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Original article

1-(2-Mercaptobenzenesulfonyl)-3-hydroxyguanidines – Novel potent antiproliferatives, synthesis and *in vitro* biological activity

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ABSTRACT

Twenty four 1-[2-alkylthio-5-(azol-2 or 5-yl)-4-chlorobenzenesulfonyl]-3-hydroxyguanidines **6a**–**x** have been synthesized in order to evaluate their biological activity. Compounds **6a**, **6c**, **6d**, **6f**, **6g**, **6i**–**p**, **6r**–**t**, and **6v**–**x** were tested for their *in vitro* anticancer activity at the US National Cancer Institute. The highest *in vitro* anticancer activity was found for compounds **6d**, **6g** and **6k** with GI_{50} average value in the range 1.62–1.86 μM, and TGI mean values 3.72–4.47 μM, whereas the remaining compounds showed broad spectrum of anticancer activity at low micromolar GI_{50} level against all tested cancer cell lines. These results were subjected to CoMSIA analysis to establish quantitative structure–activity relationships. The results evidence that potency of these compounds correlates mainly with hydrophobic and polar surface properties of substituents located both at 2 and 5 positions of 1-(4-chlorobenzenesulfonyl) moiety of investigated 3-hydroxyguanidine series.

1. Introduction

N-Hydroxyguanidine derivatives represent a unique group of compounds which has found limited attention in synthetic medicinal chemistry. Most of them are considered as NOS substrates, thus being nitric oxide donors (NO) [1–3]. Recently cytotoxicity studies of simple 1-aryl-3-hydroxyguanidine derivatives revealed that this kind of compounds can selectively kill tumor cells *via* NO dependent mechanism [4]. There is limited evidence about antitumor activity of *N*-sulfonylated hydroxyguanidine derivatives. Closely related Eli Lily's diarylsulfonylureas (Fig. 1A) have been identified with high therapeutic efficacy in various *in vitro* and *in vivo* preclinical anticancer assays [5,6] and reached clinical trials [7,8]. Earlier studies implicate mitochondria as a potential site of action [9], although their primary molecular target remains unknown. Due to discovered sulfonylurea instability in physiological conditions, bioisosteric hydroxyguanidine derivatives were developed which showed interesting activity on several human cancer cell lines (Fig. 1B) [10]. Another researchers reported tricyclic analogs with built-in 1-sulfonyl-3-hydroxyguanidine moiety (Fig. 1C) as potent antiproliferatives against KB, Colo 205, HeLa and Hepa-2 cell lines [11].

Synthesis and antitumor activity of unsubstituted 1-benzenesulfonyl-3-hydroxyguanidines appear to be unknown. Our systematic study of 2-mercaptobenzenesulfonamide derivatives led us to discover novel anticancer [12–18], anti-HIV [19–21] and antibacterial [22] agents. Our ongoing research in the synthesis and biological activity of novel 2-mercaptobenzenesulfonamides (MBSA) with five-membered heterocycles located in 5 position of MBSA scaffold [17,18] led us to an assumption that incorporation of hydroxyguanidine moiety into developed structures will be of high value to understand more about the chemistry and biological activity of this class of compounds. This paper herein describes a novel class of highly potent antitumor agents derived from MBSA scaffold, bearing the common core of 1-(4-chloro-2-mercaptobenzenesulfonyl)-3-hydroxyguanidine.

2. Results and discussion

2.1. Chemistry

The synthetic route for the preparation of target 1-[2-alkylthio-5-(azol-2 or 5-yl)-4-chlorobenzenesulfonyl]-3-hydroxyguanidines **6a**–**x** are shown in Schemes 1 and 2.

Thus, the essential intermediates **3a**–**d** for the synthesis of 3-amino-1,1-dioxo-1,4,2-benzodithiazines **4a**–**d** were obtained according to the methods described previously [17]. Subsequent new intermediates **3e**–**h** for the synthesis of novel 3-aminobenzodithiazines **4e**–**h** were synthesized as depicted in Scheme 1.

Thus, the starting 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxylic acid **1** [23] could be easily converted

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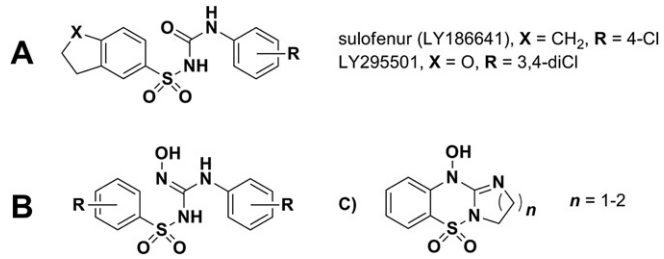


Fig. 1. (A) Diarylsulfonylureas (DSUs); (B) 1-benzenesulfonyl-2-hydroxyguanidines; (C) 1-benzenesulfonyl-3-hydroxyguanidine heterocyclic anticancer agents.

to thioamide **2** by three-step reaction sequence which involves reaction with an excess of thionyl chloride, then 12% aqueous ammonia solution in benzene mixture and finally thionation with Lawesson's reagent (LR) to give thioamide **2** in 78% yield. Subsequent reactions of **2** with the appropriate fenacyl bromides afforded the expected thiazoles **3e** and **3f** in good yields. In turn, desired 1,2,4-oxadiazoles **3g** and **3h** were synthesized in one-pot procedure starting from 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxylic acid chloride with the appropriate commercially available *N′*-hydroxybenzamidines by sequential *O*-acylation and intramolecular dehydration reaction in toluene (Scheme 1).

3-Aminobenzodithiazines **4a**–**d** [17] and **4e**–**h** were in turn obtained by the reaction of the corresponding 3-methylthiobenzodithiazines **3a**–**d** [17] and **3e**–**h** with 25% aqueous ammonia solution in methanol or methanol/toluene mixture at ambient temperature for several hours (Scheme 2). Subsequent reactions of **4a**–**h** with halomethyl electrophiles such as corresponding benzyl chloride derivatives (Scheme 2) in dry THF in the presence of an excess anhydrous potassium carbonate (K_2CO_3) furnished desired *N*-(benzenesulfonyl)cyanamide potassium salts **5a**–**d** [17] and **5e**–**x** in 57–99% yields. Finally, when potassium salts **5a**–**x** were subjected to the reactions with 3 molar equiv of hydroxylamine hydrochloride in refluxing dry acetonitrile, the desired 1-[2-alkylthio-5-(azol-2 or 5-yl)-4-chlrobenzenesulfonyl]-3-hydroxyguanidines **6a**–**x** were formed in moderate to excellent yields (51–99%) (Scheme 2).

The structure of the new compounds was evidenced by IR, ^{1}H and ^{13}C and MS spectra as well as elemental analyses. Inspection of the ^{1}H NMR spectra revealed that in DMSO solution compounds **6a**–**x** exist as a stable sulfonylamino tautomers (see Scheme 2). These spectra provided evidences for the confirmation of this sulfonylamino tautomeric form, since four separate signals of the chemically non-equivalent (D_2O exchangeable) protons of N–H and O–H groups were found. Two singlets in the region δ 7.12–7.29 and 7.34–7.40 ppm are assigned to the C=N^2H and =C–N^3H– protons, respectively. Whereas singlet signals attribute to N^3–O–H and SO_2–N^1H– groups were found downfield in the regions δ 9.44–9.47 and 10.04–10.09 ppm, respectively.

2.2. Antiproliferative activity

The antiproliferative activity of **6a**–**x** was assessed according to the NIH Developmental Therapeutics Program [26,27]. Identified by the preliminary studies of one dose screen test results (10 μM concentration of test agent) nineteen compounds were subjected to further studies in 5-dose assay (Table 1). Most of 1-(2-mercaptobenzenesulfonyl)-3-hydroxyguanidine derivatives (**6a**, **6c**, **6d**, **6f**, **6g**, **6i**–**p**, **6r**–**t**, and **6v**–**x**) show broad spectrum of cytostatic activity overall tested cancer cell lines, and compound **6k** seems to be the most potent against tested cell lines (Tables 1–3).

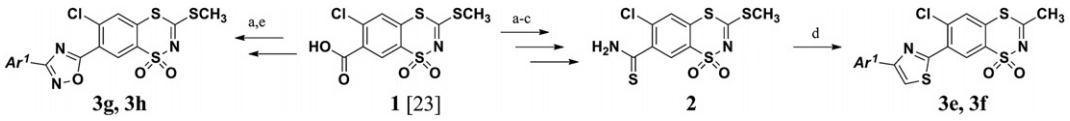
Represented mostly in 5-dose screen of the series compounds with incorporated (1,3-benzodioxol-5-yl)methylthio moiety in 2 position (**6i**–**p**: mean GI_{50} = 1.62–11.22 μM, mean TGI = 3.72–75.86 μM) and 5-aryl-1,3,4-thiadiazol-5-yl moiety in 5 position (**6c**, **6d**, **6k**, **6l**, **6s**, **6t**: mean GI_{50} = 1.62–18.62 μM, mean TGI = 3.72–70.79 μM) (Table 1) suggest that these groups increase *in vitro* anticancer activity of 1-benzenesulfonyl-3-hydroxyguanidine derivatives against cell lines in NCI-60 panel. Considering rather high efficacy toward cancer cell lines of this series of compounds, it's worth mentioning that CCRF-CEM (leukemia) is the most sensitive cell line in the panel, which growth is suppressed by *ca.* 70% of tested 1-benzenesulfonyl-3-hydroxyguanidine derivatives (**6a**, **6c**, **6d**, **6f**, **6g**, **6l**, **6o**–**s**, **6t**, **6w**) at low micromolar concentrations (GI_{50} = 0.55–1.58 μM, TGI = 3.23–7.77 μM).

In some cases was observed a slight decrease in cytostatic activity when 4-chlorophenyl substituted heterocycles are present in 5 position of the core structure (**6i** vs. **6j**, **6m** vs. **6n**, and **6s** vs. **6t**). On the other hand presence of a chlorine atom in 1,2,4-oxadiazoles increases their overall activity (**6o** vs. **6p**, **6w** vs. **6x**). Careful analysis in the series of sulfur-containing azoles with Ar^2 = 1,3-benzodioxol-5-yl substituent (**6k**–**n**) shows that chlorine introduction decreases their cytostatic effect toward SF-268, U251, MDA-MB-435, UACC-62, IGROV1, and MDA-MB-231/ATCC cell lines (Table 2). The influence of a chlorine as a modifier of the activity is more pronounced in 1,3,4-thiadiazoles (**6k** and **6l**) contrary to 1,3,4-oxadiazoles (**6i** and **6j**) (Table 1).

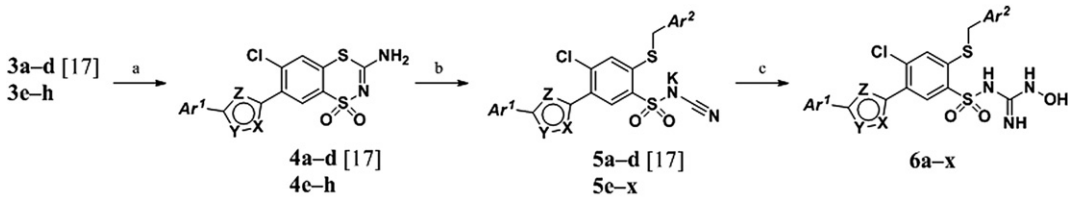
It was also found that in a series of 1,2,4-oxadiazole (**6o**, **6p**, **6w**, **6x**) as well as 1,3,4-thiadiazole (**6k**, **6l**, **6s**, **6t**) derivatives changing Ar^2 = 1,3-benzodioxol-5-yl to Ar^2 = 3,4,5-trimethoxyphenyl substituent results in substantial decrease in antiproliferative activity toward selected cell lines (Table 3).

3. Conclusions

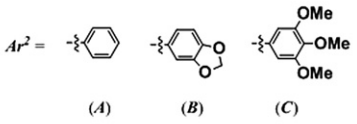
We developed 1-(2-mercaptobenzenesulfonyl)-3-hyroxyguanidine derivatives **6a**–**x** and discovered their antiproliferative activity. Most of the evaluated compounds exhibit remarkable *in vitro* anticancer activity over DTP NCI-60 test panel represented by drug sensitive and multi-drug resistant cell lines of main human cancer types (leukemia, non-small cell lung cancer, melanoma, colon, CNS, breast, ovary, prostate, and renal cancers). The COMPARE analysis of several derivatives revealed that their mean-graph screening profiles correlate with dactinomycin, bruceantin, chromomycin A3, and echinomycin profiles – nucleic acid interfering agents. Structure–activity relationship analysis implicates that potency of the series correlates mostly with hydrophobic and polar surface properties of substituents located both in 2 and 5 position of 1-(4-chlorobenzenesulfonyl)-3-hydroxyguanidine core. The most potent 1-benzenesulfonyl-3-hydroxyguanidine derivatives have (1,3-benzodioxol-5-yl)methylthio moiety in 2 position and/or 5-aryl-



Scheme 1. Reactions conditions: (a) $SOCl_2$ (excess), benzene, reflux [24]; (b) 12% NH_3 (aq)/benzene, 5–10 °C [25]; (c) 0.5 molar equiv Lawesson's reagent, toluene, 110 °C; (d) 1 molar equiv Ar^1–C(=O)CH_2Br, MeOH, reflux; (e) 1.1 molar equiv Ar^1–C(=N–OH)NH_2, toluene, 20 °C, then 110 °C.



Compd	Ar^1	X:Y:Z	Compd	Ar^1	X:Y:Z
3a	Ph	N:N:O	**4a**	Ph	N:N:O
3b	4-Cl-Ph	N:N:O	**4b**	4-Cl-Ph	N:N:O
3c	Ph	N:N:S	**4c**	Ph	N:N:S
3d	4-Cl-Ph	N:N:S	**4d**	4-Cl-Ph	N:N:S
3e	Ph	S:CH:N	**4e**	Ph	S:CH:N
3f	4-Cl-Ph	S:CH:N	**4f**	4-Cl-Ph	S:CH:N
3g	Ph	O:N:N	**4g**	Ph	O:N:N
3h	4-Cl-Ph	O:N:N	**4h**	4-Cl-Ph	O:N:N



Compd	Ar^1	Ar^2	X:Y:Z	Compd	Ar^1	Ar^2	X:Y:Z
5a,6a	Ph	*A*	N:N:O	**5m,6m**	Ph	*B*	S:CH:N
5b,6b	4-Cl-Ph	*A*	N:N:O	**5n,6n**	4-Cl-Ph	*B*	S:CH:N
5c,6c	Ph	*A*	N:N:S	**5o,6o**	Ph	*B*	O:N:N
5d,6d	4-Cl-Ph	*A*	N:N:S	**5p,6p**	4-Cl-Ph	*B*	O:N:N
5e,6e	Ph	*A*	S:CH:N	**5q,6q**	Ph	*C*	N:N:O
5f,6f	4-Cl-Ph	*A*	S:CH:N	**5r,6r**	4-Cl-Ph	*C*	N:N:O
5g,6g	Ph	*A*	O:N:N	**5s,6s**	Ph	*C*	N:N:S
5h,6h	4-Cl-Ph	*A*	O:N:N	**5t,6t**	4-Cl-Ph	*C*	N:N:S
5i,6i	Ph	*B*	N:N:O	**5u,6u**	Ph	*C*	S:CH:N
5j,6j	4-Cl-Ph	*B*	N:N:O	**5v,6v**	4-Cl-Ph	*C*	S:CH:N
5k,6k	Ph	*B*	N:N:S	**5w,6w**	Ph	*C*	O:N:N
5l,6l	4-Cl-Ph	*B*	N:N:S	**5x,6x**	4-Cl-Ph	*C*	O:N:N

Scheme 2. Reactions conditions: (a) 1.5 molar equiv 25% NH_4OH, MeOH or MeOH/toluene, 20 °C; (b) 1–1.3 molar equiv Cl–CH_2–Ar^2, 5 molar equiv K_2CO_3, dry THF, 65 °C; (c) 3 molar equiv $NH_2OH \times HCl$, dry MeCN, 83 °C.

1,3,4-thiadiazol-2-yl moiety at 5 position and render as promising antiproliferatives with broad spectrum of cytostatic activity.

4. Experimental protocols

4.1. Synthesis

Melting points were determined with Boëtius apparatus and are uncorrected. The IR spectra were taken using Thermo Mattson Satellite FTIR spectrophotometer, ^1H and ^{13}C NMR were taken with a Varian Gemini 200 MHz or Varian Unity Plus 500 MHz apparatus. Chemical shifts are reported in ppm (δ). The mass spectra were acquired on a Bruker BIFLEX III MALDI-TOF spectrometer after deposition on a 2,5-dihydroxybenzoic acid (DHB) matrix. The results of elemental analyses for C, H and N were in agreement with the calculated values within ±0.4% range (see Supplementary data).

4.1.1. 6-Chloro-3-methylthio-7-thiocarbamoyl-1,1-dioxo-1,4,2-benzodithiazine (**2**)

The suspension of 6-chloro-3-methylthio-7-carbamoyl-1,1-dioxo-1,4,2-benzodithiazine [25] (3.88 g, 12.0 mmol), Lawesson's reagent (2.66 g, 6.6 mmol) in dry toluene (60 ml) was refluxed for 1 h. The unreacted amide (about 1.92 g, 6.0 mmol) was quickly filtered out from the reaction mixture while hot and reacted with subsequent portion of Lawesson's reagent (1.36 g, 3.36 mmol) in 30 ml of fresh dry toluene for another 1 h at reflux, then filtered out. Combined filtrates were slowly cooled to −20 °C and the precipitated yellow solid was filtered off and purified by crystallization from toluene. Yield: 3.17 g (78%): m.p. 194–196 °C; IR (KBr) ν 3426, 3311, 3165, 2924, 2853, 1618, 1580, 1493 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.73 (s, 3H, SCH_3), 7.94 (s, 1H, H-5), 8.10 (s, 1H, H-8), 10.25 (br s, 2H, NH_2); Anal. $C_9H_7ClN_2O_2S_4$ (C, H, N).

4.2. General procedure for the synthesis of 7-(4-arylthiazol-2-yl)-6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazines ***3e*** and ***3f***

A suspension of **2** (1.36 g, 4.0 mmol) and the appropriate ω-bromoacetophenone (4.0 mmol) in MeOH (20 ml) was refluxed for 4–5 h. The precipitated solid was filtered off and washed with MeOH. The crude product was purified by crystallization from DMF.

4.2.1. 6-Chloro-3-methylthio-7-(4-phenylthiazol-2-yl)-1,1-dioxo-1,4,2-benzodithiazine (***3e***)

Starting from ω-bromoacetophenone (0.80 g). Yield: 1.16 g (66%): m.p. 300–302 °C; IR (KBr) ν 3100, 2926, 1497, 1479, 1335, 1163 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.76 (s, 3H, SCH_3);

Table 1
Overview of *in vitro* antiproliferative activity of 1-(2-mercaptobenzenesulfonyl)-3-hydroxyguanidines **6a**, **6c**, **6d**, **6f**, **6g**, **6i**–**p**, **6r**–**t**, and **6v**–**x**.

Compd	No. of tested cell lines[a]	GI_{50}[b] [μM]			TGI[b] [μM]		
		MG–MID[c]	No.[d]	Range[d]	MG–MID[c]	No.[d]	Range[d]
6a	60	2.88	54	1.01–5.99	9.77	55	2.99–23.50
6c	59	2.40	55	1.20–3.50	6.76	56	2.97–33.50
6d	57	1.86	55	0.83–2.57	4.07	53	2.85–4.81
6f	59	2.24	53	1.07–3.70	6.03	52	2.84–9.95
6g	57	1.86	57	1.19–3.56	4.47	55	2.71–9.03
6i	57	2.00	55	0.55–2.92	5.13	52	2.87–9.78
6j	57	2.14	55	1.41–3.78	5.50	50	2.87–9.83
6k	57	1.62	56	0.24–2.41	3.72	56	0.80–6.61
6l	59	3.39	52	1.55–7.52	9.55	58	3.13–34.40
6m	58	2.51	56	1.57–4.63	6.46	56	3.02–17.50
6n	55	11.22	50	2.84–75.40	75.86	55	19.80–100.00
6o	57	2.04	54	1.24–2.86	5.50	54	2.85–17.30
6p	56	1.95	55	1.02–2.98	4.47	53	2.46–7.70
6r	57	2.04	53	1.29–2.65	5.13	53	2.81–9.83
6s	58	3.16	53	1.16–7.89	11.48	52	2.87–45.90
6t	59	18.62	36	1.15–28.80	70.79	16	4.33–95.50
6v	58	3.39	57	0.44–6.60	16.22	48	3.76–29.50
6w	59	4.90	59	0.64–15.70	16.22	50	2.86–28.00
6x	59	3.02	58	1.36–9.78	9.55	56	2.95–34.70

[a] Number of cell lines investigated.
[b] Response parameter, describing molar concentration of the test agent causing 50% net cell growth inhibition (GI_{50}) or total growth inhibition (TGI).
[c] Mean graph mid-point − response parameter average value for all tested cell lines.
[d] Response parameter range for selected no. of cell lines.

7.42–7.45 (m, 1H, Ar), 7.52–7.55 (m, 2H, Ar), 8.09–8.10 (m, 2H, Ar), 8.32 (s, 1H, H-5), 8.52 (s, 1H, H-8), 8.99 (s, 1H, Ar). Anal. $C_{17}H_{11}ClN_2O_2S_4$ (C, H, N).

4.2.2. 6-Chloro-7-[4-(4-chlorophenyl)thiazol-2-yl]-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine (**3f**)

Starting from ω-bromo-4-chloroacetophenone (0.93 g). Yield: 1.01 g (56%): m.p. 297–299 °C; IR (KBr) ν 3107, 1492, 1474, 1458, 1339, 1165 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.76 (s, 3H, SCH_3), 7.59–7.61 (m, 2H, Ar), 8.11–8.13 (m, 2H, Ar), 8.30 (s, 1H, H-5), 8.56 (s, 1H, H-8), 8.97 (s, 1H, Ar); Anal. $C_{17}H_{10}Cl_2N_2O_2S_4$ (C, H, N).

4.3. General procedure for the synthesis of 7-(3-aryl-1,2,4-oxadiazol-5-yl)-6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazines **3g** and **3h**

A mixture of 6-chloro-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine-7-carboxylic acid (1.00 g, 3.1 mmol) and thionyl chloride (3 ml) in dry benzene (3 ml) was refluxed for 1 h. The reaction mixture was concentrated to dryness and dry toluene (50 ml) was added. The obtained solution was added dropwise at 0 °C to the suspension of the appropriate amidoxime (3.1 mmol) in dry toluene (25 ml) within 1 h and then refluxed for 4–6 h. Obtained solution was concentrated under reduced pressure to dryness and MeOH (5 ml) was added. Precipitated solid was filtered off, and purified by crystallization from EtOH.

Table 2
Comparison of cytostatic activity (GI_{50} [μM]) showing decrease in activity after chlorine introduction into 1,3-benzodioxol-5-yl derivatives **6k** and **6m**.

Cell lines:	SF-268	U251	MDA -MB-435	UACC -62	IGROV1	MDA -MB-231
6k (Ar^1 = Ph)	1.61	1.40	1.38	1.70	1.68	1.68
6l (Ar^1 = 4-Cl–Ph)	2.04	10.72	2.63	2.14	3.47	5.13
6m (Ar^1 = Ph)	1.86	1.95	2.00	2.14	2.82	2.34
6n (Ar^1 = 4-Cl–Ph)	6.46	11.22	3.89	7.76	25.12	12.59

4.3.1. 6-Chloro-3-methylthio-7-(3-phenyl-1,2,4-oxadiazol-5-yl)-1,1-dioxo-1,4,2-benzodithiazine (**3g**)

Starting from benzamidoxime (0.42 g). Yield: 0.87 g (66%): m.p. 196–198 °C; IR (KBr) ν 1595, 1506, 1445, 1337, 1169 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.75 (s, 3H, SCH_3), 7.61–7.64 (m, 3H, Ar), 8.10–8.15 (m, 2H, Ar), 8.37 (s, 1H, H-5), 8.74 (s, 1H, H-8); Anal. $C_{16}H_{10}ClN_3O_3S_3$ (C, H, N).

4.3.2. 6-Chloro-7-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]-3-methylthio-1,1-dioxo-1,4,2-benzodithiazine (**3h**)

Starting from 4-chlorobenzamidoxime (0.55 g). Yield: 0.88 g (60%): m.p.154–156 °C; IR (KBr) ν 2928, 1587, 1508, 1473, 1345, 1171 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.75 (s, 3H, SCH_3), 7.66–7.71 (m, 2H, Ar), 8.11–8.15 (m, 2H, Ar), 8.37 (s, 1H, H-5), 8.74 (s, 1H, H-8); Anal. $C_{16}H_9Cl_2N_3O_3S_3$ (C, H, N).

4.4. General procedure for the synthesis of 3-amino-7-(azol-2 or 5-yl)-6-chloro-1,1-dioxo-1,4,2-benzodithiazines **4e**–**h**

A suspension of the appropriate 3-methylthio derivative **3e**–**h** (2 mmol) in MeOH (10 ml) or MeOH/toluene (5/5 ml) was cooled to 0 °C and ammonium hydroxide solution (25%, 2.9 mmol, 0.20 g) was added dropwise. The suspension was stirred at room temperature until MeSH ceased to evolve (CAUTION: due to a high toxicity, MeSH should be trapped into an aqueous NaOH solution). Precipitated crude product was filtered off and purified by crystallization from the appropriate solvent.

4.4.1. 3-Amino-6-chloro-7-(4-phenylthiazol-2-yl)-1,1-dioxo-1,4,2-benzodithiazine (**4e**)

Starting from **3e** (0.88 g). Yield: 0.70 g (86%): m.p. >320 °C (70% DMF/H_2O); IR (KBr): ν 3398, 3314, 3199, 1632, 1583, 1541, 1531, 1481, 1461, 1354, 1306, 1161 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.39–7.42 (m, 1H, Ar), 7.49–7.52 (m, 2H, Ar), 8.05–8.07 (m, 2H, Ar), 8.20 (s, 1H, Ar), 8.45 (s, 1H, Ar), 8.86 (s, 1H, Ar), 9.31 (br s, 2H, NH_2); Anal. $C_{16}H_{10}ClN_3O_2S_3$ (C, H, N).

4.4.2. 3-Amino-6-chloro-7-[4-(4-chlorophenyl)thiazol-2-yl]-1,1-dioxo-1,4,2-benzodithiazine (**4f**)

Starting from **3f** (0.95 g). Yield: 0.71 (80%): m.p. >320 °C (70% DMF/H_2O); IR (KBr): ν 3295, 3127, 1666, 1643, 1543, 1530, 1308, 1139 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 7.55–7.60 (m, 2H, Ar), 8.07–8.11 (m, 2H, Ar), 8.20 (s, 1H, H-5), 8.51 (s, 1H, H-8), 8.85 (s, 1H, Ar), 9.28–9.36 (m, 2H, NH_2); Anal. $C_{16}H_9Cl_2N_3O_2S_3$ (C, H, N).

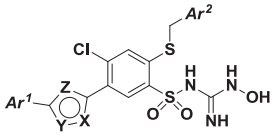
4.4.3. 3-Amino-6-chloro-7-(3-phenyl-1,2,4-oxadiazol-5-yl)-1,1-dioxo-1,4,2-benzodithiazine (**4g**)

Starting from **3g** (0.85 g). Yield: 0.64 (81%): m.p. 304–306 °C (20% DMF/MeOH); IR (KBr): ν 3416, 3283, 3188, 2924, 1620, 1596, 1565, 1539, 1521, 1474, 1350, 1302, 1159 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.60–7.66 (m, 3H, Ar), 8.12–8.13 (m, 2H, Ar), 8.30 (s, 1H, Ar), 8.65 (s, 1H, Ar), 9.42 (br s, 2H, NH_2); Anal. $C_{15}H_9ClN_4O_3S_2$ (C, H, N).

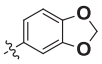
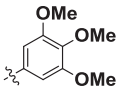
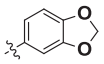
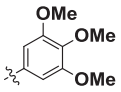
4.4.4. 3-Amino-6-chloro-7-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]-1,1-dioxo-1,4,2-benzodithiazine(**4h**)

Starting from **3h** (0.92 g). Yield: 0.67 (78%): m.p. >320 °C (20% DMF/MeOH); IR (KBr): ν 3275, 3055, 2926, 1659, 1638, 1590, 1559, 1519, 1467, 1338, 1311, 1173, 1156 cm^{-1}; ^1H NMR (500 MHz, DMSO-

Table 3
Influence of Ar^2 substitution with 1,3-benzodioxol-5-yl and 3,4,5-trimethoxyphenyl moieties on cytostatic activity (GI_{50} [μM]) of the series of 1,2,4-oxadiazoles (**6o**, **6p**, **6w**, **6x**) and 1,3,4-thiadiazoles (**6k**, **6l**, **6s**, **6t**) on selected cell lines.



Ar^1 = Ph, 4-Cl-Ph

Ar^2 =	1,2,4-oxadiazoles (X:Y:Z = O:N:N)				1,3,4-thiadiazoles (X:Y:Z = N:N:S)			
								
Cell lines	**6o**	**6p**	**6w**	**6x**	**6k**	**6l**	**6s**	**6t**
NCI-H460	2.04	2.14	4.37	2.24	1.79	2.34	3.24	34.67
NCI-H522	1.29	1.02	3.80	2.14	0.24	1.86	2.04	8.91
HT29	2.00	1.82	7.24	2.95	1.85	2.29	4.17	6.76
SF-268	1.91	1.82	6.92	2.95	1.61	2.04	2.40	9.55
SNB-75	1.55	1.74	3.98	2.95	1.07	1.78	1.86	25.70
MALME-3M	2.19	2.63	13.49	7.76	1.71	2.24	3.09	97.72
M14	1.91	1.86	6.61	2.19	1.74	2.14	4.07	40.74
UACC-257	2.00	1.91	13.18	9.33	1.67	3.55	6.03	26.30
UACC-62	1.82	1.70	3.09	1.95	1.70	2.14	3.24	16.22
IGROV1	2.57	2.14	5.62	3.80	1.68	3.47	3.72	58.88
OVCAR-3	1.70	1.70	4.79	1.91	1.70	1.62	2.19	10.23
UO-31	1.66	1.91	4.47	3.16	1.28	3.72	3.98	44.67
HS 578T	2.63	2.69	7.41	4.17	2.41	2.40	3.31	8.13

d_6): δ 7.68–7.69 (m, 2H, Ar), 8.12–8.14 (m, 2H, Ar), 8.30 (s, 1H, Ar), 8.64 (s, 1H, Ar), 9.41 (br s, 2H, NH_2); Anal. $C_{15}H_8Cl_2N_4O_3S_2$ (C, H, N).

4.5. General procedure for the synthesis of N-(benzenesulfonyl) cyanamide potassium salts ***5e–x***

Suspension of the appropriate 3-amino-1,1-dioxo-1,4,2-benzodithiazine derivative **4a–h** (0.5 mmol), anhydrous K_2CO_3 (1.5 mmol, 0.21 g) and the appropriate alkyl chloride (0.6 mmol) in dry THF (5 ml) was heated at reflux for 20–24 h, then cooled in ice-bath and filtered off. The crude product was suspended in 5 ml of water, heated gently to *ca.* 50 °C; and cooled with vigorous stirring until granular precipitate appeared. Filtering off and washing with cold water and diluted EtOH gave pure potassium salts.

4.5.1. N-[2-Benzylthio-4-chloro-5-(4-phenylthiazol-2-yl) benzenesulfonyl]cyanamide potassium salt (***5e***)

Starting from **4e** (0.204 g) and benzyl chloride (0.076 g). Yield: 0.153 g (57%): m.p. 198–199 °C; IR (KBr): ν 3092, 2926, 2170, 1602, 1578, 1528, 1494, 1476, 1452, 1350, 1284, 1140 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.40 (s, 2H, SCH_2), 7.27–7.30 (m, 1H, Ar), 7.35–7.40 (m, 3H, Ar), 7.48–7.51 (m, 4H, Ar), 7.62 (s, 1H, Ar), 8.04–8.06 (m, 2H, Ar), 8.35 (s, 1H, Ar), 8.79 (s, 1H, Ar); Anal. $C_{23}H_{15}ClKN_3O_2S_3$ (C, H, N).

4.5.2. N-{2-Benzylthio-4-chloro-5-[4-(4-chlorophenyl)thiazol-2-yl] benzenesulfonyl}cyanamide potassium salt (***5f***)

Starting from **4f** (0.221 g) and benzyl chloride (0.076 g). Yield: 0.217 g (76%): m.p. 228–230 °C; IR (KBr): ν 2923, 2175, 1577, 1527, 1494, 1471, 1453, 1350, 1275, 1139 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.40 (s, 2H, SCH_2), 7.27–7.30 (m, 1H, Ar), 7.34–7.38 (m, 2H, Ar), 7.48–7.49 (m, 2H, Ar), 7.55–7.57 (m, 2H, Ar), 7.62 (s, 1H, Ar), 8.06–8.08 (m, 2H, Ar), 8.41 (s, 1H, Ar), 8.77 (s, 1H, Ar); Anal. $C_{23}H_{14}Cl_2KN_3O_2S_3$ (C, H, N).

4.5.3. N-[2-Benzylthio-4-chloro-5-(3-phenyl-1,2,4-oxadiazol-5-yl) benzenesulfonyl]cyanamide potassium salt (***5g***)

Starting from **4g** (0.196 g) and benzyl chloride (0.076 g). Yield: 0.206 g (79%): m.p. 138–140 °C; IR (KBr): ν 3433, 2924, 2854, 2176, 1737, 1594, 1529, 1495, 1360, 1143 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 4.49 (s, 2H, SCH_2), 7.32–7.44 (m, 5H, Ar), 7.51–7.54 (m, 2H, Ar), 7.62–7.65 (m, 3H, Ar), 7.74 (s, 1H, Ar), 8.11–8.16 (m, 2H, Ar), 8.60 (s, 1H, Ar); Anal. $C_{22}H_{14}ClKN_4O_3S_2$ (C, H, N).

4.5.4. N-{2-Benzylthio-4-chloro-5-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]benzenesulfonyl}cyanamide potassium salt (***5h***)

Starting from **4h** (0.214 g) and benzyl chloride (0.076 g). Yield: 0.236 g (85%): m.p. >320 °C; IR (KBr): ν 2925, 2175, 1583, 1560, 1503, 1470, 1343, 1298, 1135 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.46 (s, 2H, SCH_2), 7.28–7.31 (m, 1H, Ar), 7.35–7.38 (m, 2H, Ar), 7.48–7.50 (m, 2H, Ar), 7.66–7.68 (m, 2H, Ar), 7.71 (s, 1H, Ar), 8.11–8.12 (m, 2H, Ar), 8.56 (s, 1H, Ar); Anal. $C_{22}H_{13}Cl_2KN_4O_3S_2$ (C, H, N).

4.5.5. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(5-phenyl-1,3,4-oxadiazol-2-yl)benzenesulfonyl}cyanamide potassium salt (***5i***)

Starting from **4a** (0.196 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.172 g (61%): m.p. 278–280 °C; IR (KBr): ν 2173, 1630, 1584, 1556, 1501, 1489, 1330, 1297, 1143 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.36 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.88–6.89 (m, 1H, Ar), 6.96–6.98 (m, 1H, Ar), 7.04 (s, 1H, Ar), 7.62–7.76 (m, 4H, Ar), 8.07–8.08 (m, 2H, Ar), 8.46 (s, 1H, Ar); Anal. $C_{23}H_{14}ClKN_4O_5S_2$ (C, H, N).

4.5.6. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]benzenesulfonyl}cyanamide potassium salt (***5j***)

Starting from **4b** (0.214 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.240 g (80%): m.p. 308–310 °C; IR (KBr): ν 3436, 3086, 2924, 2172, 1602, 1585, 1543, 1502, 1486, 1327,

1289, 1253, 1141 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.36 (s, 2H, SCH_2), 6.01 (s, 2H, OCH_2O), 6.88–7.04 (m, 5H, Ar), 7.68 (s, 1H, Ar), 7.70 (m, 2H, Ar), 8.08–8.09 (m, 2H, Ar), 8.47 (s, 1H, Ar); Anal. $C_{23}H_{13}Cl_2KN_4O_5S_2$ (C, H, N).

4.5.7. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(5-phenyl-1,3,4-thiadiazol-2-yl)benzenesulfonyl}cyanamide potassium salt (**5k**)

Starting from **4c** (0.204 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.287 g (99%): m.p. 285–287 °C; IR (KBr): ν 3443, 2924, 2853, 2179, 1652, 1623, 1577, 1501, 1489, 1357, 1129 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.35 (s, 2H, SCH_2), 6.01 (s, 2H, OCH_2O), 6.89 (d, $J = 7.81$ Hz, 1H, Ar), 6.97 (d, $J = 7.81$ Hz, 1H, Ar), 7.05 (s, 1H, Ar), 7.58–7.60 (m, 3H, Ar), 7.68 (s, 1H, Ar), 8.07–8.08 (m, 2H, Ar), 8.69 (s, 1H, Ar); Anal. $C_{23}H_{14}ClKN_4O_4S_3$ (C, H, N).

4.5.8. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl]benzenesulfonyl}cyanamide potassium salt (**5l**)

Starting from **4d** (0.222 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.175 g (57%): m.p. 265–267 °C; IR (KBr): ν 2924, 2853, 2170, 1626, 1577, 1560, 1523, 1502, 1491, 1458, 1357, 1260, 1138 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.34 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.88–6.89 (m, 1H, Ar), 6.96–6.97 (m, 1H, Ar), 7.03 (s, 1H, Ar), 7.64 (s, 1H, Ar), 7.66–7.67 (m, 2H, Ar), 8.09–8.11 (m, 2H, Ar), 8.69 (s, 1H, Ar); Anal. $C_{23}H_{13}Cl_2KN_4O_4S_3$ (C, H, N).

4.5.9. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(4-phenylthiazol-2-yl)benzenesulfonyl}cyanamide potassium salt (**5m**)

Starting from **4e** (0.204 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.209 g (72%): m.p. 263–265 °C; IR (KBr): ν 2896, 2182, 1579, 1529, 1503, 1489, 1361, 1282, 1142 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.32 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.88–6.89 (m, 1H, Ar), 6.96–6.97 (m, 1H, Ar), 7.04 (s, 1H, Ar), 7.37–7.40 (m, 1H, Ar), 7.48–7.51 (m, 2H, Ar), 7.60 (s, 1H, Ar), 8.04–8.06 (m, 2H, Ar), 8.35 (s, 1H, Ar), 8.79 (s, 1H, Ar); Anal. $C_{24}H_{15}ClKN_3O_4S_3$ (C, H, N).

4.5.10. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(4-(4-chlorophenyl)thiazol-2-yl)benzenesulfonyl}cyanamide potassium salt (**5n**)

Starting from **4f** (0.221 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.234 g (76%): m.p. 260–262 °C; IR (KBr): ν 2895, 2185, 1635, 1578, 1503, 1488, 1472, 1361, 1285, 1142 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.32 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.87–6.89 (m, 1H, Ar), 6.96–6.97 (m, 1H, Ar), 7.04 (s, 1H, Ar), 7.55–7.57 (m, 2H, Ar), 7.60 (s, 1H, Ar), 8.06–8.08 (m, 2H, Ar), 8.41 (s, 1H, Ar), 8.77 (s, 1H, Ar); Anal. $C_{24}H_{14}Cl_2KN_3O_4S_3$ (C, H, N).

4.5.11. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(3-phenyl-1,2,4-oxadiazol-5-yl)benzenesulfonyl}cyanamide potassium salt (**5o**)

Starting from **4g** (0.196 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.215 g (88%): m.p. 274–276 °C; IR (KBr): ν 2923, 2179, 1625, 1585, 1560, 1500, 1488, 1362, 1349, 1253, 1226, 1143 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.38 (s, 2H, SCH_2), 6,01 (s, 2H, OCH_2O), 6.90 (d, $J = 7.81$ Hz, 1H, Ar), 6.98 (d, $J = 7.81$ Hz, 1H, Ar), 7.05 (s, 1H, Ar), 7.59–7.63 (m, 3H, Ar), 7.70 (s, 1H, Ar), 8.10–8.11 (m, 2H, Ar), 8.59 (s, 1H, Ar); Anal. $C_{23}H_{14}ClKN_4O_5S_2$ (C, H, N).

4.5.12. N-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]benzenesulfonyl}cyanamide potassium salt (**5p**)

Starting from **4h** (0.214 g) and 1,3-benzodioxol-5-ylmethyl chloride (0.102 g). Yield: 0.255 g (85%): m.p. 283–285 °C; IR (KBr): ν 2184, 1589, 1560, 1503, 1491, 1474, 1345, 1255, 1147 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.37 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.89 (d, $J = 7.82$ Hz, 1H, Ar), 6.96 (d, $J = 7.82$ Hz, 1H, Ar), 7.02 (s, 1H, Ar), 7.66–7.68 (m, 2H, Ar), 7.69 (s, 1H, Ar), 8.11–8.12 (m, 2H, Ar), 8.56 (s, 1H, Ar); Anal. $C_{23}H_{13}Cl_2KN_4O_5S_2$ (C, H, N).

4.5.13. N-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(5-phenyl-1,3,4-oxadiazol-2-yl)benzenesulfonyl]cyanamide potassium salt (**5q**)

Starting from **4a** (0.196 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.189 g (62%): m.p. 272–274 °C; IR (KBr): ν 2926, 2851, 2180, 1591, 1552, 1507, 1452, 1331, 1294, 1145, 1126 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.77 (s, 6H, 2× OCH_3), 4.37 (s, 2H, SCH_2), 6.84 (s, 2H, Ar), 7.63–7.66 (m, 3H, Ar), 7.73 (s, 1H, Ar), 8.07–8.08 (m, 2H, Ar), 8.46 (s, 1H, Ar); Anal. $C_{24}H_{20}ClKN_4O_6S_2$ (C, H, N).

4.5.14. N-{4-Chloro-5-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-2-(3,4,5-trimethoxybenzylthio)benzenesulfonyl}cyanamide potassium salt (**5r**)

Starting from **4b** (0.214 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.258 g (80%): m.p. 313–315 °C; IR (KBr): ν 3437, 2926, 2852, 2177, 1591, 1540, 1508, 1482, 1331, 1147, 1128 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.77 (s, 6H, 2× OCH_3), 4.38 (s, 2H, SCH_2), 6.84 (s, 2H, Ar), 7.70–7.72 (m, 2H, Ar), 7.73 (s, 1H, Ar), 8.07–8.09 (m, 2H, Ar), 8.46 (s, 1H, Ar); Anal. $C_{25}H_{19}Cl_2KN_4O_6S_2$ (C, H, N).

4.5.15. N-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(5-phenyl-1,3,4-thiadiazol-2-yl)benzenesulfonyl]cyanamide potassium salt (**5s**)

Starting from **4c** (0.204 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.279 g (89%): m.p. 173–175 °C; IR (KBr): ν 2938, 2176, 1635, 1590, 1458, 1334, 1283, 1240, 1123 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.64 (s, 3H, OCH_3), 3.78 (s, 6H, 2× OCH_3), 4.37 (s, 2H, SCH_2), 6.85 (s, 2H, Ar), 7.58–7.62 (m, 3H, Ar), 7.74 (s, 1H, Ar), 8.08–8.13 (m, 2H, Ar), 8.69 (s, 1H, Ar); Anal. $C_{25}H_{20}ClKN_4O_5S_3$ (C, H, N).

4.5.16. N-{4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl]benzenesulfonyl}cyanamide potassium salt (**5t**)

Starting from **4d** (0.222 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.175 g (53%): m.p. 148–150 °C; IR (KBr): ν 2923, 2851, 2175, 1654, 1637, 1587, 1561, 1542, 1512, 1459, 1334, 1277, 1179, 1144, 1125 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.77 (s, 6H, 2× OCH_3), 4.36 (s, 2H, SCH_2), 6.84 (s, 2H, Ar), 7.65–7.66 (m, 2H, Ar), 7.72 (s, 1H, Ar), 8.09–8.11 (m, 2H, Ar), 8.68 (s, 1H, Ar); Anal. $C_{25}H_{19}Cl_2KN_4O_5S_3$ (C, H, N).

4.5.17. N-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(4-phenylthiazol-2-yl)benzenesulfonyl]cyanamide potassium salt (**5u**)

Starting from **4e** (0.204 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.310 g (99%): m.p. >320 °C; IR (KBr): ν 3078, 2935, 2176, 1593, 1529, 1506, 1452, 1329, 1289, 1142, 1123 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.77 (s, 6H, 2× OCH_3), 4.34 (s, 2H, SCH_2), 6.04 (s, 2H, Ar), 7.37–7.40 (m, 1H, Ar), 7.48–7.51 (m, 2H, Ar), 7.65 (s, 1H, Ar), 8.04–8.05 (m, 2H, Ar), 8.35 (s, 1H, Ar), 8.79 (s, 1H, Ar); Anal. $C_{26}H_{21}ClKN_3O_5S_3$ (C, H, N).

4.5.18. N-{4-Chloro-5-[4-(4-chlorophenyl)thiazol-2-yl]-2-(3,4,5-trimethoxybenzylthio)benzenesulfonyl}cyanamide potassium salt (**5v**)

Starting from **4f** (0.221 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.231 g (70%): m.p. >320 °C; IR (KBr): ν 2934, 2173, 1596, 1507, 1458, 1331, 1287, 1141, 1123 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.76 (s, 6H, 2× OCH_3), 4.34 (s, 2H, SCH_2), 6.83 (s, 2H, Ar), 7.55–7.57 (m, 2H, Ar), 7.65 (s, 1H, Ar),

8.06–8.08 (m, 2H, Ar), 8.41 (s, 1H, Ar), 8.76 (s, 1H, Ar); Anal. $C_{26}H_{20}Cl_2KN_3O_5S_3$ (C, H, N).

4.5.19. N-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(3-phenyl-1,2,4-oxadiazol-5-yl)benzenesulfonyl]cyanamide potassium salt (**5w**)

Starting from **4g** (0.196 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.266 g (87%): m.p. 283–285 °C; IR (KBr): ν 2930, 2176, 1594, 1507, 1333, 1144, 1124 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.64 (s, 3H, OCH_3), 3.78 (s, 6H, 2× OCH_3), 4.40 (s, 2H, SCH_2), 6.85 (s, 2H, Ar), 7.59–7.63 (m, 3H, Ar), 7.76 (s, 1H, Ar), 8.09–8.14 (m, 2H, Ar), 8.56 (s, 1H, Ar); Anal. $C_{25}H_{20}ClKN_4O_6S_2$ (C, H, N).

4.5.20. N-{4-Chloro-5-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]-2-(3,4,5-trimethoxybenzylthio)benzenesulfonyl}cyanamide potassium salt (**5x**)

Starting from **4h** (0.214 g) and 3,4,5-trimethoxybenzyl chloride (0.130 g). Yield: 0.316 g (98%): m.p. >320 °C; IR (KBr): ν 2937, 2838, 2180, 1591, 1561, 1506, 1460, 1334, 1301, 1148, 1121 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.76 (s, 6H, 2× OCH_3), 4.39 (s, 2H, SCH_2), 6.84 (s, 2H, Ar), 7.66–7.68 (m, 2H, Ar), 7.75 (s, 1H, Ar), 8.11–8.12 (m, 2H, Ar), 8.55 (s, 1H, Ar); Anal. $C_{25}H_{19}Cl_2KN_4O_6S_2$ (C, H, N).

4.6. General procedure for the synthesis of 1-(2-mercaptobenzenesulfonyl)-3-hydroxyguanidines **6a–x**

A suspension of the appropriate *N*-(benzenesulfonyl)cyanamide potassium salt **5a–x** (0.25 mmol) and hydroxylamine hydrochloride (0.052 g, 0.75 mmol) in dry MeCN (3 ml) was refluxed for 20–24 h. After cooling to room temperature, stirring was continued overnight. The precipitate was filtered off, washed with MeOH, 50% MeOH/H_2O, water, MeOH and cold MeCN giving pure product.

4.6.1. 1-[2-Benzylthio-4-chloro-5-(5-phenyl-1,3,4-oxadiazol-2-yl)benzenesulfonyl]-3-hydroxyguanidine (**6a**)

Starting from **5a** (0.130 g). Yield: 0.128 g (99%): m.p. 214–216 °C; IR (KBr): ν 3444, 3336, 3206, 2923, 1622, 1586, 1553, 1492, 1450, 1326, 1280, 1162, 1117 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.48 (s, 2H, SCH_2), 7.19 (br s, 1H, NH), 7.30–7.33 (m, 1H, Ar), 7.37–7.40 (m, 3H, Ar), 7.64–7.71 (m, 3H, Ar), 7.74 (s, 1H, H-3), 8.11–8.12 (m, 2H, Ar), 8.56 (s, 1H, H-6), 9.47 (s, 1H, NH), 10.08 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 35.99, 117.73, 123.32, 127.07, 127.79, 128.58, 128.92, 129.51, 129.82, 130.17, 132.59, 134.51, 135.73, 139.85, 143.62, 158.90, 161.83, 164.57 ppm; Anal. $C_{22}H_{18}ClN_5O_4S_2$ (C, H, N).

4.6.2. 1-{2-Benzylthio-4-chloro-5-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]benzenesulfonyl}-3-hydroxyguanidine (**6b**)

Starting from **5b** (0.139 g). Yield: 0.132 g (96%): m.p. 238–240 °C; IR (KBr): ν 3444, 3342, 3203, 3067, 2900, 1620, 1580, 1548, 1482, 1328, 1290, 1153, 1123 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.48 (s, 2H, SCH_2), 7,20 (br s, 1H, NH), 7.30–7.32 (m, 1H, Ar), 7.37–7.40 (m, 3H, Ar and NH), 7.50–7.51 (m, 2H, Ar), 7.72–7.73 (m, 3H, Ar), 7.73 (s, 1H, H-3), 8.11–8.13 (m, 2H, Ar), 8.56 (s, 1H, H-6), 9.47 (s, 1H, NH), 10.07 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.01, 117.57, 122.22, 127.79, 128.57, 128.86, 128.92, 129.51, 129.98, 130.18, 134.52, 135.71, 137.29, 139.84, 143.73, 158.90, 161.96, 163.79 ppm; Anal. $C_{22}H_{17}Cl_2N_5O_4S_2$ (C, H, N).

4.6.3. 1-[2-Benzylthio-4-chloro-5-(5-phenyl-1,3,4-thiadiazol-2-yl)benzenesulfonyl]-3-hydroxyguanidine (**6c**)

Starting from **5c** (0.134 g). Yield: 0.110 g (83%): m.p. 234–236 °C; IR (KBr): ν 3451, 3332, 3275, 2923, 1622, 1576, 1521, 1495, 1456, 1349, 1150 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.46 (s, 2H, SCH_2), 7.29–7.61 (m, 10H, Ar and 2× NH), 7.73 (s, 1H, H-3), 8.05–8.08 (m, 2H, Ph), 8.74 (s, 1H, H-6), 9.47 (s, 1H, NH), 10.06 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.08, 123.78, 127.77, 128.01, 128.91, 129.50, 129.62, 129.65, 129.78, 129.86, 131.91, 134.22, 135.82, 140.09, 142.61, 158.97, 162.29, 169.29 ppm; Anal. $C_{22}H_{18}ClN_5O_3S_3$ (C, H, N).

4.6.4. 1-{2-Benzylthio-4-chloro-5-[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl]benzenesulfonyl}-3-hydroxyguanidine (**6d**)

Starting from **5d** (0.143 g). Yield: 0.095 g (67%): m.p. 245–246 °C; IR (KBr): ν 3454, 3378, 3334, 3254, 2924, 2854, 1625, 1576, 1531, 1495, 1456, 1248, 1151, 1130 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.47 (s, 2H, SCH_2), 7.29–7.51 (m, 7H, Ar and 2× NH), 7.65–7.69 (m, 2H, Ar), 7.72 (s, 1H, H-3), 8.08–8.12 (m, 2H, Ar), 8.75 (s, 1H, H-6), 9.47 (s, 1H, NH), 10.07 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.08, 107.38, 123.64, 127.78, 128.02, 128.32, 128.91, 129.50, 129.68, 129.90, 134.22, 135.79, 136.53, 140.07, 142.75, 158.97, 162.59, 168.08 ppm; Anal. $C_{22}H_{17}Cl_2N_5O_3S_3$ (C, H, N).

4.6.5. 1-[2-Benzylthio-4-chloro-5-(4-phenylthiazol-2-yl)benzenesulfonyl]-3-hydroxyguanidine (**6e**)

Starting from **5e** (0.134 g). Yield: 0.116 g (87%): m.p. 225–227 °C; IR (KBr): ν 3481, 3367, 3276, 2927, 1615, 1584, 1528, 1496, 1475, 1393, 1258, 1149 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.43 (s, 2H, SCH_2), 7.12–7.40 (m, 6H, Ar and NH), 7.47–7.51 (m, 4H, Ar), 7.66 (s, 1H, Ar), 8.05–8.06 (m, 2H, Ar), 8.35 (s, 1H, Ar), 8.82 (s, 1H, Ar), 9.44 (s, 1H, NH), 10.05 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.14, 116.92, 126.52, 126.85, 127.73, 128.34, 128.71, 128.89, 129.22, 129.50, 129.67, 133.40, 133.88, 135.97, 140.12, 140.70, 154.37, 159.06, 161.28 ppm; Anal. $C_{23}H_{19}ClN_4O_3S_3$ (C, H, N).

4.6.6. 1-{2-Benzylthio-4-chloro-5-[4-(4-chlorophenyl)thiazol-2-yl]benzenesulfonyl}-3-hydroxyguanidine (**6f**)

Starting from **5f** (0.143 g). Yield: 0.109 g (77%): m.p. 225–226 °C; IR (KBr): ν 3488, 3370, 3336, 3248, 3028, 2924, 1621, 1577, 1531, 1495, 1474, 1454 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.43 (s, 2H, SCH_2), 7.12–7.44 (m, 5H, Ar and NH), 7.47–7.48 (m, 2H, Ar), 7.54–7.56 (m, 2H, Ar), 7.66 (s, 1H, Ar), 8.07–8.09 (m, 2H, Ar), 8.42 (s, 1H, Ar), 8.81 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.06 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.13, 117.57, 126.70, 127.73, 128.25, 128.89, 129.24, 129.50, 129.63, 132.73, 133.19, 133.42, 135.95, 140.13, 140.83, 153.07, 159.07, 161.52 ppm; Anal. $C_{23}H_{18}Cl_2N_4O_3S_3$ (C, H, N).

4.6.7. 1-[2-Benzylthio-4-chloro-5-(3-phenyl-1,2,4-oxadiazol-5-yl)benzenesulfonyl]-3-hydroxyguanidine (**6g**)

Starting from **5g** (0.130 g). Yield: 0.107 g (83%): m.p. 224–225 °C; IR (KBr): ν 3483, 3370, 3329, 2923, 2854, 1613, 1579, 1561, 1538, 1351, 1150 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 4.51 (s, 2H, SCH_2), 7.28–7.60 (m, 7H, Ar and NH), 7.63–7.65 (m, 3H, Ar), 7.77 (s, 1H, Ar), 8.11–8.15 (m, 2H, Ar), 8.67 (s, 1H, Ar), 9.49 (s, 1H, NH), 10.09 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.00, 117.79, 126.14, 127.47, 127.83, 128.57, 128.94, 129.52, 129.63, 131.21, 132.11, 135.19, 135.62, 139.77, 145.16, 158.84, 168.29, 173.47 ppm; Anal. $C_{22}H_{18}ClN_5O_4S_2$ (C, H, N).

4.6.8. 1-{2-Benzylthio-4-chloro-5-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]benzenesulfonyl}-3-hydroxyguanidine (**6h**)

Starting from **5h** (0.139 g). Yield: 0.111 g (81%): m.p. 253–255 °C; IR (KBr): ν 3411, 3326, 3281, 3029, 2924, 1644, 1589, 1563, 1543, 1504, 1472, 1456, 1344, 1164 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.48 (s, 2H, SCH_2), 7.29–7.49 (m, 7H, Ar and NH), 7.67–7.69 (m, 2H, Ar), 7.73 (s, 1H, Ar), 8.10–8.12 (m, 2H, Ar), 8.63 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.06 (s, 1H, OH); ^{13}C NMR (50 MHz, DMSO-d_6): δ 36.02, 117.62, 125.00, 127.83, 128.55, 128.94, 129.26, 129.52, 129.79, 131.22, 135.21, 135.59, 136.83, 139.76, 145.30, 158.84, 167.49, 173.62 ppm; Anal. $C_{22}H_{17}Cl_2N_5O_4S_2$ (C, H, N).

*4.6.9. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(5-phenyl-1,3,4-oxadiazol-2-yl)benzenesulfonyl}-3-hydroxyguanidine (**6i**)*

Starting from **5i** (0.141 g). Yield: 0.077 g (55%): m.p. 230–232 °C; IR (KBr): ν 3436, 3141, 2926, 1704, 1591, 1530, 1496, 1450, 1351, 1172 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.32 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.88–6.89 (m, 1H, Ar), 6.96–6.97 (m, 1H, Ar), 7.02 (s, 1H, Ar), 7.18 (br s, 1H, NH), 7.38 (br s, 1H, NH), 7.63–7.70 (m, 4H, Ar), 8.09–8.10 (m, 2H, Ar), 8.54 (s, 1H, H-6), 9.44 (s, 1H, NH), 10.04 (s, 1H, OH); MALDI-TOF *m/z* obsd: 560.2, $[M + H]^+$, 582.2, $[M + Na]^+$ calcd: 559.0; Anal. $C_{23}H_{18}ClN_5O_6S_2$ (C, H, N).

*4.6.10. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]benzenesulfonyl}-3-hydroxyguanidine (**6j**)*

Starting from **5j** (0.150 g). Yield: 0.125 g (84%): m.p. 240–242 °C; IR (KBr): ν 3450, 3348, 3249, 2907, 1619, 1581, 1544, 1504, 1487, 1368, 1328, 1288, 1249, 1156 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.38 (s, 2H, SCH_2), 6.01 (s, 2H, OCH_2O), 6.89 (d, $J = 7.81$ Hz, 1H, Ar), 6.96 (d, $J = 7.81$ Hz, 1H, Ar), 7.02 (s, 1H, Ar), 7.17 (br s, 1H, NH), 7.39 (br s, 1H, NH), 7.71–7.73 (m, 3H, Ar), 8.10–8.12 (m, 2H, Ar), 8.54 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.05 (s, 1H, OH); MALDI-TOF *m/z* obsd: 594.0, $[M + H]^+$, 616.0, $[M + Na]^+$ calcd: 593.0; Anal. $C_{23}H_{17}Cl_2N_5O_6S_2$ (C, H, N).

*4.6.11. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(5-phenyl-1,3,4-thiadiazol-2-yl)benzenesulfonyl}-3-hydroxyguanidine (**6k**)*

Starting from **5k** (0.145 g). Yield: 0.118 g (82%): m.p. 231–233 °C; IR (KBr): ν 3486, 3441, 3352, 3322, 3098, 2923, 2854, 1626, 1577, 1558, 1540, 1500, 1468, 1457, 1373, 1247, 1160 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.37 (s, 2H, SCH_2), 6.01 (s, 2H, OCH_2O), 6.89 (d, $J = 7.81$ Hz, 1H, Ar), 6.97 (d, $J = 7.81$ Hz, 1H, Ar), 7.03 (s, 1H, Ar), 7.16 (br s, 1H, NH), 7.40 (br s, 1H, NH), 7.59–7.60 (m, 3H, Ar), 7.71 (s, 1H, Ar), 8.06–8.07 (m, 2H, Ar), 8.74 (s, 1H, Ar), 9.46 (s, 1H, NH), 10.05 (s, 1H, OH); MALDI-TOF *m/z* obsd: 576.0, $[M + H]^+$, 598.0, $[M + Na]^+$ calcd: 575.0; Anal. $C_{23}H_{18}ClN_5O_5S_3$(C, H, N).

*4.6.12. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl]benzenesulfonyl}-3-hydroxyguanidine (**6l**)*

Starting from **5l** (0.154 g). Yield: 0.122 g (80%): m.p. 234–236 °C; IR (KBr): ν 3458, 3354, 3297, 2922, 2853, 1626, 1577, 1535, 1503, 1489, 1363, 1253, 1150, 1128 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.36 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.89–7.02 (m, 3H, Ar), 7.15 (br s, 2H, NH), 7.38 (br s, 1H, NH), 7.65–7.67 (m, 2H, Ar), 7.71 (s, 1H, Ar), 8.08–8.10 (m, 2H, Ar), 8.73 (s, 1H, Ar), 9.44 (s, 1H, NH), 10.05 (s, 1H, OH); MALDI-TOF *m/z* obsd: 610.0, $[M + H]^+$, 632.0, $[M + Na]^+$ calcd: 609.0; Anal. $C_{23}H_{17}Cl_2N_5O_5S_3$ (C, H, N).

*4.6.13. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(4-phenylthiazol-2-yl)benzenesulfonyl}-3-hydroxyguanidine (**6m**)*

Starting from **5m** (0.145 g). Yield: 0.115 g (80%): m.p. 234–236 °C; IR (KBr): ν 3438, 3336, 2921, 1637, 1579, 1531, 1501, 1488, 1362, 1278, 1254, 1151, 1125 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.34 (s, 2H, SCH_2), 6.00 (s, 2H, OCH_2O), 6.88 (d, $J = 7.81$ Hz, 1H, Ar), 6.95 (d, $J = 7.81$ Hz, 1H, Ar), 7.03 (s, 1H, Ar), 7.12–7.50 (m, 4H, Ar and NH), 7.64 (s, 1H, Ar), 8.04–8.06 (m, 2H, Ar), 8.36 (s, 1H, Ar), 8.82 (s, 1H, Ar), 9.44 (s, 1H, NH), 10.05 (s, 1H, OH); MALDI-TOF *m/z* obsd: 575.2, $[M + H]^+$, 597.2, $[M + Na]^+$ calcd: 574.0; Anal. $C_{24}H_{19}ClN_4O_5S_3$ (C, H, N).

*4.6.14. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-[4-(4-chlorophenyl)thiazol-2-yl]benzenesulfonyl}-3-hydroxyguanidine (**6n**)*

Starting from **5n** (0.154 g). Yield: 0.125 g (82%): m.p. 261–263 °C; IR (KBr): ν 3438, 3306, 3230, 2923, 1629, 1575, 1544, 1501, 1489, 1473, 1254, 1156, 1130 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 4.35 (s, 2H, SCH_2), 6.01 (s, 2H, OCH_2O), 6.87–7.03 (m, 3H, Ar), 7.12–7.42 (br s, 2H, NH), 7.54–7.58 (m, 2H, Ar), 7.65 (s, 1H, Ar), 8.07–8.12 (m, 2H, Ar), 8.43 (s, 1H, Ar), 8.82 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.06 (s, 1H, OH); MALDI-TOF *m/z* obsd: 609.1, $[M + H]^+$, 631.1, $[M + Na]^+$ calcd: 608.0; Anal. $C_{24}H_{18}Cl_2N_4O_5S_3$ (C, H, N).

*4.6.15. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-(3-phenyl-1,2,4-oxadiazol-5-yl)benzenesulfonyl}-3-hydroxyguanidine (**6o**)*

Starting from **5o** (0.141 g). Yield: 0.116 g (83%): m.p. 235–237 °C; IR (KBr): ν 3486, 3370, 3302, 3204, 2923, 2854, 1612, 1586, 1560, 1550, 1502, 1491, 1475, 1353, 1255, 1154 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.40 (s, 2H, SCH_2), 6.01 (s, 2H, OCH_2O), 6.89 (d, $J = 7.81$ Hz, 1H, Ar), 6.98 (d, $J = 7.81$ Hz, 1H, Ar), 7.03 (s, 1H, Ar), 7.17 (br s, 1H, NH), 7.39 (br s, 1H, NH), 7.60–7.64 (m, 3H, Ar), 7.72 (s, 1H, Ar), 8.10–8.11 (m, 2H, Ar), 8.64 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.05 (s, 1H, OH); Anal. $C_{23}H_{18}ClN_5O_6S_2$ (C, H, N).

*4.6.16. 1-{2-[(1,3-Benzodioxol-5-yl)methylthio]-4-chloro-5-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]benzenesulfonyl}-3-(hydroxyguanidine) (**6p**)*

Starting from **5p** (0.150 g). Yield: 0.117 g (79%): m.p. 258–260 °C; IR (KBr): ν 3436, 3321, 2923, 1624, 1586, 1558, 1502, 1491, 1472, 1342, 1253, 1158 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 4.39 (s, 2H, SCH_2), 6.01 (s, 2H, OCH_2O), 6.88 (d, $J = 7.81$ Hz, 1H, Ar), 6.96 (d, $J = 7.81$ Hz, 1H, Ar), 7.03 (s, 1H, Ar), 7.18 (br s, 1H, NH), 7.39 (br s, 1H, NH), 7.67–7.69 (m, 2H, Ar), 7.72 (s, 1H, Ar), 8.10–8.12 (m, 2H, Ar), 8.63 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.05 (s, 1H, OH); Anal. $C_{23}H_{17}Cl_2N_5O_6S_2$ (C, H, N).

*4.6.17. 1-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(5-phenyl-1,3,4-oxadiazol-2-yl)benzenesulfonyl]-3-hydroxyguanidine (**6q**)*

Starting from **5q** (0.153 g). Yield: 0.133 g (88%): m.p. 235–237 °C; IR (KBr): ν 3434, 3344, 2999, 2929, 1626, 1589, 1555, 1507, 1460, 1338, 1126 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.77 (s, 6H, 2× OCH_3), 4.39 (s, 2H, SCH_2), 6.82 (s, 2H, Ar), 7.19 (br s, 1H, NH), 7.39 (br s, 1H, NH), 7.63–7.64 (m, 3H, Ar), 7.75 (s, 1H, H-3), 8.08–8.09 (m, 2H, Ar), 8.54 (s, 1H, H-6), 9.45 (s, 1H, NH), 10.05 (s, 1H, OH); MALDI-TOF *m/z* obsd: 606.1, $[M + H]^+$, 628.1, $[M + Na]^+$ calcd: 605.0; Anal. $C_{25}H_{24}ClN_5O_7S_2$ (C, H, N).

*4.6.18. 1-{4-Chloro-5-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-2-(3,4,5-trimethoxybenzylthio)benzenesulfonyl}-3-hydroxyguanidine (**6r**)*

Starting from **5r** (0.161 g). Yield: 0.125 g (78%): m.p. 227–229 °C; IR (KBr): ν 3457, 3355, 3271, 2927, 1631, 1588, 1556, 1506, 1481, 1334, 1158 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.63 (s, 3H, OCH_3), 3.77 (s, 6H, 2× OCH_3), 4.40 (s, 2H, SCH_2), 6.82 (s, 2H, Ar), 7.19 (br s, 1H, NH), 7.42 (br s, 1H, NH), 7.71–7.73 (m, 2H, Ar), 7.76 (s, 1H, Ar), 8.10–8.11 (m, 2H, Ar), 8.54 (s, 1H, Ar), 9.46 (s, 1H, NH), 10.06 (s, 1H, OH); MALDI-TOF *m/z* obsd: 640.2, $[M + H]^+$, 662.2, $[M + Na]^+$ calcd: 639.0; Anal. $C_{25}H_{23}Cl_2N_5O_7S_2$ (C, H, N).

*4.6.19. 1-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(5-phenyl-1,3,4-thiadiazol-2-yl)benzenesulfonyl]-3-hydroxyguanidine (**6s**)*

Starting from **5s** (0.156 g). Yield: 0.107 g (69%): m.p. 187–189 °C; IR (KBr): ν 3475, 3363, 3280, 2933, 1614, 1592, 1557, 1506, 1458, 1334, 1246, 1143, 1123 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.64 (s, 3H, OCH_3), 3.78 (s, 6H, 2× OCH_3), 4.39 (s, 2H, SCH_2), 6.82 (s, 2H, Ar), 7.15–7.45 (m, 2H, 2× NH), 7.59–7.62 (m, 3H, Ar), 7.76 (s, 1H, Ar), 8.05–8.10 (m, 2H, Ar), 8.74 (s, 1H, Ar), 9.47 (s, 1H, NH), 10.06 (s, 1H, OH); MALDI-TOF *m/z* obsd: 622.0, $[M + H]^+$, 644.1, $[M + Na]^+$ calcd: 621.0; Anal. $C_{25}H_{24}ClN_5O_6S_3$ (C, H, N).

*4.6.20. 1-{4-Chloro-5-[5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl]-2-(3,4,5-trimethoxybenzylthio)benzenesulfonyl}-3-hydroxyguanidine (**6t**)*

Starting from **5t** (0.165 g). Yield: 0.131 g (80%): m.p. 228–230 °C; IR (KBr): ν 3433, 3339, 3274, 2927, 2839, 1620, 1594, 1542, 1508, 1461, 1334, 1240, 1153, 1126 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d$_6$): δ 3.63 (s, 3H, OCH$_3$), 3.77 (s, 6H, 2× OCH$_3$), 4.38 (s, 2H, SCH$_2$), 6.81 (s, 2H, Ar), 7.13 (br s, 1H, NH), 7.40 (br s, 1H, NH), 7.66–7.67 (m, 2H, Ar), 7.75 (s, 1H, Ar), 8.08–8.10 (m, 2H, Ar), 8.74 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.05 (s, 1H, OH); MALDI-TOF *m*/*z* obsd: 656.0, [M + H]$^+$, 678.1, [M + Na]$^+$ calcd: 655.0; Anal. $C_{25}H_{23}Cl_2N_5O_6S_3$ (C, H, N).

*4.6.21. 1-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(4-phenylthiazol-2-yl)benzenesulfonyl]-3-hydroxyguanidine (**6u**)*

Starting from **5u** (0.157 g). Yield: 0.124 g (80%): m.p. 233–235 °C; IR (KBr): ν 3423, 3317, 2933, 1617, 1593, 1540, 1507, 1458, 1333, 1277, 1144, 1127 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d$_6$): δ 3.63 (s, 3H, OCH$_3$), 3.77 (s, 6H, 2× OCH$_3$), 4.36 (s, 2H, SCH$_2$), 7.17–7.40 (m, 3H, Ar and NH), 7.47–7.51 (m, 2H, Ar), 7.69 (s, 1H, Ar), 8.05–8.07 (m, 2H, Ar), 8.36 (s, 1H, Ar), 8.82 (s, 1H, Ar), 9.46 (s, 1H, NH), 10.06 (s, 1H, OH); MALDI-TOF *m*/*z* obsd: 621.3, [M + H]$^+$, 643.3, [M + Na]$^+$ calcd: 620.1; Anal. $C_{26}H_{25}ClN_4O_6S_3$ (C, H, N).

*4.6.22. 1-{4-Chloro-5-[4-(4-chlorophenyl)thiazol-2-yl]-2-(3,4,5-trimethoxybenzylthio)benzenesulfonyl}-3-hydroxyguanidine (**6v**)*

Starting from **5v** (0.165 g). Yield: 0.134 g (82%): m.p. 241–243 °C; IR (KBr): ν 3429, 3314, 2931, 1617, 1597, 1540, 1508, 1461, 1334, 1274, 1129 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d$_6$): δ 3.64 (s, 3H, OCH$_3$), 3.77 (s, 6H, 2× OCH$_3$), 4.37 (s, 2H, SCH$_2$), 6.81 (s, 2H, Ar), 7.26–7.34 (br s, 2H, NH), 7.54–7.58 (m, 2H, Ar), 7.70 (s, 1H, Ar), 8.07–8.11 (m, 2H, Ar), 8.43 (s, 1H, Ar), 8.82 (s, 1H, Ar), 9.46 (s, 1H, NH), 10.07 (s, 1H, OH); MALDI-TOF *m*/*z* obsd: 655.1, [M + H]$^+$, 677.1, [M + Na]$^+$ calcd: 654.0; Anal. $C_{26}H_{24}Cl_2N_4O_6S_3$ (C, H, N).

*4.6.23. 1-[4-Chloro-2-(3,4,5-trimethoxybenzylthio)-5-(3-phenyl-1,2,4-oxadiazol-5-yl)benzenesulfonyl]-3-hydroxyguanidine (**6w**)*

Starting from **5w** (0.153 g). Yield: 0.077 g (51%): m.p. 228–230 °C; IR (KBr): ν 3440, 3334, 2933, 2837, 1622, 1592, 1545, 1506, 1461, 1380, 1351, 1334, 1286, 1247, 1159, 1128 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d$_6$): δ 3.64 (s, 3H, OCH$_3$), 3.78 (s, 6H, 2× OCH$_3$), 4.42 (s, 2H, SCH$_2$), 6.83 (s, 2H, Ar), 7.20–7.40 (m, 2H, 2× NH), 7.60–7.64 (m, 3H, Ar), 7.78 (s, 1H, Ar), 8.09–8.13 (m, 2H, Ar), 8.64 (s, 1H, Ar), 9.46 (s, 1H, NH), 10.06 (s, 1H, OH); Anal. $C_{25}H_{24}ClN_5O_7S_2$ (C, H, N).

*4.6.24. 1-{4-Chloro-5-[3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl]-2-(3,4,5-trimethoxybenzylthio)benzenesulfonyl}-3-hydroxyguanidine (**6x**)*

Starting from **5x** (0.161 g). Yield: 0.122 g (76%): m.p. 243–245 °C; IR (KBr): ν 3457, 3353, 3268, 2936, 1631, 1589, 1558, 1505, 1459, 1335, 1157 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 3.63 (s, 3H, OCH$_3$), 3.77 (s, 6H, 2× OCH$_3$), 4.40 (s, 2H, SCH$_2$), 6.81 (s, 2H, Ar), 7.19 (br s, 1H, NH), 7.40 (br s, 1H, NH), 7.67–7.69 (m, 2H, Ar), 7.77 (s, 1H, Ar), 8.10–8.12 (m, 2H, Ar), 8.63 (s, 1H, Ar), 9.45 (s, 1H, NH), 10.05 (s, 1H, OH); Anal. $C_{25}H_{23}Cl_2N_5O_7S_2$ (C, H, N).

4.7. Molecular modeling

QSAR studies were conducted to better understand the biological activity of this series of compounds.

SYBYL (Tripos Inc., St. Louis, USA, Ver. 8.0) molecular modeling software package running on Linux workstation was used to perform QSAR analysis. 3D structures of all 5-dose assayed 19 compounds were constructed using Sketch Molecule module and were energy-minimized to the putative global low-energy conformation using the standard Tripos molecular mechanics force field [28,29] with a distance-dependent (1/*r*) dielectric function and the Powell conjugate-gradient algorithm with convergence criterion of 0.05 kcal/mol. Atomic partial charges were computed using the Geisteiger–Hückel method [30,31]. In CoMSIA studies, a common probe atom with 1.0 Å radius, +1.0 formal charge and +1.0 hydrophobicity was used to evaluate three interaction fields: steric, electrostatic and hydrophobic. Because our data set is structurally consistent all molecules in the created database were aligned to the common core of 1-(4-chloro-2-mercaptobenzenesulfonyl)-3-hydroxyguanidine and **6k** as the most active compound was chosen as a template molecule (Fig. 2a). CoMSIA field descriptors used in the modeling gave some insight into 3D structure–antiproliferative activity relationship (Fig. 3).

The aligned molecules were set in a Cartesian coordinate box, a 3D regular lattice with 2 Å grid spacing, and similarity indices were calculated at each grid point using following equation:

$$A^q_{F,k}(j) = \sum_i w_{probe,k} w_{ik} e^{-\alpha r^2_{iq}}$$

A is the similarity index at grid point *q*, summed overall atoms (*i*) of the molecule *j*; $w_{probe,k}$ is the probe atom, w_{ik} is the actual value of the physicochemical property (*k*) of atom *i*; r_{iq} is the mutual distance between the probe atom at grid point *q* and atom *i* of the test molecule; α is the attenuation factor.

Because of used in this calculation Gaussian-type distance dependence, the similarity indices can be calculated at all grid points (both inside and outside the molecular surface); therefore, no cut-off values need to be defined. The only field parameter available that can be modified at this step is α, which determines the steepness of the Gaussian function, and was set to the default, yet optimal value (0.3). Here, steric indices are related to the third power of the atomic radii, electrostatic descriptors are derived from atomic partial charges, hydrophobic fields are derived from atom-

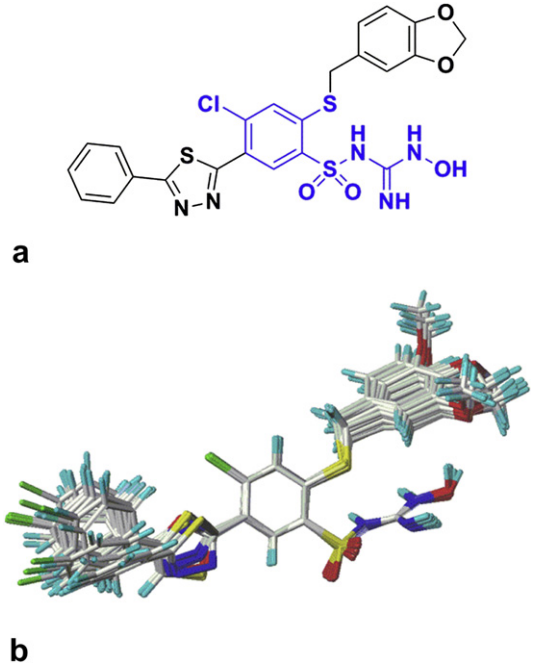


Fig. 2. (a) Template molecule (**6k**) used for alignment (common substructure shown in blue). (b) Training set compounds aligned on minimum energy conformation of compound **6k**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

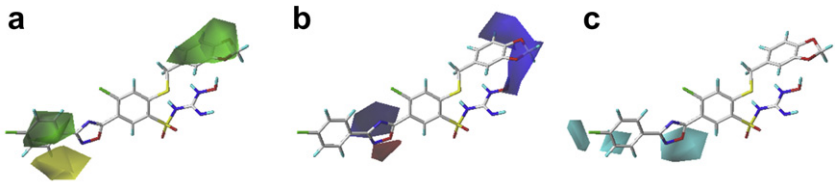


Fig. 3. Contour maps of CoMSIA fields contributing to ligand binding generated by PLS analysis in Model_B. Compound **6p** (stick model) is shown as a reference to depict the field regions. The contours of the steric map are shown in yellow and green (a), those of the electrostatic map are shown in red and blue (b) and contour map of hydrophobic field in cyan (c). Greater values of activity are correlated with: more bulk near green; less bulk near yellow; more positive charge near blue, and more negative charge near red; hydrophobic moieties near cyan. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

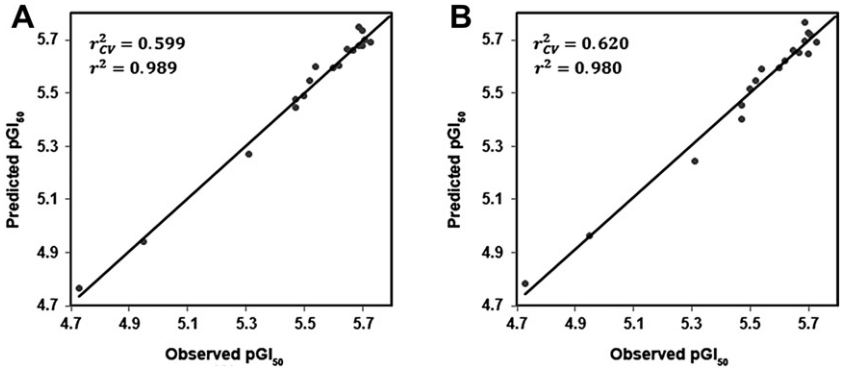


Fig. 4. Plot of observed vs. predicted activities (MG–MID pGI$_{50}$) for the training set compounds based on the best QSAR model (A) and CoMSIA region-focusing model (B).

based parameters [32]. Biological activities for this set of compounds fall within the range 10^{-5} to 10^{-6} M (data presented in supporting materials in Table 6) and this led to challenges to obtain good model of high cross-validated r^2 (q^2) value.

$$q^2 = 1 - \frac{\sum_Y \left(Y_{pred} - Y_{actual}\right)^2}{\sum_Y (Y_{actual} - Y_{mean})^2}$$

Y_{pred} is a predicted value; Y_{actual} is an actual (experimental) value; Y_{mean} is the best estimate of the mean of all values that might be predicted.

In order to get an optimal QSAR model, different descriptors were used, namely, log of the *n*-octanol/water partition coefficient (*CLogP*), molar refractivity (CMR), polar volume (PV) and polar surface area (PSA). The constructed best QSAR model (Model_A) was further optimized. The best results were obtained at a column filtering (*Cf*) of 0.5 kcal/mol. CoMSIA Hydrophobic explanatory variable contributed mostly in the obtained model (data presented in supporting materials in Table 7) and CoMSIA region-focusing method was employed to derive Model_B (Fig. 4).

The model confirms that the bulky substituent, especially (1,3-benzodioxol-5-yl)methylthio (**6i**–**p**), at 2 position of 1-benzenesulfonyl-3-hydroxyguanidine core accompanied with positive electrostatic charge projecting above and electronegative below the heterocyclic residue (1,3,4-thiadiazole ring) in 5 position of the core structure correlate with better activity (**6c**, **6d**, **6k**, **6l**, **6s**, **6t**). The model does not judge weather phenyl or 4-chlorophenyl is preferred at heterocycle moiety.

The main outcome of this complex model is that the potency of this series of compounds seems to accompany not only local steric, electrostatic or hydrophobic properties but overall structural features associated with the whole molecule attributes like molar refractivity, hydrophobicity and polar surface properties of the molecular structure, represented in the model by 0.541 (Model_A) or 0.550 (Model_B) fraction (descriptors: CMR, *CLogP*, PSA and PV) of explanatory variables (data presented in supporting materials in Table 7).

4.8. COMPARE analysis

The biological activity results (NCI-60 panel inhibition profiles) of 11 representatives were subjected to COMPARE analysis [33,34]. Some of them showed good correlation (PCC = 0.510–0.806) with nucleic acids interfering agents like dactinomycin, bruceantin, chromomycin A3 and echinomycin (Table 4). The highest correlation coefficients were identified when compounds **6d**, **6g** and **6r**

Table 4
COMPARE analysis of eleven 5-dose assayed 1-benzenesulfonyl-3-hydroxyguanidines (**6c**, **6d**, **6f**, **6g**, **6i**, **6j**, **6m**, **6p**, **6r**, **6s**, and **6v**). Compounds with the highest correlation coefficient were bolded out.

Target vector[a]	No.[b]	PCC range[c]	Correlated compounds[c]
Dactinomycin (NSC 3053)	11	0.510–0.806	6c, 6d, 6f, **6g**, 6i, 6j, 6m, 6p, 6r, 6s, 6v
Bruceantin (NSC 165563)	10	0.539–0.791	6c, **6d**, 6g, 6i, 6j, 6l, 6p, 6r, 6s, 6v
Chromomycin A3 (NSC 58514)	8	0.596–0.799	6c, 6d, 6g, 6i, 6j, **6r**, 6s, 6v
Echinomycin (NSC 526417)	8	0.555–0.760	6c, 6d, **6g**, 6i, 6j, 6p, 6s, 6v
Didemnin B (NSC 325319)	6	0.522–0.712	6c, 6d, **6g**, 6p, 6s, 6v

[a] For definitions and methods of calculation of the correlation coefficient (PCC) from the COMPARE analysis, see Ref. [33,34].
[b] Number of correlated compounds giving PCC >0.5.
[c] Pearson Correlation coefficient (PCC) range for the described compounds.
[d] Compounds in NIH-DTP STANDARD_AGENTS database, giving correlation coefficient >0.5.

were used as Seed [34]. This could indicate DNA as a potential molecular target for the synthesized 1-benzenesulfonyl-3-hydroxyguanidines and further biological studies are needed to verify the suggested by COMPARE algorithm hypothesis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ejmech.2012.07.042.

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