SUPPORTING INFORMATION

Incorporation of sulfonamide moiety into biguanide scaffold results in apoptosis induction and cell cycle arrest in MCF-7 breast cancer cells.

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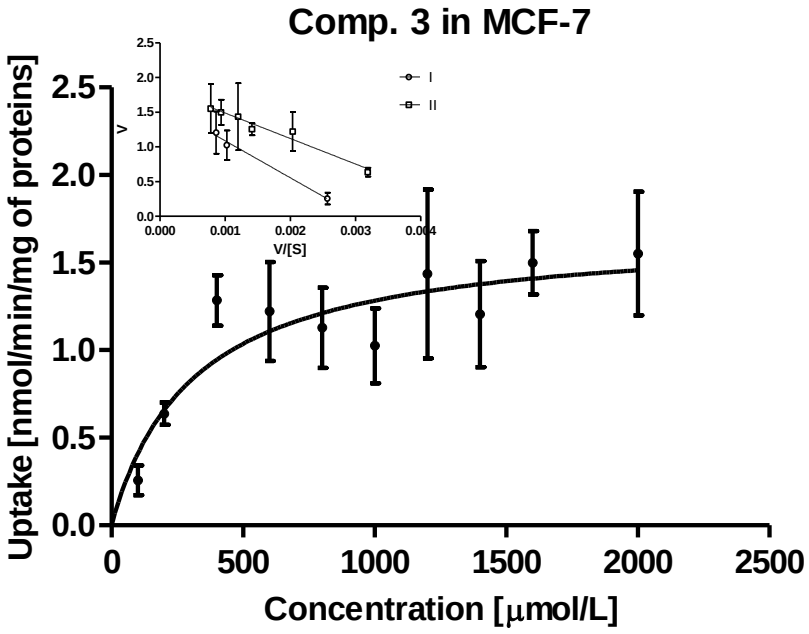
Table S1. Eadie-Hofstee analysis of the uptake of sulfonamide derivatives of metformin (**1 – 10**) in MCF-7 and MDA-MB-231 cells (K_m and V_{max} values).

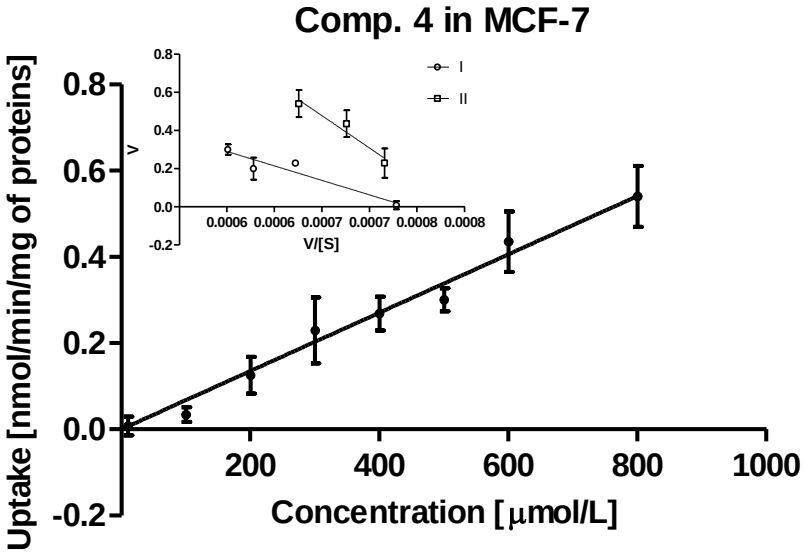
		Kinetic parameters of prodrugs uptake			
		MCF-7		**MDA-231 cells**	
Compound	**Transp.**	**K_m** [μmol/L]	**V_{max}** [nmol/min/mg]	**K_m** [μmol/L]	**V_{max}** [nmol/min/mg]
1	I	890.4 ± 216.1	0.653 ± 0.124	22.66 ± 3.0	0.476 ± 0.023
	V_{max}/K_m	0.000733		0.02101	
	II	1054 ± 556.2	1.225 ± 0.358	298.4 ± 134.0	0.714 ± 0.184
	V_{max}/K_m	0.001162		0.002393	
2	I	257.9 ± 43.28	1.147 ± 0.084	2115 ± 470.7	0.958 ± 0.149
	V_{max}/K_m	0.004447		0.000453	
	II	1907 ± 490.0	4.640 ± 0.689	590.6 ± 164.1	0.707 ± 0.079
	V_{max}/K_m	0.002433		0.001197	
3	I	532.7 ± 90.2	1.621 ± 0.151	NE	NE
	V_{max}/K_m	0.003043		NE	
	II	367.4 ± 75.1	1.856 ± 0.136	NE	NE
	V_{max}/K_m	0.005052		NE	
4	I	1518 ± 180.4	1.201 ± 0.121	144.6 ± 32.81	0.249 ± 0.022
	V_{max}/K_m	0.000791		0.001722	
	II	3389 ± 605.9	2.850 ± 0.438	2930 ± 652.4	1.504 ± 0.278
	V_{max}/K_m	0.000841		0.000513	
5	I	413.5 ± 167.2	0.491 ± 0.182	31.18 ± 9.06	0.274 ± 0.023
	V_{max}/K_m	0.001187		0.008788	
	II	760.3 ± 86.20	1.160 ± 0.080	693.8 ± 311.8	1.021 ± 0.211
	V_{max}/K_m	0.001526		0.001472	
6	I	1260 ± 178.3	2.713 ± 0.235	NE	NE
	V_{max}/K_m	0.002153		NE	
	II	1077 ± 145.1	2.705 ± 0.234	NE	NE
	V_{max}/K_m	0.002512		NE	
7	I	588.5 ± 79.7	2.223 ± 0.154	2689 ± 1945	0.569 ± 0.290
	V_{max}/K_m	0.003777		0.000212	
	II	962.7 ± 60.78	3.980 ± 0.140	349.5 ± 70.54	0.313 ± 0.029
	V_{max}/K_m	0.004134		0.000895	
8	I	358.1 ± 256.7	0.629 ± 0.223	1755 ± 418.1	38.20 ± 6.451
	V_{max}/K_m	0.001756		0.021766	
	II	896.7 ± 213.3	2.802 ± 0.276	546.5 ± 358.3	27.91 ± 6.767
	V_{max}/K_m	0.003124		0.051070	
9	I	259.7 ± 63.31	0.793 ± 0.099	630.5 ± 466.6	0.885 ± 0.412
	V_{max}/K_m	0.003053		0.001404	
	II	465.8 ± 188.3	1.296 ± 0.169	137.2 ± 240.2	1.028 ± 0.463
	V_{max}/K_m	0.002782		0.007493	
10	I	NE	NE	NE	NE

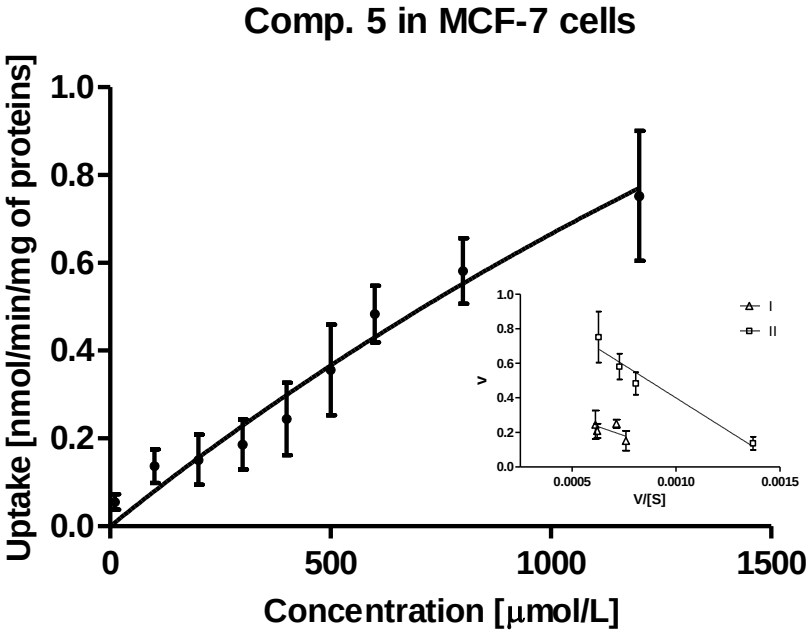
	V_{max}/K_m	NE		NE	
	II	NE	NE	NE	NE
	V_{max}/K_m	NE		NE	

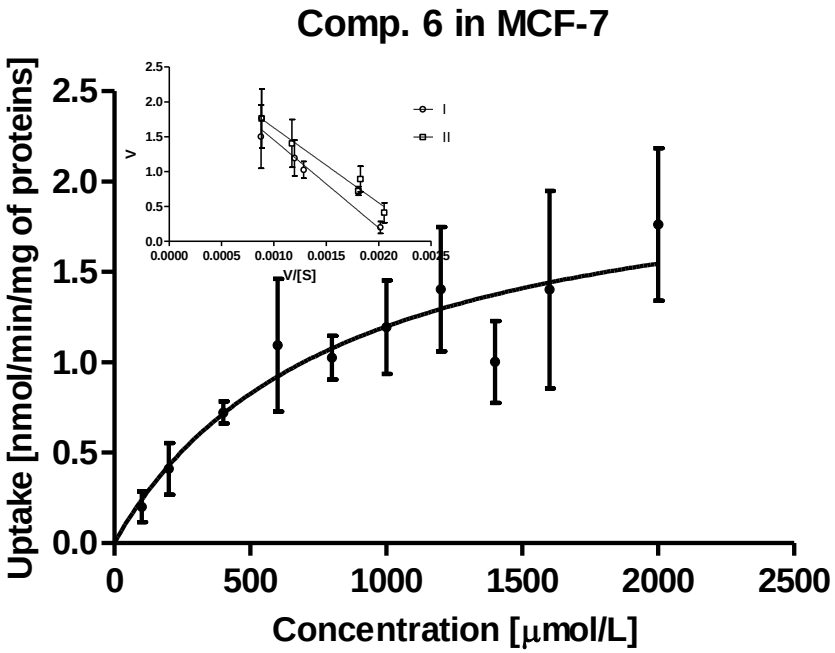
NE- not established.

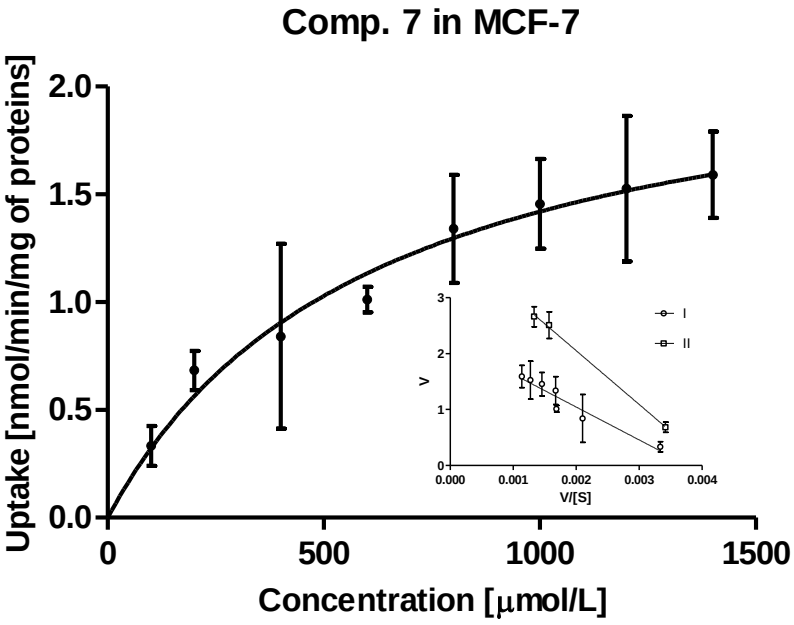
Figure S1. The uptake of compounds **3** - **10** into MCF-7 cells at the concentrations of 10 – 2000 μmol/L and Eadie–Hofstee plots for OCTs mediated transport.

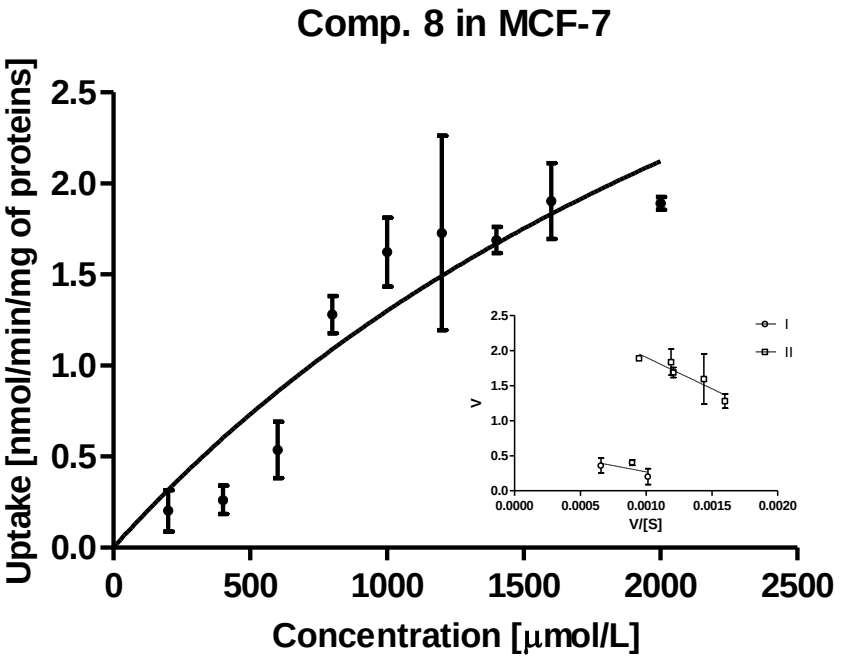


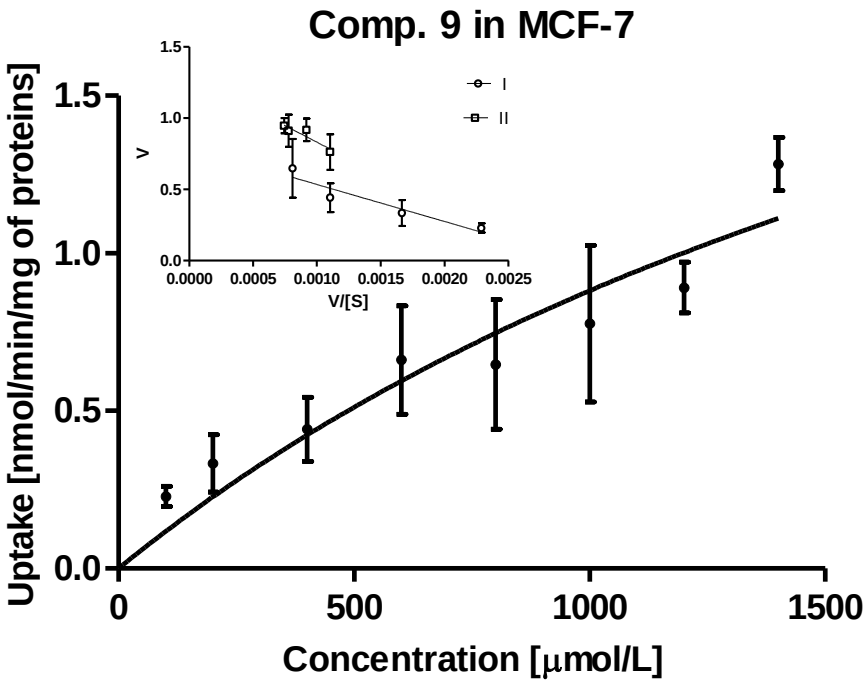

Comp. 4 in MCF-7
Uptake [nmol/min/mg of proteins]
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 5 in MCF-7 cells
Uptake [nmol/min/mg of proteins]
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 6 in MCF-7
Uptake [nmol/min/mg of proteins]
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 7 in MCF-7
Uptake [nmol/min/mg of proteins]
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 8 in MCF-7
Uptake [nmol/min/mg of proteins]
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
2500
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 9 in MCF-7
Uptake [nmol/min/mg of proteins]
1.5
1.0
0.5
0.0
0
500
1000
1500
Concentration [μmol/L]
V
V/[S]
I
II

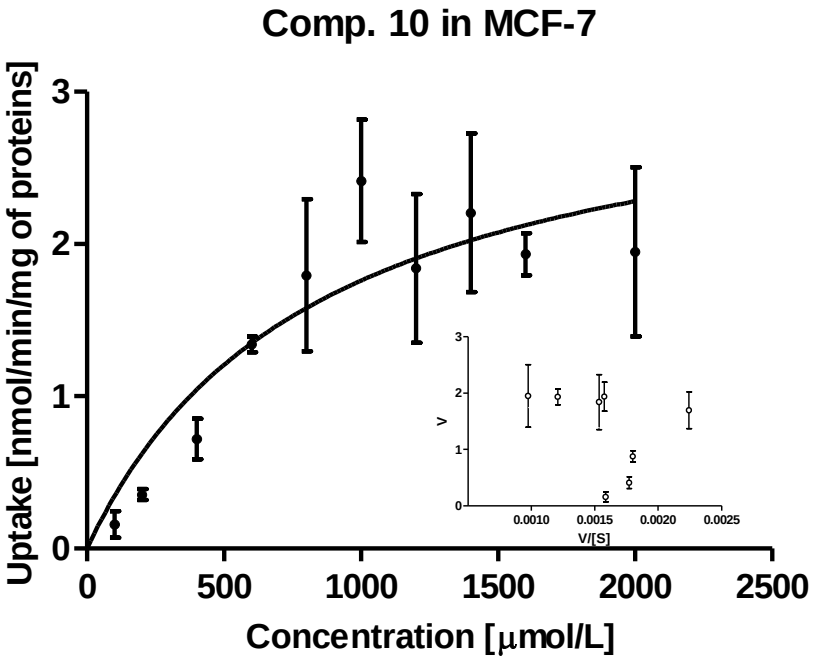
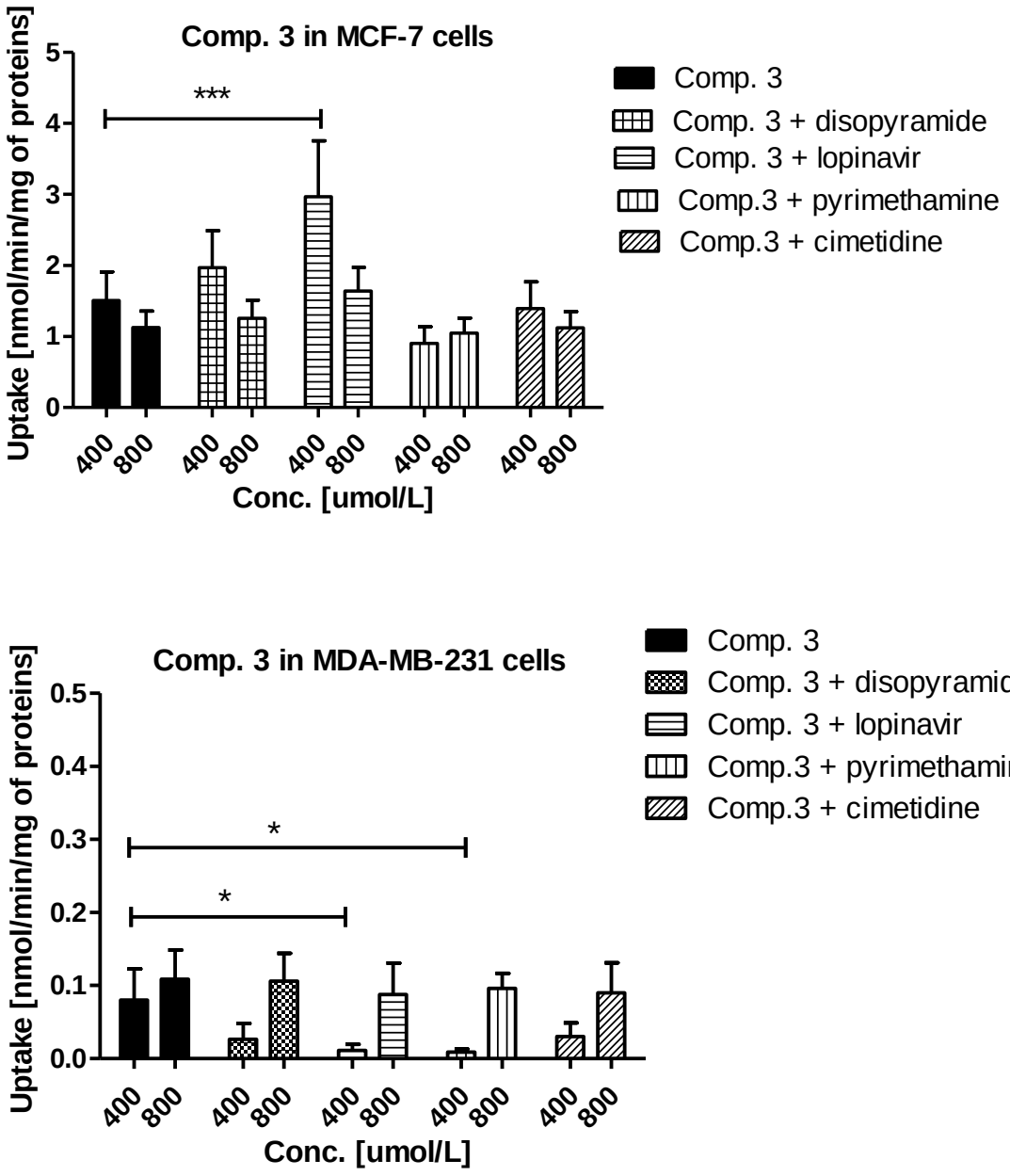
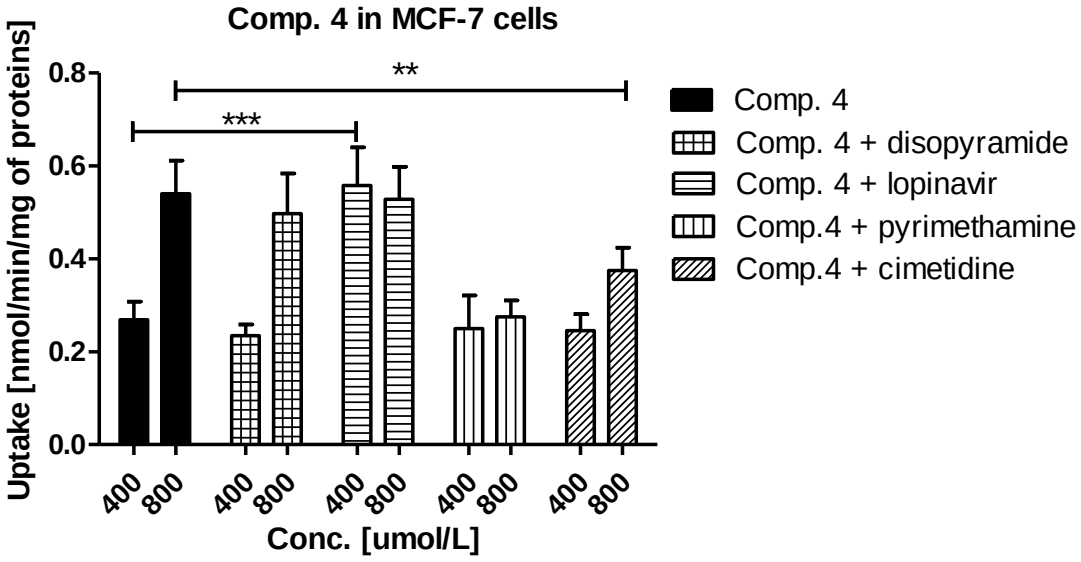
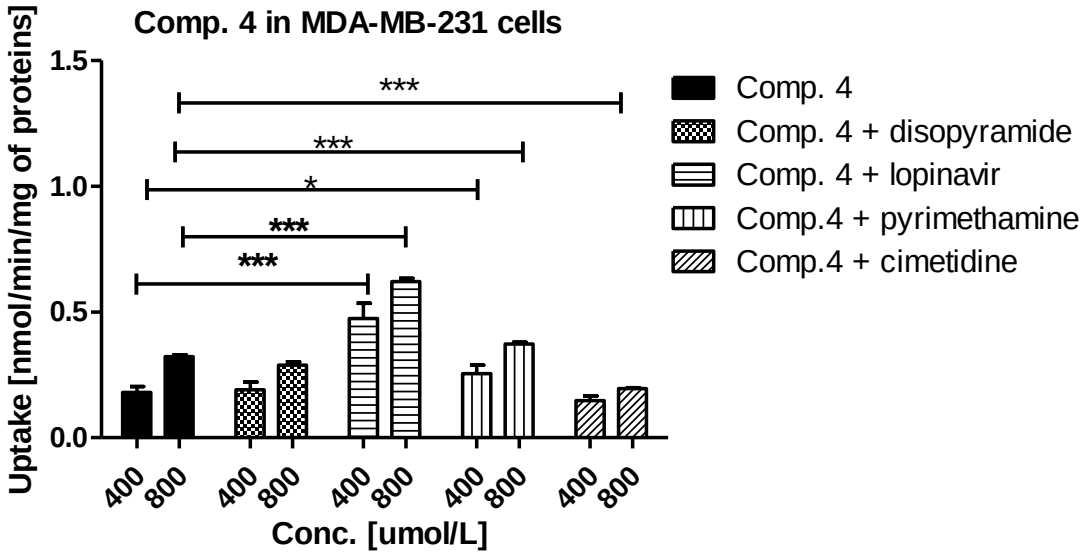
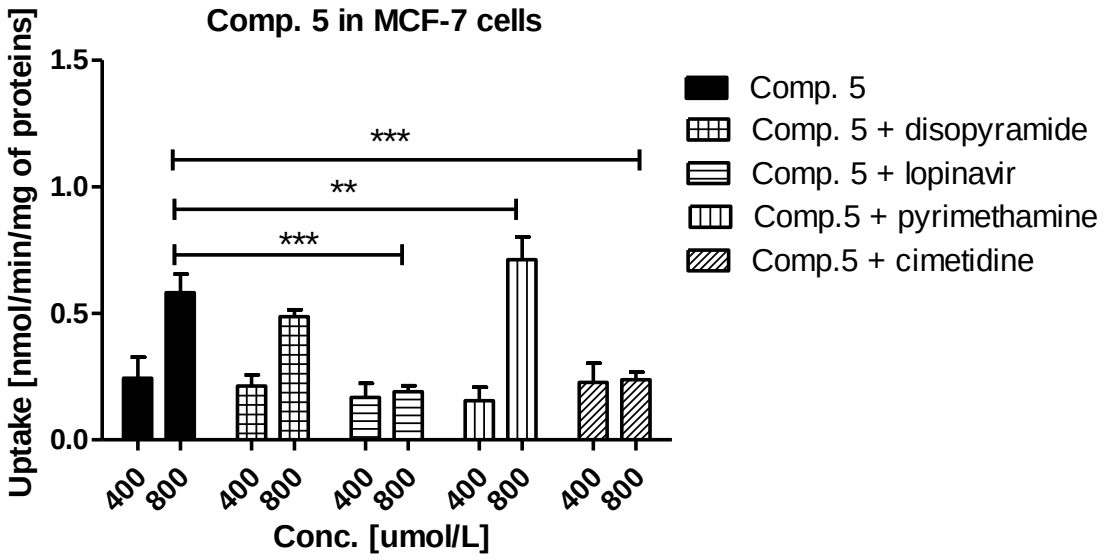

Comp. 10 in MCF-7
Uptake [nmol/min/mg of proteins]
0
1
2
3
0
500
1000
1500
2000
2500
Concentration [μmol/L]
V
0
1
2
3
0.0010
0.0015
0.0020
0.0025
V/[S]

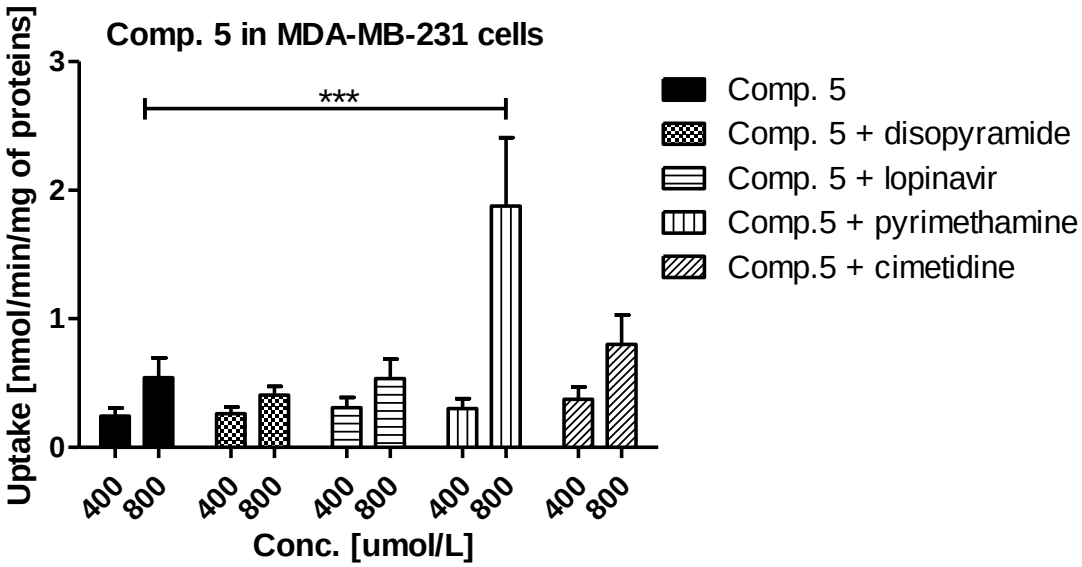
Figure S2. The uptake mechanism of compounds **3 – 10** (400 and 800 μmol/L) into MCF-7 cells and MDA-MB-231 cells. The uptake was determined in the presence of OCT and MATE inhibitors, disopyramide, lopinavir, methenamine, cimetidine (400 and 800 μmol/L) for 10 minutes at 37 °C. The significant differences between pure compounds **3 – 10** and their mixtures with inhibitors are denoted with asterisk. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

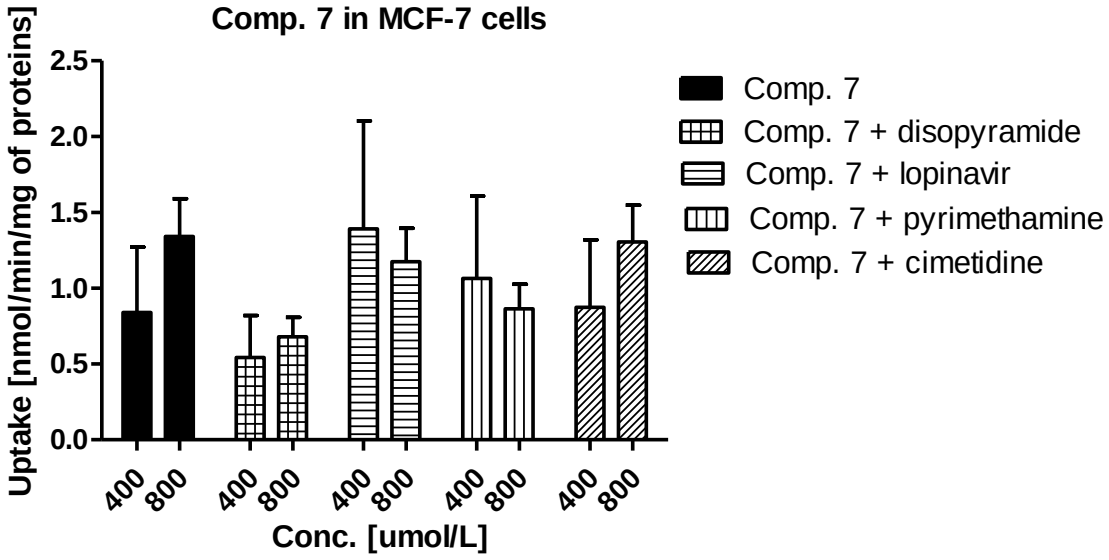


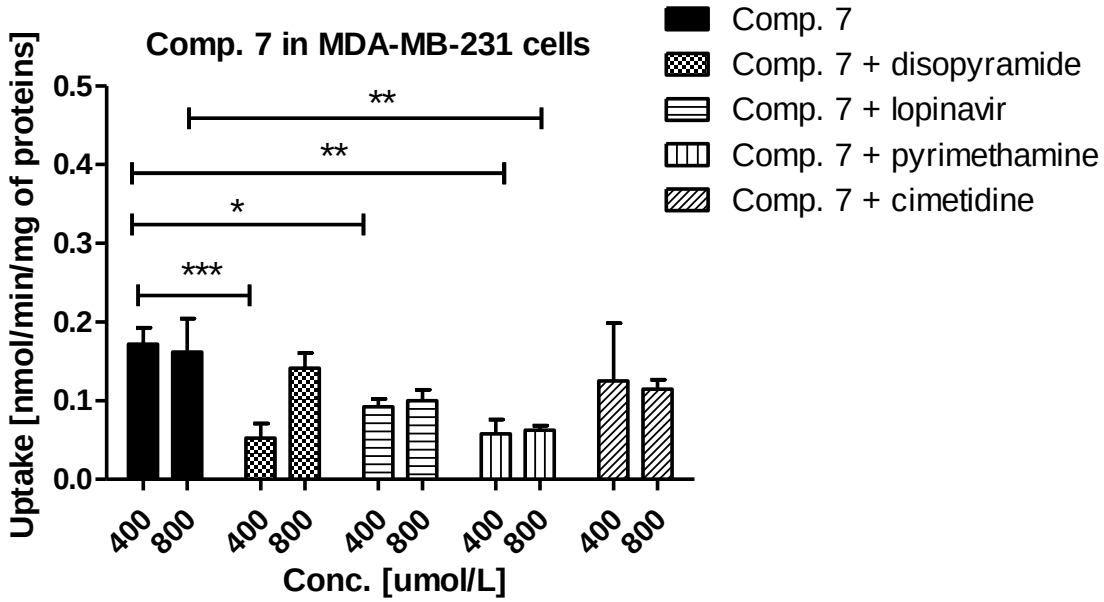

Comp. 4 in MCF-7 cells
Uptake [nmol/min/mg of proteins]
0.8
0.6
0.4
0.2
0.0
**
***
400
800
Conc. [umol/L]
Comp. 4
Comp. 4 + disopyramide
Comp. 4 + lopinavir
Comp.4 + pyrimethamine
Comp.4 + cimetidine

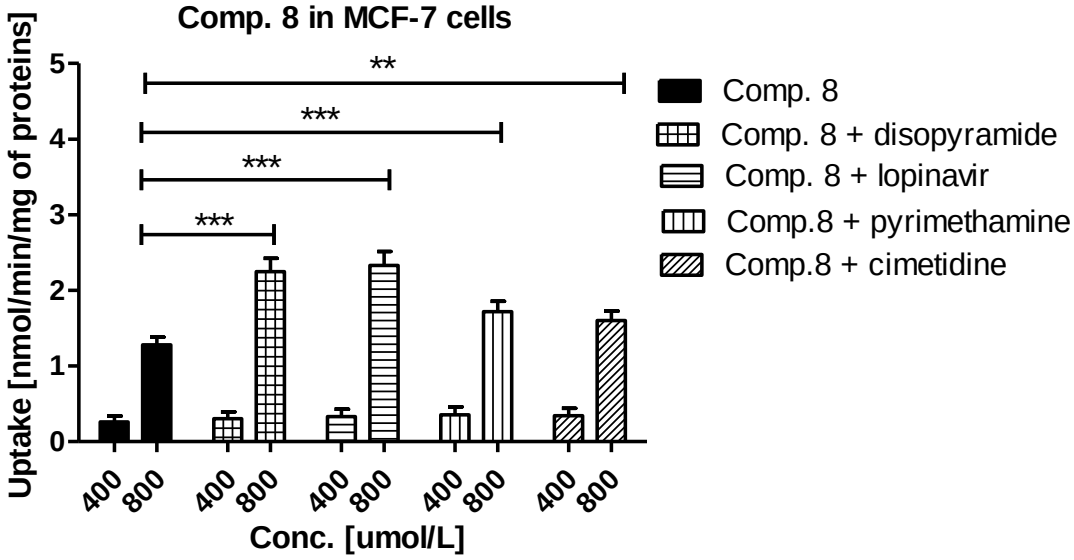

Comp. 4 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
1.5
1.0
0.5
0.0
***
***
*
***
***
400
800
Conc. [umol/L]
Comp. 4
Comp. 4 + disopyramide
Comp. 4 + lopinavir
Comp.4 + pyrimethamine
Comp.4 + cimetidine

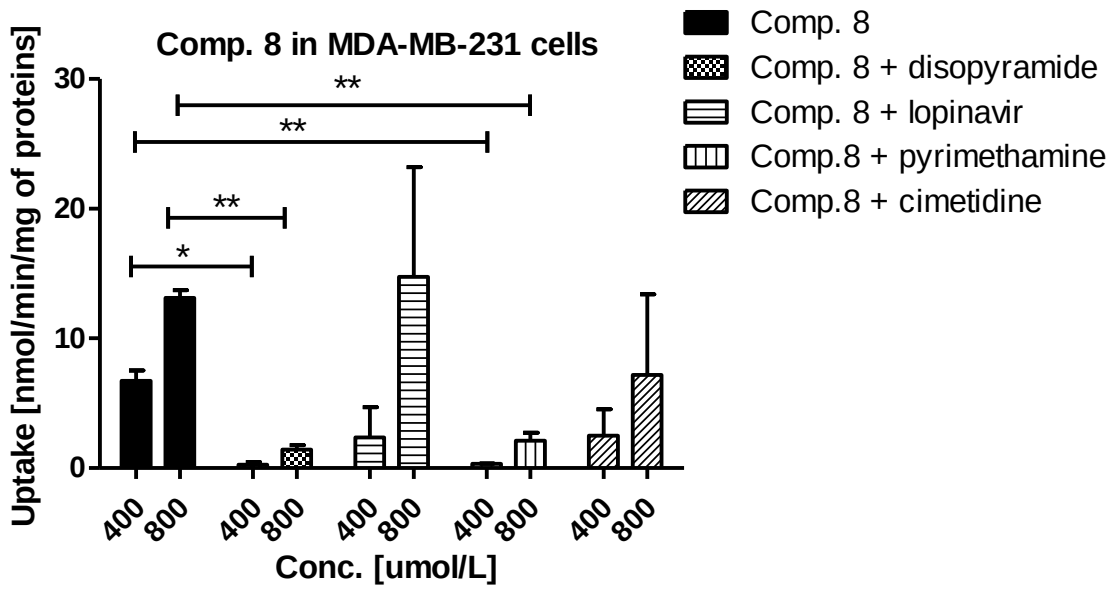

Comp. 5 in MCF-7 cells
Uptake [nmol/min/mg of proteins]
1.5
1.0
0.5
0.0
***
**
***
400
800
Conc. [umol/L]
Comp. 5
Comp. 5 + disopyramide
Comp. 5 + lopinavir
Comp.5 + pyrimethamine
Comp.5 + cimetidine

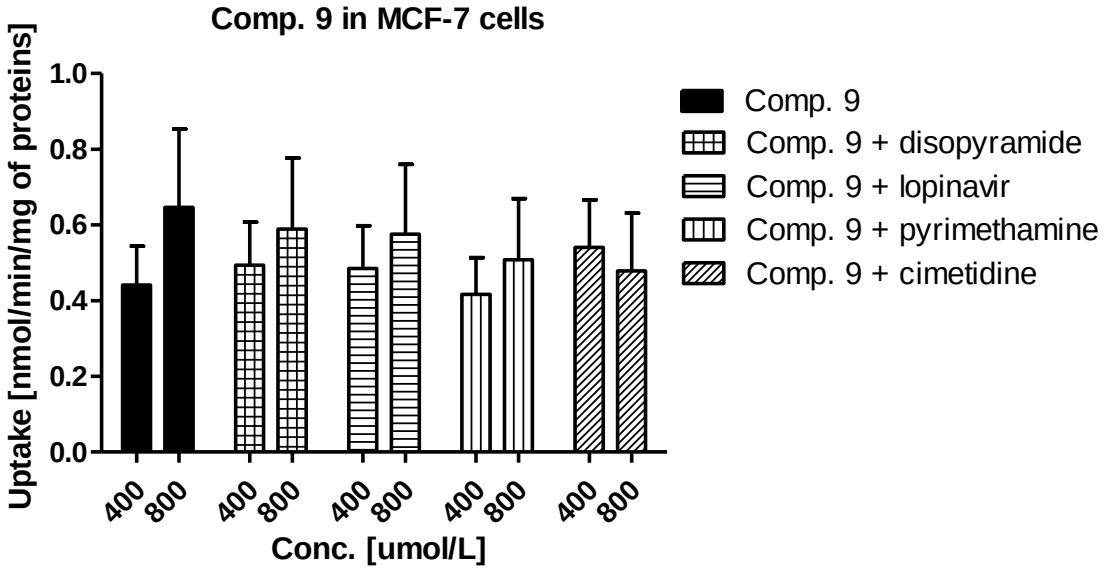

Comp. 5 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
3
2
1
0
***
400
800
Conc. [umol/L]
Comp. 5
Comp. 5 + disopyramide
Comp. 5 + lopinavir
Comp.5 + pyrimethamine
Comp.5 + cimetidine

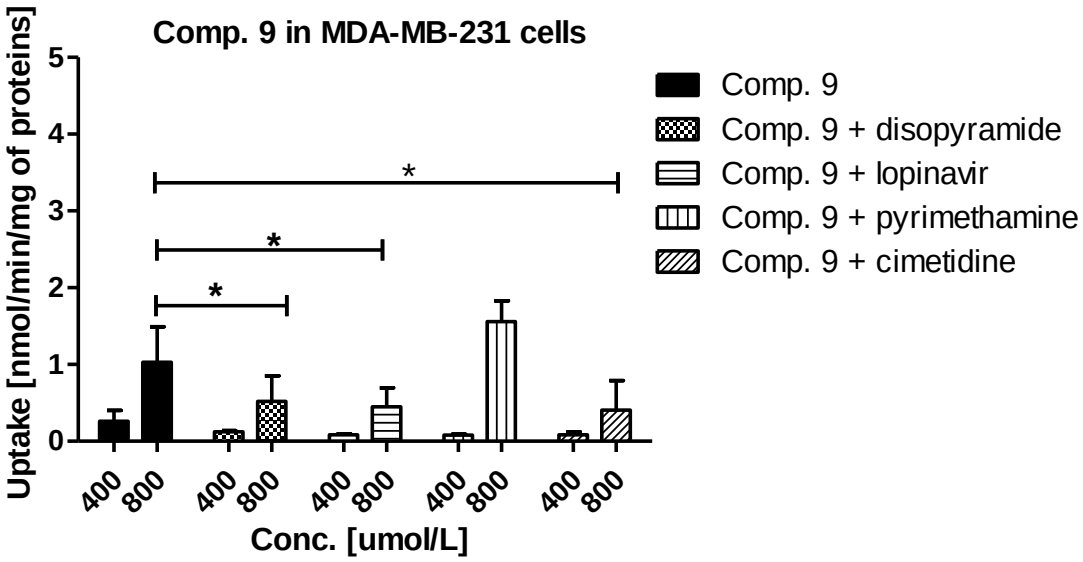

Comp. 7 in MCF-7 cells
Uptake [nmol/min/mg of proteins]
2.5
2.0
1.5
1.0
0.5
0.0
400
800
Conc. [umol/L]
Comp. 7
Comp. 7 + disopyramide
Comp. 7 + lopinavir
Comp. 7 + pyrimethamine
Comp. 7 + cimetidine

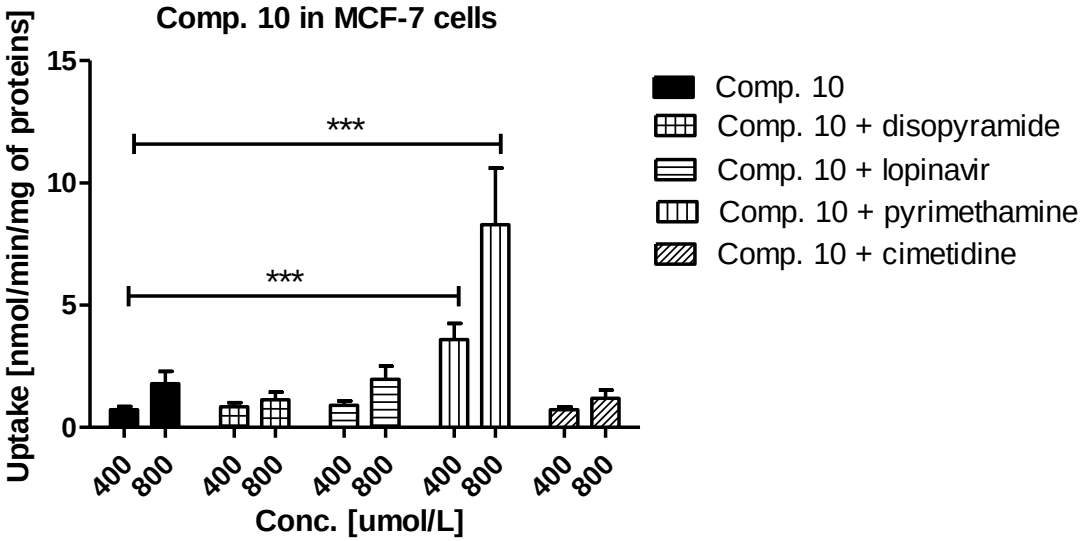

Comp. 7 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
0.5
0.4
0.3
0.2
0.1
0.0
**
**
*
***
400
800
Conc. [umol/L]
Comp. 7
Comp. 7 + disopyramide
Comp. 7 + lopinavir
Comp. 7 + pyrimethamine
Comp. 7 + cimetidine


Comp. 8 in MCF-7 cells
Uptake [nmol/min/mg of proteins]
Conc. [umol/L]
Comp. 8
Comp. 8 + disopyramide
Comp. 8 + lopinavir
Comp.8 + pyrimethamine
Comp.8 + cimetidine


Comp. 8 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
Conc. [umol/L]
Comp. 8
Comp. 8 + disopyramide
Comp. 8 + lopinavir
Comp.8 + pyrimethamine
Comp.8 + cimetidine


Comp. 9 in MCF-7 cells
Uptake [nmol/min/mg of proteins]
1.0
0.8
0.6
0.4
0.2
0.0
400
800
Conc. [umol/L]
Comp. 9
Comp. 9 + disopyramide
Comp. 9 + lopinavir
Comp. 9 + pyrimethamine
Comp. 9 + cimetidine


Comp. 9 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
5
4
3
2
1
0
*
*
*
400
800
Conc. [umol/L]
Comp. 9
Comp. 9 + disopyramide
Comp. 9 + lopinavir
Comp. 9 + pyrimethamine
Comp. 9 + cimetidine


Comp. 10 in MCF-7 cells
Uptake [nmol/min/mg of proteins]
15
10
5
0
***
***
400
800
Conc. [umol/L]
Comp. 10
Comp. 10 + disopyramide
Comp. 10 + lopinavir
Comp. 10 + pyrimethamine
Comp. 10 + cimetidine

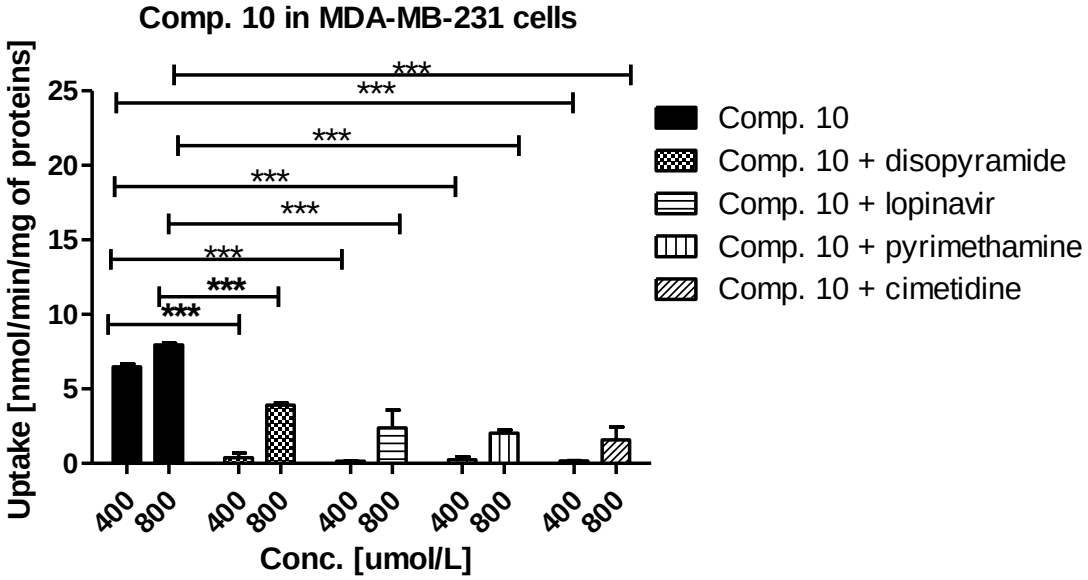
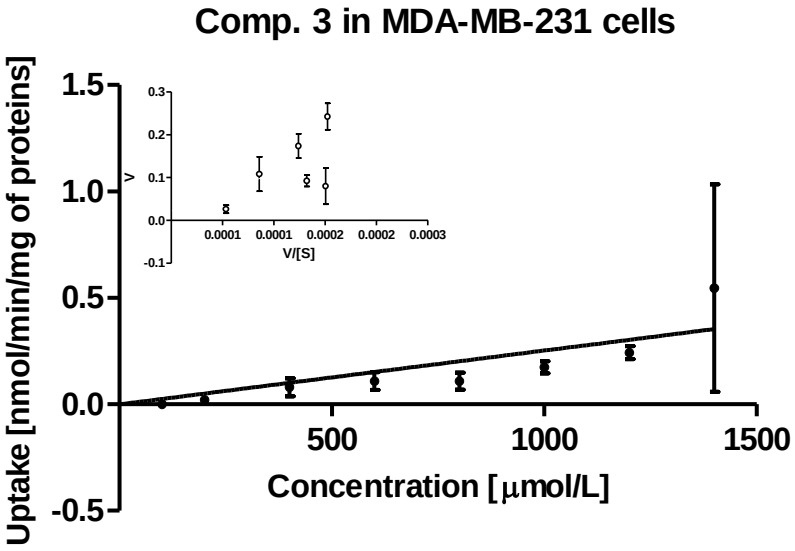
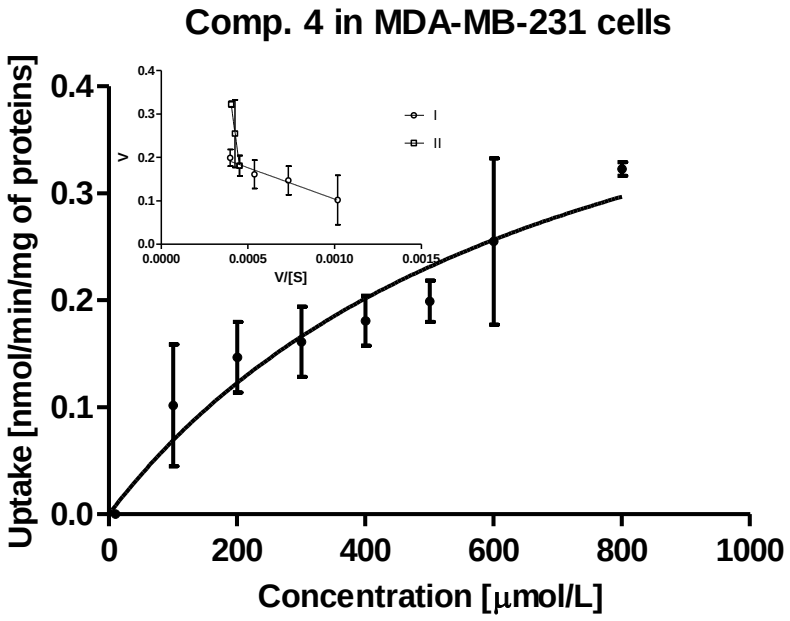
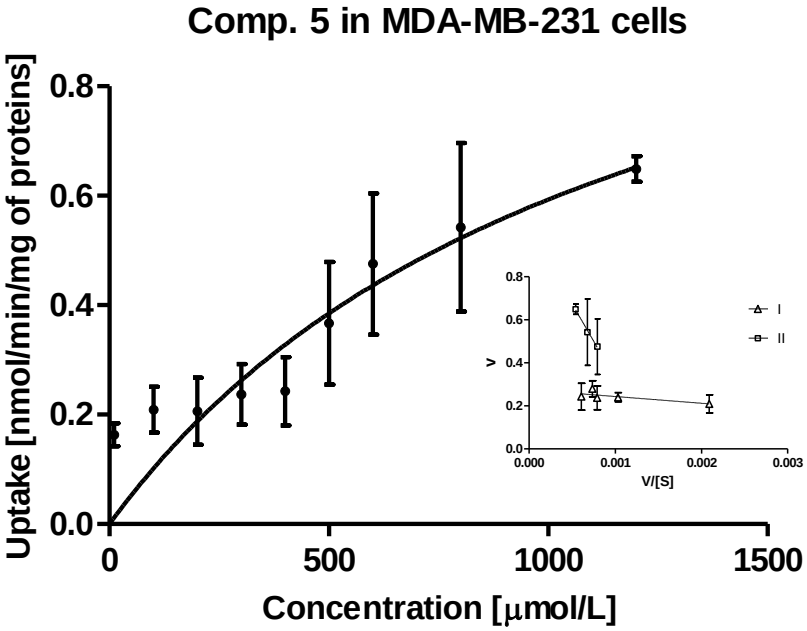
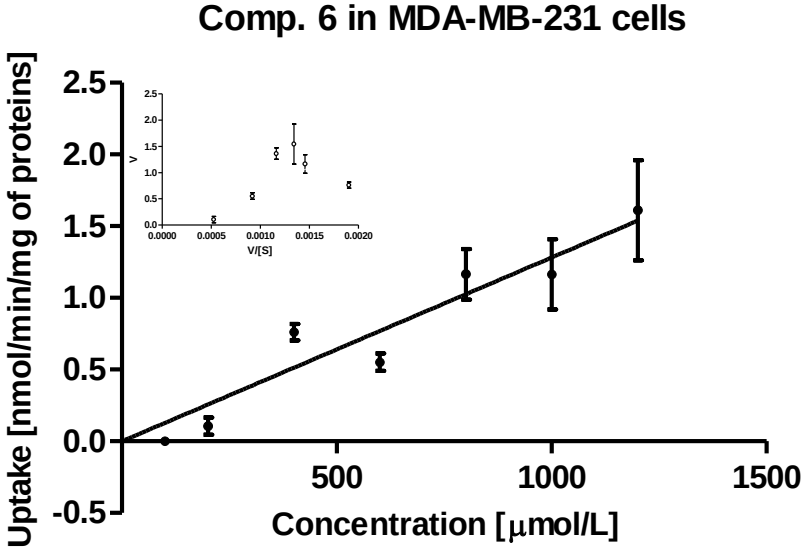

Comp. 10 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
25
20
15
10
5
0
***
***
***
***
***
***
***
400
800
Conc. [umol/L]
Comp. 10
Comp. 10 + disopyramide
Comp. 10 + lopinavir
Comp. 10 + pyrimethamine
Comp. 10 + cimetidine

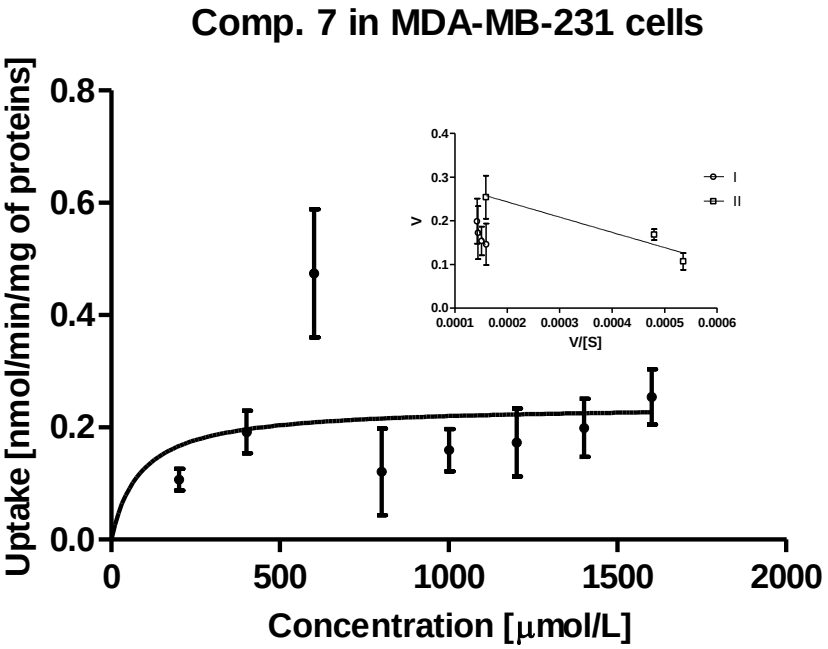
Figure S3. The uptake of compounds **3** - **10** into MDA-MB-231 cells at the concentrations of 10 – 2000 μmol/L and Eadie–Hofstee plots for OCTs mediated transport.

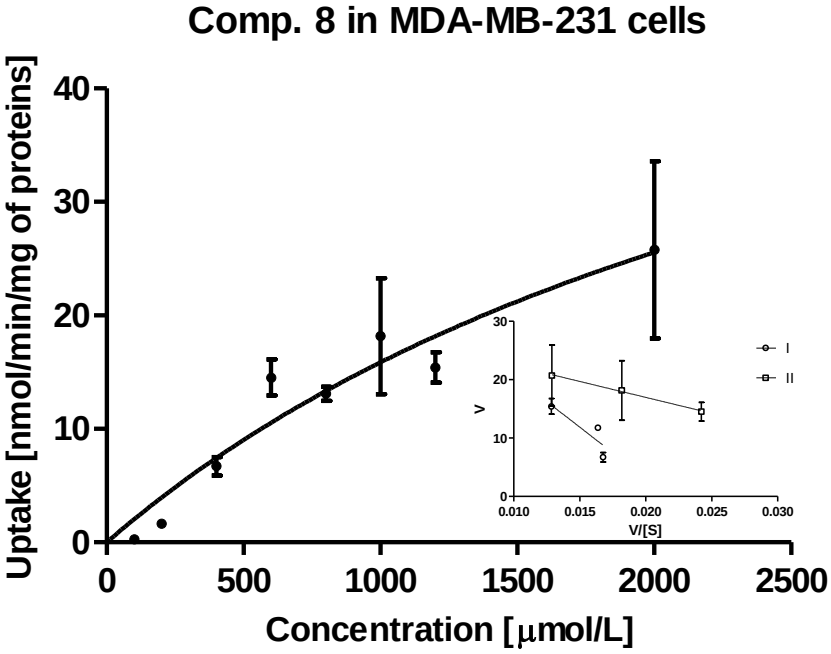


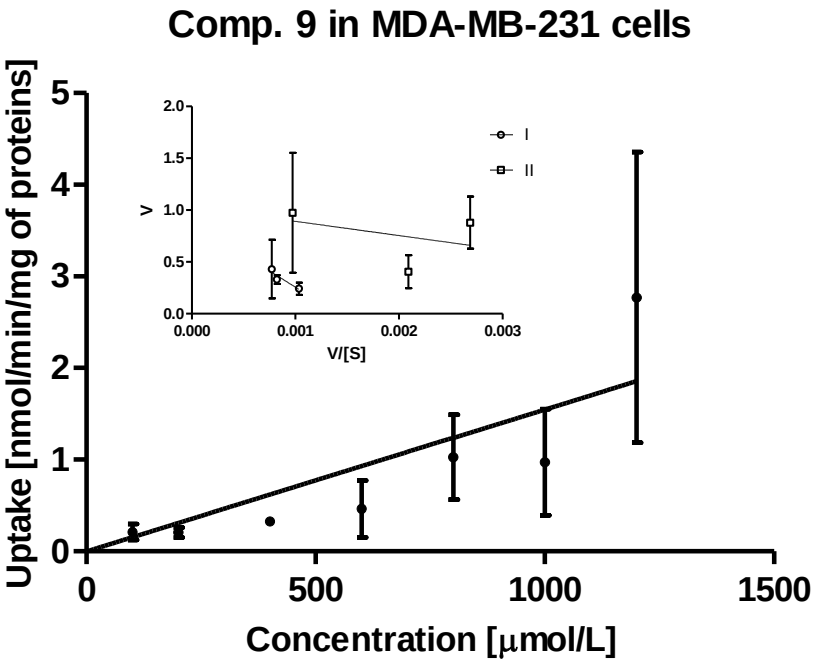



Comp. 5 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
0.0
0.2
0.4
0.6
0.8
0
500
1000
1500
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 6 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
500
1000
1500
Concentration [μmol/L]
V
V/[S]


Comp. 7 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
0.0
0.2
0.4
0.6
0.8
0
500
1000
1500
2000
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 8 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
0
10
20
30
40
0
500
1000
1500
2000
2500
Concentration [μmol/L]
V
V/[S]
I
II


Comp. 9 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
Concentration [μmol/L]
0
500
1000
1500
0
1
2
3
4
5
V
V/[S]
I
II
0.000
0.001
0.002
0.003
0.0
0.5
1.0
1.5
2.0

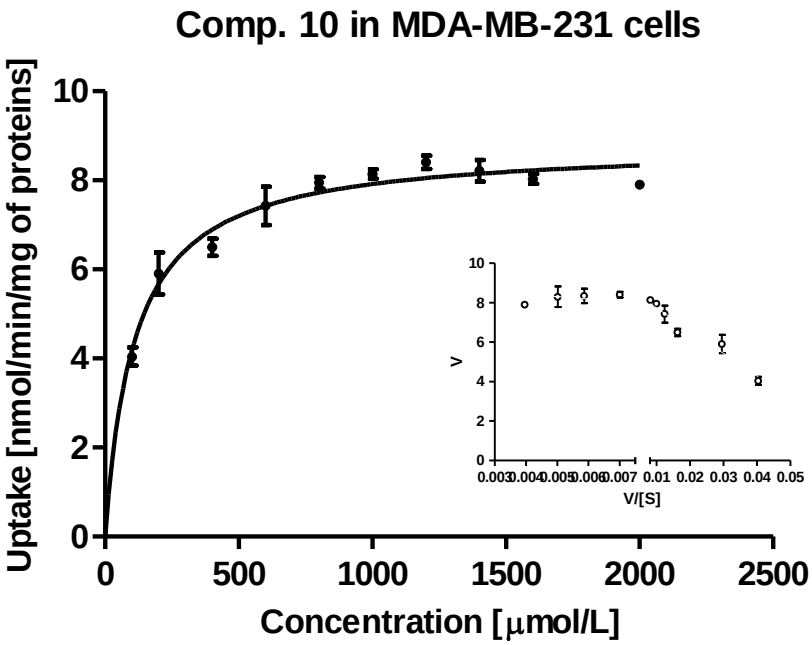

Comp. 10 in MDA-MB-231 cells
Uptake [nmol/min/mg of proteins]
Concentration [μmol/L]
0
500
1000
1500
2000
2500
0
2
4
6
8
10
V
V/[S]
0.003
0.004
0.005
0.006
0.007
0.01
0.02
0.03
0.04
0.05

Table S2. The summary of interactions of metformin derivatives 1 – 10 with OCT, PMAT and MATE1 transporters in MCF-7 and MDA-MB-231 cells.

COMPOUND	MCF-7	MDA-MB-231
1	OCT3 (non-sign.)	OCT3, PMAT
2	PMAT, MATE1	OCT1, PMAT
3	PMAT	PMAT, MATE1
4	OCT3, PMAT	OCT3, PMAT, MATE1
5	OCT3, PMAT, MATE1	MATE1
6	OCT1, OCT3, PMAT, MATE1	OCT1, MATE1
7	OCT1 (non-sign.)	OCT1, PMAT, MATE1
8	OCT1, OCT3, PMAT, MATE1	OCT1, MATE1
9	OCT3 (non-sign.)	OCT1, OCT3, PMAT
10	MATE1	OCT1, OCT3, PMAT, MATE1

Table S3. Effects of selected biguanides on the production of intracellular reactive oxygen species in MCF-7 and MDA-MB-231 cells.

Compound [μmol/L]	Median fluorescence [RFU]
Control MCF-7	**204 422.3 ± 17 741.1**
AAPH [100 μmol/L]	328 183.3 ± 95 164.4*
Metformin [500 μmol/L]	226 427.2 ± 60 332.4
Comp. **2** [44 μmol/L]	40 907.4 ± 10 098.9**
Comp. **3** [72.5 μmol/L]	391 177.4 ± 42 746.8*
Comp. **4** [678.5 μmol/L]	179 072.7 ± 22 957.6
Comp. **8** [7.8 μmol/L]	418 376.2 ± 272 653.5
Comp. **10** [24.2 μmol/L]	377 063.6 ± 18 351.2**
Control MDA-MB-231	**184 715.4 ± 23 171.9**
AAPH [100 μmol/L]	344 061.3 ± 65 766.2*
Metformin [500 μmol/L]	305 732.8 ± 36 879.5*
Comp. 4 [678.5 μmol/L]	198 801.63 ± 25 103.0
Comp. 8 [452 μmol/L]	252 568.2 ± 22 955.4
Comp. 10 [496 μmol/L]	171 794.2 ± 23 831.0

The experiments were performed using 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), a fluorescent indicator for ROS in cells. The fluorescence of stained cells was measured by flow cytometry. 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was used as positive control. Results are presented as median intensity of fluorescence of 10000 events (n = 3). Statistically significant differences between the samples and respective controls are depicted by asterisks ($p < 0.05$; ** $p < 0.01$).

Table S4. The effects of metformin and its sulfonamide derivatives on MCF-7 cell migration.

Compound	Conc. [μmol/L]	T_0 [μm]	T_4 [μm]	T_{24} [μm]	T_{48} [μm]
Control	-	77.05 ± 13.80	70.79 ± 10.80	56.84 ± 5.13	38.70 ± 8.11
Metformin	500	76.90 ± 4.48	78.67 ± 3.97	72.73 ± 3.89*	60.97 ± 3.07***
	1000	77.77 ± 5.53	76.56 ± 3.56	73.11 ± 2.28*	63.84 ± 7.12***
Comp. 2	22	83.74 ± 15.63	82.52 ± 10.61*	83.29 ± 10.98***	76.68 ± 15.27***
	44	83.84 ± 11.67	82.46 ± 10.98*	81.88 ± 10.47***	73.80 ± 12.07***
Comp. 3	36.2	83.44 ± 9.38	80.47 ± 7.09	71.36 ± 9.06**	64.78 ± 15.91***
	72.5	72.65 ± 4.38	71.03 ± 5.54	68.74 ± 9.65*	60.94 ± 16.82***
Comp. 4	339.25	83.99 ± 12.23	82.77 ± 9.49*	66.12 ± 8.57	73.00 ± 12.01***
	678.5	76.76 ± 14.23	75.99 ± 13.63	76.62 ± 12.27***	71.00 ± 14.41***
Comp. 8	3.9	79.24 ± 13.03	78.09 ± 12.85	77.35 ± 15.31***	70.08 ± 15.92***
	7.8	80.79 ± 12.02	78.52 ± 11.69	70.98 ± 16.39**	65.23 ± 16.19***
Comp. 10	12.1	86.52 ± 9.93	80.54 ± 10.81	73.10 ± 17.41**	69.66 ± 16.38***
	24.2	73.21 ± 10.13	75.42 ± 13.37	73.69 ± 18.32***	67.71 ± 17.69***

Results are presented as mean ± SD of the wound width, n = 8-12 at various time points (T_0, 4 hours (T_4), 24 hours (T_{24}), and 48 hours (T_{48})). Asterisks represent statistically significant changes versus control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

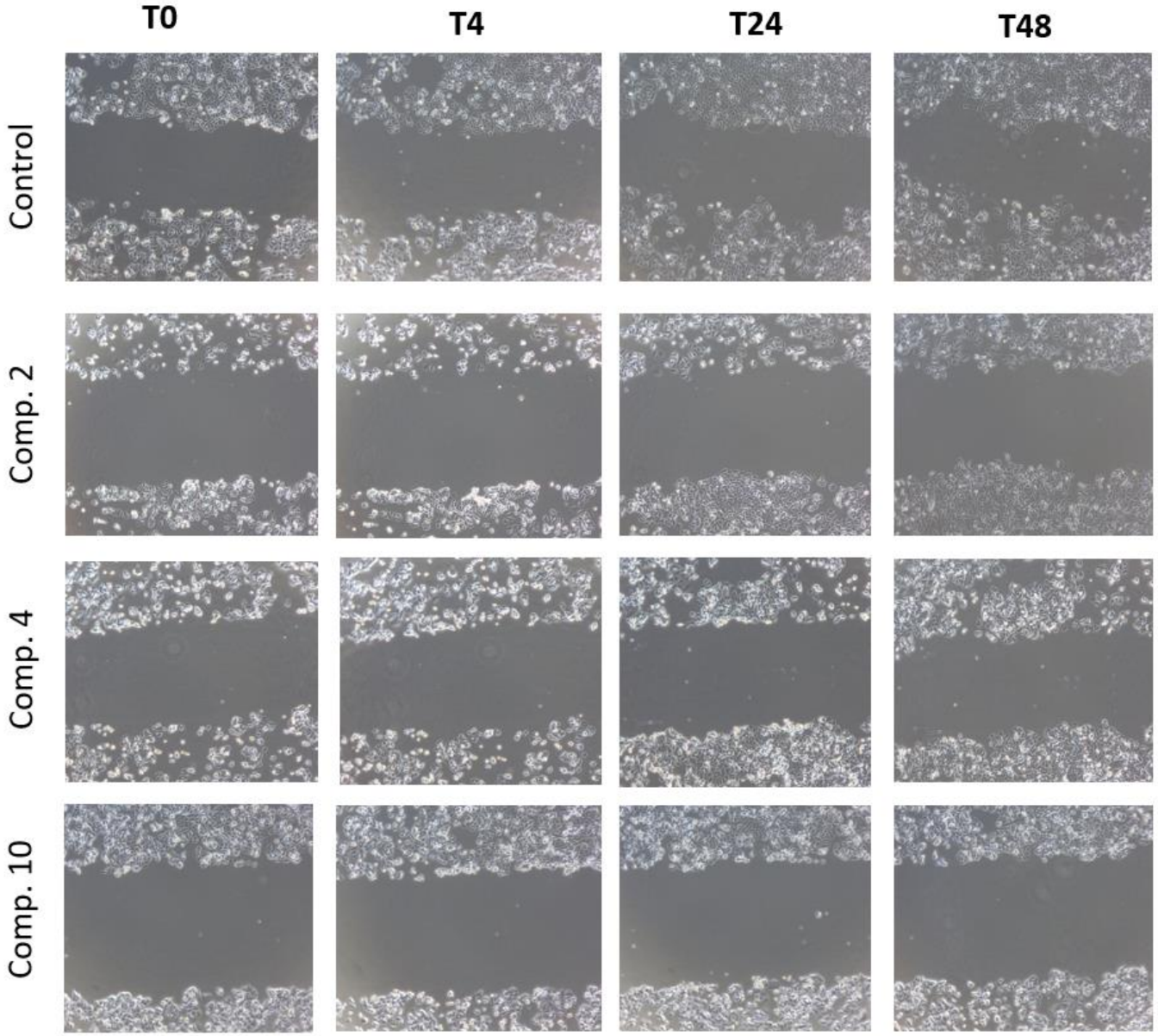
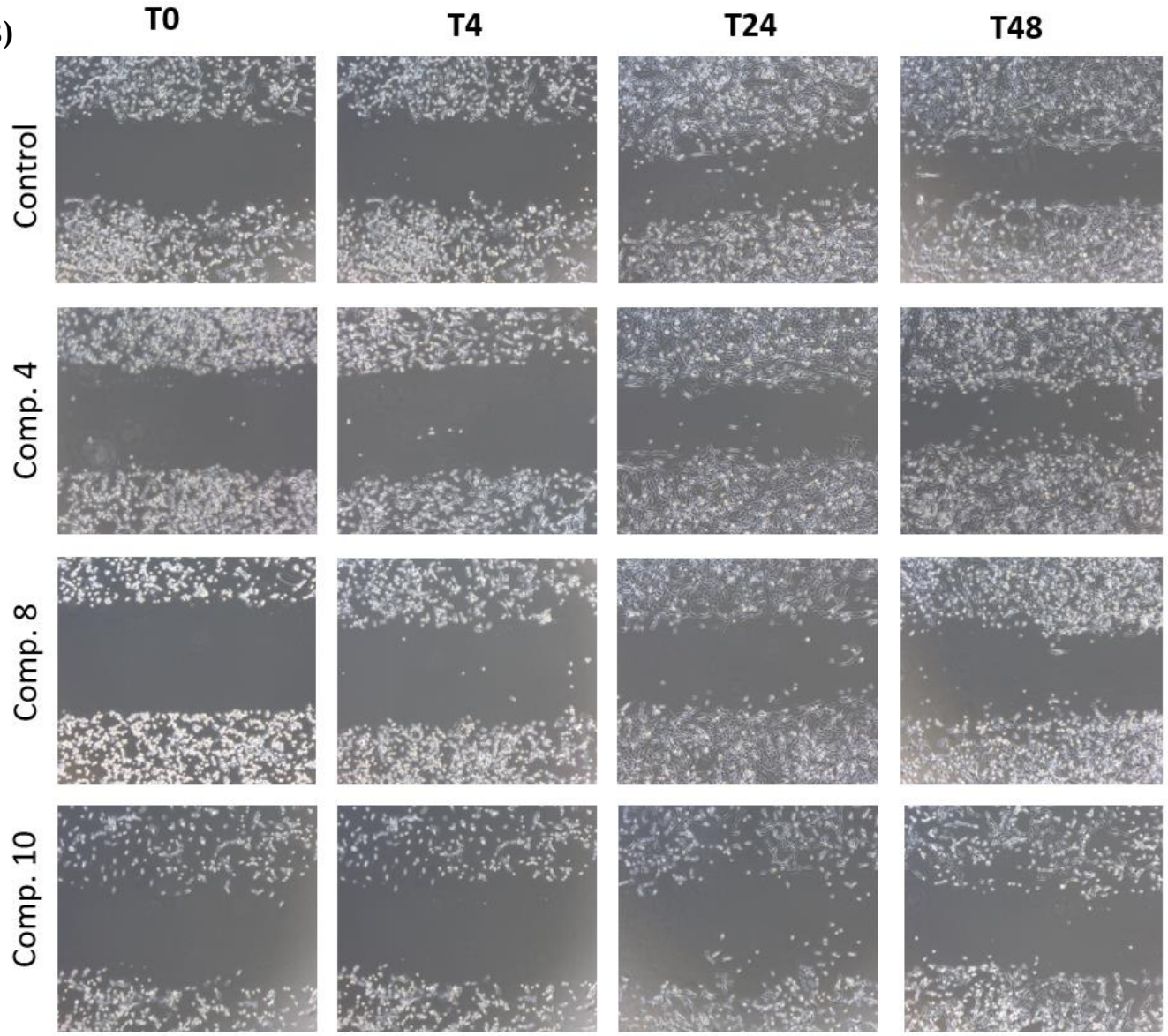
Table S5. The effects of metformin and its sulfonamide derivatives on MDA-MB-231 cell migration.

Compound	Conc. [μmol/L]	T_0 [μm]	T_4 [μm]	T_{24} [μm]	T_{48} [μm]
Control	-	84.73 ± 11.95	75.38 ± 11.82	55.42 ± 10.66	35.01 ± 9.28
Metformin	500	83.26 ± 3.90	78.51 ± 5.50	72.44 ± 5.35***	62.87 ± 4.62***
	1000	80.97 ± 4.53	76.91 ± 2.95	71.10 ± 2.43***	64.74 ± 5.04***
Comp. 4	327	85.89 ± 5.09	80.13 ± 4.79	49.57 ± 8.52	36.09 ± 9.91
	654	80.50 ± 7.82	74.86 ± 9.03	50.14 ± 11.35	41.25 ± 14.28
Comp. 8	226	87.88 ± 9.50	82.53 ± 13.39	59.55 ± 7.19	52.73 ± 11.76***
	452	80.20 ± 15.48	77.14 ± 11.12	65.09 ± 9.72**	50.01 ± 13.74***
Comp. 10	248	91.03 ± 11.53	89.98 ± 9.28**	69.47 ± 7.39***	58.85 ± 13.74***
	496	83.63 ± 12.41	87.55 ± 13.48**	65.35 ± 10.54**	56.99 ± 15.74***

Results are presented as mean ± SD of the wound width, n = 8-12 at various time points (T_0, 4 hours (T_4), 24 hours (T_{24}), and 48 hours (T_{48})). Asterisk represent statistically significant changes versus control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S4. Inhibition of cell migration in the presence of selected biguanides. MCF-7 (A) and MDA-MB-231 (B) cells migration was evaluated using wound healing assay. Representative cell images are shown for control samples and biguanides at the concentrations corresponding to 1/2xIC_{50} value. Cells were photographed using an inverted microscope at the indicated times (t = 0, 4, 24 and 48 h); 100 × magnification.

A)



B)