Electronic Supplementary Material (ESI) for Dalton Transactions

A functionalized heterobimetallic ^{99m}Tc/Re complex as a potential bimodal imaging probe: synthesis, photophysical properties, cytotoxicity and cellular imaging investigations

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Electronic Supporting Information

I. Selected spectra

II. Stability assays

III. Cytotoxicity studies

IV. Confocal microscopic study

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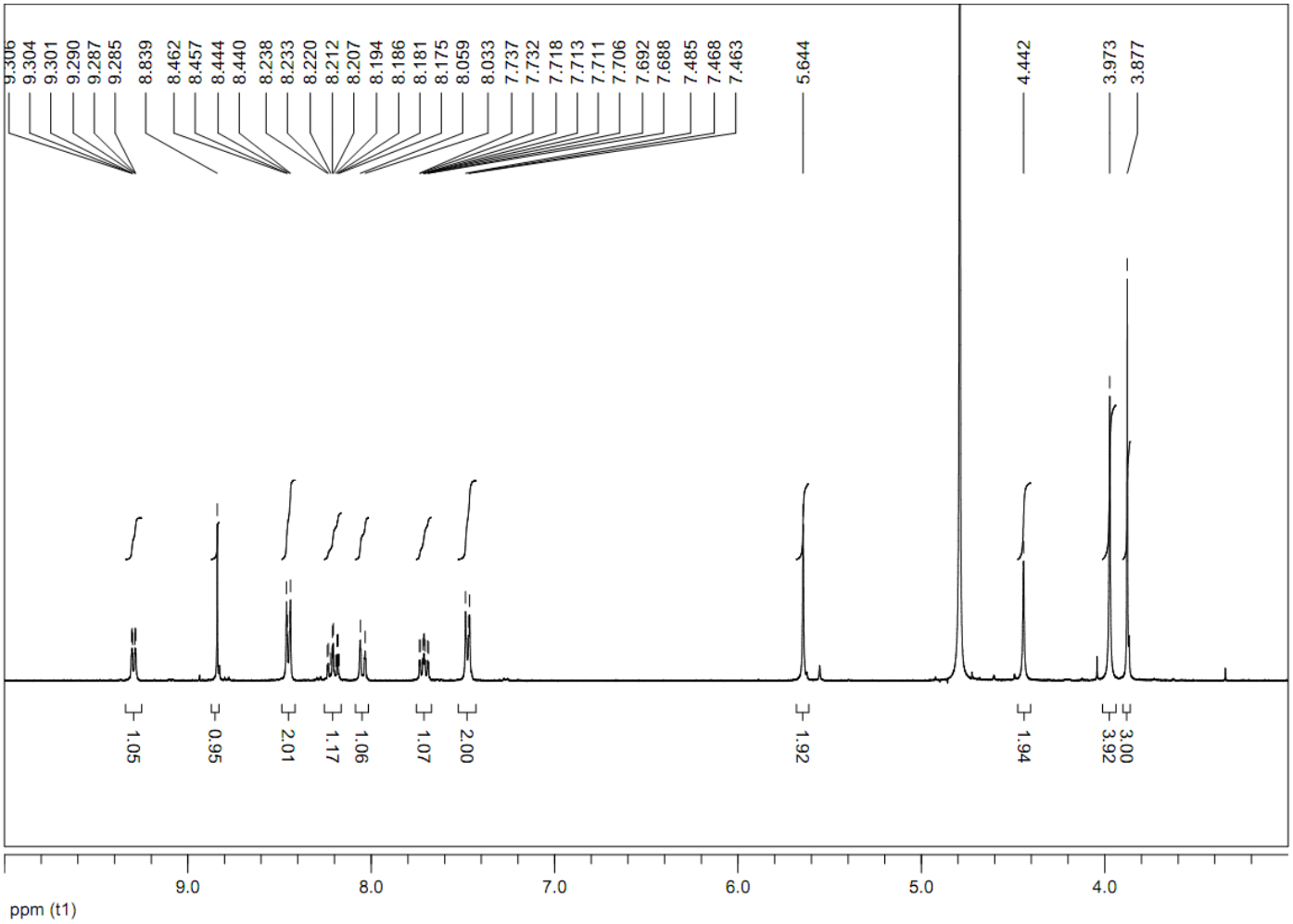
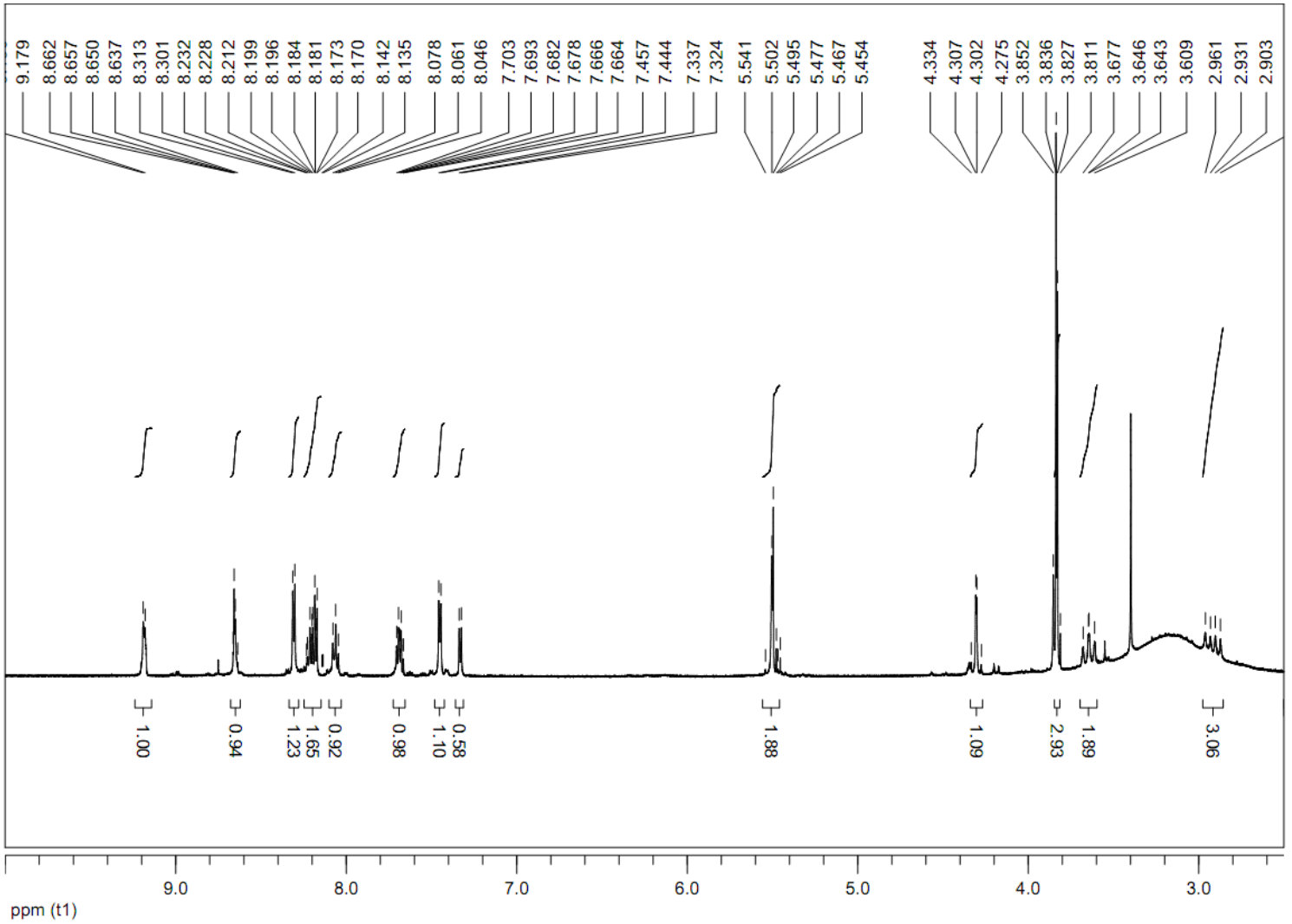
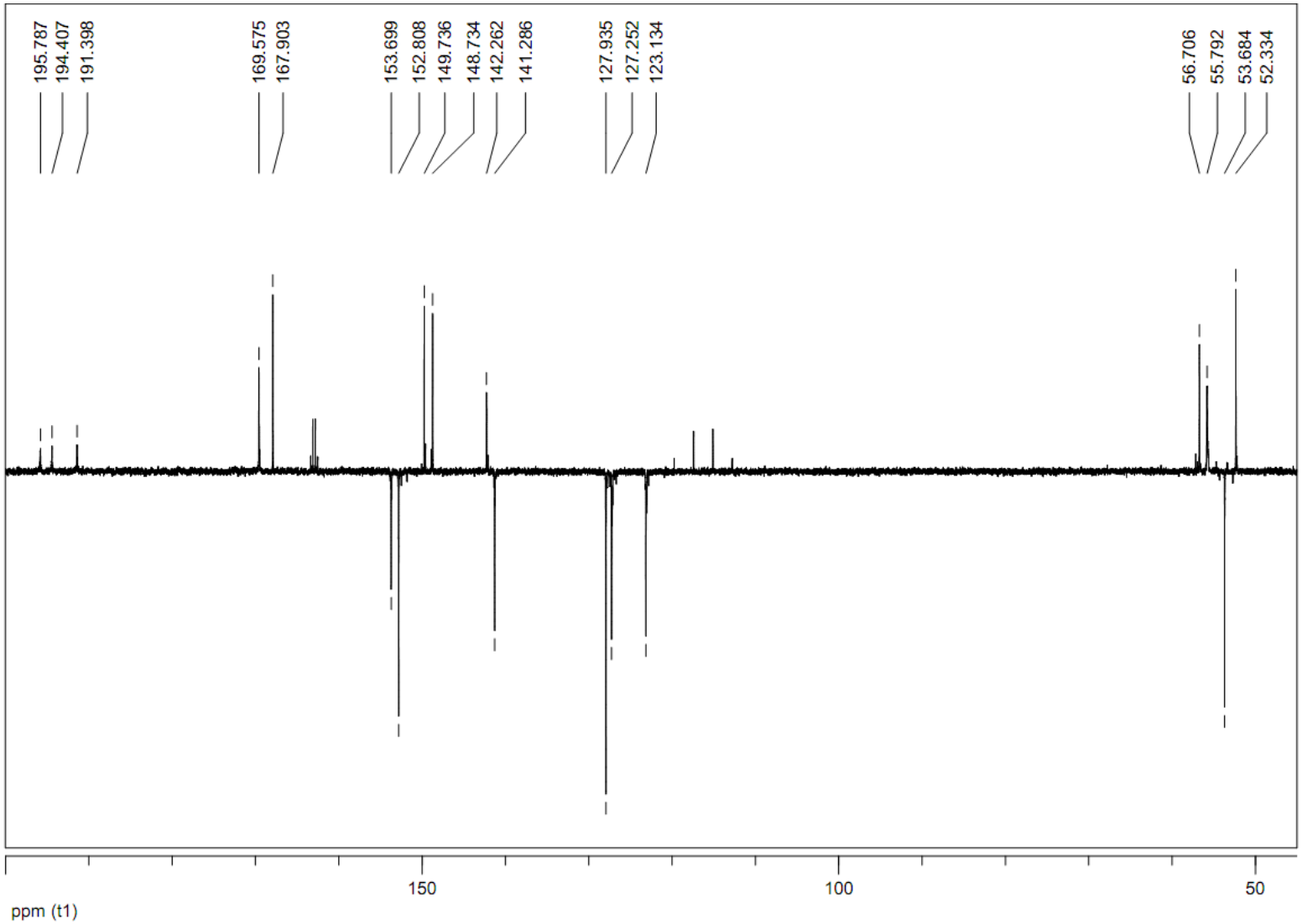
I. Selected spectra



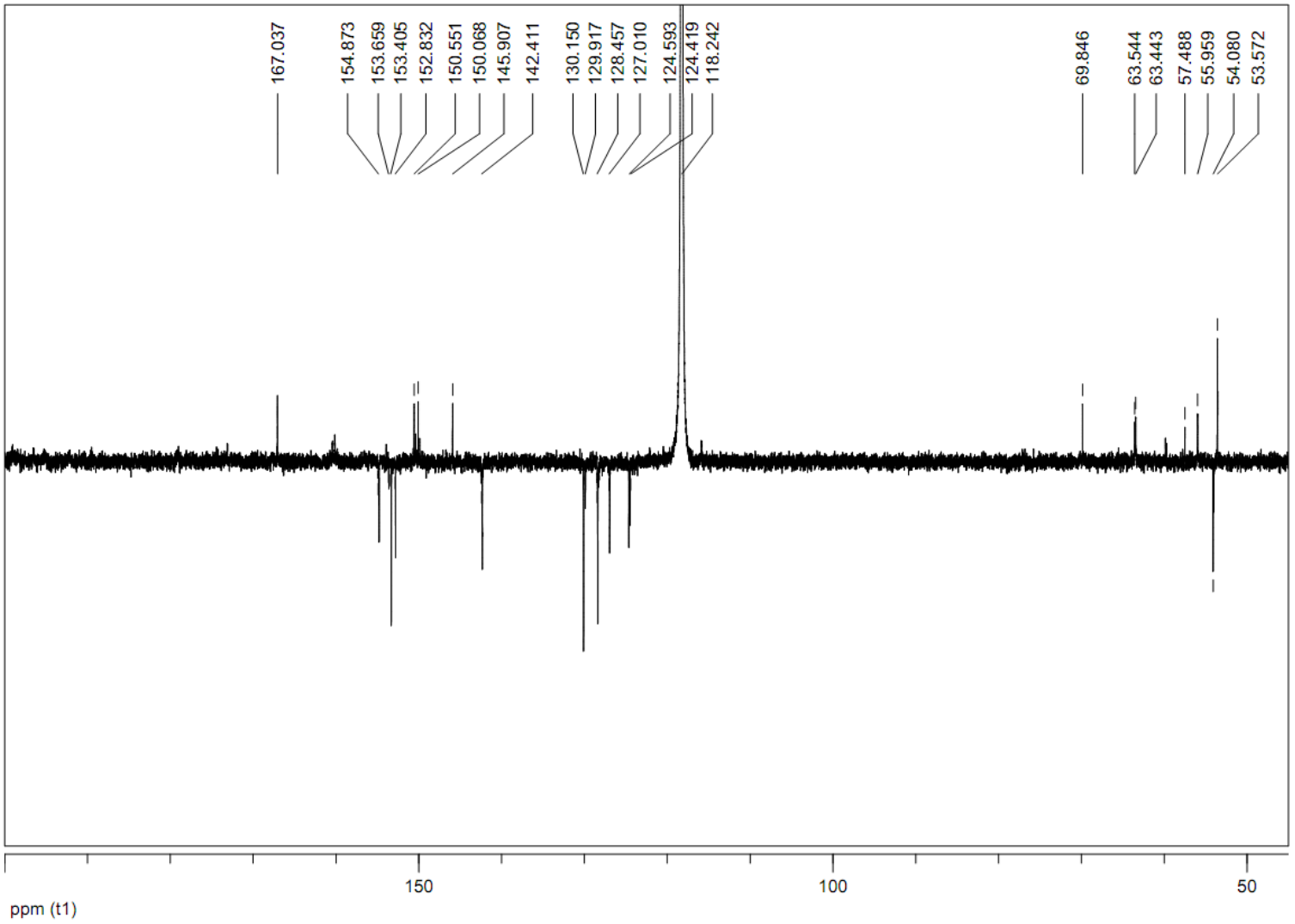
^{1}H NMR spectrum of **5** (D_2O, 300 MHz)



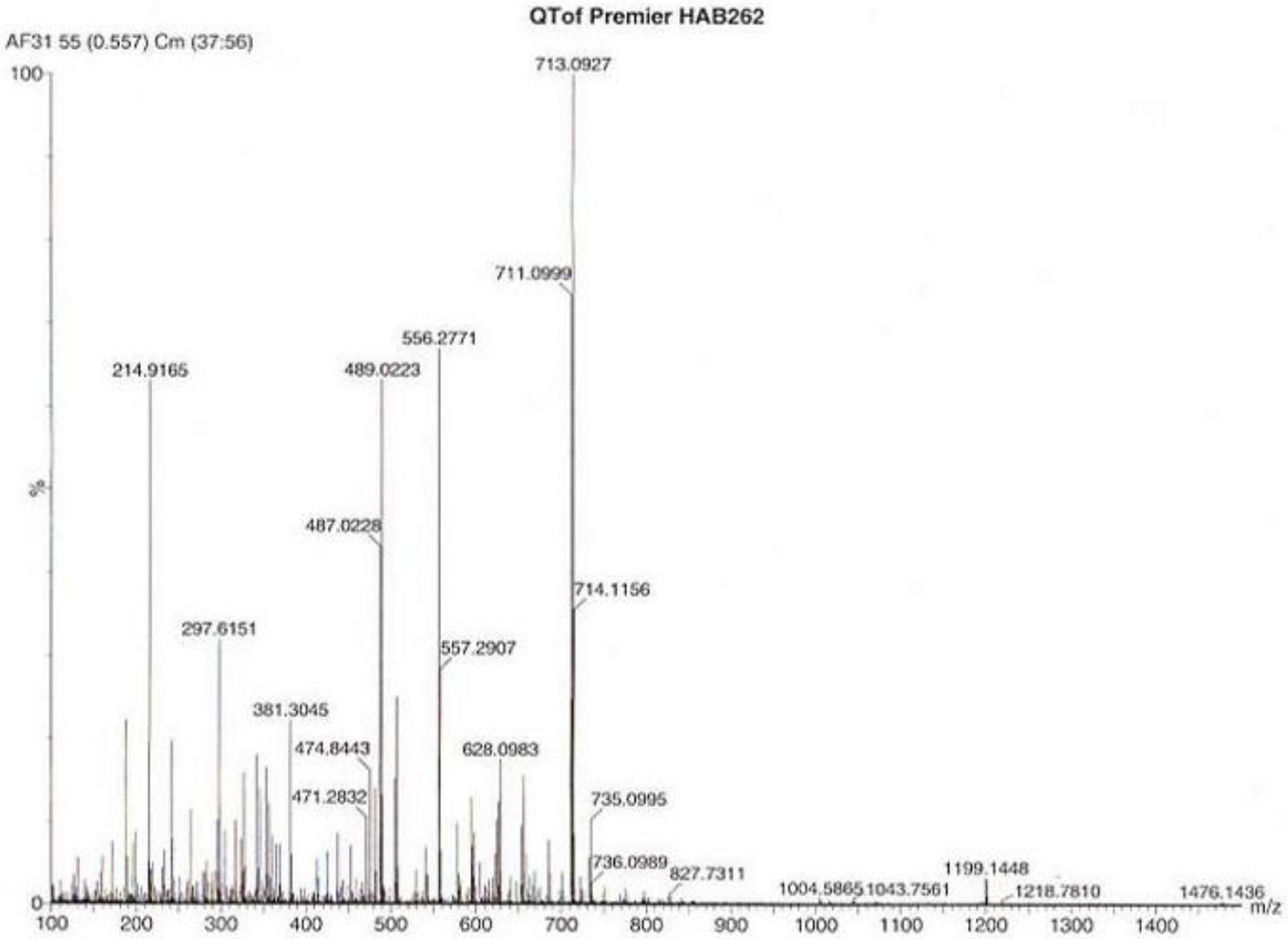
^{1}H NMR spectrum of **7** (CD_3CN, 500 MHz)



^{13}C-JMOD NMR spectrum of **5** (D_2O, 125 MHz)



^{13}C-JMOD NMR spectrum of **7** (CD_3CN, 125 MHz)



Mass Spectrum of **5** (ESI^+ mode)

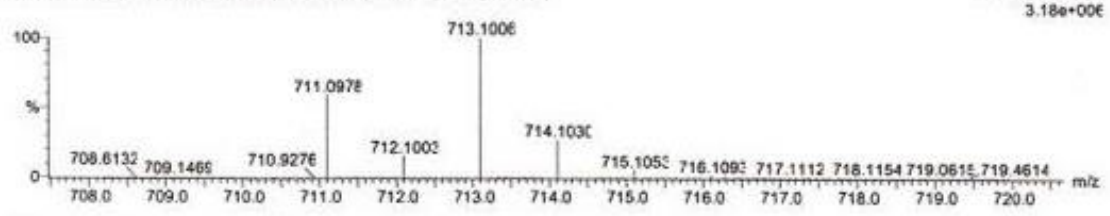
Elemental Composition Report

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 3.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd Electron Ions
3182 formula(e) evaluated with 16 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-100 N: 0-6 O: 0-9 185Re: 0-1 187Re: 0-1

AF31
UniT_11 453 (0.896) AM2 (Ar,22000.0,556.28,0.00,LS 10); Cm (441:517)

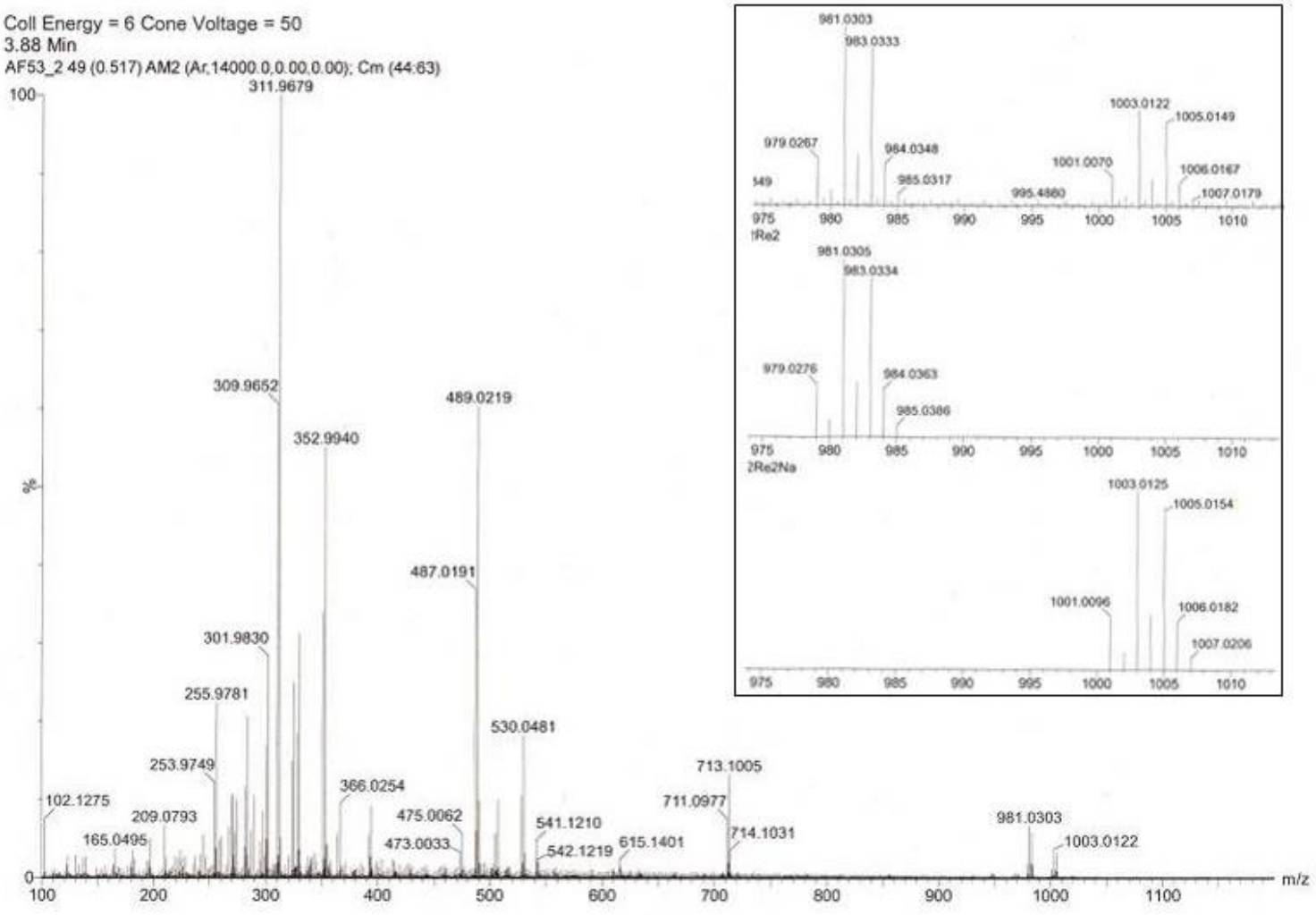
1: TOF MS ES+
3.18e+006



Minimum: 50.00 -1.5
Maximum: 100.00 3.0 10.0 50.0

Mass	RA	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
711.0978	58.36	711.0978	0.0	0.0	16.0	406.2	1.347	28.00	C23 H22 N6 O9 185Re
		711.0980	-0.2	-0.3	1.0	406.2	1.444	23.60	C14 H29 N O8 185Re 187Re
		711.0994	-1.6	-2.3	6.0	406.8	2.043	12.97	C15 H25 N5 O4 185Re 187Re
		711.0968	1.0	1.4	44.0	407.2	2.426	8.84	C47 H13 N5 O4
		711.0959	1.9	2.7	29.0	407.3	2.454	8.60	C35 H18 N4 O2 185Re
		711.0954	2.4	3.4	39.0	407.5	2.698	6.74	C46 H17 N O8
		711.0970	0.8	1.1	29.0	407.7	2.884	5.59	C38 H20 O3 187Re
		711.1002	-2.4	-3.4	21.0	407.7	2.932	5.33	C27 H20 N6 O6 187Re
		711.1000	-2.2	-3.1	33.0	408.6	3.753	2.35	C40 H18 N2 185Re
713.1006	100.00	713.1006	0.0	0.0	16.0	337.6	0.001	99.91	C23 H22 N6 O9 187Re
		713.0979	2.7	3.8	14.0	344.8	7.221	0.07	C23 H23 N3 185Re 187Re
		713.0998	0.8	1.1	1.0	347.1	9.498	0.01	C11 H27 N5 O7 185Re 187Re
		713.0987	1.9	2.7	29.0	347.9	10.389	0.00	C35 H18 N4 O2 187Re
		713.1003	0.3	0.4	28.0	348.1	10.525	0.00	C36 H20 N2 O3 185Re
		713.1012	-0.6	-0.8	43.0	351.2	13.627	0.00	C48 H15 N3 O5
		713.1027	-2.1	-2.9	33.0	351.2	13.662	0.00	C40 H18 N2 187Re

High Resolution Mass Spectrum of **5** (ESI^+ mode)



Mass Spectrum of **7** (ESI^+ mode).
In the window: experimental MS zoom (up), $[M+H]^+$ (middle) and $[M+Na]^+$ (bottom) theoretical isotopic signatures

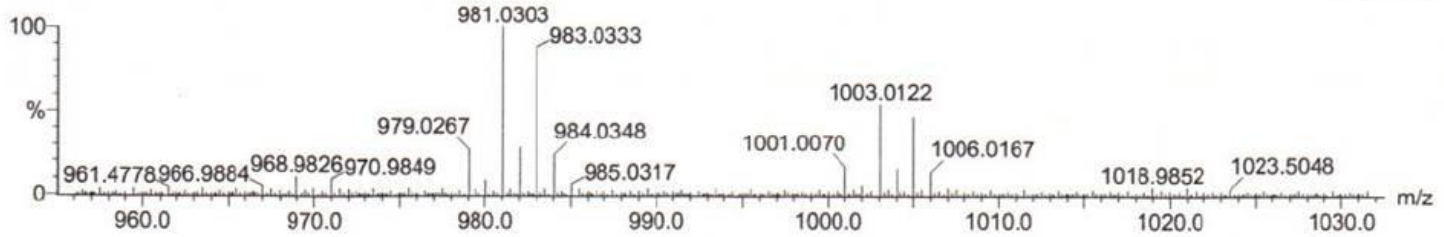
Elemental Composition Report

Single Mass Analysis

Tolerance = 2.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

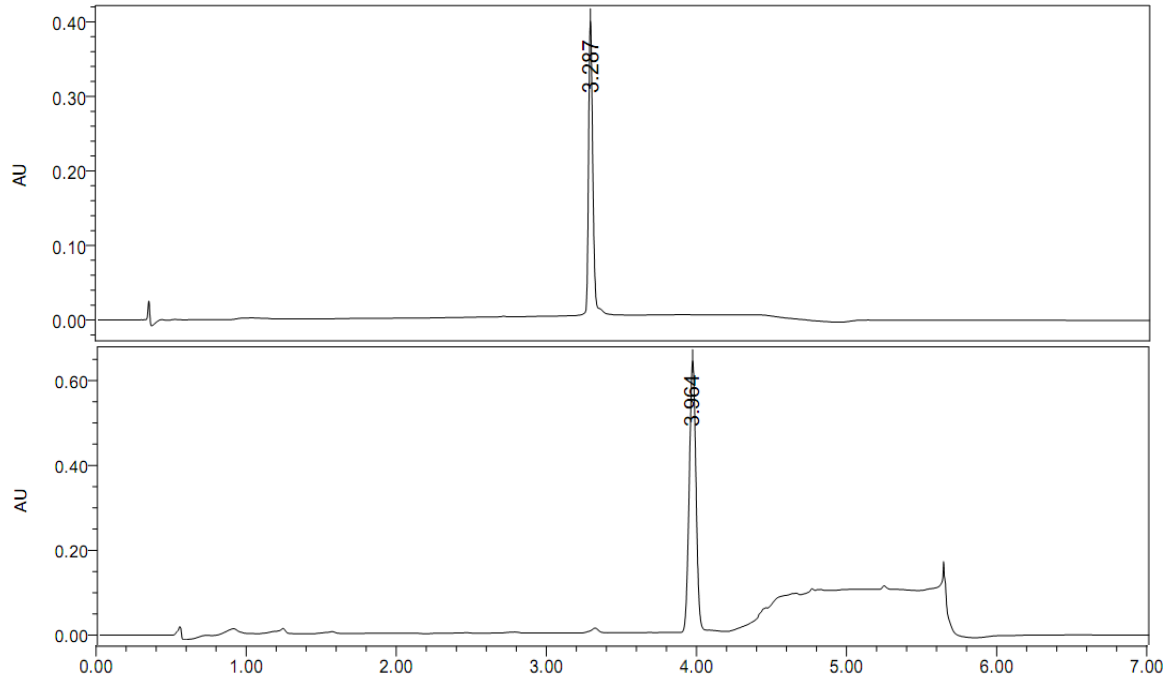
Monoisotopic Mass, Odd and Even Electron Ions
762 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-50 H: 0-50 N: 0-10 O: 0-15 185Re: 2-2

Coll Energy = 6 Cone Voltage = 50 XEVO-G2QTOF#YCA210
3.88 Min
AF53_2 49 (0.517) AM2 (Ar,14000.0,0.00,0.00); Cm (44:63)
2: TOF MS ES+
3.08e+005



Minimum:				-1.5				
Maximum:		5.0	2.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
979.0267	979.0263	0.4	0.4	20.0	140.5	1.022	35.97	C24 H19 N9 O11 185Re2
	979.0250	1.7	1.7	15.0	140.6	1.197	30.22	C23 H23 N5 O15 185Re2
	979.0258	0.9	0.9	32.5	141.4	1.981	13.80	C38 H17 N4 O5 185Re2
	979.0271	-0.4	-0.4	37.5	142.0	2.597	7.45	C39 H13 N8 O 185Re2
→	979.0277	-1.0	-1.0	19.5	142.1	2.707	6.68	C26 H21 N6 O12 185Re2
	979.0271	-0.4	-0.4	32.0	142.6	3.186	4.13	C40 H19 N O6 185Re2
	979.0285	-1.8	-1.8	37.0	143.5	4.046	1.75	C41 H15 N5 O2 185Re2

High Resolution Mass Spectrum of **7** (ESI^+ mode)



Analytical Chromatograms of **5** (top) and **7** (bottom) after HPLC purification
(*Column: Acquity BEH UPLC C18 column, 1.7 µm 2.1x50 mm, 0.3 mL/min,*
Solvent (A/B): 0.1% TFA in water / 0.1% TFA in CH_3CN, Gradient: from A:B 90:10 to 0:100 in 4.5 min, λ=239 nm)

II. Stability assays

Procedure

Radiocomplex ^{99m}Tc/Re (8) stability vs. Histidine

The histidine challenge experiment was performed on purified radiocomplex **8** using a large excess of histidine (1:20 molar ratio). In a vial containing 100 µL of freshly prepared histidine solution (Histidine monohydrate monohydrochlorate PROLABO; 4.77 10^{-3} M in water), 20 µL of purified ^{99m}Tc/Re complex **8** (1.2 10^{-3} M) were added. The mixture was stirred at 37°C from 1 to 6h. After this incubation time, 100 µL CH_3CN were added, the mixture was centrifuged 5 min at 300 rpm. The supernatant was then analyzed by HPLC, under the previous conditions.

Results

Table 1. Stability of complexes **7** and **8** in buffer solution, and against ligand exchange with histidine

	Complex **7**[a]			Complex **8**[a]		
Conditions	0.5 h	2 h	6 h	0.5 h	2 h	6 h
Aqueous[b]	>99%	>99%	>99%	>99%	>99%	>99%
Histidine[c]	*n.d.*	*n.d.*	*n.d.*	99%	95%	88%

a: Percent of complex remaining at the indicated time, b: Tris buffer, pH 7.4 at ambient temperature; c: ligand exchange with an excess of histidine at 37°C, *n.d.*: not determined

III. Cytotoxicity studies

Material and methods:

Human lung alveolar (A549), human breast (MCF7) and human colon (HT29) adenocarcinoma cells were obtained from the Developmental Therapeutics Branch of the National Cancer Institute, Bethesda, MD (USA). Cells were grown with RPMI-1640 GlutaMax medium, 10% fetal bovine serum (GIBCO, Grand Island, USA), at 37°C and 5% CO_2 in flasks purchased from Nunc (Denmark). Cell passaging was performed twice a week. Adherent cells cultures were washed once with phosphate-buffer saline (PBS, 10x, pH 7.2, GIBCO) and harvested by stripping of flasks with trypsin (0.25% Trypsin-EDTA, 1X, Phenol Red, GIBCO) after 5 minutes incubation period at 37°C. Cells were counted on Countess Automatic Cells Counter, using trypan blue, before being plated into a 96-well plate (800 MCF7 or HT29 cells / 1,000 A549 cells per well). 24 h later, the medium was removed and replaced by 200 µL dilutions of **7** in RPMI, 1% DMSO.[1] After a 72 h incubation time, the number of surviving cells was estimated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay[2]: after removing the medium, MTT reagent (200 µL per well, 0.5 mg/mL in RPMI) was added. After 4h, it was removed and DMSO was added (150 µL per well) to dissolve formazan crystals. Absorbance of the purple solutions was evaluated on a microplate spectrophotometer (Bio-Tek Instruments; measurements at 570 and 630 nm). Wells treated with solvent only were used as a control, with 100% viability. Absorbance of treated wells was compared to this control value, to estimate cell viability. All experiments were performed in triplicate.

[1] (a) K.K.W. Lo, M.W. Louie, K.S. Sze, J.S.Y. Lau, *Inorg. Chem.,* 2008, **47**, 602-611, (b) M.W. LouieT.T.H. Fong, K.K.W. Lo, *Inorg. Chem*., 2011, **50**, 9465-9471.

[2] T. Mosmann, *J. Immunol. Methods*, **1983**, *65*, 55-63.

Cytotoxicity studies of Paclitaxel, Cisplatin and complex 7 on A549, MCF7 and HT29 cells (standard deviation between three replicates)

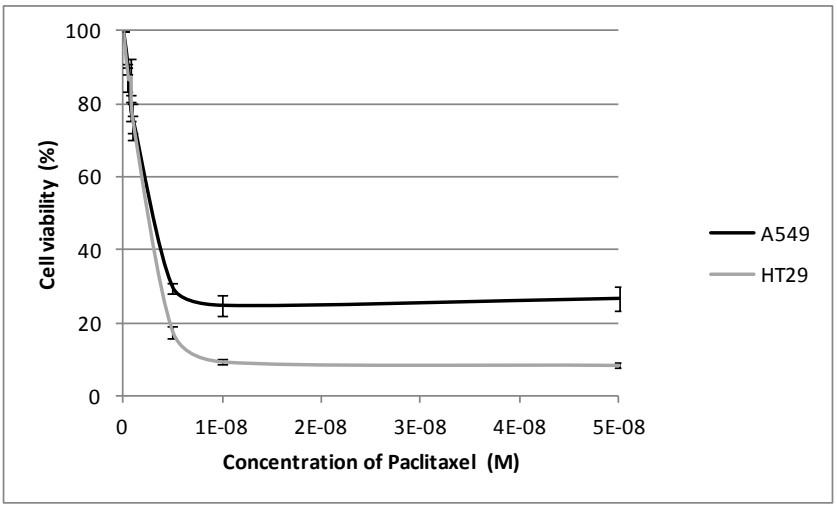


Figure 1. Cytotoxicity positive assay with Paclitaxel (nanomolar IC_{50})

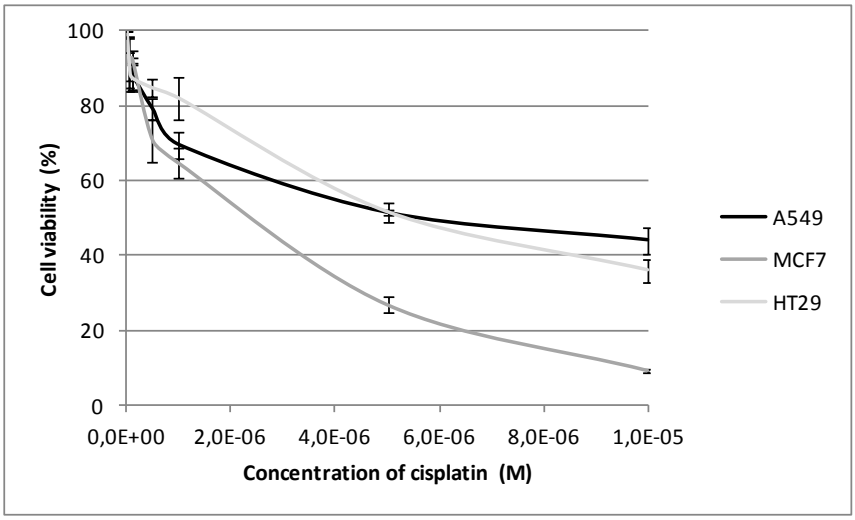


Figure 2. Cytotoxicity positive assay with Cisplatin (micromolar IC_{50})

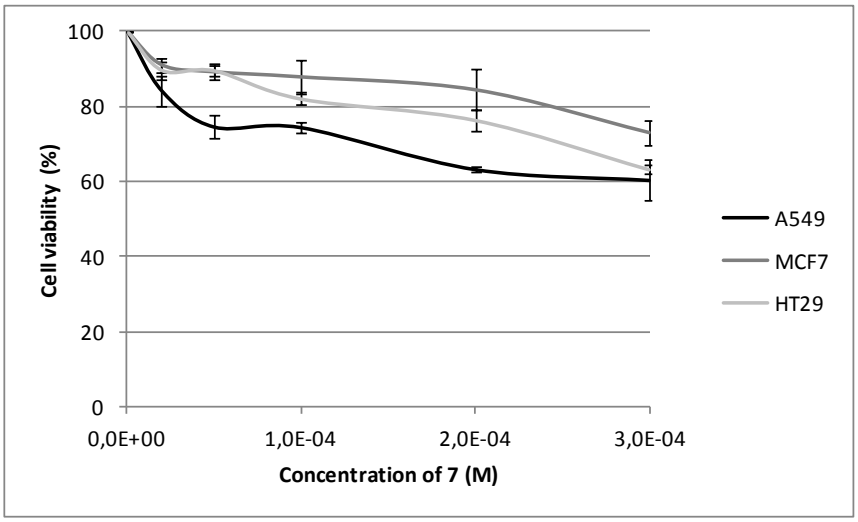


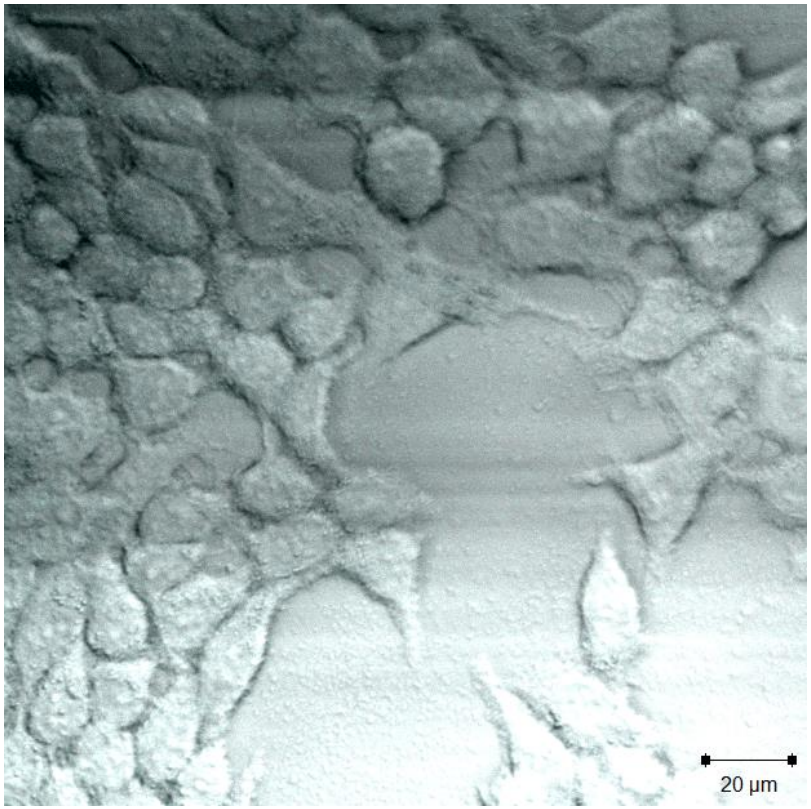
Figure 3. Cytotoxicity studies of complex **7** (micromolar IC_{50})

IV. Confocal microscopic study

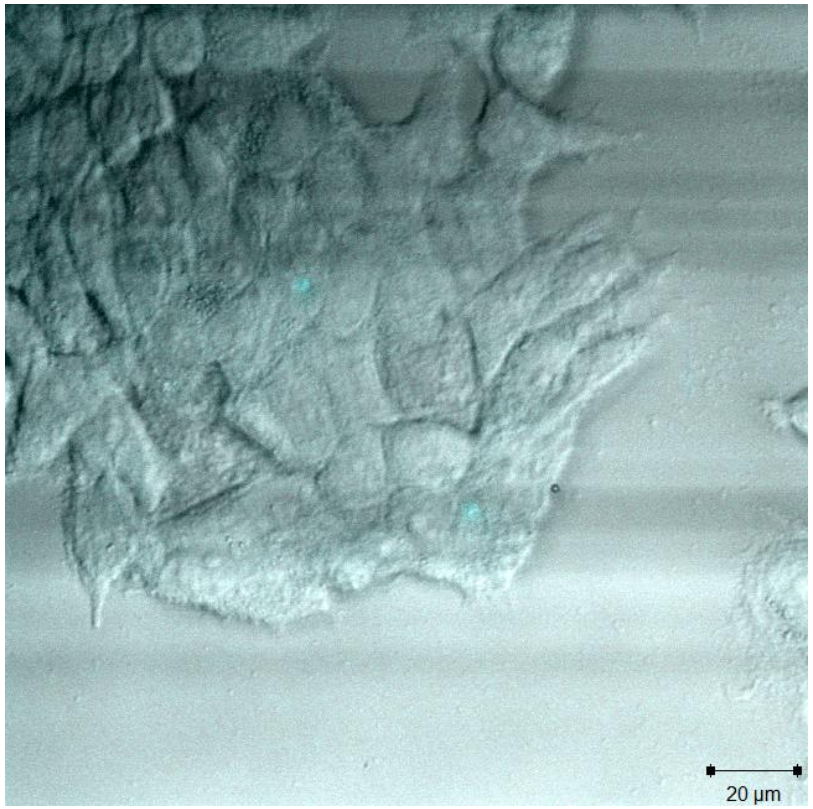
Material and methods

5,000 cells of A549 were plated onto 96-black well plates and incubated at 37°C, 5% CO_2 for 24 h, in 200 µL RPMI medium. After this incubation time, the medium was removed and replaced by 200 µL solutions of **7** (0, 50, 100 and 200 µM) in RPMI + 1% DMSO medium. The compound was incubated 72h and then removed. Cells were washed twice with PBS and viewed, still in PBS, under a Zeiss LSM 510 confocal microscope (Carl Zeiss AG, Oberkochen, Germany) with LSM Image Browser software, equipped with a diode laser, amongst others. Fluorescence was observed at room temperature with a 25x objective, with an excitation at 405 nm, using the DAPI configuration (emission in blue area; Dye (emission): DAPI; laser (excitation): 405 nm at 25 mW). Wells without compound **7** were used as a control, to ensure that no background fluorescence was recorded.

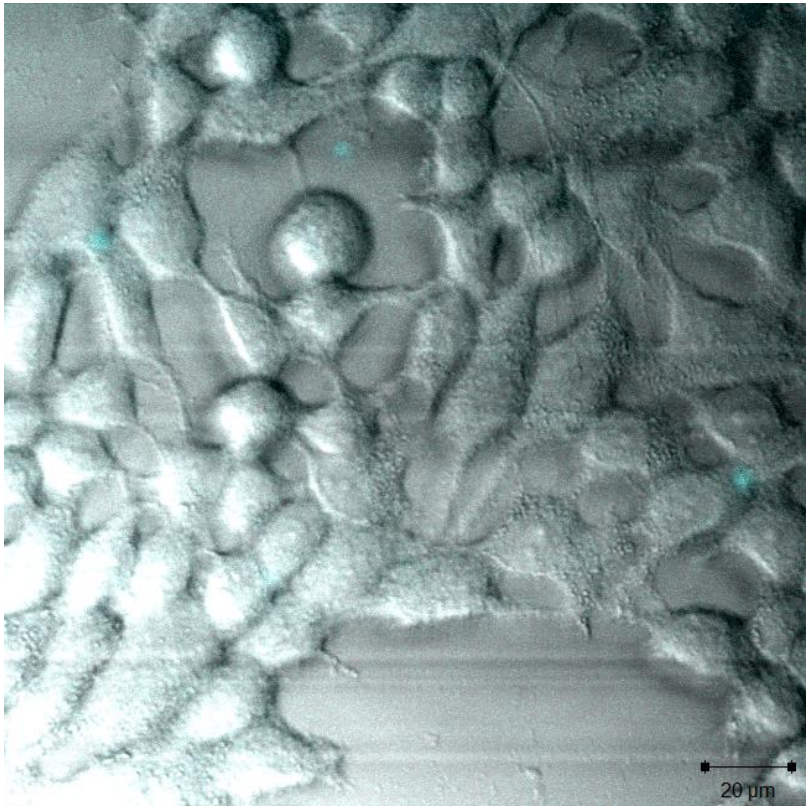
Confocal microscopic study of A549 cells after treatment with complex 7 : dose-response relationship
(various dose concentrations, λ_{ex} = 405 nm, 72h incubation time)



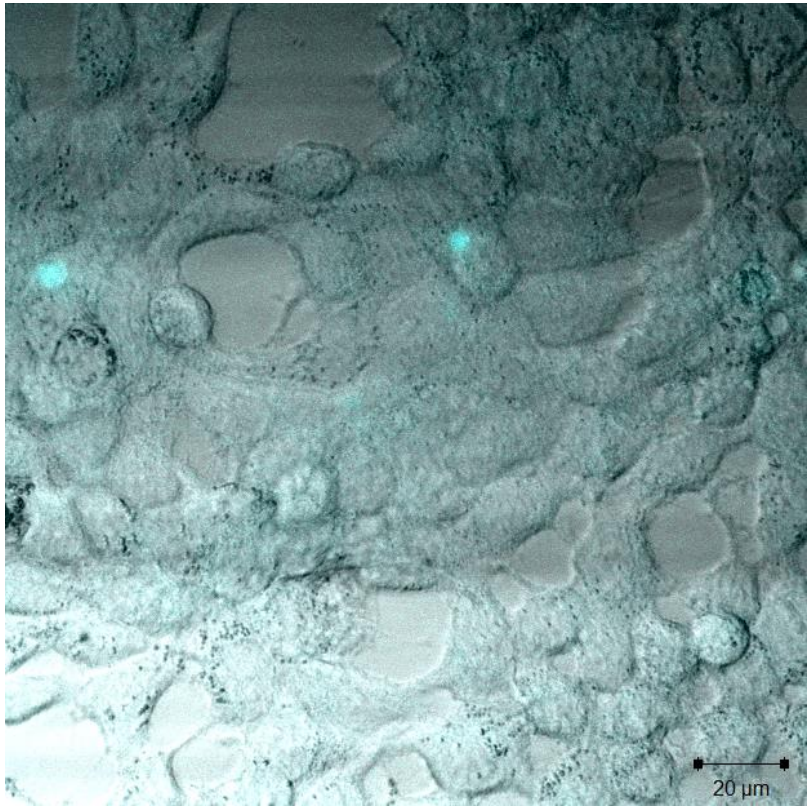
Confocal microscopic image of A549 cells without incubation of complex **7** and two washes with PBS, as a reference.



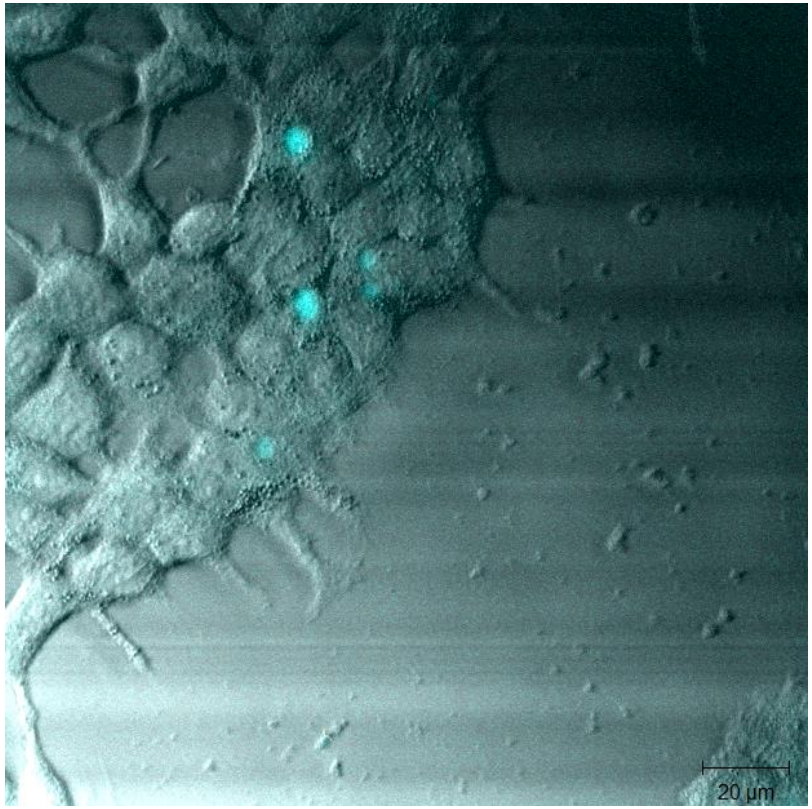
Confocal microscopic image of A549 after a 72h-incubation time with ***5 10^{-5} M*** complex **7** and two washes with PBS.



Confocal microscopic image of A549 after a 72h-incubation time with ***1 10^{-4} M*** complex **7** and two washes with PBS.



Confocal microscopy image of A549 after a 72h-incubation time with ***1.5 10^{-4} M*** complex **7** and two washes with PBS.



Confocal microscopy image of A549 after a 72h-incubation time with **2 10^{-4} M** complex **7** and two washes with PBS.