Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Synthesis and biological evaluation of novel analogues of batracylin with synthetic amino acids and adenosine: an unexpected effect on centromere segregation in tumor cells through a dual inhibition of topoisomerase IIα and Aurora B

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Experimental part

Chemistry

Determination of the structure of selected analogues **3d** and **3h** by ^{1}H NMR (500 MHz) and ^{13}C NMR. Assigned carbon atom numbering sequence in the structures obtained analogues (Fig. 1 and 2) for which a description is given of ^{1}H NMR and ^{13}C NMR spectra.

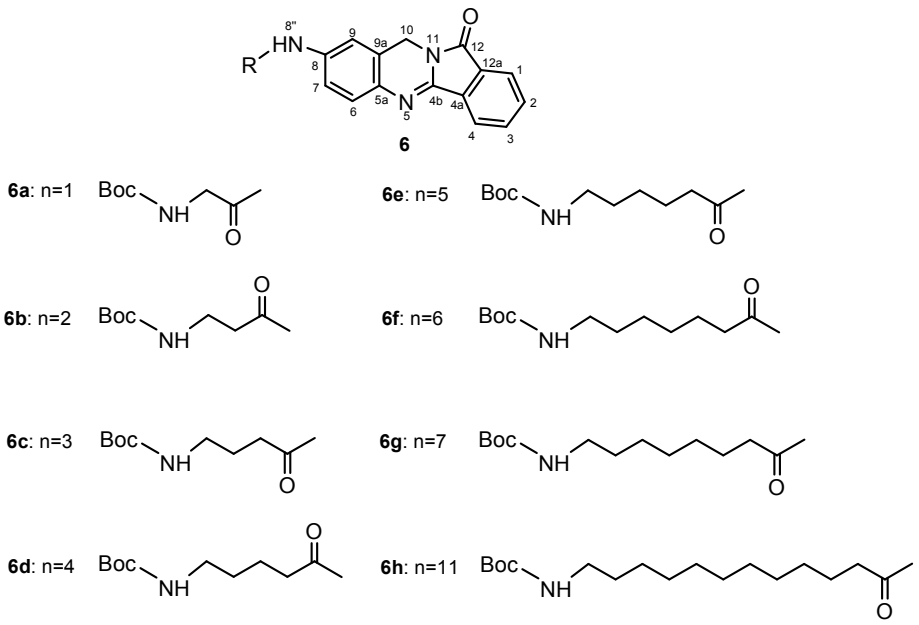


Fig. 1 The structure of *C*^{8}-substituted batracylin derivatives, **6a–h**.

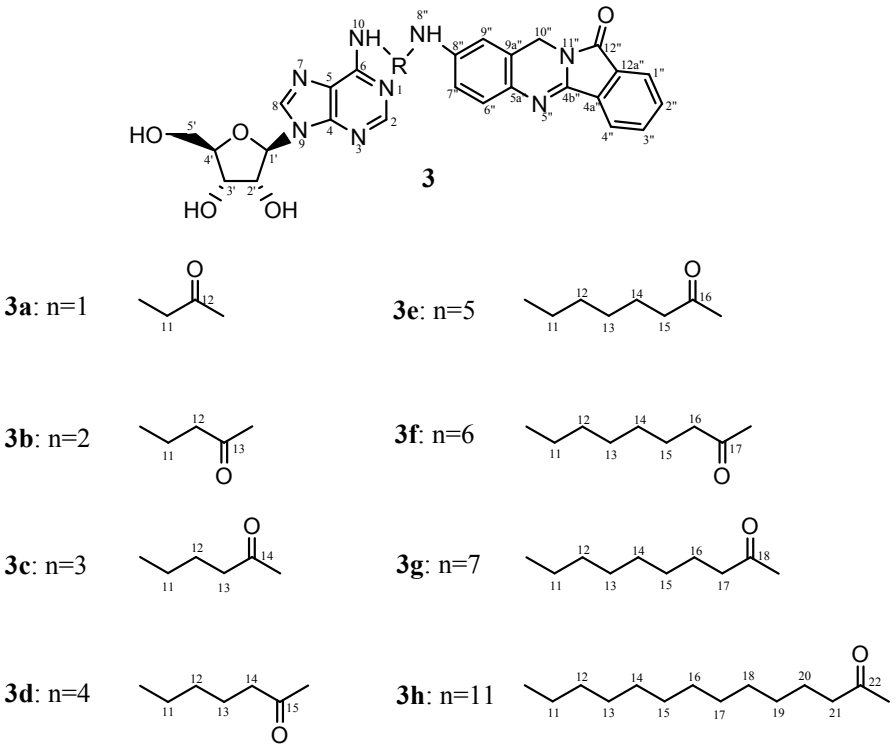


Fig. 2 The structure of analogues **3a–h**.

Table 3 Samples concentration.

Compound no	Concentration [mg mL^{-1}]
3a	8
3b	8
3c	10
3d	17
3e	21
3f	20
3g	21
3h	21
6a	21
6d	21
6f	18
6g	21
6h	17

Table 4 ROE contacts for compound **3d** and **3h** in DMSO-d_6 at 298 K.

Proton no		ROE contacts	
		3d	**3h**
adenosine moiety			
1’		8, 2’, 3’, 4’	8, 2’, 3’, 4’
2’		8, 1’, 3’, 4’, 5a’, 5b’	8, 1’, 3’, 4’, 5a’, 5b’, 2’OH*
3’		8, 1’, 2’, 4’, 5a’, 5b’	8, 1’, 2’, 4’, 5a’, 5b’
4’		1’, 2’, 3’, 5a’, 5b’	1’, 2’, 3’, 5a’, 5b’
5a’		1’, 2’, 3’, 4’, 5b’	1’, 2’, 3’, 4’, 5b’
5b’		1’, 2’, 3’, 4’, 5a’	1’, 2’, 3’, 4’, 5a’
2’OH			2’*
3’OH			
5’OH		5a’, 5b’	5a’, 5b’
2			
8		1’, 2’, 3’	1’, 2’, 3’
10-NH		11*, 12, 13	11*, 12, 13
linker			
3d	**3h**		
11	11	12, 13, 10-NH*	12, 13, 10-NH*
12/13	12/20	11, 14	11, 21, 10-NH
14	13-19	13, 8”-CONH	11, 12, 21, 10-NH
	21		19, 20, 8”-CONH
batracylin			
1”		2”*	2”*
2”		1”*, 3”*	1”*, 3”*
3”		4”*, 2”*	4”*, 2”*
4”		3”*	3”*
6”		7”*	7”*
7”		6”*, 8”-CONH	6”*, 8”-CONH
9”		6”, 10”, 8”-CONH	6”, 10”, 8”-CONH
10”		7”, 9”	7”, 9”
8”-CONH		21, 7”, 9”	21, 7”, 9”
HHT – Hartmann Hahn Transfer			

Table 5 ^{13}C NMR (500 MHz) chemical shifts for compound **3d** in DMSO-d_6 at 298 K.

^{13}C atom no.	*^{13}C δ (ppm)*	*^{13}C atom no.*	*^{13}C δ (ppm)*
adenosine moiety		**batracylin**	
C-1’	88.64	C-1”	122.49
C-2’	74.18	C-2”	133.90
C-3’	71.35	C-3”	132.92
C-4’	86.60	C-4”	123.54
C-5’	62.36	C-4a”	134.79
C-2	153.07	C-4b”	148.10
C-4	148.93	C-5a”	139.37

C-5	120.43	C-6”	128.59
C-6	155.34	C-7”	119.41
C-8	140.34	C-8”	135.96
linker		C-9”	118.8
C-11	39.70	C-9a”	123.10
C-12	29.40	C-10”	41.10
C-13	23.30	C-12”	166.87
C-14	36.89	C-12a”	130.73
C-15	172.07		

Table 6 ^{1}H-NMR (500 MHz) chemical shifts for compound **3d** in DMSO-d_6 at 298 K.

Proton no.	*^{1}H δ (ppm)*	*$J_{H,H}$ (Hz)*	*ROE contacts*
adenosine moiety			
1’	5.86	*d (5.0Hz)*	8, 2’, 3’, 4’
2’	4.59	*t (5.0Hz)*	8, 1’, 3’, 4’, 5a’, 5b’
3’	4.14	*t (5.0Hz)*	8, 1’, 2’, 4’, 5a’, 5b’
4’	3.95	*dd (5.0Hz, 3.5Hz)*	1’, 2’, 3’, 5a’, 5b’
5a’	3.65	*dd (12.0Hz, 3.5Hz)*	1’, 2’, 3’, 4’, 5b’
5b’	3.55	*dd (12.0Hz, 3.5Hz)*	1’, 2’, 3’, 4’, 5a’
2’OH	5.51	*bs*	
3’OH	5.26	*bs*	
5’OH	5.43	*bs*	5a’, 5b’
2	8.19	*bs*	
8	8.34	*bs*	1’, 2’, 3’
10-NH	7.93	*bs*	11*, 12, 13
linker			
11	3.49	*bs*	12, 13, 10-NH*
12/13	1.62	*m*	11, 14
14	2.37	*t (7.0Hz)*	13, 8”-CONH
batracylin			
1”	7.98	*d (7.0Hz)*	2”*
2”	7.79	*t (7.0Hz)*	1”*, 3”*
3”	7.74	*t (7.0Hz)*	4”*, 2”*
4”	7.87	*d (7.0Hz)*	3”*
6”	7.33	*bd (8.0Hz)*	7”*
7”	7.53	*bd (8.0Hz)*	6”*, 8”-CONH
9”	7.57	*bs*	6”, 10”, 8”-CONH
10”	4.89	*s*	7”, 9”
8”-CONH	10.15	*s*	21, 7”, 9”
HHT – Hartmann Hahn Transfer			

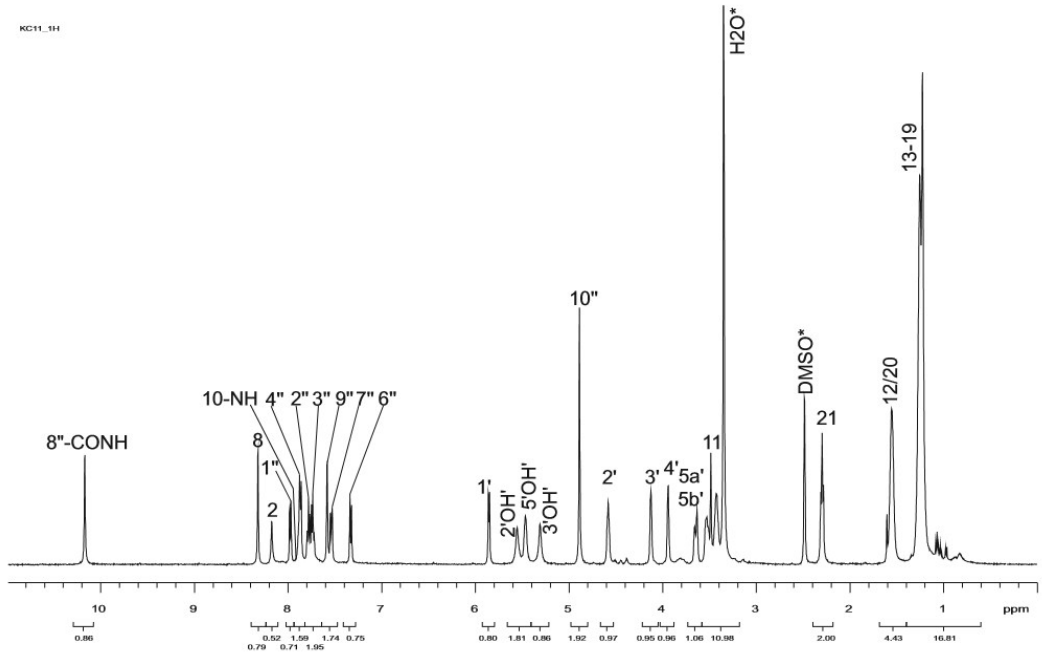
Table 7 ^{13}C NMR (500 MHz) chemical shifts for compound **3h** in DMSO-d_6 at 293 K.

^{13}C atom no.	*^{13}C δ (ppm)*	*^{13}C atom no.*	*^{13}C δ (ppm)*
adenosine moiety		**batracylin**	
C-1’	88.63	C-1”	122.48
C-2’	74.17	C-2”	133.89
C-3’	71.34	C-3”	132.91
C-4’	86.59	C-4”	123.54
C-5’	62.36	C-4a”	134.78
C-2	153.07	C-4b”	148.07
C-4	148.84	C-5a”	139.42
C-5	120.39	C-6”	128.58
C-6	155.31	C-7”	119.38
C-8	140.30	C-8”	135.91
linker		C-9”	118.04
C-11	39.67	C-9a”	123.07
C-12	29.70	C-10”	41.10
C-13-19	26.0-32.0	C-12”	166.87
C-20	25.80	C-12a”	130.73
C-21	37.09		
C-22	172.19		

Table 8 ^{1}H NMR (500 MHz) chemical shifts for compound **3h** in DMSO-d_6 at 293 K.

Proton no.	*^{1}H δ (ppm)*	*$J_{H,H}$ (Hz)*	*ROE contacts*
adenosine moiety			
1’	5.86	*d (5.0Hz)*	8, 2’, 3’, 4’
2’	4.58	*t (5.0Hz)*	8, 1’, 3’, 4’, 5a’, 5b’, 2’OH*
3’	4.12	*t (5.0Hz)*	8, 1’, 2’, 4’, 5a’, 5b’
4’	3.95	*dd (5.0Hz, 3.5Hz)*	1’, 2’, 3’, 5a’, 5b’
5a’	3.65	*dd (12.0Hz, 3.5Hz)*	1’, 2’, 3’, 4’, 5b’
5b’	3.55	*dd (12.0Hz, 3.5Hz)*	1’, 2’, 3’, 4’, 5a’
2’OH	5.56	*bs*	2’*
3’OH	5.31	*bs*	
5’OH	5.47	*bs*	5a’, 5b’
2	8.18	*bs*	
8	8.33	*bs*	1’, 2’, 3’
10-NH	7.90	*bs*	11*, 12, 13
linker			
11	3.49	*bs*	12, 13, 10-NH*
12/20	1.56	*m*	11, 21, 10-NH
13-19	1.25	*m*	11, 12, 21, 10-NH
21	2.30	*t (7.0Hz)*	19, 20, 8”-CONH
batracylin			
1”	7.98	*d (7.0Hz)*	2”*
2”	7.78	*t (7.0Hz)*	1”*, 3”*

3”	7.73	*t (7.0Hz)*	4”*, 2”*
4”	7.87	*d (7.0Hz)*	3”*
6”	7.33	*bd (8.0Hz)*	7”*
7”	7.54	*bd (8.0Hz)*	6”*, 8”-CONH
9”	7.58	*bs*	6”, 10”, 8”-CONH
10”	4.89	*s*	7”, 9”
8”-CONH	10.17	*s*	21, 7”, 9”
**** HHT – Hartmann Hahn Transfer***			



Biological assays

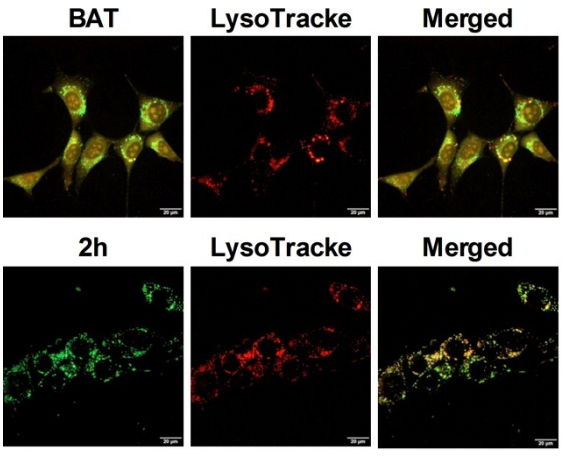


Fig. 3 Intracellular localization of BAT and its 2h derivative in living A549 cells.

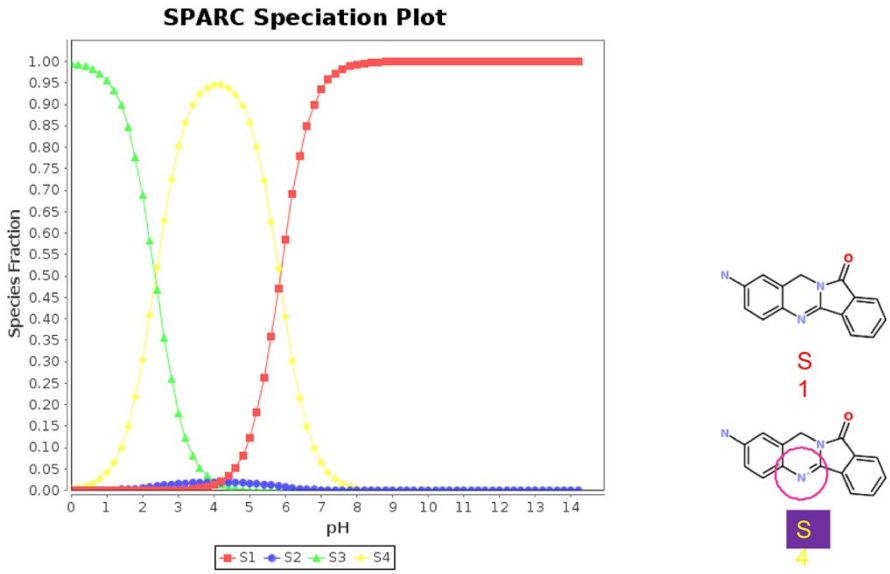


Fig. 4A Protonation profile of batracylin

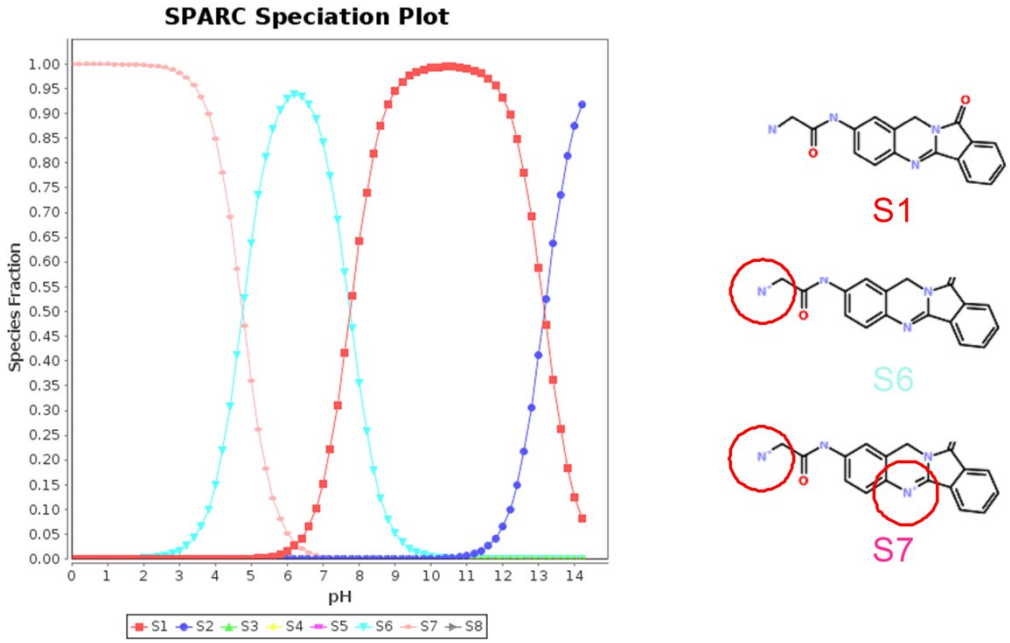


Fig. 4B Protonation profile of compound **2a**

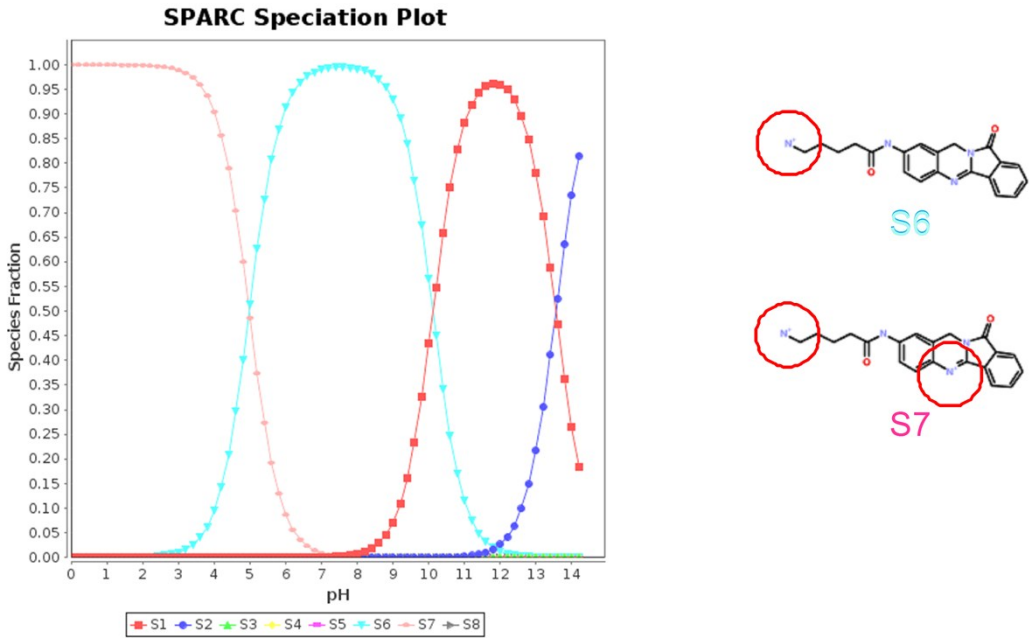


Fig. 4C Protonation profile of compound **2d**

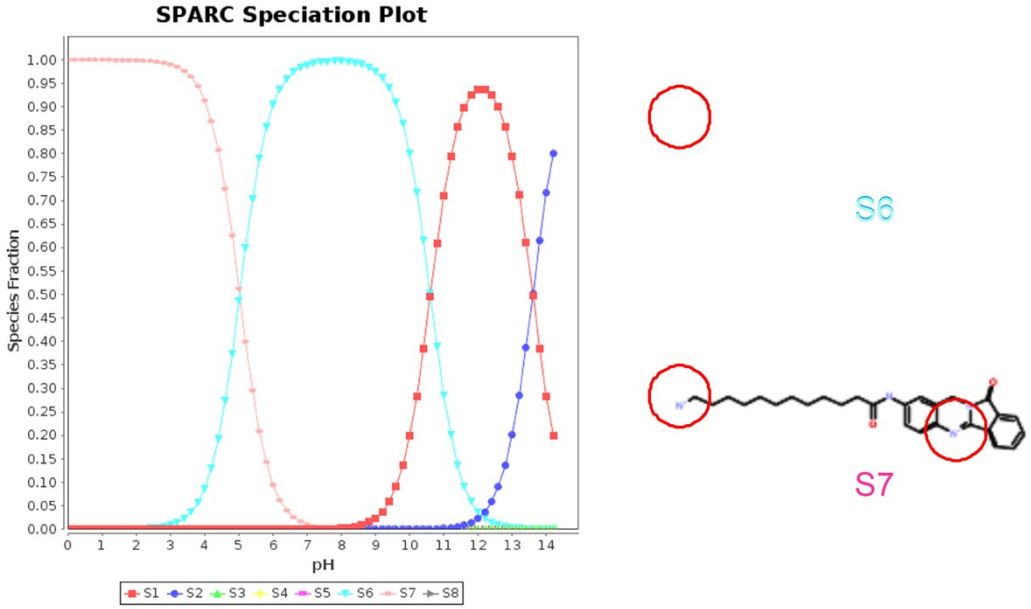


Fig. 4D Protonation profile of compound **2h**

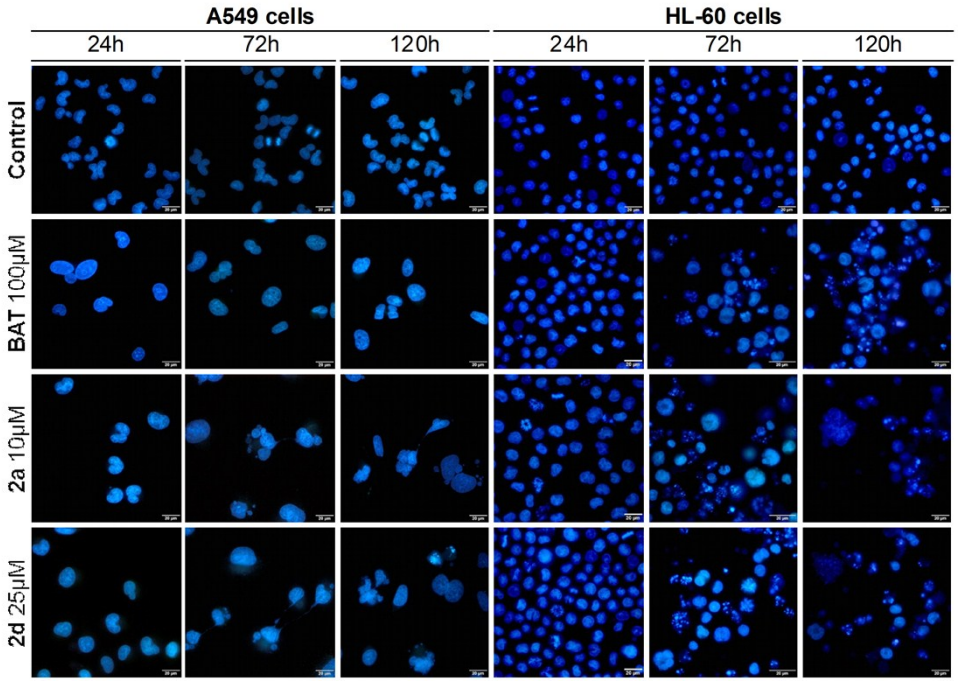


Fig. 5 Nuclear morphology of cells treated with BAT and its amino acid conjugates for the time indicated. Cells were treated with studied compounds, stained with Hoechst 33342 and analyzed by fluorescence microscopy.

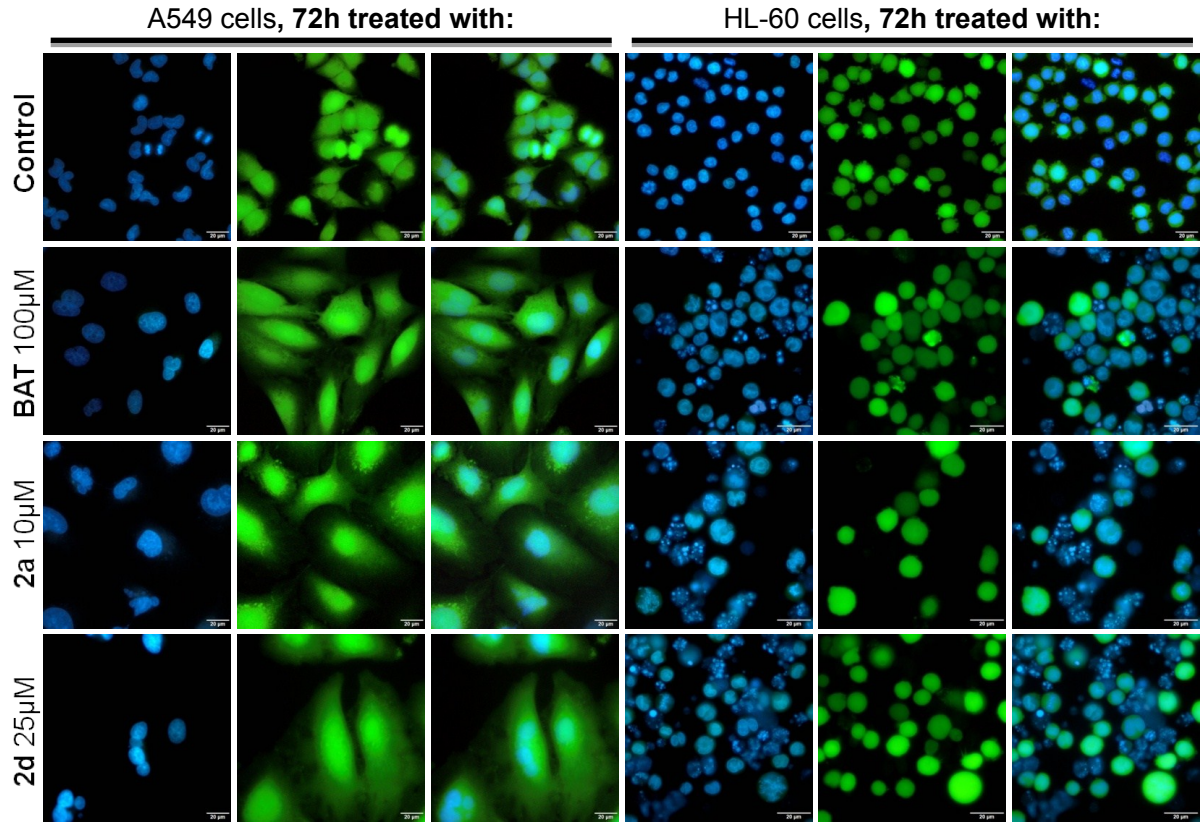


Fig. 6 Nuclear morphology of A549 and HL-60 cells treated with BAT and its amino acid conjugates. Cells were treated with studied compounds for 72h, stained with Hoechst 33342 (blue), acridine orange (green) and analyzed by fluorescence microscopy.

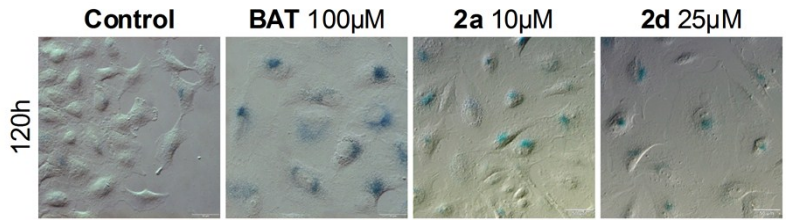


Fig. 7 Induction of premature cellular senescence in A549 cells treated with studied compounds for 120h. After treatment cells were fixed and stained for SA-β-Gal activity as described in Materials and Methods and analyzed by light microscopy.

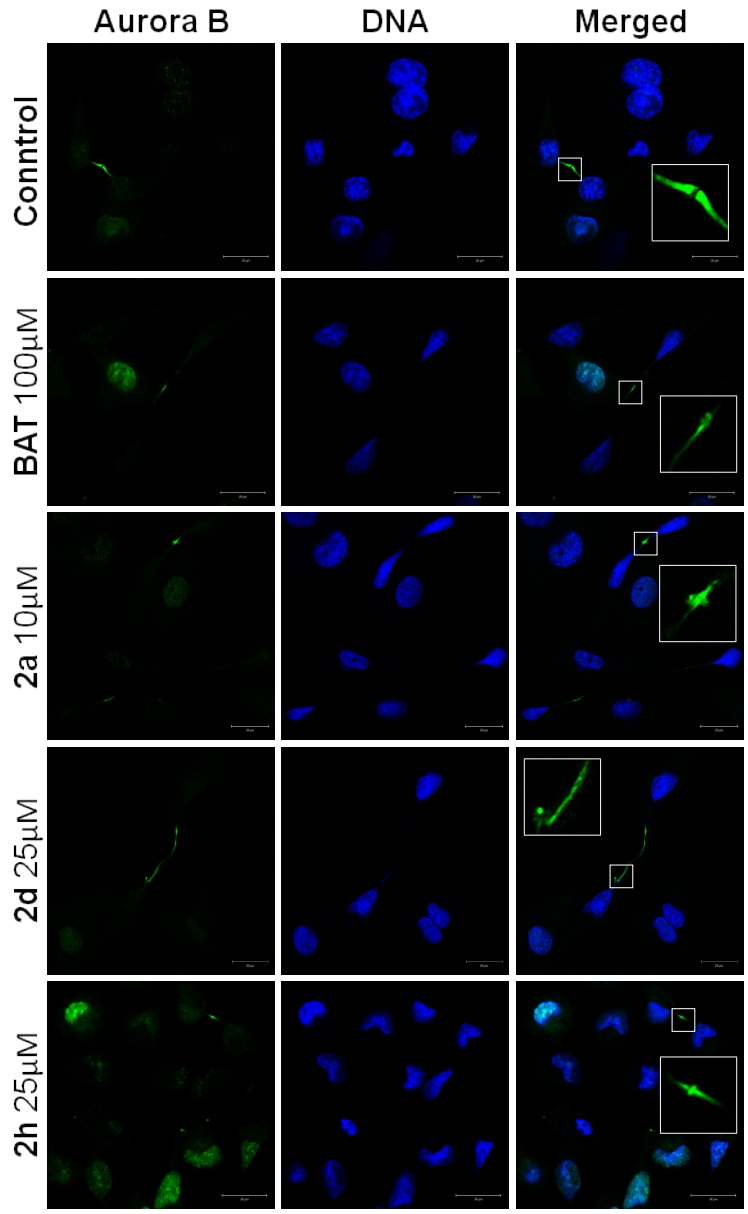


Fig. 8 Intracellular localization of AuroraB in A549 cells treated with studied compounds for 3h. After treatment cells were fixed, stained with Aurora B specific antibodies (green) as described in Materials and Methods and analyzed by fluorescence microscopy. DNA was counterstained with DAPI (blue). Insert: enlarged image of the mid-body structure containing Aurora B.