Electronic Supplementary Information

Luminescent rhenium(I) perfluorobiphenyl complexes as site-specific labels for peptides to afford photofunctional bioconjugates†

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Experimental

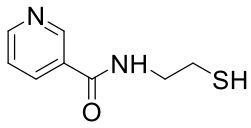
Materials and Reagents

All solvents were of analytical reagent grade and purified according to published procedures.[1] All buffer components were of biological grade and used as received. Autoclaved Milli-Q water was used for the preparation of the aqueous solutions. 1,10-Phenanthroline (phen), 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4-phen), 4,7-diphenyl-1,10-phenanthroline (Ph_2-phen), silver trifluoromethanesulfonate ($AgCF_3SO_3$), nicotinoyl chloride hydrochloride, cysteamine hydrochloride, decafluorobiphenyl, ethylamine, triethylamine (Et_3N) and trifluoroacetic acid (TFA) were purchased from Acros. Rhenium(I) pentacarbonyl bromide and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was supplied by Sigma-Aldrich. The peptides FCPF, GCPG, Ac-FCPFPKKKRKV-$CONH_2$ and H_2N-DEVDFCPF-$CONH_2$ were purchased from GL Biochem (Shanghai) and used as received. Caspase-3 inhibitor drug screening kit (active caspase-3, caspase reaction buffer and Z-VAD-FMK), caspase-7 inhibitor drug screening kit (active caspase-7, caspase reaction buffer and Z-VAD-FMK) and 5-((*S*)-(+)-2-(methoxymethyl)pyrrolidino)sulfonylisatin (MPS) were supplied by BioVision Inc. QSY-7 carboxylic acid succinimidyl ester (QSY7-NHS), Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), phosphate buffer saline (PBS), trypsin-EDTA and penicillin/streptomycin were purchased from Invitrogen. KYSE-510 cells were obtained from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). Unless otherwise specified, the growth medium for cell culture contained RPMI with 10% FBS and 1% penicillin/streptomycin. The rhenium(I) precursor complexes $[Re(N^N)(CO)_3(CH_3CN)](CF_3SO_3)$ (N^N = phen, Me_4-phen, Ph_2-phen) were prepared by reported methods.[2] The control

complexes [Re(N^N)$(CO)_3$(py-Et)](CF_3SO_3) (N^N = phen, Me_4-phen, Ph_2-phen; py-Et = *N*-ethyl-(3-pyridyl)formamide) were synthesised according to methods reported in the literature.[3]

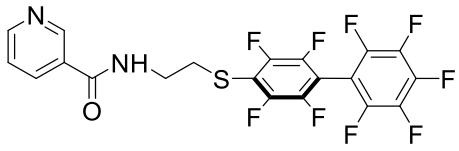
Synthesis

3-(2-Mercaptoethyl)aminocarbonylpyridine (py-SH)



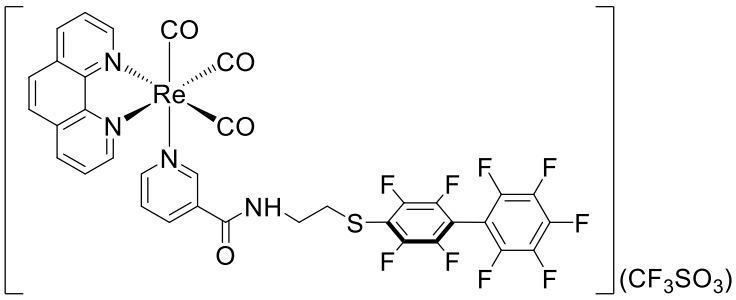
To a mixture of nicotinoyl chloride hydrochloride (500 mg, 2.81 mmol) and cysteamine hydrochloride (638 mg, 5.62 mmol) in CH_2Cl_2 (30 mL), Et_3N (1.96 mL) was added dropwise. The resulting mixture was stirred at room temperature under an inert atmosphere of nitrogen for 18 h. The white precipitate was filtered and the filtrate was evaporated to dryness under reduced pressure to give a pale yellow solid, which was purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (40:1, *v*/*v*). The solvent was removed under reduced pressure to afford the product as a white solid. Yield: 425 mg (83%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 9.07 (s, 1H, H2 of pyridine), 8.71 (d, 1H, *J* = 4.8 Hz, H6 of pyridine), 8.19 (d, 1H, *J* = 7.9 Hz, H4 of pyridine), 7.38 (dd, 1H, *J* = 7.7 and 5.0 Hz, H5 of pyridine), 7.35 (br, 1H, N*H*CH_2CH_2SH), 3.66 (q, 2H, *J* = 6.2 Hz, NHC*H*$_2CH_2SH$), 2.81 (q, 2H, *J* = 7.1 Hz, $NHCH_2CH_2SH$), 1.49 (*t*, 1H, *J* = 8.4 Hz, $NHCH_2CH_2SH$). MS (ESI, positive-ion mode): *m*/*z* 183 $[M + H^+]^+$.

3-((2-((Perfluoro-(1,1'-biphenyl)-4-yl)thio)ethyl))aminocarbonylpyridine (py-PFBP)



A mixture of py-SH (100 mg, 0.55 mmol), decafluorobiphenyl (917 mg, 2.74 mmol) and Et_3N (383 μL) in CH_2Cl_2 (10 mL) was stirred at room temperature under an inert atmosphere of nitrogen for 18 h. The solvent was removed by rotary evaporation and the resulting white solid was purified by column chromatography on silica gel. The desired product was eluted with CH_2Cl_2/MeOH (10:1, *v*/*v*). The solvent was evaporated under reduced pressure to afford the product as a white solid. Yield: 172 mg (63%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K, TMS): δ 9.02 (s, 1H, H2 of pyridine), 8.77 (d, 1H, *J* = 4.5 Hz, H6 of pyridine), 8.14 (d, 1H, *J* = 7.9 Hz, H4 of pyridine), 7.43 (dd, 1H, *J* = 7.9 and 4.9 Hz, H5 of pyridine), 6.87 (br, 1H, N*H*CH_2CH_2S), 3.71 (q, 2H, *J* = 6.0 Hz, NHC*H*$_2CH_2S$), 3.32 (t, 2H, *J* = 6.1 Hz, $NHCH_2CH_2S$). MS (ESI, positive-ion mode): *m*/*z* 497 $[M + H^+]^+$.

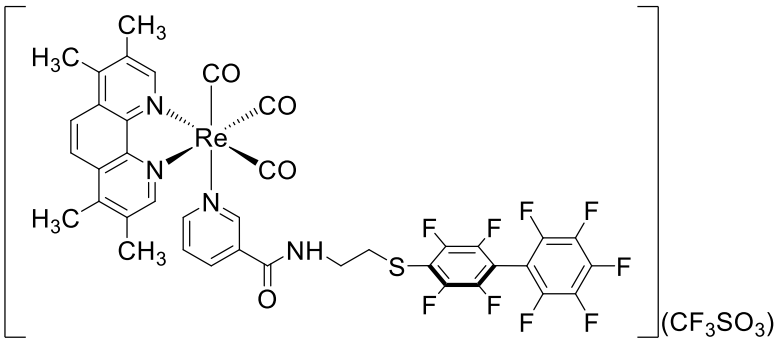
[Re(phen)(CO)$_3$(py-PFBP)](CF$_3$SO$_3$) (**1**)



A mixture of [Re(phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (50 mg, 0.08 mmol) and py-PFBP (40 mg, 0.08 mmol) in THF (30 mL) was refluxed under an inert atmosphere of nitrogen for 4 h. The mixture was evaporated to dryness under reduced pressure to give a yellow solid, which was purified by column chromatography on alumina using CH_2Cl_2/CH_3OH (40:1, *v*/*v*) as the eluent. The solvent was removed under reduced pressure to yield a yellow solid. Subsequent recrystallisation of the solid from CH_2Cl_2/Et_2O afforded the complex as yellow crystals. Yield: 62 mg (71%). ^{1}H NMR (300 MHz, CD_3COCD_3, 298 K, TMS): δ9.95 (d, 2H, *J* = 4.0 Hz, H2 and H9 of phen), 9.11 (dd, 2H, *J* = 8.3 and 1.2 Hz, H4 and H7 of phen), 8.89 (s, 1H, H2 of pyridine), 8.75 (d, 1H, *J* = 5.1 Hz, H6 of pyridine), 8.40 – 8.35 (m, 5H, H3, H5, H6 and H8 of phen and CON*H* of pyridine), 8.25 (d, 1H, *J* = 8.1 Hz, H4 of pyridine), 7.45 (dd, 1H, *J* = 7.9 and 5.7 Hz, H5 of pyridine), 3.58 (q, 2H, *J* = 6.3 Hz, NHCH_2CH$_2$S of pyridine), 3.29 (t, 2H, *J* = 6.6 Hz, NHCH$_2$CH_2S of pyridine). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K, TMS): δ194.58, 190.53, 163.17, 154.32, 154.21, 153.41, 153.20, 150.72, 146.54, 140.17, 139.04, 133.29, 131.23, 128.45, 127.52, 126.60, 126.52, 121.46, 119.34, 117.11, 116.98, 105.67, 65.85, 39.61, 34.48, 33.17, 15.26. ^{19}F NMR (376 MHz, $CDCl_3$, 298 K): δ–78.52 (s), –132.64 (q, *J* = 11.9 Hz), –136.72 – –137.02 (m), –138.13 – –138.25 (m), –150.30 (t, *J* = 21.0 Hz), –160.57 – –160.70 (m). IR (KBr) $\tilde{\nu}$/cm^{-1}: 2031 (C≡O), 1934 (C≡O), 1734 (C=O), 1119 ($CF_3SO_3^-$), 1032 ($CF_3SO_3^-$). MS (ESI, positive-ion mode): *m*/*z* 947 [M

– $CF_3SO_3^-]^+$. Elemental analysis calculated (%) for $ReC_{36}H_{17}N_4O_7S_2F_{12}\cdot H_2O$: C, 38.82; H, 1.72; N, 5.03; found: C, 38.53; H, 1.80; N, 5.19.

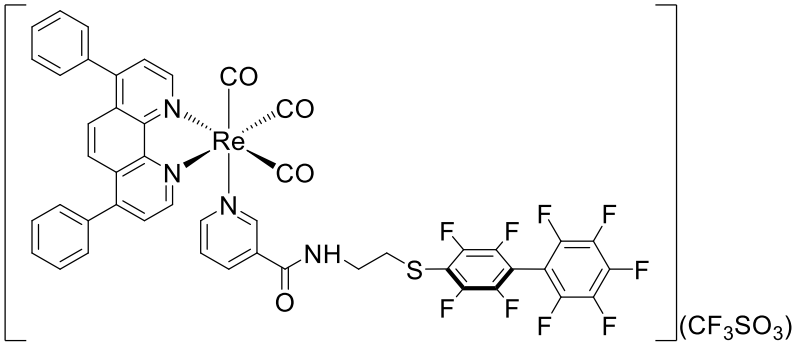
[Re(Me$_4$-phen)(CO)$_3$(py-PFBP)](CF$_3$SO$_3$) (**2**)



A mixture of [Re(Me$_4$-phen)(CO)$_3$(CH$_3$CN)](CF$_3$SO$_3$) (50 mg, 0.07 mmol) and py-PFBP (35 mg, 0.07 mmol) in THF (30 mL) was refluxed under an inert atmosphere of nitrogen for 4 h. The mixture was evaporated to dryness under reduced pressure to give a yellow solid, which was purified by column chromatography on alumina using CH_2Cl_2/CH_3OH (20:1, *v*/*v*) as the eluent. The solvent was removed under reduced pressure to yield a yellow solid. Subsequent recrystallisation of the solid from CH_2Cl_2/Et_2O afforded the complex as pale yellow crystals. Yield: 23 mg (29%). ^{1}H NMR (300 MHz, CD_3COCD_3, 298 K): δ 9.67 (s, 2H, H2 and H9 of Me$_4$-phen), 8.89 (s, 1H, H2 of pyridine), 8.74 (d, 1H, *J* = 5.6 Hz, H6 of pyridine), 8.47 (s, 2H, H5 and H6 of Me$_4$-phen), 8.34 (t, 1H, *J* = 5.3 Hz, CON*H* of pyridine), 8.23 (d, 1H, *J* = 8.1 Hz, H4 of pyridine), 7.42 (dd, 1H, *J* = 7.8 and 5.7, H5 of pyridine), 3.57 (q, 2H, *J* = 6.2 Hz, NHC*H*$_2$CH$_2$S of pyridine), 3.29 (t, 2H, *J* = 6.6 Hz, NHCH$_2$C*H*$_2$S of pyridine), 2.90 (s, 6H, CH_3 at C4 and C7 of Me$_4$-phen), 2.81 (s, 6H, CH_3 at C3 and C8 of Me$_4$-phen). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K, TMS): δ 195.32, 191.29, 163.62, 153.91, 153.83, 152.87, 151.11, 148.68, 148.58, 145.32, 143.26, 138.73, 136.19, 133.37, 129.82, 126.46, 124.24, 121.39, 119.27, 117.15, 117.02, 65.85, 39.60,

33.16, 18.03, 15.43, 15.26. ^{19}F NMR (376 MHz, $CDCl_3$, 298 K): δ–78.62 (s), –132.65 (q, *J* = 11.9 Hz), –136.93 – –136.99 (m), –138.05 – –138.16 (m), –150.37 (t, *J* = 21.0 Hz), –160.60 – –160.73 (m). IR (KBr) $\tilde{\nu}$/cm^{-1}: 2031 (C≡O), 1933 (C≡O), 1734 (C=O), 1118 ($CF_3SO_3^-$), 1031 ($CF_3SO_3^-$). MS (ESI, positive-ion mode): *m*/*z* 1003 [M – $CF_3SO_3^-$]$^+$. Elemental analysis calculated (%) for $ReC_{40}H_{25}N_4O_7S_2F_{12}$: C, 41.71; H, 2.19; N, 4.86; found: C, 41.41; H, 2.33; N, 4.88.

[Re(Ph$_2$-phen)(CO)$_3$(py-PFBP)](CF_3SO_3) (**3**)



A mixture of [Re(Me$_4$-phen)(CO)$_3$(CH_3CN)](CF_3SO_3) (50 mg, 0.06 mmol) and py-PFBP (30 mg, 0.06 mmol) in THF (30 mL) was refluxed under an inert atmosphere of nitrogen for 4 h. The mixture was evaporated to dryness under reduced pressure to give a yellow solid, which was purified by column chromatography on alumina using CH_2Cl_2/CH_3OH (60:1, *v*/*v*) as the eluent. The solvent was removed under reduced pressure to yield a yellow solid. Subsequent recrystallisation of the solid from CH_2Cl_2/Et_2O afforded the complex as yellow crystals. Yield: 39 mg (52%). ^{1}H NMR (300 MHz, CD_3COCD_3, 298 K): δ 10.01 (d, 2H, *J* = 5.4 Hz, H2 and H9 of Ph$_2$-phen), 8.98 (s, 1H, H2 of pyridine), 8.88 (d, 1H, *J* = 5.8 Hz, H6 of pyridine), 8.37 – 8.35 (m, 1H, CON*H* of pyridine), 8.33 (d, 2H, *J* = 5.4 Hz, H3 and H8 of Ph$_2$-phen), 8.28 (d, *J* = 8.1, H4 of pyridine), 8.24 (s, 2H, H5 and H6 of Ph$_2$-phen), 7.74 – 7.67 (m, 10H, C_6H_5 at C4 and C7 of Ph$_2$-phen), 7.51 (dd, 1H, *J* = 8.0 and 5.7 Hz, H5 of pyridine), 3.60 (q, 2H, *J* = 6.2 Hz, CONHC*H*$_2$CH$_2$S),

3.30 (t, 2H, *J* = 6.4 Hz, $CONHCH_2CH_2S$). ^{13}C NMR (150 MHz, $CDCl_3$, 298 K, TMS): δ194.90, 190.90, 163.47, 153.47, 153.39, 153.14, 153.06, 150.95, 150.88, 147.88, 147.43, 143.22, 139.31, 139.18, 134.79, 133.60, 130.40, 129.71, 129.46, 129.34, 127.40, 126.56, 126.42, 121.45, 119.33, 117.20, 117.06, 65.85, 39.63, 33.24, 15.26. ^{19}F NMR (376 MHz, $CDCl_3$, 298 K): δ–78.49 (s), –132.55 (q, *J* = 11.8 Hz), –136.67 – –136.99 (m), –138.16 – –138.25 (m), –150.41 (t, *J* = 21.0 Hz), –160.57 – –160.72 (m). IR (KBr) $\tilde{\nu}$/cm^{-1}: 2031 (C≡O), 1933 (C≡O), 1733 (C=O), 1119 ($CF_3SO_3^-$), 1031 ($CF_3SO_3^-$). MS (ESI, positive-ion mode): *m*/*z* 1099 [M – $CF_3SO_3^-$]$^+$. Elemental analysis calculated (%) for $ReC_{48}H_{25}N_4O_7S_2F_{12}$: C, 46.19; H, 2.02; N, 4.49; found: C, 45.89; H, 2.13; N, 4.51.

Physical Measurements and Instrumentation

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on Bruker 300, 400 and 600 MHz AVANCE III spectrometers at 298 K using deuterated solvents. Chemical shifts (δ, ppm) were reported relative to tetramethylsilane (TMS). Positive-ion electrospray ionization (ESI) mass spectra were recorded on an AB Sciex API 3200 QTRAP mass spectrometer at 298 K. IR spectra of the samples in KBr pellets were recorded in the range of 4000 – 400 cm^{-1} using a Perkin Elmer Spectrum 100 FTIR spectrometer. Elemental analyses were carried out on an Elementar Analysensysteme GmbH Vario MICRO elemental analyzer. Electronic absorption spectra were recorded on an Agilent 8454 diode array spectrophotometer. Steady-state emission spectra of the complexes and the conjugates were recorded on a HORIBA Jobin Yvon FluoroMax-4 spectrofluorometer. Unless specified otherwise, all 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[4] using an aerated aqueous solution of [Re(phen)(CO)$_3$(pyridine)](CF$_3$SO$_3$) (Φ_{em} = 0.18, λ_{ex} = 355 nm) as the standard solution.[5] The concentrations of the standard and sample solutions were adjusted until the absorbance at 355 nm was 0.1. The emission lifetimes were measured on an Edinburgh Instruments LP920-KS Laser Flash Photolysis spectrometer in the kinetic mode with the 355 nm output of the Spectra-Physics Quanta-Ray Lab-150 pulsed Nd:YAG laser as the excitation source. In singlet oxygen ($^{1}O_2$) measurement, the steady-state emission of $^{1}O_2$ of the sample solutions was recorded on an Edinburgh Instruments FLS980 spectrometer equipped with an R5509-73 NIR photomultiplier tube and C9940-02 exclusive coolers. High-performance liquid chromatography (HPLC) was performed on an Agilent 1260 Infinity II system coupled with a

diode array detector WR using H_2O containing 0.1% (*v*/*v*) TFA (solvent A) and MeOH containing 0.1% (*v*/*v*) TFA (solvent B) as the solvents and the detector was set at 220, 250 or 350 nm.

Crystal Structure Determination of Complex 2

Single crystals of complex **2** suitable for X-ray crystallographic studies were obtained by slow diffusion of diethyl ether vapour into a concentrated dichloromethane solution of the complex. X-ray data were collected on an Oxford Diffraction Gemini S Ultra single-crystal X-ray diffractometer with graphite-monochromatised Cu Kα radiation (λ = 1.54178 Å). Data collection, cell refinement and data reduction were performed using CrysAlisPro-1.171.37.35h (Agilent Technologies, 2015) program. The structure was solved using SHELXT-2014/5 (Sheldrick, 2014) program and refined by SHELXL-2016/6 (Sheldrick, 2016) program. All estimated standard deviations (e.s.d.'s) (except the e.s.d. in the dihedral angle between two least-squares planes) were estimated using a full covariance matrix. Dichloromethane solvent molecules were found to co-crystallise and diffuse in the lattice. The X-ray crystallographic data for complex **2** have been deposited at the Cambridge Crystallographic Data Centre (CCDC), under the deposition number CCDC 2103172. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk.

Preparation of Rhenium(I)–Peptide Conjugates

A mixture of the rhenium(I) PFBP complex (8.0 μmol), a peptide containing the π-clamp sequence (12.0 μmol) and Et_3N (40.0 μmol) in DMF (500 μL) was stirred at 37°C under an inert

atmosphere of nitrogen in the dark for 2 h. The solvent was removed under reduced pressure and the residual solid was dissolved in 60% B and purified by semi-preparative RP-HPLC. The HPLC purification was carried out using an Agilent semi-preparative column (ZORBAX Eclipse XDB-C18 column: 9.4 × 250 mm, 5 µm) with a linear gradient of 60 – 100% B over 20 min and a flow rate of 4 mL min^{-1}. Fractions containing the product were combined and lyophilised. The purified product was characterised by analytical RP-HPLC and ESI-MS. The HPLC analyses were carried out using an Agilent analytical column (ZORBAX Eclipse Plus C18: 4.6 × 150 mm, 5 µm) with a linear gradient of 60 – 100% B over 20 min and a flow rate of 1 mL min^{-1}. **3-FCPF**. Yield: 12.4 mg (91%). t_R = 12.15 min. MS (ESI, positive-ion mode): *m*/*z* 796 $[M + H^+ – CF_3CO_2^–]^{2+}$, 1591 $[M – CF_3CO_2^–]^+$. **3-CPP**. Yield: 12.7 mg (61%). t_R = 7.01 min. MS (ESI, positive-ion mode): *m*/*z* 883 $[M + 2H^+ – CF_3CO_2^–]^{3+}$, 1249 $[M + H^+ – CF_3CO_2^–]^{2+}$. **DEVD-3**. Yield: 5.0 mg (29%). t_R = 13.74 min. MS (ESI, positive-ion mode): *m*/*z* 1025 $[M + H^+ – CF_3CO_2^–]^{2+}$.

For the QSY7-containing conjugate, a mixture of the purified **DEVD-3** (1 µmol), QSY7-NHS (2 µmol) and Et_3N (5 µmol) in DMF (500 µL) was stirred at 37°C under an inert atmosphere of nitrogen in the dark for 18 h. The solvent was removed under reduced pressure and the residual solid was further purified by semi-preparative RP-HPLC. **QSY7-DEVD-3**. Yield: 2.1 mg (71%). t_R = 15.70 min. MS (ESI, positive-ion mode): *m*/*z* 898 $[M + H^+ – CF_3CO_2^–]^{3+}$, 1346 $[M – 2CF_3CO_2^–]^{2+}$.

Determination of Lipophilicity

The lipophilicity (log $P_{o/w}$) of the complexes and the conjugate was measured using the shake-flask method.[6] An aliquot of stock solution of the complex or the conjugate in octan-1-ol (saturated with 0.9% NaCl, *w*/*v*) was added to an equal volume of aqueous NaCl solution (0.9% *w*/*v* and saturated with octan-1-ol). The mixture was shaken at 60 rpm for 2 h to allow partitioning at 298 K. The solution was then centrifuged and the amount of the complex in the organic layer ($[Re]_o$) was determined by emission spectroscopy. The partition coefficient ($P_{o/w}$) for each complex was calculated as the ratio of $[Re]_o/[Re]_w$, where $[Re]_w$ was obtained by subtraction of the total amount of complex by the amount in the organic phase after partitioning.

Conjugation Selectivity Studies

The complex (6.25 nmol) in anhydrous DMSO (50 µL) was added to a mixture of FCPF (0.63 µmol) and/or GCPG (0.63 µmol) in potassium phosphate buffer (200 mM, pH 8.0) (450 µL) containing TCEP (10 mM). The mixture was incubated in the dark at 37°C for 1 h. An aliquot of the reaction mixture (50 µL) was analysed by RP-HPLC.

Determination of $^1O_2$ Generation Quantum Yields

$^1O_2$ generation quantum yields (Φ_Δ) were measured by the optically dilute method[4] using phenalenone in benzene (Φ_Δ = 0.97)[7] as a reference for $^1O_2$ photosensitisation. An air-equilibrated CH_3CN solution (2 mL) containing the rhenium(I) complex was introduced to a quartz cuvette of 1 cm path length. The concentrations of the reference and sample solutions

were adjusted until the absorbance at the excitation wavelength (355 nm) was 0.15. The emission spectra of $^1O_2$ at 1200 – 1350 nm were recorded and the Φ_Δ values of the complexes were determined using the following equation:

$$\Phi_s = \Phi_r \left(\frac{I_r}{I_s}\right)\left(\frac{B_r}{B_s}\right)\left(\frac{n_s}{n_r}\right)^2\left(\frac{D_s}{D_r}\right)$$

where the subscripts *s* and *r* refer to the sample and reference solutions, respectively: Φ is emission quantum yield; *I* is excitation intensity; *B* is $1 - 10^{-AL}$; *A* is the absorbance at the excitation wavelength; *L* is path length in cm; *n* is the refractive index of the solvent and *D* is integrated intensity.

Förster Distance Measurements

The Förster distance (R_o) between the rhenium donor (D) and QSY-7 acceptor (A) was calculated according to the following equation:

$$R_{\mathrm{o}}(\text{in Å}) = 0.211 \times \left(\kappa^2 \times n^{-4} \times \Phi_{\mathrm{D}} \times J(\lambda)\right)^{\frac{1}{6}}$$

where κ^2 is a factor describing the relative orientation in space of the transition dipoles of the D and the A and is assumed to be 2/3; *n* is the refractive index of the solvent; Φ_{D} is the emission quantum yield of **DEVD-3**; $J(\lambda)$ is the overlap integral of the donor **DEVD-3** emission and the acceptor QSY-7 absorption spectra.

Calculation of $J(\lambda)$ is based on the equation below:

$$J(\lambda) = \int_0^\infty F_{\mathrm{D}}(\lambda) \times \varepsilon_{\mathrm{A}}(\lambda) \times \lambda^4 \, \mathrm{d}\lambda$$

where F_D is the corrected emission intensity of the donor **DEVD-3** with the emission intensity normalised to unity and ε_A is the absorption coefficient of the acceptor.

Calculated energy transfer efficiency (E_{calc}) based on Förster's theory was determined according to the following equation:

$$E_{\mathrm{calc}} = \frac{R_0^6}{R_0^6 + r^6}$$

where r is the distance between the rhenium(I) metal centre and the QSY-7 moiety, which was estimated by the three-dimensional structures of the conjugate **QSY7-DEVD-3** modulated by Chem3D 16.0.

Experimentally determined energy transfer efficiency (E_{expt}) was determined on the basis of the emission quantum yields of **DEVD-3** and **QSY7-DEVD-3** according to the following equation:

$$E_{\mathrm{expt}} = 1 - (\Phi_{\mathrm{em,\ QSY7\text{-}DEVD\text{-}3}} / \Phi_{\mathrm{em,\ DEVD\text{-}3}})$$

Live-cell Confocal Imaging

KYSE-510 cells in growth medium were seeded on a sterilised coverslip in a 35-mm tissue culture dish and grown at 37°C under a 5% CO_2 atmosphere for 48 h. The medium was replaced with medium/DMSO (99:1, *v*/*v*) containing the complex or the conjugate (10 μM). After incubation for 1, 3 or 6 h, the cells were washed with PBS (1 mL × 3). The coverslip was then mounted onto a sterilised glass slide and imaging was performed using a Leica TCS SPE (inverted configuration) confocal microscope with an oil immersion 63x objective and an excitation wavelength at 405 nm.

In colocalisation experiments, KYSE-510 cells were treated with the complex (10 μM) for 30 min or the conjugate (10 μM) for 3 h, then further incubated with ER-Tracker Green (1 μM, 20 min), MitoTracker Deep Red FM (100 nM, 20 min) or LysoTracker Deep Red (100 nM, 1 h) in the medium. The cells were washed with PBS (1 mL x 3) and then mounted onto a sterilised glass slide for imaging. The excitation wavelength of the complex or conjugate, ER-Tracker Green, MitoTracker Deep Red FM and LysoTracker Deep Red were 405, 488, 635 and 635 nm, respectively. The Pearson's correlation coefficients (PCC) were determined using the program ImageJ (Version 1.4.3.67).

For imaging of caspase-3 activity, KYSE-510 cells were incubated with the conjugate **DEVD-3** (10 μM) for 1 h and then treated with cisplatin (10 μM) for 3 h to induce apoptosis of the cells.[8] The cells were washed with PBS (1 mL x 3) and then mounted onto a sterilised glass slide for imaging with an excitation wavelength of 405 nm. Cells were pretreated with the caspase-3/7 inhibitor MPS (5 μM) for 2 h in the control experiments.

Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS)

KYSE-510 cells were grown in a 60-mm tissue culture dish and incubated at 37°C under a 5% CO_2 atmosphere for 48 h. The growth medium was then replaced with a mixture of medium/DMSO (99:1, *v*/*v*) containing the complex or the conjugate (10 μM). After incubation for 1, 3 and 6 h, the medium was removed and the cells were washed thoroughly with PBS (1 mL × 3). The cells were trypsinised and harvested with PBS. The resultant solution (1 mL) was digested with 65% HNO_3 (1 mL) at 60°C for 2 h and cooled to room temperature. The concentration of iridium in the solution was measured using a Perkin Elmer PE NexION 2000 ICP-MS system.

Photocytotoxicity Assays

KYSE-510 cells were seeded in two 96-well flat-bottomed microplates (*ca.* 10,000 cells/well) in growth medium (100 μL) and incubated at 37°C under a 5% CO_2 atmosphere for 24 h. The growth medium was replaced with 10 μM of the complexes, conjugates and cisplatin in medium/DMSO (99:1, *v*/*v*) at 37°C under a 5% CO_2 atmosphere for 1 h. Wells containing untreated cells were used as blank control. After the treatment, the medium was removed, the cells were washed with PBS (100 μL) and phenol red-free growth medium were added to each wall (100 μL). One of the microplates was irradiated at 365 nm (3 mW cm^{-2}) for 30 min in a LED cellular photocytotoxicity irradiators (PURI Materials, Shenzhen, China) and the other microplate was kept in the dark. The culture medium was then replaced with fresh medium and the cells were incubated at 37°C under a 5% CO_2 atmosphere. After incubation for 20 h, the medium in each well was replaced with fresh medium (90 μL) and 10 μL of MTT (5 mg/mL)

in PBS was added. The medium was removed after incubation at 37°C for 3 h and DMSO (200 µL) was added to each well. The absorbance of the solutions at 570 nm was measured with a BioTek Powerwave XS MQX200R microplate spectrophotometer.

Table S1 Crystal and structural determination data of complex **2**.

Formula	**$ReC_{41}H_{27}N_4O_7F_{12}S_2Cl_2$**
Formula weight	1236.88
T/K	173
Crystal system	Triclinic
Space group	*P*–1
a/Å	11.2012(4)
b/Å	15.0828(5)
c/Å	15.7471(6)
α/°	116.793(4)
β/°	107.386(3)
γ/°	91.672(3)
Volume/Å^3	2223.96(16)
Z	2
Density/g cm^{-3}	1.847
F(000)	1212
Crystal size/mm^3	0.62 x 0.31 x 0.12
Radiation	Cu Kα (λ = 1.54178 Å)
2θ range for data collection/°	3.36 – 68.24
Index ranges	$-8 \le h \le 13$, $-18 \le k \le 17$, $-18 \le l \le 17$
Reflections collected	15971
Independent reflections	8089
Data/restraints/parameters	8089/0/626
Goodness-of-fit on F^2	1.068
Final R indexes [$I \ge 2\sigma(I)$]	R_1 = 0.0353, wR_2 = 0.0912
Final R indexes [all data]	R_1 = 0.0360, wR_2 = 0.0917

Table S2 Selected bond lengths (Å) and bond angles (°) for complex **2**.

Re(1)–C(1)	1.927(4)	Re(1)–C(2)	1.927(4)
Re(1)–C(3)	1.932(4)	Re(1)–N(1)	2.169(3)
Re(1)–N(2)	2.174(3)	Re(1)–N(3)	2.228(3)
C(1)–Re(1)–C(2)	86.52(16)	C(1)–Re(1)–C(3)	88.68(17)
C(1)–Re(1)–N(1)	173.39(14)	C(1)–Re(1)–N(2)	98.38(14)
C(1)–Re(1)–N(3)	94.83(14)	C(2)–Re(1)–C(3)	88.83(17)
C(2)–Re(1)–N(1)	99.66(14)	C(2)–Re(1)–N(2)	173.12(13)
C(2)–Re(1)–N(3)	92.36(14)	C(3)–Re(1)–N(1)	93.76(15)
C(3)–Re(1)–N(2)	96.09(15)	C(3)–Re(1)–N(3)	176.36(14)
N(1)–Re(1)–N(2)	75.26(12)	N(1)–Re(1)–N(3)	82.65(11)
N(2)–Re(1)–N(3)	82.45(11)		

Table S3 Electronic absorption spectral data of complexes **1**–**3** and **1-Et**–**3-Et** in CH_2Cl_2 and CH_3CN at 298 K.

Complex	Solvent	λ_{em}/nm
1	CH_2Cl_2	277 (35,410), 293 sh (21,115), 336 sh (5,045), 386 sh (2,935)
	CH_3CN	276 (36,450), 293 sh (20,390), 328 sh (6,100), 373 sh (3,190)
2	CH_2Cl_2	240 sh (47,070), 282 (51,125), 293 sh (35,335), 328 sh (11,685), 375 sh (3,675)
	CH_3CN	249 sh (42,705), 281 (51,410), 293 sh (34,675), 328 sh (11,610), 373 sh (3,520)
3	CH_2Cl_2	288 (48,520), 342 sh (15,040), 390 sh (7,595)
	CH_3CN	288 (48,985), 335 sh (15,815), 387 sh (6,790)
1-Et	CH_2Cl_2	257 sh (26,635), 276 (29,280), 299 sh (13,895), 334 sh (6,105), 386 sh (3,605)
	CH_3CN	254 sh (24,050), 275 (28,765), 292 sh (15,750), 327 sh (6,525), 369 sh (3,650)
2-Et	CH_2Cl_2	254 sh (26,635), 282 (31,250), 310 sh (13,675), 327 sh (9,845), 377 sh (3,230)
	CH_3CN	248 sh (29,040), 281 (31,500), 310 sh (13,930), 327 sh (9,490), 370 sh (3,060)
3-Et	CH_2Cl_2	262 sh (26,940), 293 (41,440), 343 sh (15,730), 396 sh (7,465)
	CH_3CN	260 sh (25,295), 291 (39,640), 336 sh (14,570), 390 sh (5,960)

Table S4 Photophysical data of complexes **1**–**3** and **1-Et**–**3-Et**.

Complex	Medium (*T*/K)	λ_{em}/nm	Φ_{em}	τ_o/μs
1	CH_2Cl_2 (298)	531	0.50	3.25
	CH_3CN (298)	541	0.19	1.88
	Buffer (298)[a]	542	0.20	1.46
	Glass (77)[b]	477 sh, 495, 518 sh		
2	CH_2Cl_2 (298)	488 sh, 510	0.76	13.20
	CH_3CN (298)	485 sh, 512	0.61	12.32
	Buffer (298)[a]	486 sh, 513	0.60	12.19
	Glass (77)[b]	466 (max), 498, 537 sh, 577 sh		
3	CH_2Cl_2 (298)	540	0.44	9.43
	CH_3CN (298)	554	0.30	5.94
	Buffer (298)[a]	554	0.15	3.56
	Glass (77)[b]	510, 530 sh		
1-Et	CH_2Cl_2 (298)	532	0.51	3.26
	CH_3CN (298)	546	0.22	1.74
	Buffer (298)[a]	544	0.16	1.32
	Glass (77)[b]	474 sh, 494		
2-Et	CH_2Cl_2 (298)	488 sh, 511	0.81	15.05
	CH_3CN (298)	486 sh, 515	0.56	12.49
	Buffer (298)[a]	485 sh, 515	0.56	11.91
	Glass (77)[b]	466 (max), 498, 537 sh, 577 sh		
3-Et	CH_2Cl_2 (298)	543	0.46	9.69
	CH_3CN (298)	557	0.27	5.15
	Buffer (298)[a]	557	0.15	3.32
	Glass (77)[b]	510, 537 sh		

[a] KPi (50 mM, pH 7.4)/MeOH (7:3, *v*/*v*).

[b] EtOH/MeOH (4:1, *v*/*v*).

Table S5 $^1O_2$ generation quantum yields (Φ_Δ) of complexes **1**–**3** and **1-Et**–**3-Et** in aerated CH_3CN at 298 K.

Complex	Φ_Δ[a]
1	0.49
2	0.56
3	0.70
1-Et	0.47
2-Et	0.53
3-Et	0.59

[a] λ_{ex} = 355 nm and phenalenone in benzene was adopted as the reference.

Table S6 Lipophilicity (log $P_{o/w}$) and time-dependent cellular uptake of complexes **1**–**3** and the conjugates of complex **3** by KYSE-510 cells.

Compound	log $P_{o/w}$[a]	Amount of rhenium/fmol[b]		
		1h	3h	6h
1	0.74	2.10 ± 0.01	3.47 ± 0.07	4.29 ± 0.07
2	0.81	5.37 ± 0.05	5.82 ± 0.17	6.33 ± 0.11
3	0.97	5.69 ± 0.06	6.25 ± 0.27	8.12 ± 0.12
3-FCPF	0.59	0.20 ± 0.004	0.34 ± 0.01	0.77 ± 0.01
3-CPP	0.26	0.54 ± 0.01	0.86 ± 0.02	1.16 ± 0.03
QSY7-DEVD-3	0.49	0.032 ± 0.001	0.042 ± 0.001	0.064 ± 0.001

[a] Log $P_{o/w}$ is defined as the logarithmic ratio of the concentration of the complex in saturated octan-1-ol with NaCl to that in an aqueous solution of NaCl (0.9%, *w*/*v*). [b] Amount of rhenium of complexes **1**–**3** and the conjugates of complex **3** associated with an average KYSE-510 cell upon incubation with the complexes and the conjugates (10 μM) at 37°C for 1, 3 and 6 h, as determined by ICP-MS.

Table S7 Photophysical data of the conjugates of complex **3** in degassed KPi (50 mM, pH 7.4)/MeOH (7:3, *v*/*v*) at 298 K.

Conjugates	λ_{em}/nm	Φ_{em}	τ_o/μs
3-FCPF	562	0.17	3.68
3-CPP	563	0.18	4.07
DEVD-3	564	0.18	4.21
QSY7-DEVD-3	555, 595 sh	< 0.009	3.83

Table S8 FRET parameters of conjugate **DEVD-3**.

Donor	Acceptor	$J(\lambda)^a$/nm^4 M^{-1} cm^{-1}	R_o/Å	d^b/Å	E_{calc}	E_{expt}
DEVD-3	QSY-7	4.45 x 10^{15}	49.7	29.3	0.96	0.95

[a] Overlap integral of the emission spectrum of the QSY-7-free conjugate **DEVD-3** and the absorption spectrum of QSY-7 (acceptor).

[b] Distance between the rhenium(I) atom and QSY-7 in **QSY7-DEVD-3**.

Table S9 Emission enhancement factors (I/I_o) and lifetimes (τ) of **QSY7-DEVD-3** (50 μM) upon incubation with caspase-3 (1 unit) and caspase-7 (1 unit) in the absence and presence of the inhibitor Z-VAD-FMK (20 μM) in aerated caspase reaction buffer/DMSO (95:5, *v*/*v*) at 37°C.

	I/I_o	τ
Conjugate only	1.0	0.33
Conjugate + caspase-3	3.0	0.57
Conjugate + caspase-3 + Z-VAD-FMK	1.1	0.32
Conjugate + caspase-7	2.1	0.41
Conjugate + caspase-7 + Z-VAD-FMK	1.0	0.32

Fig. S1 Perspective view of four neighbouring cations of complex **2** showing C–H···F–C interactions (magenta).

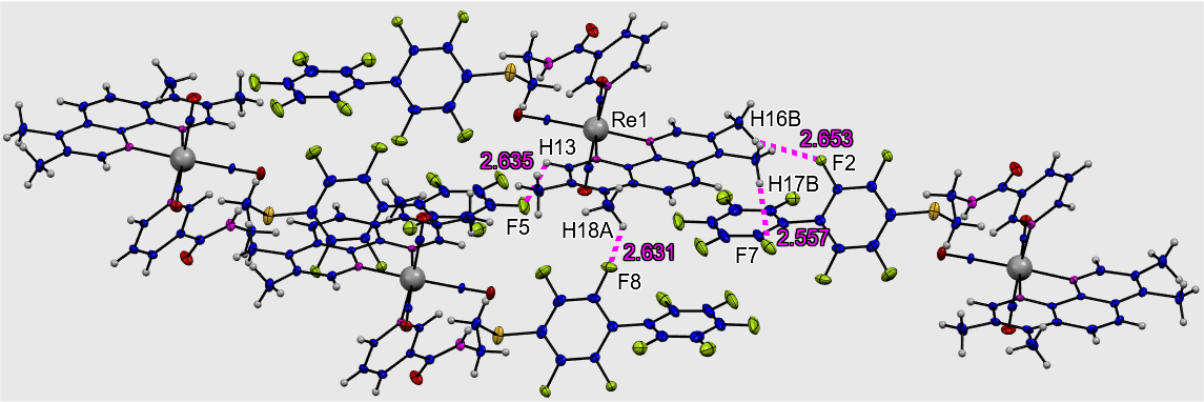


Fig. S2 Electronic absorption spectra of complexes **1**–**3** and **1-Et**–**3-Et** in CH_2Cl_2 (black) and CH_3CN (red) at 298 K.

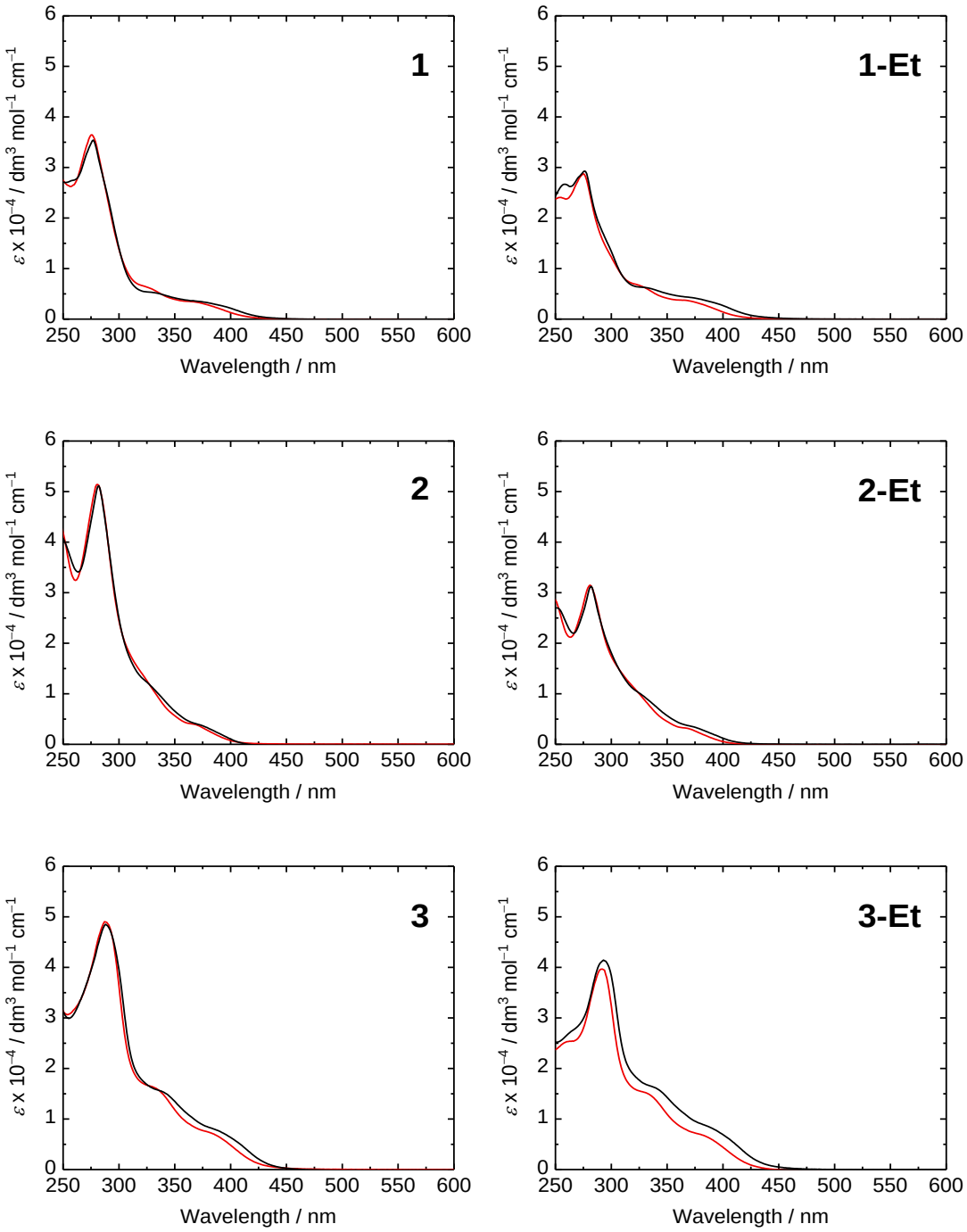


Fig. S3 Emission spectra of complexes **1**–**3** and **1-Et**–**3-Et** in degassed CH_2Cl_2 (black) and CH_3CN (red) at 298 K. Excitation wavelength = 355 nm.

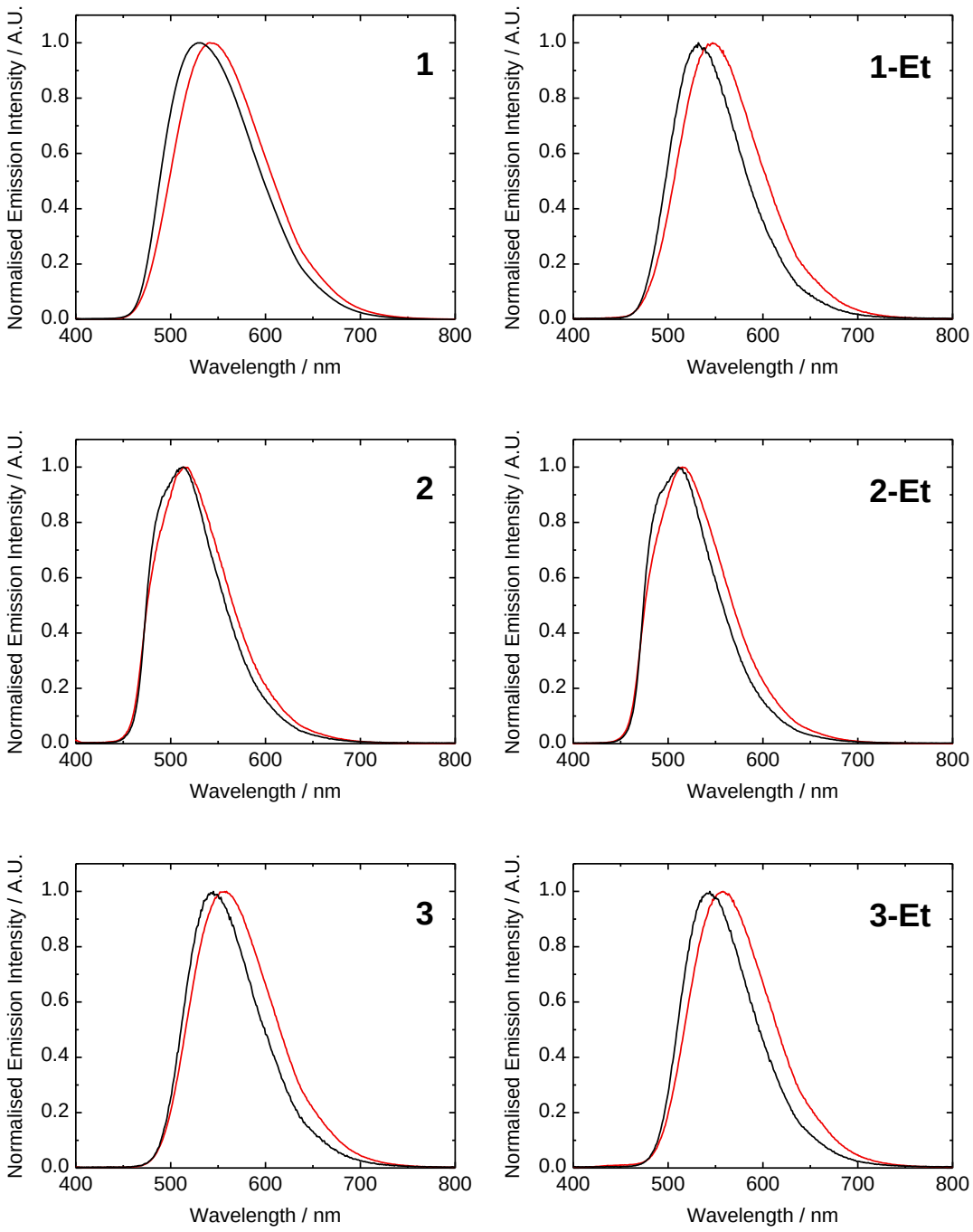


Fig. S4 LSCM images of KYSE-510 cells incubated with complexes **1**–**3** (10 μM, 30 min, λ_{ex} = 405 nm, λ_{em} = 525 – 575 nm) and then ER-Tracker Green (1 μM, 20 min, λ_{ex} = 488 nm, λ_{em} = 500 – 530 nm) at 37°C. Pearson's correlation coefficients were 0.88, 0.88 and 0.90 for complexes **1**, **2** and **3**, respectively. Scale bar = 25 μm.

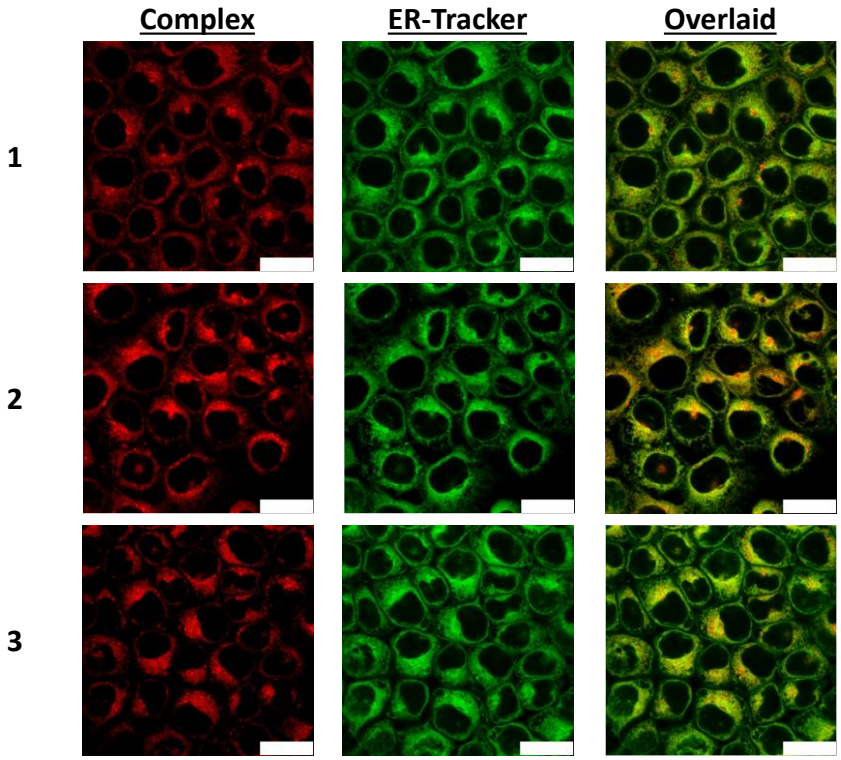


Fig. S5 HPLC chromatograms of (a) the π-clamp (FCPF) (1.25 mM), (b) a double glycine mutant of π-clamp (GCPG) (1.25 mM), (c) complex **3** (12.5 μM), (d) a reaction mixture of complex **3** (12.5 μM) and FCPF (1.25 mM), (e) a reaction mixture of complex **3** (12.5 μM) and GCPG (1.25 mM), (f) a reaction mixture of complex **3** (12.5 μM), FCPF (1.25 mM) and GCPG (1.25 mM) in potassium phosphate buffer (200 mM, pH 8.0)/DMSO (9:1, *v*/*v*) containing TCEP (10 mM) after incubation at 37°C for 1 h. The absorbance was monitored at 220 nm and the emission was monitored at 560 nm (λ_{ex} = 355 nm).

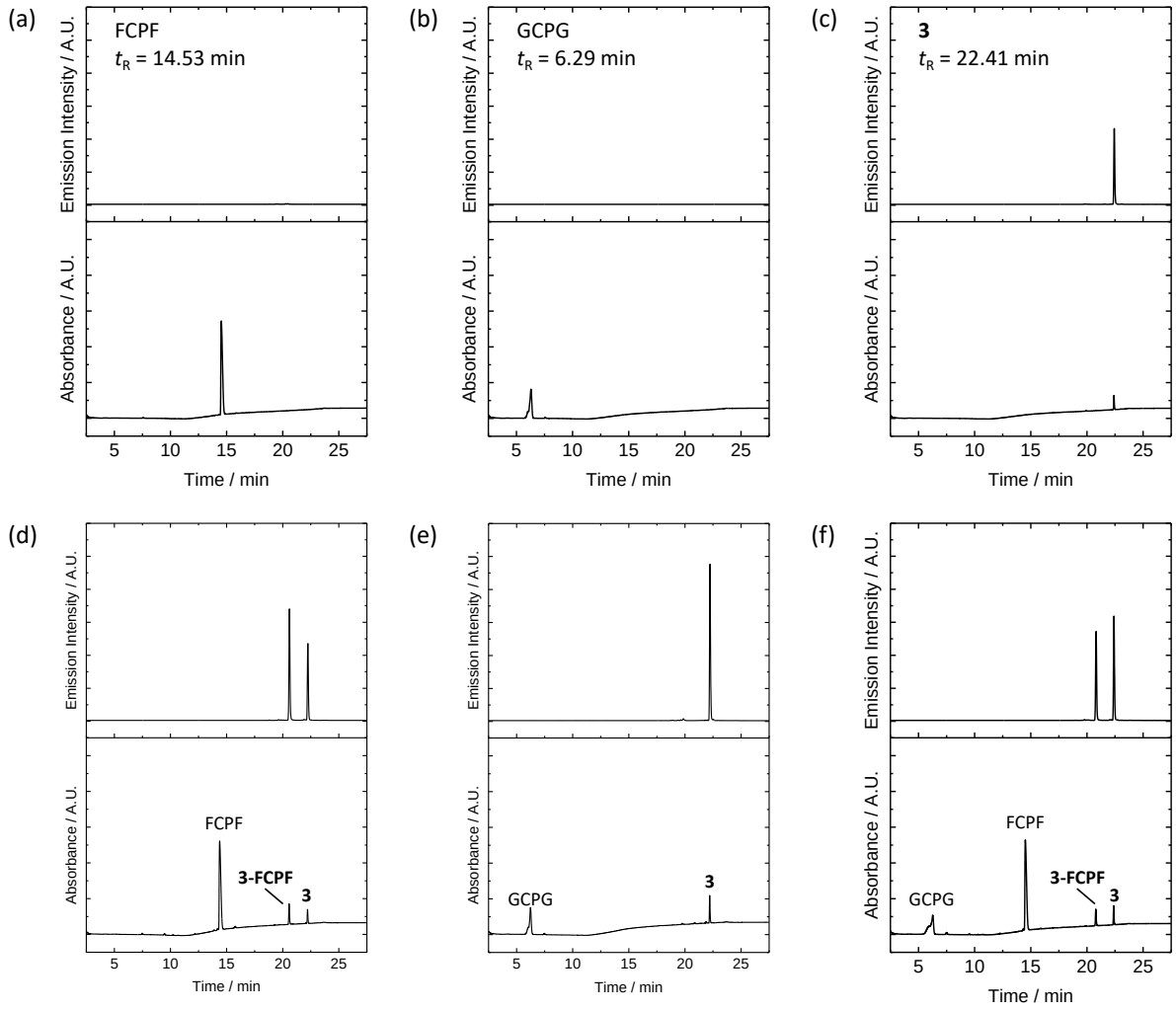
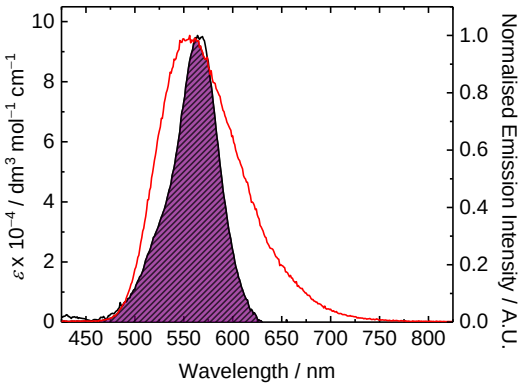


Fig. S6 Spectral overlap of the absorption spectrum of the acceptor QSY-7 and normalised emission spectrum of the donor conjugate **DEVD-3** in KPi (50 mM, pH 7.4, /MeOH (7:3, *v*/*v*) at 298 K. The extinction coefficient of QSY-7 was 103,565 M^{-1} cm^{-1} at 566 nm.



References

1. D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, Elsevier, Oxford, U. K., 2009.
2. K. K.-W. Lo and W.-K. Hui, *Inorg. Chem.*, 2005, **44**, 1992.
3. (a) K. K.-W. Lo, K. H.-K. Tsang, W.-K Hui and N. Zhu, *Chem. Commun.*, 2003, 2704; (b) K. K.-W. Lo, K. H.-K. Tsang, W.-K. Hui and N. Zhu, *Inorg. Chem.*, 2005, **44**, 6100.
4. J. N. Demas and G. A. Crosby, *J. Phys. Chem.*, 1971, **75**, 991.
5. L. Wallance and D. P. Rillema, *Inorg. Chem.*, 1993, **32**, 3836.
6. M. J. McKeage, S. J. Berners-Price, P. Galettis, R. J. Bowen, W. Brouwer, L. Ding, L. Zhuang and B. C. Baguley, *Cancer Chemother. Pharmacol.*, 2000, **46**, 343.
7. R. W. Redmond and J. N. Gamlin, *Photochem. Photobiol.*, 1999, **70**, 391.
8. K. M. Henkels and J. J. Turchi, *Cancer Res.*, 1999, **59**, 3077.