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Synthesis and Characterisation of Zirconium Complexes for Cell Tracking with Zr-89 by Positron Emission Tomography

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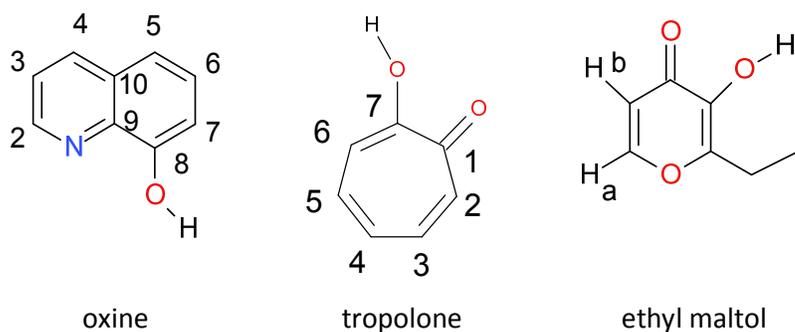
Key words: Zirconium , oxine, tropolone, ethyl maltol, zirconium-89, PET

Abstract

The increasing availability of the long half-life positron emitter Zr-89 (half life 78.4 h) suggests that it is a strong candidate for cell labelling and hence cell tracking using positron emission tomography. The aim was to produce a range of neutral ZrL₄ lipophilic complexes for cell labelling which could be prepared under radiopharmaceutical conditions. This was achieved when the ligand was oxine, tropolone or ethyl maltol. The complexes can be prepared in high yield from zirconium(IV) precursors in hydrochloric or oxalic acid solution. The oxinate and tropolonate complexes were the most amenable to chromatographic characterisation, and HPLC and ITLC protocols have been established to monitor their radiochemical purity. The radiochemical synthesis and quality control of ⁸⁹Zr(oxinate)₄ is reported as well as preliminary cell labelling data for the oxinate, tropolonate and ethyl maltolate complexes which indicates that ⁸⁹Zr(oxinate)₄ is the most promising candidate for further evaluation.

Introduction

The increasing availability of the positron emitter zirconium-89 combined with its half life of 78.4 h suggests that it is a strong candidate for cell labelling and hence cell tracking *in vivo* using positron emission tomography (PET), affording the opportunity to exploit the advantages of PET over conventional gamma camera imaging of cells labelled with the gamma emitter indium-111. Although the favoured oxidation state of zirconium is 4+ (compared to 3+ for indium) there are parallels between the two metals in their reactivity and preferred ligand types. This raises the possibility that the mechanism exploited to label cells with In-111 (i.e. use of metastable chelates that enter cells by virtue of their lipophilicity followed by dissociation within cells leading to intracellular trapping of the radiometal¹⁻⁴) might be exploited in the case of zirconium-89. Tetravalent zirconium is known to form complexes with monobasic bidentate ligands such as oxinate,⁵ tropolonate⁶ and hydroxamates⁷ of the stoichiometry ZrL_4 , bearing analogy to InL_3 .^{8,9} Here we describe methodologies for the synthesis and characterisation of Zr(IV) complexes containing oxine, tropolone and ethyl maltol ligands. Conditions compatible with the radiopharmacy, where zirconium-89 is typically available in hydrochloric or oxalic acid solution, were investigated. The characterisation of these compounds underpins understanding of their utility in radiolabelled form for cell labelling, and provides chromatographic standards for identification and quality control of the radioactive species.



The synthesis of $Zr(\text{oxinate})_4$, by reacting $ZrCl_4$ with oxine either in THF or in the absence of solvent was first reported in 1968¹⁰ and the X-ray crystal structure of $Zr(\text{oxinate})_4 \cdot (\text{PhMe})_3$ was reported in 1974.¹¹ There has been renewed interest in $Zr(\text{oxinate})_4$ in recent years

because of its potential applications in organic light emitting diodes (OLEDs) and two new crystalline forms of $\text{Zr}(\text{oxinate})_4$ have since been reported.⁵ The molecular structures are very similar in the various crystal structures: the metal centre is eight coordinated in approximately dodecahedral geometry by four chelating N,O pairs from four oxinate ligands. Tropolonate zirconium complexes have also been known for over fifty years and the crystal structure of $\text{Zr}(\text{tropolonate})_4 \cdot (\text{CHCl}_3)_{2.25}$ was published in 1978 displaying again a neutral eight coordinate complex of approximately dodecahedral geometry.¹² $\text{ZrCl}_2(\text{ethyl maltolate})_2$ has been shown to be a catalyst for ethylene polymerisation,¹³ but we can find no previous reports of $\text{Zr}(\text{ethyl maltolate})_4$.

Results and Discussion

Synthesis and Characterisation

$\text{Zr}(\text{oxinate})_4$ was initially prepared in 71% yield by the reaction of ZrCl_4 with 4 molar equivalents of oxine and an excess of piperidine followed by Soxhlet extraction with 1, 4-dioxane, in a similar manner to the reported synthesis of beta- $\text{Zr}(\text{oxinate})_4$ (Method 1).⁵ NMR spectroscopic data and elemental analysis were in accord with the formation of the desired compound and previous reports (see experimental).⁵ IR and Raman spectra were assigned by comparison with $\text{Ga}(\text{oxinate})_3$.¹⁴ The characteristic $\nu(\text{OH})$ stretching band, located at 3176 cm^{-1} in the IR spectrum of oxine disappears in the complex due to deprotonation of the ligand and the band assigned to the $\nu(\text{C-O})$ vibration moves to lower energy in the complex (from 1093 to 1054 cm^{-1} in the IR and from 1101 to 1054 cm^{-1} in the Raman spectrum).

The UV-visible spectrum (see Table 1) of $\text{Zr}(\text{oxinate})_4$ in acetonitrile has an absorption at 380 nm (previously reported in toluene at 387 nm)⁵ assigned to a $\pi \rightarrow \pi^*$ transition in the ligand by comparison with related complexes.¹⁵ The molar absorptivity is approximately four times that observed for oxine in accord with the chromophore being ligand-based rather than involving the zirconium.

Table 1. UV-visible spectroscopic data for ligands and complexes

	Solvent	$\lambda_{\text{max}} / \text{nm}$ ($\epsilon / \text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$)
Oxine	MeCN	240 (35000 ± 2000), 308 (2570 ± 10)
$\text{Zr}(\text{oxinate})_4$	MeCN	250 (47000 ± 500), 380 (9430 ± 30)
Tropolone	MeOH	307 (sh), 320 (6500 ± 600), 354 (5900 ± 200), 370 (4700 ± 100)

Zr(tropolonate) ₄	MeOH	333 (63000 ± 5000), 369 (33000 ± 1000), 380 (sh), 390 (sh)
Ethyl maltol	MeOH	268 (10200 ± 300)
Zr(ethyl maltolate) ₄	MeOH	224 (64000 ± 5000), 312 (21000 ± 1000)

In order to prepare Zr(oxinate)₄ from zirconium (IV) chloride in 1M HCl the acidic solution was first neutralised with NaOH followed by addition of aqueous oxine resulting in the precipitation of Zr(oxinate)₄ after reduction of the solvent volume (Method 2).

Spectroscopic data confirmed that the complex isolated was the desired compound.

Attempts to prepare Zr(oxinate)₄ from a solution of zirconium (IV) chloride in 1M HCl failed unless the solution was first neutralised. A similar approach was taken to the synthesis from K₄[Zr(C₂O₄)₄] in 1M oxalic acid, the neutralisation being effected with Na₂CO₃ (Method 3).

Importantly these results demonstrate the formation of Zr(oxinate)₄ in the presence of a high excess of chloride or oxalate, as would be encountered in a radiopharmaceutical synthesis.

Zr(tropolonate)₄ was prepared by adapting the published method of synthesis of zirconium tetrakis isopropyltropolone (Method 4).⁶ ZrCl₄ and 4 molar equivalents of tropolone were reacted in chloroform and the product isolated by evaporation of the chloroform. ¹H and ¹³C NMR spectra were in accord with the proposed product showing ligand resonances perturbed from their positions in tropolone. ¹H NMR spectroscopy and elemental analysis data suggest inclusion of CHCl₃. NMR integration indicates approximately 0.85 CHCl₃ while elemental analysis fits best for 0.55 CHCl₃. The previously reported crystal structure contained 2.25 CHCl₃.¹² IR and Raman spectra were assigned by comparison with bistrropolonato vanadium, cobalt, copper and zinc complexes,¹⁶ and tristrropolonato lanthanum, neodymium, samarium and ytterbium complexes.¹⁷

Electronic absorptions for free tropolone in methanol were in accord with those previously observed in DMSO.¹⁶ The absorptions are due to π→π* transitions and analogous transitions are observed in Zr(tropolonate)₄ with increased extinction coefficients attributed to the presence of four tropolone rings with enhancement by the contribution of a charge transfer transition.¹⁶ Zr(tropolonate)₄ was also successfully prepared from ZrCl₄ in 1M HCl or K₄[Zr(C₂O₄)₄] in 1M oxalic acid if the acidic solutions were first neutralised (Method 5).

Zr(ethyl maltolate)₄ was prepared in 91% yield by the reaction of 4 equivalents of ethyl maltol with ZrCl₄ in tetrahydrofuran (method 6). Elemental analysis data were in accord with the desired complex (see experimental). As expected the ¹H NMR spectrum shows no resonance due to free ligand OH in the region of 8.8 ppm and the shift of the H_a and H_b protons to lower frequencies in relation to the ligand demonstrates the loss of aromaticity due to the donation of electron density to the metal. IR and Raman spectra were assigned by comparison with bis(maltolato) vanadium¹⁸ and zinc¹⁹ and tris(maltolato) gallium²⁰ complexes. An electronic absorption for ethyl maltol in methanol was recorded as 268 nm having been observed previously in toluene at 286 nm and interpreted as a π→π* transitions of the β unsaturated enone.¹³ In Zr(ethyl maltolate)₄ this band disappears due to the loss of conjugation of the enone, demonstrating the complexation by the carbonyl group. Absorption bands appear in the UV spectrum at 224 and 312 nm. The latter may be attributed to a ligand-to-metal charge transfer from a π ligand orbital to an empty zirconium d orbital. Zr(ethyl maltolate)₄ was also successfully prepared from K₄[Zr(C₂O₄)₄] in 1M oxalic acid if the acidic solution was first neutralised (Method 7).

High Performance Liquid Chromatography

The HPLC of oxinate complexes has previously been reported when oxine was used as a pre-column chelating agent for reverse-phase HPLC multi-element (Zn, Al, Co, Cr, Cu, Ga, In and Fe) determinations.²¹ For ions other than the kinetically inert Co(III) and Cr(III), excess ligand was required in the mobile phase to observe good chromatographic properties, presumably due to ready ligand dissociation occurring during passage through the column. A large range of columns and solvents were evaluated in order to arrive at conditions suitable for using these compounds as standards for chromatographic identification of their radioactive analogues.

For Zr(oxinate)₄ using a XBD-C8 eclipse column very poor results were obtained without excess ligand in the mobile phase. However addition of *ca.* 1000 ppm ligand to the mobile phase gives chromatograms (MeCN:H₂O 75:25, 1.0 mL min⁻¹, 380 nm) with a retention time of 2.96 min. With a Altima C18 5 μm column (MeOH:H₂O 50:50, 0.1% formic acid, 2.0 mL min⁻¹), monitored at 245 nm to detect ligand and complex simultaneously, separation was obtained with retention times of oxine and Zr(oxinate)₄ being 1.27 and 1.00 min respectively without the use of excess ligand in the mobile phase (figure 1).

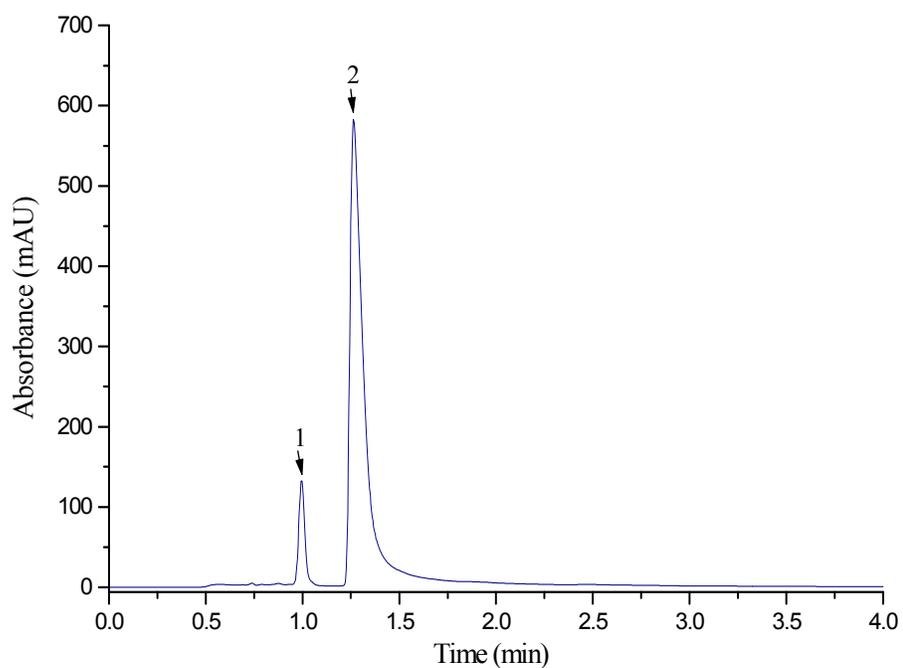


Figure 1: HPLC of a 1:1 mixture of $\text{Zr}(\text{oxinate})_4$ (1) and oxine (2). Altima C18 5 μm column (MeOH:H₂O 50:50, 0.1% formic acid, 2.0 mL min⁻¹, 245 nm)

Chromatograms of $\text{Zr}(\text{tropolonate})_4$ (figure 2) were obtained using a Luna 3 silica column (MeCN:H₂O 90:10, 1.0 mL min⁻¹, 333 nm). A retention time of 1.78 min was observed, clearly resolvable from the free ligand at 2.04 min.

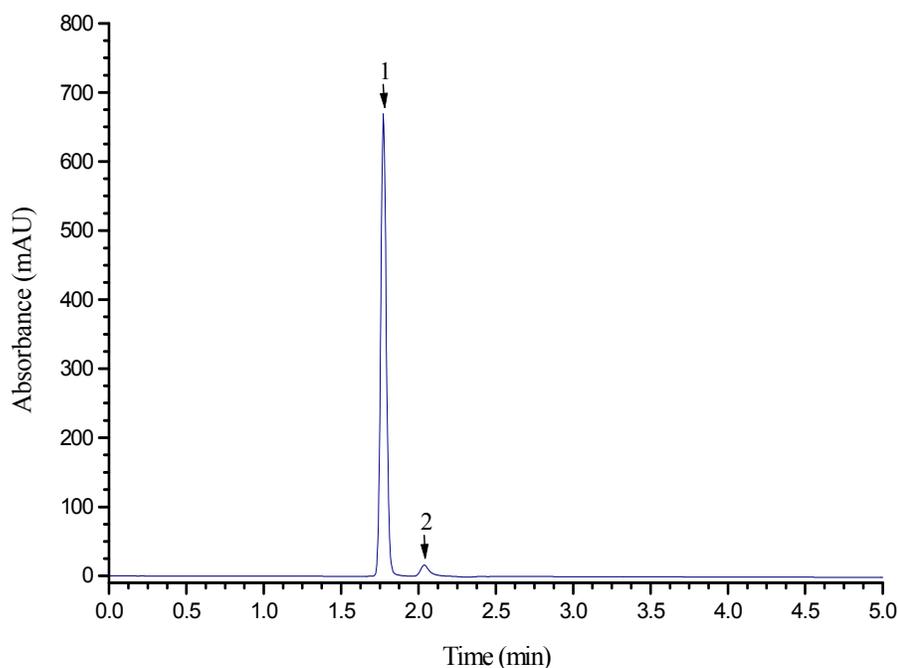


Figure 2: HPLC of a 1:1 mixture of $\text{Zr}(\text{tropolonate})_4$ (1) and tropolone (2). Luna 3 silica column (MeCN:H₂O 90:10, 1.0 mL min⁻¹, 333 nm)

Numerous conditions were attempted to produce chromatograms demonstrating separation of $\text{Zr}(\text{ethyl maltolate})_4$ /ethyl maltol without success. In all cases the chromatograms were indistinguishable from those of the free ligand.

Instant Thin Layer Chromatography (ITLC)

Instant thin layer chromatography (ITLC) can provide a quick and accurate test for radiochemical purity and hence its use was explored for the zirconium complexes (see Table 2). The oxine and tropolone ligands and their zirconium complexes were visualised by UV light, and ZrCl_4 by staining with silver nitrate, however it was not possible to find a method of visualising $\text{K}_4[\text{Zr}(\text{C}_2\text{O}_4)_4]$. The R_f value could only be determined by radio-ITLC of $\text{K}_4[{}^{89}\text{Zr}(\text{C}_2\text{O}_4)_4]$.

Table 2: ITLC Results

Compound	Solvent	Visualisation	Rf Value
ZrCl ₄	20 mM sodium citrate	AgNO ₃	1.0
K ₄ [Zr(C ₂ O ₄) ₄]	20 mM sodium citrate	Zr-89	0.9
K ₄ [Zr(C ₂ O ₄) ₄]	Ethyl acetate	Zr-89	0.0
Oxine	20 mM sodium citrate	UV 254 nm	0.8
Zr(oxinate) ₄	20 mM sodium citrate	UV 254 nm	0.0
Oxine	Ethyl acetate	UV 254 nm	0.2
Zr(oxinate) ₄	Ethyl acetate	UV 254 nm/ Zr-89	0.9
Tropolone	20 mM sodium citrate	UV 254 nm	1.0
Zr(tropolonate) ₄	20 mM sodium citrate	UV 254 nm	0.0

Oxine and Zr(oxinate)₄ were applied to the ITLC sheets as 1 mg/mL solutions in acetonitrile. Eluting with 20 mM sodium citrate solution provided clearly distinguishable chromatographic behaviour with the ligand displaying a spot at Rf 0.8 with some streaking, the complex remaining at the origin (Rf = 0) compared with the precursors ZrCl₄ moving with the solvent front and K₄[Zr(C₂O₄)₄] having a Rf of 0.9. Alternatively eluting with ethyl acetate results in Rf for Zr(oxinate)₄ of 0.9 and K₄[Zr(C₂O₄)₄] zero.

Similar results were obtained eluting with 20 mM sodium citrate solution when tropolone and Zr(tropolonate)₄ were applied to the ITLC sheets as 1 mg/mL solutions in methanol. Non-radioactive visualisation methods could not be established for ethyl maltol and Zr(ethyl maltolate)₄. Performing the synthesis of ⁸⁹Zr(oxinate)₄ from ⁸⁹Zr in 0.1 M oxalic acid by adapting method 3 (see experimental) resulted in a yield of *ca.* 60% in the chloroform phase and a radiochemical purity of 99% as determined by ITLC with ethyl acetate, exhibiting a Rf of 0.9 in agreement with that of the spectroscopically and analytically characterised sample of Zr(oxinate)₄. This ITLC method was therefore adopted as the main quality control of the ⁸⁹Zr(oxinate)₄ complex performed before each biological evaluation, as reported elsewhere.^{22, 23}

Cell Labelling

The uptake of ⁸⁹Zr(oxinate)₄ in breast cancer cell line MDA-MB-231 was found to be *ca.* 16% after 1 min rising to *ca.* 20% after 1h, in contrast to ⁸⁹Zr(oxalate)₄ which rose to *ca.* 5% after 1 h. Retention of ⁸⁹Zr(oxinate)₄ was high with 82% retention after 24 h. Similar results were

obtained with mouse macrophage J447 with *ca.* 5% uptake of $^{89}\text{Zr}(\text{oxalate})_4$ and *ca.* 22% uptake of $^{89}\text{Zr}(\text{oxinate})_4$ after 1 h. Approximately 91% of $^{89}\text{Zr}(\text{oxinate})_4$ was retained after 24 h. Uptake of $^{89}\text{Zr}(\text{tropolonate})_4$ in this cell line was found to be *ca.* 22% after 1 h, with *ca.* 49% being retained after 24 h. Uptake of $^{89}\text{Zr}(\text{ethyl maltolate})_4$ by colon cancer cells HTC-116 was found to be *ca.* 43% after 1 h, with *ca.* 26% retention after 24 h. These cell labelling experiments were conducted with around one million cells per sample, a much smaller number than typically used with clinical samples, and therefore the % labelling yields reported here are expected to translate into much higher yields when used in clinical samples. These preliminary experiments indicate that $^{89}\text{Zr}(\text{oxinate})_4$ is a promising candidate for cell tracking and detailed evaluation is currently ongoing.

Conclusions

The aim was to produce a range of neutral ZrL_4 complexes for cell labelling which could be prepared under radiopharmaceutical conditions. This was achieved using oxinate, tropolonate or ethyl maltolate as uninegative bidentate ligands to prepare neutral eight coordinate Zr(IV) complexes. The complexes can be prepared in high yield from zirconium precursors supplied in high concentrations of hydrochloric or oxalic acid following neutralisation. The oxine and tropolone complexes were the most amenable to chromatographic characterisation with both HPLC and ITLC conditions established to monitor their purity. $^{89}\text{Zr}(\text{oxinate})_4$ complex is able to radiolabel cells with good yields and good retention over 24 h; it is currently undergoing detailed evaluation for cell labelling and *in vivo* tracking by PET imaging.

Experimental

NMR analysis was undertaken using a JEOL NMR ECS-400 and JEOL Delta v5.02 software. FTIR spectra were obtained using a Thermo Scientific Nicolet 380 FT-IR spectrometer with a Smart Orbit high performance diamond single bounce ATR accessory. CHN analyses were obtained at the University of Kent utilising an A EMA Syst 1106 elemental analyser and at the Science Centre, London Metropolitan University utilising a Carlo Erba Flash 2000 elemental analyser. Raman spectra were obtained using a Horiba LabRAM-HR Raman

spectrometer utilising a laser operating at 784 nm. A $\times 50$ objective lens was used giving a beam diameter of approximately 2 μm on the sample. The spectrometer was calibrated against the silicon line at 520.6 cm^{-1} . UV-visible spectra were obtained with a Shimadzu UV-1800 spectrometer.

HPLC was performed using a Dionex Ultimate 3000 system with data processing using Chromeleon 7. For ITLC a 20 mM citrate elutant solution (pH 4.0) was made by the addition of 0.262 g of citric acid and 0.193 g of tri sodium citrate to 100 mL of millipore water. ITLC-SG plates were obtained from Varian. $\text{K}_4[\text{Zr}(\text{C}_2\text{O}_4)_4]$ was prepared by a literature method.²⁴

Synthesis of $\text{Zr}(\text{oxinate})_4$

Method 1

A solution of oxine (5.00 g; 34.5 mmol) in ethanol (35 mL) was added dropwise to a solution of zirconium (IV) chloride (2.01 g; 8.63 mmol) in ethanol (25 mL) which had been cooled to 10°C with an ice-water bath. The resulting mixture was stirred for 20 min at room temperature (20°C). The solution was then heated to 50°C in a water bath and piperidine (10 mL; 100 mmol) was added dropwise resulting in the formation of a yellow precipitate. This suspension was then refluxed for 1 h and allowed to cool to room temperature. The precipitate was collected by filtration and washed with ethanol (15 mL), tetrahydrofuran (15 mL) and diethyl ether (15 mL) before drying at 70°C for 1 h affording a crude yield 5.06 g (89%). To further purify the compound Soxhlet extraction was undertaken with 1, 4-dioxane for 8 h. Concentration of the extract resulted in a yellow precipitate which was collected with a Büchner funnel and washed with ethanol (15 mL). The product was then dried for 2 h in an oven at 50°C and stored in a glass air tight screw top bottle. Yield 4.01 g (71%). Found C 64.1, H 3.8, N 8.6%; $\text{C}_{36}\text{H}_{24}\text{N}_4\text{O}_4\text{Zr}$ requires 64.8, H 3.6, N 8.7%.

δ_{H} (400 MHz, d^6 -DMSO), 8.60 (1 H, b s, 2-H), 8.15 (1 H, d, $J_{3,4}$ 7.3, 4-H), 7.33-7.26 (2 H, m, 5-H and 6-H), 7.02 (1 H, d, $J_{6,7}$ 8.2, 7-H), 6.69 (1 H, d, $J_{3,4}$ 7.3, 4-H)

δ_{C} (100 MHz, d^6 -DMSO) 162.84 (C8), 145.88 (C2), 141.75 (C9), 138.39 (C4), 129.92 (C10), 129.63 (C6), 122.19 (C3), 114.17 (C5), 112.39 (C7)

IR ν_{max} (cm^{-1}): 3045 w (ν_{CH}), 1604 m ($\nu_{\text{C=N}}$), 1573 s ($\nu_{\text{C=C}}$), 1495 vs, 1462 vs, 1423 m, 1378 vs, 1318 vs ($\nu_{\text{C-C}}$), 1274 s, 1227 m, 1173 w, 1107 vs (δ_{CH} in plane), 1054 m ($\nu_{\text{C-O}}$), 1030 m, 910 w,

822 s, 806 m (δ_{CH} out of plane), 785 s, 737 vs (ring breathing), 643 m, 616 s, 560 w, 514 vs (ring deformations).

Raman ν_{max} (cm^{-1}): 1606 w ($\nu_{\text{C=N}}$), 1589 m, 1578 m 1572 sh ($\nu_{\text{C=C}}$), 1498 m, 1463 w, 1421 m, 1388 vs, 1378 s, 1327 w ($\nu_{\text{C-C}}$), 1284 m, 1209 w, 1175 w, 1143 w, 1137 w, 1112 w (δ_{CH} in plane), 1054 w ($\nu_{\text{C-O}}$), 1032 w, 907 m, 824 w, 805 m (δ_{CH} out of plane), 754 m, 736 w (ring breathing), 650 w, 617 m, 558 w, 524 vs, 500 sh, 492 m, 469 w, 449 w (ring deformations).

Method 2

Zirconium(IV) tetrachloride (1.01 g; 4.31 mmol) was dissolved in 1M HCl (20 mL). The pH was then raised to 7.60 by addition of 1 M NaOH (28 mL). A solution of oxine (2.50 g; 17.3 mmol) in reverse osmosis water (150 mL) was then added drop wise. The solution was brought to the boil and reduced in volume by 90%. After cooling to room temperature the resulting precipitate was then collected by the use of a Büchner funnel and washed with ethanol (15 mL), tetrahydrofuran (15 mL) and diethyl ether (15 mL) and dried in an electric oven at a 100 °C for 10 minutes. Yield 2.55 g (91%).

Method 3

$\text{K}_4[\text{Zr}(\text{C}_2\text{O}_4)_4]$ (2.00 g; 2.89 mmol) was dissolved in 1 M oxalic acid (20 mL) and then neutralised with 1 M sodium carbonate (Na_2CO_3) (19 mL) before diluting to 50 mL with water. A solution of oxine (2.10 g; 14.5 mmol) in chloroform (50 mL) was then added drop wise with stirring. The mixture was stirred for 20 minutes at room temperature and then the pale yellow chloroform layer separated. The product was isolated by evaporation at 60 °C. Yield 1.70 g (88%).

Synthesis of $\text{Zr}(\text{tropolonate})_4$

Method 4

A solution of tropolone (3.80 g; 31.0 mmol) in chloroform (75 mL) was added drop wise to a solution of zirconium (IV) chloride (1.78 g; 7.64 mmol) in chloroform (75 mL). The solution was then stirred for 20 min during which time it became pale yellow in colour. The solution was then heated in a water bath at between 50°C and 55°C for 2 h. The volume of the solution was reduced to ~40 mL during this period. The remaining chloroform was removed

by use of a rotary evaporator. The compound was then dried at 40°C for 2 h in an electric oven. Yield 4.18 g (83%)

Found C 53.33, H 3.51, N 0.0%; $C_{28}H_{20}O_8Zr \cdot 0.55CHCl_3$ requires C 53.47, H 3.23, N 0.0%

δ_H (400 MHz, d^6 -DMSO), 8.27 (0.125H, s, $CHCl_3$), 7.59 (2H, dd, J 9.6, 11.0, H3 & 5), 7.19 (2 H, d, $J_{2,3} J_{5,6}$ 11.0, H2 & 6), 7.13 (1 H, t, J 9.6, H4)

δ_C (100 MHz, d^6 -DMSO), 181.72 (C1 & C7), 140.31 (C2 & C6), 128.28 (C4), 125.05 (C3 & C5)

IR ν_{max} (cm^{-1}): 3013 m (ν_{CH}), 1590 vs ($\nu_{C=C}$ and $\nu_{C=O}$), 1516 vs ($\nu_{C=O}$ and $\nu_{C=C}$), 1418 sh, 1408 vs, ($\nu_{C=O}$, ν_{C-C} and δ_{CH}), 1349 vs ($\nu_{C=C}$), 1223 vs (ν_{C-O}), 1079 m (ν_{C-C} and δ_{CH}), 968 m, 934 m, 919 m (ν_{C-C}), 875 s, 728 vs (ν_{C-C} and δ_{CH}), 712 vs (ν_{CH}), 661 m (ν_{C-C}), 586 m, 530 vs (ν_{Zr-O})

Raman ν_{max} (cm^{-1}): 1593 vs ($\nu_{C=C}$ and $\nu_{C=O}$), 1533 w, 1521 w ($\nu_{C=O}$ and $\nu_{C=C}$), 1471 m (δ_{CH}), 1422 m, 1408 m ($\nu_{C=O}$, ν_{C-C} and δ_{CH}), 1390 w, 1350 w ($\nu_{C=C}$), 1227 m (ν_{C-O}), 1082 m (ν_{C-C} and δ_{CH}), 975 s (ν_{C-C}), 879 m (ν_{C-C} and δ_{CH}), 722 vs (ν_{CH}), 662 w (ν_{C-C}), 575m, 538 m (ν_{Zr-O})

Method 5

Tropolone (3.60 g; 29.8 mmol) was dissolved in chloroform (50 mL). $K_4[Zr(C_2O_4)_4]$ (5.00g; 7.25 mmol) was dissolved in 1 M oxalic acid (20 mL) and then neutralised with 1 M sodium carbonate (19 mL) before diluting to 50 mL with water. The solution of tropolone was added to the solution of $K_4[Zr(C_2O_4)_4]$ drop wise with stirring. The mixture was stirred for 20 mins before separating the pale yellow chloroform phase. The product was isolated by evaporation at 60°C. Yield 4.64 g (97%).

Synthesis of Zr(ethyl maltolate)₄

Method 6

A solution of ethyl maltol (4.00 g; 28.6 mmol) in tetrahydrofuran (100 mL) was added drop wise to solution of zirconium (IV) chloride (1.66 g; 7.12 mmol) in tetrahydrofuran (50 mL) and then stirred for 2 h. The solution was then heated in a water bath at between 50°C and 55°C for 3 h during which time the volume was reduced to ~30 mL. Hexane (50mL) was added to the solution and a pale yellow precipitate was formed. The resulting precipitate was collected by the use of a Büchner funnel. The product was washed with hexane (4 x 20 mL) and dried at 55°C for 3 h in an electric oven. Yield 4.12 g (91%).

Found C 51.8, H 4.3, N 0%; $C_{28}H_{28}O_{12}Zr$ requires 51.9, H 4.4, N 0%

δ_{H} (400 MHz, d^6 -DMSO), 8.24 (1H, d, $J_{\text{a,b}}$ 5.2, Ha), 6.64 (1 H, d, $J_{\text{a,b}}$ 4.8, Hb), 2.50 (2 H, q, J 7.3, CH₂), 0.91 (3 H, t, J 7.6, CH₃)

δ_{C} (100 MHz, d^6 -DMSO), 180.3 (CO), 155.97 (Ca), 154.36 (C2), 153.79 (C3), 109.94 (Cb), 21.46 (CH₂), 11.42 (CH₃)

IR ν_{max} (cm⁻¹): 3065 w, 2986 m, 2943 w, 2885 w, 1628 sh ($\nu_{\text{C=O}}$), 1606 sh ($\nu_{\text{C=O}}$), 1556 vs ($\nu_{\text{C=C}}$), 1519 s, 1507 sh, 1473 vs, 1456 sh, 1433 sh ($\nu_{\text{C-C}}$), 1393 m, 1368 m, 1331 s, 1262 vs, 1235 s, 1187 vs, 1102 w

Raman ν_{max} (cm⁻¹): 1599 vs ($\nu_{\text{C=O}}$), 1582 sh ($\nu_{\text{C=O}}$), 1518 s, 1476 m, 1428 w ($\nu_{\text{C-C}}$), 1327 w, 1264 w, 1048 s, 938 w, 845 m, 769 w, 725 s, 621 w, 603 m, 546 m, 539 m, 525 m, 511 m

Method 7

Ethyl maltol (4.20 g; 30.0 mmol) was dissolved in chloroform (50 mL). K₄[Zr(C₂O₄)₄] (5.00 g; 7.25 mmol) was dissolved in oxalic acid (20 mL) and then neutralised with 1 M Na₂CO₃ before diluting to 50 mL with water. The solution of ethyl maltol was added to the solution of K₄[Zr(C₂O₄)₄] drop wise whilst stirring and then stirred for a further 20 mins. The chloroform layer became off white in colour as the Zr(ethyl maltolate)₄ was extracted into the chloroform phase which was separated and heated on an electric hotplate at 60°C until the product had precipitated and the remaining chloroform evaporated. Yield 4.22 g (90%).

Synthesis of ⁸⁹Zr(oxinate)₄

⁸⁹Zr was supplied as Zr⁴⁺ (20-90 MBq, measured with a Capintec CRC-25 dose calibrator) in 0.1 M oxalic acid (Perkin-Elmer, Seer Green, UK), brought to pH 7 with 1M Na₂CO₃ and diluted to 500 μ L with distilled water. Oxine in chloroform (500 μ L, 1 mg/mL) was added and the vessel was vortexed (1000 RPM) for 15 mins to facilitate phase transfer. The two phases were then allowed to separate and the aqueous phase was transferred into a separate vessel. The chloroform extract was evaporated at 60 °C and the residue containing ⁸⁹Zr(oxinate)₄ was dissolved in 10-20 μ L of DMSO and diluted to 1-3 mL with phosphate buffered saline (PBS). Samples were analysed on silica gel impregnated ITLC strips (ITLC-SG; Agilent, UK) with ethyl acetate mobile phase. 8-9 cm long strips were developed in 50 mL centrifuge tubes. Chromatograms were evaluated on a LabLogic MINI-SCAN radio TLC linear

scanner connected to a LabLogic B-FC-3200 NaI detector for gamma photon detection.

$^{89}\text{Zr}(\text{tropolonate})_4$ and $^{89}\text{Zr}(\text{ethyl maltolate})_4$ were prepared similarly.

Cell Cultures

HCT116: colon cancer, J774: mouse macrophage and MDA-MB-231 breast cancer cell lines were cultured as adhesion cells at 37°C with CO₂ at 5 % under a humidified atmosphere.

Uptake Experiments

$^{89}\text{Zr}(\text{oxinate})_4$, $^{89}\text{Zr}(\text{tropolonate})_4$ and $^{89}\text{Zr}(\text{ethyl maltolate})_4$ were diluted to an activity of 0.05 MBq in 50 µL of a serum free medium. This was then added to glass test tubes containing the cell line under investigation ($\sim 1 \times 10^6$ cells) in 500 µL of a serum free medium. The percentage of uptake was determined in triplicate at 1, 15, 30, 45 and 60 minutes after adding the tracer to the cell line under investigation. Percentage uptake was determined at each of the time points by centrifuging the sample for 5 minutes at 2500 RPM. 450 µL of supernatant was collected using a micro pipette and placed into a clean glass vial. The supernatant (S) and cell pellet (C) were placed separately into a gamma counter to calculate the percentage of radioactivity in the labelled cells using the equation; $(C / (C + S)) \times 100$. Controls were the uptake of neutralised $^{89}\text{Zr}(\text{oxalate})_4$ and the determination of the degree of binding of the tracers to the glass test tubes.

Efflux Experiments

Following the method reported above the ^{89}Zr tracers were allowed 30 minutes for uptake to occur in the respective cell lines. Efflux was measured at time points of 1, 2, 3, 20 and 24 hrs depending on the tracer. Samples were centrifuged at ~ 2000 RPM for 5 mins. 450 µL of supernatant was then removed from the glass reaction vial. Cell pellets were washed with phosphate buffered saline (PBS) 500 µL x 2 to remove any excess tracer. Fresh media (with serum, 500 µL) was added to each reaction vial. Samples were incubated at 37°C until the required time point. At the required time point the sample was centrifuged at 3000 RPM for 5 mins to obtain a cell pellet. Supernatant (~ 400 -500 µL) from each reaction vial was placed into a new vial and matched to its cell pellet and both were analysed for radioactivity

utilising a gamma counter. Percentage retention of the tracers in the labelled cells was calculated by the comparison of the total activity in the cells and the supernatant.

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Footnote

Electronic supplementary information (ESI) available: Contains NMR, IR, Raman and UV-visible spectra.

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Table of Contents Entry

Preparation of tetravalent zirconium complexes containing monobasic bidentate ligands under radiopharmaceutical conditions has been evaluated. $\text{Zr}(\text{oxinate})_4$ and $\text{Zr}(\text{tropolonate})_4$ have been identified as candidates for cell tracking using the positron emitter Zr-89.

