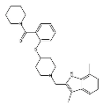
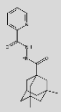
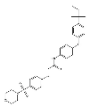
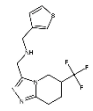
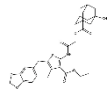
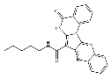
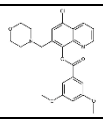
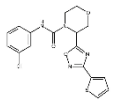
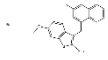
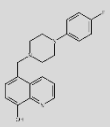
Supplements for the Manuscript:

Novel High-Throughput Screen Identified S100A4 Inhibitors for anti-Metastatic Therapy

Table S1: S100A4 expression levels after treatment with the HTS selected compounds after first screening with low concentration range (1 µM, 10 µM, 30 µM). S100A4 mRNA expression normalized to the DMSO control is represented in [%] after treatment with 10 µM of each compound for 24 h and 48 h in HCT116 cells and 48 h in SW620 cells. Selected compounds E2, E10 E12 are marked in light grey with highlighting their strongest reduction of S100A4 mRNA levels for the respective concentration in dark grey.

Compound	S100A4 mRNA Expression Normalized to DMSO control [%]		
	HCT116 24 h	HCT116 48 h	SW620 24 h
E1 	89	116	107
E2 	85	**37**	167
E3 	105	56	115
E4 	122	136	75
E5 	102	90	82
E6 	78	50	126
E7 	81	94	65
E8 	118	202	214
E9 	208	95	99
E10 	49	**32**	91

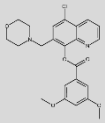
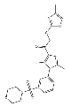
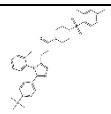
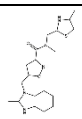
E11 	98	103	98
E12 	4	**18**	41
E13 	97	48	109
E14 	89	73	100
E15 	81	49	75

Table S2 and S3: S100A4 expression levels after treatment with the HTS selected compounds after first screening with low concentration range (Table S2: 1 µM, Table S3: 30 µM). S100A4 mRNA expression normalized to the DMSO control is represented in [%] after treatment with 1 or 30 µM of each compound for 24 h and 48 h in HCT116 cells and 48 h in SW620 cells. Selected compounds E2, E10 and E12 are marked in light grey with highlighting their strongest reduction of S100A4 mRNA levels for the respective treatment concentration in dark grey.

Table S2: 1 µM treatment with HTS identified compounds.

Compound	S100A4 mRNA Expression Normalized to DMSO control [%]		
	HCT116 24 h	HCT116 48 h	SW620 24 h
E1	80	92	83
E2	86	**91**	94
E3	116	106	110
E4	89	110	77
E5	92	98	85
E6	89	105	81
E7	91	93	88
E8	104	110	117
E9	271	129	90
E10	92	**61**	77
E11	91	82	98
E12	103	**81**	102
E13	86	75	101
E14	95	89	97
E15	86	53	92

Table S3: 30 µM treatment with HTS identified compounds.

Compound	S100A4 mRNA Expression Normalized to DMSO control [%]		
	HCT116 24 h	**HCT116 48 h**	**SW620 24 h**
E1	83	95	60
E2	55	**22**	113
E3	67	68	107
E4	93	87	80
E5	81	50	48
E6	63	43	117
E7	47	9	104
E8	208	128	207
E9	149	34	67
E10	40	**17**	99
E11	93	110	104
E12	2	**0**	34
E13	104	46	95
E14	65	67	88
E15	67	43	121

Table S4: Overview of HTS identified clusters. The S100A4 expression in the reporter construct of the HTS is shown in % and each compound was tested with 10 µM for 72 h. For compounds not included in the HTS but found through the clustering process an ND for not determined is shown.

Cluster	Compound Name	Supplier	Supplier ID	MW [Da]	S100A4 expression inhibition in HTS [%]
E1	E1	ChemBridge	21374804	445.596	95.1
	E1.2		26693136	393.522	94.08
	E1.3		38184601	412.952	75.81
E2	E2	Enamine	Z27728028	327.421	97.92
	E2.2		Z27728746	299.368	96.46
	E2.3		Z286454988	313.394	86.39
E3	E3	Enamine	T0503-7182	538.655	81.65
	E3.2		T5337738	444.544	72.21
E4	E4	Enamine	Z1268662935	316.345	88.65
	E4.2		Z1268662690	324.344	86.37
E5	E5	Enamine	T5243227	555.639	70.81
	E5.2		T5271961	430.492	70.17
E6	E6	Enamine	Z1033301156	399.445	82.26
	E6.2		Z1089877546	385.419	74.18
E7	E7	Enamine	Z56836338	442.892	97.94
	E7.2		Z56174876	400.831	94
	E7.3		Z56836325	417.285	96.96
E8	E8	Enamine	Z2034819477	390.844	88.56
	E8.2		Z2034819411	381.408	82.12
	E8.3		Z2234920098	408.834	64.17
E9	E9	Enamine	Z56777163	336.431	88.56
	E9.2		Z56777161	350.457	81.89
E10	E10	Enamine	Z44113219	337.391	96.01
	E10.2		Z44283769	333.427	91.15
E11	E11	Enamine	Z1147801836	353.504	98.93
	E11.2		Z1101327871	339.478	98.14
E12	E12	Enamine	T0505-9066	455.525	82.4
	E12.2 (A1)		T0506-7625	467.561	35.2
	E12.3		T0505-7380	522.017	14.9
	E12.4		T0507-7068	512.515	-3.63
	E12.5 (A2)		T0510-2106	497.562	69.91
	E12.6 (A3)		T5401236	401.481	2.34
E13	E13	Enamine	T5320044	491.647	52
E14	E14	Enamine	T5454969	617.824	27
E15	E15	Chembridge	45775314	381.451	51
	E15.2		56093530	379.412	ND
	E15.3		12793122	326.35	19.26
	E15.4		92172055	370.469	-21.3
	E15.5		90937896	379.436	ND
	E15.6		89711317	336.388	28.2
	E15.7		64458800	361.397	23.85
	E15.8		12264395	364.401	6.31
	E15.9		77568677	338.285	29.37

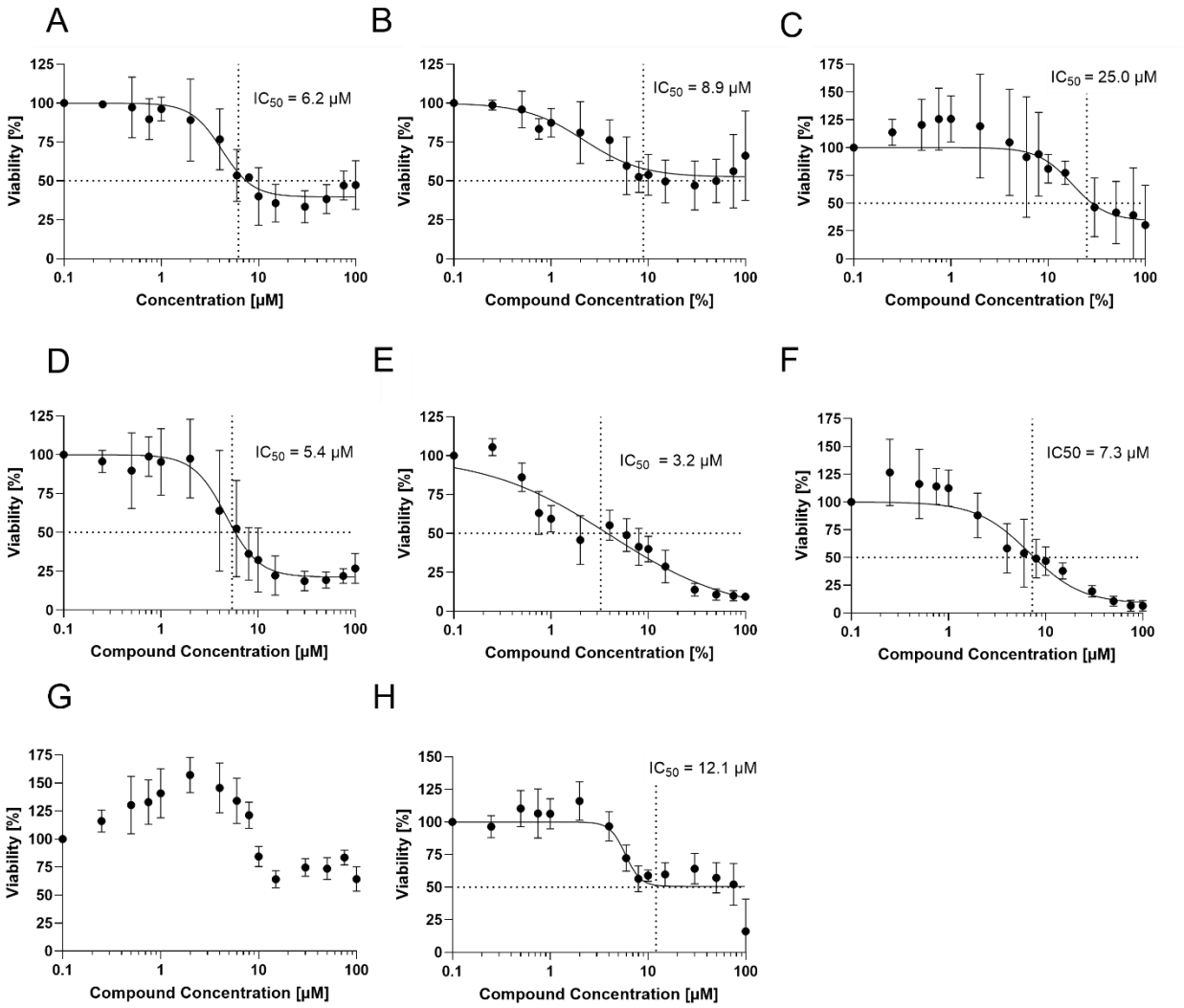


Figure S1. To evaluate the effect of the compounds on the cell's viability, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was conducted, investigating cellular metabolic activity under compound treatment of E2, E10 and E12 for 24 h (**A-C**) and 48 h (**D-F**) in HCT116 cells and for E12 in SW620 for 24 h (**G**) and 48 h (**H**). For the calculation of the Half Maximal Inhibitory Concentration (IC_{50}), a sigmoidal dose-response – Inhibition curve fit was performed, with a baseline defining 0% viable cells determined via using a 10% DMSO positive control. Analysis was conducted with GraphPad Prism version 10.4.1.

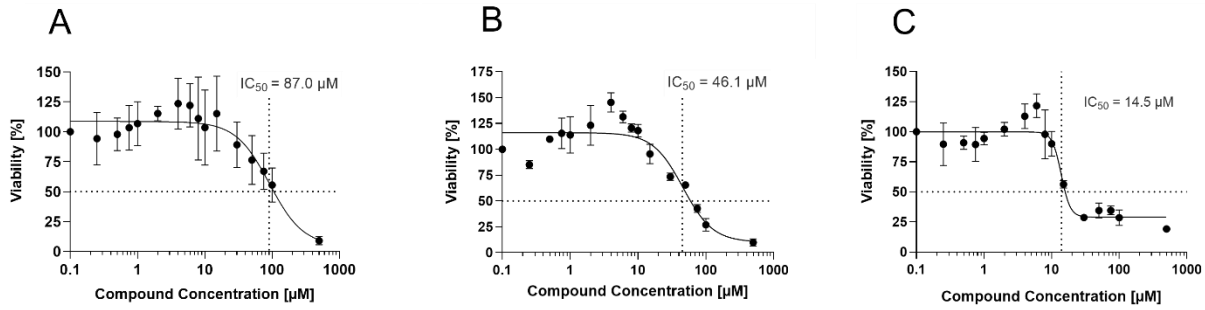


Figure S2. To evaluate the effect of the compounds on the cell's viability, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was conducted, investigating cellular metabolic activity under compound treatment of A1, A2 and A3 (**A-C**) for 24 h in HCT116 cells. For the calculation of the Half Maximal Inhibitory Concentration (IC_{50}), a sigmoidal dose-response – Inhibition curve fit was performed, with a baseline defining 0% viable cells determined via using a 10% DMSO positive control. Analysis was conducted with GraphPad Prism version 10.4.1.