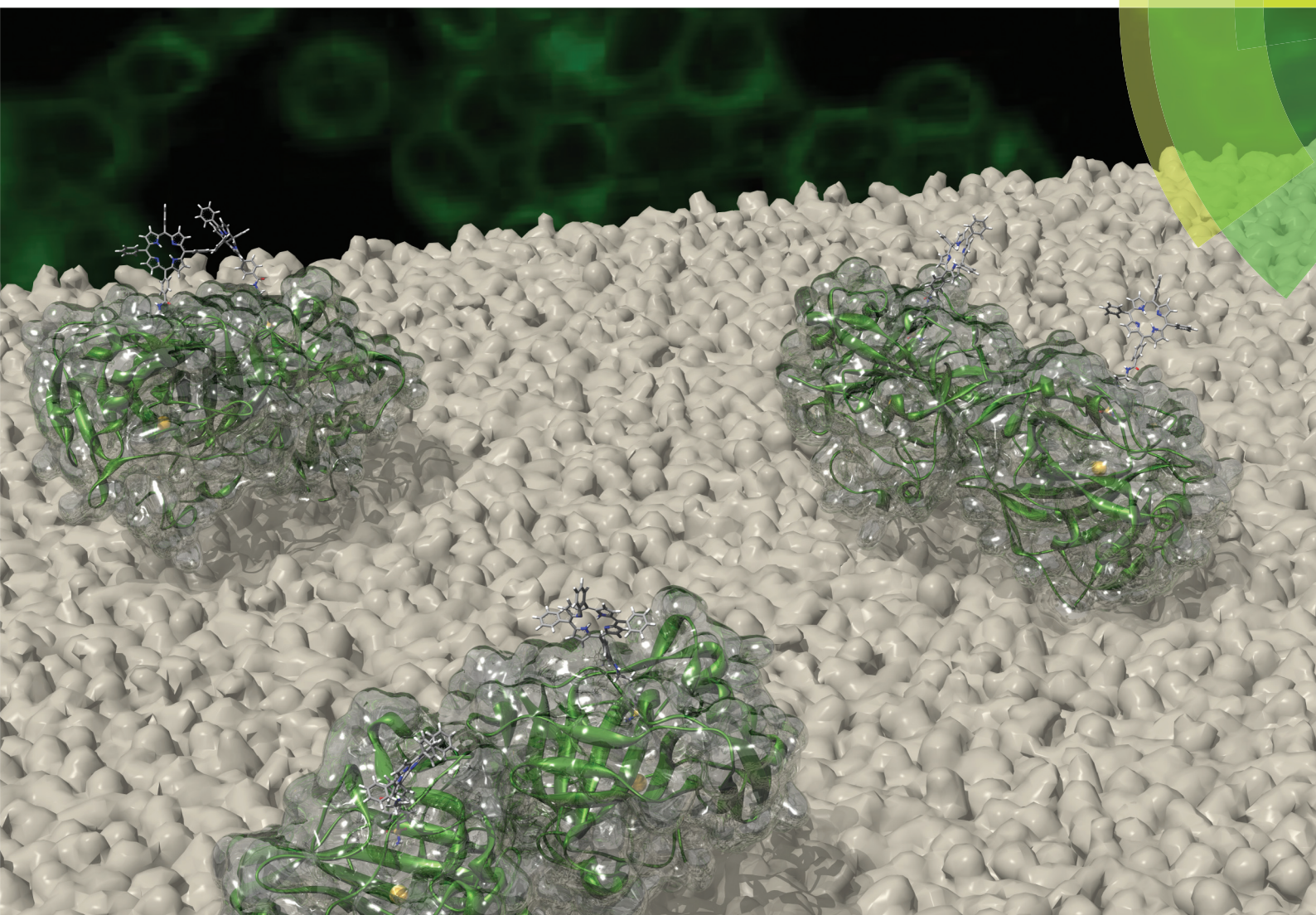


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PAPER

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Synthesis of sulfonamide conjugates of Cu(II), Ga(III), In(III), Re(V) and Zn(II) complexes: carbonic anhydrase inhibition studies and cellular imaging investigations



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Synthesis of sulfonamide conjugates of Cu(II), Ga(III), In(III), Re(V) and Zn(II) complexes: carbonic anhydrase inhibition studies and cellular imaging investigations†

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Carbonic anhydrase IX (CA IX) is currently generating great interest as a marker of tumour hypoxia and a potential chemotherapeutic target. In order to test the principle that a CA IX inhibitor could be used for targeting PET or SPECT metallic radioisotopes to tumours we have prepared a number of conjugates involving aryl-sulfonamides or an acetazolamide derivative linked to a range of copper, indium, rhenium, ^{99m}Tc and zinc complexes. Radiolabelled ⁶⁴Cu and ^{99m}Tc analogues of the 'cold' Cu and some of the Re complexes were prepared in good radiochemical incorporation. Inhibition of various human carbonic anhydrase isoforms (I, II, IX and XII) was tested with the 'cold', non-radiolabelled complexes, and compared with an acetazolamide standard (**AZA**). The molecular structure of a new, tri-sulfonated porphyrin-labeled sulfonamide was determined using synchrotron X-ray crystallography.

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Introduction

Carbonic anhydrases (CA) are zinc metalloenzymes that catalyse the interconversion between CO₂ and HCO₃[−], maintaining pH balance in blood plasma and transporting carbon dioxide out of tissue. Sixteen isoforms of carbonic anhydrase exist to date, located in the cytosol, mitochondria and cellular membranes.¹ Four transmembrane isozymes are implicated in the control of cell proliferation and cellular transformation. Particular interest surrounds the transmembrane CA IX isozyme, which has been shown to be overexpressed in certain cancer lines including renal, cervical squamous, ovarian, colorectal, bladder and non-small cell lung carcinoma. Strong association between CA IX expression and intra-tumoural hypoxia has also been demonstrated.² At a molecular level the response to hypoxia is the stabilization and activation of the transcription

factor HIF-1α and its targets. Under normoxic conditions HIF-1α is hydroxylated and binds the ubiquitin ligase von Hippel-Lindau (VHL) protein, targeting the HIF-1α for degradation. Loss or mutation in VHL triggers the hypoxic phenotype. The tumour associated upregulation of CA IX is the result of a strong transcriptional activation of the CA9 gene by HIF-1α.³ Studies on non-small cell lung carcinomas have shown CA IX association with proteins involved in angiogenesis, apoptosis inhibition and cell-cell adhesion disruption suggesting a strong relationship of this enzyme to a poor clinical outcome.⁴ Therefore, current research interest focuses in the development of CA inhibitors as an approach to the treatment of cancers where CA IX is over-expressed. Similar interest surrounds CA XII, a second tumour associated CA isozyme which has been identified in renal cell carcinoma.⁵ It is similarly regulated by tumour hypoxia but by different biochemical pathways to the CA IX isozyme.³ Although CA XII is a less effective catalyst for the CO₂ hydration with respect to CA IX, its catalytic activity is associated with the acidification of the tumour environment under hypoxia. The inhibition of these tumour associated CA isozymes could represent a novel therapeutic approach for the management of hypoxic tumours which over-express these proteins.^{6,7}

Selectivity for CA IX over the other CA enzymes has been investigated through docking studies/molecular modeling and X-ray crystal structure analysis⁸ and it was found that the guanidine moiety of an arginine residue is specific to the CA IX enzyme, which also has a larger active site than the CA I and CA II isozymes.⁸ Supuran *et al.* have carried out some compre-

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† Electronic supplementary information (ESI) available. CCDC 1028969. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4dt03206c

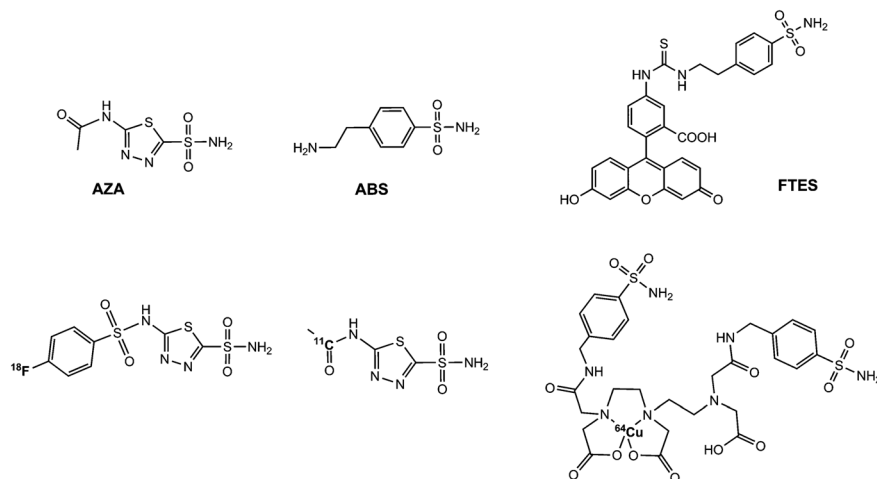


Fig. 1 Schematic representations of: acetazolamide (AZA), 4-(2-aminoethyl) benzenesulfonamide (ABS), (4-sulfamoylphenylethyl thioureido) fluorescein and of three known reagents radiolabelled with [^{18}F], [^{11}C] or [^{64}Cu] for PET imaging *in vivo*.

hensive screening studies on libraries of sulfonamides to find the most selective compounds for CA binding. Inhibition studies with a series of aromatic and heterocyclic sulfonamides showed that CA IX and CA XII are sulfonamide avid.^{9,10} 4-(2-Aminoethyl) benzenesulfonamide (ABS) was shown to be an efficient and specific CA IX inhibitor with an approximate selectivity of more than 60 fold for CA IX over the CA I isozyme. Another sulfonamide derivative acetazolamide (AZA), a known inhibitor of CA IX, is a clinically approved drug (Diamox)¹¹ with a strong binding affinity for CA IX and CA XII in comparison with the CA I and CA II enzymes.^{3,9}

Derivatives of AZA and ABS have been investigated as bifunctional chelators for imaging. However only very few examples of these derivatives labelled with radioisotopes have been reported thus far (Fig. 1).¹² Preliminary radiolabelling studies with 2-[^{18}F]-3,5,6-trifluor-3'-sulphamoylbenzanilide have demonstrated good correlation between ^{18}F retention and the level of the CA IX expression *in vitro* and favourable tumour to muscle uptake ratios for CA IX expressing HT-29 xenograft models *in vivo*.¹³ Bifunctional compounds of these sulfonamides have been developed to incorporate a fluorescent tag in the form of fluorescein¹⁴ to enable *in vitro* fluorescence imaging. The compound (4-sulfamoylphenylethyl thioureido) fluorescein has already been shown to stain selectively cells which up-regulate the CA IX isozyme. *In silico* docking studies of the enzyme suggested that hydrogen bonding accounts for the increased affinity for CA IX over CA II with a guanidine residue specific to CA IX participating in hydrogen bonding with the carbonyl oxygen of the fluorescein tail of the inhibitor.⁸ Upon incorporation of a linker such as EDTA or DTPA for the binding of copper (Fig. 1) interest could be focused on PET radiolabelling studies.¹⁵ Metal-complexed sulfonamides are particularly interesting as in some instances the binding affinity of the conjugate has been shown to exceed that of the parent sulfonamide due to the simultaneous binding of the sulfonamide to the catalytic zinc centre within the CA active site, and of the metal ion to a histidine residue critical to the

catalytic cycle of CO_2 processing.^{16–18} Recently, the Supuran group and collaborators also reported on the organometallic [(Cp-R)M(CO)₃] (M = Re or $^{99\text{m}}\text{Tc}$) arylsulfonamide conjugates which were found to be selective for targeting of human Carbonic Anhydrase IX.^{8c} This paper reports on the synthesis and characterisation of new conjugates of arylsulfonamides (grouped under three different ligand classes: thiosemicarbazone-, amidothiolate- and porphyrin-based) and their corresponding metal complexes, *i.e.* complexes of technetium, rhenium, gallium, indium copper and zinc in their non-radioactive or radioactive form (for ^{64}Cu and $^{99\text{m}}\text{Tc}$). Cellular imaging tests were also performed on three (fluorescent) porphyrin ligands and one indium porphyrin complex, aiming to shed light into the cell inclusion of these conjugates and explore the effect of these sulfonamide derivatives on CA-mediated mechanisms. Their capacity to inhibit a series of carbonic anhydrase isozymes were performed *via* CA enzyme inhibition assays: the observed binding constants were used to assess potential candidates for use as SPECT or PET imaging agents for tumour hypoxia.

Results and discussion

Arylsulfonamides conjugated with 64-copper(II) or zinc(II) bis(thiosemicarbazone) complexes

The complex diacetyl-bis(N^4 -methylthiosemicarbazonato)-copper(II), CuATSM, Fig. 2, has been shown to be selective for hypoxic tissue in imaging studies, where the PET radioisotopes ^{60}Cu , ^{61}Cu or ^{64}Cu were used,^{19–21} and, as such it has been used clinically for the detection of hypoxia in cancer.^{22,23} It has been shown²⁴ that Cu(II) bis(thiosemicarbazones) are stable *in vitro* in serum and can also be used to radiolabel bombesin at room temperature without compromising the biological targeting capabilities of the protein. Here, analogous bis(thiosemicarbazide) system was used to form new conjugates with arylsulfonamides (see Fig. 2 and Experimental section). The possible impact on enzyme inhibition of the

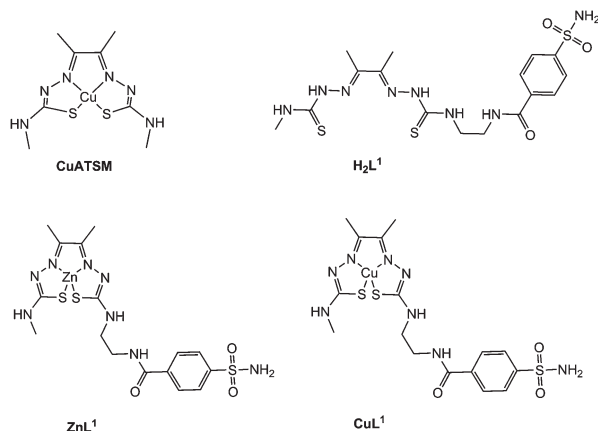


Fig. 2 Schematic representations of CuATSM and the new conjugates emerging from the H₂ATSM/en precursor, H₂L¹, ZnL¹ and CuL¹.

coordination of zinc or copper to the bis(thiosemicarbazone) ligand conjugate was explored. Synthesis of the bis(thiosemicarbazone)-sulfonamide **H₂L¹** was accomplished in an 82% yield *via* the carbodiimide-mediated coupling of 4-sulfamoylbenzoic acid (ABS) with the previously described precursor H₂ATSM/en.²⁰ The complexes **ZnL¹** and **CuL¹** were produced in yields of 81% and 44% respectively by room temperature reaction of **H₂L¹** in methanol solution with zinc(II) acetate and copper(II) acetate respectively. ⁶⁴**CuL¹** was prepared in excellent radiochemical incorporation (>95%) by reaction of a DMSO solution of **H₂L¹** with ⁶⁴Cu(II) in aqueous acetate buffer.

Arylsulfonamides conjugated to 99m-technetium(v) or 'cold' rhenium(v) complexes

In the case of Tc and Re labelling we used the known **MAG₃**, **DADS** and **MAGpy** ligands (see Fig. 3) to study the effect of overall charge and ligand structures on CA enzyme inhibition. The **MAG₃** and **DADS** ligands have been used extensively with 99m-Tc and the complexes are rapidly and specifically excreted from the body *via* the kidneys,^{25,26} a desirable attribute for radiotracers. The technetium(v) or rhenium(v) complexes of **MAG₃** and **DADS** share the same charge (−1) but have structural differences, while the neutral complex of **MAGpy** is different in both charge and structure.^{25,27} For the sulfonamide conjugates, high efficiency of CA IX binding requires a favourable steric fit into the inhibitor binding site of the CA IX enzyme²⁸ and secondary interactions such as H-bonding may be important.

In this study, three known ligands for Tc and Re (**MAG₃**, **DADS** and **MAGpy**), all bearing a pendant carboxylic acid group were synthesised and are reported. **ABS** (Fig. 1) and glycinated acetazolamide (**Gly-AZA**) containing a reactive amino group available for linking the sulfonamide were first synthesised. The proligands **H₃L²Trt**, **H₂L³Trt** and **H₂L⁴Trt** (where Trt = trityl, Fig. 3 and 4) were obtained *via* standard amide formation using a coupling agent (EDC or BOP, Experimental section). These conjugates were then successfully labelled with ^{99m}Tc as the Tc(v) mono-oxo derivatives. Because

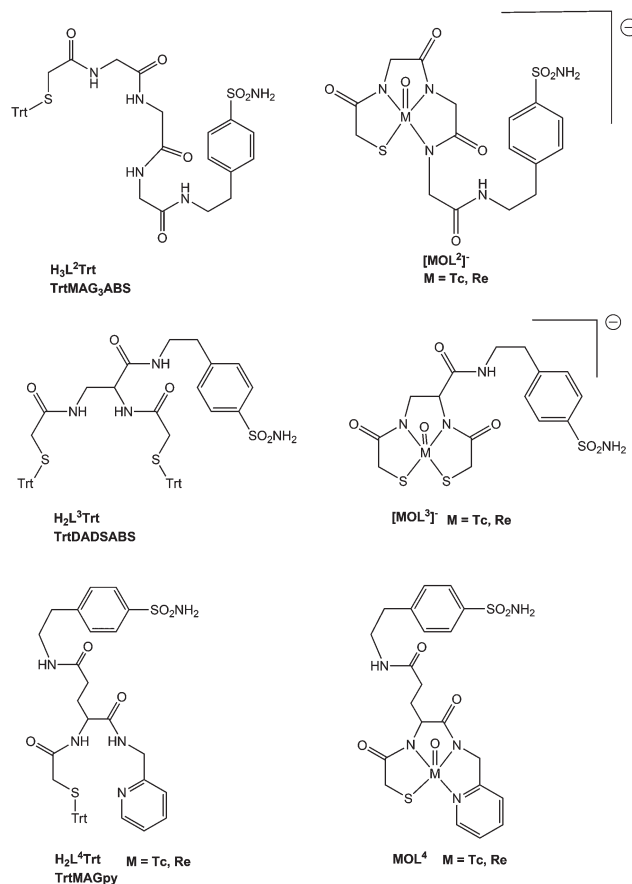


Fig. 3 Schematic representations of new protected arylsulfonamide conjugated ligands and complexes with Re and ^{99m}Tc.

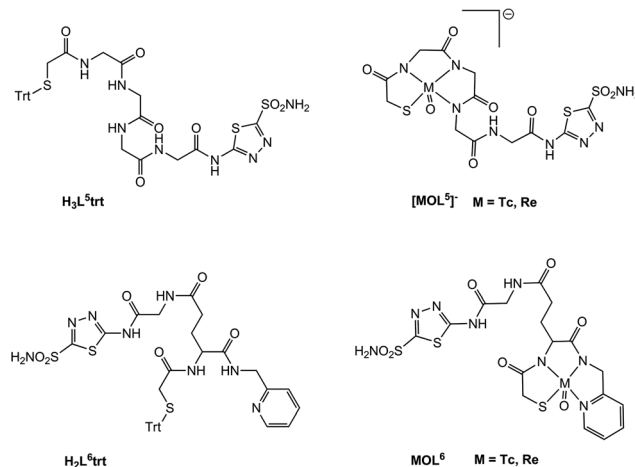


Fig. 4 Schematic representations of the new protected acetazolamide conjugated ligands and complexes with Re(v) and ^{99m}Tc(v).

technetium and rhenium are in the same group of the periodic table and share similar chemical and biological properties,²⁹ the bioassay results of the rhenium and technetium complexes are directly comparable. Therefore, due to the need to use a 'cold' HPLC confirmation method for the ^{99m}Tc complexes

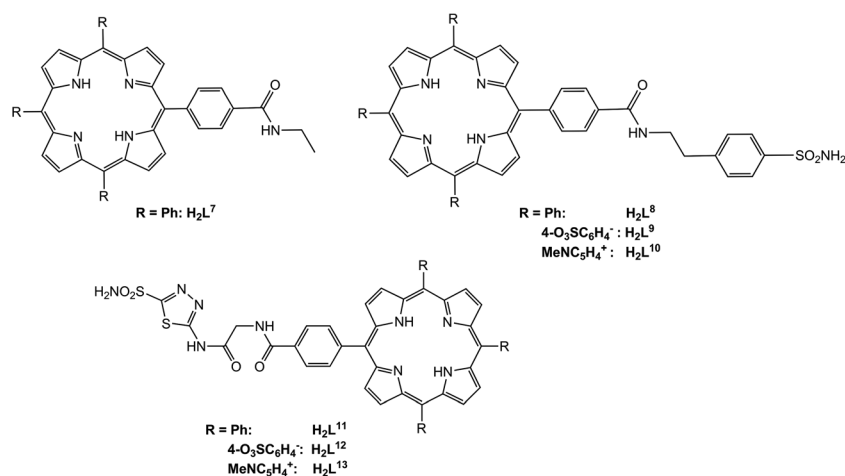


Fig. 5 Structural representations of the free base porphyrin derivatives investigated.

and to proceed to further studies on CAs inhibition studies the isostructural rhenium complexes were also synthesised and fully characterised, as described in Experimental section.

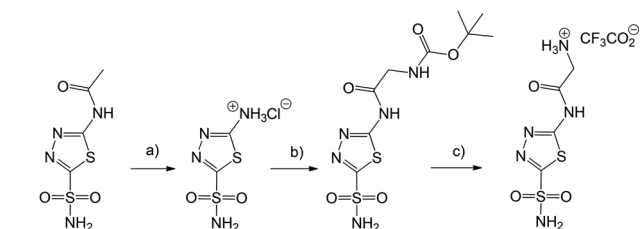
Arylsulfonamide conjugates with porphyrins and metalloporphyrins

To explore if metalloporphyrins conjugated to sulfonamides would also target CA IX a range of porphyrins (Fig. 5) conjugated to **ABS** or **GlyAZA** were synthesised together with their indium(III) and gallium(III) complexes. These were of interest as they provide the possibility of using the intrinsic fluorescence of the porphyrins to study cellular uptake mechanisms and tissue distributions *in vivo*. The peripheral porphyrin substituents were selected deliberately aiming to vary the overall charge and water solubility. The amide and sulfonamide monofunctionalised tetraarylporphyrin or tripyridyl-arylporphyrin ligands (H₂L⁷ through to H₂L¹³) were synthesised from the carboxylate using standard coupling reactions with the appropriate amino sulfonamide. Whilst **ABS** is commercially available as the free amine, the structure of **AZA** is not suitable for direct amide coupling. To achieve a reliable nucleophilic amine source a Boc-glycine linker was introduced according to Scheme 1. Deprotection of the Boc group with TFA yielded a final functionalised **AZA** derivative suitable for

coupling to the porphyrin scaffold using either the NHS activated ester or using BOP as a coupling agent with the porphyrin precursor of choice, see Experimental section and ESI.† Fig. 5 gives the schematic representation of the new free base porphyrin derivatives synthesised.

Molecular structure of H₂L⁹ by synchrotron X-ray diffraction

Crystals of trisulfonated porphyrin H₂L⁹ were grown by the slow infusion of acetone into an aqueous THF solution (1 : 1 v/v THF–water) of the compound. The crystals produced extremely weak but promising X-ray diffraction patterns only when a synchrotron X-ray source was used (SRS Daresbury, Station 9.8). The small crystal size (0.02 × 0.02 × 0.35 mm), the large size of the unit cell and asymmetric unit, the weak scattering of the atoms involved and the extensive disorder of solvating water molecules made solving and refining this data set extremely challenging. The molecular structure is shown in Fig. 6 (CCDC 1028969). The sulfonamide bond lengths and angles



Scheme 1 Schematic representation for the synthesis of derivatised acetazolamide for the aryl carboxylate-functionalised porphyrin coupling: (a) conc. HCl, EtOH, reflux 3 h; (b) Boc-glycine, NEt₃, t-Butyl chloroformate, MeCN, -10 °C to rt. 12 h; (c) TFA, rt. 3 h.

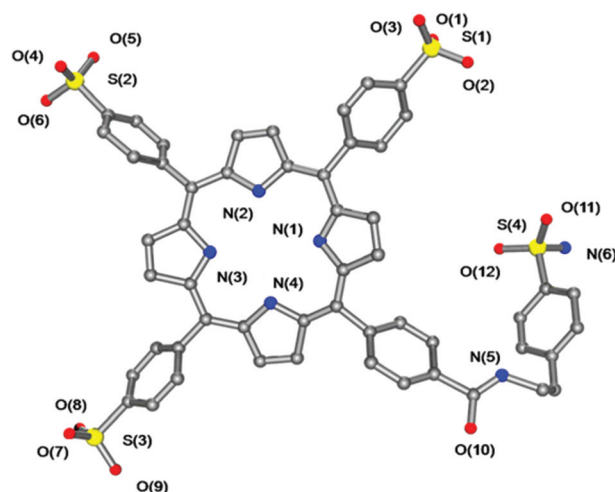


Fig. 6 Ball and stick representation of the molecular structure of H₂L⁹.

are all in accordance with published X-ray crystal structures for aromatic sulfonamide structures.³⁰ Though the data set was extremely weak, a solution was found and the structure determination allowed the confirmation of the connectivity and thus the identity of this compound, in close agreement with ¹H and ¹³C NMR, and ESI-MS data, and thus the formation of the desired functionalised porphyrin complex. The short amide C–N bond (C(45)–N(5) 1.26 Å) found in **H₂L**⁹ is indicative of significant delocalization over the amide group, and extensive disorder in the sulfonamide arm necessitated modeling over several sites during refinement. Despite this, the overall connectivity is clear and shed light into the molecular structure of the first water soluble porphyrin-sulfonamide derivative. The crystal packing is described by a series of H-bonding interactions (Fig. S1 in ESI†) which network between solvent water molecules, sulfonate groups and sulfonamide amine groups, with H-bonding distances ranging between *ca.* 2.15 Å (between the sulfonamide amine nitrogen and water molecule) for (N(6)–O(13)) and *ca.* 2.77 Å (between a sulfonate oxygen and water molecule) for (O(8)–O(14)). The intermolecular interactions observed include some weak aromatic interactions (with short range interactions of *ca.* 2.75 Å) whereby π – π stacking takes place *via* the T-shape stacking of phenyl rings on adjacent porphyrins (Fig. S2 in ESI†).

CA IX inhibition assays

Enzyme inhibition data was determined, for all those compounds which could be isolated in satisfactory yields and

which showed aqueous solubility, for physiologically dominant hCA I and II and cancer-associated hCA IX and XII by assaying the CA catalysed hydration of CO₂³¹ and the data obtained is summarised in Table 1. The entry numbers 1–20 used in the following text refer to those listed in Table 1.

In the cases of the bis(thiosemicarbazones) studied (*i.e.* with Compound entries with no's 1–3 in Table 1), the free ligand and the metal complexes all show low *K_i* values for CA IX and CA XII inhibition, that for the Cu complex being almost equivalent to **ABS** (given at the bottom of the table, and used as a control) itself. However, the discrimination between CA IX and the other isoforms is poor. The anionic Re complexes under entries 4–6 appear to be active, and promising substrates to be used in this context however they show selectivity differences that are difficult to rationalise on the basis of structure or targeting group. The rhenium complex under entry 5 with a pendant sulfonamide has the best overall performance in this assay. Intriguingly, the free base porphyrin **H₂L**⁷ and its corresponding indium complex, which possess no sulfonamide functionality, both show reasonably strong binding affinities for the CA enzymes but in both cases show poor selectivity for CA IX and XII over both CA I and II. Introduction of the sulfonamide entity **ABS** (to give compound **H₂L**⁸) increases the binding affinity for the CA IX and XII enzymes and dramatically increases the selectivity for these enzymes over the non-target CA I and II. In terms of CA IX inhibition with the porphyrins and metal complexes it is reassuring that the porphyrin derivative (see entry 7 in Table 1) with no sulfonamide shows no selectivity for CA IX whereas

Table 1 Enzyme inhibition studies (binding affinity data *K_i* (nM)) for sulfonamide series with calculated selectivity ratios^{a,b}

No	Compound	<i>K_i</i> (nM)				Selectivity ratios		
		CA I	CA II	CA IX	CA XII	CA I/CA IX	CA II/CA IX	CA XII/CA IX
1	H₂L ¹	335	470	62	73	5.4	7.5	0.9
2	ZnL ¹	11.4	9.3	28	6.8	0.4	0.33	0.2
3	CuL ¹	33.1	9.0	9.0	6.1	3.6	1.0	0.7
4	[ReL ²] [−]	432	1180	105	87	4.1	11.2	0.8
5	[ReL ³] [−]	1030	1110	89	88	11.6	12.5	1.0
6	[ReL ³] [−]	1020	1190	1270	85	0.8	0.9	0.1
7	H₂L ⁷	78	46	82	84	1.0	0.5	1.0
8	InClL ⁷	91	111	87	80	1.0	15.1	1.0
9	H₂L ⁸	1080	818	54	46	20.0	15.2	1.0
10	GaClL ⁸	442	841	38	71	11.6	22.1	1.9
11	InClL ⁸	126	46	68	72	1.9	0.7	1.0
12	H₂L ⁹	2650	5750	435	720	6.1	13.2	1.7
13	InClL ⁹	442	841	38	71	11.6	22.1	1.9
14	H₂L ¹⁰	451	770	49	66	9.2	15.7	1.3
15	H₂L ¹¹	820	651	52	73	15.8	12.5	1.4
16	GaClL ¹¹	433	84	59	42	7.3	1.4	0.7
17	InClL ¹¹	391	39	86	35	4.6	1.0	1.0
18	H₂L ¹²	2970	3986	518	792	5.7	7.7	1.5
19	H₂L ¹³	655	520	39	45	16.8	13.3	1.1
20	InClL ¹³	77	81	79	97	1.0	1.0	1.2
Control	ABS	2100	160	33	3.2	63.6	4.9	0.1
Ref. 14b	AZA	250	12	25	5.7	10	0.5	0.02
Control	FTES	1300	45	24	—	54.2	1.9	—

^a **ABS** = arenebenzylsulfonamide, **AZA** = acetazolamide, **FTES** = (4-sulfamoylphenylethyl thioureido) fluorescein. ^b Errors in the range of ± 5 –10% of the reported value, from three different determinations. CA I and CA II are Human (cloned) isozymes. CA IX is the catalytic domain of the human, cloned isozyme. CA XII is the catalytic domain of the human recombinant isozyme, CO₂ hydrase assay method.

the free base compound shown under entry 9 (having one aryl replaced by a pendant arylsulfonamide, H_2L^8) shows strong discrimination between CA IX and other isoforms and is one of the most effective compounds of those screened in this study. However the introduction of either indium or gallium to give the metal complexes results in a loss in selectivity particularly with the indium complexes. Disappointingly, from the perspective of using these compounds for PET or SPECT imaging incorporation of a metal-halide centre (e.g. In(III)-Cl or Ga(III)-Cl) this generally appears to reduce the selectivity for CA IX over the other isoforms significantly. Free-base sulfonated porphyrin H_2L^9 (entry 12) and its corresponding In-Cl complex (entry 13) provide an exception to this with the In complex showing both stronger inhibition and higher selectivity. As with the metal complexes mentioned above there is no obvious correlation of the observed binding/selectivity characteristics with structure. It is likely that subtle factors involving secondary interactions within the binding pocket are dictating the overall inhibition patterns. Based on these results complexes under entries 5 and 13 are under current investigations in our laboratories, and are being taken forward for *in vitro* and *in vivo* studies using the $^{99\text{m}}\text{Tc}$ and ^{111}In labelled variants respectively. For the **AZA**-derivatised metallo-porphyrin compounds (H_2L^{11} , H_2L^{12} and H_2L^{13}) we observed a lower binding affinity to the CA XI and XII enzymes, whilst for the **ABS** metallo-porphyrin species we see an increased binding affinity for the CA XII enzyme. The shift from H_2TPP to the pyridinium-functionalised porphyrin species results in a reduction in the selectivity for CA IX and XII over both CA I and II but the binding affinity to CA IX and CA XII is still favourable for both **AZA**- (H_2L^{13}) and **ABS**- (H_2L^{10}) functionalised species.

Introduction of indium to the tetrapyrrole ring results in complete lack of selectivity towards CA IX and XII with a diminished binding affinity to both enzymes. This suggests that either the free base porphyrin provides additional non-covalent bonding interactions that enhance the overall binding affinity and selectivity towards the CA IX and CA XII enzymes or that the ubiquitous CA enzymes I and II provide additional interactions with the metallo porphyrins which enhance the binding interactions with these enzymes more so than with CA IX and CA XII. Shifting from H_2TPP to the sulfonate species (H_2L^9) still shows a strong affinity for CA IX and XII over both CA I and II but the binding affinity for all the enzymes is dramatically reduced suggesting that the sulfonate groups act to inhibit binding, conversely introduction of the metal ion dramatically increases the binding affinity and selectivity for CA IX and XII over CA I and II. In general, all of the porphyrin species show a uniform medium strength binding affinity to CA IX and CA XII enzymes. All sulfonamide species show an enhanced selectivity for CA IX and CA XII over CA I and CA II in comparison to the porphyrin species alone indicating that the presence of the sulfonamide porphyrin conjugate has a positive effect towards improving selectivity. The selectivity ratios and binding affinities however are much poorer than the control sulfonamides **ABS** and **AZA** on their own and the **FTES** ligand suggesting that the intrinsic affinity that the porphyrin

group has for the CA enzymes prevents the sulfonamide groups from binding preferentially to the CA IX and CA XII enzyme isoforms. Several of the compounds do however show particularly favourable binding affinities such as the free base porphyrins H_2L^8 and the sulfonate variant H_2L^9 .

Docking studies, in line with those already reported by Supuran *et al.* will be carried out in future investigations to elucidate in full the structure activity relationships and the CA inhibition mechanism.^{8b}

Confocal fluorescence imaging tests

To assess the binding potential of the sulfonamide groups to the CA IX receptors, confocal fluorescence studies were performed with compounds H_2L^8 , H_2L^9 and H_2L^{10} on the HCT 116 colon carcinoma cell line. The HCT 116 line has been transfected to over-express the CA IX enzyme (without the need for hypoxic culturing) and denoted CA IX positive. The empty-vector cell line, *i.e.* showing no expression of the CA IX enzyme, was denoted CA IX negative and was used as a control.

Cells lines were cultured according to known protocols⁴⁰ and as described below. Cells were seeded on to glass cover slips and left to adhere for 12 h overnight. Cells were incubated with free-base porphyrin compounds H_2L^8 , H_2L^9 , H_2L^{10} at 10 μM concentrations for 2 h at 37 °C. After 2 h, the cells were washed three times with PBS and confocal images recorded using a Zeiss LSM 510 META microscope, irradiating at 405 nm with emission collected above 565 nm. In each case a sample of 5 images were recorded. Images shown in Fig. 7 and 8 and ESI† represent a fair sample of the recorded images. The two most notable observations are: (a) the different porphyrin species investigated, whereby R = Ph (H_2L^8), 4- $\text{O}_3\text{SC}_6\text{H}_4$ (H_2L^9) and $\text{MeNC}_5\text{H}_4^+$ (H_2L^{10}) show distinct cellular distributions; and (b) there is no distinguishable difference between the uptake in the positive CA IX expressing cell line and the empty vector cell line. Cellular imaging suggests that the uptake and localisation is strongly driven by the porphyrin unit and not by the sulfonamide groups. The H_2TPP -type derivative H_2L^8 shows strong cytosol uptake with no nuclear staining.

The sulfonate species H_2L^9 shows only very weak internalisation, as may be expected from the negative charge associated with the conjugate, whilst the pyridinium analogue H_2L^{10} shows strong nuclear uptake in both cell lines. For compound H_2L^{10} , nuclear uptake was confirmed by colocalisation with the nuclear stain DAPI (as shown in Fig. 8). The nuclear uptake of the pyridinium species mirrors the nuclear localisation observed by confocal fluorescence in the case of a positively charged tetra(methylquinolinium) porphyrin identified as a G-quadruplex interactive agent.⁴² In line with observations from the binding affinities measurements, it appears that the porphyrin unit dominate the cellular uptake of the conjugate. The porphyrin sulfonamides still had some affinity for the CA enzymes. However, the precise discrimination in terms of specific selectivity for the tumour expressing CA IX (and CA XII) enzyme(s) with respect to the other isoforms is not supported by this study.

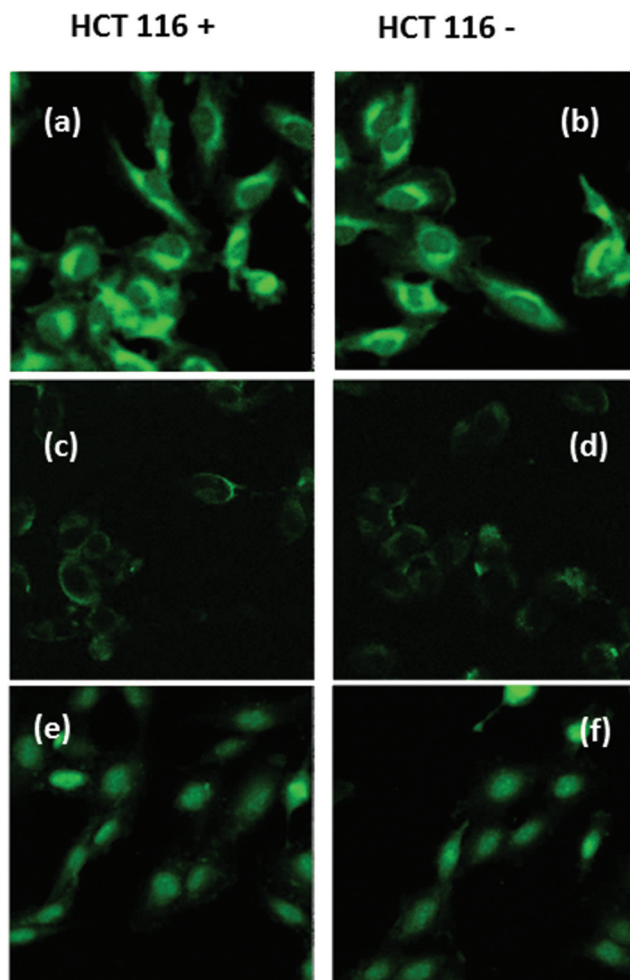


Fig. 7 Confocal fluorescence imaging: cellular uptake images of H_2L^8 (a) and (b); H_2L^9 (c) and (d); H_2L^{10} (e) and (f) in HCT 116 (CA IX positive, denoted HCT 116+ and CA IX negative denoted HCT 116-) cell lines. Typical bright-field images for the confocal fluorescence micrographs are provided in ESI†. Conditions are described in the text.

Radiolabelling tests for CA IX binding by the porphyrin $^{111}\text{In}(\text{Cl})\text{L}^9$

Whilst the porphyrin species do not show the same favourable differentials between CA IX and XII over both CA I and II as the small sulfonamide groups on their own, the medium strength binding affinities and reasonable selectivity ratios are not discouraging for all of the derivatives. Of particular interest is the sulfonate derivative which when present as the metal complex InClL^9 shows between 5–10 fold increased selectivity for CA IX over CA I and CA II. Whilst the H_2TTP and pyridinium species show by confocal fluorescence rapid cellular uptake by a mechanism independent of CA IX expression, the sulfonate species displayed less cellular association. This is probably a result of the negative charge on the porphyrin. This may make it more suitable for CA binding in the same manner as the fluorescein derivative. **FTES** shows little cellular uptake as a result of its negatively charged free acid group but binds

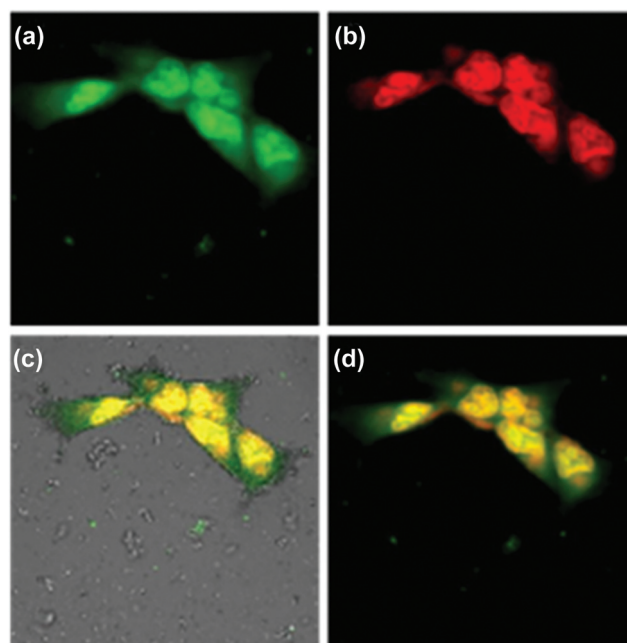


Fig. 8 Confocal fluorescence imaging showing the cellular uptake at 37 °C and 2 h incubation time: (a) H_2L^{10} ($\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} > 565 \text{ nm}$); (b) DAPI nuclear stain ($\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 465\text{--}535 \text{ nm}$); (c) overlay showing the simultaneous imaging with H_2L^{10} and DAPI nuclear stain; (d) overlay of H_2L^{10} , DAPI nuclear stain and brightfield image. Scalebar = micrograph width = 150 μm .

selectively to the membrane bound CA IX enzymes. The selectivity of the fluorescein derivative is only observed by confocal fluorescence after incubation for 24 h with a clear delineation of the cell membrane in CA IX expressing cells. As such uptake studies with the sulfonate compounds were repeated with 24 h incubation at 37 °C for H_2L^9 and InClL^9 . After 24 h it is observed that internalisation is now strongly evident with uniform uptake throughout the cytoplasm. No apparent difference between the HCT 116 CA IX positive and negative cell lines is observed. However it was noted that the cell lines after 24 h incubation showed clear signs of cell death, and uptake mechanisms independent of CA IX expression cannot be ruled out. Further studies will be required to measure the toxicity of these compounds. Incubation at concentrations orders of magnitude lower to reduce toxicity results in much weaker fluorescence and subsequently cellular uptake is not discernible from background autofluorescence. Whilst the porphyrins are fluorescent their quantum yields are still very weak (0.06 and 0.03 for H_2L^9 and its corresponding indium derivative InClL^9 , respectively) when compared to fluorescein (0.95) and so low concentrations (nanomolar range) of the compounds cannot be efficiently detected by fluorescence. To investigate further the porphyrins at lower concentrations radiolabelling studies have been performed.

Compound H_2L^9 was radiolabelled using an adapted method with respect to previously published conditions⁴² with $^{111}\text{InCl}_3$ and *in vitro* uptake studies carried out in the same two cell lines as used for confocal fluorescence tests. After 1, 3,

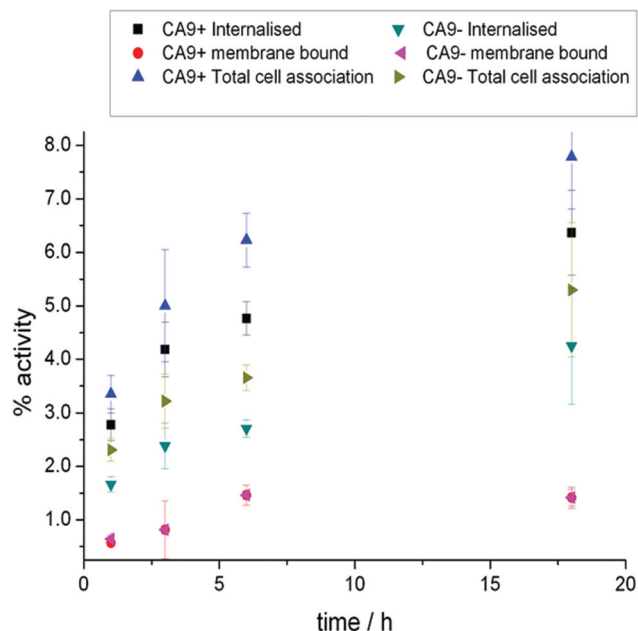


Fig. 9 Uptake of [111-In]-labelled H_2L^9 species in HCT116 (CA IX positive) and HCT116 (CA IX negative) cell lines showing % activity associated with the cell membrane and internalised for each cell line. Experiments were carried out in triplicate and error bars indicate one standard deviation of the mean.

6 and 18 h incubation (37 °C) of compound H_2L^9 with both the CA IX positive and CA IX negative cell lines the cells were washed with PBS (to remove activity in the supernatant), 1 M HCl (to remove activity associated with the cell membrane) and 1 M NaOH (to lyse the cells of internalised activity) sequentially, and the supernatant, membrane associated activity and internalised associated activity isolated respectively and measured separately. Cell percentage uptake is given as the amount of activity internalised *i.e.* the activity in the 1 M NaOH wash with respect to the total activity and the amount of activity associated with the membrane *i.e.* the activity in the 1 M HCl wash with respect to the total activity. The uptake data suggests low uptake in line with the fluorescence images. Identical weak membrane associated activity is measured for both cell lines suggesting that no sulfonamide binding occurs in the CA IX positive cell line (Fig. 9).

Cell uptake is observed which may be mediated by sulfonamide binding but is more likely to arise from the intrinsic uptake of the porphyrin conjugate. A higher percentage of radioactivity is associated with the CA IX positive cell line, which might suggest some positive sulfonamide response, but there is potential for variation in the amount of protein in the different cell population, despite the same initial cell concentration. Detailed protein assays are beyond the scope of this work and were not completed so the difference in internalised activity as a result of difference in cell population cannot be immediately ignored. Comparable changes in cell uptake in both cell lines over the time course are observed for the total cell associated activity with a difference in associated activity

over 18 h of only 1.5%. This again suggests that there is no CA mediated mechanism of cell inclusion of these porphyrin conjugates.

A closer inspection of the CA binding data (Table 1) suggested that the sulfonamide species **InCIL⁹** has an increased binding affinity of 5–10 fold in a CA IX expressing cell line based on binding affinities alone. This should lead to increased membrane and potentially increased internalised associated activity. On the basis of radiolabelling studies no such increase in uptake or binding is observed, which is in agreement with the confocal fluorescent studies and suggests strongly that uptake and/or binding is not receptor-mediated *via* initial carbonic anhydrase recognition. Furthermore, the anionic sulfonamides show no apparent recognition for the CA IX receptors and the weak uptake is most probably driven by the intrinsic accumulation of the porphyrin entity. Given the rapid and strong cellular uptake of the cationic and neutral porphyrin species it was felt unlikely that they would confer any better differential, with their cellular uptake dominated purely by the porphyrin entity and by a pathway independent of sulfonamide binding to CA IX enzymes.

Conclusions

A range of new sulfonamide conjugated metal complexes involving bis(thiosemicarbazone), amidothiolate and porphyrin ligands have been prepared with a view to developing PET and SPECT agents based on metallic radionuclides to target CA IX expression *in vivo*. These compounds were all fully characterised and successful, radiolabelling protocols resulting in a high radiochemical incorporation were established for the 64-Copper and 99m-Techneium complexes. Those complexes with solubility and stability in an aqueous environment suitable for the CA enzyme inhibition assay were screened for activity, by testing their ability to inhibit CA IX and other isoforms of the enzyme *in vitro*. A series of sulfonamide porphyrin conjugates has been synthesised and fully characterised. Water soluble analogues have been developed for greater biocompatibility with no sign of acid hydrolysis of the amide bonds under sulfonating conditions and no sign of alkylation of the thiadiazole ring under methylating conditions. A library of sulfonamide derivatives has been studied for their binding affinities to specific CA receptors to establish their affinity for the poor prognostic CA IX and CA XII enzymes. Our findings for the rhenium derivatives are comparable with those recently reported for organometallic [(Cp-R)-M(CO)₃] (M = Re or 99m-Tc) Arylsulfonamide conjugates investigated for the selective targeting of human Carbonic Anhydrase IX.^{8c} In general the porphyrin sulfonamide conjugates displayed positive selectivity for the CA IX and CA XII enzymes though metal insertion into the porphyrin resulted in loss of selectivity and reduced binding affinity. Conversely the sulfonate porphyrins showed the reverse effect showing improved binding and increased selectivity for CA IX and CA XII on indium complexation. Fluorescent studies in a CA IX positive

and CA IX negative cell line indicate (after 2 h and 24 h incubation at 37 °C) that there is no obvious difference in the cellular binding or uptake between the two cell lines for the sulfonyl porphyrin species. A strong structural correlation with cellular uptake is observed with the neutral tetraphenyl porphyrins showing strong cytoplasm uptake, the cationic porphyrins showing nuclear accumulation whilst the sulfonyl porphyrins display only weak cell uptake. Radiolabelling studies with an indium(III)-labelled sulfonated **ABS** porphyrin identified identical weak membrane binding for the CA IX positive and CA IX negative cell lines and comparable cell internalisation suggesting that the conjugates cellular distribution and uptake was dominated by the porphyrin core. Although these results showed no direct correlations between the structures and physical properties of the complexes and the observed activities, two compounds based on the porphyrin motif were identified as being good candidates to take forward to cellular and *in vivo* investigations.

Experimental procedures

General

All reagents were purchased from Sigma-Aldrich, Merck Chemicals or Alfa-Aesar and were used as received unless otherwise stated. $\text{Na}^{99\text{m}}\text{TcO}_4$ was obtained from a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator (GE Healthcare, UK). H_2ATSM and $\text{H}_2\text{ATSM/en}$ were prepared according to published methods.^{32,33} ESI contains further details on HPLC and radioHPLC methods A–C used and intermediates' syntheses.

Diacetyl(*N*-4-methylthiosemicarbazone)(*N*-4-aminoethylamidophenyl-4-sulphamoylthiosemicarbazone), H_2L^1 . $\text{H}_2\text{ATSM/en}$ (150 mg, 0.51 mmol), 4-sulfamoylbenzoic acid (104 mg, 0.51 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (119 mg, 0.62 mmol) and hydroxybenzotriazole (84 mg, 0.62 mmol) were placed under N_2 in a schlenk flask and DMF (5 ml) added. The yellow mixture was stirred for 18 h at room temperature and water (50 ml) added generating a yellow precipitate. This was collected by filtration, washed with water and diethylether, and dried *in vacuo* to provide H_2L^1 (197 mg, 0.42 mmol, 82%).

^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 10.34 (s, 1H, $\text{NHN}=\text{C}$), 10.24 (s, 1H, $\text{NHN}=\text{C}$), 8.88 (t, $J = 5.3$ Hz, 1H, $\text{CH}_2\text{NHC}=\text{S}$), 8.60 (t, $J = 5.3$ Hz, 1H, $\text{CH}_2\text{NHC}=\text{O}$), 8.39 (q, $J = 4.40$ Hz, 1H, $\text{CH}_3\text{NHC}=\text{S}$), 8.00 (d, $J = 8.50$ Hz, 2H, *ArH*), 7.89 (d, $J = 8.50$ Hz, 2H, *ArH*), 7.49 (s, 2H, SO_2NH_2), 3.78 (td, $J = 5.7$, 5.3 Hz, 2H, $\text{CH}_2\text{NHC}=\text{S}$), 3.54 (td, $J = 5.7$, 5.3 Hz, 2H, $\text{CH}_2\text{NHC}=\text{O}$), 3.02 (d, $J = 4.51$ Hz, 2H, $\text{CH}_3\text{NHC}=\text{S}$), 2.23 (s, 3H, $\text{CH}_3\text{C}=\text{N}$), 2.21 (s, 3H, $\text{CH}_3\text{C}=\text{N}$). $^{13}\text{C}\{^1\text{H}\}$ NMR (75.5 MHz, $\text{DMSO}-d_6$) δ ppm 178.5 ($\text{C}=\text{S}$), 178.1 ($\text{C}=\text{S}$), 165.9 ($\text{C}=\text{O}$), 148.4 ($\text{C}=\text{N}$), 148.0 ($\text{C}=\text{N}$), 146.3 (*Ar*), 137.2 (*Ar*), 128.0 (*Ar*), 125.6 (*Ar*), 43.9 (CH_2), 38.9 (CH_2), 31.3 (CH_3NH), 11.8 ($\text{CH}_3\text{C}=\text{N}$), 11.7 ($\text{CH}_3\text{C}=\text{N}$). MS ES^+ : found 495.1, calc. for $[\text{M} + \text{Na}]^+$ ($\text{C}_{16}\text{H}_{24}\text{N}_8\text{NaO}_3\text{S}_3$) 495.1; ES^- : found 471.1, calc. for $[\text{M} - \text{H}]^-$ ($\text{C}_{16}\text{H}_{23}\text{N}_8\text{O}_3\text{S}_3$) 471.1. HPLC $R_t = 10.86$ min.

Diacetyl(*N*-4-methylthiosemicarbazone)(*N*-4-aminoethylamidophenyl-4-sulphamoylthiosemicarbazone)zinc(II), ZnL^1 . H_2L^1 (40 mg, 85 μmol) and $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ (22 mg, 100 μmol) were stirred together in methanol (5 ml) at room temperature for 2 h forming a bright yellow solution. Distilled water (20 ml) was added and the solution evaporated to remove the methanol. The solution was extracted with ethyl acetate (3×10 ml) and the combined organic fractions dried (MgSO_4), evaporated and dried under vacuum to give ZnL^1 as a bright yellow solid (37 mg, 69 μmol , 81%).

^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ ppm 8.72 (t, $J = 4.5$ Hz, 1H, $\text{CH}_2\text{NHC}=\text{O}$), 7.97 (d, $J = 8.5$ Hz, 2H, *ArH*), 7.88 (d, $J = 8.5$ Hz, 2H, *ArH*), 7.49 (s, 2H, SO_2NH_2), 3.51–3.44 (m, 2H, CH_2), other CH_2 obscured by water peak, 3.16 (d, $J = 5.0$ Hz, 2H, $\text{CH}_3\text{NHC}=\text{S}$), 2.20 (s, 3H, $\text{CH}_3\text{C}=\text{N}$), 2.18 (s, 3H, $\text{CH}_3\text{C}=\text{N}$). HRMS ES^+ : found 535.0320, calc. for $[\text{M} + \text{H}]^+$ ($\text{C}_{16}\text{H}_{23}\text{N}_8\text{O}_3\text{S}_3\text{Zn}$) 535.0347. HPLC $R_t = 10.12$ min.

Diacetyl(*N*-4-methylthiosemicarbazone)(*N*-4-aminoethylamidophenyl-4-sulphamoylthiosemicarbazone)copper(II), CuL^1 . H_2L^1 (40 mg, 85 μmol) and $\text{Cu}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ (22 mg, 100 μmol) were stirred together in methanol (5 ml) at room temperature for 2 h forming a brown solution. Distilled water (20 ml) was added and the solution evaporated to 1/3 volume to produce CuL as a brown precipitate, which was collected by filtration and dried under vacuum (20 mg, 38 μmol , 44%). HRMS ES^+ : found 534.0364, calc. for $[\text{M} + \text{H}]^+$ ($\text{C}_{16}\text{H}_{23}\text{N}_8\text{O}_3\text{S}_3\text{Cu}$) 534.0351. HPLC $R_t = 11.98$ min.

Syntheses of technetium and rhenium conjugates

***N*-(2-Oxo-2-((2-oxo-2-((4-sulfamoylphenylethyl)amino)ethyl)-amino)ethyl)-2-(tritylthio)acetamide TrtMAG₃-ABS, TrtH₃L².** Succinimido-2-((triphenylmethyl)thio)acetate (**1**) was prepared following literature procedures.^{3,34} To a solution of (**1**) (3.44 g, 7.98 mmol) in 1,2-dimethoxyethane (35 mL) and DMF (18 mL) was added a solution of triglycine (1.52, 8.04 mmol) and NaHCO_3 (1.38 g) in distilled water (18 mL). The resulting solution was stirred at room temperature for 3 h and then concentrated *in vacuo* to remove 1,2-dimethoxyethane. Dilution with distilled water (35 mL) and treatment with 50% aqueous citric acid (6 mL) gave a white solid of Trt-MAG₃ (**2**), which was recrystallized in ethanol (2.90 g, 71.9%).

To a solution of (**2**) (393 mg, 0.777 mmol) in DMF (5 mL) was added **ABS** (155 mg, 0.774 mmol), EDC (149 mg, 0.777 mmol), HOBT (104 mg, 0.770 mmol) and TEA (24 μL). The reaction was left in ice bath for 1 h and then at RT for 1 day. The solvent was evaporated thereafter. The residue was re-dissolved in ethyl acetate (10 mL) and washed with 10% citric acid, 3% NaHCO_3 and saturated brine, 20 mL each. The solids, which precipitated when washed with NaHCO_3 and saturated brine, were collected. The organic solution was collected and dried under vacuum. The solutions of the raw product were combined and recrystallized from ethanol–water to give TrtH₃L² (440 mg, 82.6%).

^1H NMR (d_6 -DMSO, δ , 300 MHz): 2.75 (t, $J = 7.2$ Hz, 2H), 2.84 (s, 2H), 3.25 (m, 2H), 3.61 (m, 4H), 3.68 (d, $J = 4.9$ Hz,

2H), 7.21–7.37 (m, 17H), 7.71 (d, J = 8.3 Hz, 2H), 7.92 (t, J = 5.3 Hz, 1H), 8.12 (t, J = 5.7 Hz, 1H), 8.22–8.25 (m, 2H). ^{13}C NMR (d_6 -DMSO, δ , 75 MHz): 35.2, 36.4, 42.5, 42.6, 42.8, 42.8, 66.4, 126.1, 127.3, 128.5, 129.5, 129.5, 142.5, 144.0, 144.4, 168.2, 169.1, 169.5, 169.6. ES/MS(+): m/z = 688.2915 (Calc. m/z = 688.2264) $[\text{M} + \text{H}]^+$. HPLC (Method B): R_t = 11.88 min.

***N,N'*-(3-Oxo-3-((4-sulfamoylphenylethyl)amino)propane-1,2-diyl)bis(2-(tritylthio)acetamide, TrtDADS-ABS, $\text{H}_3\text{L}^3\text{Trt}$.** Ethyl 2,3-bis(triphenylmethylthioacetamino)propanoate (**3**) was prepared following the literature procedure.³⁵ To **3** (1.05 g, 1.33 mmol) in a 1 : 1 mixture of THF and water (80 mL), NaOH (100 mg, 2.5 mmol) was added. The mixture was heated to reflux for 3 h under N_2 . The mixture was adjusted to pH = 2 by addition of 6 M HCl and was concentrated to 30 mL under reduced pressure. The precipitate that formed was collected by filtration and dried under vacuum to give Trt-DADS (**4**) (825 mg, 84.2%). To a solution of **4** (147 mg, 0.199 mmol) in DMF (5 mL) was added ABS (40.0 mg, 0.200 mmol), EDC (37.8 mg, 0.200 mmol), HOBT (26.7 mg, 0.198 mmol) and TEA (6 μL). The reaction was left in ice bath for 1 h and then at RT overnight. The solvent was evaporated and the residue re-dissolved in ethyl acetate (20 mL). The solution was washed with 10% citric acid, 3% NaHCO_3 and saturated brine, 20 mL each. The organic layer was collected and dried under vacuum. The raw product was combined and recrystallized from ethanol-water to give TrtH_3L^3 (108 mg, 59.0%).

^1H NMR (d_6 -DMSO, δ , 300 MHz): 2.70 (m, 2H), 2.74 (s, 2H), 2.80 (s, 2H), 3.11 (m, 2H), 3.21 (m, 2H), 4.11 (m, 1H), 7.19–7.34 (m, 32H), 7.71 (d, J = 8.4 Hz, 2H), 7.86 (t, J = 5.7 Hz, 1H), 8.00–8.07 (m, 2H). ^{13}C NMR (d_6 -DMSO, δ , 75 MHz): 35.2, 36.4, 36.5, 40.4, 41.1, 53.3, 66.3, 126.1, 127.2, 128.5, 129.5, 142.5, 144.0, 144.4, 144.5, 167.8, 168.1, 169.6. ES/MS(+): m/z = 941.541 (Calc. m/z = 941.284) $[\text{M} + \text{Na}]^+$. HPLC (Method B in ESI $^+$): R_t = 15.37 min.

***N'*-(Pyridine-2-ylmethyl)-*N*⁵-(4-sulfamoylphenylethyl)-2-(2-tritylthio)acetamido)pentanediamide, TrtMAGpy-ABS, $\text{H}_2\text{L}^4\text{Trt}$.** Trt-MAGpy (**5**) was synthesized following the literature procedure.³⁶ Compound **5** (111 mg, 0.200 mmol) was added to DMF (5 mL) and mixed with ABS (40.0 mg, 0.200 mmol), EDC (37.8 mg, 0.200 mmol), HOBT (26.7 mg, 0.198 mmol) and TEA (6 μL). The reaction was left at RT for 1 day. The solvent was evaporated and the residue was washed with 0.1 M HCl (3 \times 10 mL) and ethyl acetate (2 \times 10 mL). The solid was dried under vacuum to give $\text{H}_2\text{L}^4\text{Trt}$ (37.8 mg, 25.7%).

^1H NMR (d_6 -DMSO, δ , 300 MHz): 1.77 (m, 2H), 1.96 (t, J = 7.7 Hz, 2H), 2.65 (t, J = 6.8 Hz, 2H), 2.76 (s, 2H), 3.14 (td, J_A = 6.6 Hz, J_B = 19.3 Hz, 2H), 4.09 (td, J_A = 5.4 Hz, J_B = 7.8 Hz, 1H), 4.24 (d, J = 5.7 Hz, 2H), 7.08–7.33 (m, 18H), 7.63 (d, J = 8.0 Hz, 2H) Ar-*H*; 7.86 (t, J = 5.5 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 8.36 (d, J = 3.9 Hz, 1H), 8.41 (t, J = 5.7 Hz, 1H). ^{13}C NMR (d_6 -DMSO, δ , 75 MHz): 26.9, 31.5, 34.8, 35.7, 40.0, 41.8, 53.6, 66.9, 123.9, 124.3, 125.9, 126.7, 127.7, 129.1, 129.3, 141.6, 143.2, 143.8, 143.9, 144.1, 155.3, 170.2, 172.8, 173.2. ES/MS(+): m/z = 736.2386 (Calc. m/z = 736.2628) $[\text{M} + \text{H}]^+$. HPLC (Method A): R_t = 12.04 min.

***N*-(2-Oxo-2-((2-oxo-2-((2-oxo-2-((5-sulfamoyl-1,3,4-thiadiazol-2-yl)amino)ethyl)amino)ethyl)amino)ethyl)amino)-2-(tritylthio)acetamide, TrtMAG₃-glyAZA, $\text{H}_3\text{L}^5\text{Trt}$.** Trt-MAG₃ (**2**) (50.5 mg, 0.100 mmol) was added to DMF (5 mL) and mixed with glyAZA (35.1 mg, 0.100 mmol), EDC (20.0 mg, 0.105 mmol), HOBT (13.5 mg, 0.100 mmol) and TEA (20 μL). The reaction was left in ice bath for 1 h and then at room temperature for 1 day. The solvent was evaporated and the residue was re-dissolved in ethyl acetate (10 mL). The solution was washed with 1 M HCl, 3% NaHCO_3 and saturated brine, 20 mL each. The organic solution was collected and dried under vacuum. The raw product was recrystallized from ethanol-water to give $\text{H}_3\text{L}^5\text{Trt}$ (41.0 mg, 56.6%).

^1H NMR (d_6 -DMSO, δ , 300 MHz): 2.84 (s, 2H), 3.63 (d, J = 5.6 Hz, 2H), 3.71 (d, J = 5.0 Hz, 2H), 3.77 (d, J = 5.9 Hz, 2H), 4.08 (d, J = 5.5 Hz, 2H), 7.24–7.34 (m, 15H), 8.11–8.19 (m, 4H), 8.35 (s, 2H), 13.11 (s, 1H). ^{13}C NMR (d_6 -DMSO, δ , 75 MHz): 36.3, 42.2, 42.4, 42.7, 66.4, 127.3, 128.5, 129.5, 144.5, 161.5, 164.9, 168.2, 169.4, 169.6, 170.0, 171.5. ES/MS(–): m/z = 723.1169 (Calc. m/z = 723.1478) $[\text{M} - \text{H}]^-$. HPLC (Method A): R_t = 9.88 min.

***N*⁵-(2-Oxo-2-((5-sulfamoyl-1,3,4-thiadiazol-2-yl)amino)ethyl)-*N*¹-(pyridine-2-ylmethyl)-2-(2-(tritylthio)acetamido)pentanediamide, TrtMAGpy-glyAZA or $\text{H}_2\text{L}^6\text{Trt}$.** Trt-MAGpy (**5**) (79.7 mg, 0.144 mmol) was mixed with BOP (76.4 mg, 0.173 mmol) and DIPEA (90 μL , 0.52 mmol) in DMF (5 mL) in ice bath for 20 min., then glyAZA (synthesised as above, (c) (60.7 mg, 0.173 mmol) was added. The mixture was stirred at RT overnight. The solvent was evaporated, and 3 M HCl (20 mL) and ethyl acetate (20 mL) were used consecutively to wash off the impurities. The solid was further purified by silica gel chromatography using DCM–MeOH (9/1 by volume) to give $\text{H}_2\text{L}^6\text{Trt}$ (32.3 mg, 29.0%).

^1H NMR (d_4 -CD₃OD, δ , 500 MHz): 1.87–2.15 (m, 2H), 2.29–2.42 (m, 2H), 3.08 (d, J = 1.26 Hz, 2H), 4.15–4.25 (m, 2H), 4.27 (dd, J_A = 7.88, J_B = 5.99 Hz, 1H), 4.53 (s, 2H), 4.61 (br, 2H), 7.09–7.52 (m, 17H), 7.80 (td, J_A = 7.72, J_B = 1.58 Hz, 1H), 8.48 (d, J = 4.41 Hz, 1H). ^{13}C NMR (d_4 -CD₃OD, δ , 125 MHz): 28.7, 32.6, 37.2, 43.6, 45.4, 54.7, 68.5, 123.0, 123.9, 128.2, 129.2, 130.8, 139.0, 145.6, 149.7, 158.9, 163.3, 166.6, 170.7, 171.4, 173.6, 175.7. ES/MS(+): m/z = 795.1804 (Calc. m/z = 795.1812) $[\text{M} + \text{Na}]^+$. HPLC (Method A): R_t = 12.04 min.

Syntheses of rhenium complexes

Sodium *N*-(2-oxo-2-((2-oxo-2-((4-sulfamoylphenylethyl)amido)ethyl)amido)ethyl)-2-thioacetamido-oxorhenium(v), Na[ReOMAG₃-ABS], Na[ReOL²]. Trt-MAG₃ (**5**) (240 mg, 0.474 mmol) and sodium acetate (258 mg, 3.14 mmol) were mixed in dry methanol (60 mL), followed by addition of precursor $[\text{ReOCl}_3(\text{PPh}_3)_2]$ (395 mg, 0.474 mmol). After being refluxed for 4 h, the solution turned orange. After cooling, the solution was filtered and evaporated to dryness. The residue was chromatographed on silica gel with DCM–methanol. The orange eluent was collected and dried to give Na[ReO-MAG₃] (**6**) (185 mg, 80.6%). After **6** (48.4 mg, 0.100 mmol) was dissolved in phosphate buffer (10 mL, 0.1 M, pH = 6.0), ABS (20.0 mg, 0.100 mmol) in

acetonitrile (1 mL) was added to the solution, followed by EDC (19.1 mg, 0.100 mmol) and TEA (14 μ L). The mixture was stirred at room temperature overnight. After the solvent was evaporated, the residue was chromatographed on silica gel with DCM–methanol (3/1 by volume). The orange eluent was collected and dried to give Na[ReOMAG₃-ABS] (37.1 mg, 55.6%).

¹H NMR (*d*₄-CD₃OD, δ , 500 MHz): 2.79–2.91 (m, 2H), 3.41 (t, *J* = 7.3 Hz, 2H), 3.78 (d, *J* = 17.2 Hz, 1H), 4.02 (d, *J* = 17.3 Hz, 1H), 4.26 (d, *J* = 18.0 Hz, 1H), 4.28 (d, *J* = 18.1 Hz, 1H), 4.40 (d, *J* = 18.0 Hz, 1H), 4.65 (d, *J* = 16.6 Hz, 1H), 4.74 (d, *J* = 18.2 Hz, 1H), 4.99 (d, *J* = 16.6 Hz, 1H), 7.39 (d, *J* = 8.3, 2H), 7.82 (d, *J* = 8.2, 2H), ¹³C NMR (*d*₄-CD₃OD, δ , 125 MHz): 34.3, 41.1, 41.5, 43.5, 49.9, 54.5, 57.7, 127.8, 130.5, 142.7, 144.0, 191.4, 194.7, 195.8. ES/MS(–): *m/z* = 644.0281 (Calc. *m/z* = 643.9832) [M][–]. IR: 978 cm^{–1} (Re=O), 1616 cm^{–1} (C=O). UV-Vis: $\lambda_{\text{abs}}(\text{MeOH})/\text{nm}$ ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$) = 224 (2.52 $\times 10^4$), 274 (sh, 5.73 $\times 10^3$), 339 (sh, 3.23 $\times 10^2$), 402 (1.27 $\times 10^2$), 485 (22). HPLC (Method C): *R*_t = 3.03 min.

***N,N'*-(3-Oxo-3-((4-sulfamoylphenylethyl)amido)propane-1,2-diyl)bis(2-thioacetamido)oxorhenium(v) Na[ReODADS-ABS], or Na[ReOL³].** Na[ReODADS] (7) was synthesized from (4) according to the method described for Na[ReOMAG₃] (6); (50.1 mg, 70.5%). The title compound was synthesized by coupling 7 with ABS using the same method as described above for the MAG₃ system (35.8 mg, 51.5%). ¹H NMR (*d*₄-CD₃OD, δ , 500 MHz): 3.03 (m, 2H), 3.20 (t, *J* = 7.4, 2H), 3.78 (d, *J* = 17.4 Hz, 1H), 3.84 (d, *J* = 17.1 Hz, 1H), 4.14 (d, *J* = 17.4 Hz, 1H), 4.43 (d, *J* = 16.9 Hz, 1H), 4.75 (d, *J* = 8.4 Hz, 1H), 5.00 (d, *J* = 12.9 Hz, 1H), 7.50 (d, *J* = 8.6, 2H), 7.91 (d, *J* = 8.5, 2H). (N.B.: the ¹H resonance of the other NCH₂CHN proton was overlapped with other peaks). ¹³C NMR (*d*₄-CD₃OD, δ , 125 MHz): 32.8, 40.1, 42.9, 43.5, 59.2, 69.6, 126.3, 129.1, 141.3, 142.4, 194.2, 195.8, 196.4. ES/MS(–): *m/z* = 632.9900 (Calc. *m/z* = 632.9941) [M][–]. IR: 962 cm^{–1} (Re=O), 1581 cm^{–1} (C=O). UV-Vis: $\lambda_{\text{abs}}(\text{MeOH})/\text{nm}$ ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$) = 226 (2.93 $\times 10^4$), 249 (sh, 1.10 $\times 10^4$), 300 (sh, 3.15 $\times 10^3$), 332 (sh, 1.58 $\times 10^3$), 402 (1.83 $\times 10^2$), 489 (11). HPLC (Method 3): *R*_t = 4.27 min.

***N*-(2-Oxo-2-((2-oxo-2-((2-oxo-2-((5-sulfamoyl-1,3,4-thiadiazol-2yl)amido)ethyl)amido)ethyl)amido)ethyl)amido)-ethyl-2-(thio)acetamido Na[ReOMAG₃-glyAZA] or Na[ReOL⁵].** The title compound was synthesized by coupling 6 with glyAZA using an analogous method to that described for the synthesis of (45.1 mg, 64.1%). ¹H NMR (*d*₄-CD₃OD, δ , 500 MHz): 2.91 (d, *J* = 6.9, 2H), 3.82 (d, *J* = 17.2 Hz, 1H), 4.05 (d, *J* = 17.2 Hz, 1H), 4.29 (d, *J* = 18.6 Hz, 1H), 4.32 (d, *J* = 18.8 Hz, 1H), 4.42 (d, *J* = 18.2 Hz, 1H), 4.73 (d, *J* = 17.1 Hz, 1H), 4.79 (d, *J* = 18.1 Hz, 1H), 5.05 (d, *J* = 17.1 Hz, 1H). ¹³C NMR (*d*₄-CD₃OD, δ , 125 MHz): $\delta(\text{ppm})$ = 41.1, 43.6, 54.5, 56.1, 57.7, 164.61, 191.5, 194.7, 195.8. ES/MS(–): *m/z* = 680.9280 (Calc. *m/z* = 680.9650) [M][–]. IR: 977 cm^{–1} (Re=O), 1629 cm^{–1} (C=O). UV-Vis: $\lambda_{\text{abs}}(\text{MeOH})/\text{nm}$ ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$) = 221 (1.43 $\times 10^4$), 270 (sh, 4.61 $\times 10^3$), 337 (sh, 3.18 $\times 10^2$), 397 (1.03 $\times 10^2$), 491 (18). HPLC (Method A): *R*_t = 6.62 min.

5-[4-(*N*-Propyl benzamide)]-10,15,20-tri(4-pyridyl)-porphyrin, [TPyP-propyl, H₂L⁷]. 4-(10,15,20-Triphenylporphyrin-5-yl)-benzoic acid³⁷ and 5-(4-carboxyphenyl)-10,15,20-tris(4-pyridyl)-porphyrin,³⁸ were synthesised according to literature methods with analytical data in accord with the literature values (ESI[†]).

5-[4-(*N*-Propyl benzamide)]-10,15,20-tri(4-pyridyl)-porphyrin was synthesised employing general amide coupling method B (ESI[†]). 5-(4-Carboxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin **9** (see ESI[†]) (75 mg, 0.113 mmol), DIPEA (38.7 mg, 52.3 μ L, 0.300 mmol), propylamine (7.97 mg, 8.04 μ L, 0.135 mmol), BOP (67.5 mg, 0.146 mmol), CHCl₃ (10 mL). 5-[4-(*N*-propyl benzamide)]-10,15,20-tri(4-pyridyl)-porphyrin was purified *via* silica gel chromatography (0% to 5% MeOH in CHCl₃). Yield: 65.2 mg, 0.093 mmol, 82%.

¹H NMR (300 MHz, *d*₆-DMSO, 25 °C): δ 9.40 (d, 6H, *J* = 6.1, CH_{py}), 9.14 (m, 8H, CH_{pyrr}), 8.91 (m, 6H, CH_{py}), 8.65 (t, 1H, *J* = 5.5, CONH), 8.27 (d, 2H, *J* = 7.9, ArH), 8.15 (d, 2H, *J* = 8.1, ArH), 3.52 (m, 2H, CONHCH₂CH₂CH₃), 1.81 (m, 2H, CONHCH₂CH₂CH₃), 1.07 (t, 2H, *J* = 6.9, CONHCH₂CH₂CH₃), –2.95 (br s, 2H, ring NH). ESI-MS calcd for C₄₄H₃₅N₈O [M + H]⁺ 703.2928, found 703.2935.

5-[4-(*N*-Propyl benzamide)]-10,15,20-tri(4-pyridyl)-porphyrin-chloroindium(III), InClL⁷. This was prepared using general metal complexation method 1. H₂L⁷ (50.0 mg, 0.072 mmol), NaOAc (35.0 mg, 0.429 mmol), InCl₃ (32.0 mg, 0.143 mmol), HOAc (10 mL). Yield: 51.2 mg, 0.061 mmol, 84%.

¹H NMR: (300 MHz, *d*₆-DMSO, 25 °C): 8.99 (s, 8H, CH_{pyrr}), 8.50 (t, 1H, *J* = 5.4, CONH), 8.31 (2 \times d, 2 \times 2H, ArH), 8.22 (m, 6H, *o*-Ph), 7.85 (m, 9H, *o,m,p*-Ph), 3.43 (m, 2H, CONHCH₂CH₂CH₃), 1.75 (m, 2H, CONHCH₂CH₂CH₃), 1.02 (t, 3H, CONHCH₂CH₂CH₃). ESI-MS Calculated for C₄₈H₃₆InN₅O [M – Cl]⁺, 812.1875, found 812.1855; HPLC: (method A in ESI[†]): *R*_t = 10.64 min.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-triphenylporphyrin, [H₂TPP-ABS], H₂L⁸. The title compound was synthesised employing general peptide coupling method 1. **8** (see ESI[†]) (250 mg, 0.331 mmol), 4-(2-aminoethyl)benzenesulfonamide (100 mg, 0.500 mmol), DIPEA (64.6 mg, 87.1 μ L, 0.500 mmol), DMF (10 mL). Purification was achieved by silica gel chromatography (5% MeOH in CHCl₃). Yield: 236 mg, 0.281 mmol, 85%.

¹H NMR (300 MHz, *d*₆-DMSO, 25 °C): δ 8.99 (t, 1H, *J* = 5.5, CONH), 8.81 (m, 8H, CH_{pyrr}), 8.29 (d, 2H, *J* = 8.1, ArH), 8.23 (d, 2H, *J* = 8.4, ArH), 8.17 (m, 6H, *o*-ArH), 7.84 (d, 2H, *J* = 8.4, ArH), 7.77 (m, 9H, *m,p*-ArH), 7.55 (d, 2H, *J* = 8.4 ArH), 7.37 (s, 2H, SO₂NH₂), 3.69 (m, 2H, CONHCH₂CH₂), 3.06 (t, 1H, *J* = 7.2, CONHCH₂CH₂). ¹³C NMR (125 MHz, *d*₆-DMSO, 25 °C): δ 166.3, 144.0, 143.9, 142.2, 141.2, 134.2, 134.1, 131.0 (bs), 129.3, 128.1, 127.0, 125.9, 120.3, 120.2, 119.1, 34.9, 25.3. Mass Spectrum ESI-MS calcd for C₅₃H₄₀N₆O₃S [M + H]⁺ 841.2961, found 841.2768. HPLC: (Method A) *R*_t = 11.81 min. Elem. Anal.: Found: C; 75.2%, H; 4.9%, N; 10.1%. Calc.: C; 75.7%, H; 4.8%, N; 10.0%.

5-[4-(*N*-(2-Oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)-ethyl)benzamide)]-10,15,20-triphenylporphyrin, [H₂TPP-AZA], H₂L¹¹. The title compound was synthesised employing general amide coupling method 1. **8** (330 mg, 0.436 mmol), glyAZA (180 mg, 0.512 mmol), DIPEA (66.2 mg, 89.2 μ L, 0.512 mmol), DMF (10 mL). Purification was achieved *via* silica gel chromatography (2 to 8% MeOH in CHCl₃). Yield: 256 mg, 0.292 mmol, 67%.

^1H NMR (500 MHz, d_6 -DMSO, 25 °C): δ 13.39 (s, 1H, CONH-thiadiazole), 9.44 (t, 1H, $J = 5.5$, CONH), 8.84 (m, 8H, CH_{pyrr}), 8.40 (s, 2H, SO_2NH_2), 8.37 (m, 4H, ArH), 8.23 (m, 6H, o -ArH), 7.84 (m, 9H, m , p -ArH), 4.45 (d, 2H, $J = 5.6$, $\text{CONHCH}_2\text{CONH}$), -2.90 (s, 2H, ring NH). ^{13}C NMR (125 MHz, d_6 -DMSO, 25 °C): δ 169.6, 166.8, 164.5, 161.3, 144.5, 141.1, 134.2, 134.2, 133.0, 131.5, 128.1, 127.0, 126.1, 120.3, 120.1, 118.9, 43.0. ESI-MS calcd for $\text{C}_{49}\text{H}_{36}\text{N}_9\text{O}_4\text{S}_2$ [$\text{M} + \text{H}$] $^+$ 878.2326, found 878.2275. HPLC: (Method A) $R_t = 10.82$ min. Elem. Anal.: Found: C; 66.8%, H; 3.8%, N; 14.4%. Calc.: C; 67.0%, H; 4.0%, N; 14.4%.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-triphenylporphyrin(chloroindium(III)), [In(Cl)TPP-ABS] or [InCl] 8 . The title compound was synthesised employing general metal complexation method 1. H_2L^8 (74.5 mg, 0.087 mmol), NaOAc (43.6 mg, 0.532 mmol), InCl_3 (39.2 mg, 0.177 mmol), AcOH (10 mL). A maroon coloured solid was obtained. Yield: 69.7 mg, 0.070 mmol, 81%.

^1H NMR (300 MHz, d_6 -DMSO, 25 °C): δ 9.11 (t, 1H, $J = 5.6$, CONH), 9.13–9.01 (m, 8H, CH_{pyrr}), 8.26 (d, 2H, $J = 7.8$, ArH), 8.19 (m, 8H, o -Ph, ArH), 7.95 (d, 2H, $J = 8.2$, ArH), 7.89 (m, 9H, m , p -Ph), 7.51 (d, 2H, $J = 8.0$, ArH), 3.71 (m, 2H, $\text{CONHCH}_2\text{CH}_2$), 3.04 (t, 2H, $J = 7.6$, $\text{CONHCH}_2\text{CH}_2$). ^{13}C NMR (75.5 MHz, d_6 -DMSO, 25 °C): δ 166.1, 148.0, 147.8, 147.7, 147.4, 144.5144.1, 141.9, 141.3, 134.4, 134.2, 133.9, 132.54, 132.51, 132.4, 130.5, 128.1, 127.1, 125.9, 125.8, 121.5, 121.3, 120.1, 38.2, 27.4. ESI-MS calcd for $\text{C}_{53}\text{H}_{38}\text{InN}_6\text{O}_3\text{S}$ [$\text{M} - \text{Cl}$] $^+$ 953.1765, found 953.1789. HPLC: (Method A) $R_t = 10.12$ min. Elem. Anal.: Found: C; 63.9%, H; 4.0%, N; 8.7%. Calc.: C; 64.4%, H; 3.9%, N; 8.5%.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-triphenylporphyrin(chlorogallium(III)), [Ga(Cl)TPP-ABS] or [GaCl] 8 . The title compound was synthesised employing general metal complexation method 1. H_2L^8 (68.5 mg, 0.082 mmol), NaOAc (40.1 mg, 0.489 mmol), GaCl_3 (28.7 mg, 0.163 mmol), AcOH (10 mL). A maroon coloured solid was obtained. Yield: 57.3 mg, 0.061 mmol, 74%.

^1H NMR (500 MHz, d_6 -DMSO, 25 °C): δ 8.92 (m, 8H, CH_{pyrr}), 8.76 (t, 1H, $J = 5.5$, CONH), 8.32 (d, 2H, $J = 7.6$, ArH), 8.21 (d, 2H, $J = 7.7$, ArH), 8.26 (m, 6H, o -Ph), 7.88 (d, 2H, $J = 8.1$, ArH), 7.85 (m, 9H, m , p -Ph), 7.49 (d, 2H, $J = 7.9$, ArH), 3.75 (m, 2H, $\text{CONHCH}_2\text{CH}_2$), 3.15 (t, 2H, $J = 7.3$, $\text{CONHCH}_2\text{CH}_2$). ESI-MS calcd for $\text{C}_{53}\text{H}_{38}\text{GaN}_6\text{O}_3\text{S}$ [$\text{M} - \text{Cl}$] $^+$ 907.1982, found 907.1965. HPLC: (Method A) $R_t = 10.05$ min. Elem. Anal.: Found: C; 67.8%, H; 4.3%, N; 8.7%. Calc.: C; 67.5%, H; 4.1%, N; 8.9%.

5-[4-(*N*-(2-Oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethyl)benzamide)]-10,15,20-triphenyl-porphyrin(chloroindium(III)), [In(Cl)TPP-AZA] or [InCl] 11 . The title compound was synthesised employing general metal complexation method 1 (ESI $^+$). H_2L^{11} (40.0 mg, 0.046 mmol), NaOAc (22.0 mg, 0.273 mmol), InCl_3 (19.7 mg, 0.091 mmol), AcOH (10 mL). A purple coloured solid was obtained. Yield: 34.0 mg, 0.033 mmol, 72%.

^1H NMR (500 MHz, d_6 -DMSO, 25 °C): δ 9.06–9.03 (m, 2H, CH_{pyrr}), 8.99 (m, 7H, CH_{pyrr} , CONH), 8.43 (d, 2H, $J = 8.1$, ArH), 8.36 (d, 2H, $J = 8.3$, ArH), 8.25 (m, 6H, o -ArH), 7.90 (m, 9H, m , p -ArH), 4.20 (d, 2H, $J = 5.9$, CONHCH_2). ^{13}C NMR (125 MHz, d_6 -DMSO, 25 °C): δ 175.8, 175.0, 172.1, 165.9, 148.12, 148.09,

148.06, 147.8, 144.1, 141.4, 134.5, 134.4, 134.2, 132.6, 132.5, 132.5, 128.2, 127.0, 125.9, 121.4, 121.3, 120.3, 45.5. Mass Spectrum ESI-MS calcd for $\text{C}_{49}\text{H}_{33}\text{InN}_9\text{O}_4\text{S}_2$ [$\text{M} - \text{Cl}$] $^+$ 990.1130, found 990.1090. HPLC: (Method A) $R_t = 9.99$ min.

5-[4-(*N*-(2-Oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethyl)benzamide)]-10,15,20-triphenyl-porphyrin(chlorogallium(III)), [Ga(Cl)TPP-AZA] or [GaCl] 11 . The title compound was synthesised employing general metal complexation method 1. H_2L^{11} (50.0 mg, 0.057 mmol), NaOAc (28.0 mg, 0.341 mmol), GaCl_3 (21.0 mg, 0.114 mmol), AcOH (10 mL). A maroon coloured solid was obtained. Yield: 45.3 mg, 0.046 mmol, 81%.

^1H NMR (500 MHz, d_6 -DMSO, 25 °C): δ 9.13–8.91 (m, 8H, CH_{pyrr}), 8.76 (br s, 1H, CONH), 8.40 (d, 2H, $J = 7.3$, ArH), 8.31 (d, 2H, $J = 7.5$, ArH), 8.22 (m, 6H, o -ArH), 7.86 (m, 9H, m , p -ArH), 4.20 (d, 2H, $J = 4.9$, CONHCH_2). ^{13}C NMR (75.5 MHz, d_6 -DMSO, 25 °C): δ 165.9, 147.3, 147.0, 143.6, 140.8, 134.2, 132.3, 132.2, 128.4, 127.2, 126.1, 120.4, 119.4, 45.1. ESI-MS calcd for $\text{C}_{49}\text{H}_{33}\text{GaN}_9\text{O}_4\text{S}_2$ [$\text{M} - \text{Cl}$] $^+$ 944.1347, found 944.1334. HPLC: (Method A) $R_t = 10.11$ min. Elem. Anal.: Found: C; 60.5%, H; 3.6%, N; 12.2%. Calc.: C; 60.0%, H; 3.4%, N; 12.8%.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-tri-(4-sulfonylphenyl)porphyrin, [H $_2$ TSPP-ABS], H_2L^9 . The title compound was synthesised employing the general sulfonation method (ESI $^+$). H_2L^7 (70.0 mg, 0.083 mmol), conc. H_2SO_4 (2 mL). Yield: 66.6 mg, 0.058 mmol, 70%.

^1H NMR (500 MHz, d_6 -DMSO, 25 °C): δ 9.05 (t, 1H, $J = 5.7$, CONH), 8.82 (m, 8H, CH_{pyrr}), 8.29 (d, 2H, $J = 8.0$, ArH), 8.21 (m, 6H, $\text{ArH}_{\text{SO}_3\text{Na}}$), 8.17 (d, 2H, $J = 8.1$ Hz, ArH), 8.05 (m, 6H, $\text{ArH}_{\text{SO}_3\text{Na}}$), 7.87 (d, 2H, $J = 8.1$, ArH), 7.61 (d, 2H, $J = 7.8$, ArH), 7.37 (s, 2H, SO_2NH_2), 3.71 (m, 2H, $\text{CONHCH}_2\text{CH}_2$), 3.14 (t, 1H, $J = 7.5$, $\text{CONHCH}_2\text{CH}_2$). ^{13}C NMR (75.5 MHz, d_6 -DMSO, 25 °C): δ 166.1, 147.8, 144.1, 143.8, 142.5, 141.1, 134.24, 134.16, 133.7, 131.4 (broad), 131.2, 129.1, 125.9, 124.6, 120.1, 119.8, 118.9, 35.6, 26.4. ESI-MS calcd for $\text{C}_{53}\text{H}_{37}\text{N}_6\text{O}_{12}\text{S}_4$ [$\text{M} - 3\text{Na}$] $^{3-}/3$ 359.0451, found 359.0467. HPLC: (Method B, ESI $^+$) $R_t = 15.20$ min.

5-[4-(*N*-(2-Oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)ethyl)benzamide)]-10,15,20-tri-(4-sulfonylphenyl)-porphyrin, [H $_2$ TSPP-AZA] or H_2L^{12} . The title compound was synthesised employing the general sulfonation method (ESI $^+$). H_2L^{10} (50.0 mg, 0.057 mmol), conc. H_2SO_4 (2 mL). Yield: 48.6 mg, 0.041 mmol, 72%.

^1H NMR (500 MHz, d_6 -DMSO, 25 °C): δ 8.90 (t, 1H, $J = 5.7$, CONH), 8.88 (m, 8H, CH_{pyrr}), 8.37 (m, 4H, ArH), 8.21 (d, 6H, $J = 8.1$, $\text{ArH}_{\text{SO}_3\text{Na}}$), 8.07 (d, 6H, $J = 8.0$, $\text{ArH}_{\text{SO}_3\text{Na}}$), 4.20 (d, 2H, $J = 5.6$, $\text{CONHCH}_2\text{CONH}$), -2.91 (s, 2H, ring NH). ^{13}C NMR (125 MHz, d_6 -DMSO, 25 °C): 174.9, 171.9, 165.9, 165.3, 147.8, 143.9, 141.2, 134.3, 134.2, 133.7, 131.6 (broad), 125.9, 124.2, 119.8, 119.7, 119.3, 45.5. ESI-MS calcd for $\text{C}_{49}\text{H}_{32}\text{N}_9\text{O}_{13}\text{S}_5$ [$\text{M} - 3\text{Na}$] $^{3-}/3$ 371.3580, found 371.3576. HPLC: (Method B, ESI $^+$) $R_t = 16.11$ min.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-tri-(4-*N*-methylpyridinium phenyl)-porphyrin tri-iodide, [H $_2$ TMePyP-ABS], H_2L^{10} . H_2L^{10} was synthesised employing the general alkylation method (ESI $^+$). The tripyridylporphyrin ABS conjugate [H $_2$ TPyP-ABS] synthesis described in ESI $^+$ (65.0 mg,

0.077 mmol), MeI (109 mg, 47.9 μ L, 0.77 mmol), DMF. Yield: 114 mg, 0.090 mmol, 92%.

^1H NMR (500 MHz, d_6 -DMSO, 25 $^\circ\text{C}$): 9.46 (m, 6H, CH_{py}), 9.11 (m, 8H, CH_{pyrr}), 9.02 (t, 1H, $J = 5.4$, CONH), 8.99 (m, 6H, CH_{py}), 8.32 (d, 2H, $J = 8.7$, ArH), 8.30 (d, 2H, $J = 8.8$, ArH), 7.81 (d, 2H, $J = 8.3$, ArH), 7.56 (d, 2H, $J = 8.4$, ArH), 7.34 (s, 2H, SO_2NH_2), 4.70 (m, $3 \times 3\text{H}$, $3 \times \text{N-CH}_3$), 3.70 (m, 2H, $\text{CONHCH}_2\text{CH}_2$), 3.07 (t, 2H, $J = 6.9$, $\text{CONHCH}_2\text{CH}_2$), -3.06 (m, 1H) ^{13}C NMR (125 MHz, d_6 -DMSO, 25 $^\circ\text{C}$): δ 166.2, 156.6, 156.5, 144.3, 144.2, 143.9, 143.1, 142.1, 134.4, 134.3, 132.1, 129.3, 126.0, 125.8, 124.1, 121.9, 115.4, 114.7, 114.5, 111.8, 47.9, 34.5, 25.3. ESI-MS calcd for $\text{C}_{53}\text{H}_{44}\text{N}_9\text{O}_3\text{S} [\text{M} - 3\text{I}]^{3+}/3$ 269.1143, found 269.1143, ESI-MS calcd for $\text{C}_{53}\text{H}_{44}\text{N}_9\text{O}_3\text{S} [\text{M} - 3\text{I}]^{3+}/2$ 444.1717, found 444.1708. HPLC: (Method C) $R_t = 12.22$ min. Elem. Anal.: Found: C; 50.3%, H; 3.3%, N; 9.8%. Calc. [$\text{H}_2\text{L}^{10} + \text{MeOH}$]: C; 49.9%, H; 3.7%, N; 9.7%.

5-[4-(*N*-(2-Oxo-2-(5-sulphamoyl-1,3,4-thiadiazol-2-ylamino)-ethyl)benzamide)]-10,15,20-tri(4-*N*-methylpyridiniumyl)-porphyrin tri-iodide, [$\text{H}_2\text{TMePyP-AZA}$] I_3 or H_2L^{13} . Compound H_2L^{13} was synthesised employing the general alkylation method described in ESI† [$\text{H}_2\text{TPyP-AZA}$] (50.0 mg, 0.038 mmol), MeI (54.3 mg, 23.8 μ L, 0.38 mmol), DMF (see ESI†). Yield: 52.9 mg, 0.040 mmol, 88%.

^1H NMR (300 MHz, d_6 -DMSO, 25 $^\circ\text{C}$): δ 9.46 (d, 6H, $J = 6.6$, CH_{py}), 9.36 (t, 1H, $J = 5.4$, CONH), 9.26–9.05 (m, 8H, CH_{pyrr}), 8.93–9.03 (m, 6H, CH_{py}), 8.40 (d, 2H, $J = 8.0$, ArH), 8.36 (d, 2H, $J = 7.8$, ArH), 4.71 (2 s, $3 \times 3\text{H}$, $3 \times \text{N-CH}_3$), 4.43 (d, 2H, $J = 5.8$, $\text{CONHCH}_2\text{CONH}$), -3.05 (br s, 1H, ring NH). Mass Spectrum ESI-MS calcd for $\text{C}_{49}\text{H}_{41}\text{N}_{12}\text{O}_4\text{S}_2 [\text{M} - 3\text{I}]^{3+}/3$ 308.0640, found 308.0621, ESI-MS calcd for $\text{C}_{49}\text{H}_{41}\text{N}_{12}\text{O}_4\text{S}_2 [\text{M} - 3\text{I}]^{3+}/2$ 462.0960, found 462.0993. HPLC: (Method C) $R_t = 13.08$ min.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-tri(4-sulfonylphenyl)-porphyrin(chloroindium(III)), [$\text{In}(\text{Cl})\text{-TSPP-ABS}$], [InCl] 9 . The title compound was synthesised employing general metal complexation method 3. H_2L^9 (40.0 mg, 0.035 mmol), InCl_3 (15.5 mg, 0.070 mmol) in pH 4.5 NaOAc buffer (5 mL). Yield: 29.9 mg, 0.023 mmol, 66%.

^1H NMR (300 MHz, d_6 -DMSO, 25 $^\circ\text{C}$): δ 9.07 (t, 1H, $J = 5.4$, CONH), 8.94 (s, 8H, CH_{pyrr}), 8.29 (s, 4H, ArH), 8.18 (d, 6H, $J = 8.0$, $\text{ArH}_{\text{SO}_3\text{Na}}$), 8.08 (d, 6H, $J = 8.2$, $\text{ArH}_{\text{SO}_3\text{Na}}$), 7.60 (d, 2H, $J = 8.0$, ArH), 7.32 (d, 2H, $J = 8.0$, ArH), 3.65 (m, 2H, $\text{CONHCH}_2\text{CH}_2$), 3.00 (t, 2H, $J = 7.4$, $\text{CONHCH}_2\text{CH}_2$) ^{13}C NMR (75.5 MHz, d_6 -DMSO, 25 $^\circ\text{C}$): δ 166.3, 147.72, 147.69, 147.5, 147.4, 145.9, 144.1, 141.7, 140.3, 134.2, 134.0, 132.6 (broad), 128.1, 125.9, 125.7, 124.2, 120.8, 120.4, 35.0, 25.0. Mass Spectrum ESI-MS calcd for $\text{C}_{53}\text{H}_{35}\text{ClInN}_6\text{O}_{12}\text{S}_4 [\text{M} - 3\text{Na}]^{3-}/3$ 408.3313, found 408.3345. HPLC: (Method B) $R_t = 13.23$ min.

5-[4-(*N*-(4-Sulphamoylphenethyl)benzamide)]-10,15,20-tri(4-*N*-methylpyridiniumylphenyl)-porphyrin (chloroindium(III)) trichloride [$\text{In}(\text{Cl})\text{TMePyP-ABS}$] Cl_3 , [InCl] 10 . The title compound was synthesised employing general metal complexation method 3. H_2L^{10} (50.0 mg, 0.039 mmol), InCl_3 (17.4 mg, 0.079 mmol) in pH 4.5 NaOAc buffer (5 mL). Yield: 30.3 mg, 0.027 mmol, 68%.

^1H NMR (500 MHz, d_6 -DMSO, 25 $^\circ\text{C}$): δ 9.41–9.67 (m, 6H, CH_{py}), 9.23–9.35 (br s, 4H CH_{pyrr}), 9.13–9.23 (br s, 3H, CONH,

CH_{pyrr}), 9.02–9.11 (br s, 2H, CH_{pyrr}), 8.87–9.00 (br s, 6H, CH_{py}), 8.36 (d, 2H, $J = 7.5$, ArH), 8.30 (d, 2H, $J = 7.5$, ArH), 7.81 (d, 2H, $J = 8.0$, ArH), 7.55 (d, 2H, $J = 8.0$, ArH), 7.36 (s, 2H, SO_2NH_2), 4.66–4.87 (br s, 9H, $3 \times \text{CH}_3$), 3.69 (m, 2H, $\text{CONHCH}_2\text{CH}_2$), 3.09–3.16 (t, 2H, $J = 5.7$, $\text{CONHCH}_2\text{CH}_2$). ^{13}C NMR (75.5 MHz, d_6 -DMSO, 25 $^\circ\text{C}$): δ 166.7, 156.6, 156.5, 147.8, 146.5, 146.4, 144.8, 144.3, 144.2, 142.9, 133.8, 133.3, 132.4, 132.3, 129.8, 126.2, 126.1, 117.3, 116.8, 48.1, 47.9, 35.1, 26.2. Mass Spectrum ESI-MS calcd for $\text{C}_{53}\text{H}_{42}\text{ClInN}_9\text{O}_3\text{S} [\text{M} - 3\text{Cl}]^{3+}/3$ 344.7281, found 344.7298. HPLC: (Method C in ESI†) $R_t = 12.05$ min.

General method for copper-64 radiolabelling

^{64}Cu -ATSM was prepared from H_2 -ATSM according to a published method.^{2,39} ^{64}CuL was prepared by adding ^{64}Cu -(OAc) $_2$ (aq) (70 μL of a 1 MBq μL^{-1} solution) to a solution of H_2L in DMSO (100 μL , 1 mg mL^{-1}). After 20 min stirring at room temperature HPLC analysis was carried out using a Platinum C18 column (5 μm particle size, dimensions 250 \times 3.0 mm). A 20 min gradient method with acetonitrile–water as the mobile phase was used: 0 min 5% MeCN; 10 min 95% MeCN; 15 min 95% MeCN; 18 min 5% MeCN, 20 min 5% MeCN. Flow rate = 1.0 mL min^{-1} . The radiochemical purity of ^{64}CuL ($R_t = \text{mins}$) was 99%. 0.9% saline solution (to <5% v/v ethanol) for use in PET imaging experiments. The specific activity of the administered tracer was approximately 20 MBq mL^{-1} (0.2 MBq μg^{-1} of H_2L).

General method for indium-111 radiolabelling

The ligand was prepared as a 1.0 mg mL^{-1} solution in DMSO or distilled water. For indium labeling of water soluble porphyrins 10 μL of the stock porphyrin solution was diluted with 90 μL of pH 4.5 sodium acetate buffered solution and heated at 115 $^\circ\text{C}$ for 30 min in an Eppendorf tube in the presence of 20 μL $^{111}\text{InCl}_3$ (<10 MBq per experiment). For indium labelling of tetraphenyl porphyrin derivative 10 μL of the stock solution was diluted with 90 μL of AcOH and heated at 120 $^\circ\text{C}$ for 1 h in the presence 10 μL of a 0.1 M NaOAc (aq.) solution and 20 μL $^{111}\text{InCl}_3$ (<10 MBq per experiment). AcOH was then driven off under nitrogen and the residue re-suspended in 50 μL of CHCl_3 . The CHCl_3 layer was washed repeatedly with 100 μL aliquots of distilled water until no further activity was associated with the aqueous washings. For all porphyrin-based compounds the 20 min HPLC gradient methods (analytical HPLC) of was performed by one of three methods below:

General method for technetium-99m radiolabelling

The radiolabelling was carried out according to the procedure described previously,³⁵ using [$^{99\text{m}}\text{TcO}_4$] $^-$ with SnCl_2 as the reducing agent in sodium carbonate buffer at a temperature of 95 $^\circ\text{C}$. Briefly, the ligand (L^2 to L^6 , 0.1 mg) was dissolved in TFA (190 μL) and treated with triethylsilane (10 μL) to remove the Trt-group. After removal of the solvent under a stream of N_2 , sodium carbonate solution (80 μL , 0.1 mol L^{-1} , pH = 9.5)

was added to the residue. Next, SnCl_2 solution (3 μL , 1 mg mL^{-1}) in citrate buffer (0.1 mol L^{-1} , $\text{pH} = 5.0$) and $[\text{}^{99\text{m}}\text{TcO}_4]^-$ (200 μL , 20 MBq) solution were added, and the reaction mixture was vigorously stirred and allowed to react at 95 $^\circ\text{C}$ for 1 h. The radiochemical purity and labelling efficiency were determined using radio-HPLC (Method C in ESI†) or radio-TLC. For radio-TLC, acetonitrile–water (1/1 by volume) was used for the determination of $^{99\text{m}}\text{Tc}$ -colloid in the silica gel TLC ($R_f = 0$) and 2-butanone–ethyl acetate (2/3 by volume) was used for the determination of $^{99\text{m}}\text{Tc}$ -pertechnetate ($R_f = 1.0$).⁴⁰ In the cases when the radiochemical purity of the radiotracer did not exceed 95%, the raw product was purified using a C18 Sep-Pak™ cartridge. The cartridge was pre-treated using ethanol (5 mL), followed by water (5 mL), before the raw product was loaded. The loaded cartridge was rinsed twice with water (2×5 mL) to release the $^{99\text{m}}\text{Tc}$ -pertechnetate and any other hydrophilic impurities, and the technetium-labelled product was eluted with ethanol (1 mL). The radiochemical purity of the purified radiotracer was then determined using the method described above. All the $^{99\text{m}}\text{Tc}$ complexes had RCP's of >98% after purification.

Confocal fluorescence microscopy

HCT116 cells were seeded as monolayers in T75 tissue culture flasks, and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum, L-glutamine, penicillin and streptomycin. Cells were maintained at 37 $^\circ\text{C}$ in a 5% CO_2 humidified atmosphere and grown to approximately 85% confluence before being split using 2.5% trypsin. For microscopy, cells were seeded onto chambered cover-glass slides and incubated⁴¹ for 12 h to ensure adhesion.

Porphyrin complexes were prepared as 10 mM solution in DMSO or distilled water, and diluted to 10 μM with DMEM, and incubated with the cells at 37 $^\circ\text{C}$. Prior to imaging, the solution was replaced with 1 mL fresh DMEM. Background autofluorescence was measured by imaging the cells in 1 mL of DMEM medium only. The fluorescent uptake of porphyrin complexes was imaged by laser-scanning confocal microscopy using a Zeiss LSM 510 META microscope irradiating at 405 nm with emission filtered between 565 and 615 nm.

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