A Phosphorescent Rhenium(I) Tricarbonyl Polypyridine Com-plex Appended with a Fructose Pendant that Exhibits Photo-cytotoxicity and Enhanced Uptake by Breast Cancer Cells

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Supporting Information

Materials and Synthesis. All solvents were of analytical reagent grade and purified according to standard procedures.[1] Silver(I) trifluoromethanesulfonate, cisplatin, *n*-decylamine, dibenzylamine, *N*,*N*'-dicyclohexylcarbodiimide (DCC), D-glucose, ethylamine, glacial acetic acid, *N*-hydroxysuccinimide (NHS), nicotinic acid, Ph_2-phen, rhenium standard in H_2O (1 mg per mL), and triethylamine were purchased from Acros. Cytochalasin B, fasentin, fructose, and MTT were purchased from Sigma. Acetophenone, anthracene, *tert*-butylbenzene, 4-methoxyaniline, 4-methoxyphenol, 3-morpholinopropanesulfonic acid (MOPS), naphthalene, *n*-octanol, phenol, pyrene, palladium (5 wt. %) on activated carbon, DHN, and $Re(CO)_5Cl$ were purchased from Aldrich. All these chemicals were used without further purification. 1-Amino-1-deoxy-D-fructose acetic acid salt (fru-NH_2-AcOH),[2] nicotinic acid *N*-hydroxysuccinimidyl ester,[3] $[Re(Ph_2\text{-phen})(CO)_3(CH_3CN)](CF_3SO_3)$,[4] and $[Re(Ph_2\text{-phen})(CO)_3(py\text{-}3\text{-}Et)](CF_3SO_3)$,[3] were prepared as described previously. Autoclaved Milli-Q water was used for preparation of the aqueous solutions. MCF-7, MDA-MD-231, A549, HEK293T, HepG2, and NIH/3T3 cells were obtained from American Type Culture Collection. High glucose Dulbecco's modified Eagle's medium (DMEM), phosphate-buffered saline (PBS), fetal bovine serum (FBS), trypsin-EDTA, and penicillin/streptomycin were purchased from Invitrogen.

Py-fructose. 1-Amino-1-deoxy-D-fructose acetic acid salt (fru-NH_2-AcOH) (150 mg, 0.53 mmol) was dissolved in warm Milli-Q water (1 mL) and the solution was added dropwise to a mixture of nicotinic acid *N*-hydroxysuccinimidyl ester (141 mg, 0.64 mmol) and triethylamine (1 mL, 7.17 mmol) in DMF (40 mL) at room temperature under an inert atmosphere of nitrogen. The mixture was stirred at room temperature in the dark for 24 h, after which the solution was evaporated to dryness

under reduced pressure to give pale yellow oil. The crude product was purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (4:1, v/v) and isolated as colorless oil. Yield: 80 mg (53%). ^{1}H NMR (300 MHz, CD_3OD, 298 K, relative to Me_4Si): δ = 8.85 (s, 1H, H2 pyridine), 8.52 (d, 1H, J = 3.3 Hz, H6 pyridine), 8.52 (d, 1H, J = 7.8 Hz, H4 pyridine), 7.39 (t, 1H, J = 4.8 Hz, H5 pyridine), 4.06 – 3.37 ppm (m, 7H, H fructose ring); (KBr): ν = 3373 (O–H, N–H), 2930 (C–H), 1649 (C=O); positive-ion ESI-MS ion cluster at m/z 285 $[M + H^+]^+$.

Physical Measurements and Instrumentation. The instrumentation for physical measurements has been described previously.[3] Electronic absorption and steady-state emission spectra were recorded on a Hewlett-Packard 8453 diode array spectrophotometer and a SPEX FluoroLog 3-TCSPC spectrophotometer, respectively. Emission lifetimes were measured in the Fast MCS mode with a NanoLED N-375 as the excitation source. Unless specified, all the solutions for photophysical studies were degassed with no fewer than four successive freeze–pump–thaw cycles and stored in a 10 cm^3 round-bottomed flask equipped with a side arm 1 cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper. Luminescence quantum yields were measured by the optically dilute method[5] with a degassed acetonitrile solution of $[Re(phen)(CO)_3(py)](CF_3SO_3)$ (λ_{ex} = 355 nm, $A_{355\ nm}$ = 0.1)[6] as the standard. The lipophilicity of the complexes was determined from the log k'_w values (k'_w = chromatographic capacity factor at 100% aqueous solution). The log k'_w values were determined by reversed-phase HPLC on a C-18 column according to the method described by Minick.[7]

Cell Cultures. MCF-7, MDA-MD-231, A549, HEK293T, HepG2, and NIH/3T3

cells were cultured in growth medium (high glucose DMEM supplemented with 10% FBS and 1% penicillin-streptomycin). The cells were cultured in a humidified chamber with 5% CO_2 at 37°C and were subcultured every 3 to 4 days.

ICP-MS. Cells were grown to 80% confluency in a 60-mm tissue culture dish. Unless specified, the cells were incubated with the rhenium(I) polypyridine complex (100 μM) in glucose-free medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 1 h. The medium was then removed and washed thoroughly with PBS (1 mL × 5). The cells were trypsinized and harvested. The culture dish was further washed with PBS (1 mL × 3) and the PBS was collected. The concentration of the cells was then determined using a BioRad TC10 automated cell counter. The harvested cells, together with the collected PBS, were digested with 65% HNO_3 at 60°C. The digested solution was filtered and the concentration of rhenium in the filtrate was measured using an Elan 6100 DRC-ICP-MS system (PerkinElmer SCIEX Instruments, USA) equipped with a peristaltic pump, Meinhard quartz nebulizer, cyclonic spray chamber, nickel skimmer and sample cones.

MTT Assays. Cells were seeded in a 96-well flat-bottomed microplate (*ca.* 10,000 cells per well) in growth medium (100 μL) and incubated at 37°C under a 5% CO_2 atmosphere for 24 h. The rhenium(I) polypyridine complex was dissolved in the growth medium with 1% DMSO and the solutions were added to the wells. After the microtiter plate was incubated for 48 h, MTT in PBS (5 mg per mL, 10 μL) was added to each well. The microplate was incubated for another 3 h. The medium was removed carefully and DMSO (200 μL) was added to each well. The microplate was further incubated for 5 min. All the assays were run in parallel with a positive control, in which cisplatin was used as a cytotoxic agent. The absorbance

of the solutions at 570 nm was measured with a SPECTRAmax 340 microplate reader (Molecular Devices Corp., Sunnyvale, CA). The IC_{50} values of the complexes were determined from dose dependence of surviving cells after exposure to the complexes for 48 h and were calculated using MicroCal ORIGIN 6.0 (MicroCal Software).

Live-Cell Confocal Imaging. Cells in growth medium were seeded on a sterilized coverslip in a 60-mm tissue culture dish and grown at 37°C under a 5% CO_2 atmosphere for 48 h. The growth medium was then removed and replaced with sugar-free medium/DMSO (99:1, v/v) containing the rhenium(I) polypyridine complex (50 μM). After incubation for 1 h, the medium was removed and the cell layer was washed with PBS (1 mL × 5). The coverslip was mounted onto a sterilized glass slide and then imaged using a Leica TCS SPE confocal microscope with an oil immersion 40× or 63× objective and an excitation wavelength at 405 nm. The emission was measured using a long-pass filter at 532 nm. In the colocalization experiments, MCF-7 cells were treated with the complexes (50 μM) for 1 h, washed with PBS (1 mL × 5), and then incubated with MitoTracker Deep Red FM (100 nM) in FBS-free medium for 20 min, and finally washed with PBS (1 mL × 5). The excitation wavelength for MitoTracker Deep Red FM was 633 nm. The Pearson's colocalization coefficient was determined by the program Image J (Version 1.4.3.67).

Photocytotoxicity Experiments. MCF-7 cells were incubated with the rhenium(I) complexes for 2 h, and then the medium was removed. The cells were irradiated in PBS with the long wave (> 365 nm) of a UV lamp (Spectronics ENF-260C/FE) for 30 min, and then incubated in medium in the dark for further 45.5 h before being analyzed by the MTT assay.

Singlet Oxygen Sensitizing Measurements. The rhenium(I) complex (7.5 μM) and DHN (150 μM) in air-saturated acetonitrile/2-propanol (4:1, v/v) were irradiated at 350 nm using a SPEX FluoroLog 3-TCSPC spectrophotometer equipped with a xenon lamp for 4 h. The electronic absorption spectra were recorded before and after the excitation on an Agilent 8453 diode array spectrophotometer. The production yield of photooxidation of DHN to Juglone was calculated using the extinction coefficient of Juglone at 427 nm (ε = 3811 M^{-1} cm^{-1}).

Scheme S1. Synthetic route for py-fructose

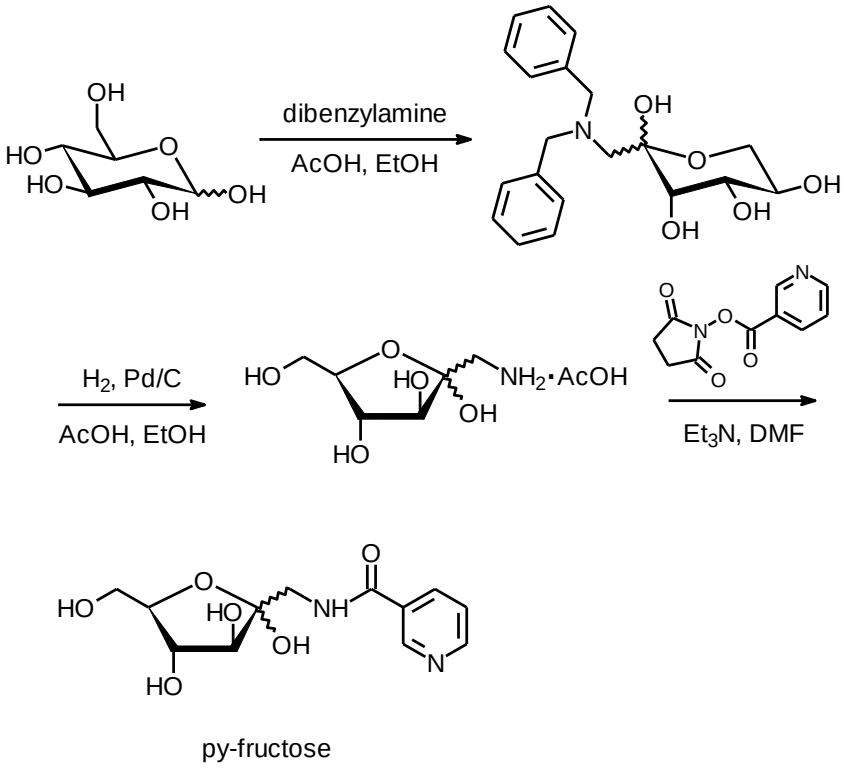


Table S1. Electronic absorption spectral data of complex 1 at 298 K[a]

Solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
CH_2Cl_2	264 sh (18 950), 293 (28 600), 338 sh (11 305), 387 sh (5825)
CH_3CN	257 sh (19 055), 292 (30 870), 338 sh (11 950), 389 sh (5110)

[a] Assignments: intense bands at 257 – 338 nm: intraligand (^{1}IL) ($\pi \rightarrow \pi^*$)(Ph_2-phen) transitions; less intense shoulders at 387 – 389 nm: metal-to-ligand charge-transfer (^{1}MLCT) (dπ(Re) $\rightarrow \pi^*$(Ph_2-phen)).

Table S2. Photophysical data of complexes 1 and 2

Complex	Medium (T/K)	λ_{em}/nm	τ_o/μs	Φ_{em}
1	CH_2Cl_2 (298)	540	8.87	0.39
	PBS (298)[a]	553	2.91	0.18
	glass (77)[b]	505, 535 sh	15.18	
2	CH_2Cl_2 (298)	540	8.81	0.42
	PBS (298)[a]	553	2.90	0.18
	glass (77)[b]	506, 535 sh	15.90	

[a] Phosphate-buffered saline pH 7.2/DMSO(99:1, v/v). [b] EtOH/MeOH (4:1, v/v).

Figure S1. The simulated isotope pattern for complex **1** (top) and the expanded ion cluster from the parent peak (*m*/*z* = 887) in the ESI-mass spectrum (bottom).

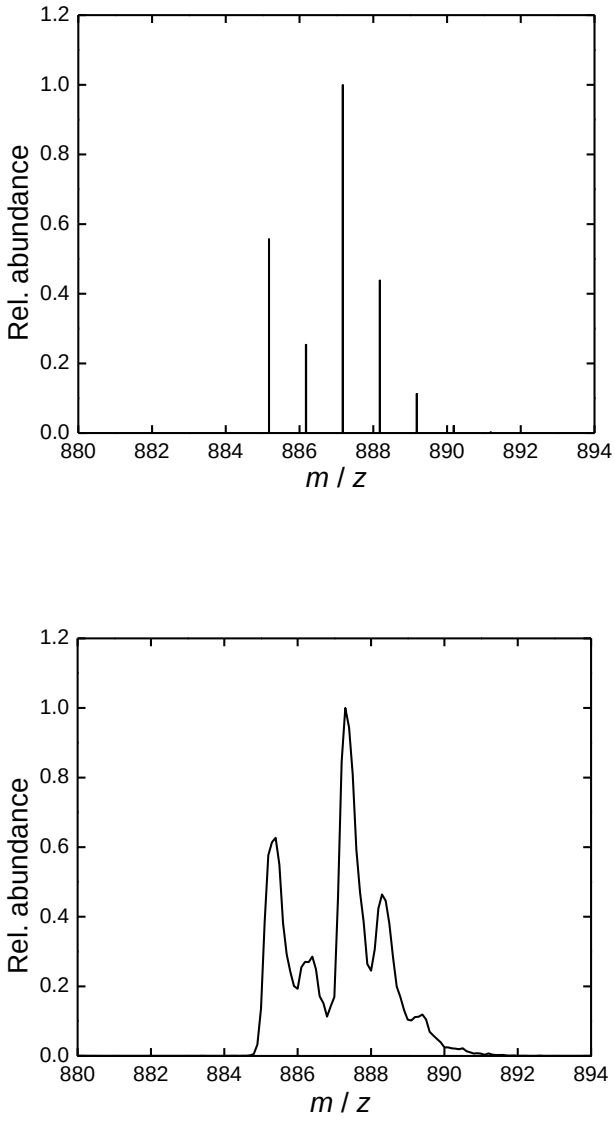


Figure S2. Electronic absorption spectra of complex **1** in CH_2Cl_2 (blue) and CH_3CN (red) at 298 K.

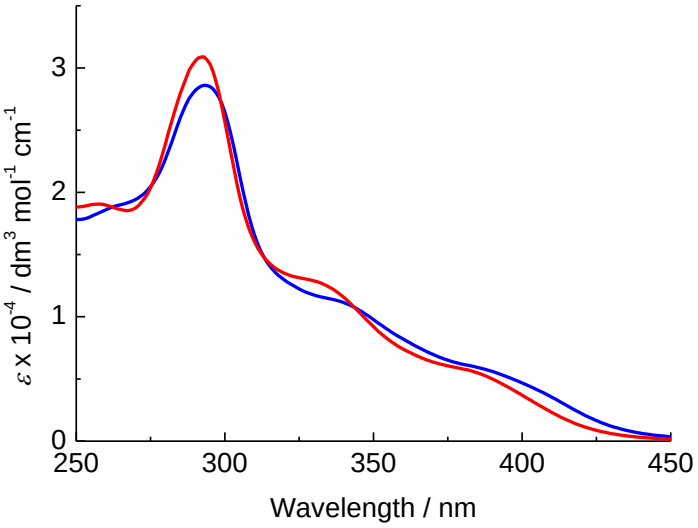


Figure S3. Emission spectra of complex **1** in CH_2Cl_2 (green) and phosphate-buffered saline (red) at 298 K and in EtOH/CH_3OH (4:1, v/v) at 77 K (blue).

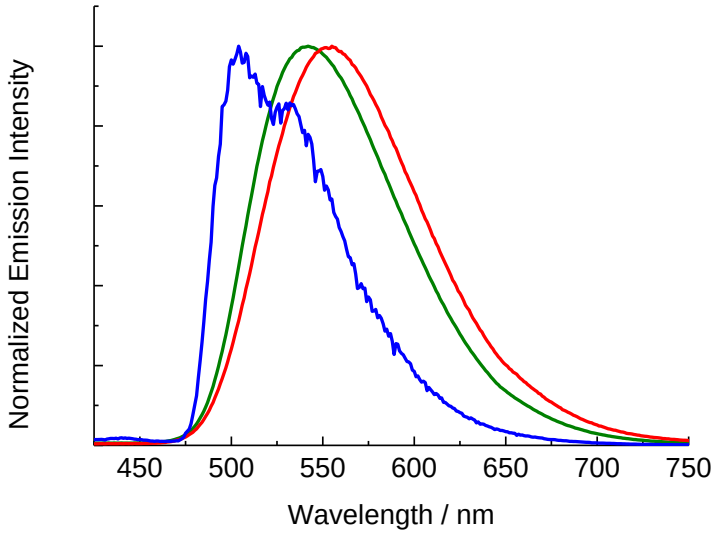


Figure S4. Electronic absorption spectra of a solution of complex **1** or **2** (10 μM) and DHN (150 μM) in air saturated acetonitrile/2-propanol (4:1, v/v) before (black) and after (red) irradiation at 350 nm for 4 h.

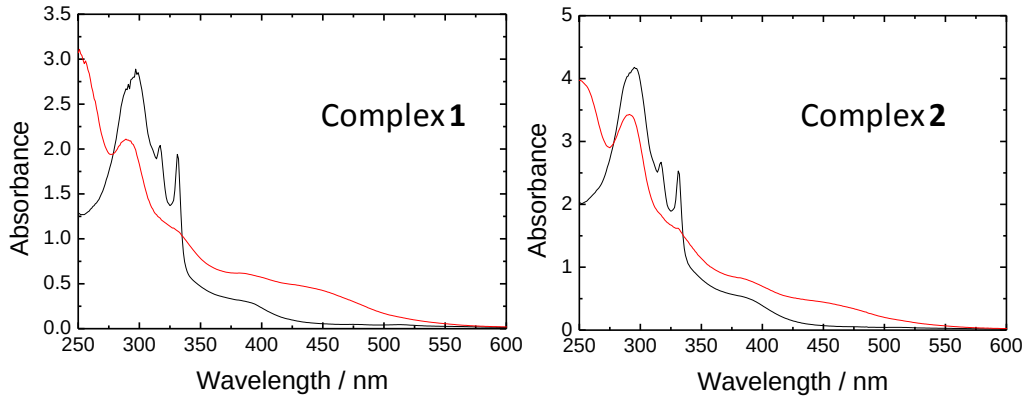


Figure S5. Dependence of percentage of living (a) MCF-7, (b) MDA-MB-231, (c) A549, (d) HepG2, (e) NIH/3T3, and (f) HEK293T cells on different concentrations of complex **1** upon incubation for 48 h.

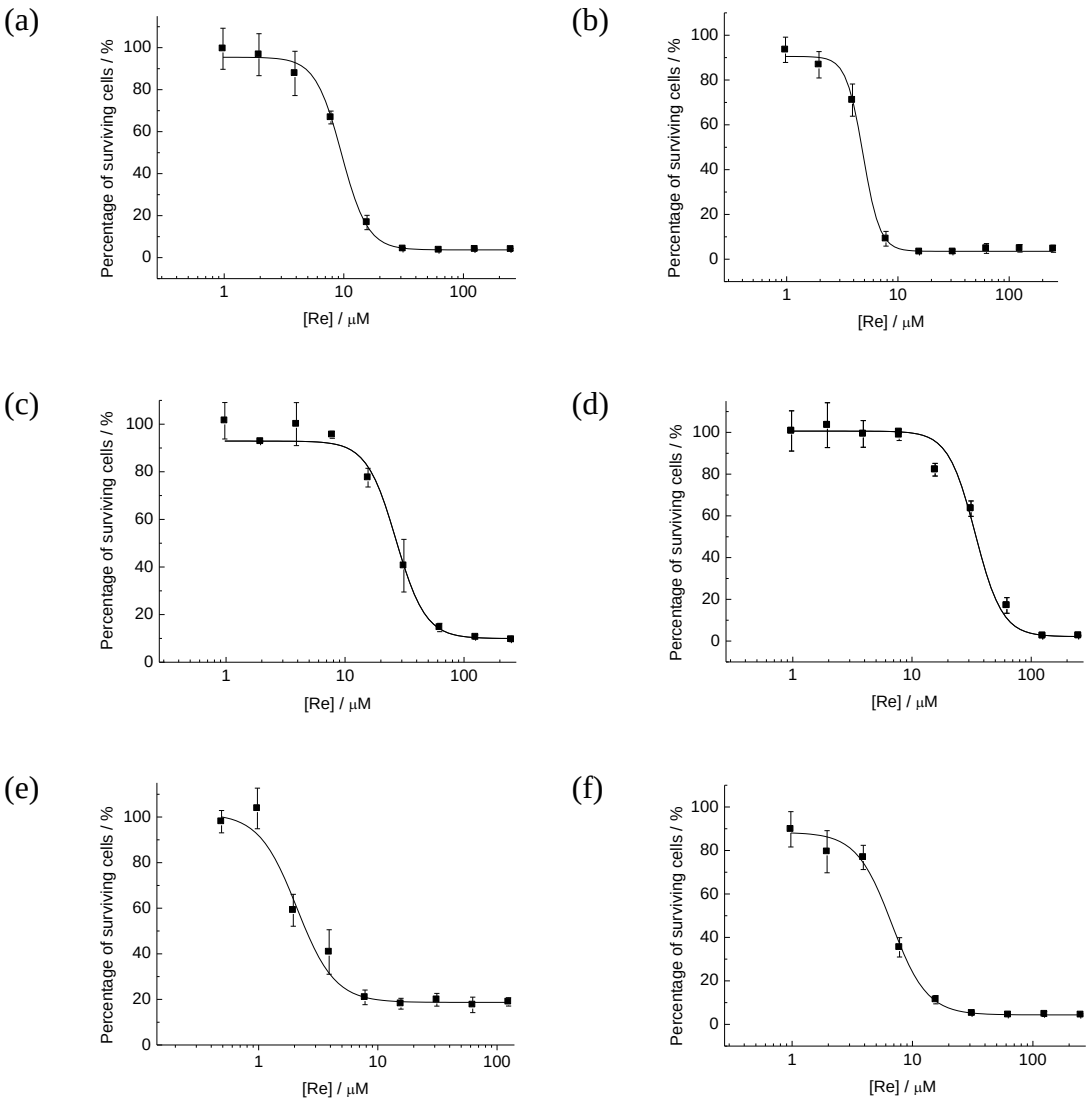


Figure S6. Dependence of percentage of living (a) MCF-7, (b) MDA-MB-231, (c) A549, (d) HepG2, (e) NIH/3T3, and (f) HEK293T cells on different concentrations of complex **2** upon incubation for 48 h.

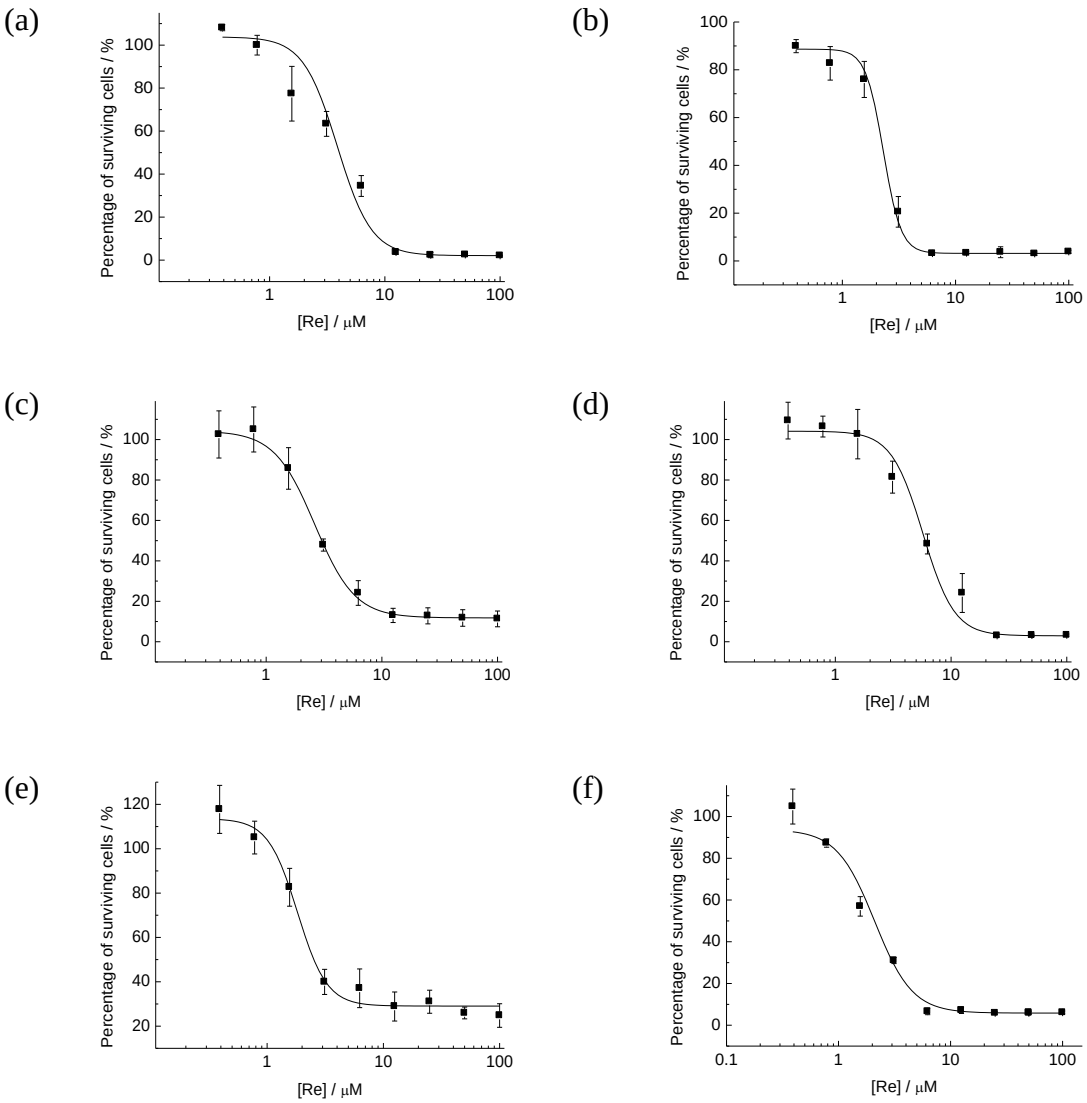


Figure S7. Laser-scanning confocal microscopy images of (a) MCF-7, (b) MDA-MB-231, (c) A549, (d) HepG2, (e) NIH/3T3, and (f) HEK293T cells upon incubation of complex **2** (50 μM, 1 h) in the absence (left) or presence (right) of 50 mM fructose.

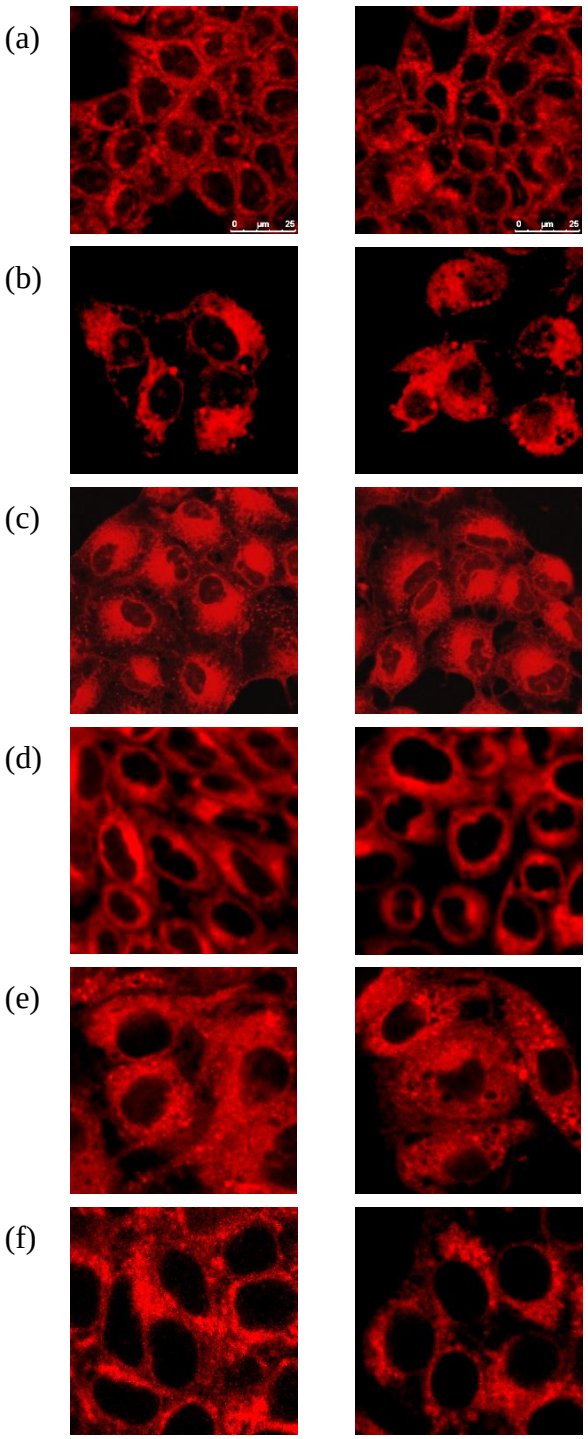
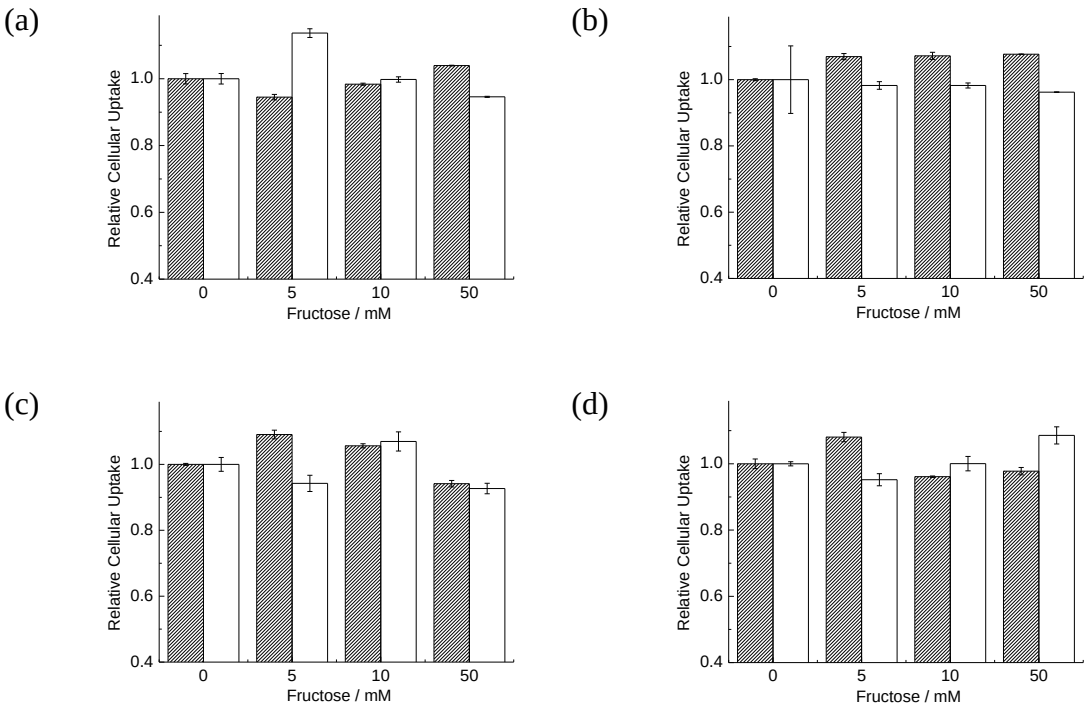


Figure S8. Relative cellular uptake of rhenium by an average (a) A549, (b) HepG2, (c) NIH/3T3, and (d) HEK293T cell upon incubation with complexes **1** (shaded) and **2** (empty) (50 μM, 1 h) at 37°C in a medium containing different concentrations of fructose. The uptake of the complexes was relative to their corresponding uptake at [fructose] = 0 mM.



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