Electronic Supplementary Materials

Synthesis of Sulfonamide Conjugates of Cu, Ga, In, Re and Zn Complexes: Carbonic Anhydrase Inhibition Studies

Jonathan R. Dilworth,*[1] Sofia I. Pascu*[2], Philip A. Waghorn,*[1] Daniela Vullo,[3] Simon R. Bayly,[1] Martin Christlieb,[4] Xin Sun[1] and Claudiu T. Supuran[3]

General methods

NMR spectroscopy was performed on a Varian Mercury VX300 spectrometer (300 MHz) at 298 K or on a Varian Unity spectrometer (500 MHz) at 298 K, and spectra were referenced to the residual solvent peak. Proton chemical shifts are expressed as parts per million (ppm) versus tetramethylsilane. High resolution mass spectrometry was performed using a Bruker Micromass LCT instrument or Bruker MicroTOF. Low resolution mass spectrometry was performed using Micromass LCT Premier Open Access. HPLC analysis of 'cold' compounds was carried out using a Platinum C18 column (5 µm particle size, dimensions 250 x 3.0 mm). In general, unless specified otherwise, gradient methods with acetonitrile/water as the mobile phase were used: 0 min 5% MeCN; 15 min 95% MeCN; 20 min 95% MeCN; 25 min 5% MeCN, 30 min 5% MeCN. Flow rate = 1.0 mL/min

HPLC methods:

Method (A) was performed using a Waters C-18 column (4.6 x 250 mm) with UV/Vis detection at λobs = 254nm and 410 nm with a 0.9 mL/min gradient elution method (Solvent A: THF with 0.1 % TFA *v/v*, Solvent B: water with 0.1% TFA *v/v*): start 5 % A, gradient over 12 min reaching 95 % A, hold to 15 min at 95 % A, reverse gradient till 18 min reaching 5 % A, then hold to 20 min at 5 % A.
Method (B) was performed using a Waters C-18 column (4.6 x 250 mm) with UV/Vis detection at λobs = 254nm and 410 nm with a 1.0 mL/min gradient elution method (Solvent A: acetonitril, Solvent B: water with 0.1% NH4OAc *v/v*): start 5 % A, gradient over 12 min reaching 65 % A, hold to 15 min at 65 % A, reverse gradient till 18 min reaching 5 % A, then hold to 20 min at 5 % A.

Method (C) was performed using a Waters C-18 column (4.6 x 250 mm) with UV/Vis detection at λobs = 254nm and 410 nm with a 1.0 mL/min gradient elution method (Solvent A: acetonitrile with 0.1 % TFA *v/v*, Solvent B: water with 0.1% TFA *v/v*): start 5 % A, gradient over 12 min reaching 65 % A, hold to 15 min at 65 % A, reverse gradient till 18 min reaching 5 % A, then hold to 20 min at 5 % A.

General coupling procedures

Method 1: Amine was added to a solution of carboxyphenylporphyrin in DMF. The mixture was stirred at room temperature until TLC ($CHCl_3$:MeOH, 95:5) indicated complete consumption of porphyrin starting material. The solvent was removed under reduced pressure and the residue re-dissolved in $CHCl_3$. The solution was washed with water and brine and dried over anhydrous magnesium sulphate. The $CHCl_3$ was reduced to minimum volume under reduced pressure and the crude amide was purified by silica gel chromatography ($CHCl_3$:MeOH).

Method 2: DIPEA was added to a solution of carboxyphenylporphyrin in DMF and the mixture cooled to 0 ºC. BOP was added and the solution stirred at 0 ºC for 30 min. The appropriate amine was added and the solution allowed to warm to rt and stirred for 12 h. DMF was removed under reduced pressure and the residue re-dissolved in $CHCl_3$. The solution was washed with 1M HCl, saturated aqueous Na_2CO_3, water, brine and dried over anhydrous magnesium sulphate. The $CHCl_3$ was reduced

to minimum volume under reduced pressure and the crude amide was purified by silica gel chromatography ($CHCl_3$:MeOH).

General sulphonation procedure

The carboxyphenyltriphenylporphyrin was dissolved in conc. H_2SO_4 and heated at 75 °C for 4 h. The reaction mixture was cooled to rt and acetone added to precipitate out a green solid. The solid was filtered and washed with further acetone. The residue was dissolved in a minimum amount of water and the pH adjusted to pH 7 by addition of 2M NaOH(aq.) A C18 Sep-Pak cartridge supplied by Millipore Ltd. was then pre-conditioned by elution with MeOH (2 mL) and water (5 mL) and the aqueous porphyrin solution loaded on to the cartridge. Water soluble impurities were removed from the cartridge by elution with water (5 x 2 mL) and the absorbed porphyrin was eluted using MeOH (3 x 3mL). The solvent was removed under reduced pressure to leave the sodium form of the sulphonated porphyrin as a purple solid.

General pyridine alkylation procedure

The carboxyphenyltripyridylporphyrin was dissolved in DMF and MeI added. The solution was stirred with heating at 35 °C for 1 h. The reaction mixture was cooled to rt and Et_2O added to precipitate out a brown solid. The solid was redissolved in MeOH and reprecipitated with Et_2O. The solid was filtered, washed with Et_2O and hexane and dried under vacuum to leave the tri-iodide salt of the carboxyphenyltri(methylpyridinium)porphyrin as a brown solid.

General metal complexation

Method 1: Sodium acetate was added to a solution of the porphyrin in acetic acid. Metal salt was added and the solution stirred under reflux for 12 h. The reaction mixture was cooled to rt and neutralized to pH 7 with saturated aqueous Na_2CO_3 and extracted with $CHCl_3$. The organic layer was washed with saturated aqueous Na_2CO_3, water and brine and dried over anhydrous magnesium sulphate. The $CHCl_3$ was removed under reduced pressure and the solid recrystalised from $CHCl_3$:MeOH (9:1)/hexane.

Method 2: Metal salt was added to a solution of the porphyrin in DMF and the solution stirred under reflux for 12 h. The reaction mixture was cooled to room temperature and DMF removed under reduced pressure. The residue was re-dissolved in $CHCl_3$ and washed with water and brine and dried over anhydrous magnesium sulphate. The $CHCl_3$ was removed under reduced pressure and the solid recrystallised from $CHCl_3$:MeOH (9:1)/hexane.

Method 3: The water soluble porphyrin was dissolved in pH 4.5 NaOAc buffer and $InCl_3$ added. The solution was then heated under reflux for 2 h. The reaction mixture was cooled to room temperature and the solution filtered. The filtrate was reduced under vacuum to minimum volume and loaded onto a pre-conditioned C18 Sep-Pak cartridge (see above). Water soluble impurities were removed from the cartridge by elution with water (5 x 2 mL) and the absorbed porphyrin was eluted using MeOH (3 x 3mL). The solvent was removed under reduced pressure to leave a solid residue.

Synthesis of 2-oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethanaminium, trifluoroacetic acid, [glyAZA].

(a) 5-Amino-1,3,4-thiadiazole-2-sulphonamide

A mixture of acetazolamide (1.00 g, 4.50 mmol), conc. HCl (2 mL) and ethanol (15 mL) was heated under reflux for 3 h. The solvent was evaporated to near dryness, and the remaining suspension allowed to cool slowly to room temperature. The solid was filtered and dried *in vacuo.* Yield: 528 mg, 2.93 mmol, 65%.

^{1}H NMR (300 MHz, d_6 –DMSO, 20 ºC): 8.05 (s, 2H, SO_2NH_2), 7.79 (s, 2H, thiadiazole-NH_2). Mass Spectrum ESI-MS calcd for $C_2H_4N_4O_2S_2$ [M - H]$^-$ 178.9776, found 178.9758.

(b) ^{t}Butyl-2-oxo-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethylcarbamate

Boc-glycine (87.5 mg, 0.500 mmol) and NEt_3 (70 µL, 0.50 mmol) in MeCN (1.5 mL) was cooled to -10 ºC and ^{i}Butyl chloroformate (65.3 µL, 0.50 mmol) was added with stirring. After 20 min at -10 ºC a shaken suspension of 5-amino-1,3,4-thiadiazole-2-sulphonamide (90 mg, 0.50 mmol) in MeCN (1.5 mL) containing NEt_3 (70 µL, 0.50 mmol) was added. The mixture was stirred at rt overnight and the solvent evaporated. The solid was recrystallised from water/EtOH mix to give a crystalline white solid. Yield: 102.0 mg, 0.30 mmol, 60.6 %.

^{1}H NMR (300 MHz, d_6 –DMSO, 20 ºC): 8.28 (s, 2H, SO_2NH_2), 7.31 (t, 1H, *J* = 6.3, BocN*H*CH_2CONH), 3.87 (d, 2H, *J* = 6.1, BocNHC*H*$_2$CONH), 1.32 (s, 9H, *Boc*NH). Mass Spectrum ESI-MS calcd for $C_9H_{15}N_5O_5S_2$ [M - H]$^-$ 336.0515, found 336.0524

(c) 2-Oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethanaminium, trifluoroacetic acid, [glyAZA]

^{t}Butyl-2-oxo-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethylcarbamate (122 mg, 0.362 mmol) was added to TFA (5 mL) at rt and stirred for 3 h. The TFA was evaporated under reduced pressure and the residue dried under vacuum. Yield: 110 mg, 0.313 mmol, 86.5 %.

^{1}H NMR (300 MHz, d_6 –DMSO, 25 ºC): 8.38 (s, 2H, SO_2NH_2), 5.24 (m, 3H, N*H*$_3$CH$_2$CONH), 3.96 (m, 2H, NH_3CH_2CONH). **Mass Spectrum** ESI-MS calcd for $C_4H_8N_5O_2S_2$ [M – CF_3CO_2]$^+$ 239.0063, found 239.0081.

4-(10,15,20-Triphenylporphyrin-5-yl)-benzoic acid (8)

^{1}H NMR (300 MHz, $CDCl_3$, 20 ºC): , 8.75 (br s, 8H, C*Hpyrr*), 8.36 (d, 2H, *J* = 8.1, Ar*H*), 8.16 (d, 2H, *J* = 7.9, Ar*H*), 8.13 (m, 6H, *o*-Ph), 7.82 (m, 9H, *m-,p*-Ph), -2.91 (s, 2H, ring N*H*). Mass Spectrum ESI-MS calcd for $C_{44}H_{31}N_5$ [M - H]$^-$ 657.2296, found 657.2314.

5-(4-Carboxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin (9)

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$, 20 ºC): 8.17 (d, 6H, *J* = 8.0, Ar*H*), 7.62 (m, 4H, Ar*H*), 7.47 (d, 6H, *J* = 8.1, Ar*H*), -3.31 (s, 2H, ring N*H*). Mass Spectrum ESI-MS calcd for $C_{42}H_{26}N_7O_2$ [M - H]$^-$ 660.21, found 660.22.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-tri(4-pyridylphenyl)-porphyrin, [H_2TPyP-ABS]

The tripyridylporphyrin ABS conjugate was synthesised employing general amide coupling method B. **9** (130 mg, 0.184 mmol), DIPEA (67.4 mg, 90.9 µL 0.522 mmol), 4-(2-aminoethyl)benzene sulphonamide (110 mg, 0.552 mmol), BOP (244 mg, 0.552 mmol), DMF (3 mL). The product was purified via silica gel chromatography (2 to 8 % MeOH in $CHCl_3$). Yield: 126 mg, 0.149 mmol, 81 %.

^{1}H NMR (500 MHz, d_6 –DMSO, 25 ºC): δ 9.08 (t, 1H, *J* =5.4, CON*H*), 9.02 (m, 6H, CH_{py}), 8.88 (m, 8H, CH_{pyrr}), 8.29 (d, 2H, *J* = 8.3, Ar*H*), 8.27 (d, 2H, *J* = 8.3, Ar*H*), 8.25 (m, 6H, CH_{py}), 7.81 (d, 2H, *J* = 8.2, Ar*H*), 7.55 (d, 2H, *J* = 8.2, Ar*H*), 7.35 (s, 2H, SO_2NH_2), 3.68 (m, 2H, CONHCH_2CH$_2$), 3.07 (t, 2H, *J* = 7.1, CONHCH$_2$CH_2), -3.05 (s, 2H, ring N*H*). **^{13}C NMR** (125 MHz, d_6 –DMSO, 25ºC): δ 166.9, 149.5, 149.0, 144.5, 144.3, 142.8, 134.9, 131.3 (broad) 129.9, 129.8, 126.6, 126.5, 120.8, 118.3, 118.1, 35.6, 25.6. **Mass Spectrum** ESI-MS calcd for $C_{50}H_{37}ClN_9O_3S$ [M + Cl]$^-$ 878.2434, found 878.2449.

5-[4-(N-(2-Oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethyl)benzamide)]-10,15,20-tri(4-pyridyl)-porphyrin, [H_2TPyP-AZA].

Compound **[H_2TPyP-AZA]** was synthesised employing general peptide coupling method B. Precursor **9** (100 mg, 0.151 mmol), DIPEA (49.1 mg, 66.2 µL 0.380 mmol), **glyAZA** (64.0 mg, 0.181 mmol), BOP (90.0 mg, 0.196 mmol), DMF (3 mL) **[H_2TPyP-AZA]** was purified *via* silica gel chromatography (1 to 10 % MeOH in $CHCl_3$). Yield: 95.8 mg, 0.109 mmol, 72 %.

^{1}H NMR (500 MHz, d_6 –DMSO, 25 ºC): δ 9.50 (t, 1H, *J* = 5.6, CON*H*), 9.01 (m, 6H, CH_{py}), 8.88 (m, 8H, CH_{pyrr}), 8.36 (d, 2H, *J* = 8.2, Ar*H*), 8.31 (d, 2H, *J* = 7.9, Ar*H*), 8.24 (m, 6H, CH_{py}), 4.43 (d, 2H, *J* = 5.2, CONHCH_2CONH), -3.05 (s, 2H, ring N*H*). **Mass Spectrum** ESI-MS calcd for $C_{46}H_{31}N_{12}O_4S_2$ $[M - H]^-$ 879.2038, found 879.2030. **HPLC:** (Method A) R_t = 13.85 min

.

Table S1: Selected bond lengths (Å) and bond angles (°) for H_2L^9

S(1)-C(24)	1.781(12)	S(4)-O(11)	1.566(18)
S(2)-C(30)	1.819(16)	S(4)-O(12)	1.48(2)
S(3)-C(36)	1.830(12)	S(4)-N(6)	1.47(2)
C(42)-C(45)	1.61(2)	C(42)-C(45)-N(5)	116.0(15)
C(45)-C(10)	1.225(19)	C(42)-C(45)-O(10)	112.1(15)
C (45)-N(5)	1.26(2)	O(10)-C(45)-N(5)	130.8(18)

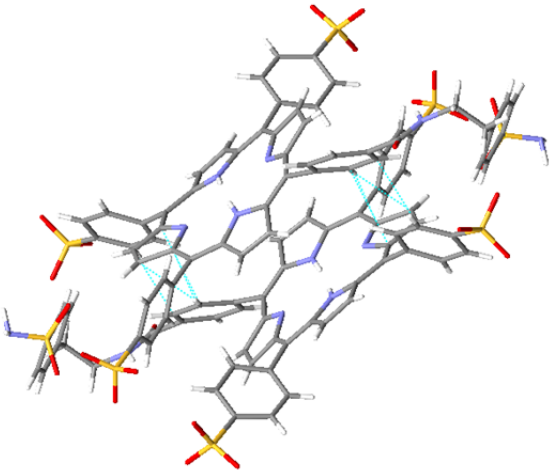


Figure S1: Packing diagram of **H_2L^9** showing H-bonding interactions between solvent water molecule, sulphonamide and aryl-sulphonate groups.

Colour code: blue = N, yellow = S, red = O, grey = C

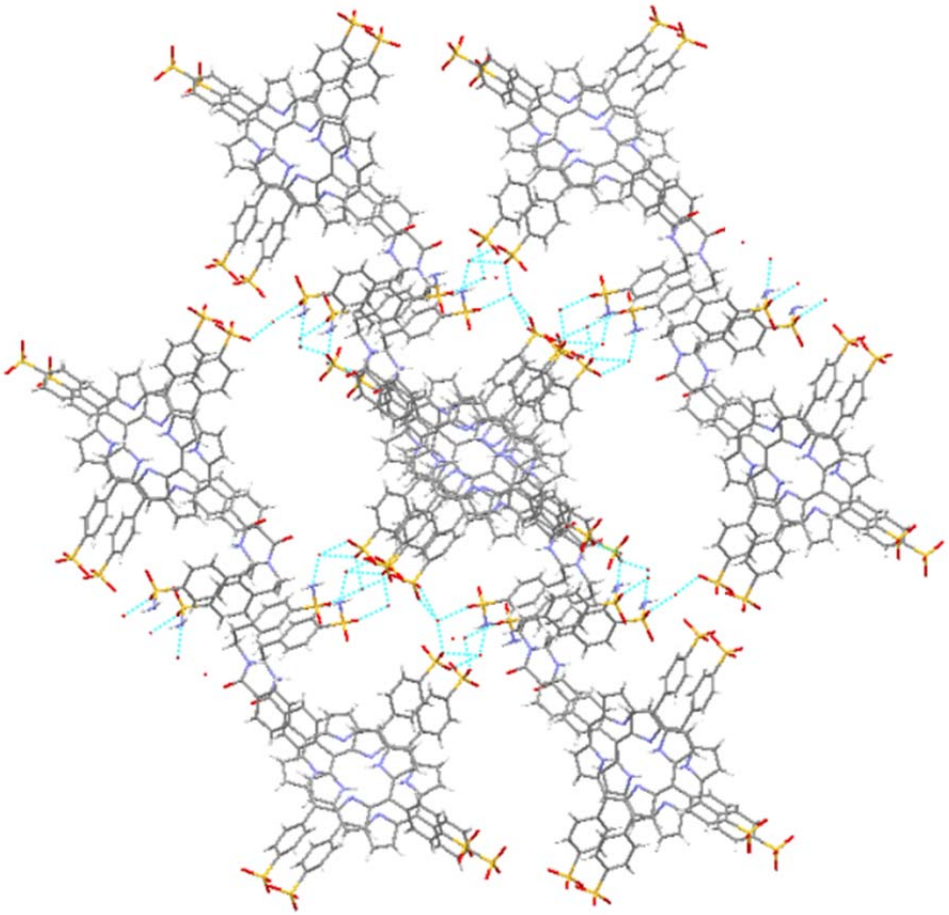


Figure S2: Packing diagram showing additional short range interactions, i.e. aromatic stacking between porphyrin rings.

Colour code: blue = N, yellow = S, red = O, grey = C

Confocal fluorescence imaging:

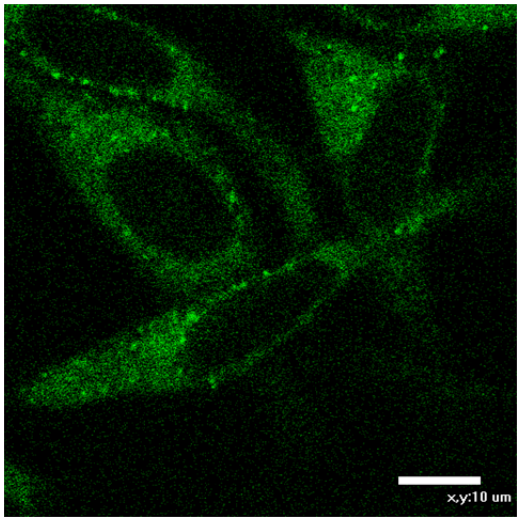


Figure S3. Confocal fluorescence imaging of CA IX positive HCT116 cell line after a 4 hr incubation of compound **InClL**[9] , λ_{ex} 410 nm, λ_{em} >600.

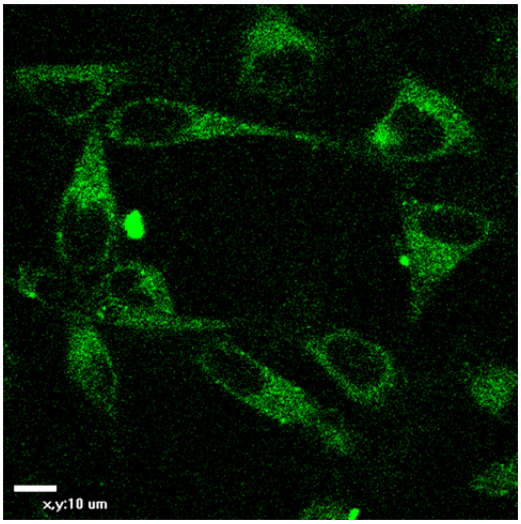


Figure S4. Confocal fluorescence imaging of CA IX negative HCT116 cell line after a 4hr incubation at 37 ^{o}C with compound **InClL^{9}** , λ_{ex} 410 nm, λ_{em} >600.

HCT 116 +

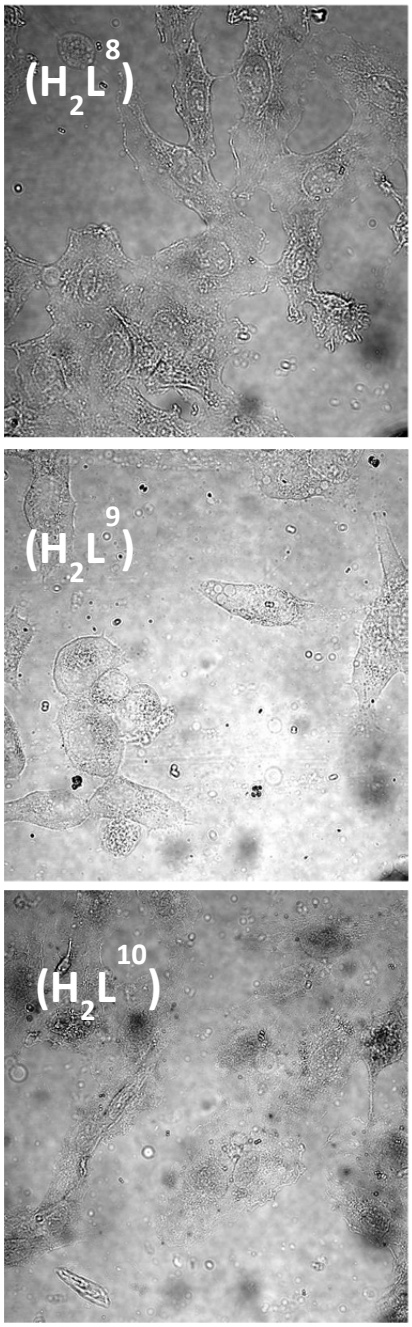
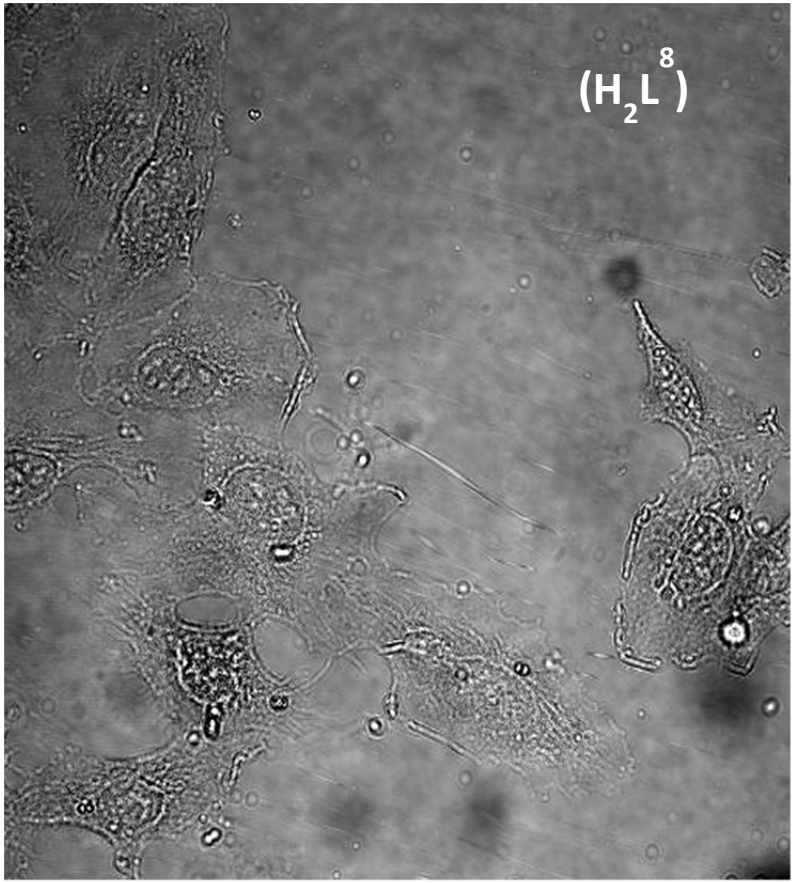


Figure S5. Typical bright-field imaging micrographs, corresponding to the confocal fluorescence imaging at the cellular uptake images of H_2**L^{8}**, H_2**L^{9}** and H_2**L^{10}** in HCT116 cells which are CA IX positive (denoted HCT 116+). Conditions are described in main manuscript.

HCT 116 -



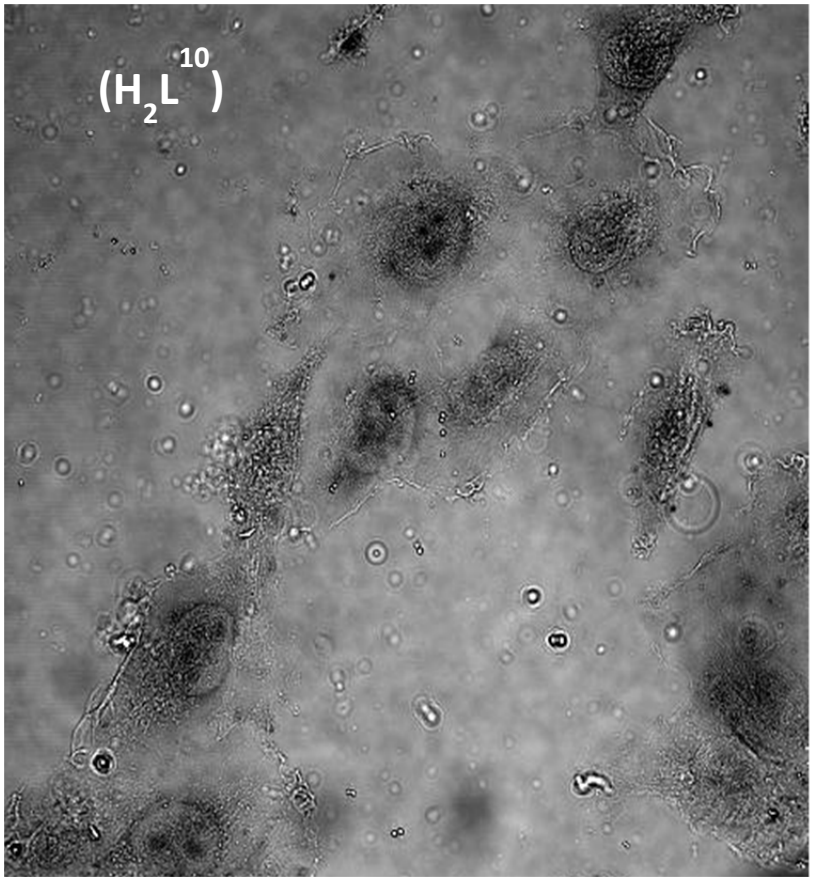


Figure S6. Expansions of typical bright-field imaging micrographs, corresponding to the confocal fluorescence imaging at the cellular uptake images of $H_2\mathbf{L}^{8}$ and $H_2\mathbf{L}^{10}$ in HCT116 cells which are CA IX negative (denoted HCT 116-). Conditions are described in main manuscript.