Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information for

A novel Gd^{3+} DTPA-bisamide complex with high relaxivity as an MRI contrast agent

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Materials and Instrumentation.

All reagents and solvents were obtained commercially and used without further purification unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were recorded on JNM-ECS-400 MHz spectrometers and referenced to the solvent signals. Mass spectra (ESI) were performed on Bruker Daltonics Esquire6000 mass spectrometers.

Determination of the association constant of L- Gd^{3+} complex system.

The total binding constant of **L** complexing with Gd^{3+} was studied by the absorbance curve at 270 nm, which was obtained from the absorbance titration spectra of **L** with the increase of $Gd(NO_3)_3 \cdot 6H_2O$ in water. The absorbance titration for **L**-Gd^{3+} system exhibited 1:1 stoichiometry. The equilibrium is given by following equation:

$$Gd + L \leftrightarrow GdL$$

The association constant, *K*, is expressed as:

$$K = \frac{[GdL]}{[Gd]eq\,[L]eq} = \frac{[GdL]}{([Gd][GdL])\,\epsilon_L[GdL]}$$

where $[Gd]_{eq}$, $[L]_{eq}$ and [GdL] are the equilibrium concentrations of free Gd^{3+}, ligand **L** and **L**- Gd^{3+}, respectively. [Gd] and c_L are the initial concentrations of Gd^{3+} and ligand **L**, respectively. The equation is transformed to:

$$[GdL] = \frac{(c_L + [Gd] + 1/K) - \sqrt{(c_L + [Gd] + 1/K^2) - 4c_L[Gd]}}{2}$$

Absorbance is given by the Lambert-Beer law as follows:

$$A_0 = \varepsilon_0\, c_L\, l$$

$$A = \varepsilon_0\, [L]_{eq}\, l + \varepsilon\, [GdL] l$$

$$A_{max} = \varepsilon\, [GdL]_{max}\, l = \varepsilon c_L\, l$$

A_0 is the absorbance of **L** at 270 nm without Gd^{3+}, A is the absorbance of **L** at 270 nm obtained with Gd^{3+}, and A_{max} is the absorbance of **L** at 270 nm in the presence of excess amount of Gd^{3+}. These relations together with $c_L = [L]_{eq} + [GdL]$ lead to:

$$\frac{A - A_0}{A_{max} - A_0} = \frac{[GdL]}{c_L}$$

Thus, the following equation is obtained:

$$A = A_0 + \frac{A_{max} - A_0}{2}\{(1 + \frac{[Gd]}{c_L} + \frac{1}{c_L K}) - \sqrt{(1 + \frac{[Gd]}{c_L} + \frac{1}{c_L K}^2) - 4\frac{[Gd]}{c_L}}\}$$

The equation was used for fitting of the absorption titration data with Gd^{3+}. The obtained curve is shown in Figure S1.

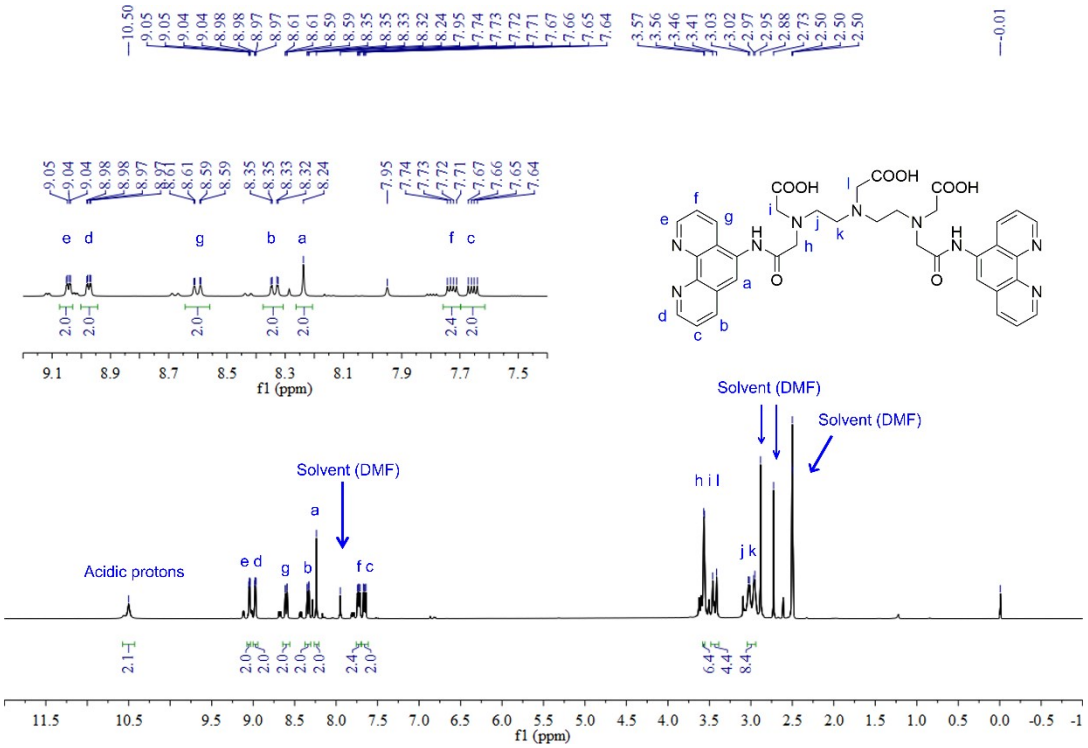

Fig. S1 ^{1}H NMR Spectrum of **L** in DMSO-d_6.

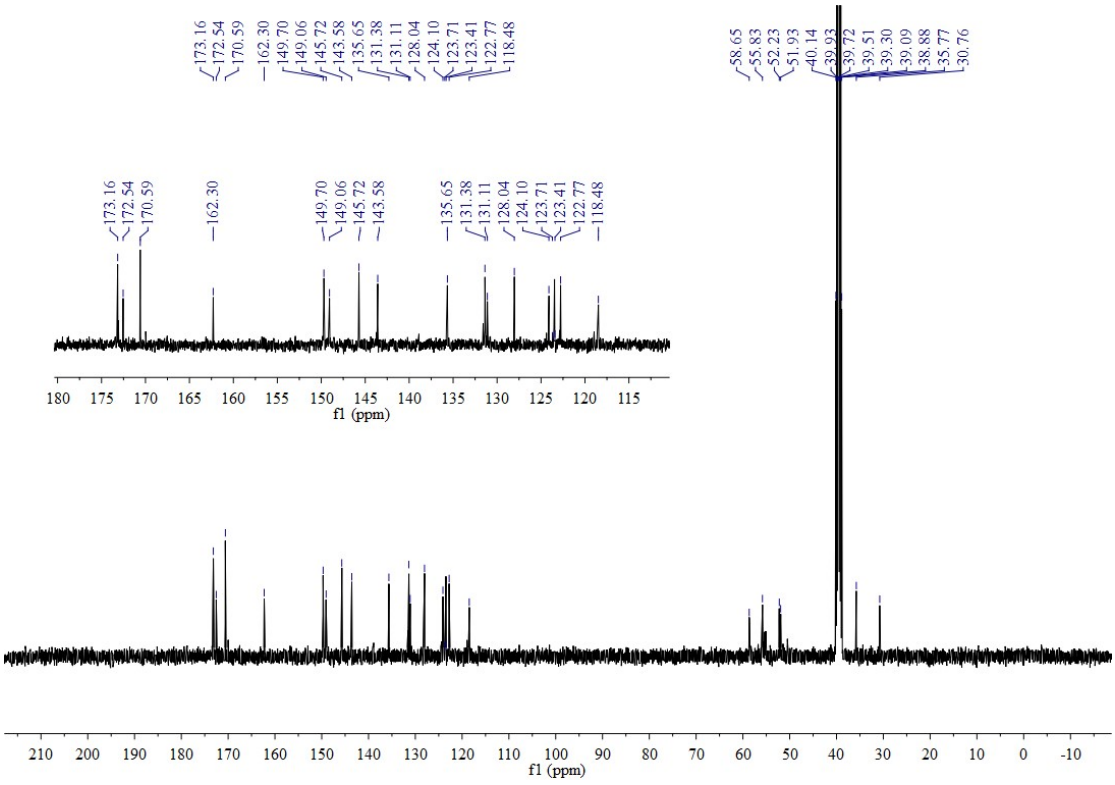

Fig. S2 ^{13}C NMR Spectrum of **L** in DMSO-d_6.

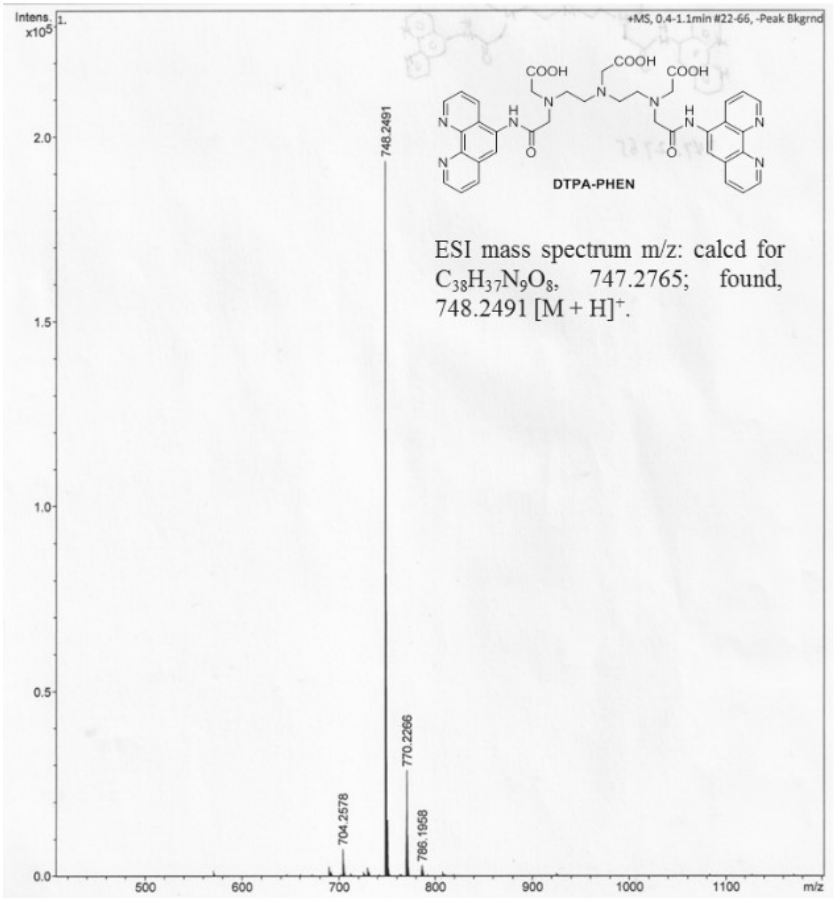

Fig. S3 ESI mass spectrum of **L**.

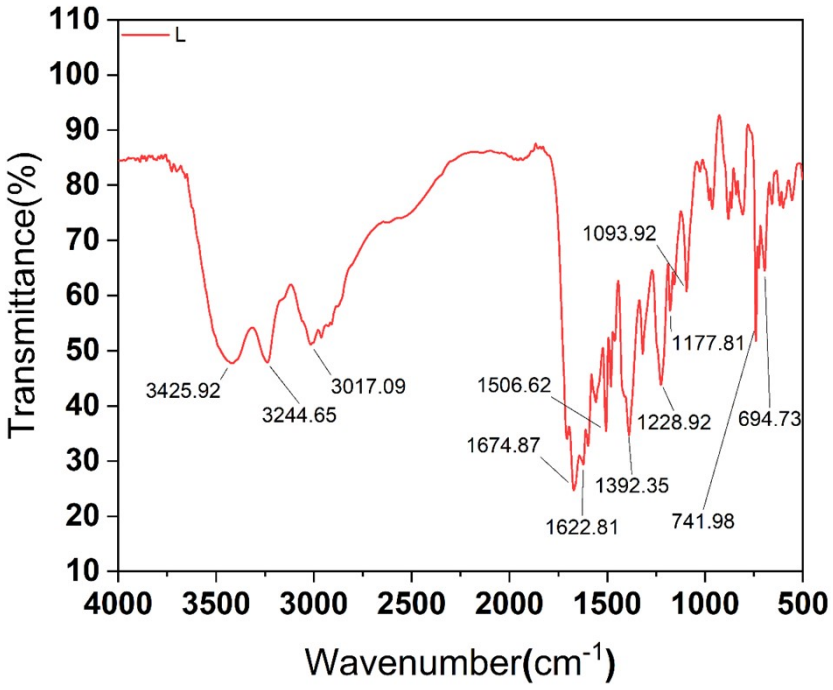

Fig.S4 FT-IR spectrum of **L.**

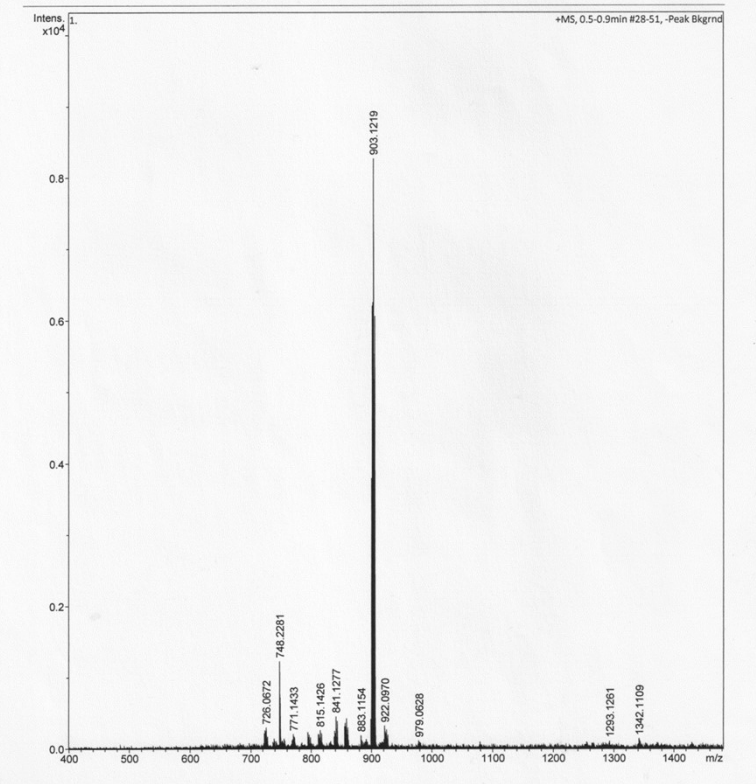

Fig. S5 ESI mass spectrum of **GdL**.

Fig. S6 ESI mass spectrum of **GdL**. (The original magnification was in a range from 858 to 962).

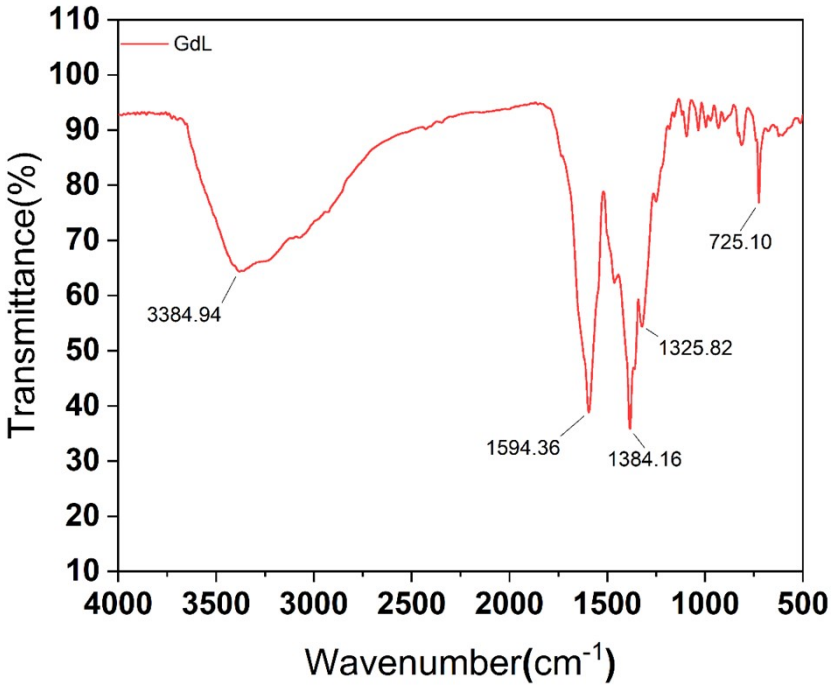

Fig.S7 FT-IR spectrum of **GdL.**

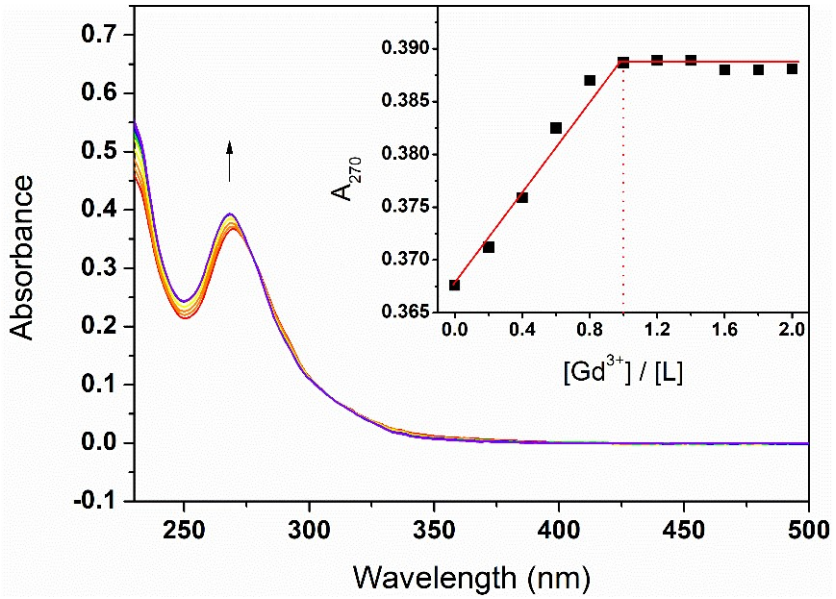

Fig. S8 UV-Vis absorbance spectrum of **L** (10 μM) upon titration with Gd^{3+} (0−20 μM) in water. Inset: titration curve of **L** with Gd^{3+}.

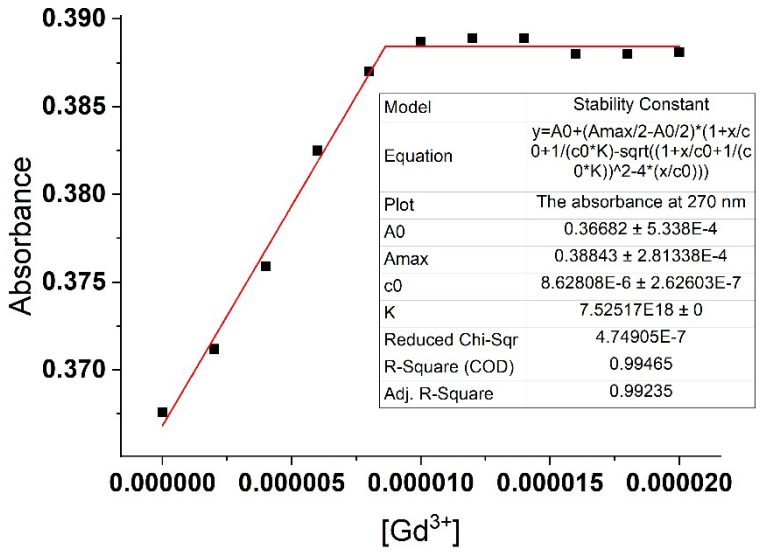

Fig. S9 Determination of the association constant by UV-Vis absorbance spectrum. The absorbance change of **L** at 270 nm in water with the increase of $Gd(NO_3)_3 \cdot 6H_2O$. The red line is the nonlinear fitting curve obtained assuming a 1:1 association between **L** and Gd^{3+}. [**L**] = 1.0×10^{-5} M.

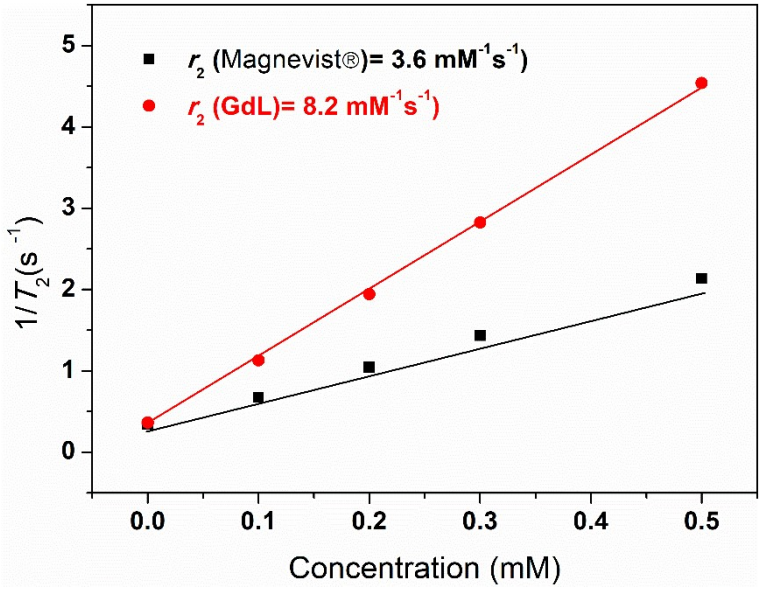

Fig. S10 Relaxivitys r_2 ($1/T_2$) versus different concentrations of **GdL** and Magnevist® in water at 37 °C.

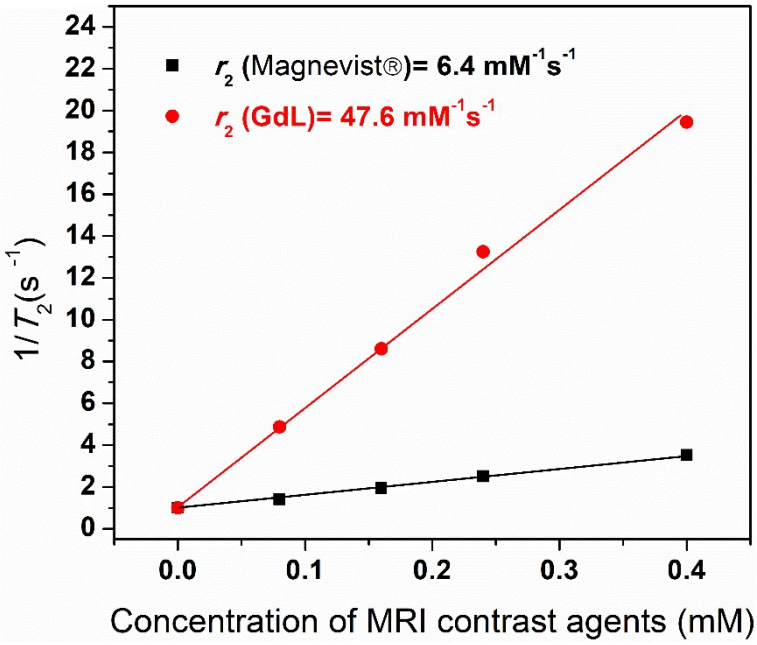

Fig. S11 Relaxivitys r_2 (1/ T_2) versus different concentrations of **GdL** and Magnevist® in 4.5% BSA solution at 37 ℃.

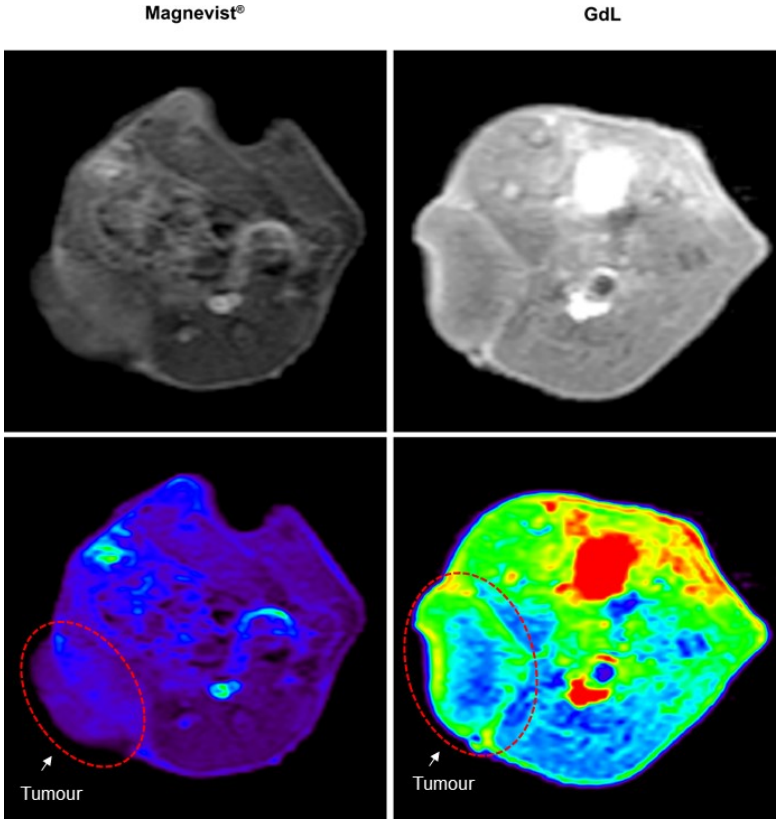

Fig. S12 The transversal cross-sectional images and color-mapped images of mouse tumour after intravenous injection of **GdL** and Magnevist® at 27 min post-injection.

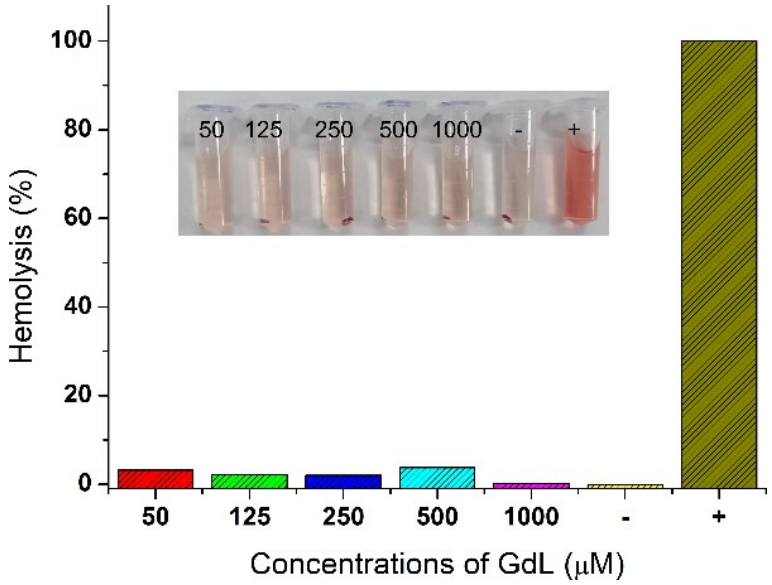

Fig. S13 The hemolytic ratio of the control and **GdL** (50 – 1000 μM). Deionized water served as the positive control and PBS served as the negative control.

Table S1 DFT optimized coordinates for **GdL** in the ground state.

Number	Atom	Coordinates		
		X	Y	Z
1	C	-1.680154	1.516815	2.388965
2	H	-2.562313	1.331336	3.020154
3	H	-0.903881	0.807586	2.688179
4	C	-1.205860	2.940484	2.624532
5	H	-0.999977	3.067221	3.696107
6	H	-2.007415	3.644323	2.390758
7	N	-1.973328	1.263488	0.967794
8	C	-3.131232	2.021935	0.469058
9	H	-3.203360	2.972137	1.004781
10	H	-4.068701	1.482008	0.649610
11	C	-2.984586	2.387023	-1.012346
12	O	-1.800825	2.753889	-1.351489
13	O	-3.974189	2.354788	-1.744931
14	N	-0.017984	3.308039	1.817837
15	C	1.201264	3.014077	2.602095
16	H	1.242222	3.679743	3.477931
17	H	1.113764	1.988821	2.976599
18	C	2.492454	3.156909	1.815034

19	H	2.566984	4.151804	1.370036
20	H	3.338407	3.067857	2.512359
21	N	2.573356	2.158713	0.744392
22	C	-0.055706	4.733204	1.443692
23	H	-1.096161	5.006098	1.236607
24	H	0.290289	5.375199	2.264558
25	C	3.409360	2.554898	-0.388928
26	H	3.162013	3.587361	-0.654379
27	H	4.482507	2.503170	-0.160610
28	C	2.983265	0.843097	1.239883
29	H	3.952913	0.548110	0.832140
30	H	3.103597	0.862343	2.331423
31	C	-2.193412	-0.163444	0.724355
32	H	-1.431591	-0.731844	1.267015
33	H	-3.180701	-0.490890	1.068480
34	C	-1.946485	-0.493465	-0.733934
35	O	-0.941964	-0.043299	-1.307008
36	C	1.916091	-0.193686	0.983591
37	O	0.720896	0.144000	1.075780
38	C	3.101977	1.687326	-1.614501
39	O	1.915736	1.174434	-1.639243
40	O	3.964514	1.534723	-2.476519
41	C	0.714856	5.073563	0.163947
42	O	0.809471	4.118666	-0.696671
43	O	1.141377	6.215547	0.018956
44	Gd	0.136639	1.979471	-0.416333
45	N	-2.776434	-1.336382	-1.366460
46	H	-2.489388	-1.587999	-2.308035
47	N	2.251976	-1.468949	0.758389
48	H	1.464842	-2.108947	0.697816
49	C	7.072440	-1.844865	-2.943563
50	C	5.836004	-1.399056	-2.537301
51	C	5.292213	-1.903211	-1.338838
52	C	6.054605	-2.835867	-0.593356
53	C	7.739682	-2.786960	-2.139136
54	C	4.008473	-1.487696	-0.879386
55	C	5.517768	-3.333267	0.662997
56	C	4.261206	-2.855067	1.120165
57	C	3.524405	-1.926367	0.312589
58	C	3.788344	-3.317573	2.362959
59	H	2.841484	-2.952319	2.748896
60	C	4.543188	-4.224459	3.070767
61	C	5.764546	-4.650291	2.520757
62	H	3.418062	-0.814212	-1.492912

63	H	7.534264	-1.484104	-3.856742
64	H	5.275398	-0.656822	-3.099472
65	H	8.717384	-3.157024	-2.444601
66	H	4.213366	-4.605974	4.031306
67	H	6.376189	-5.372376	3.059452
68	N	6.245053	-4.224048	1.366806
69	N	7.263923	-3.268759	-1.004302
70	C	-8.700664	-0.500672	-0.184130
71	C	-7.438394	-0.127136	-0.583425
72	C	-6.405385	-1.085760	-0.576334
73	C	-6.719449	-2.401239	-0.151386
74	C	-8.905436	-1.834039	0.214504
75	C	-5.080471	-0.752248	-0.989717
76	C	-5.663703	-3.399952	-0.127014
77	C	-4.351170	-3.030924	-0.520619
78	C	-4.093553	-1.686943	-0.952527
79	C	-3.341147	-4.010126	-0.459154
80	H	-2.324449	-3.751980	-0.739074
81	C	-3.664493	-5.279750	-0.039077
82	C	-4.997911	-5.545732	0.317180
83	H	-4.865588	0.259356	-1.333510
84	H	-9.524731	0.204924	-0.173091
85	H	-7.221222	0.888941	-0.901914
86	H	-9.896864	-2.153614	0.532255
87	H	-2.915672	-6.062475	0.021238
88	H	-5.278468	-6.543953	0.649769
89	N	-5.966154	-4.648342	0.282581
90	N	-7.961237	-2.757925	0.235361