



Supplementary Materials: Novel Benzenesulfonate Scaffolds with a High Anticancer Activity and G2/M Cell Cycle Arrest

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1. Chemistry Data

¹H and ¹³C NMR spectra of target compounds:



Figure S2. ¹³C NMR plot of BS1.











Figure S8. ¹³C NMR plot of BS4.







Figure S12. ¹³C NMR plot of BS6.

HR-ESI spectra of target compounds:











Figure S16. HR-ESI spectrum of BS4.





Figure S18. HR-ESI spectrum of BS6.

2. Biological Studies

Cell Line Comp.	K562	HCT 116 p53+/+	HCT 116 p53 -/-	MCF-7	A549	U-251	PANC-1
BS1	72.91	14.25	22.27	1.51	1.00	6.61	3.15
BS2	63.70	13.06	11.94	0.93	1.00	6.80	5.39
BS3	120.71	25.94	39.39	2.05	1.23	5.36	97.06
BS4	144.51	15.95	6.71	2.74	2.20	13.11	106.38
BS5	2.45	1.00	1.00	1.00	1.00	1.00	1.00
BS6	9.26	1.00	1.15	2.55	1.00	1.00	1.00
CP-31398	3.97	0.66	0.47	0.45	0.49	0.65	0.49
Imatinib	187.97	0.56	0.49	1.00	1.00	1.00	1.00

Table S1. Selectivity index of tested derivatives.



Table S2. Sequences of primer pairs used in determining the mRNA expression of tested genes.

Gene	GenBank Accesion no.	Forward Primer (5'→3')	Reverse Primer (3'→5')
IDH1	NM_001282387	TCCGTCACTTGGTGTGTAGG	GGCTTGTGAGTGGATGGGTA
GADD45a	NM_001924.3	AGTCAGCGCACGATCACTGT	GGATCAGGGTGAAGTGGATCT
calreticulin	NM_004343.3	CTGCCGTCTACTTCAAGGA	GAACTTGCCGGAACTGAGAAC
<i>p</i> 62	NM_003900.5	AGGACGGGGACTTGGTTG	GGCGGGAGATGTGGGTAC
LC3	NM_022818.5	CAGCATCCAACCAAAATCCC	CACTGACAATTTCATCCCGAAC
GAPDH	NM_002046	GAGTCAACGGATTTGGTCGTA	GCCCCACTTGATTTTGGAG



Figure S19. Effect of the treatment with the paclitaxel at various concentrations on regulating the cell cycle in U-251 cells. The representative histograms with the distribution of the cells in the respective phases of their cycles for one of several independent experiments (**A**). Data chart with the statistical analysis using a one-way ANOVA with Bonferroni's posthoc test: * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.001 compared to the untreated cells (control) (**B**).

NHDF cells



Figure S20. Effect of the treatment with the selected compounds (**BS1**, **BS4**) at various concentrations on regulating the cell cycle in NHDF cells. The representative histograms with the distribution of the cells in the respective phases of their cycles for one of several independent experiments (**A**). Data chart with the statistical analysis using a one-way ANOVA with Bonferroni's post-hoc test: *** p < 0.001, **** p < 0.001 compared to the untreated cells (control) (**B**).

NHDF cells



Figure S21. Assessment of the effect on the induction of apoptosis in the NHDF cells after a 48-hour incubation with the tested compounds (**BS1**, **BS4**) at various concentrations. The representative histograms from one of several independent experiments include the percentage of live and apoptotic cells (**A**). Data chart with the statistical analysis using a one-way ANOVA with Bonferroni's post-hoc test: * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.001 compared to the untreated cells (control) (**B**).

The uncropped Western blots and densitometric readings/intensity ratio data:

Images of the gels prepared during this study. All gels uncropped and unmodified. Proteins relevant to this study are marked in boxes along with reference proteins. Colors used to distinguish appropriate protein pairs.



Α.







F.





D.



The band intensity of tested proteins (p53, cyclin E1, cdc2, p21, HIF-1 α , cathepsin b, PARP, caspase-9, BID, AIF) were analyzed by densitometry readings/intensity ratio, using ImageJ software (NIH) and were normalized to the corresponding reference proteins (GAPDH, vinculin or β -actin) value.

The densitometric readings/intensity ratio data from four/five independent experiments (compared to control):

	p53/ Refer- ence	p21/ Refer- ence	cdc2/ Refer- ence	Cyclin E1/ Reference	HIF-1α/ Reference	Cathepsin b/Reference		rence
	53 kDa	21 kDa	34 kDa	48 kDa	120 kDa	44 kDa	27 kDa	22 kDa
Control	1.000	1.000	1.000	1.000	1.000	1.000	2.837	2.748
BS1 4µM	1.357	1.932	1.986	0.576	0.550	2.467	2.726	2.940
BS2 6µM	1.448	2.783	2.282	0.392	0.661	2.641	3.172	3.717
BS2 4µM	1.698	2.205	2.272	0.465	0.587	1.805	2.625	2.893

	PARP/Reference		BID/ Reference	Caspase-9/Reference	
	116 kDa	89 kDa	22 kDa	47 kDa	37-35 kDa
Control	1.000	0.330	1.000	1.000	0.330
BS1 4µM	0.871	1.044	0.675	1.167	1.485
BS2 6µM	0.940	0.698	1.227	1.035	1.188
BS2 4µM	0.953	0.763	0.701	1.007	1.071

K562 cell line

	cdc2/ Reference	cyclin E1/ Reference	AIF/ Reference	BID/ Reference
	34 kDa	48 kDa	67 kDa	22 kDa
Control	1.000	1.000	1.000	1.000
BS1 0.5μM	1.122	1.110	2.737	0.695
BS1 0.35μM	1.234	1.273	3.809	1.049
BS2 0.5μM	1.206	1.270	4.160	0.762
BS2 0.35μM	1.338	1.640	4.141	0.965

	PARP/ R	eference	caspase-9	/ Reference
-	116 kDa	89 kDa	47 kDa	37-35 kDa
Control	1.000	0.167	1.000	0.167
BS1 0.5μM	1.646	2.156	1.627	1.368
BS1 0.35μM	1.793	2.111	1.109	1.287
BS2 0.5μM	1.665	0.811	1.104	1.113
BS2 0.35µM	1.556	0.494	1.108	1.268

Figure S22. The uncropped Western blots.