Supplementary Information

New insights on ruthenium(II) metallodendrimers as anticancer drugs nanocarriers: from synthesis to preclinic behaviour

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Content

Fig. CDCl₃.	S2	a)	¹ H-NMR	and	b)	³¹ P-NMR	spectra	of	the	GORu	metallodendrimer	performed	in 4
Fig. CDCl₃.	S3	a)	¹ H-NMR	and	b)	³¹ P-NMR	spectra	of	the	G1Ru	metallodendrimer	performed	in 5
Fig. CDCl₃.	S4	a)	¹ H-NMR	and	b)	³¹ P-NMR	spectra	of	the	G2Ru	metallodendrimer	performed	in 6
Fig. SS cells a	5 FT-I nd G(R speo DRu in	ctra of the KBr pellets	GOCN d	endrir	mer (above)	and GORu	meta	llodenc	drimer (b	elow). GOCN was perf	ormed using I	NaCl 7
Fig. Se cells a	5 FT-l nd G	R speo LRu in	ctra of the (KBr pellets	G1CN d	endrin	ner (above)	and G1Ru	metal	lodend	lrimer (be	elow). G1CN was perfo	ormed in the I	NaCl 7
Fig. S7 cells a	Fig. S7 FT-IR spectra of the G2CN dendrimer (above) and G2Ru metallodendrimer (below). G2CN was performed in the NaCl cells and G2Ru in KBr pellets												
Fig. S8	M as	s spec	tra of GORu	mtallo	dendr	imer							9
Fig. S9	Mas	s spec	tra of G1Ru	metallo	odend	rimer							10
Fig. S1	. 0 Ma	ss spe	ectrum of G	2Ru me	tallode	endrimer							11
Table repres	S1.	Size, : d	zeta poten [.] as	tial, an mea	d poly an	/dispersity i ±	index meas SD	surem of	ients c	of the ru five	thenium metallodeno independent	drimers. Data experim	are ents 11
Fig. S1 out at	1 ¹ H-	NMR obe te	and ³¹ P-NN emperature	IR spect	tra of C. a) ¹	G0Ru metal H-NMR: † =	lodendrime proton sig	er wei inal fr	re perfo om the	ormed in e cyclope	DMSO-d ₆ . Stability stunt ntadienyl of the meta	udies were car allodendrimer,	ried , * =

out at a probe temperature of 25°C. a) ¹H-NMR: \dagger = proton signal from the cyclopentadienyl of the metallodendrimer, * = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: \dagger = phosphorous signal from the phosphines of the metallodendrimer, * = phosphorous signal from the phosphines of the RuCp complex. 12

Fig. S12 ¹H-NMR and ³¹P-NMR spectra of G1Ru metallodendrimer were performed in DMSO- d_6 . Stability studies were carried out at a probe temperature of 25°C. a) ¹H-NMR: [†] = proton signal from the cyclopentadienyl of the metallodendrimer, ^{*} = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: [†] = phosphorous signal from the phosphines of the

metallodendrimer,	*	=	phosphorous	signal	from	the	phosphines	of	the	RuCp
complex										12

Fig. S13 ¹H-NMR and ³¹P-NMR spectra of G1Ru metallodendrimer were performed in DMSO- d_6 . Stability studies were carried out at a probe temperature of 37°C. a) ¹H-NMR: [†] = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: [†] = phosphorous signal from the phosphines of the metallodendrimer, ^{*} = phosphorous signal from the phosphines of the RuCp complex. 13

Fig. S14 ¹H-NMR and ³¹P-NMR spectra of G2Ru metallodendrimer were performed in DMSO-*d*₆. Stability studies were carried out at a probe temperature of 25°C. a) ¹H-NMR: ⁺ = proton signal from the cyclopentadienyl of the metallodendrimer, ^{*} = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: ⁺ = phosphorous signal from the phosphines of the metallodendrimer, ^{*} = phosphorous signal from the phosphines of the RuCp complex. 13

Fig. S15 ¹H-NMR and ³¹P-NMR spectra of G2Ru metallodendrimer were performed in DMSO- d_6 . Stability studies were carried out at a probe temperature of 37°C. a) ¹H-NMR: [†] = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: [†] = phosphorous signal from the phosphines of the metallodendrimer, ^{*} = phosphorous signal from the phosphines of the RuCp complex. 14



Fig. S1 a) Synthesis of the ruthenium metallodendrimers and b) Structural representation of the prepared GORu – G2Ru metallodendrimers.





Fig. S2 a) ¹H-NMR and b) ³¹P-NMR spectra of the GORu metallodendrimer performed in CDCl₃.



Fig. S3 a) ¹H-NMR and b) ³¹P-NMR spectra of the G1Ru metallodendrimer performed in CDCl₃.



Fig. S4 a) ¹H-NMR and b) ³¹P-NMR spectra of the G2Ru metallodendrimer performed in CDCl₃.



Fig. S5 FT-IR spectra of the GOCN dendrimer (above) and GORu metallodendrimer (below). GOCN was performed using NaCl cells and GORu in KBr pellets.



Fig. S6 FT-IR spectra of the G1CN dendrimer (above) and G1Ru metallodendrimer (below). G1CN was performed in the NaCl cells and G1Ru in KBr pellets.



Fig. S7 FT-IR spectra of the G2CN dendrimer (above) and G2Ru metallodendrimer (below). G2CN was performed in the NaCl cells and G2Ru in KBr pellets.



Fig. S8 Mass spectra of G0Ru metallodendrimer.



Fig. S9 Mass spectra of G1Ru metallodendrimer.



Fig. S10 Mass spectrum of G2Ru metallodendrimer.

Table S1. Size, zeta potential, and polydispersity index measurements of the ruthenium metallodendrimers. Data are represented as mean ± SD of five independent experiments.

-	Size (nm)	Zeta Potential (mV)	Polydispersity index
G0Ru	115.5 ± 4.6	29.9 ± 2.0	0.177 ± 0.013
G1Ru	60.2 ± 4.5	40.7 ± 0.6	0.267 ± 0.049
G2Ru	208.7 ± 7.3	41.7 ± 1.6	0.247 ± 0.007



Fig. S11 ¹H-NMR and ³¹P-NMR spectra of GORu metallodendrimer were performed in DMSO- d_6 . Stability studies were carried out at a probe temperature of 25°C. a) ¹H-NMR: \dagger = proton signal from the cyclopentadienyl of the metallodendrimer, * = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: \dagger = phosphorous signal from the phosphines of the metallodendrimer, * = phosphorous signal from the phosphines of the RuCp complex.



Fig. S12 ¹H-NMR and ³¹P-NMR spectra of G1Ru metallodendrimer were performed in DMSO- d_6 . Stability studies were carried out at a probe temperature of 25°C. a) ¹H-NMR: ⁺ = proton signal from the cyclopentadienyl of the metallodendrimer, ^{*} = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: ⁺ = phosphorous signal from the phosphines of the metallodendrimer, ^{*} = phosphorous signal from the phosphines of the RuCp complex.



Fig. S13 ¹H-NMR and ³¹P-NMR spectra of G1Ru metallodendrimer were performed in DMSO-d₆. Stability studies were carried out at a probe temperature of 37°C. a) ¹H-NMR: \dagger = proton signal from the cyclopentadienyl of the metallodendrimer, * = proton signal from the cyclopentadienyl of the RuCp complex. b) ³¹P-NMR: \dagger = phosphorous signal from the phosphines of the metallodendrimer, * = phosphorous signal from the phosphines of the RuCp complex.



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Commound	N.(a)	[Ru, μM]						
Compound	IVI ^{*/} -	A2780	A2780cisR	CAL-72	U-87MG	Caco-2	MCF-7	hMSC
RuCp	1	0.5	0.7	0.6	0.5	0.6	0.4	3.9
GORu	4	1.2	1.0	1.6	1.4	1.5	1.3	3.3
G1Ru	8	1.2	1.8	2.4	1.4	2.1	1.8	6.6
G2Ru	16	1.1	1.1	1.8	1.3	1.3	1.4	6.4

Table S2. Cytotoxic concentration of ruthenium in the metallodendrimers against A2780, A2780cisR, CAL-72, U-87MG, Caco-2, and MCF-7 cancer cell lines and hMSCs as a non-cancer cell line.



Fig. S16 Apoptosis rates in different groups by quantification of the TUNEL-positive tumour cells in tumour sections.

Table S3. The *in vitro* IC₅₀ values of the metallodendrimers using MCF-7 and hMSCs and the *in vivo* concentration of the metallodendrimers, and the corresponding ruthenium concentration.

	Metal centres	in	vitro	in vivo		
Compound		MCF-7	hMSC	[metallodendrimer,	[Pu uM]	
Compound		(IC ₅₀ , μM)	(IC ₅₀ , μM)	μM]	[1,0, [1,1]	
RuCp	1	0.37 ± 0.03	3.89 ± 0.3	650	650	
G0Ru	4	0.33 ± 0.02	0.83 ± 0.07	162.5	650	
G1Ru	8	0.23 ± 0.05	0.83 ± 0.04	162.5	1310	
G2Ru	16	0.09 ± 0.01	0.39 ± 0.3	162.5	2620	