Supplementary material

A strong perspective on antimicrobial and anticancer application of silver(I) dipeptide complexes

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Figure S1. IR spectra of ligand HGlyGly, HGlyAla, H_2GlyAsp and their AgGlyGly, AgGlyAla and AgGlyAsp complexes.

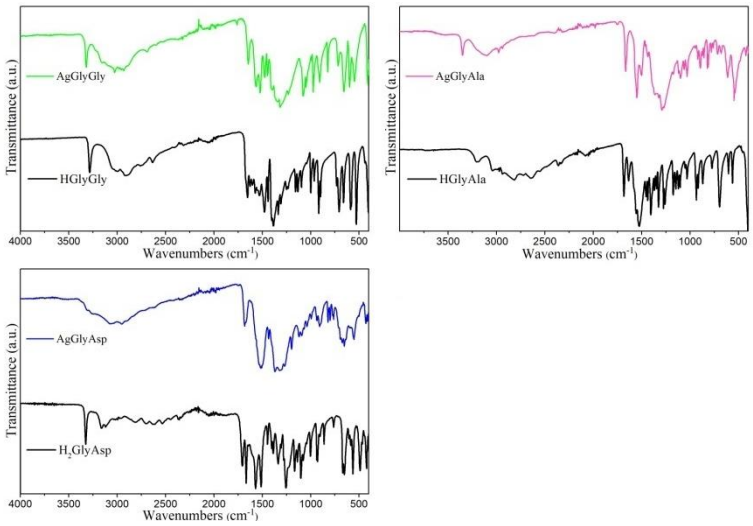
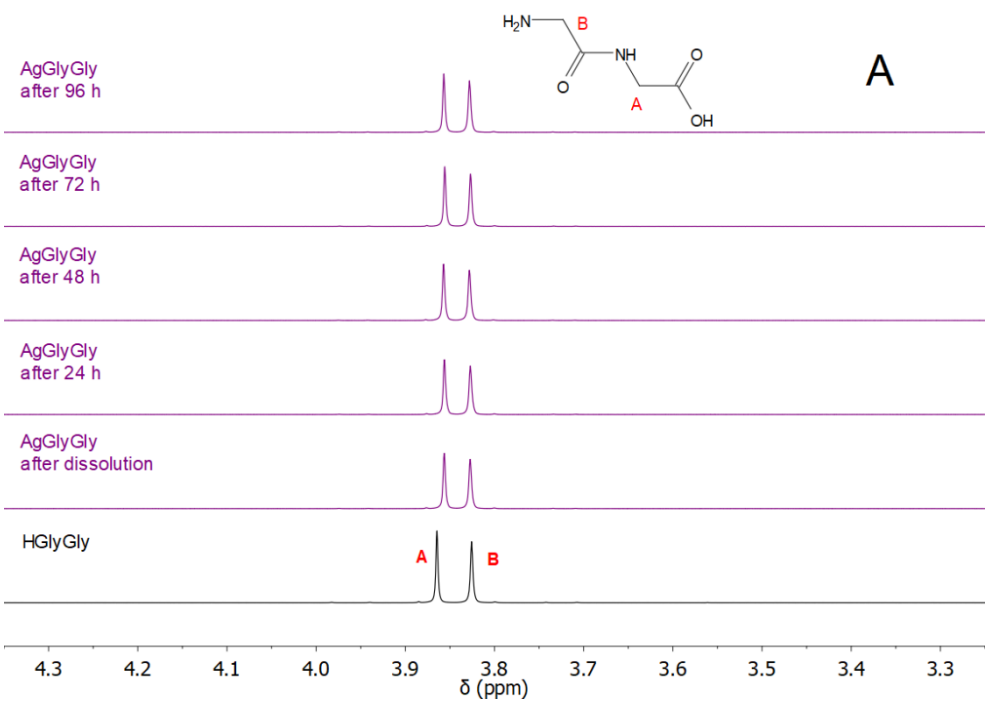
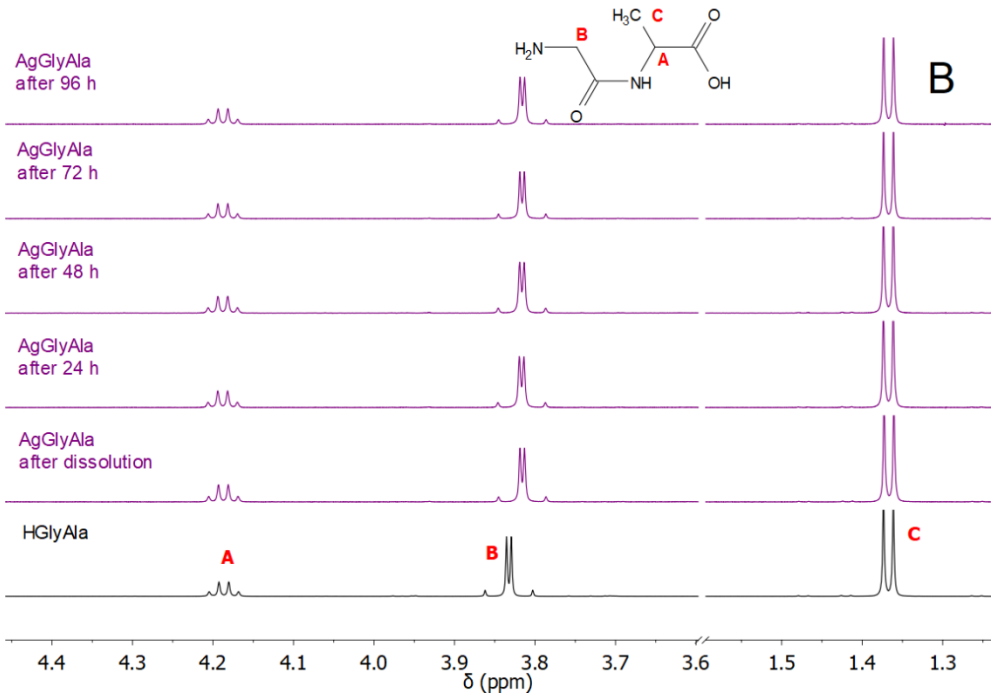


Figure S2A-C. ^{1}H NMR spectra of HGlyGly ligand and time dependent spectra of AgGlyGly complex recorded in 1% DMSO d_6/D_2O (A), HGlyAla ligand and time dependent spectra of AgGlyAla complex (B) and H_2GlyGly ligand and time dependent spectra of AgGlyAsp complex (C).




AgGlyAla
after 96 h
AgGlyAla
after 72 h
AgGlyAla
after 48 h
AgGlyAla
after 24 h
AgGlyAla
after dissolution
HGlyAla
A
B
C
B
4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 1.5 1.4 1.3
δ (ppm)

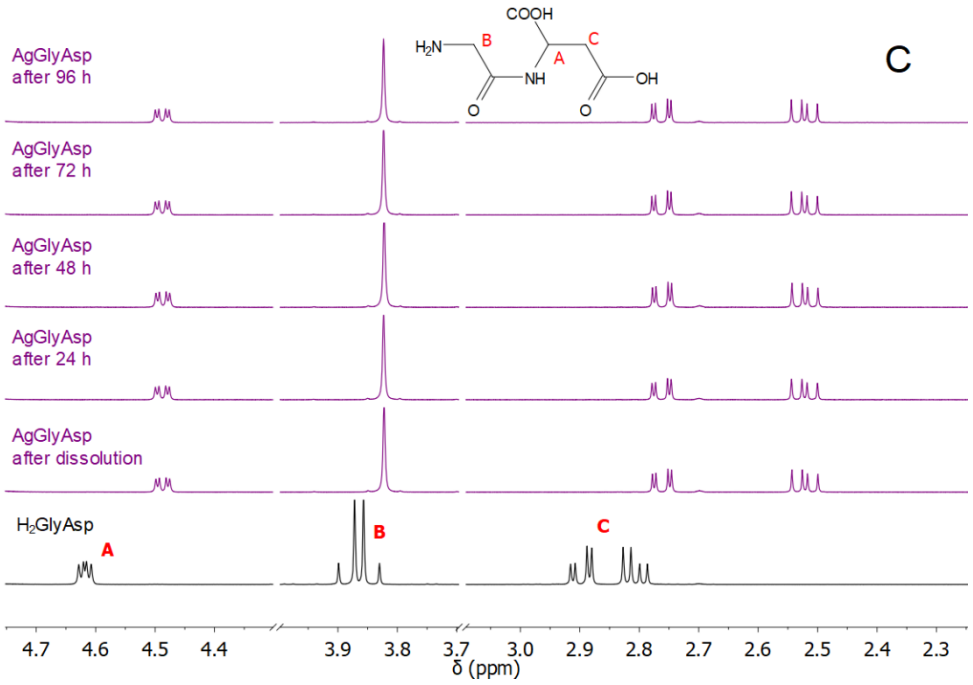

AgGlyAsp
after 96 h
AgGlyAsp
after 72 h
AgGlyAsp
after 48 h
AgGlyAsp
after 24 h
AgGlyAsp
after dissolution
H2GlyAsp
A
B
C
C
4.7 4.6 4.5 4.4 3.9 3.8 3.7 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3
δ (ppm)

Figure S3. Spectroscopic titration in Tris-HCl solution for the AgGlyGly complex during titration with increasing amounts of CT DNA.

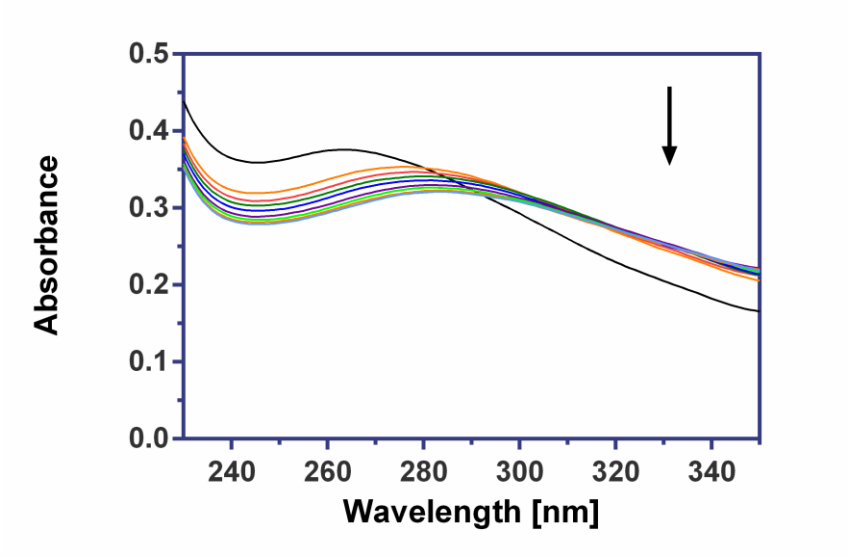
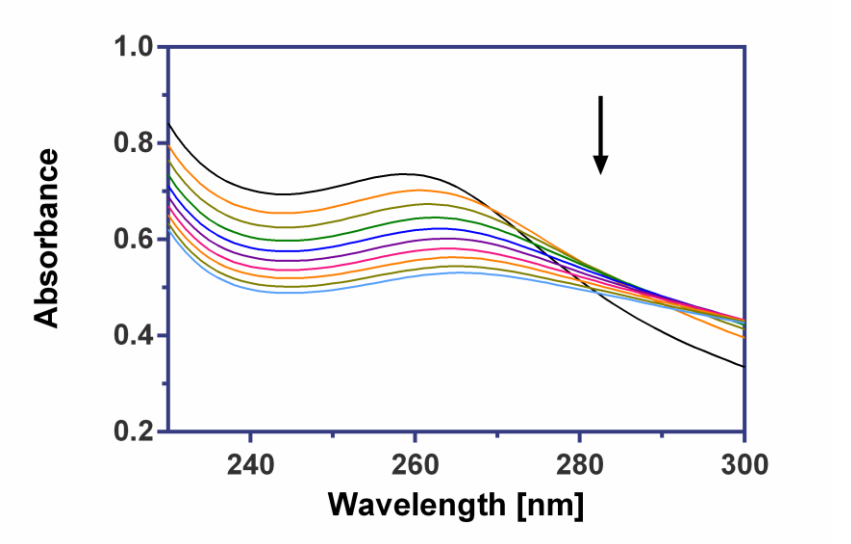


Figure S4. Spectroscopic titration in Tris-HCl solution for the AgGlyAla complex during titration with increasing amounts of CT DNA.



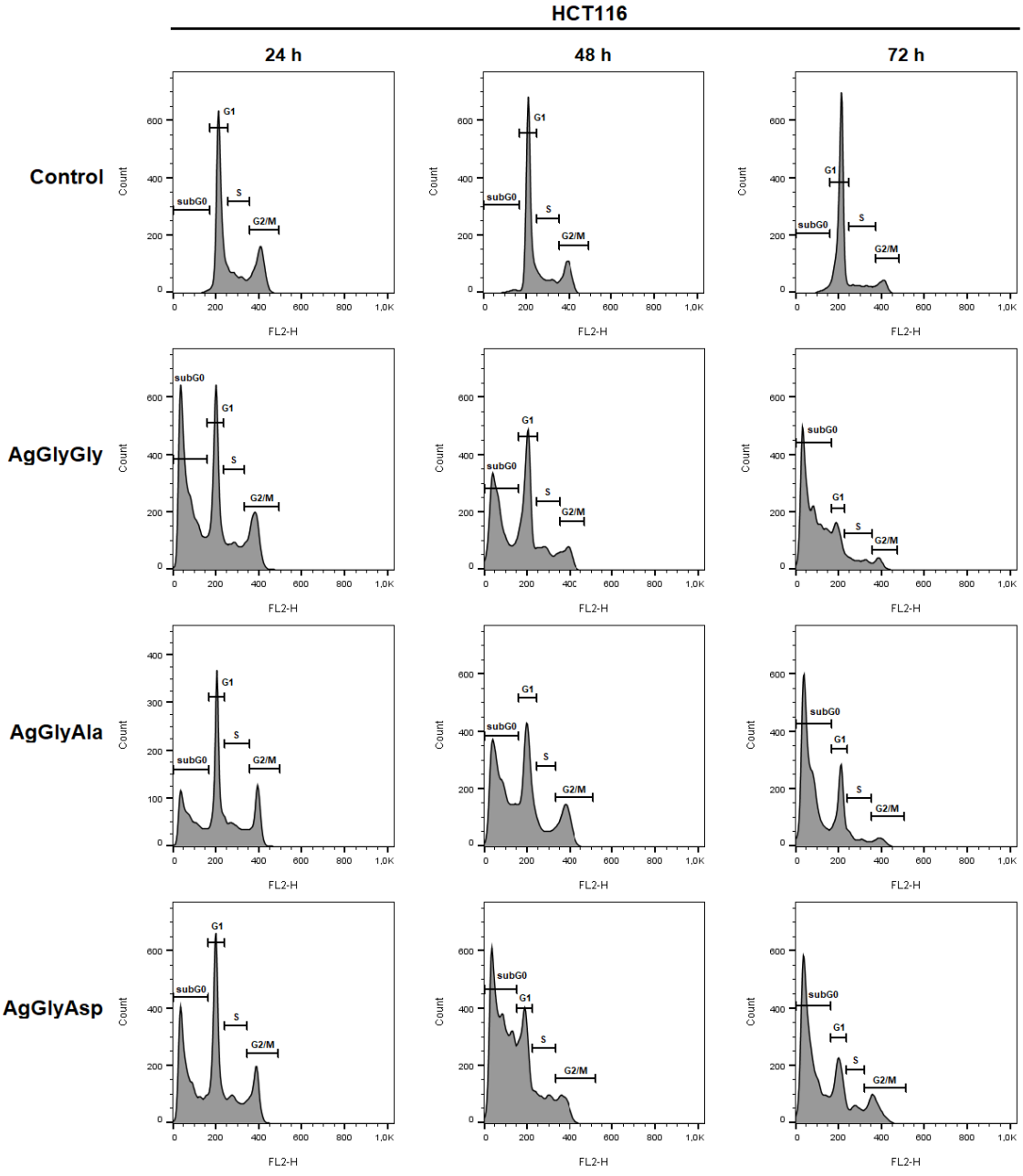


Figure S5. Histogram representation of cell cycle distribution in HCT116 cells treated with IC_{50} AgGlyGly, AgGlyAla, AgGlyAsp after 24 h, 48 h, and 72 h. Representative data of three independent experiments are presented.

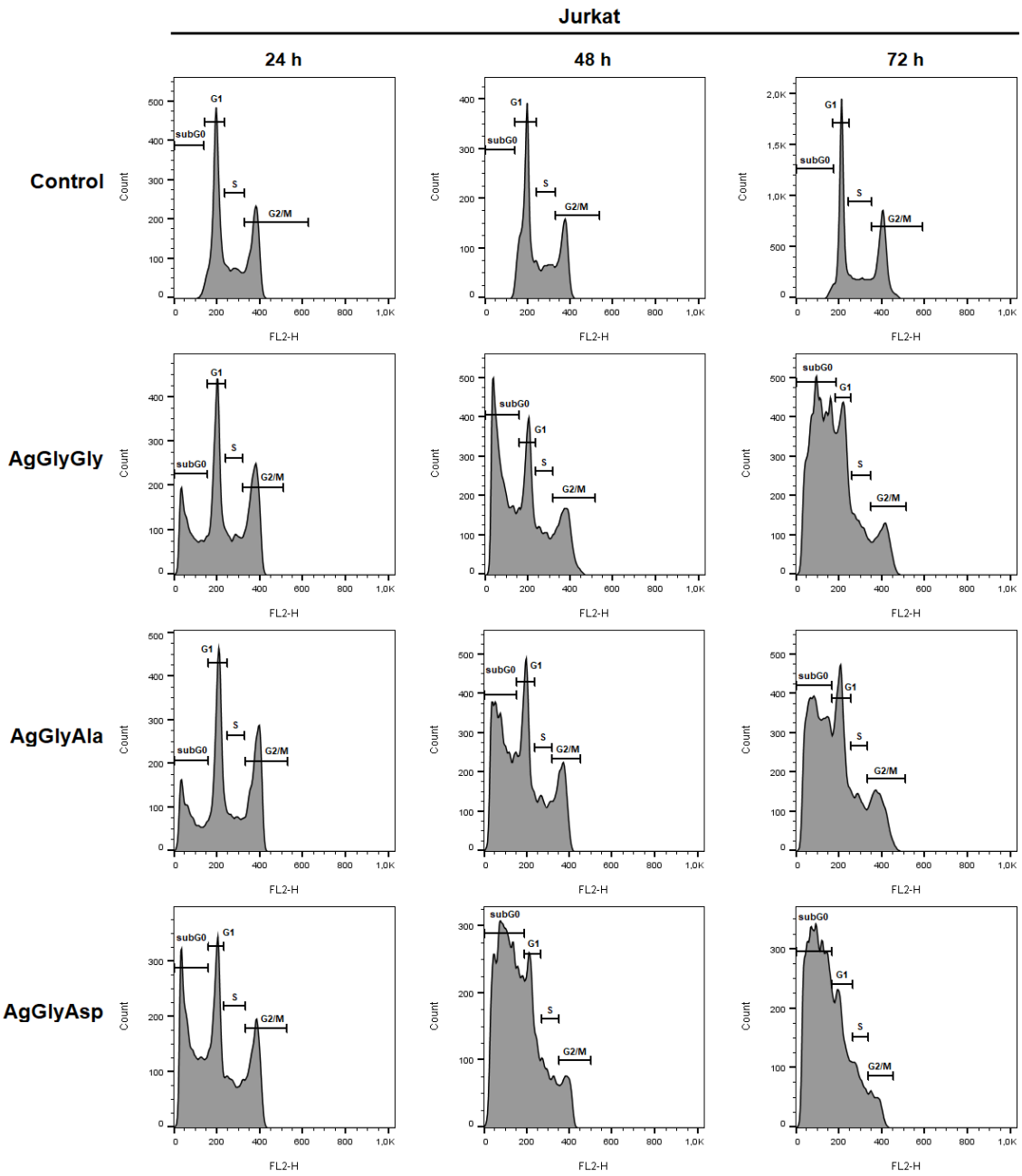


Figure S6 Histogram representation of cell cycle distribution in Jurkat cells treated with IC_{50} AgGlyGly, AgGlyAla, AgGlyAsp after 24 h, 48 h, and 72 h. Representative data of three independent experiments are presented.

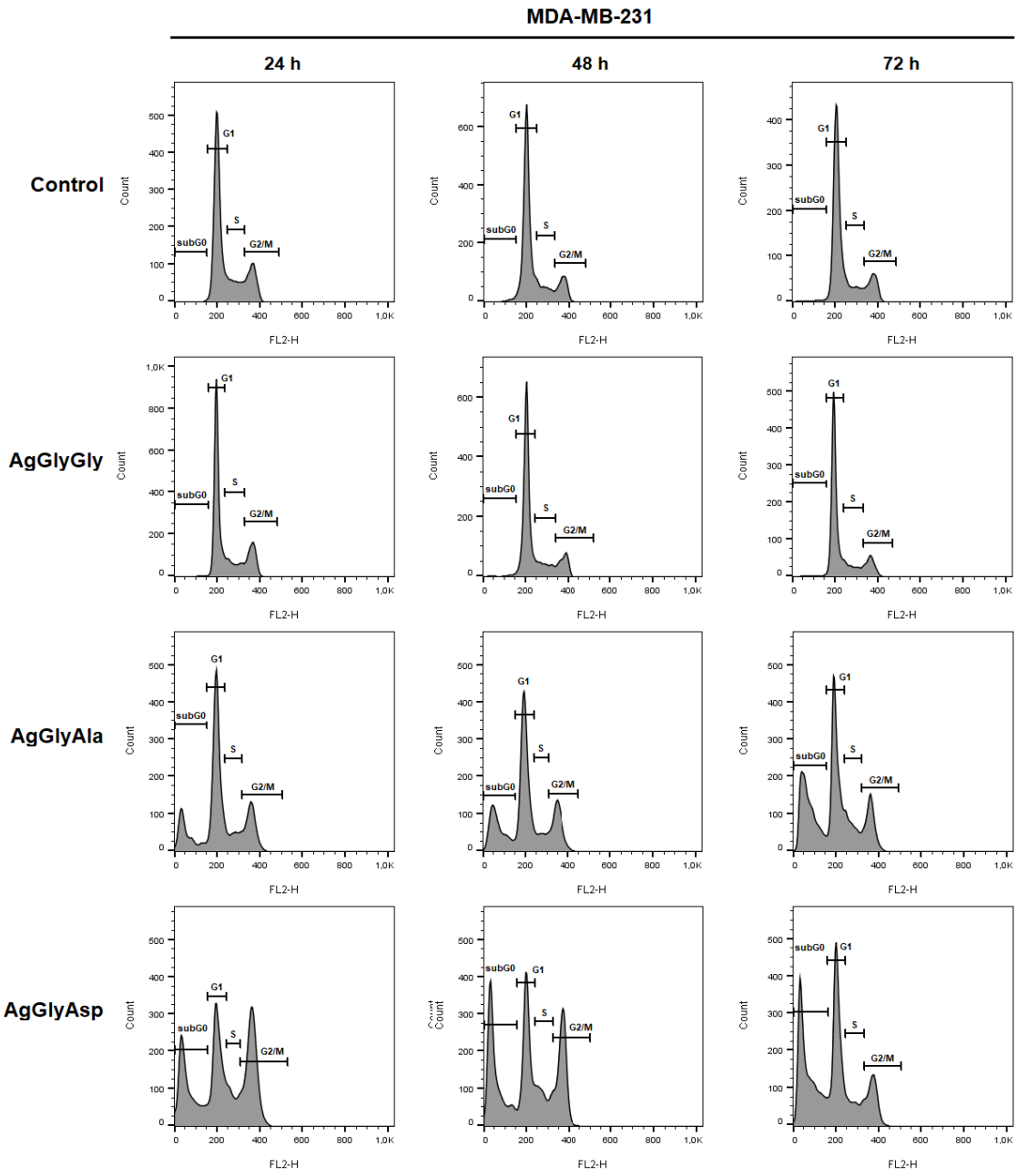


Figure S7. Histogram representation of cell cycle distribution in MDA-MB-231 cells treated with IC_{50} AgGlyGly, AgGlyAla, AgGlyAsp after 24 h, 48 h, and 72 h. Representative data of three independent experiments are presented.

Figure S8. Mass spectra of the aqueous solution of AgGlyGly complex measured in positive mode.

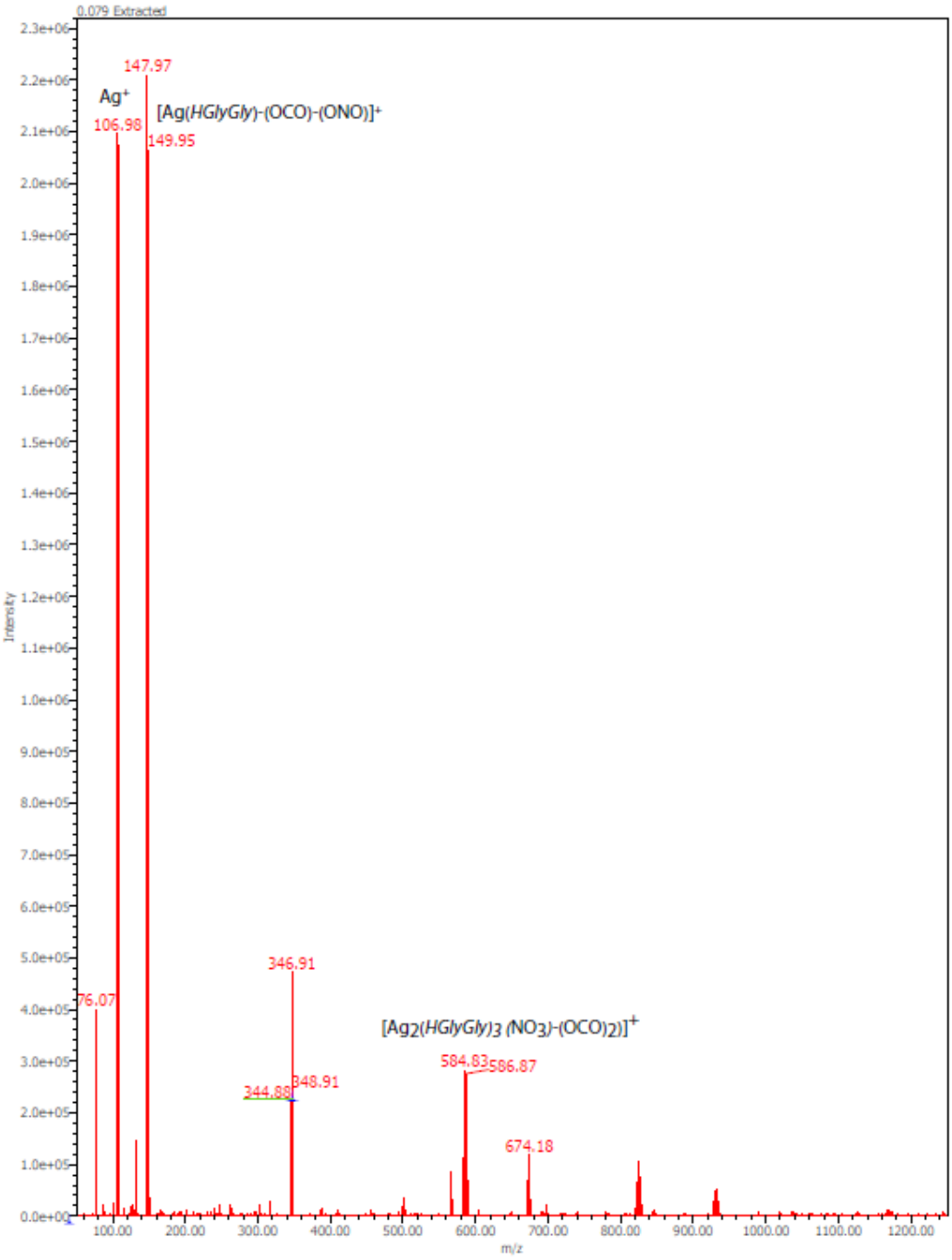


Figure S9. Mass spectra of the aqueous solution of AgGlyAla complex measured in positive mode.

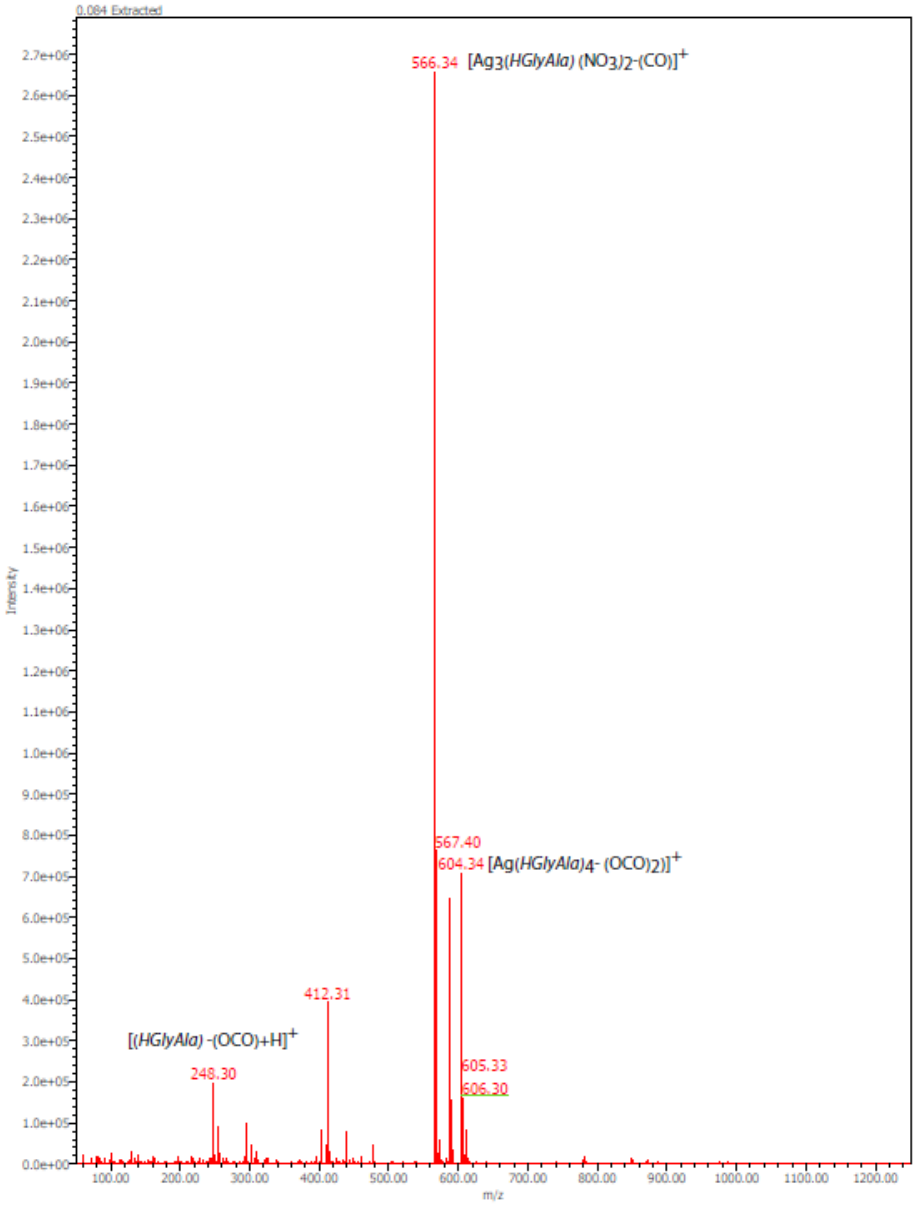


Figure S10. Mass spectra of the methanolic solution of AgGlyAsp complex measured in positive mode.

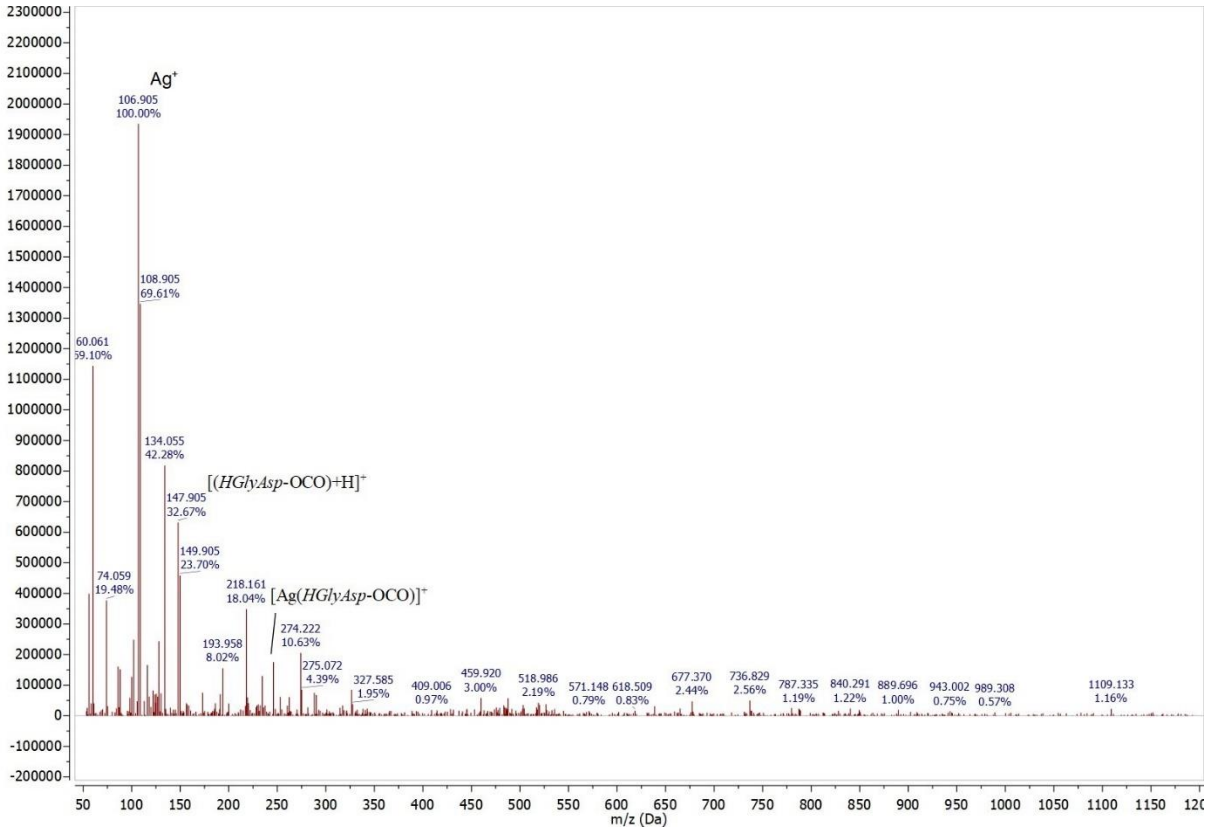


Table S1. Crystal data and structure refinement summary for complex AgGlyGly

Formula	$[Ag(HGlyGly)]_n(NO_3)_n$
Acronym	AgGlyGly
Empirical formula	$C_4\ H_8\ Ag\ N_3\ O_6$
Formula weight	301.99
Crystal parameters	
Crystal size (mm)	0.981 x 0.580 x 0.194
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimension	
a (Å)	4.9778 (7)
b (Å)	27.387 (4)
c (Å)	5.9015 (8)
α (°)	90
β (°)	94.022 (2)
γ (°)	90
V_c (Å^3)	802.6(2)
Molecules per cell, *Z*	4
D_{calc} (g.cm^{-3})	5.574
Measurement of intensity data	
Temperature (K)	120(2)
Wavelength (Å)	0.71073
θ range (°)	1.487 to 26.072
Reflections collected / unique	10247 / 1580 [R(int) = 0.0284]
Data	1580
F(000)	592
Indices limit	
H	-6 to 6
K	-33 to 33
L	-7 to 7
Refinement	
Refinement method	full-matrix least-squares on F^2
Parameters	133
Goodness-of-fit on F^2	1.256
R_1	0.035
wR_2	0.093
Maximum and minimum height in final difference synthesis (e.Å^3)	2.042 and -0.769

Table S2. Selected bond distances (Å) and angles (°) for AgGlyGly.

Bond distances			
Ag1—O2^{i}	2.198 (4)	C3—O3	1.229 (7)
Ag1—O1	2.206 (4)	C3—C4	1.521(7)
Ag1—O2^{ii}	2.595 (4)	C4—N2	1.488 (7)
Ag1—Ag^{i}	2.8928 (9)	C4—H4A	0.9900
O1—C1	1.252 (7)	C4—H4B	0.9900
C1—O2	1.265 (7)	N2—H2C	0.9100
C1—C2	1.518 (7)	N2—H2D	0.9100
C2—N1	1.450 (7)	N2—H2E	0.9100
C2—H2A	0.9900	N3—O5	1.236 (6)
C2—H2B	0.9900	N3—O4	1.262 (6)
N1—C3	1.342 (7)	N3—O6	1.269 (6)
N1—H3N	0.88 (7)		

Bond angles			
O2^{i}—Ag1—O1	161.57 (15)	O2^{ii}—Ag1—Ag1^{i}	119.35 (9)
O2^{i}—Ag1—O2^{ii}	85.64 (14)	C1—O1—Ag1	116.0 (3)
O1—Ag1—O2^{ii}	97.66 (14)	O1—C1—O2	126.9 (5)
O2^{i}—Ag1—Ag1^{i}	74.58 (10)	O1—C1—C2	118.7 (5)
O1—Ag1—Ag1^{i}	88.14 (10)	O2—C1—C2	114.4 (5)
C1—O2—Ag1^{i}	132.9 (4)	N2 —C4—H4A	110.0
C1—O2—Ag1^{iii}	118.1 (3)	C3—C4—H4A	110.0
Ag1^{i}—O2—Ag1^{iii}	94.36 (14)	N2—C4—H4B	110.0
N1—C2—C1	112.7 (5)	C3—C4—H4B	110.0
N1—C2—H2A	109.0	H4A—C4—H4B	108.3
C1—C2—H2A	109.0	C4—N2—H2C	109.5
N1—C2—H2B	109.0	C4—N2—H2D	109.5
C1—C2—H2B	109.0	H2C—N2—H2D	109.5
H2A—C2—H2B	107.8	C4—N2—H2E	109.5
C3—N1—C2	120.6 (5)	H2C—N2—H2E	109.5
C3—N1—H3N	118 (4)	H2D—N2—H2E	109.5
C2—N1—H3N	120 (4)	O5—N3—O4	120.7 (5)
O3—C3—N1	123.7 (5)	O5—N3—O6	120.6 (5)
O3—C3—C4	121.8 (5)	O4—N3—O6	118.7 (4)
N1—C3—C4	114.5 (5)		
N2—C4—C3	108.7 (4)		

Symmetry codes: (i) -x,-y,-z+3; (ii) x-1,y,z ; (iii) x+1,y,z

Table S3. Antibacterial activity of silver complex and free ligands (IC_{50}, MIC_{90}: µmol.l^{-1}).

	E. coli		***S. aureus***		***S. aureus* L12**		***S.epidermidis***	
	IC_{50}	MIC_{90}	IC_{50}	MIC_{90}	IC_{50}	MIC_{90}	IC_{50}	MIC_{90}
Gly	N		N		N		N	
Ala	N		N		N		N	
GlyGly	N		N		N		N	
GlyAla	N		N		N		N	
GlyAsp	N		N		N		N	
AgSD	1	4	1	2	1	4	2	5
AgGly	**3**	**5**	**3**	**5**	**5**	**10**	**5**	**10**
AgAla	**3**	**5**	**4**	**6**	**5**	**10**	**3**	**10**
AgGlyGly	6	10	12	22	15	38	10	20
AgGlyAla	**6**	**9**	**7**	**11**	**9**	**20**	**8**	**10**
AgGlyAsp	17	23	51	96	31	49	25	50

Note: N- not active IC_{50} and MIC_{90} values > 2 mmol.l^{-1}, Gly – glycine, Ala - alanine, SD-sulfadiazine

Table S4. Antifungal activity of silver complexes and free ligands.

	C. parapsilosis		***C. albicans***		***Rhizopus oryzae***		***A. alternata***		***A.flavus***		***M.gypseum***	
	IC_{50}	**MIC_{80}**	**IC_{50}**	**MIC_{80}**	**IC_{50}**	**MIC_{100}**	**IC_{50}**	**MIC_{100}**	**IC_{50}**	**MIC_{100}**	**IC_{50}**	**MIC_{100}**
Gly	N		N		N		N		N		N	
Ala	N		N		N		N		N		N	
GlyGly	N		N		N		N		N		N	
GlyAla	N		N		N		N		N		N	
GlyAsp	N		N		N		N		N		N	
AgSD	1	5	7	23	**3**	**10s**	**8**	**>100**	**46**	**89s**	**30**	**50s**
AgGly	**2,5**	**5**	**7**	**10**	**3**	**5s**	**5**	**10s**	**20**	**100s**	**100**	**>100**
AgAla	**25**	**50**	**7**	**10**	30	50s	>100	>100	NA		**100**	>100
AgGlyGly	30	70	70	100	30	50s	71	>100	NA		NA	
AgGlyAla	**30**	**60**	**50**	**87**	30	50s	42	>100	NA		**75**	100s
AgGlyAsp	50	100	73	100	73	100s	35	>100	NA		NA	

Note: N- not active IC_{50} and MIC values > 2 $mmol.l^{-1}$, NA – not active IC_{50} and MIC values >100$\mu mol.l^{-1}$, Gly – glycine, Ala - alanine, SD-sulfadiazine,s-fungistatic

Table S5. Cell cycle analysis of BLM cells after 24 h, 48 h, and 72 h incubation with dipeptides complexes.

BLM	Sub-G0	G1	S	G2/M
CTRL 24 h	2.2 ± 0.7	49.2 ± 0.1	18.6 ± 0.9	30.1 ± 0.3
AgGlyGly	17.3 ± 4.2 **	32.5 ± 2.8 **	14.1 ± 1.0 *	36.2 ± 1.3 *
AgGlyAla	13.5 ± 1.1 *	32.6 ± 0.1 **	17.9 ± 2.6	36.1 ± 0.9 *
AgGlyAsp	51.7 ± 0.5 ***	19.8 ± 2.7 ***	13.8 ± 3.0 *	14.8 ± 0.2 **
CTRL 48 h	2.0 ± 0.7	56.3 ± 0.6	20.5 ± 0.4	21.3 ± 0.4
AgGlyGly	18.8 ± 0.1 **	33.2 ± 0.9 **	15.3 ± 1.5 *	32.7 ± 2.4 **
AgGlyAla	13.7 ± 3.3 *	38.0 ± 0.5 **	19.5 ± 0.1	28.9 ± 2.7 *
AgGlyAsp	56.1 ± 0.9 ***	22.2 ± 2.8 ***	10.8 ± 2.5 *	10.9 ± 2.8 *
CTRL 72 h	1.6 ± 0.5	53.2 ± 2.0	22.3 ± 2.3	23.0 ± 0.9
AgGlyGly	22.8 ± 2.8 **	34.1 ± 2.9 **	16.0 ± 2.5 *	27.1 ± 2.1 *
AgGlyAla	13.8 ± 0.1 *	40.1 ± 1.6 **	18.8 ± 0.4	27.3 ± 1.3
AgGlyAsp	59.3 ± 3.4 ***	16.9 ± 3.5 ***	14.6 ± 0.9 *	9.4 ± 0.7 *

The data are presented from three independent experiments after 24 h, 48 h, and 72 h treatment as average percentage ± SD. Significantly different * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs untreated cells (control).

Table S6. Cell cycle analysis of HCT116 cells after 24 h, 48 h, and 72 h incubation with dipeptides complexes.

HCT116	Sub-G0	G1	S	G2/M
CTRL 24 h	0.8 ± 0.2	50.9 ± 3.6	18.5 ± 0.7	29.8 ± 3.2
AgGlyGly	44.3 ± 2.0 ***	26.3 ± 1.2 **	13.6 ± 2.4 *	15.8 ± 0.8 *
AgGlyAla	32.4 ± 0.9 **	37.8 ± 2.4 **	18.9 ± 0.8	10.9 ± 2.5 *
AgGlyAsp	37.2 ± 1.3 **	29.8 ± 3.4 **	16.3 ± 2.3	16.8 ± 2.4 *
CTRL 48 h	3.7 ± 0.8	56.2 ± 3.9	20.0 ± 2.4	20.3 ± 0.7
AgGlyGly	50.8 ± 2.0 ***	24.8 ± 1.1 **	13.7 ± 0.7 *	10.8 ± 2.5 *
AgGlyAla	46.2 ± 0.4 ***	32.9 ± 1.2 **	7.0 ± 0.3 *	14.0 ± 0.4 *
AgGlyAsp	57.1 ± 0.4 ***	21.0 ± 1.7 ***	10.3 ± 1.3 *	11.7 ± 3.4 *
CTRL 72 h	3.5 ± 0.1	69.4 ± 0.9	13.5 ± 0.5	13.6 ± 0.4
AgGlyGly	62.9 ± 5.3 ***	20.3 ± 2.9 ***	9.8 ± 0.1 *	7.1 ± 2.3 *
AgGlyAla	56.8 ± 4.3 ***	27.6 ± 2.6 ***	8.1 ± 1.2 *	7.6 ± 1.7 *
AgGlyAsp	62.7 ± 1.6 ***	20.3 ± 0.3 ***	7.7 ± 0.1 *	9.3 ± 2.0 *

The data are presented from three independent experiments after 24 h, 48 h, and 72 h treatment as average percentage ± SD. Significantly different * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs untreated cells (control).

Table S7. Cell cycle analysis of Jurkat cells after 24 h, 48 h, and 72 h incubation with dipeptides complexes.

Jurkat	Sub-G0	G1	S	G2/M
CTRL 24 h	1.0 ± 0.1	50.1 ± 0.2	16.2 ± 1.3	32.8 ± 1.5
AgGlyGly	25.9 ± 0.3 **	34.5 ± 0.8 **	12.8 ± 0.1 *	26.9 ± 0.4 *
AgGlyAla	20.4 ± 1.9 **	36.1 ± 0.4 **	12.7 ± 0.1 *	30.9 ± 1.5
AgGlyAsp	46.9 ± 3.2 ***	23.6 ± 1.9 **	14.0 ± 0.6	15.5 ± 2.7 *
CTRL 48 h	0.7 ± 0.4	53.0 ± 1.2	22.1 ± 1.5	24.3 ± 0.6
AgGlyGly	46.1 ± 0.1 ***	25.6 ± 0.8 **	11.8 ± 0.5 *	16.6 ± 1.3 *
AgGlyAla	39.7 ± 3.7 ***	29.9 ± 1.3 **	12.6 ± 0.5 *	17.9 ± 1.8 *
AgGlyAsp	57.4 ± 4.2 ***	24.0 ± 3.4 **	12.4 ± 1.1 *	6.3 ± 0.3 *
CTRL 72 h	2.3 ± 0.3	46.8 ± 2.9	16.6 ± 1.2	34.4 ± 1.3
AgGlyGly	57.0 ± 0.6 ***	23.0 ± 0.1 **	11.1 ± 0.8 *	8.9 ± 0.1 **
AgGlyAla	53.2 ± 3.6 ***	24.4 ± 2.3 **	12.1 ± 1.8 *	10.3 ± 3.1 **
AgGlyAsp	59.4 ± 1.8 ***	23.8 ± 0.1 **	11.1 ± 1.8 *	5.8 ± 0.2 **

The data are presented from three independent experiments after 24 h, 48 h, and 72 h treatment as average percentage ± SD. Significantly different * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs untreated cells (control).

Table S8. Cell cycle analysis of MDA-MB-231 cells after 24 h, 48 h, and 72 h incubation with dipeptides complexes.

MDA-MB-231	Sub-G0	G1	S	G2/M
CTRL 24 h	1.0 ± 0.6	66.4 ± 1.3	14.1 ± 1.4	18.6 ± 0.5
AgGlyGly	1.8 ± 0.4	62.3 ± 0.2	16.6 ± 0.3	19.3 ± 0.9
AgGlyAla	17.7 ± 3.0 *	48.3 ± 0.2 **	11.2 ± 2.0	22.8 ± 2.5 *
AgGlyAsp	24.7 ± 2.0 **	37.2 ± 4.2 **	7.6 ± 2.5 *	30.6 ± 2.4 *
CTRL 48 h	1.9 ± 0.4	68.5 ± 1.7	14.4 ± 1.3	15.3 ± 0.7
AgGlyGly	2.1 ± 0.2	68.4 ± 0.1	14.7 ± 0.2	14.9 ± 0.3
AgGlyAla	21.1 ± 2.5 **	48.5 ± 1.8 **	11.1 ± 0.7	19.4 ± 0.1 *
AgGlyAsp	35.8 ± 2.2 **	27.8 ± 0.8 ***	10.6 ± 1.9 *	25.8 ± 0.5 *
CTRL 72 h	2.2 ± 0.3	69.2 ± 0.1	13.8 ± 0.9	14.9 ± 0.5
AgGlyGly	2.3 ± 0.1	68.5 ± 0.1	14.4 ± 0.1	14.9 ± 0.1
AgGlyAla	40.5 ± 3.3 ***	35.0 ± 2.3 **	10.9 ± 1.3	13.7 ± 1.8
AgGlyAsp	55.9 ± 0.5 ***	27.4 ± 2.3 ***	7.1 ± 2.4 *	9.6 ± 1.2 *

The data are presented from three independent experiments after 24 h, 48 h, and 72 h treatment as average percentage ± SD. Significantly different * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs untreated cells (control).