Data S1. Supplementary Materials and methods

Chemistry. Detailed physicochemical information of the synthesized benzenesulphonohydrazones 1-9 is presented below.

N-[(2,3-difluorophenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 1). Yield: 31%; m.p.: 218˚C. IR: 3157 (NH), 3012 (CH, arom.), 2842 (CH, aliph.), 1568 (C=N), 1317, 1185 (SO_2); Proton nuclear magnetic resonance (^{1}H NMR) (DMSO-*d*$_6$) δ (ppm) =2.34 (s, 3H, CH_3), 7.03-7.05 (m, 2H, ArH), 7.31-7.33 (m, 1H, ArH), 7.38-7.40 (d, 2H, ArH, *J*=6 Hz), 7.91-7.93 (d, 2H, ArH, *J*=6 Hz), 8.74 (s, 1H, =CH), 10.60 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.1 (CH_3), 118.9, 122.5, 126.1, 128.5, 129.2, 129.4, 138.1 ($9C_{ar}$), 140.2 (=CH), 141.7, 151.5, 152.5 ($3C_{ar}$). HR-MS: 310.0582 (M^+). Analysis for $C_{14}H_{12}F_2N_2O_2S$ (310.32), calculated: C: 54.19%, H: 3.90%, N: 9.03%; observed: C: 54.26%, H: 3.96%, N: 8.90%.

N-[(2-chloro-3-methoxyphenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 2). Yield: 44%; m.p.: 152˚C. IR: 3157 (NH), 3012 (CH, arom.), 2842 (CH, aliph.), 1568 (C=N), 1317, 1185 (SO_2); ^{1}H NMR (DMSO-*d*$_6$) δ (ppm) =2.36 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 7.13-7.19 (m, 1H, ArH), 7.31-7.35 (m, 2H, ArH), 7.40-7.43 (d, 2H, ArH, *J*=9 Hz), 7.75-7.78 (d, 2H, ArH, *J*=9 Hz), 8.27 (s, 1H, =CH), 11.75 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.1 (CH_3), 56.8 (CH_3), 113.4, 121.5, 124.1, 126.8, 128.5, 129.4, 135.1, 138.1 ($10C_{ar}$), 140.2 (=CH), 141.7, 157.5 ($2C_{ar}$). HR-MS: 338.0486 (M^+). Analysis for $C_{15}H_{15}ClN_2O_3S$ (338.81), calculated: C: 53.17%, H: 4.46%, N: 8.27%; observed: C: 53.22%, H: 4.52%, N: 8.20%.

N-[(2-bromo-3-hydroxy-4-methoxyphenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 3). Yield: 38%; m.p.: 144˚C. IR: 3203 (NH), 3015 (CH, arom.), 2970 (CH, aliph.), 1594(C=N), 1349, 1186 (SO_2), ^{1}H NMR (DMSO-*d*$_6$) δ (ppm) =2.34 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 6.70-6.72 (d, 1H, ArH, *J*=6 Hz), 7.03-7.05 (d, 1H, ArH, *J*=6 Hz), 7.37-7.40 (d, 2H, ArH, *J*=9 Hz), 7.84-7.87 (d, 2H, ArH, *J*=9 Hz), 8.63 (s, 1H, OH), 8.75 (s, 1H, =CH), 11.10 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.1 (CH_3), 56.8 (CH_3), 108.1, 115.0, 119.9, 126.2, 128.5, 129.4, 138.1 ($9C_{ar}$), 139.4 (=CH), 141.7, 145.0, 148.9 ($3C_{ar}$). Analysis for $C_{15}H_{15}BrN_2O_4S$ (399.26), calculated: C: 45.12%, H: 3.79%, N: 7.02%; observed: C: 45.20%, H: 3.71%, N: 7.08%.

N-[(4-bromo-2-fluorophenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 4). Yield: 43%; m.p.: 180˚C. IR: 3184 (NH), 3031 (CH, arom.), 2970 (CH, aliph.), 1600 (C=N), 1330, 1182 (SO_2); ^{1}H NMR (DMSO-*d*$_6$) δ (ppm) =3.01 (s, 3H, CH_3), 7.40-7.43 (d, 2H, ArH, *J*=9 Hz), 7.46-7.47 (d, 1H, ArH, *J*=3 Hz), 7.60-7.66 (m, 2H, ArH), 7.74-7.77 (d, 2H, ArH, *J*=9 Hz), 8.01 (s, 1H, =CH), 11.75 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.5 (CH_3), 119.8, 120.1, 121.1, 124.0, 127.6, 128.3, 130.3, 136.4, 139.2, 143.9 ($10C_{ar}$), 144.1 (=CH), 158.8, 162.2 ($2C_{ar}$). Analysis for $C_{14}H_{12}BrFN_2O_2S$ (371.22), calculated: C: 45.30%, H: 3.26%, N: 7.55%; observed: C: 45.39%, H: 3.22%, N: 7.63%.

N-[(2,3-dimethoxyphenyl)methylidene]-4-methylbenzenesulfonohydrazide (compound 5). Yield: 37%; m.p.: 216˚C. IR: 3203 (NH), 3065 (CH, arom.), 2929 (CH, aliph.), 1596 (C=N), 1329, 1185 (SO_2); ^{1}H NMR (DMSO-*d*$_6$) δ (ppm) =2.40 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 7.06-7.08 (m, 1H, ArH), 7.18-7.21 (m, 1H, ArH), 7.38-7.41 (d, 2H, ArH, *J*=9 Hz), 7.63-7.66 (d, 2H, ArH, *J*=9 Hz), 7.74-7.76 (d, 1H, ArH, *J*=6Hz), 9.59 (s, 1H, =CH), 11.45 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.1 (CH_3), 56.8 (CH_3), 60.6 (CH_3), 114.4, 122.0, 127.5, 128.3, 128.5, 129.4, 129.9, 135.9, 138.1 ($9C_{ar}$), 141.6 (=CH), 141.7, 149.7, 149.9 ($3C_{ar}$). Analysis for $C_{16}H_{18}N_2O_4S$ (334.39), calculated: C: 57.47%, H: 5.43%, N: 8.38%; observed: C: 57.53%, H: 5.39%, N: 8.42%.

N-[(3-chloro-4-methoxyphenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 6). Yield: 55%; m.p.: 158˚C. IR: 3197 (NH), 3002 (CH, arom.), 2970, 2839 (CH, aliph.), 1599 (C=N), 1321, 1185 (SO_2); ^{1}H

NMR (DMSO-*d6*) δ (ppm) =2.36 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 7.15-7.18 (d, 1H, ArH, *J*=9 Hz), 7.40-7.42 (d, 2H, ArH, *J*=6 Hz), 7.49-7.52 (d, 1H, ArH, *J*=9 Hz), 7.60-7.66 (m, 1H, ArH), 7.74-7.77 (d, 2H, ArH, *J*=9 Hz), 7.84 (s, 1H, =CH), 11.42 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.5 (CH_3), 56.8 (CH_3), 113.4, 121.9, 127.7, 128.0, 128.3, 129.9, 130.1, 135.9, 136.6, 143.9 (11C_{ar}), 146.0 (=CH), 156.2 (C_{ar}). Analysis for $C_{15}H_{15}ClN_2O_3S$ (338.81), calculated: C: 53.17%, H: 4.46%, N: 8.27%; observed: C: 53.22%, H: 4.50%, N: 8.31%.

N-[(3-bromo-4-methoxyphenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 7). Yield: 39%; m.p.: 152˚C. IR: 3204 (NH), 3018 (CH, arom.), 2967, 2928 (CH, aliph.), 1596 (C=N), 1317, 1187 (SO_2); ^{1}H NMR (DMSO-*d6*) δ (ppm) =2.36 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 7.12-7.15 (d, 2H, ArH, *J*=9 Hz), 7.38-7.42 (m, 1H, ArH), 7.53-7.56 (d, 1H, ArH, *J*=9 Hz), 7.64-7.66 (d, 2H, ArH, *J*=6 Hz), 7.74-7.77 (m, 1H, ArH), 7.84 (s, 1H, =CH), 11.42 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.5 (CH_3), 56.9 (CH_3), 111.5, 113.2, 127.7, 128.2, 128.3, 129.9, 130.8, 131.1, 135.9, 136.6, 143.9 (11C_{ar}), 145.9 (=CH), 157.1 (C_{ar}). Analysis for $C_{15}H_{15}BrN_2O_3S$ (383.26), calculated: C: 47.01%, H: 3.94%, N: 7.31%; observed: C: 47.11%, H: 3.98%, N: 7.26%.

N-[(3-bromo-4-hydroxyphenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 8). Yield: 42%; m.p.: 145˚C. IR: 3203 (NH), 3038 (CH, arom.), 2869 (CH, aliph.), 1595 (C=N), 1319, 1162 (SO_2); ^{1}H NMR (DMSO-*d6*) δ (ppm) =2.40 (s, 3H, CH_3), 6.73-6.75 (d, 1H, ArH, *J*=6 Hz), 7.35-7.37 (m, 1H, ArH), 7.38-7.40 (d, 2H, ArH, *J*=6 Hz), 7.63-7.66 (d, 2H, ArH, *J*=9 Hz), 7.91-7.92 (d, 1H, ArH, *J*=3 Hz), 8.69 (s, 1H, =CH), 9.24 (s, 1H, OH), 10.57 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =21.3 (CH_3), 109.9, 116.3, 127.2, 128.5, 129.4, 130.4, 138.1, 141.7 (11C_{ar}), 144.7 (=CH), 153.7 (C_{ar}). Analysis for $C_{14}H_{13}BrN_2O_3S$ (369.23), calculated: C: 45.54%, H: 3.55%, N: 7.59%; observed: C: 45.63%, H: 3.51%, N: 7.63%.

N-[(3-ethoxy-4-hydroxyphenyl)methylidene]-4-methylbenzenesulphonohydrazide (compound 9). Yield: 44%; m.p.: 218˚C. IR: 3204 (NH), 3065 (CH, arom.), 2970 (CH, aliph.), 1596 (C=N), 1330, 1185 (SO_2); ^{1}H NMR (DMSO-*d6*) δ (ppm) =1.39-1.41 (t, 3H, CH_3, *J*=3 Hz), 2.34 (s, 3H, CH_3), 4.02-4.08 (q, 2H, CH_2, *J*=6 Hz), 6.78-6.80 (d, 1H, ArH, *J*=6 Hz), 7.03-7.06 (m, 2H, ArH), 7.36-7.38 (d, 2H, ArH, *J*=6 Hz), 7.84-7.86 (d, 2H, ArH, *J*=6 Hz), 8.60 (s, 1H, =CH), 8.94 (s, 1H, OH), 11.00 (s, 1H, NH); ^{13}C NMR (DMSO) δ (ppm) =13.8 (CH_3), 21.1 (CH_3), 64.5 (CH_2), 113.5, 114.5, 122.1, 127.4, 128.5, 129.4, 138.1, 141.7 (10C_{ar}), 144.7 (=CH), 147.8, 150.3 (2C_{ar}). Analysis for $C_{16}H_{18}N_2O_4S$ (334.39), calculated: C: 57.47%, H: 5.43%, N: 8.38%; observed: C: 57.59%, H: 5.40%, N: 8.44%.

Lipophilicity. Experimental lipophilicity of synthesized benzenesulphonohydrazide derivatives 1-9 was determined using reversed-phase thin-layer chromatography (TLC). The examined compounds 1-9 and the reference substances with known lipophilicity (2-aminophenol, 8-hydroxyquinoline, napht-2-ol, diphenylamine and 3,4-benzopyrene) were dissolved in methanol to obtain the required concentrations (2.0 mg/ml) and the solutions (0.2 µl) were applied to 10x20 cm HPTLC plates coated with C18 silica F254 (Merck KGaA). The plates were developed to a distance of 9 cm from the origin in a horizontal teflon chamber with an eluent distributor (DS; Chromdes) at 23±1˚C. The mobile phases were prepared by mixing appropriate amounts of water and polar modifier (55-75% acetone, 50-80% acetonitrile, 50-75% 1,4-dioxane, 60-85% methanol). All solvents were analytical grade from POCh; Avantor Performance Materials Poland S.A.

The spots of the substances were located under UV illumination (254 nm) and retardation coefficients (R_F) were measured. R_F is defined as the distance travelled by the compound divided by the distance travelled by the mobile phase.

On the basis of R_F values for the tested compounds and for the reference substances, the R_M values were calculated using the following formula:

$$R_M = \log \frac{1 - R_F}{R_F}$$

The R_{M0} values (equivalent to the retention of a solute extrapolated to pure water as a mobile phase) were calculated using the equation: $R_M = R_{M0} - S \times \varphi$, where φ is the volume fraction of the organic modifier in the mobile phase (Tables SI and SII) (1,2).

Using Pearson's correlation test, the calculated R_{M0} values for the reference substances were correlated with the values of log P in the literature, and appropriate calibration curves were obtained with satisfactory linearity.

Acetone: log P_{EXP} = 1.0892 x R_{M0} - 0.1515; r^2=0.8822; P=0.002

Acetonitrile: log P_{EXP} = 2.2419 x R_{M0} - 2.6948; r^2=0.9500; P=0.005

1,4-Dioxane: log P_{EXP} = 1.3075 x R_{M0} - 0.8322; r^2=0.8597; P=0.003

Methanol: log P_{EXP} = 1.8499 x R_{M0} - 2.1728; r^2=0.7810; P=0.008

Experimental lipophilicity (log P_{EXP}) of the synthesized benzenesulphonohydrazide derivatives 1-9 was calculated using the calibration equations and R_{M0} values from the TLC method. Additionally, log P_{CAL} values of the compounds 1-9 were calculated using ALOGPS 2.1 software (3,4) and are presented in Table SIII.

References

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Table SI. Log P and R_{M0} values of reference substances (1).

A, Acetone-water					
Substance	Log P	R_{M0}	*S*	r^2	φ
2-aminophenol	0.62	1.65	-0.02	0.9768	67.11
8-hydroxyquinoline	2.02	1.41	-0.02	0.9233	69.02
napht-2-ol	2.7	2.09	-0.03	0.9787	72.71
diphenylamine	3.5	3.65	-0.05	0.9259	80.33
3,4-benzopyrene	6.04	5.56	-0.07	0.9681	85.49
B, Acetonitrile-water					
Substance	Log P	R_{M0}	*S*	r^2	φ
2-aminophenol	0.62	1.28	-0.02	0.9722	62.82
8-hydroxyquinoline	2.02	2.39	-0.03	0.9900	81.73
napht-2-ol	2.7	2.47	-0.04	0.9884	59.65
diphenylamine	3.5	2.79	-0.04	0.9969	74.75
3,4-benzopyrene	6.04	3.72	-0.04	0.9782	103.21
C, 1,4-Dioxane-water					
Substance	Log P	R_{M0}	*S*	r^2	φ
2-aminophenol	0.62	1.99	-0.03	0.9919	57.24
8-hydroxyquinoline	2.02	1.87	-0.03	0.9949	61.51
napht-2-ol	2.7	2.05	-0.03	0.9667	60.29
diphenylamine	3.5	3.46	-0.05	0.9902	70.90
3,4-benzopyrene	6.04	5.19	-0.07	0.9861	79.29
D, Methanol-water					
Substance	Log P	R_{M0}	*S*	r^2	φ
2-aminophenol	0.62	2.40	-0.03	0.9843	84.90
8-hydroxyquinoline	2.02	1.93	-0.03	0.9858	76.82
napht-2-ol	2.7	2.32	-0.03	0.9847	76.45
diphenylamine	3.5	2.87	-0.03	0.8599	86.43
3,4-benzopyrene	6.04	4.39	-0.04	0.8387	118.68

φ, amount of organic modifier in the mobile phase; R_{M0}, intercept of the plot; *S*, slope of the plot; *r*, correlation coefficient.

Table SII. R_{M0} values of the synthesized benzenesulphonohydrazones.

A, Acetone-water				
Compound no.	R_{M0}	S	r^2	φ
1	2.03	-0.03	0.9564	69.38
2	3.20	-0.04	0.9742	72.03
3	3.57	-0.05	0.9810	70.83
4	4.16	-0.06	0.9747	73.83
5	2.84	-0.04	0.9762	67.92
6	3.29	-0.05	0.9766	69.62
7	3.53	-0.05	0.9699	70.04
8	2.19	-0.03	0.9839	64.29
9	2.13	-0.03	0.9846	64.16
B, Acetonitrile-water				
Compound no.	R_{M0}	S	r^2	φ
1	2.63	-0.04	0.9553	60.71
2	3.13	-0.05	0.9881	69.44
3	2.99	-0.04	0.9902	69.05
4	3.03	-0.04	0.9932	73.60
5	2.78	-0.04	0.9900	65.34
6	2.77	-0.04	0.9850	67.18
7	2.82	-0.04	0.9899	68.65
8	2.76	-0.05	0.9970	57.67
9	2.97	-0.05	0.9917	57.55
C, 1,4-Dioxane-water				
Compound no.	R_{M0}	S	r^2	φ
1	3.99	-0.06	0.9786	64.22
2	3.66	-0.06	0.9972	64.71
3	3.59	-0.06	0.9959	63.56
4	4.32	-0.06	0.9953	68.10
5	3.28	-0.05	0.9909	61.81
6	3.51	-0.06	0.9926	63.04
7	3.65	-0.06	0.9985	63.52
8	2.36	-0.04	0.9882	58.23

9	2.33	-0.04	0.9845	58.05
D, Methanol-water				
Compound no.	R_{M0}	*S*	r^2	φ
1	2.80	-0.04	0.9962	70.04
2	4.18	-0.05	0.9898	80.53
3	4.38	-0.05	0.9885	80.73
4	4.72	-0.06	0.9942	84.00
5	3.63	-0.05	0.9876	76.43
6	4.02	-0.05	0.9909	80.14
7	4.09	-0.05	0.9935	81.06
8	2.44	-0.04	0.9934	67.14
9	2.39	-0.04	0.9901	67.15

φ, amount of organic modifier in the mobile phase; R_{M0}, intercept of the plot; *S*, slope of the plot; *r*, correlation coefficient.

Table SIII. Log P_{CAL} values of the synthesized benzenesulphonohydrazides 1-9.

Compound no.	R	log P_{CAL}
1	2,3-diF	3.34
2	2-Cl-3-OMe	3.83
3	2-Br-3-OH-4-OMe	3.95
4	2-F-4-Br	3.79
5	2,3-diOMe	3.20
6	3-Cl-4-OMe	3.78
7	3-Br-4-OMe	3.80
8	3-Br-4-OH	3.82
9	3-OEt-4-OH	3.69

R, substituent; log P_{CAL}, log P values obtained with ALOGPS 2.1 software.

Table SIV. Determination of IC_{50} values for the tested compounds (1-9) in cancer and normal cell lines; data from the concentration-response curves.

Compound no.	R	Regression equations and correlation coefficients (r^2)			
		769-P	HepG2	H2170	Vero
1	2,3-diF	y = 5.6466ln(x) - 8.9487; r^2 = 0.9603	y = 17.073ln(x) - 61.095; r^2 = 0.8723	y = 17.3ln(x) - 90.583; r^2 = 0.9365	y = 13.081ln(x) - 22.689; r^2 = 0.7802
2	2-Cl-3-OMe	y = 31.689ln(x) - 83.508; r^2 = 0.7789	y = 6.4767ln(x) + 17.111; r^2 = 0.861	y = 28.895ln(x) - 129.66; r^2 = 0.9412	y = 11.333ln(x) - 33.936; r^2 = 0.7985
3	2-Br-3-OH-4-OMe	y = 61.531ln(x) - 255.92; r^2 = 0.8899	y = 15.773ln(x) - 33.577; r^2 = 0.7511	y = 17.383ln(x) - 53.586; r^2 = 0.9591	y = 20.089ln(x) - 70.173; r^2 = 0.9151
4	2-F-4-Br	y = 16.661ln(x) - 21.624; r^2 = 0.7424	y = 15.672ln(x) - 23.133; r^2 = 0.9624	y = 19.096ln(x) - 62.918; r^2 = 0.8868	y = 14.065ln(x) - 46.652; r^2 = 0.9226
5	2,3-diOMe	y = 7.4578ln(x) + 45.048; r^2 = 0.7672	y = 4.3131ln(x) + 20.869; r^2 = 0.9878	y = 11.312ln(x) - 60.228; r^2 = 0.7948	y = 16.407ln(x) - 26.961; r^2 = 0.8257
6	3-Cl-4-OMe	y = 7.0979ln(x) + 26.77; r^2 = 0.9475	y = 10.531ln(x) - 12.13; r^2 = 0.9929	y = 25.213ln(x) - 107.55; r^2 = 0.8273	y = 12.929ln(x) - 44.184; r^2 = 0.9496
7	3-Br-4-OMe	y = 25.752ln(x) - 68.754; r^2 = 0.9687	y = 6.139ln(x) + 7.4308; r^2 = 0.7323	y = 25.179ln(x) - 94.808; r^2 = 0.9754	y = 22.557ln(x) - 110.09; r^2 = 0.9837
8	3-Br-4-OH	y = 12.429ln(x) - 3.585; r^2 = 0.7685	nd	y = 4.7569ln(x) - 26.772; r^2 = 0.8306	y = 45.663ln(x) - 180.94; r^2 = 0.941
9	3-OEt-4-OH	y = 21.199ln(x) - 34.653; r^2 = 0.9389	y = 32.425ln(x) - 133.39; r^2 = 0.7764	y = 5.2416ln(x) - 15.631; r^2 = 0.7212	y = 28.596ln(x) - 92.635; r^2 = 0.9045

nd, not determined.