Supporting Information

Phosphorescent Heavy-metal Complexes for Bio-imaging

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Table S1. Summaries of photophysical properties and bio-imaging characteristics of complexes.

Com-plex	Photophysical properties in solution				λ_{ex} (nm) for imaging	Media for bio-imaging	Staining compartment	Uptake mechanism	Cytotoxicity	Ref
	λ_{abs} (nm)	λ_{em} (nm)	φ	solvent						
1	485	491, 524	0.60	CH_2Cl_2	355, 488, 758[a]	PBS/DMSO (99:1, v/v)	nucleolus	diffusion control	low (≤ 100 μM) (MTT)	36
2	388	643	0.035	PBS/DMSO (49/1, v/v)	405	PBS/DMSO (49:1, v/v)	cytoplasm	–	low (≤ 100 μM) (MTS)	37
3	500	601	0.051	CH_2Cl_2	–	–	–	–	830.4±54.5 μM (IC_{50}, 48h, HeLa cell)	39
4	530	562, 586sh	0.19	CH_2Cl_2	488	PBS/DMSO (99:1, v/v)	Cytoplasm and Golgi	–	565.9±49.4 μM (IC_{50}, 48h, HeLa cell)	39
5	452	532, 566sh	0.26	CH_2Cl_2	–	–	–	–	1050.0±64.9 μM (IC_{50}, 48h, HeLa cell)	39
6	501	603	0.062	CH_2Cl_2	–	–	–	–	1180.0±70.5 μM (IC_{50}, 48h, HeLa cell)	39
7	530	561sh, 583	0.16	CH_2Cl_2	–	–	–	–	286.5±35.2 μM (IC_{50}, 48h, HeLa cell)	39

8	360	459, 484	0.061	PBS/DMSO (49/1, v/v)	405	PBS/DMSO (49:1, v/v)	cytoplasm	–	low (≤ 100 μM) (MTS)	42
9	360	530	0.10	PBS/DMSO (49/1, v/v)	405	PBS/DMSO (49:1, v/v)	cytoplasm	–	low (≤ 100 μM) (MTS)	42
10	342	570	0.071	PBS/DMSO (49/1, v/v)	405	PBS/DMSO (49:1, v/v)	cytoplasm	–	low (≤ 100 μM) (MTS)	42
11	371	593	0.042	PBS/DMSO (49/1, v/v)	405	PBS/DMSO (49:1, v/v)	cytoplasm	–	low (≤ 100 μM) (MTS)	42
12	425	732	0.003	CH_2Cl_2	488	PBS/DMSO (49:1, v/v)	cytoplasm	–	low (≤ 100 μM) (MTS)	42
13	443	635	0.004	PBS	405	PBS	–	–	–	43
14	380	651	0.004	PBS	405	PBS	–	–	–	43
15	357	580	0.106	PBS	405	PBS	cytoplasm	–	–	43
16	436	594sh, 633	0.015	PBS	405	PBS	cytoplasm	–	–	43
17	461	594	0.036	PBS	405	PBS	cytoplasm	–	–	43
18	437	607	0.019	PBS/MeOH (7:3, v/v)	488	PBS/DMSO (99:1, v/v)	cytoplasm	energy-requiring endocytosis	–	44

19	437	609	0.02	PBS/MeOH (7:3, v/v)	–	–	–	–	2.3±0.4 μM (IC_{50}, 48h, HeLa cell line)	44
20	437	608	0.015	PBS/MeOH (7:3, v/v)	405	PBS/DMSO (99:1, v/v)	perinuclear region	energy-independent diffusion	3.8±0.5 μM (IC_{50}, 48h, HeLa cell line)	44, 45
21	464	626	0.0069	PBS/MeOH (7:3, v/v)	405	PBS/DMSO (99:1, v/v)	perinuclear region	energy-independent diffusion	18.1±2.3 μM (IC_{50}, 48h, HeLa cell line)	44, 45
22	468	594	0.0061	PBS/MeOH (9:1, v/v)	405	PBS/DMSO (99:1, v/v)	perinuclear region and Golgi	energy-requiring endocytosis	5.2±0.3 μM (IC_{50}, 48h, HeLa cell line)	45
23	468	588	0.011	PBS/MeOH (9:1, v/v)	405	PBS/DMSO (99:1, v/v)	perinuclear region and Golgi	energy-requiring endocytosis	3.3±0.1 μM (IC_{50}, 48h, HeLa cell line)	45
24	467	592	0.0067	PBS/MeOH (9:1, v/v)	405	PBS/DMSO (99:1, v/v)	Golgi and perinuclear region	energy-requiring endocytosis	2.1±0.2 μM (IC_{50}, 48h, HeLa cell line)	45
25	438	563sh, 597	0.0034	PBS/MeOH (9:1, v/v)	405	PBS/DMSO (99:1, v/v)	perinuclear region and Golgi	energy-requiring endocytosis	2.7±0.1 μM (IC_{50}, 48h, HeLa cell line)	45
26	438	567sh, 597	0.0056	PBS/MeOH (9:1, v/v)	405	PBS/DMSO (99:1, v/v)	perinuclear region and Golgi	energy-requiring endocytosis	2.1±0.1 μM (IC_{50}, 48h, HeLa cell line)	45
27	438	572, 601sh	0.011	PBS/MeOH (9:1, v/v)	405	PBS/DMSO (99:1, v/v)	Golgi and perinuclear region	energy-requiring endocytosis	1.4±0.1 μM (IC_{50}, 48h, HeLa cell line)	45

28	–	558, 595sh	0.28	PBS/MeOH (9:1, v/v)	–	–	perinuclear region	energy-requiring uptake pathway	4.0 μM (IC_{50}, 48h, HeLa cell)	46
29	–	558, 594sh	0.48	PBS/MeOH (9:1, v/v)	–	–	perinuclear region weak uptake	–	12.0 μM (IC_{50}, 48h, HeLa cell)	46
30	–	558, 595sh	0.50	PBS/MeOH (9:1, v/v)	–	–	perinuclear region weak uptake	–	10.5 μM (IC_{50}, 48h, HeLa cell)	46
31	460	605	0.067	PBS	458	PBS	no cell uptake	–	–	49
32	460	607	0.06	PBS	458	PBS	cytoplasm	energy-dependent endocytosis.	–	49
33	460	607	0.06	PBS	458	PBS	no cell uptake	–	–	49
34	–	–	–	–	488	culture medium	cytoplasm	endocytosis	–	50
35	–	–	–	–	488	culture medium	cytoplasm, nucleus, nucleoli	non-endocytic uptake	–	50
36	–	–	–	–	488	culture medium	no cell uptake	–	–	50
37	370	563	0.0061	PBS	–	–	–	–	–	51
38	352	557	0.013	PBS	360	PBS	whole cells	–	–	51
39	459	567	0.014	solid state	458	RPMI 1640	cytoplasm	–	–	52

40	475	615	0.063	DMSO	488	MEM solution	cytoplasm	energy-dependent endocytosis	low (MTT)	53
41	372	661	0.0078	PBS/MeOH (9/1, v/v)	405	PBS/DMSO (99:1, v/v)	cytoplasm	endocytosis and diffusion pathway	3.2±0.4 μM (IC_{50}, 48h, HeLa cell)	58
42	360	554	–	MeCN	405	PBS/DMSO (20:1, v/v)	membranes	–	–	59
43	355	554	–	MeCN	405	PBS/DMSO (20:1, v/v)	membranes	–	–	59
44	358	552	–	MeCN	405	PBS/DMSO (20:1, v/v)	membranes	–	–	59
45	365	556	–	MeCN	405	PBS/DMSO (20:1, v/v)	membranes	–	–	59
46	488	600-620	–	MeCN	488	Tris buffer	cytoplasm	passive diffusion	–	56,62
47	488	600-620	–	MeCN	488	Tris buffer	cytoplasm	passive diffusion	–	50,62
48	488	600-620	–	MeCN	488	Tris buffer	cytoplasm	–	–	62
49	488	600-620	–	MeCN	488	Tris buffer	cytoplasm	passive diffusion	–	50, 62
50	488	600-620	–	MeCN	488	Tris buffer	cytoplasm	–	–	62

51	–	557, 594sh	0.16	PBS/MeOH (9:1, v/v)	–	–	perinuclear region	energy-requiring uptake pathway	2.9 μM (IC_{50}, 48h, HeLa cell)	46
52	550	577, 624sh	0.27	MeCN	488	culture medium / DMSO (99:1, v/v)	perinuclear region	energy-requiring pathway	4.0 ± 0.2 μM (IC_{50}, 48h, HeLa cell)	63
53	420	509	0.014	CH_2Cl_2	405	–	cytoplasm	–	34.7±5.6 μM (IC_{50}, 48h, HeLa cell)	64
54	415	509	0.01	CH_2Cl_2	405	–	cytoplasm	–	57.8±13.6 μM (IC_{50}, 48h, HeLa cell line)	64
55	486	563	0.01	H_2O	–	culture medium	perinuclear region	–	80 μM (IC_{50}, 48h, HeLa cell line)	65
56	390	514	0.10	HEPES/DMSO (95:5, v/v)	400,730[b]	culture medium	cytoplasm	–	low (20 ug/mL, MTT)	66
57	–	630	–	–	455	DMEM	nucleus	–	–	68
58	–	630	–	–	455	DMEM	nucleus	–	low	68
59	–	600	–	–	455	DMEM	poor uptake	–	–	68
60	–	600	–	–	455	DMEM	poor uptake	–	–	68
61	–	514	–	$CHCl_3$	390	culture medium / DMF (1000:1, v/v)	nucleus	–	2.6±0.4 μM (IC_{50}, 48h, HT-29 cell line)	69

62	–	–	–	–	390	culture medium/ DMF (1000:1, v/v)	nucleus	–	2.0±0.2 μM (IC_{50}, 48h, HT-29 cell line)	69
63	438	510	–	$CHCl_3$	390	culture medium/ DMF (1000:1, v/v)	nucleus	–	3.2±1.6 μM (IC_{50}, 48h, HT-29 cell line)	69
64	390	non-emissive	–	all solvents	488	PBS/DMSO (99:1, v/v)	nucleus	energy-requiring pathway	low (≤ 100 μM) (MTT)	72
65	450	566	0.34	CH_2Cl_2	488	culture medium /DMSO (99:1, v/v)	cytoplasm and nucleolus	–	15.3±3.4 μM (IC_{50}, 48h, MDCK cell line)	74
66	470	572	0.31	CH_2Cl_2	488	culture medium /DMSO (99:1, v/v)	cytoplasm and nucleolus	–	25.2±3.9 μM (IC_{50}, 48h, MDCK cell line)	74
67	495	590	0.30	CH_2Cl_2	488	culture medium /DMSO (99:1, v/v)	cytoplasm and nucleolus	–	4.6±0.4μM (IC_{50}, 48h, MDCK cell line)	74
68	496	601	0.13	CH_2Cl_2	488	culture medium /DMSO (99:1, v/v)	cytoplasm and nucleolus	–	8.0±1.0 μM (IC_{50}, 48h, MDCK cell line)	74
69	517	555, 598sh	0.47	CH_2Cl_2	488	culture medium /DMSO (99:1, v/v)	cytoplasm and nucleolus	–	4.0±0.3 μM (IC_{50}, 48h, MDCK cell line)	74
70	438	578	0.18	CH_2Cl_2	488	culture medium /DMSO (99:1, v/v)	cytoplasm and nucleolus	–	7.4±1.0 μM (IC_{50}, 48h, MDCK cell line)	74

71	380-410	510	0.10	CH_3CN	700[c]	culture medium	nucleolus	–	more toxic (100 μM, MTT)	75
72	355	496	0.98	aqueous solution	351	–	lysosomes	–	52 μM (IC_{50}, 48h, RAW264.7 cell line)	76
73	364	551	–	–	405	EMEM	mitochondria	–	no evidence of toxicity	78
74	378	563	–	H_2O	405	–	vacuole	–	low	59
75	385	530	0.023	CH_2Cl_2	405	culture medium	cytoplasm	–	4.0±0.5 μM (IC_{50}, 48h, HeLa cell)	81
76	372	489sh, 512	0.015	CH_2Cl_2	405	culture medium	cytoplasm	–	2.3±0.2 μM (IC_{50}, 48h, HeLa cell)	81
77	380	545	.028	CH_2Cl_2	405	culture medium	cytoplasm	–	0.7±0.1 μM (IC_{50}, 48h, HeLa cell)	81
78	435	563	0.016	CH_3CN	405	PBS	cytoplasm	–	low (≤ 100 μM) (MTT)	85
79	450	610	–	SiO_2 nanoparticles	488	–	cytoplasm	–	–	89
80	450	608	–	Coating into nanoparticles	514	–	cytoplasm	–	–	90

81	534	650	–	H_2O	380	–	–	–	–	92
82	455	610	0.0013	borate buffer/	–	culture medium / DMSO (1000:1, v/v)	cytosol and nucleus	–	–	95
83	430	547, 586sh	0.0188	DMSO/HEPES (9:1,v/v)	405	–	cytoplasm	–	low (≤ 100 μM) (MTT)	101
84	455	non-emissive	–	CH_3CN/H_2O	488	–	–	–	–	103
85	442	non-emissive	–	H_2O	458	–	nucleus	non-endocytotic	138 μM (IC_{50}, 24h, MCF-7 cell)	106
86	458, 530	610	0.016	Tris buffer	454	–	cytoplasm and nucleolus	–	–	108
87	463	620	0.074	PBS	488	culture medium /DMSO (99:1, v/v)	–	–	141.6±4.0 μM (IC_{50}, 48h, HeLa cell)	109
88	465	619	0.10	PBS	488	culture medium /DMSO (99:1, v/v)	cytoplasm	energy-requiring endocytosis	83.1±2.2 μM (IC_{50}, 48h, HeLa cell)	109
89	469	600	0.0032	PBS	488	culture medium /DMSO (99:1, v/v)	–	–	6.3±0.4 μM (IC_{50}, 48h, HeLa cell)	110
90	495	596	0.0010	PBS	488	culture medium /DMSO (99:1, v/v)	–	–	3.3±0.4 μM (IC_{50}, 48h, HeLa cell)	110

91	450	556sh, 595	0.0049	PBS	488	culture medium /DMSO (99:1, v/v)	cytoplasm	energy-requiring endocytosis	2.4±0.2 μM (IC_{50}, 48h, HeLa cell)	110
92	468	569	0.19	PBS	405	culture medium /DMSO (99:1, v/v)	cytoplasm	–	>400 μM (IC_{50}, 48h, HeLa cell)	58
93	471	600	0.02	PBS	405	culture medium /DMSO (99:1, v/v)	cytoplasm	–	>400 μM (IC_{50}, 48h, HeLa cell)	58
94	487	670	0.0021	PBS	405	culture medium /DMSO (99:1, v/v)	cytoplasm	–	>400 μM (IC_{50}, 48h, HeLa cell)	58
95	486	670	0.0020	PBS	405	culture medium /DMSO (99:1, v/v)	cytoplasm	–	>400 μM (IC_{50}, 48h, HeLa cell)	58
96	416	490max, 518	0.017	PBS	405	–	perinuclear region	energy-independent passive diffusion	37.4±5.0 μM (IC_{50}, 48h, HeLa cell)	64
97	449	535, 570sh	0.48	CH_2Cl_2	405	–	perinuclear region	–	61.4±4.4 μM (IC_{50}, 48h, HeLa cell)	64
98	392	560	0.010	PBS	420-490	culture medium /DMSO (99:1, v/v)	perinuclear region	energy-requiring endocytosis	28.5 μM (IC_{50}, 48h, HeLa cell)	112
99	314	–	–	MeCN/H_2O	–	–	FR-overexpress A2780/AD cells	–	78.2 μM (IC_{50}, 24h, A2780/AD cell line)	117

100	462	610e	–	MEM/DMSO (200 : 1, v/v)	515	MEM/DMSO (500 : 1, v/v)	cytoplasm	–	low (≤ 100 μM) (MTT)	120
101	366	425, 580	0.003	$CHCl_3$	360	–	cytoplasm	–	–	121
102	460	649	0.54	CH_2Cl_2	760[c]	–	–	–	low (MTT)	124
103	691	903	0.01	micellar dispersion	650-700	–	–	–	low (MTS)	125
104	450	610	0.048	CH_3CN/DMSO	458	–	throughout the cell	endocytosis	30% cell death (48 h)	126
105	450	610	0.025	CH_3CN/DMSO	458	–	outer membrane	endocytosis	30% cell death (48 h)	126
106	488	615	–	–	488	–	–	–	–	130
108	460	595	0.62	H_2O	–	–	cytoplasm	–	low (MTS)	132
109	476	496, 521	0.48	CH_2Cl_2	355	culture medium /DMSO (99:1, v/v)	lysosomes	–	>200 μM (IC_{50}, 48h, CHO cell)	133

λ_{abs}: the maximum wavelength in absorption spectrum; λ_{em}: the maximum wavelength in emission spectrum; φ: emission quantum yield; PBS: phosphate buffered solution MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC_{50}: value of the complex determined from the dose dependence of surviving cells after exposure to the complex for 48 h; HEPES: N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid; DMSO: dimethyl

sulfoxide; EMEM: Eagle's Minimum Essential Medium; RPMI 1640: Roswell Park Memorial Institute's medium; MEM: modified Eagle's medium.

a: The excitation wavelengths of complex are 355, 488, 758 nm for time-gated imaging, confocal imaging, and two-photon emission imaging, respectively.

b: The excitation wavelengths of complex are 400 and 730 nm for confocal imaging and two-photon emission imaging, respectively.

c: The excitation wavelength for confocal imaging based on complex

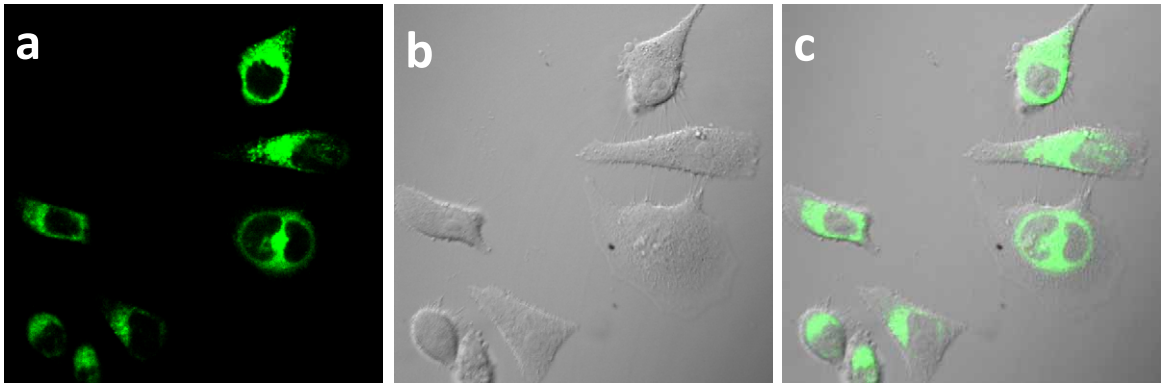

Fig. S1 Confocal luminescence (*a*) and brightfield images (*b*) of living HeLa cells incubated with 10 μM complex **9** in PBS (pH 7) for 15 min at 25 °C. Overlay of luminescence and brightfield images is shown in (*c*) for complex **9.** (λ_{ex} = 405 nm, λ_{em} = 475-575 nm).