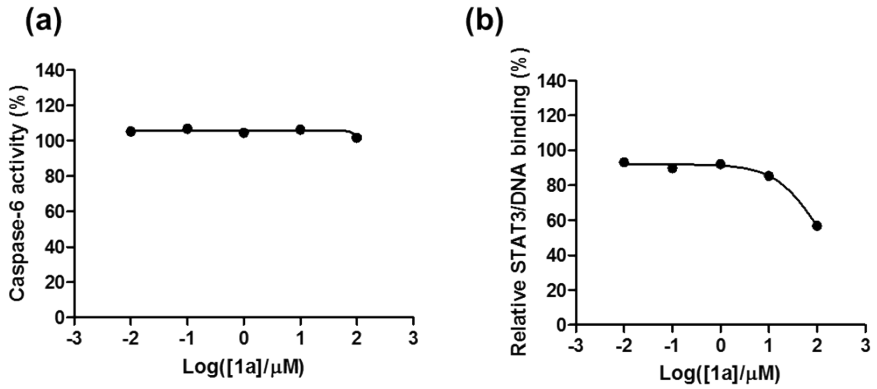

Fig. S4 (a) The level of inhibition of caspase-6 activity was determined by fluorometric methods. **1a** showed no activity to caspase-6. (b) The inhibition of STAT3 DNA-binding activity by **1a** was assayed using ELISA method. **1a** slightly inhibited STAT3 DNA-binding activity *in vitro*.

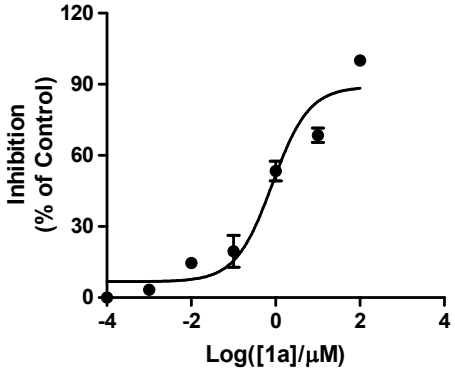

Fig. S5 AlphaScreen assay monitoring the displacement of a tetra-acetylated histone H4 peptide from BRD4(1) by **1a**. Binding of a tetraacetylated histone H4 peptide to BRD4(1) was strongly inhibited by **1a**, with half-maximum inhibitory concentration (IC_{50}) value of 0.83 μM.

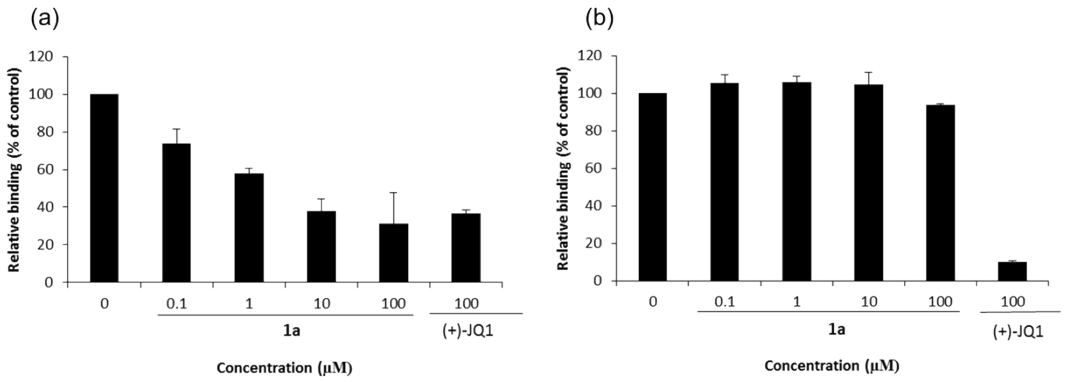

Fig. S6 Displacement of a tetra-acetylated H4 peptide from (a) BRD4(1) and (b) BRD4(2) by complex **1a** in fluorescence polarization assay. Error bars represent the standard deviations of the results from three independent experiments.

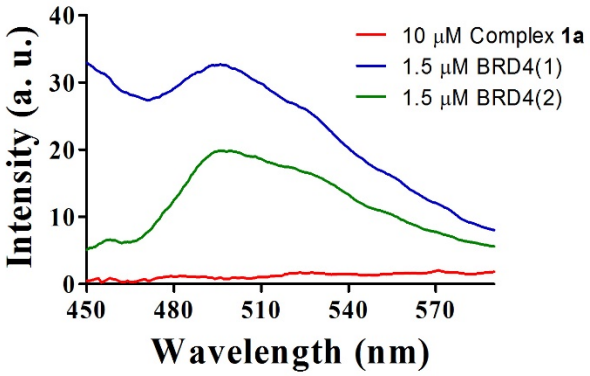

Fig. S7 Emission spectra of complex **1a** (10 μM) in HEPES buffer in the presence or absence of BRD4(1) or BRD4(2).

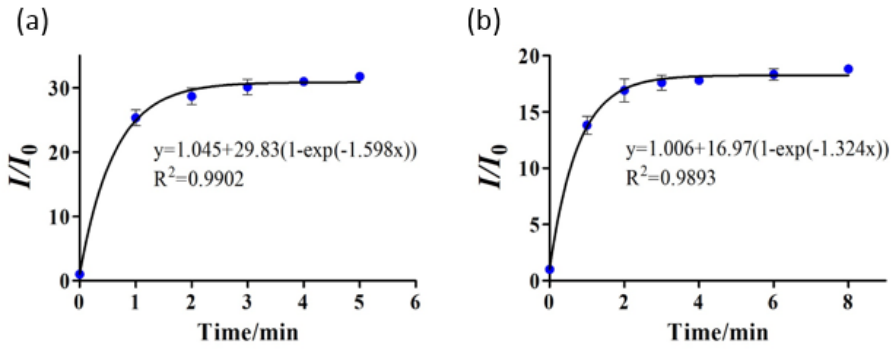

Fig. S8 The kinetic nonlinear fitting for the luminescence increasing of complex **1a** (10 μM) by (a) BRD4(1) (1.5 μM) and (b) BRD4(2) (1.5 μM), respectively.

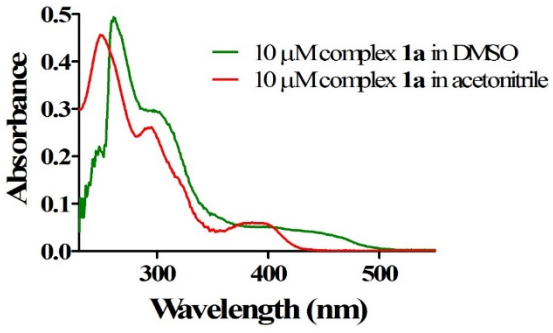

Fig. S9 The absorbance of complex **1a** in DMSO and acetonitrile, respectively.

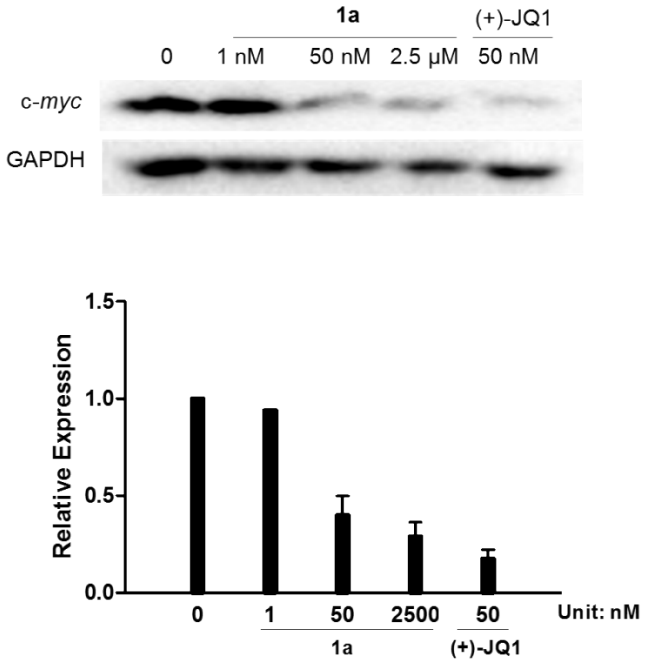

Fig. S10 Immunoblotting analysis of the effect of **1a** and (+)-JQ1 treatment on c-*myc* expression in A2058 cells. Densitometry analysis revealed that **1a** inhibited c-*myc* expression in a dose-dependent manner with an IC_{50} value of 9.0 nM. Error bars represent the standard deviations of the results from three independent experiments.

Table S1. Photophysical properties of complexes **1**–**27** and **1a**–**1j**.

Complex	Quantum yield	λ_{em}/ nm	Lifetime/ μs	UV/vis absorption λ_{abs} / nm (ε/ dm^3mol^{-1} cm^{-1})
1	Could not be obtained[a]	448	0.147	280 (2.06 × 10^4), 371 (3.12 ×10^3)
2	Could not be obtained[a]	Non-emissive	/	259 (4.64 × 10^4), 311 (1.68 ×10^4), 350 (3.6 ×10^3)
3	Could not be obtained[a]	Non-emissive	/	235 (5.0 × 10^4), 277 (5.65 ×10^4), 300 (1.86 × 10^4)
4	Could not be obtained[a]	Non-emissive	/	245 (5.2 × 10^4), 276 (3.55 ×10^4), 371 (4.16 ×10^3)
5	Could not be obtained[a]	628	/	276 (1.03 × 10^5), 384 (1.28 × 10^4)
6	0.22	568	1.27	275 (2.13 × 10^4), 383 (3.2 × 10^3)
7	0.48269	617	3.4	212 (8.07 × 10^4), 266 (8.61 × 10^4)
8	0.201	543	4.42	241 (5.34 × 10^4), 274 (4.73 ×10^4), 367 (2.92 ×10^3)
9	0.062	580	4.676	258 (5.57 × 10^4), 288 (6.0 ×10^4), 425 (2.8 ×10^3)
10	0.095	586	4.506	259 (4.55 × 10^4), 302 (2.8 ×10^4), 423 (2.0 ×10^3)
11	0.05722	577	0.74	261 (3.3 × 10^4), 268 (3.2 × 10^3), 296 (1.9 × 10^4), 371 (9.05 × 10^3)
12	0.067	571	4.437	233 (6.5 × 10^4), 267 (sh) 280 (8.05 × 10^4), 335 (2.1 × 10^4)
13	0.037	648	4.542	216 (1.55 × 10^5), 264 (9.8 × 10^4), 371 (3.36 × 10^4)
14	0.384	560	4.829	258 (4.70 × 10^4), 332 (2.08 × 10^4), 438 (4.30 × 10^3)
15	0.35	651	3.61	260 (4.57 × 10^4), 324 (3.74 × 10^4), 429 (5.24 × 10^3)
16	Could not be obtained[a]	Non-emissive	/	289 (9.36 × 10^4), 371 (1.94 × 10^4), 389 (2.77 × 10^4)
17	Could not be obtained[a]	Non-emissive	/	289 (9.31 × 10^4), 372 (2.04 × 10^4), 390 (2.21 × 10^4)
18	0.081	510	4.475	246 (6.4 × 10^4), 277 (5.56 × 10^4), 366 (3.3 × 10^3)
19	0.27	583	4.31	280 (3.6 × 10^4), 429(5.9 × 10^3)
20	Could not be obtained[a]	456	7900	266 (2 × 10^4)

21	Could not be obtained[a]	Non-emissive	/	295 (1.07×10^4), 374 (2.47×10^3)
22	Could not be obtained[a]	448	0.147	280 (2.06×10^4), 371 (3.12×10^3)
23	Could not be obtained[a]	Non-emissive	/	291 (5.2×10^4), 373 (2.8×10^3)
24	Could not be obtained[a]	Non-emissive	/	220 (8.57×10^4), 276 (5×10^4), 396 (2.7×10^3)
25	Could not be obtained[a]	602	/	220 (1.09×10^5), 246 (6.4×10^4), 286 (5.6×10^4), 393 (3.7×10^3)
26	Could not be obtained[a]	Non-emissive	/	247 (4.7×10^4), 265 (4.7×10^4), 297 (3.0×10^4), 370 (6.9×10^3)
27	Could not be obtained[a]	544	80	261 (3.3×10^4), 268 (3.2×10^4), 296 (1.9 × 104), 371 (9.05×10^4)
1a	Could not be obtained[a]	Non-emissive	/	257 (1.7×10^4), 306 (5.6×10^3), 400 (2.8×10^3)
1b	Could not be obtained[a]	Non-emissive	/	244 (3.7×10^4), 312 (8.0×10^3), 355 (2.2×10^3)
1c	Could not be obtained[a]	Non-emissive	/	264 (7.9×10^3)
1d	Could not be obtained[a]	601	/	261 (1.76×10^4), 281 (1.5×10^4), 340 (3.6×10^3), 435 (0.9×10^3)
1e	Could not be obtained[a]	Non-emissive	/	250 (3.16×10^4), 382 (1.28×10^3)
1f	0.044	595	4.327	235 (3.76×10^4),288 (3.0×10^4), 355 (1.16×10^4), 429 (3.8×10^3)
1g	Could not be obtained[a]	527	/	246 (2.72×10^4),318 (2×10^3)
1h	Could not be obtained[a]	551	/	217 (2.2×10^4), 242 (1.7×10^4), 295 (5.8×10^3)
1i	Could not be obtained[a]	Non-emissive	/	231 (9.3×10^3), 255 (1.2×10^4), 300 (4.8×10^3), 346 (1.2×10^3)
1j	Could not be obtained[a]	Non-emissive	/	218 (2.4×10^4), 243 (1.8×10^4), 296 (6.0×10^3)

[a]The emission of this complex was too low for its quantum yield to be determined.

Table S2. Microarray analysis of mouse xenografts. Tumours were removed 16 days after

administration of A375 cells to nude mice. Total RNA from tumours from the treatment and control groups were extracted and gene expression profiles were examined. The criterion for affected genes was a significant difference in tumour RNA levels between the treatment and vehicle tumors ($p < 0.05$), with at least a 2-fold difference between the groups. Gene ontology analysis was performed using PANTHER. Each group contained three mice. The table shows signaling pathways that contain multiple >2-fold up-regulated genes and <-2-fold down-regulated genes in the treatment group compared to the control group.

Signaling Pathway	**> 2-Fold Up-Regulated Genes**
ECM pathway	LAMA1, CD36, TNXB, COMP, ITGB6, ITGA10, ITGA2, COL5A3, THBS2
Signaling Pathway	**<-2-Fold Down-Regulated Genes**
VEGF signaling pathway	PLA2G4A, PLA2G10, VEGFA, PLA2G2A, PIK3R5, NFATC4, BAD, PLA2G4E, KDR