

Supplementary Materials

Carborane-Containing Folic Acid *bis*-Amides: Synthesis and *In Vitro* Evaluation of Novel Promising Agents for Boron Delivery to Tumour Cells

Dmitry A. Gruzdev ^{1*}, Angelina A. Telegina ¹, Galina L. Levit ¹, Olga I. Solovieva ^{2,3}, Tatiana Ya. Gusel'nikova ^{3,4}, Ivan A. Razumov ^{2,3}, Victor P. Krasnov ¹ and Valery N. Charushin ^{1,5}

¹ Postovsky Institute of Organic Synthesis, Russian Academy of Sciences (Ural Branch), Ekaterinburg 620108, Russia

² Institute of Cytology and Genetics, Russian Academy of Sciences (Siberian Branch), Novosibirsk 630090, Russia

³ Novosibirsk State University, Novosibirsk 630090, Russia

⁴ Nikolaev Institute of Inorganic Chemistry, Russian Academy of Sciences (Siberian Branch), Novosibirsk 630090, Russia

⁵ Institute of Chemical Engineering, Ural Federal University, Ekaterinburg 620002, Russia

NMR Spectra

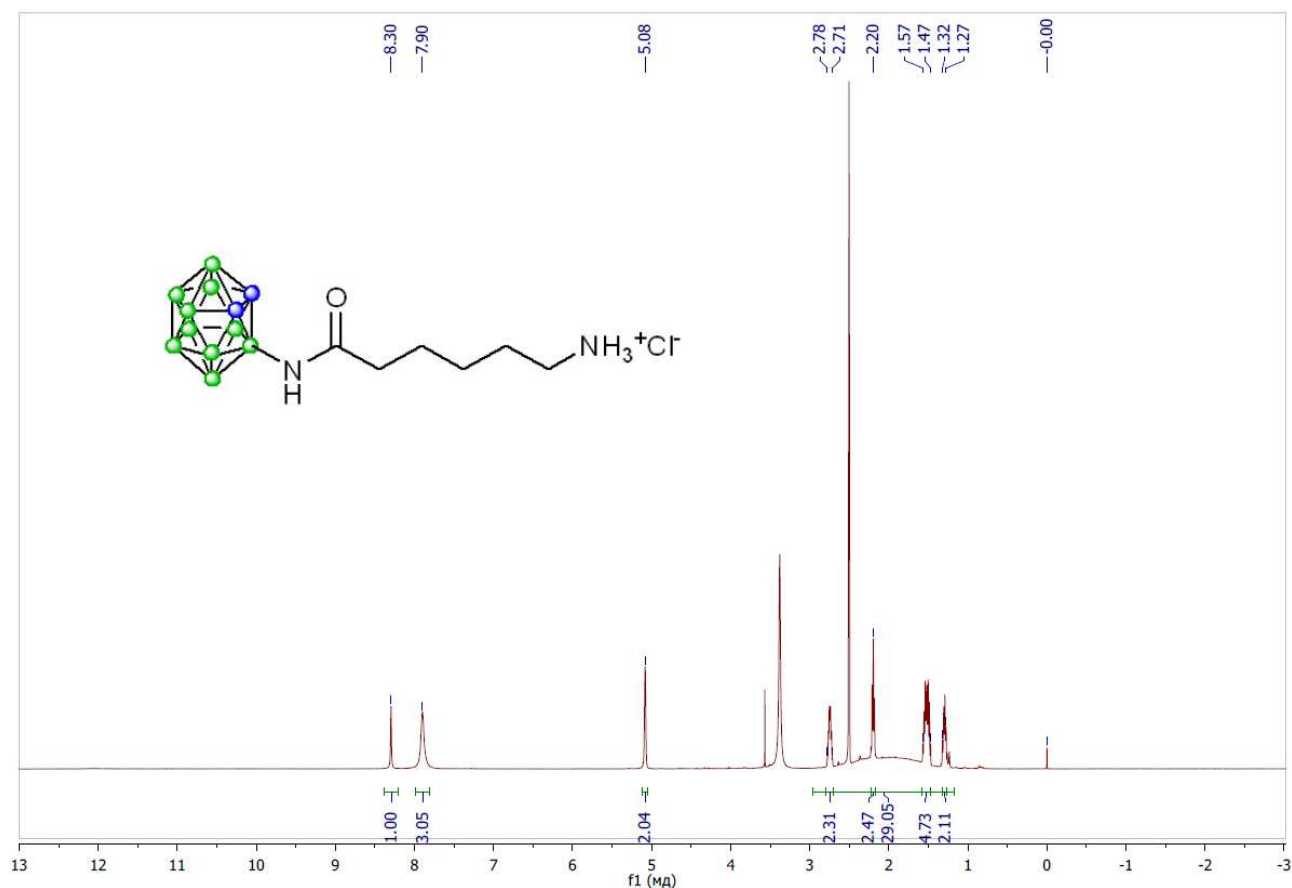


Figure S1. ¹H NMR spectrum of compound **1c** (DMSO-*d*₆, 500 MHz)

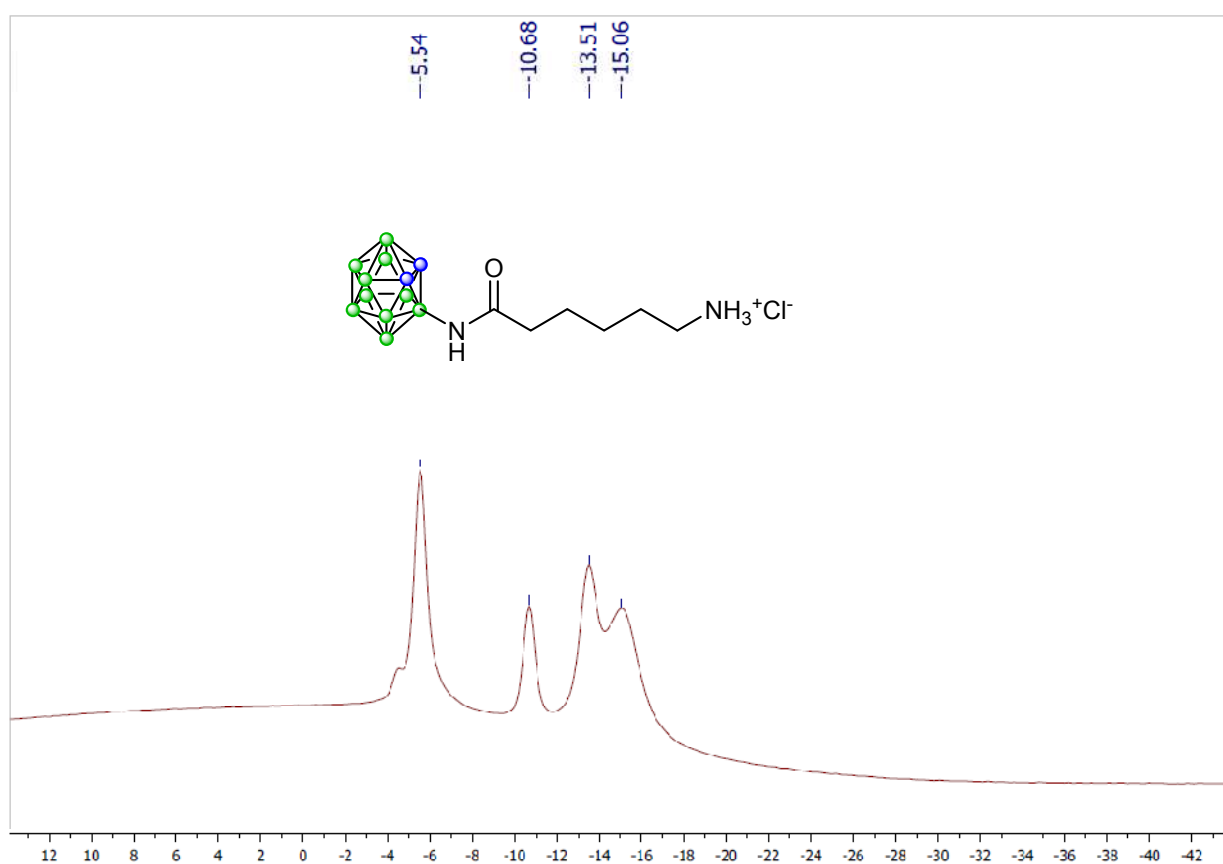


Figure S2. ¹¹B NMR spectrum of compound **1c** (DMSO-*d*₆, 160 MHz)

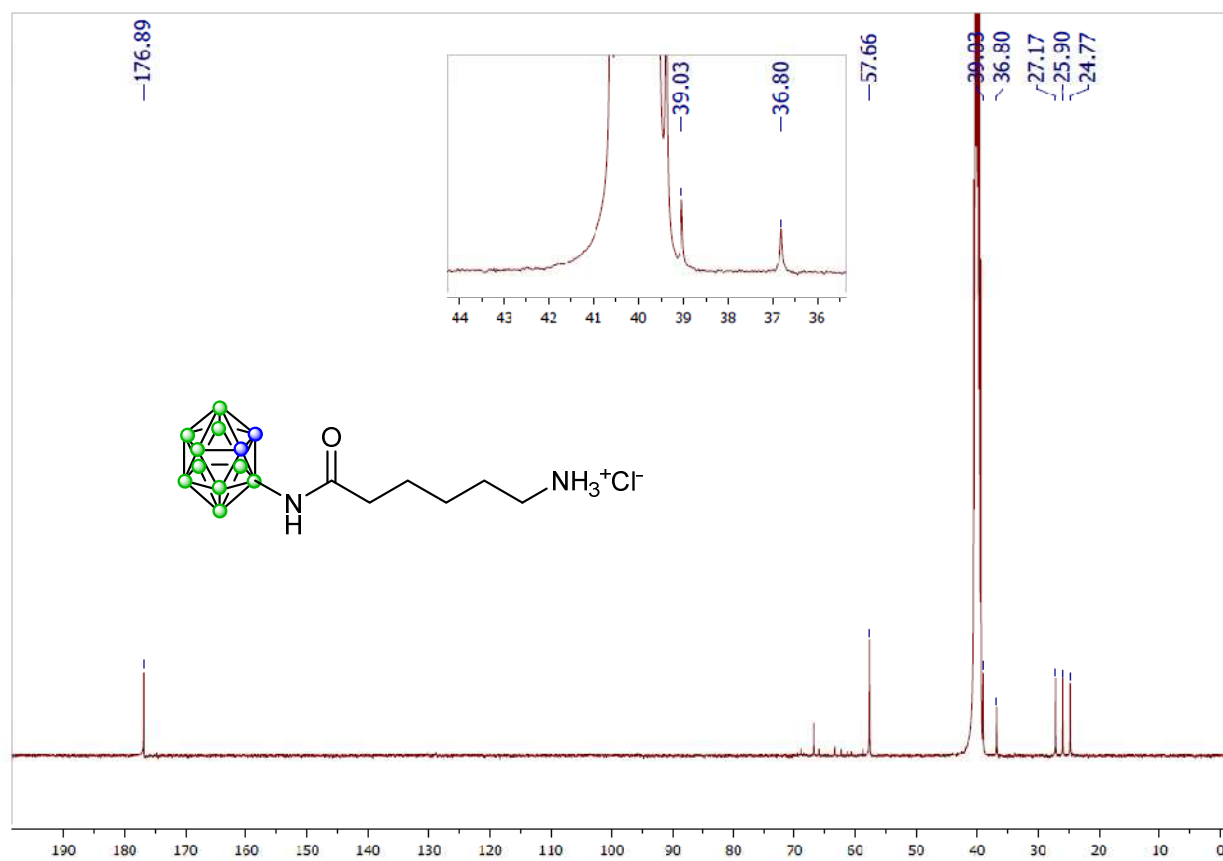


Figure S3. ¹³C NMR spectrum of compound **1c** (DMSO-*d*₆, 100 MHz)

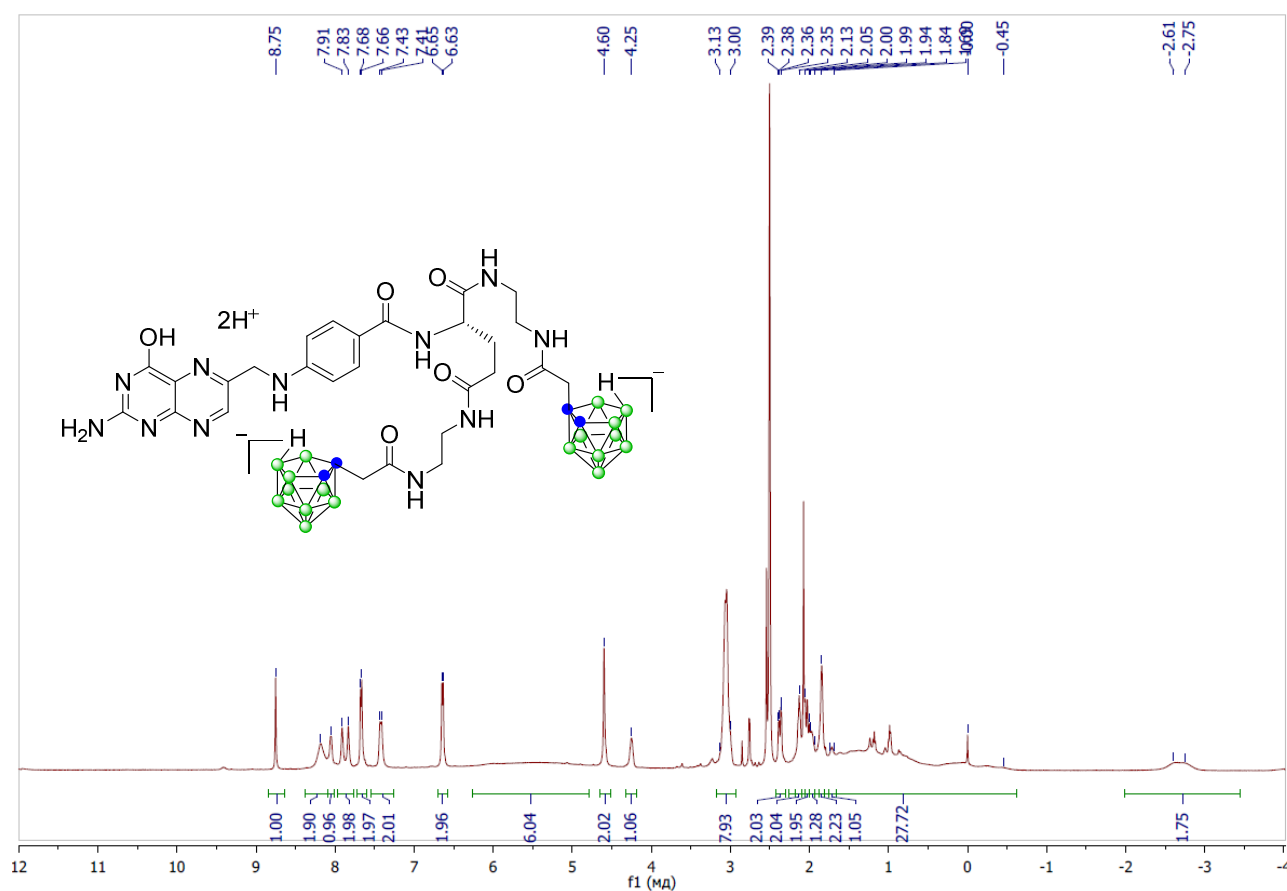


Figure S4. ¹H NMR spectrum of compound **3a** (DMSO-*d*₆, 500 MHz)

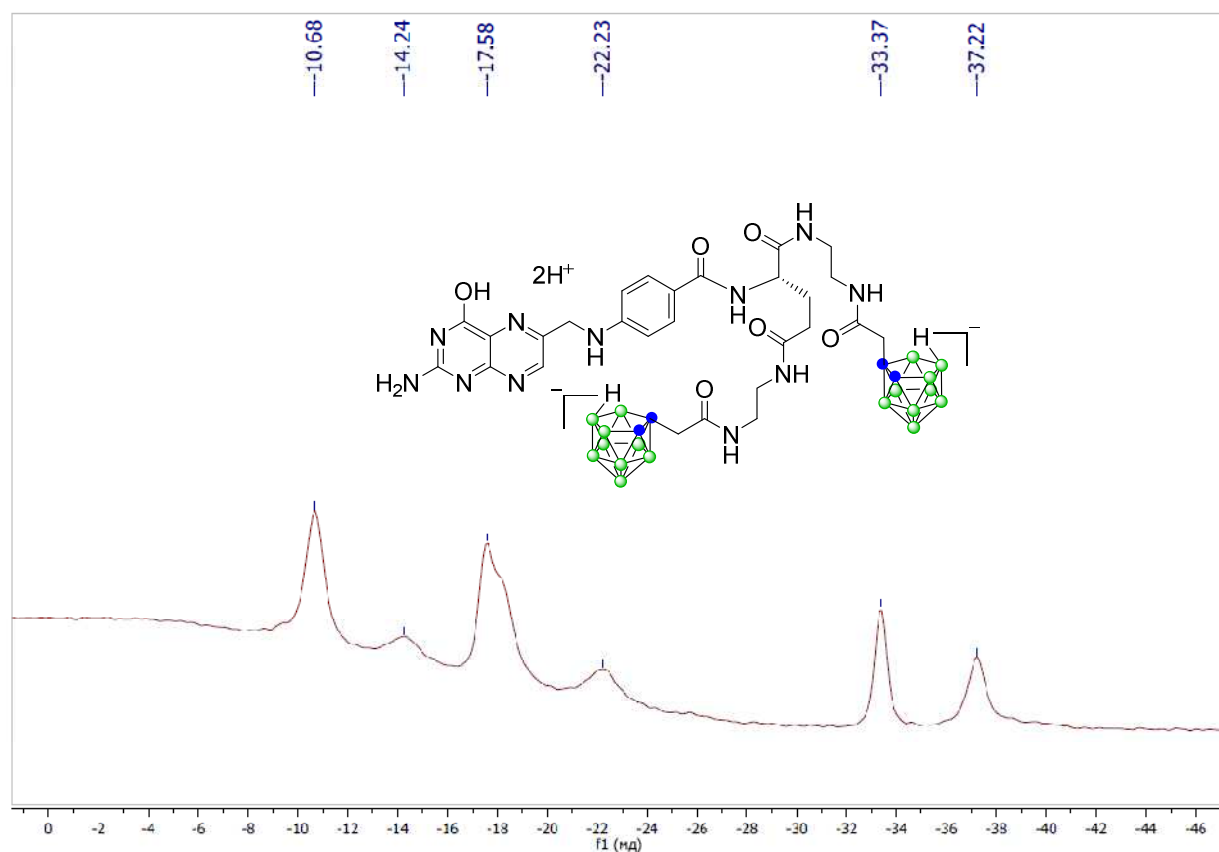


Figure S5. ¹¹B NMR spectrum of compound **3a** (DMSO-*d*₆, 160 MHz)

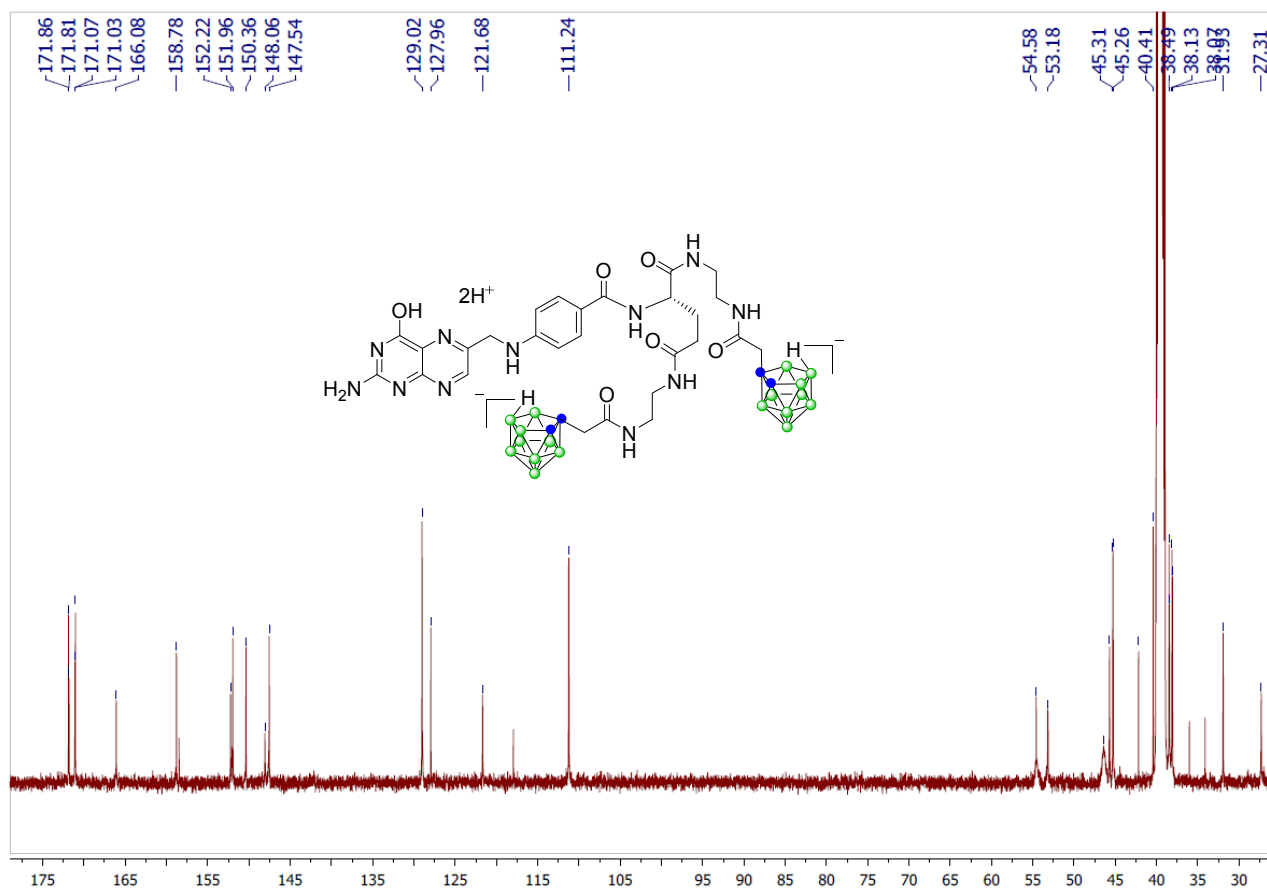


Figure S6. ¹³C NMR spectrum of compound **3a** (DMSO-*d*₆, 126 MHz)

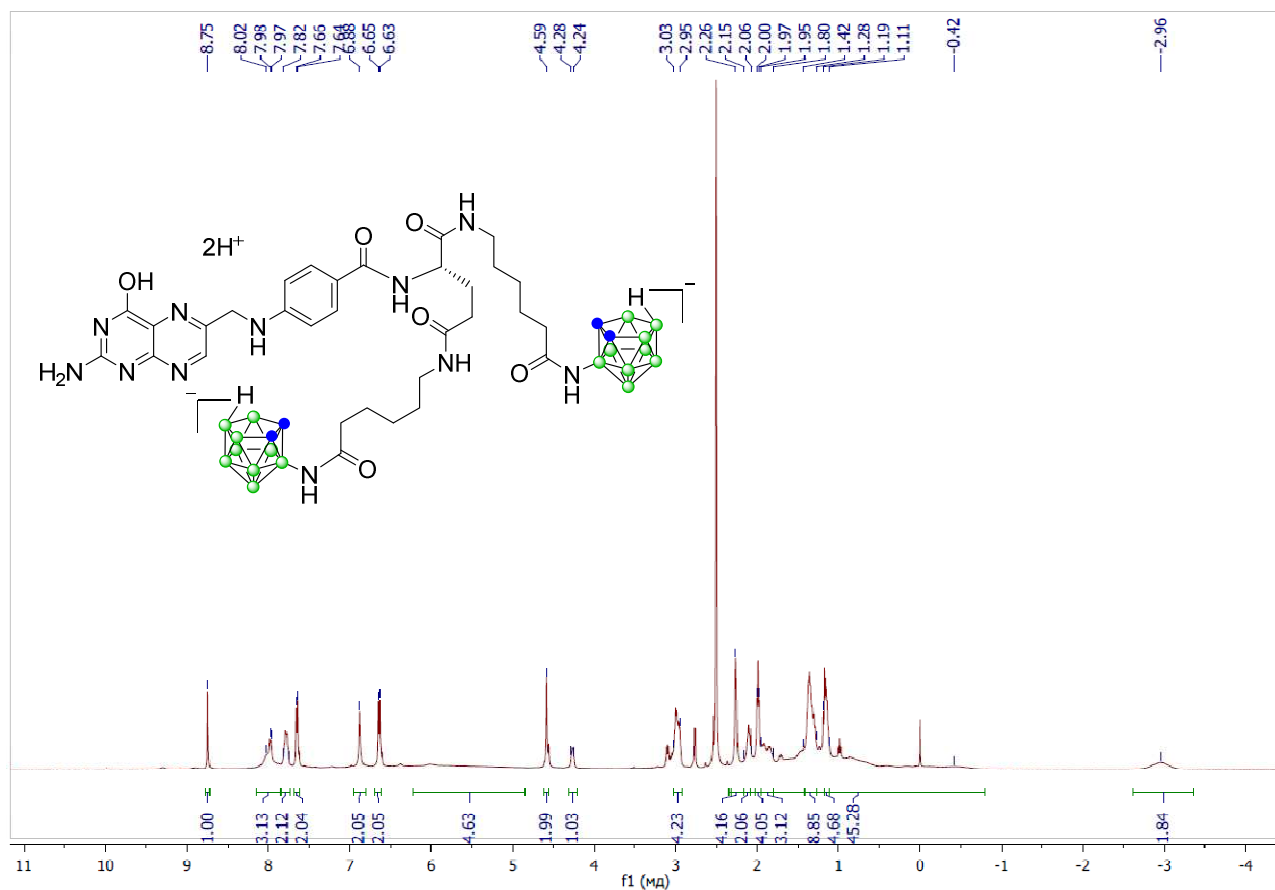


Figure S7. ¹H NMR spectrum of compound **3b** (DMSO-*d*₆, 500 MHz)

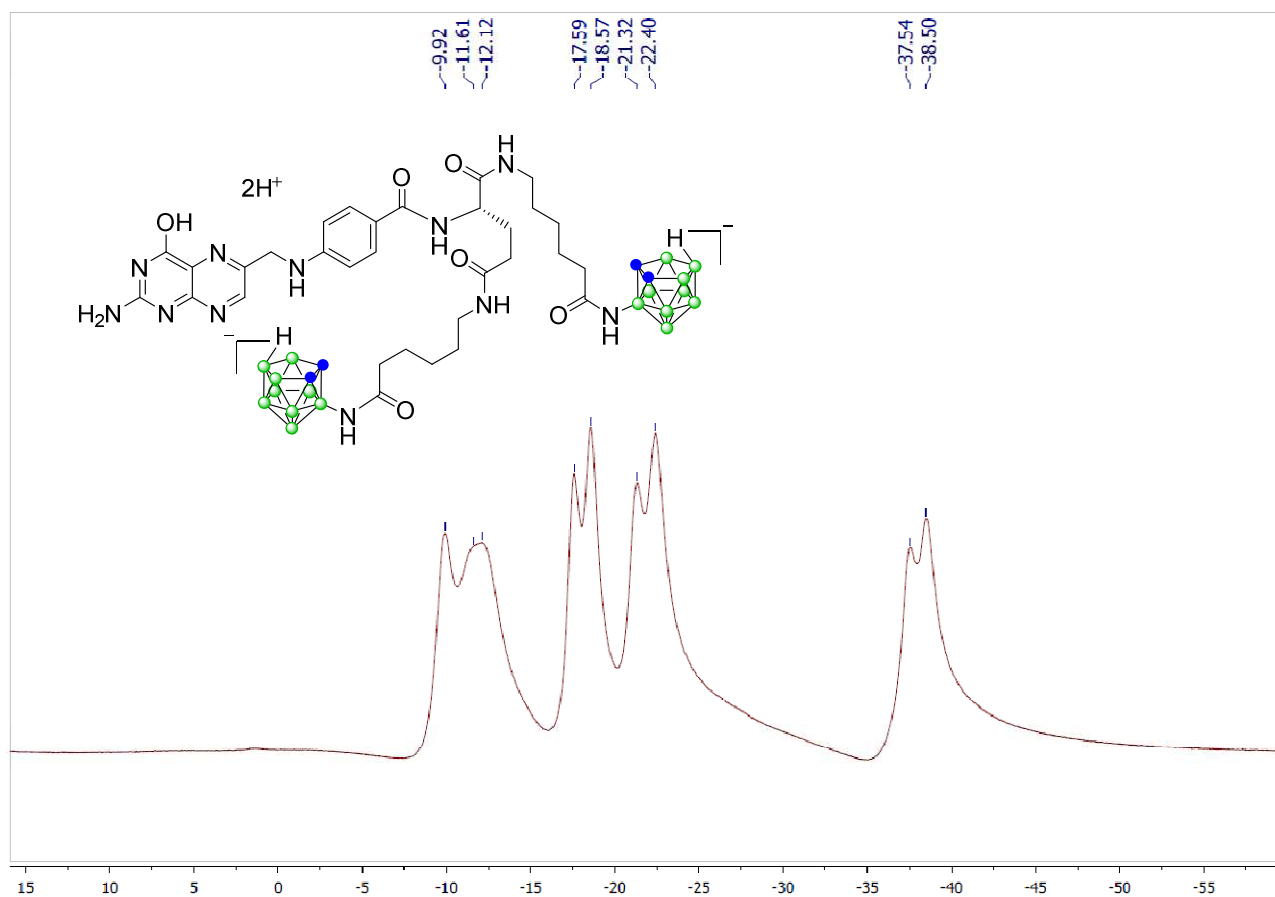
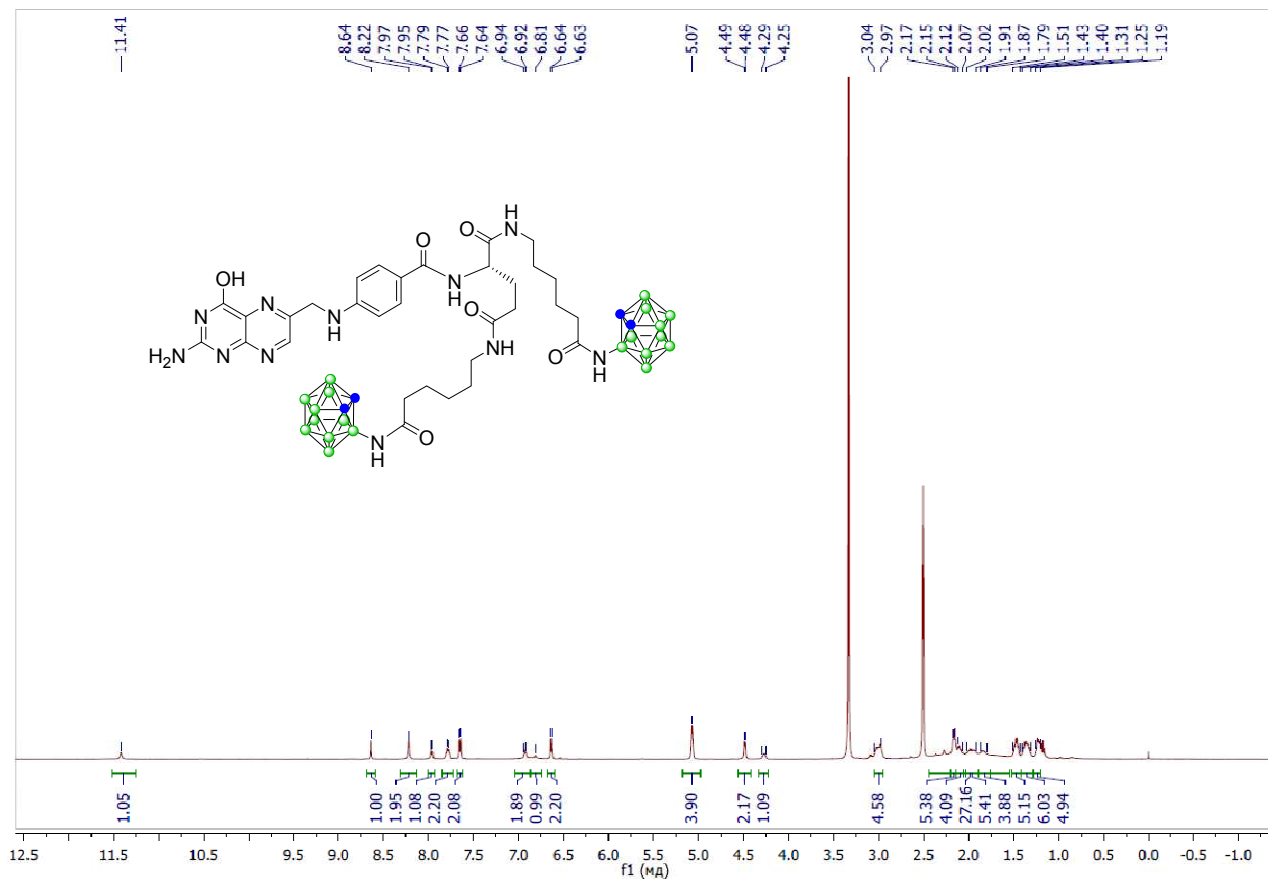
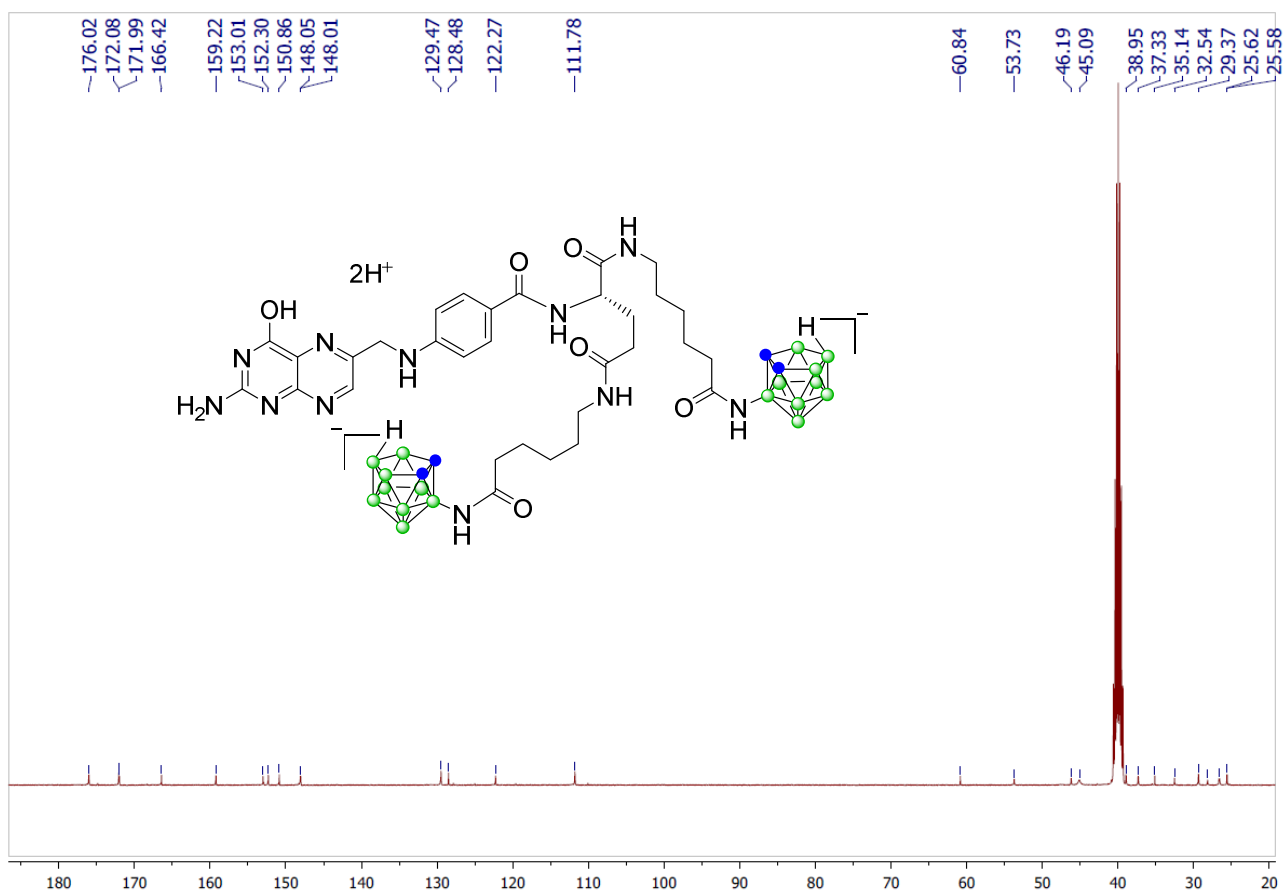


Figure S8. ¹¹B NMR spectrum of compound **3b** (DMSO-*d*₆, 128 MHz)



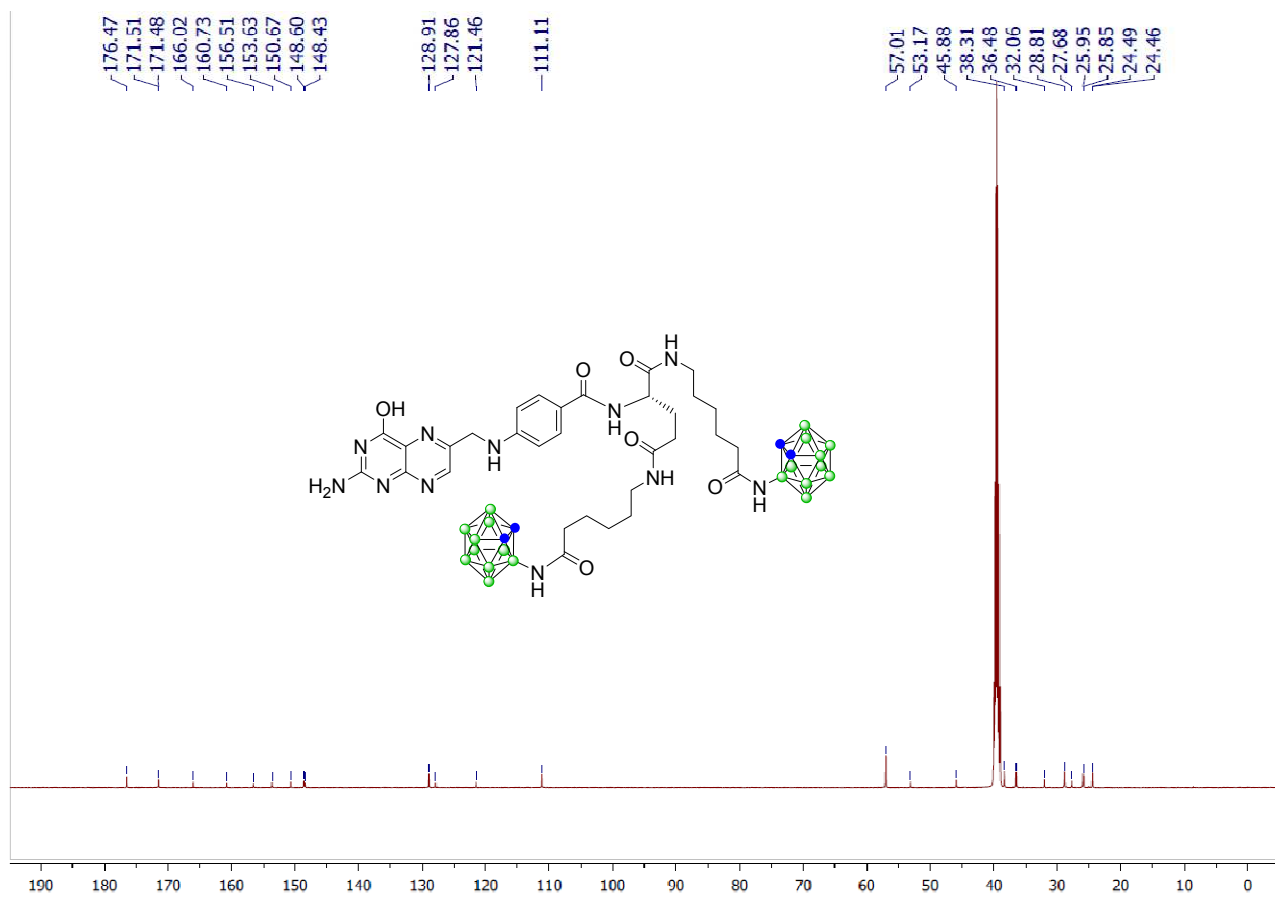
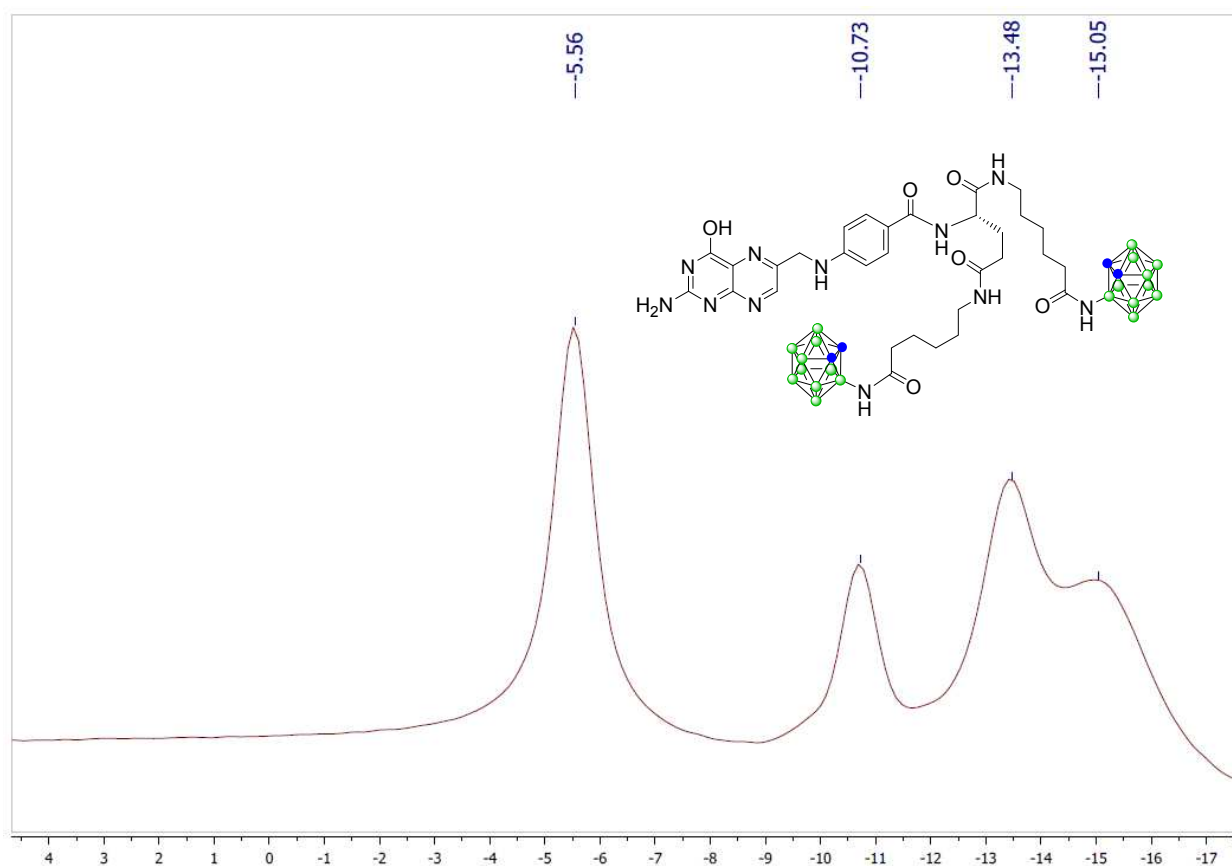


Table S1. Cell viability of various cell lines after 72-h co-incubation with compounds **3a** and **3b** and Cisplatin as a positive control^a

Cell line	Compound	Concentration, mg/mL	Cell viability, %	SE, % ^b
BJ-5ta	3a	0.008	101.6	3.3
		0.016	102.8	2.5
		0.031	100.5	2.6
		0.063	104.0	0.9
		0.125	105.6	0.3
		0.250	99.2	2.6
		0.500	95.7	1.7
		1.000	76.2	2.1
	3b	0.008	110.6	0.8
		0.016	102.5	3.4
		0.031	99.2	5.5
		0.063	120.1	3.5
		0.125	136.9	4.1
		0.250	120.6	1.6
		0.500	87.9	3.1
		1.000	70.6	2.8
	Cisplatin	0.0008	87.5	1.6
		0.0016	86.9	4.5
		0.0031	82.3	2.0
		0.0063	79.7	1.9
		0.0125	71.3	3.2
		0.0250	44.9	3.7
		0.0500	28.5	2.6
		0.1000	18.5	0.2
DU 145	3a	0.008	96.3	4.6
		0.016	92.3	2.6
		0.031	89.6	2.9
		0.063	85.9	1.2
		0.125	80.7	2.8
		0.250	60.8	1.7
		0.500	40.0	1.9
		1.000	17.9	0.7
	3b	0.008	102.3	3.0
		0.016	101.3	5.9
		0.031	103.5	3.3
		0.063	110.5	8.3
		0.125	91.8	3.6
		0.250	79.9	3.2
		0.500	67.9	3.3
		1.000	19.8	1.4
	Cisplatin	0.0008	53.7	6.9
		0.0016	41.4	2.0
		0.0031	30.5	1.0
		0.0063	22.3	1.5
		0.0125	17.1	0.6
		0.0250	16.8	1.7
		0.0500	8.3	0.8
		0.1000	7.5	0.4

MDA-MB-231	3a	0.008	90.2	4.1
		0.016	87.7	2.2
		0.031	88.4	3.0
		0.063	85.6	2.8
		0.125	81.3	1.6
		0.250	60.0	2.6
		0.500	38.7	4.5
		1.000	30.7	2.5
	3b	0.008	95.0	2.5
		0.016	86.2	2.6
		0.031	86.8	1.3
		0.063	81.7	1.0
		0.125	76.0	1.8
		0.250	65.0	4.9
		0.500	43.3	2.3
		1.000	28.1	1.9
	Cisplatin	0.0008	85.0	4.1
		0.0016	77.3	2.4
		0.0031	71.0	2.9
		0.0063	55.9	5.8
		0.0125	33.4	1.6
		0.0250	20.5	1.1
		0.0500	13.9	0.4
		0.1000	12.8	0.5
SK-Mel-28	3a	0.008	104.7	1.6
		0.016	104.4	2.3
		0.031	104.4	2.2
		0.063	101.5	3.7
		0.125	101.5	3.5
		0.250	96.9	4.9
		0.500	91.1	2.6
		1.000	57.9	3.8
	3b	0.008	108.0	5.8
		0.016	109.8	5.0
		0.031	118.8	4.0
		0.063	133.1	3.6
		0.125	152.6	4.8
		0.250	121.4	5.4
		0.500	93.3	4.4
		1.000	59.1	3.6
	Cisplatin	0.0008	93.0	1.0
		0.0016	93.7	2.3
		0.0031	76.9	2.1
		0.0063	58.2	1.8
		0.0125	35.9	0.8
		0.0250	25.0	0.5
		0.0500	15.6	1.3
		0.1000	14.3	1.1

T98G	3a	0.008	103.5	7.1
		0.016	99.6	5.0
		0.031	92.4	2.2
		0.063	90.1	2.5
		0.125	81.3	2.6
		0.250	62.8	3.9
		0.500	32.8	1.4
		1.000	27.6	1.4
	3b	0.008	98.9	2.3
		0.016	99.4	2.6
		0.031	96.4	2.5
		0.063	104.2	2.8
		0.125	113.0	2.6
		0.250	103.1	1.4
		0.500	82.3	2.0
		1.000	48.0	1.5
	Cisplatin	0.0008	86.4	3.6
		0.0016	81.9	1.5
		0.0031	66.8	2.9
		0.0063	39.1	2.2
		0.0125	21.4	1.4
		0.0250	12.3	0.2
		0.0500	9.7	0.3
		0.1000	9.3	0.5
U87 MG	3a	0.008	66.8	2.1
		0.016	60.4	0.9
		0.031	50.8	3.4
		0.063	42.6	3.1
		0.125	46.5	2.2
		0.250	39.4	2.5
		0.500	31.3	1.2
		1.000	19.9	2.4
	3b	0.008	80.0	1.9
		0.016	74.1	5.8
		0.031	63.0	2.0
		0.063	53.5	2.3
		0.125	62.1	4.0
		0.250	60.6	2.2
		0.500	40.9	1.3
		1.000	19.1	1.9
	Cisplatin	0.0008	68.2	3.4
		0.0016	59.0	2.5
		0.0031	42.8	3.0
		0.0063	21.7	2.2
		0.0125	18.0	1.1
		0.0250	15.0	1.0
		0.0500	10.6	1.9
		0.1000	6.1	0.5

^a Results of three independent experiments are presented

^b SE is the standard error calculated according to equation: $SE = s/\sqrt{3}$, where s is the standard deviation

Boron Accumulation Assay

Boron accumulation in U87 MG, MDA-MB-231, SK-Mel-28, DU 145, T98G, and BJ-5ta cells was assessed after incubation in the presence of compounds **3a** and **3b** (0.25 mg/mL in the case of U87 MG cells; 0.5 mg/mL in other cases) for 10 min, 30 min, 1 h, 3 h, 6 h and 8 h. For analysis, 1 mL of the medium was taken, the number of cells was determined on a Countess automatic cell counter (Invitrogen, Waltham, MA, USA) and the boron content was analyzed using ICP-OES as described in [Tsygankova, A.R.; Kanygin, V.V.; Kasatova, A.I.; et al. Determination of boron by inductively coupled plasma atomic emission spectroscopy. Biodistribution of ^{10}B in tumour-bearing mice. *Russ. Chem. Bull.* **2020**, *69*, 601–607. <https://doi.org/10.1007/s11172-020-2805-8>]. The relative standard deviation (RSD) did not exceed 25% for the determination of boron by the ICP-AES method. Results of three independent experiments are presented.

Table S2. Boron accumulation by U87 MG human glioblastoma cells

Compound	Number of cells, $\times 10^6$	Incubation time	Boron content in cells, $\mu\text{g B}/10^6$ cells
3a	3.0	10 min	0.01
			0.01
			0.01
	2.5	30 min	0.13
			0.15
			0.13
	2.0	1 h	0.12
			0.09
			0.09
	3.0	3 h	0.08
			0.07
			0.07
	3.0	6 h	0.10
			0.11
			0.10
	2.5	8 h	0.09
			0.07
			0.07
3b	1.0	10 min	0.63
			0.59
			0.58
	1.0	30 min	3.80
			3.60
			3.78
	1.0	1 h	3.22
			3.20
			3.90
	1.0	3 h	3.40
			4.17
			3.21
	1.0	6 h	5.50
			5.81
			5.80
	1.0	8 h	7.70
			6.62
			6.70

Table S3. Boron accumulation by MDA-MB-231 human breast carcinoma cells

Compound	Number of cells, $\times 10^6$	Incubation time	Boron content in cells, $\mu\text{g B}/10^6$ cells
3a	1.0	10 min	0.50
			0.53
			0.44
	0.7	30 min	0.74
			0.76
			0.80
	1.0	1 h	0.50
			0.46
			0.54
	1.0	3 h	0.42
			0.42
			0.37
	1.0	6 h	0.69
			0.73
			0.72
	1.4	8 h	0.46
			0.46
			0.51
3b	0.7	10 min	2.00
			2.14
			1.86
	0.9	30 min	1.67
			1.56
			2.11
	1.0	1 h	2.60
			2.50
			2.70
	1.0	3 h	1.40
			1.60
			1.40
	1.0	6 h	1.30
			1.40
			1.80
	1.0	8 h	2.00
			2.60
			3.00

Table S4. Boron accumulation by SK-Mel28 human melanoma cells

Compound	Number of cells, $\times 10^6$	Incubation time	Boron content in cells, $\mu\text{g B}/10^6$ cells
3a	2.0	10 min	0.02
			0.02
			0.02
	0.9	30 min	0.04
			0.03
			0.04
	1.2	1 h	0.06
			0.06
			0.09
	0.8	3 h	0.08
			0.06
			0.07
	0.8	6 h	0.09
			0.02
			0.07
	1.3	8 h	0.05
			0.05
			0.04
3b	0.9	10 min	0.05
			0.07
			0.06
	1.2	30 min	0.13
			0.16
			0.13
	1.0	1 h	0.11
			0.11
			0.10
	0.9	3 h	0.28
			0.29
			0.26
	0.9	6 h	0.87
			1.07
			0.51
	2.0	8 h	0.15
			0.15
			0.14

Table S5. Boron accumulation by DU145 human prostate carcinoma cells

Compound	Number of cells, $\times 10^6$	Incubation time	Boron content in cells, $\mu\text{g B}/10^6$ cells
3a	3.2	10 min	0.06
			0.05
			0.04
	2.8	30 min	0.10
			0.09
			0.08
	3.0	1 h	0.09
			0.10
			0.14
	2.4	3 h	0.27
			0.28
			0.28
	1.6	6 h	0.69
			0.65
			0.72
	3.4	8 h	0.22
			0.20
			0.20
3b	2.2	10 min	0.25
			0.24
			0.25
	2.8	30 min	0.20
			0.18
			0.18
	3.6	1 h	0.12
			0.12
			0.12
	2.4	3 h	0.31
			0.35
			0.34
	2.8	6 h	0.27
			0.28
			0.23
	3.0	8 h	0.42
			0.43
			0.42

Table S6. Boron accumulation by T98G human glioblastoma cells

Compound	Number of cells, $\times 10^6$	Incubation time	Boron content in cells, $\mu\text{g B}/10^6$ cells
3a	2.8	10 min	0.08
			0.05
			0.05
	2.8	30 min	0.06
			0.06
			0.05
	1.3	1 h	0.22
			0.23
			0.22
	1.0	3 h	0.29
			0.36
			0.34
	1.1	6 h	0.34
			0.37
			0.35
	1.3	8 h	0.35
			0.39
			0.39
3b	2.8	10 min	0.14
			0.14
			0.10
	1.9	30 min	0.27
			0.31
			0.30
	1.0	1 h	0.89
			1.00
			0.89
	0.9	3 h	0.54
			0.54
			0.52
	1.4	6 h	0.61
			0.49
			0.56
	1.2	8 h	0.79
			0.81
			0.83

Table S7. Boron accumulation by BJ-5ta human foreskin fibroblasts

Compound	Number of cells, $\times 10^6$	Incubation time	Boron content in cells, $\mu\text{g B}/10^6$ cells
3a	1.0	10 min	0.03
			0.04
			0.08
	0.6	30 min	0.30
			0.17
			0.20
	1.5	1 h	0.05
			0.06
			0.04
	1.3	3 h	0.24
			0.24
			0.24
	1.8	6 h	0.10
			0.09
			0.09
	0.8	8 h	0.51
			0.50
			0.52
3b	0.7	10 min	0.76
			0.82
			0.86
	1.5	30 min	0.67
			0.74
			0.74
	0.9	1 h	0.82
			0.98
			0.98
	1.0	3 h	0.66
			0.75
			0.68
	1.8	6 h	0.28
			0.31
			0.37
	1.8	8 h	0.32
			0.33
			0.35