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Cytotoxic Activity and Protein Binding through an unusual oxidative mechanism by an Iridium(I)–NHC Complex

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Content

General: Reactions were carried out under N_2 by using standard Schlenk techniques. All solvents were of analytical grade. Chemicals were obtained from commercial sources and used without further purification.

^1H and ^{13}C NMR spectra were recorded at room temperature on a Bruker DRX 400 in deuterated solvents which were also used as internal reference. The chemical shifts are reported in ppm (parts per million) relative to TMS. Coupling constants, J, are reported in Hz, multiplicities being marked as: singlet (s), doublet (d), triplet (t) or multiplet (m). ESI mass spectra were measured on a Bruker Esquire 6000 mass spectrometer. HR-ESI mass spectra were recorded with an LTQ Orbitrap high-resolution mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with a conventional ESI source. FAB mass spectrometry was performed with a Fisons VG Instruments Autospec spectrometer. The mass to charge relation (m/z) is given as a dimensionless number. Elemental analyses carried out on a Vario EL (Elementar Analysensysteme GmbH, Hanau, D) in C, H, N mode.

Synthesis [1]*:* A Schlenk flask was charged with the imidazolium salt (130.0 mg, 466 μmol), and 0.5 eq. of silver oxide (54.0 mg, 233 μmol) in dry dichloromethane and stirred at r.t for 1 h. 1 0.5 eq. of $[Ir(cod)Cl]_2$ (156.6 mg, 233 μmol) was added and the resulting mixture was stirred for 24 h. The resulting suspension was filtered through celite, and the filtrate was concentrated to dryness. The crude product was purified by column chromatography on silica using mixtures of dichloromethane and methanol and gave **1** (226.0 mg, 83.9%) as a yellow solid. ^1H NMR (400 MHz, CD_2Cl_2) δ = 6.72 (d, *J* = 2.0 Hz, 1H, N-C**H**=CH-N), 6.24 (d, *J* = 2.0 Hz, 1H, N-CH=C**H**-N), 5.79 (d, *J* = 14.5 Hz, 1H, N-C**H**$_2$), 5.32 (d, *J* = 14.5 Hz, 1H, N-C**H**$_2$), 4.55 – 4.49 (m, 2H, COD), 3.96 (s, 3H, H_{NMe}), 3.17 (td, *J* = 7.1, 2.8 Hz, 1H, COD), 3.09 (td, *J* = 6.8, 2.8 Hz, 1H, COD), 2.34 – 2.21 (m, 4H, COD), 2.27 (s, 3H, Me), 2.24 (s, 6H, Me), 2.21 (s, 6H, Me), 1.79 – 1.71 (m, 2H, COD), 1.70 – 1.60 (m, 2H, COD). ^{13}C NMR (101 MHz, CD_2Cl_2) δ 180.91 (N**C**N), 136.35 (C_{Ar-1}), 134.54 (C_{Ar-2}), 133.65 (C_{Ar-3}), 128.76 (C_{Ar-4}), 121.17 (N-**C**H=CH-N), 119.45 (N-CH=**C**H-N), 84.47 (COD), 84.46 (COD), 51.94 (COD), 51.41 (COD), 50.13 (N-**C**H_2), 38.13 (C_{NMe}), 34.22 (COD), 34.15 (COD), 30.16 (COD), 30.15 (COD), 17.41 (Me), 17.13 (Me). MS (ESI^+): *m/z* = 542.97 $[M\text{-}Cl]^+$. MS (FAB^+): *m/z* = 578.0 $[M]^+$, 543.1 $[M\text{-}Cl]^+$, 470.0 $[M\text{-}COD]^+$. Anal. calc. for $C_{24}H_{34}ClIrN_2$: C, 49.85; H, 5.93; N, 4.84. Found: C, 49.47; H, 6.19; N, 4.68.

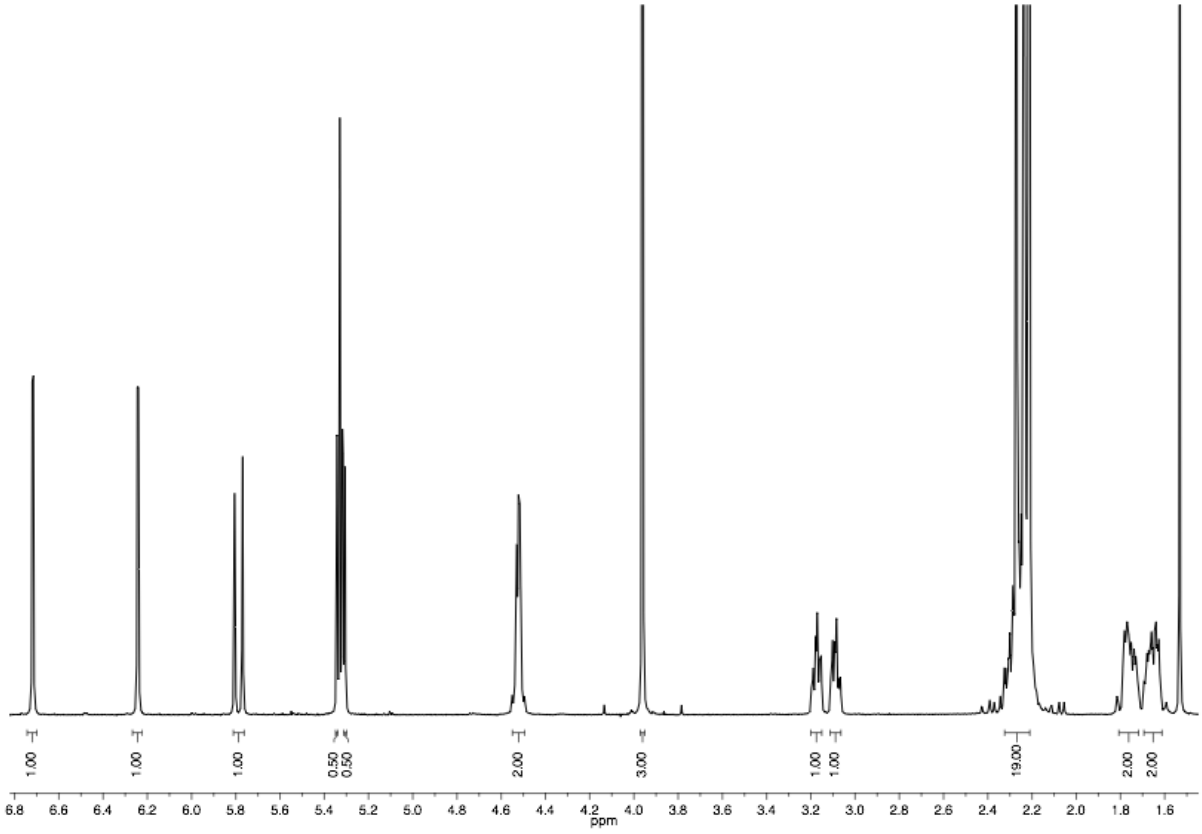

Figure S1. ^{1}H NMR spectrum of **1** recorded in CD_2Cl_2 at 400 MHz.

Crystallographic data for 1	
formula	$C_{25}H_{36}Cl_3IrN_2$
molecular mass ($g \cdot mol^{-1)}$	66.31
temperature [K]	173 (2)
wavelength [Å]	0.71073
crystal system	triclinic
space group	P-1
a / Å	9.516(6)
a / Å	9.914(6)
a / Å	14.152(8)
α / deg	95.215(7)
β / deg	95.676(10)
γ / deg	105.908(5)
V / $Å^3$	1267.9(13)
Z	2
calculated density ($g \cdot cm^{-3}$)	1.737
F(000)	656
θ_{max}/deg	24.99
reflections collected/unique	10805/4452
data/parameters	4452/275
S (F^2)	1.082
R_1/wR_2 ($I > 2\sigma$)	0.0442/0.1082
R_1/wR_2 (all data)	0.0455/0.1093

UV/Vis spectrophotometric studies

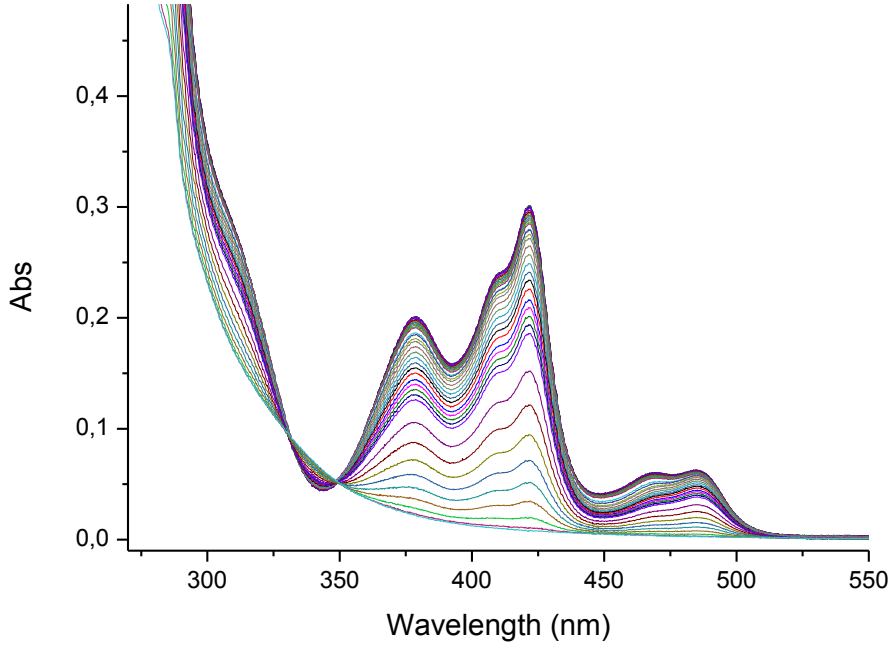

Figure S2. UV/Vis spectrum of **1** (200 μM) in DMSO. Spectrum was recorded in 10 min intervals during the first 60 min and hourly for additional 47h.

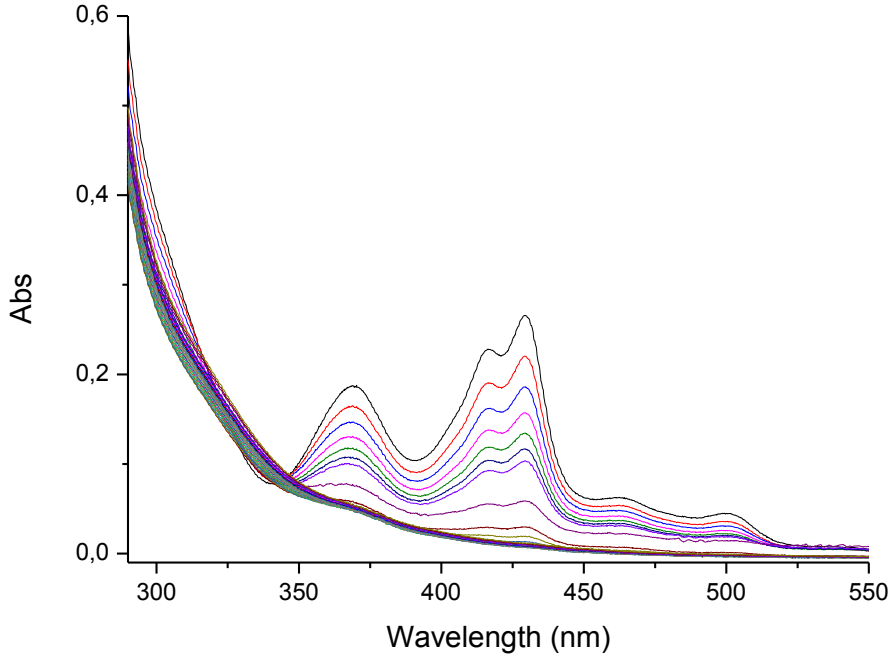

Figure S3. UV/Vis spectrum of **1** (200 μM) in ammonium acetate buffer (50% DMSO, pH 6.8). Spectrum was recorded in 10 min intervals during the first 60 min and hourly for additional 47h.

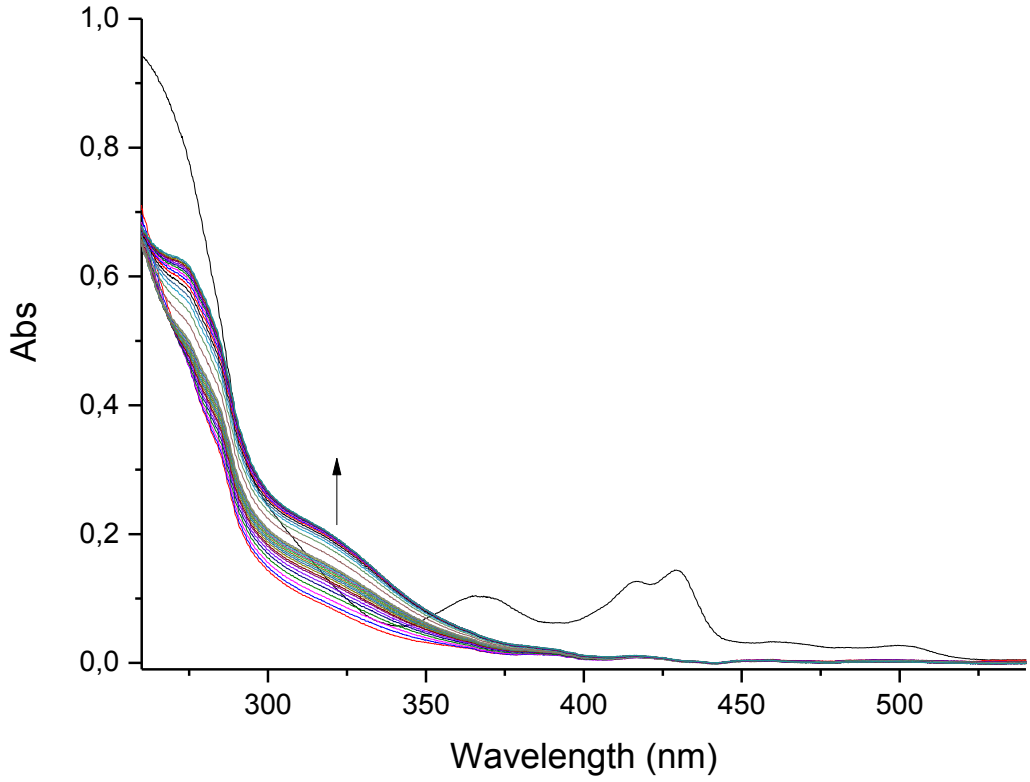

Figure S4. UV/Vis spectrum of **1** (100 µM) in ammonium acetate buffer (50% DMSO, pH 6.8). After the first run, 10 eq. of H_2O_2 were added. The spectrum was recorded in 10 min intervals during the first 60 min and hourly for additional 47h.

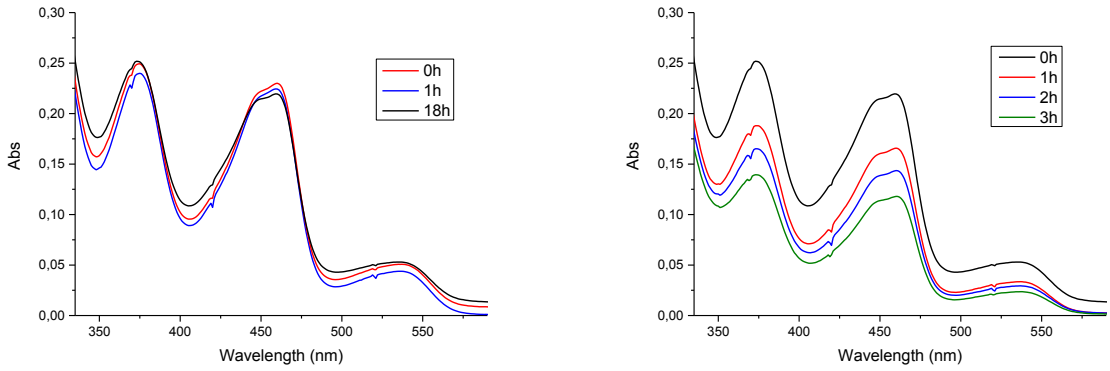

Figure S5. Left: UV/Vis spectrum of **1** (200 µM) in H_2O/DMSO (50:50) in the absence of oxygen. Spectrum was recorded after 1h and 18h. Right: UV/Vis spectrum of **1** (200 µM) in H_2O/DMSO (50:50) in the presence of oxygen. Spectrum was recorded hourly for 3h.

Interaction with Cytochrome c (Lysozyme). Solutions of **1** (100 μM) with Cytochrome c (Lysozyme) (10:1 complex/protein molar ratio) in 50% ammonium acetate buffer (20 μM, pH=6.8) and 50% DMSO were incubated at 37° C. After 24 h (48 h, 72 h) and 20-fold dilution with water, ESI-MS spectra were recorded by direct introduction at 5 μL min^{-1} flow rate in an Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. The working conditions were the following: spray voltage 3.1 kV, capillary voltage 45 V, capillary temperature 220°C, tube lens voltage 230 V. The sheath and the auxiliary gases were set, respectively, at 17 (arbitrary units) and 1 (arbitrary units). For acquisition, Xcalibur 2.0. software (Thermo) was used and monoisotopic and average deconvoluted masses were obtained by using the integrated Xtract tool. For spectrum acquisition a nominal resolution (at m/z 400) of 100,000 was used.

Cell Culture and Cytotoxicity

Dulbecco's Modified Eagle's Medium (DMEM), containing 10% fetal calf serum, 1% penicillin and streptomycin, was used as growth medium. MCF-7, HT-29 and HEK-293T cells were detached from the wells with trypsin and EDTA, harvested by centrifugation and resuspended again in cell culture medium. The assays have been carried out on 96 well plates with 6000 (10000) cells per well for MCF-7 and HT-29 (HEK-293T). After 24 h of incubation at 37°C and 10% CO_2, the cells were treated with the compounds (with DMSO concentrations of 0.5%) with a final volume of 200 µl per well. For a negative control, one series of cells was left untreated. The cells were incubated for 48 h followed by adding 50 µl MTT (2.5 mg/ml). After an incubation time of 2 h, the medium was removed and 200 µl DMSO were added. The formazan crystals were dissolved and the absorption was measured at 550 nm, using a reference wavelength of 620 nm. Each test was repeated in quadruplicates in three independent experiments for each cell line.

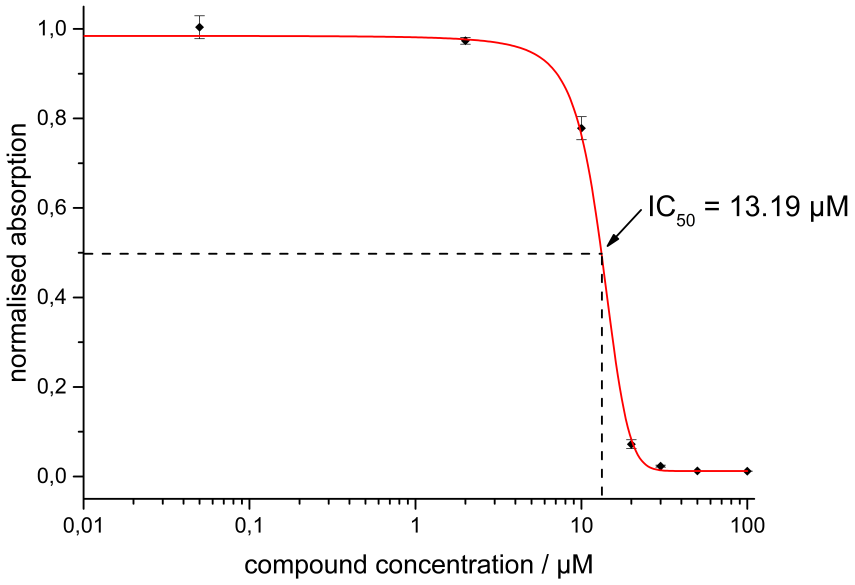

Figure S6. Example of a fitted dose-response curve for the determination of the IC_{50} value of **1** on MCF-7 cells.

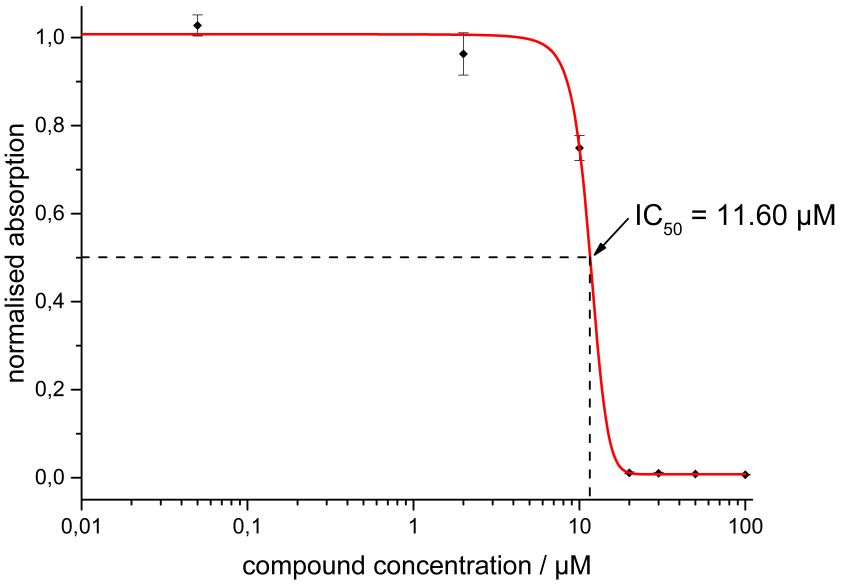

Figure S7. Example of a fitted dose-response curve for the determination of the IC_{50} value of **1** on HEK-293T cells.

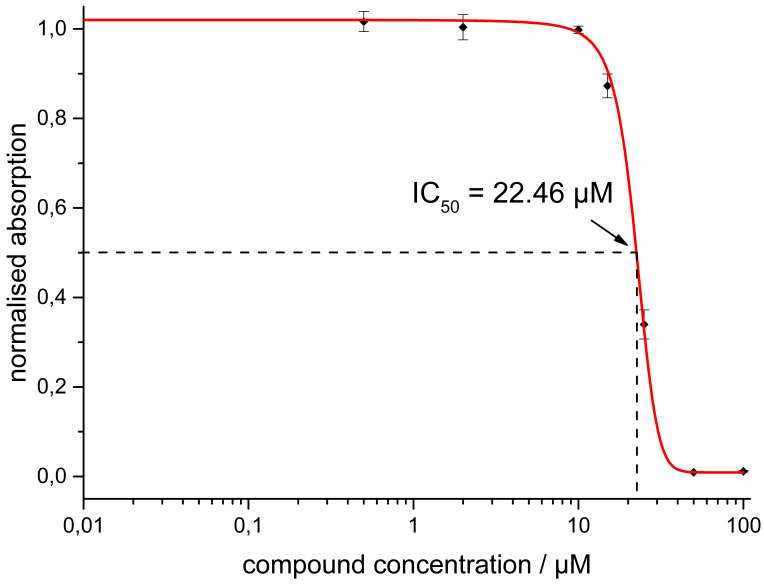

Figure S8. Example of a fitted dose-response curve for the determination of the IC_{50} value of **1** on HT-29 cells.

Time dependent cell growth studies. Cell growth was monitored continuously using the xCELLigence RTCA (Real Time Cell Analyser) system (Roche). The background of the E-plates was determined in 50 µL of medium. Subsequently, 50 µl of a suspension of the HEK-293T cells (10000 cells per well) were added and the cells were incubated for 24 h at 37°C and 10% CO_2. The cells were then treated with the compound (10 µM, 20 µM and 30 µM with DMSO concentrations of 0.5%) with a final volume of 200 µl per well. For a negative control, one series of cells was treated with media alone (0.5% DMSO). Each treatment was performed in triplicates. The impedance was monitored every 15 minutes for additional 72 h. The electronic readout, cell-sensor impedance, is displayed as "cell index", as a dimensionless unit, as suggested by the instrument maker, see also (http://icob.sinica.edu.tw/pubweb/data/xCelligence_%B2%D3%A5%CDpdf)