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Novel 2-(2-alkylthiobenzenesulfonyl)-3-(phenylprop-2-ynylideneamino)guanidine derivatives as potent anticancer agents e Synthesis, molecular structure, QSAR studies and metabolic stability

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a b s t r a c t

A series of new 2-(2-alkylthiobenzenesulfonyl)-3-(phenylprop-2-ynylideneamino)guanidine derivatives have been synthesized and evaluated in vitro by MTT assays for their antiproliferative activity against cell lines of colon cancer HCT-116, cervical cancer HeLa and breast cancer MCF-7. The obtained results indicated that these compounds display prominent cytotoxic effect. The best anticancer properties have been observed for derivatives 44 (IC_{50} ¼ 6e18 μM) and 45 (IC_{50} ¼ 8e14 μM). Very good results of antiproliferative assays have been also shown for compounds 26, 36, and 46 and noticeable anticancer profile has been found for set of derivatives 34e39. Based on results of MTT assays the structure-activity relationships have been drawn. More in-depth biological research revealed that compounds 26, 33, 37, 39, 41 and 43 display cytotoxic effect only against cancer cells and do not inhibit the growth of non-malignant HaCaT cells. Furthermore, the novel series of derivatives have shown good metabolic stabil-ity, especially among the pharmacologically active compounds. To obtain a deeper insight into the molecular description of compounds activity the QSAR studies have been applied. Support vector ma-chines (SVM) have been used to developed QSAR models for predicting the anti-proliferative activity of novel derivatives. The obtained SVM models have shown prognostic ability for HCT-116 and HeLa cell lines and as a result these models may be useful for further development of structurally similar de-rivatives with better biological properties.

Keywords: Benzenesulfonamides, Synthesis Anticancer, activity QSAR studies, Metabolic stability

1. Introduction

The twentieth century recorded the greatest advance in the control of human disease connected with microbial infections. At the present time however, despite technological and social development, neoplastic diseases have become one of the most common healthcare problem and a leading cause of human suffering and death [1]. Increasing drug-resistance of mammalian tumors as well as severe side-effects of chemotherapeutic agents reduce the clinical efficacy of currently used anticancer drugs and therapies. Thus, there is always a constant need to develop new chemotherapeutics with an unique mechanism of action.

Sulfonamides constitute an interesting class of novel anticancer agents. Many of these compounds have been recognized as unique therapeutics with a novel antitumor mechanism. For instance, indisulam is regarded as breakthrough in the search for sulfonamides with strong antineoplastic properties and belongs to a class of novel cell cycle inhibitors that block cell cycle progression at multiple points, although its target remains unclear [2–5]. However, since the last decade, when indisulam was investigated at

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different phase of clinical research, a lot of novel anticancer sulfonamides have been introduced into either clinical trials or approved to treat different types of cancer (Fig. 1) [6–18].

Pazopanib, a small molecule inhibitor of multiple protein tyrosine kinases, is approved to treat advanced renal cell carcinoma [6] and advanced soft tissue sarcoma in patients who received prior chemotherapy [7]. Another drug, dabrafenib, which inhibits the activity of B-raf (BRAF) protein, is accepted to treat patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation [12]. In 2014, FDA has also granted accelerated approval to a novel hydroxamic acid-type histone deacetylase (HDAC) inhibitor, belinostat, for the treatment of patients with relapsed or refractory peripheral T-cell lymphoma (PTCL) [13]. Apart from all medical use mentioned already, those three compounds are also investigated at different phases of clinical trials (see Table 1) and so too are novel sulfonamides, especially any of the following: MK-2461, R547 or XL-147 (Fig. 1). MK-2461 is ATP-competitive multitargeted inhibitor of the activated c-Met, a member of a receptor tyrosine kinase subfamily, which is involved in proliferation of several gastric and lung cancer cell lines [19]. R547 effectively blocks a cell cycle progression at G(1) and G(2) phases and induces apoptosis of tumor. Interestingly enough, the growth-inhibitory activity is independent of multidrug resistant status, histologic type, retinoblastoma protein, or p53 status and is related with the inhibition of the cyclin-dependent protein kinases (CDK) [16]. XL-147 (SAR245408) acts as selective PI3K inhibitor and inhibits tumor growth and survival [20]. All of these compounds are investigated in comprehensive research regarding wide range of cancers. Due to a lot of clinical trials concerning above mentioned sulfonamides, the current state of only several is summarized in Table 1 [21].

According to anticancer activity of compounds containing sulfonamide moiety as well as our previous study for 2-mercaptobenzenesulfonamides with cytostatic properties [22–26] we have designed novel derivatives of 2-(2-alkylthiobenzenesulfonyl)-3-(3-phenylpro-2-ynylideneamino) guanidine of type **I** (Fig. 1). This modification is motivated by fact that both the tautomeric amidinohydrazone group and alkynyl scaffold are important moieties modulating anticancer properties of compounds [27–31].

2. Results and discussion

2.1. Chemistry

The starting substrates, 3-amino-6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazine **1** [32], *N*-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)cyanamide potassium salt (**2**, **5**, **7**, **8**, **11**–**13**) [32–35], as well as 1-amino-2-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)guanidines (**14, 17, 19–20, 23–25**) [33,36,37] were obtained as previously described. Similarly, novel substrates have been synthesized. According to Scheme 1, 3-aminobenzodithiazine **1** reacts with potassium carbonate and appropriate alkyl chloride giving novel potassium salts **3**–**4**, **6** and **9**–**10** which are further reacted with hydrazine monohydrochloride and converted into aminoguanidines **15**–**16**, **18** and **21**–**22**. The final products, 2-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-(3-phenylprop-2-ynylideneamino)guanidine derivatives **26**–**49** were obtained by treatment of aminoguanidines **14**–**25** with 4-phenyl-3-butyn-2-one or phenylpropiolaldehyde diethyl acetal in the presence of *p*-toluenesulfonic acid (PTSA) as catalyst.

The structures of novel monopotassium salts **3**–**4**, **6** and **9**–**10** as well as aminoguanidines **15**–**16**, **18** and **21**–**22** were confirmed by elemental analyses, IR and ^{1}H NMR spectroscopy. IR spectra of potassium salts showed the most characteristic absorption band derived from C≡N group that appeared in the range of 2169–2179 cm^{-1}. In the case of aminoguanidines signals in ^{1}H NMR spectra derived from two groups NH_2 are the most representative. These protons are observed as two singlets, the first due to C-NH_2 ranging from 4.44 to 4.49 ppm and the second assign to NNH_2 in

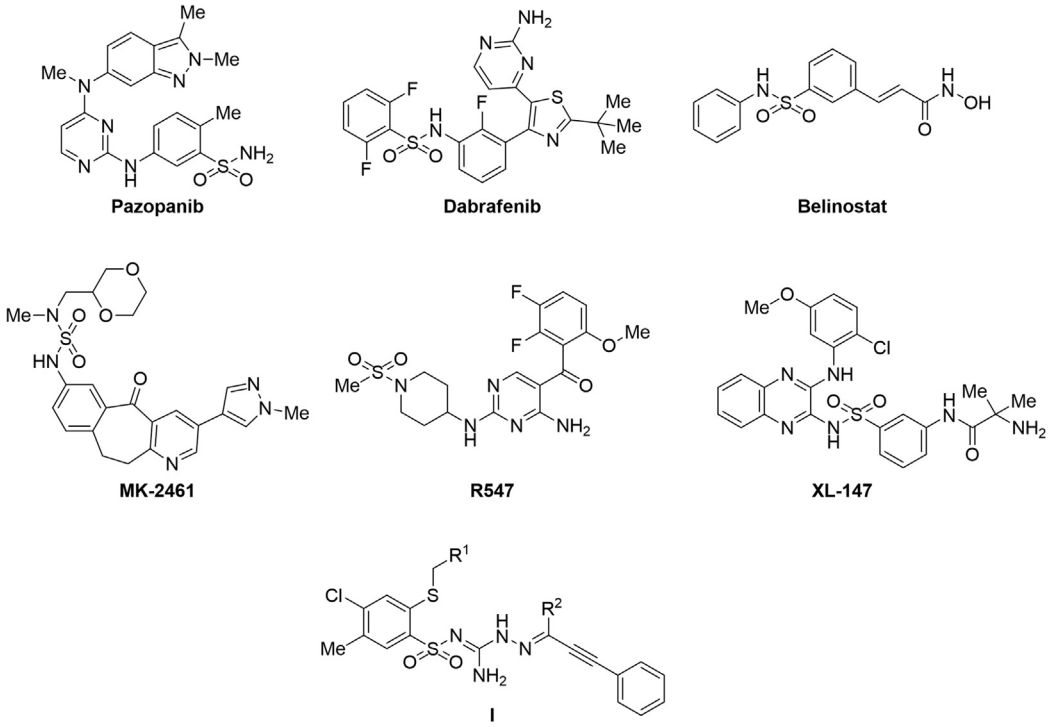


Fig. 1. General structures of known compounds containing sulfonamide moiety with anticancer activity (**pazopanib**, **dabrafenib**, **belinostat**, **MK-2461**, **R547** and **XL-147**) and novel 2-(2-alkylthiobenzenesulfonyl)-3-(3-phenylpro-2-ynylideneamino)guanidines I.

Table 1
Clinical Trial Information about pazopanib, dabrafenib, belinostat, MK-2461, R547 and XL-147 [21].

Drug	Condition	Study Start Date–Study Completion Date	Phase
Pazopanib(GW786034)	Thyroid Carcinoma	2013–2016	II
	Stromal Tumors (GIST)	2011–2016	II
	Neoadjuvant Breast Cancer	2009–2013	II
	Non-Small Cell Lung Cancer	2008-ongoing	II/III
	Small Cell Lung Cancer	2013-ongoing	II
	Sarcoma	2014-ongoing	II
	Liver Cancer	2006–2009	I
	Lymphoma	2008–2013	I
	Hormone-Resistant Prostate Cancer and Recurrent Prostate Cancer	2007-ongoing	II
	Ovarian, Fallopian Tube or Primary Peritoneal Cancer	2009-ongoing	III
	Osteosarcoma Metastatic to the Lung	2013-ongoing	II
	Advanced Angiosarcoma	2011-ongoing	II
	Chondrosarcoma	2011-ongoing	II
	Advanced and/or Metastatic Liposarcoma	2013-ongoing	II
	Solitary Fibrous Tumor and Extraskeletal Myxoid Chondrosarcoma	2014-ongoing	II
	Metastatic Kidney Cancer	2013-ongoing	II
	Recurrent Glioblastoma	2007-ongoing	II
	Recurrent or Persistent Uterine Cancer	2011-ongoing	II
	Metastatic Melanoma	2009-ongoing	II
	Nasopharyngeal Cancer	2007–2010	II
	Advanced Breast Cancer	2012-ongoing	II
	Gastrointestinal Stromal Tumors	2012–2015	II
	Salivary GlandCarcinoma	2013-ongoing	II
	Adenocarcinoma of the Prostate	2013-ongoing	II
	Metastatic Urothelial Cancer	2008–2013	II
	Metastatic Urethral Cancer or Bladder Cancer	2009–2012	II
	Advanced Neuroendocrine Cancer	2007–2014	II
		2010–2013	II
Dabrafenib (GSK2118436)	Brain Neoplasms	2013-ongoing	I
	BRAF Mutated Ameloblastoma	2015-ongoing	pilot
	High-grade glioma	2016-ongoing	II
	Melanoma and Brain Metastases	2011-ongoing	II
	BRAF V600E Mutation Positive Metastatic Non-Small Cell Lung Cancer	2011–2012	II
Belinostat (PXD101)	Liver Cancer	2006-ongoing	I/II
	Lymphomas	2010-ongoing	I
	Malignant Mesothelioma of the Chest	2006–2009	II
	Thymic Carcinoma	2007–2014	II
	Relapsed or Refractory Aggressive B-Cell Non-Hodgkin's Lymphoma	2006–2010	II
MK-2461	Advanced Solid Tumors	2007–2008	I/II
	Advanced Cancer	2007–2008	I
R547	Advanced Solid Tumors	2005–2008	I
XL-147 (SAR245408)	Endometrial Cancer	2010–2013	II
	Solid Tumors	2007–2012	I
	Solid Tumors or Lymphoma	2013–2015	I

the range of 6.94–6.98 ppm.

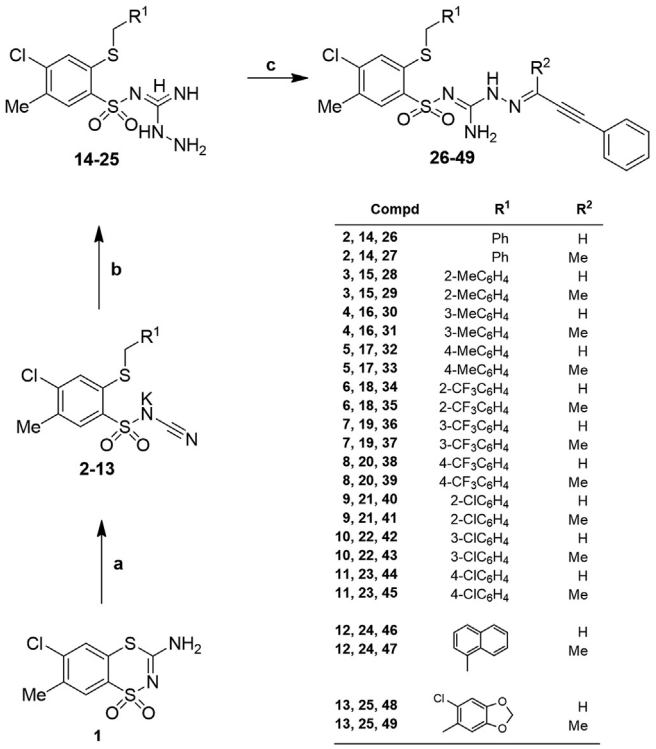
Confirmation of the structures of final compounds **26**–**49** was obtained with spectroscopic methods IR, ^{1}H NMR and ^{13}C NMR as well as HRMS spectrometry and elemental analyses. All synthesized compounds can occur in three tautomeric forms (Fig. 2).

According to the proton NMR spectra of the starting aminoguanidines it was expected to show for compounds **26**–**49** two signals due to NH protons from both CNH_2 and NHN groups. However, ^{1}H NMR revealed that NH protons can be integrated in three separate signals: the first in the range of 7.42–7.77 ppm, the second which is often seen as a broad singlet ranging from 7.66 to 7.89 ppm and the third as a sharp singlet in the range of 10.87–11.35 ppm. These could suggest that the form **II** is the right structure. However, NMR spectra were obtained in DMSO-d_6 which may affect tautomeric equilibrium of the obtained derivatives. In order to confirm the structures in the solid state, X-ray analyses of compounds **28**, **35**, **42** and **43** were done. Details on data collection, structure solution and refinement for all structures are given in the Supplementary Material (Tables 1S-2S). The results of these studies revealed that sulfonamidic nitrogen atom is deprotonated in the solid state indicating that the tautomer **I** is correct (Figs. 3–8).

Compound **28** crystallizes in the monoclinic system, the space group $P2_1/n$, with one molecule in the asymmetric unit, and four in the unit cell, $Z = 4$. Atom numbering scheme is presented in Fig. 3.

The S1 sulfur atom coordination is close to tetrahedral and all nitrogen atoms and C8 are arranged in a plane. The structure of the sulfonamide motif is confirmed by a typical S1-N1 bond length (*ca* 1.6 Å) and by participation of N1, as an acceptor, in formation of intermolecular hydrogen bond with H2A-N2 group from a neighbour molecule (see the Supplementary Material, Table 3S). It creates a robust ring hydrogen bond motif, $R_2^2(8)$ [38], around a local inversion centre and is accompanied by (and fused with) another centrosymmetric ring $R_4^2(8)$, based on four donors and O1. These all together form a chain of hydrogen bonding interactions spreading along the crystallographic axis (Fig. 4). Moreover it should be noticed that rings C1-C6 and C19-C24 are almost parallel with dihedral angle 18.94° (see Table 2).

Compound **35** crystallizes in the triclinic system, the space group $P\bar{1}$ with one molecule in the asymmetric unit, and two in the unit cell, $Z = 2$. Atom numbering scheme is presented in Fig. 5. In order to keep equivalence of atoms with the same label, the methyl group at C9 in **35** was given the highest number (C26).



Compd	R^1	R^2
2, 14, 26	Ph	H
2, 14, 27	Ph	Me
3, 15, 28	2-MeC_6H_4	H
3, 15, 29	2-MeC_6H_4	Me
4, 16, 30	3-MeC_6H_4	H
4, 16, 31	3-MeC_6H_4	Me
5, 17, 32	4-MeC_6H_4	H
5, 17, 33	4-MeC_6H_4	Me
6, 18, 34	2-$CF_3C_6H_4$	H
6, 18, 35	2-$CF_3C_6H_4$	Me
7, 19, 36	3-$CF_3C_6H_4$	H
7, 19, 37	3-$CF_3C_6H_4$	Me
8, 20, 38	4-$CF_3C_6H_4$	H
8, 20, 39	4-$CF_3C_6H_4$	Me
9, 21, 40	2-ClC_6H_4	H
9, 21, 41	2-ClC_6H_4	Me
10, 22, 42	3-ClC_6H_4	H
10, 22, 43	3-ClC_6H_4	Me
11, 23, 44	4-ClC_6H_4	H
11, 23, 45	4-ClC_6H_4	Me
12, 24, 46		H
12, 24, 47		Me
13, 25, 48		H
13, 25, 49		Me

Scheme 1. Reagents and conditions: **a**) anhydrous K_2CO_3 (5.075 eq.), alkyl chloride (1.05 eq.), dry THF, reflux, 18–26 h; **b**) $NH_2NH_2 \cdot HCl$ (1 eq.), dry toluene, reflux, 3–5 h; **c**) phenylpropiolaldehyde diethyl acetal (1 eq.) or 4-phenylbut-3-yn-2-one (1 eq.), PTSA (0.1 eq.), EtOH, reflux, 2–20 h.

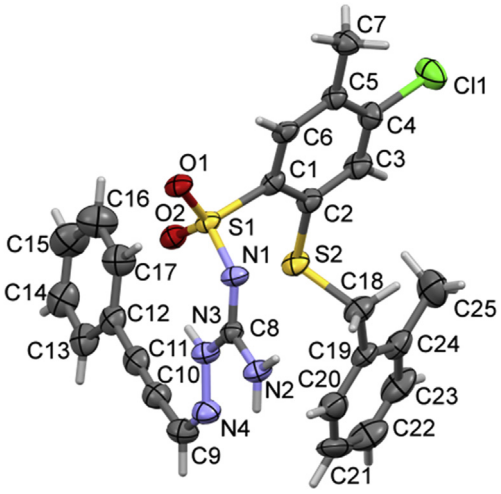


Fig. 3. Molecular structure of **28**, showing atom numbering scheme. Displacement ellipsoids drawn at 50% probability level. Rings C1-C6 and C19-C24 are almost parallel (dihedral angle 18.94°, see Table 2).

Table 2
Selected dihedral angles (acute) [°] in **28**, **35**, **42** and **43**. Definitions of the mean square planes: R1 = C1– C6 (sulfonyl phenyl), R2 = C12– C17 (phenylethynyl) R3 = C19– C24 (thiobenzyl ring), R4 = C8, N1-N4 (nitrogen-base plane). The angle values most relevant for the structural differences between the compounds of R^2 = Me and R^2 = H are bold.

Dihedral angles	**28**	**35**	**42**	**43**
R1– R2	78.62	79.44	84.88	88.98
R1– R3	**18.94**	**77.95**	**13.46**	**48.50**
R2– R3	59.79	66.82	86.74	48.85
R1– R4	83.66	88.21	81.57	89.33
R2– R4	6.36	12.90	30.05	19.15

Coordination environment of sulfur atom S1 is close to tetrahedral. Similar robust ring hydrogen bond motif, $R_2^2(8)$, around local inversion centre (compare hydrogen bond details given in the Supplementary Material Table 4S, and Fig. 6). One more strong hydrogen bond is formed inside the molecule between N3-H3A and O1. Those NH···N and NH···O interactions give supramolecular dimers which in turn are packed together using weaker NH···S interactions. The triple character of C10-C11 bond is confirmed by short length of the bond and general shape of molecule. All the "guanidine" residues are flat, so atoms C8, N1-N4 together with C9 are aligned in a well-defined plane. Naturally, the -S-CH_2- group provides a bent link to the phenyl ring with a valence angle on S2 (C2-S2-C18) equal to 102.52°. Dihedral angle between rings C1-C6 and C19-C24 is rather wide, almost reaching 78°. Conformation of the molecule can be conveniently described by dihedral angles among their stiff flat fragments (data gathered in Table 2).

Compound **42** crystallizes in the triclinic system, the space group $P\bar{1}$, with one molecule in the asymmetric unit, and two in the unit cell, $Z = 2$. It shares many features with **28**, especially in the hydrogen bonding (Table 5S in the Supplementary Material) which also influence on the packing mode (compare Figs. 6 and 7; for comparison of bond lengths and angles see Table 2S in the Supplementary Material). One can notice distinctive twist of phenylethynyl group in relation to the "nitrogen atoms containing" plane of ca 32° (Table 2).

Compound **43** crystallizes in the triclinic system, the space group $P\bar{1}$, with one molecule in the asymmetric unit, and two in the unit cell, $Z = 2$. It shares many features with the previous structures, such as the formation of dimers linked by cyclic N2–H2A … N hydrogen bonding, motif $R_2^2(8)$, and intermolecular N3H3···O2. Differently, the second donor (N2–H2B) forms N–H···Cl bonds to a Cl1 chlorine atom from the neighbour molecule (Table 6S in the Supplementary Material). One can notice also rather high twist of phenylethynyl group in relation to the "nitrogen atoms containing" plane of *ca* 19°, but in this case N3 and N4 are almost coplanar with the phenylethynyl residue (Table 2).

The main difference in conformations (see Table 2) are in twist of *S*-benzyl group in relation to the benzenesulfonyl group (R1– R3 dihedral). Methylated structures **35** and **43**, exhibit wide dihedral angle between sulfonamidic ring and –S-$CH_2C_6H_4R$ (R = CF_3, CH_3 or Cl) fragment (*ca* 78° and 48.5° respectively) while in both **28** and **42** the dihedral angle is much lower (*ca* 19° and 16.5°, respectively). Moreover, as it was shown in Fig. 8, the distance between R2 (C12–

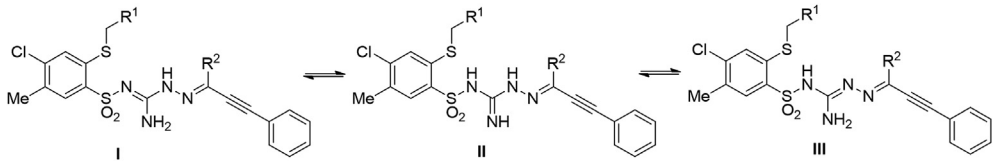


Fig. 2. Three possible tautomeric forms of compounds **26**–**49**.

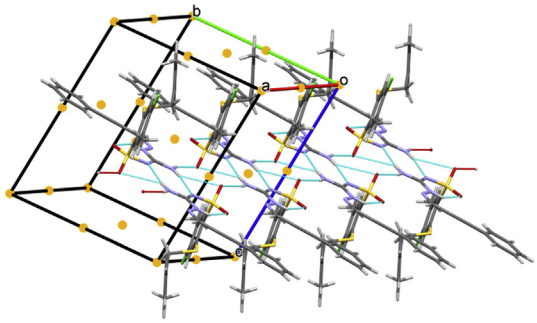


Fig. 4. Packing of **28**. Hydrogen bonding motifs are extending in crystallographic **a** direction. Inversion centres are marked as yellow balls. Exterior of the substructure is hydrophobic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

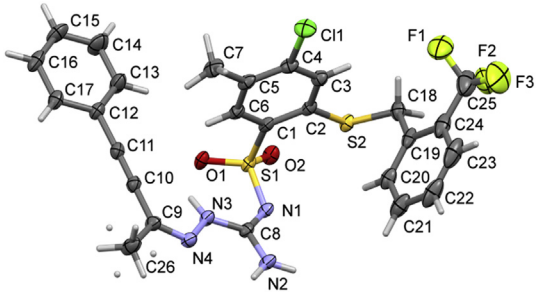


Fig. 5. Atom numbering scheme for **35** with displacement ellipsoids drawn at 50% probability level. Dihedral angle between rings C1-C6 and C19-C24 is wide, 77.95°. Methyl group at C26 is disordered.

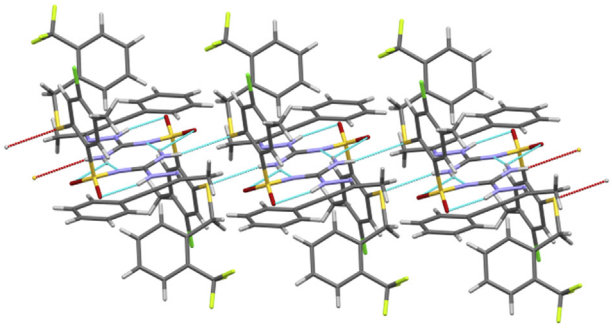


Fig. 6. Chain of dimers of molecules of **35** linked by NH···S hydrogen bonding.

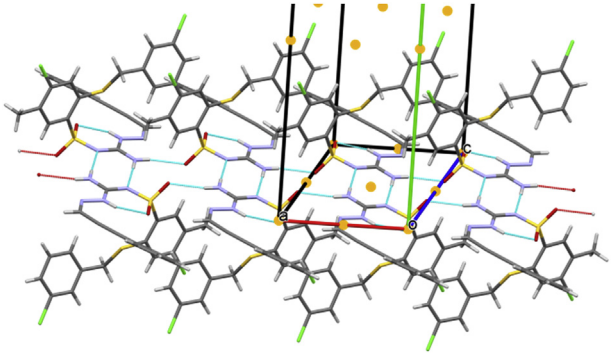


Fig. 7. Crystal packing diagram for **42**, inversion centres shown as yellow balls. Ladders of hydrogen bonding spread along [1 0 0] direction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

C17; phenylethynyl) and R3 (C19– C24; benzylthio ring) is much smaller for compounds with R^2 = H (*ca* 6.9 Å and 7.7 Å for **28** and **42**, respectively) in comparison with derivatives containing methyl group as R^2 substituent (*ca* 11.1 Å and 13 Å for **35** and **43**, respectively).

2.2. Cytotoxic activity

Compounds **26**–**49** were evaluated *in vitro* for their antiproliferative activity towards three human cancer cell lines: MCF-7 (breast cancer), HCT-116 (colon cancer) and HeLa (cervical cancer). Cytotoxic evaluations were performed in a 5-dose MTT assay and subsequently IC_{50} values have been obtained (Table 3). Among studied cell lines, HCT-116 and HeLa were the most sensitive while MCF-7 had slightly reduced susceptibility to the derivatives.

Results in Table 3 indicate the most active compounds belong to the series possess either chlorobenzylthio (**40**–**45**) or benzylthio (**26**–**27**) groups attached at position 2 of benzenesulfonylguanidine scaffold. Among those, compounds **44** and **45** with chlorine atom at *para* position in benzyl moiety display the strongest cytotoxic effects. The obtained IC_{50} values were in the range of 6, 8 μM and 18 μM for **44** or 8, 10 μM and 14 μM for **45** against HeLa, HCT-116 and MCF-7, respectively.

Very good results of antiproliferative assays was also observed for compounds **26** (R^1 = Ph, R^2 = H; IC_{50} ranging from 5 to 24 μM), **36** (R^1 = 3-$CF_3C_6H_4$, R^2 = H; IC_{50} ranging from 8 to 14 μM) and **46** (R^1 = 1-naphthyl, R^2 = H; IC_{50} ranging from 8 to 22 μM). Aside from above, derivatives with trifluoromethylphenyl substituent as R^1 (**34**–**39**) display noticeable anticancer profile too. On the other hand, compounds with methylphenyl moiety (**28**–**33**) attached at this position show decreasing level of activity. In conjunction with data obtained for chlorophenyl series, this suggest that high electronegativity of substituent (R^1) incorporated at methylenethio group might play an important role by which the compounds exert their anticancer activity.

Moreover it seems the bulky groups in phenyl scaffold are less favorable especially for *para* substitution. It is easy to observed comparing the results obtained for compounds **32**, **38** and **44** which indicate IC_{50} values against cancer cell lines in the range of 11–24 μM (**32**, R^1 = 4-MeC_6H_4), 70–105 μM (**38**, R^1 = 4-$CF_3C_6H_4$), and 6–18 μM (**44**, R^1 = 4-ClC_6H_4), respectively.

It should be noticed that hydrogen atom attached at the imine carbon atom (R^2 moiety) is the preferable substituent for growth inhibition of cancer cells. However, the replacement of R^2 with methyl group often lead to better selectivity. Particularly it is evident in the comparison of antiproliferative effect observed for analogical series of compounds **32** and **33** (R^1 = 4-MeC_6H_4) or **38** and **39** (R^1 = 4-$CF_3C_6H_4$). In the first case, derivative **32** displayed high or moderate anticancer activity with IC_{50} = 11 μM for HeLa and 24 μM for both HCT-116 and MCF-7 while **33** was selective only against HCT-116 with IC_{50} = 13 μM. In turn compound **38** exhibited weak antiproliferative effects (IC_{50} ranging from 70 to 105 μM in all tested cell lines) whereas **39** strongly and selectively inhibited growth of HeLa cells with IC_{50} = 8 μM.

For the compounds with the strongest ability to inhibit the growth of all cancer cell lines as well as derivatives with selective activity, an investigation of cytotoxic effect against non-carcinogenic cell line HaCaT was done. Very promising results have been obtained for six derivatives **26**, **33**, **37**, **39**, **41** and **43**. The most prominent anticancer profile was obtained for compounds **26** and **37** with interesting selectivity toward tested cancer cell lines. The activity against HCT-116, HeLa and MCF-7, respectively, was

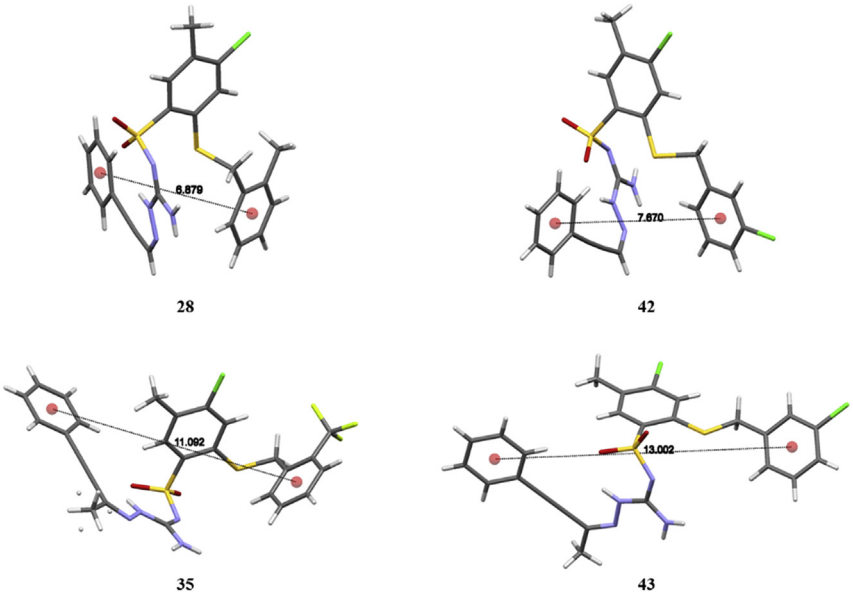
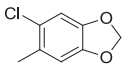
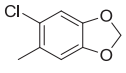


Fig. 8. Distance between R2-R3 for compounds **28**, **35**, **42** and **43**.

Table 3
IC_{50} values for compounds **26**–**49**.

Compd	R^1	R^2	HCT-116	HeLa	MCF-7	HaCaT
26	Ph	H	8 ± 0.2	5 ± 0.2	24 ± 0.5	145 ± 3.0
27	Ph	Me	10 ± 0.2	6 ± 0.1	110 ± 1.0	38 ± 1.0
28	2-MeC_6H_4	H	19 ± 1.0	19 ± 0.5	13 ± 0.5	17 ± 0.5
29	2-MeC_6H_4	Me	142 ± 3.0	270 ± 10.0	150 ± 3.0	nt
30	3-MeC_6H_4	H	18 ± 0.5	11 ± 0.4	16 ± 0.5	9 ± 0.5
31	3-MeC_6H_4	Me	160 ± 2.0	105 ± 4.0	130 ± 4.0	nt
32	4-MeC_6H_4	H	11 ± 0.2	24 ± 1.0	24 ± 0.1	50 ± 1.0
33	4-MeC_6H_4	Me	13 ± 0.1	110 ± 2.0	125 ± 1.0	270 ± 5
34	2-$CF_3C_6H_4$	H	18 ± 0.5	16 ± 0.5	14 ± 0.1	23 ± 0.5
35	2-$CF_3C_6H_4$	Me	23 ± 0.5	21 ± 0.2	19 ± 0.5	18 ± 1.0
36	3-$CF_3C_6H_4$	H	8 ± 0.1	14 ± 0.3	14 ± 0.5	16 ± 0.5
37	3-$CF_3C_6H_4$	Me	14 ± 0.3	18 ± 0.2	20 ± 1.0	105 ± 1.0
38	4-$CF_3C_6H_4$	H	70 ± 1.0	93 ± 3.0	105 ± 2.0	nt
39	4-$CF_3C_6H_4$	Me	160 ± 5.0	8 ± 0.1	135 ± 8.0	120 ± 1.0
40	2-ClC_6H_4	H	27 ± 1.0	14 ± 0.2	15 ± 0.6	25 ± 0.5
41	2-ClC_6H_4	Me	129 ± 5.0	25 ± 0.2	114 ± 1.0	340 ± 7.0
42	3-ClC_6H_4	H	17 ± 0.5	13 ± 0.4	15 ± 0.3	17 ± 1.0
43	3-ClC_6H_4	Me	119 ± 2.0	21 ± 0.4	80 ± 2.0	155 ± 8.0
44	4-ClC_6H_4	H	8 ± 0.1	6 ± 0.1	18 ± 0.4	5 ± 0.1
45	4-ClC_6H_4	Me	10 ± 0.1	8 ± 0.2	14 ± 0.1	16 ± 1.0
46	1-naphthyl	H	10 ± 0.1	8 ± 0.1	22 ± 0.2	5 ± 0.1
47	1-naphthyl	Me	105 ± 4.0	125 ± 1.0	210 ± 4.0	nt
48		H	10 ± 0.4	20 ± 0.4	20 ± 1.0	20 ± 0.5
49		Me	80 ± 2.0	190 ± 8.0	205 ± 4.0	nt
Cisplatin			3.8 ± 0.2	2.2 ± 0.1	3.0 ± 0.1	nt

nt – not tested.

either 18-fold, 29-fold and 6-fold for **26** or 7.5-fold, 5.8-fold and 5.25-fold for **37** more potent when compared with HaCaT. In the case of compounds **39**, **41** and **43**, strong and selective growth inhibition of HeLa cells have been observed. It should be emphasized that the inhibitory effect against cancer cells was approximately 15, 13.6 and 7.4 times bigger, respectively for **39**, **41** and **43**, in comparison with this one obtained for HaCaT. In turn, compound **33** displayed strong growth inhibition of HCT-116 and it was almost 21-fold more active against cancer than non-carcinogenic cell line. Good selectivity was also observed in the case of compounds **27** and **32**. Derivative **27** displayed high activity against both HCT-116 and HeLa with IC_{50} = 10 μM and IC_{50} = 6 μM, respectively, while IC_{50} obtained for HaCaT was 38 μM. Compound **32** was active against all tested cancer cell lines with IC_{50} in the range of 11–24 μM when growth inhibition for HaCaT cells was 50 μM.

2.3. QSAR studies

The support vector machines (SVM) were used to develop QSAR model for predicting the anticancer activity of derivatives **26**–**49**. Descriptors used to create SVM based regression model have been chosen by data mining variable selection feature of Statistica software. Variable selection has been performed separately for each cell line. For HCT-116 cell line, chosen descriptors were RDF125 m, VE1_Dt, JGI3, Mor13u and E1e. For HeLa cell line, chosen descriptors were ATSC1i, ATSC2p, Mor01i and SpMin8_Bh(s). For MCF-7 cell line, chosen descriptors were R4s, Eig05_AEA(ri), R5m+, SM03_EA(dm) and SM11_EA(dm). As all these descriptors belong to various groups, it is hard to develop a sound chemical interpretation of developed model. However, even though they do not provide any clear suggestions regarding structure-activity relationship, they can still be used as an entry data for predictive QSAR model.

After successful establishment of SVM model, predicted activity values and observed activity values for all 3 cell lines were plotted against each other for both sets (Figs. 9–11) to visualize accuracy of the model. Correlation coefficients were as follow:

1. For HCT-116 cell line: R' = 0.9369 for training set, R" = 0.8440 for test set and R‴ = 0.9130 for both sets (Fig. 9). Mean squared error was MSE' = 0.01 for training set, MSE" = 0.316 for test set and MSE‴ = 0.087 for both sets.
2. For HeLa cell line: R' = 0.947 for training set, R" = 0.902 for test set and R‴ = 0.930 for both sets (Fig. 10). Mean squared error

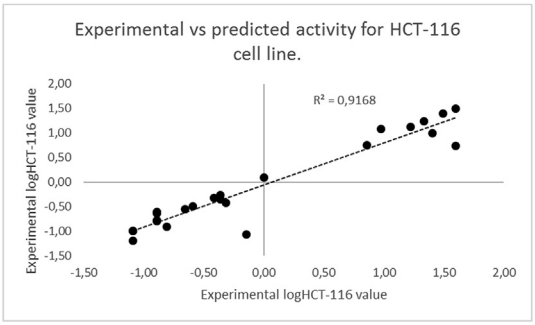


Fig. 9. Comparison plot of experimental vs predicted logHCT-116 values for developed SVM model. Presented correlation coefficient (R value) is for both the training and test set.

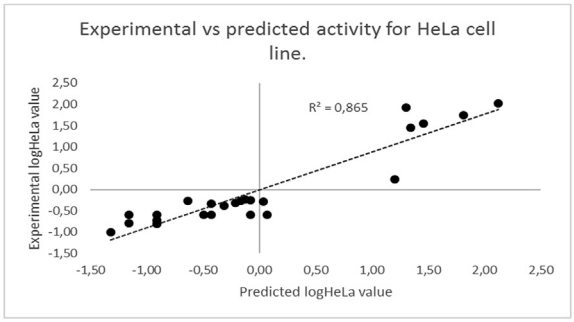


Fig. 10. Comparison plot of experimental vs predicted logHeLa values for developed SVM model. Presented correlation coefficient (R value) is for both the training and test set.

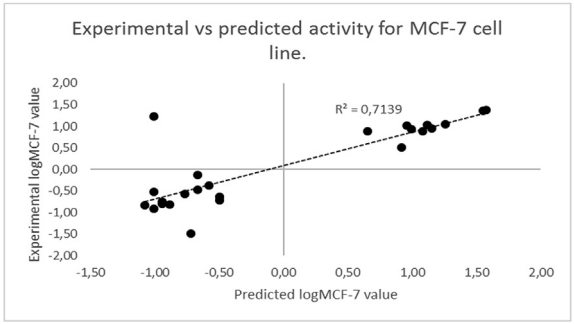


Fig. 11. Comparison plot of experimental vs predicted logMCF-7 values for developed SVM model. Presented correlation coefficient (R value) is for both the training and test set.

was MSE' = 0.325 for training set, MSE" = 0.606 for test set and MSE‴ = 0.368 for both sets.

3. For MCF-7 cell line: R' = 0.980 for training set, R" = 0.542 for test set and R‴ = 0.845 for both sets (Fig. 11). Mean squared error was MSE' = 0.234 for training set, MSE" = 1.033 for test set and MSE‴ = 0.540 for both sets.

Developed QSAR model showed good predictability for HCT-116 and HeLa cell lines. Correlation coefficients for both training and test sets prove to be high, and mean square errors show that models predictions are valid. Such thing can not be said about MCF-7 cell line model, as both its correlation coefficients and mean square errors for test set are not satisfactory. Predictions are unfortunately far from valid.

Presented models show, that Support Vector Regression can be successfully used to develop predictive models for anti-cancer activity of studied derivatives for HCT-116 and HeLa cell lines. Even though descriptors chosen by Feature selection are hard to interpret, predictive value of presented models is high.

2.4. Metabolic stability

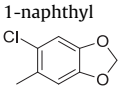
The results of compound incubations in the presence of pooled human liver microsomes and NADPH are presented in Table 4. Metabolic stability is presented as a biological half-life value, which enables easy comparison of compounds structures and their susceptibility to phase 1 biotransformation reactions (the result of incubation on the presence of human liver microsomes).

Tested derivatives show good metabolic stability, especially among the pharmacologically active compounds. Compounds **26**, **36** and **44**, as the most active ones against all three cell lines return *in vitro* half-lives of 28.14 min, 58.05 min and 39.89 min respectively. Less active compounds, such as **27**, **32**, **45**, **46**, and **48** also prove to be in a preferred half-life value range. Among the studied compounds, metabolic stability of **35** could not be assessed, due to lack of repeatability. This might because either by superior stability, far beyond incubation conditions, or problems with solubility, that could have caused a precipitation of compound in phosphate buffer, therefore decreasing initial concentration and lowering achieved *in vitro* half-life value. Considering, that a perfect drug should be stable enough to reach its pharmacological target, but it should also undergo biotransformation to prevent adverse drug reactions, results seem to be promising. As such, even though no clear structure-metabolic activity relationships can be observed, studied derivatives represent a group of compounds that are not only active, but also resistant to biotransformation in *in vitro* conditions.

3. Conclusions

We have developed methods for the preparation of novel series of 2-(2-alkylthiobenzenesulfonyl)-3-(phenylprop-2-ynylideneamino)guanidine derivatives. The crystallographic analysis revealed that depend on R^2 substituent compounds could take different conformation. The main difference were observed in values of dihedral angles between rings of benzenesulfonamide and benzylthio groups. Compounds **35** and **43** (R^2 = Me) exhibited

Table 4
Experimental $t_{1/2}$ values along with corresponding SD and RSD%.

Compd	R^1	R^2	Average $t_{1/2}$ [min](n = 2)	SD [min]	RSD%
26	Ph	H	28.14	4.26	15.14
27	Ph	Me	27.96	5.45	19.49
28	2- MeC_6H_4	H	51.72	7.45	14.40
30	3- MeC_6H_4	H	52.75	9.19	17.42
32	4-MeC_6H_4	H	32.18	0.21	0.65
33	4-MeC_6H_4	Me	10.70	5.84	54.58
34	2-$CF_3C_6H_4$	Me	39.16	8.25	21.07
35	2-$CF_3C_6H_4$	H	6.42	4.26	66.36
36	3-$CF_3C_6H_4$	H	58.05	4.10	7.06
37	3-$CF_3C_6H_4$	Me	7.80	1.99	25.51
39	4-$CF_3C_6H_4$	Me	84.54	19.28	22.81
40	2-ClC_6H_4	H	15.23	0.34	2.23
41	2-ClC_6H_4	Me	85.33	7.83	9.18
42	3-ClC_6H_4	H	41.56	0.53	1.27
43	3-ClC_6H_4	Me	35.73	5.32	14.89
44	4-ClC_6H_4	H	39.89	5.15	12.91
46	1-naphthyl	H	71.82	5.00	6.96
48		H	26.47	2.08	7.86

SD – standard deviation, RSD% – relative standard deviation, expressed as SD/average*100%.

wide dihedral angle (*ca* 78° and 48.5° respectively) while in both **28** and **42** the dihedral angle was significantly narrower (*ca* 19° and 16.5°, respectively). Important difference was also observed in the distance between ring planes of phenylethynyl and benzylthio residues which was much smaller for compounds with R^2 = H (*ca* 6.9 Å and 7.7 Å for **28** and **42** respectively) when compared to **35** and **43** containing methyl group as R^2 (*ca* 11.1 Å and 13 Å respectively).

The compounds were evaluated *in vitro* by MTT assay for their antiproliferative activity against three cancer cell lines: colon HCT-116, cervical HeLa and breast MCF-7. We have found that novel compounds displayed prominent cytotoxic effect which can be due to their structural differences. Compounds with R^2 = H usually displayed meaningfully greater antiproliferative effect. On the other hand, the replacement of R^2 with methyl group often led to better selectivity. Based on variability of R^1 moiety incorporated at methylenethio group we found, that its high electronegativity as well as steric hindrance might play an important role for the compounds anticancer activity. It must be emphasized that six compounds **26**, **33**, **37**, **39**, **41** and **43** specifically affected *in vitro* only the cancer cells and not the non-cancerous human keratinocyte cells (HaCaT). Furthermore, metabolic stability research revealed that prominent cytotoxic activity go hand in hand with their high biological half-life value.

Support vector machines (SVM) were applied to build up the QSAR model for predicting the anti-proliferative activity of novel derivatives, based on descriptors calculated from molecular structure. The results showed that the SVM models were able to establish a fitting relationship between the molecular descriptors and the anticancer activity against HCT-116 and HeLa cell lines. Difficulty in practical interpretation of models descriptors for both cell lines could be due to a complex nature of studied derivatives mode of action. However, developed models already showed a good predictability and therefore might be useful for further development of structurally similar derivatives with better pharmacological properties.

4. Experimental protocols

4.1. Synthesis

Melting points were determined with a Boethius PHMK apparatus. Infrared (IR) spectra were recorded with a Thermo Mattson Satellite FTIR spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) experiments were carried out on a Varian Gemini 200 apparatus at 200 MHz (^{1}H NMR) and 50 MHz (^{13}C NMR) or on a Varian Unity 500 Plus apparatus at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR); chemical shifts are expressed in parts per million (ppm) relative to TMS as an internal standard. HRMS analyses were performed on a TripleTOF 5600 + System (AB SCIEX, USA) in positive ion mode. The elemental analyses were measured using PerkinElmer 2400 Series II CHN Elemental Analyzer and the results for C, H and N were in agreement with the theoretical values within ±0.4% range.

Commercially unavailable substrates were obtained according to the following methods described previously: 3-amino-6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazine (**1**) [32], *N*-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)cyanamide potassium salt (**2**) [32], *N*-{4-chloro-5-methyl-2-[(4-methylbenzyl)thio]benzenesulfonyl}cyanamide potassium salt (**5**) [33], *N*-{4-chloro-5-methyl-2-[(3-trifluoromethylbenzyl)thio]benzenesulfonyl}cyanamide potassium salt (**7**) [34], *N*-{4-chloro-5-methyl-2-[(4-trifluoromethylbenzyl)thio]benzenesulfonyl}cyanamide potassium salt (**8**) [34], *N*-{4-chloro-2-[(4-chloromethylbenzyl)thio]-5-methylbenzenesulfonyl}cyanamide potassium salt (**11**) [33], *N*-[4-chloro-5-methyl-2-(naphthalene-1-ylmethylthio)benzenesulfonyl] cyanamide potassium salt (**12**) [34], *N*-[4-chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]cyanamide potassium salt (**13**) [35], 1-amino-2-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)guanidine (**14**) [36], 1-amino-2-{4-chloro-5-methyl-2-[(4-methylbenzyl)thio]benzenesulfonyl}guanidine (**17**) [33], 1-amino-2-{4-chloro-5-methyl-2-[(3-trifluoromethylbenzyl)thio]benzenesulfonyl}guanidine (**19**) [33], 1-amino-2-{4-chloro-5-methyl-2-[(4-trifluoromethylbenzyl)thio]benzenesulfonyl}guanidine (**20**) [33], 1-amino-2-{4-chloro-2[(4-chlorobenzyl)thio]-5-methylbenzenesulfonyl}guanidine (**23**) [33], 1-amino-2-[4-chloro-5-methyl-2-(naphthalene-1-ylmethylthio)benzenesulfonyl}guanidine (**24**) [37], 1-amino-2-[4-chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]guanidine (**25**) [33].

4.1.1. Procedures for the preparation of N-(2-alkylthio-4-chloro-5-methyl-benzenesulfonyl)cyanamide potassium salts ***3*–*4*, *6* and *9*–*10***

The mixture of 3-amino-6-chloro-7-methyl-1,1-dioxo-1,4,2-benzodithiazine **1** (1.051 g, 4 mmol), anhydrous K_2CO_3 (2.81 g, 20.3 mmol) and the appropriate alkyl chloride (4.2 mmol) in dry THF (28 mL) was stirred at reflux for 18–26 h. The solid was filtered off and isolated by extraction with boiling ethanol (50 mL). In this manner the following potassium salts were obtained.

*4.1.1.1. N-{4-Chloro-5-methyl-2-[(2-methylbenzyl)thio]benzenesulfonyl}cyanamide potassium salt (**3**).* Starting from **1** and 2-methylbenzyl chloride (0.55 mL) in dry THF with stirring for 26 h, the title compound **3** was obtained (1.361 g, 84%): m.p. 195–198 °C; IR (KBr) ν_{max} 3065, 2923 (C-H), 2176 (C≡N), 1343, 1142 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 4.22 (s, 2H, SCH_2), 7.13–7.17 (m, 1H, arom.), 7.19–7.20 (m, 2H arom.), 7.33 (d, J = 6.9 Hz, 2H, arom.), 7.42 (s, 1H, H-3), 7.74 (s, 1H, H-6) ppm. Anal. ($C_{16}H_{14}ClKN_2O_2S_2$) C, H, N.

*4.1.1.2. N-{4-Chloro-5-methyl-2-[(3-methylbenzyl)thio]benzenesulfonyl}cyanamide potassium salt (**4**).* Starting from **1** and 3-methylbenzyl chloride (0.55 mL) in dry THF with stirring for 20 h, the title compound **4** was obtained (1.264 g, 78%): m.p. 158–161 °C; IR (KBr) ν_{max} 2920 (CH), 2177 (C≡N), 1343, 1142 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.29 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 4.21 (s, 2H, SCH_2), 7.07–7.08 (m, 1H, arom.), 7.19–7.22 (m, 2H, arom.), 7.25 (s, 1H, arom.), 7.38 (s, 1H, H-3), 7.73 (s, 1H, H-6) ppm. Anal. ($C_{16}H_{14}ClKN_2O_2S_2$) C, H, N.

*4.1.1.3. N-{4-Chloro-5-methyl-2-[(2-trifluoromethylbenzyl)thio]benzenesulfonyl}cyanamide potassium salt (**6**).* Starting from **1** and 2-trifluoromethylbenzyl chloride (0.52 mL) in dry THF with stirring for 18 h, the title compound **6** was obtained (1.155 g, 62%): m.p. 212–215 °C; IR (KBr) ν_{max} 3071, 2977, 2925 (CH), 2169 (C≡N), 1343, 1164 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.38 (s, 2H, SCH_2), 7.30 (s, 1H, H-3), 7.50 (t, 1H, arom.), 7.63 (t, 1H, arom.), 7.73–7.76 (m, 3H, H-6, arom.) ppm. Anal. ($C_{16}H_{11}ClF_3KN_2O_2S_2$) C, H, N.

*4.1.1.4. N-[4-Chloro-2-[(2-chlorobenzyl)thio]-5-methylbenzenesulfonyl}cyanamide potassium salt (**9**).* Starting from **1** and 2-chlorobenzyl chloride (0.53 mL) in dry THF with stirring for 21 h, the title compound **9** was obtained (1.106 g, 65%): m.p. 192–196 °C; IR (KBr) ν_{max} 3019, 2922 (CH), 2179 (C≡N), 1342, 1140 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.31 (s, 3H, CH_3), 4.33 (s, 2H, SCH_2), 7.29–7.33 (m, 3H, arom.), 7.47–7.49 (m, 1H, arom.), 7.54–7.55 (m, 1H, arom.), 7.75 (s, 1H, H-6) ppm. Anal. ($C_{15}H_{11}Cl_2KN_2O_2S_2$) C, H, N.

*4.1.1.5. N-[4-Chloro-2-[(3-chlorobenzylthio]-5-methylbenzenesulfonyl}cyanamide potassium salt (**10**).* Starting from **1** and 3-chlorobenzyl chloride (0.43 mL) in THF with stirring for 21 h, the title compound **10** was obtained (1.225 g, 72%): m.p. 158–161 °C; IR (KBr) ν_{max} 2923 (CH), 2173 (C≡N), 1342, 1142 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.29 (s, 2H, SCH_2), 7.31–7.35 (m, 2H, arom.), 7.37 (s, 1H, H-3), 7.41 (d, $J = 7.3$ Hz, 1H, arom.), 7.50 (s, 1H, arom.), 7.74 (s, 1H, H-6) ppm. Anal. ($C_{15}H_{11}Cl_2KN_2O_2S_2$) C, H, N.

*4.1.2. Procedures for the preparation of aminoguanidines **15**–**16**, **18** and **21**–**22***

A mixture of the appropriate cyanamide potassium salt **3**, **4**, **6**, **9** or **10** (2 mmol) and hydrazine monohydrochloride (0.137 g, 2 mmol) in anhydrous toluene (4 mL) was refluxed with stirring for 3–5 h. After cooling to room temperature, the reaction mixture was left in the freezer overnight. The precipitate was collected by filtration, dried and suspended in water (9 mL). The solid was separated and crystallized from acetonitrile. In this manner, the following aminoguanidines were obtained.

*4.1.2.1. 1-Amino-2-{4-chloro-5-methyl-2-[(2-methylbenzyl)thio]benzenesulfonyl}guanidine (**15**).* Starting from **3** (0.810 g) with stirring for 4 h, the title compound **15** was obtained (0.758 g, 95%): m.p. 218–221 °C; IR (KBr): 3468, 3361, 3310, 3205 (NH), 2947 (CH), 1647, 1595 (NH), 1567, 1446 (C=N, C=C), 1343, 1135 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.32 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 4.23 (s, 2H, SCH_2), 4.46 (s, 2H, C-NH_2), 6.94 (s, 2H, NNH_2), 7.13–7.17 (m, 1H, arom.), 7.19 (m, 2H, arom.), 7.31 (d, $J = 7.2$ Hz, 1H, arom.), 7.42 (s, 1H, H-3 arom.), 7.83 (s, 1H, H-6 arom.), 8.37 (s, 1H, NH) ppm. Anal. ($C_{16}H_{19}ClN_4O_2S_2$) C, H, N.

*4.1.2.2. 1-Amino-2-{4-chloro-5-methyl-2-[(3-methylbenzyl)thio]benzenesulfonyl}guanidine (**16**).* Starting from **4** (0.810 g) with stirring for 3 h, the title compound **16** was obtained (0.734 g, 92%): m.p. 177–180 °C; IR (KBr): 3456, 3348, 3330, 3212 (NH), 3056, 3010, 2971, 2918 (CH), 1659, 1607 (NH), 1570, 1532, 1485, 1450 (C=N, C=C), 1345, 1130 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.29, (s, 6H, 2 × CH_3), 4.22 (s, 2H, SCH_2), 4.44 (s, 2H, C-NH_2), 6.96 (s, 2H, NNH_2), 7.07–7.08 (m, 1H, arom.), 7.21–7.22 (m, 3H, arom.), 7.40 (s, 1H, H-3 arom.), 7.81 (s, 1H, H-6 arom.), 8.39 (s, 1H, NH) ppm. Anal. ($C_{16}H_{19}ClN_4O_2S_2$) C, H, N.

*4.1.2.3. 1-Amino-2-{4-chloro-5-methyl-2-[(2-trifluoromethylbenzyl)thio]benzenesulfonyl}guanidine (**18**).* Starting from **6** (0.918 g) with stirring for 5 h, the title compound **18** was obtained (0.843 g, 93%): m.p. 221–224 °C; IR (KBr): 3455, 3357, 3338, 3221 (NH), 2952 (CH), 1660, 1601 (NH), 1571, 1491, 1451 (C=C, C=N), 1339, 1137 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 4.39 (s, 2H, SCH_2), 4.49 (s, 2H, C-NH_2), 6.98 (s, 2H, NNH_2), 7.30 (s, 1H, H-3 arom.), 7.50 (t, 1H, arom.), 7.64 (t, 1H, arom.), 7.70 (d, $J = 7.8$ Hz, 1H, arom.), 7.74 (d, $J = 7.4$ Hz, 1H, arom.), 7.84 (s, 1H, H-6 arom.), 8.41 (s, 1H, NH) ppm. Anal. ($C_{16}H_{16}ClF_3N_4O_2S_2$) C, H, N.

*4.1.2.4. 1-Amino-2-{4-chloro-2-[(2-chlorobenzyl)thio]-5-methylbenzenesulfonyl}guanidine (**21**).* Starting from **9** (0.851 g) with stirring for 5 h, the title compound **21** was obtained (0.763 g, 91%): m.p. 201–204 °C; IR (KBr): 3474, 3366, 3314, 3207 (NH), 3055, 2954 (CH), 1649, 1596 (NH), 1473, 1447 (C=N, C=C), 1343, 1135 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 4.33 (s, 2H, SCH_2), 4.48 (s, 2H, C-NH_2), 6.96 (s, 2H, NNH_2), 7.30–7.32 (m, 2H, arom.), 7.34 (s, 1H, H-3 arom.), 7.46–7.50 (m, 2H, arom.), 7.83 (s, 1H, H-6 arom.), 8.39 (s, 1H, NH) ppm. Anal. ($C_{15}H_{16}Cl_2N_4O_2S_2$) C, H, N.

*4.1.2.5. 1-Amino-2-{4-chloro-2-[(3-chlorobenzyl)thio]-5-methylbenzenesulfonyl}guanidine (**22**).* Starting from **10** (0.851 g) with stirring for 3 h, the title compound **22** was obtained (0.780 g, 93%): m.p. 191–193 °C; IR (KBr): 3464, 3352, 3332 (NH), 2985 (CH), 1663, 1607 (NH), 1572, 1531, 1475, 1444 (C=N, C=C), 1343, 1128 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 4.30 (s, 2H, SCH_2), 4.49 (s, 2H, C-NH_2), 6.97 (s, 2H, NNH_2), 7.31–7.32 (m, 1H, arom.), 7.34–7.38 (m, 2H, arom.), 7.40 (s, 1H arom.), 7.48 (s, 1H, H-3 arom.), 7.81 (s, 1H, H-6 arom.), 8.41 (s, 1H, NH) ppm. Anal. ($C_{15}H_{16}Cl_2N_4O_2S_2$) C, H, N.

*4.1.3. Procedures for the preparation of 2-(2-alkylthiobenzenesulfonyl)-3-(3-phenylprop-2-ynylideneamino) guanidines **26**–**49***

To a suspension of the appropriate 1-amino-2-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)guanidine (**14**–**25**) (0.5 mmol) and PTSA (0.010 g, 0.05 mmol) in ethanol (3 mL) phenylpropiolaldehyde diethyl acetal (0.103 mL, 0.5 mmol) (**26**, **28**, **30**, **32**, **34**, **36**, **38**, **40**, **42**, **44**, **46**, **48**) or 4-phenylbut-3-yn-2-one (0.073 mL, 0.5 mmol) (**27**, **29**, **31**, **33**, **35**, **37**, **39**, **41**, **43**, **45**, **47**, **49**) was added. A reaction mixture was stirred at reflux for 2–20 h, the precipitate was filtered off, dried and crystallized from ethanol (**26**, **27**, **38**, **39**, **44**–**47**), acetonitrile (**28**–**37**, **40**–**43**), glacial acetic acid (**48**) or benzene (**49**). In this manner the following compounds were obtained.

*4.1.3.1. 2-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(3-phenylprop-2-ynylideneamino)guanidine (**26**).* Starting from **14** (0.192 g) and phenylpropiolaldehyde diethyl acetal with stirring for 9 h, the title compound **26** was obtained (0.204 g, 82%): m.p. 196–198 °C; IR (KBr): 3456, 3242 (NH), 3107, 2923 (CH), 2193 (C≡C), 1632 (NH), 1566, 1499, 1448 (C=N, C=C), 1332, 1170 (SO_2) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 4.31 (s, 2H, SCH_2), 7.13 (s, 1H, N=CH), 7.22–7.29 (m, 3H, arom.), 7.33–7.35 (m, 2H, arom.), 7.42–7.52 (m, 4H, NH, H-3, arom.), 7.68–7.76 (m, 3H, NH, arom.), 7.85 (s, 1H, H-6 arom.), 11.29 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{21}ClN_4O_2S_2$ [M + H^+] 497.0867, found 497.0855. Anal. ($C_{24}H_{21}ClN_4O_2S_2$) C, H, N.

*4.1.3.2. 2-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**27**).* Starting from **14** (0.192 g) and 4-phenylbut-3-yn-2-one with stirring for 12 h, the title compound **27** was obtained (0.235 g, 92%): m.p. 194–196 °C; IR (KBr): 3451, 3247 (NH), 3104, 2922 (CH), 2182 (C≡C), 1633 (NH), 1565, 1494, 1443 (C=N, C=C), 1341, 1170 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.14 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 4.23 (s, 2H, SCH_2), 7.19–7.26 (m, 3H, arom.), 7.33 (d, $J = 6.8$ Hz, 2H, arom.), 7.43–7.50 (m, 4H, NH, rom), 7.54 (brs, 1H, NH), 7.70 (d, $J = 6.8$ Hz, 2H, arom.), 7.76 (brs, 1H, NH), 7.82 (s, 1H, H-6 arom.), 10.97 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{23}ClN_4O_2S_2$ [M + H^+] 511.1024, found 511.1028. Anal. ($C_{25}H_{23}ClN_4O_2S_2$) C, H, N.

*4.1.3.3. 2-{4-Chloro-5-methyl-2-[(2-methylbenzyl)thio]benzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**28**).* Starting from **15** (0.199 g) and phenylpropiolaldehyde diethyl acetal with stirring for 6 h, the title compound **28** was obtained (0.199 g, 78%): m.p. 179–182 °C; IR (KBr): 3468, 3362, 3309, 3206 (NH), 2947 (CH), 2194 (C≡C), 1634, 1595 (NH), 1565, 1508, 1490, 1445 (C=N, C=C), 1342, 1167 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.25 (s, 2H, SCH_2), 7.03 (s, 1H, N=CH), 7.06–7.11 (m, 2H, arom.), 7.13–7.19 (m, 2H, arom.), 7.23 (d, $J = 7.9$ Hz, 1H, arom.), 7.42–7.46 (m, 2H, NH, arom.), 7.48–7.51 (m, 2H, H-3, arom.), 7.66–7.68 (m, 2H arom.), 7.79 (brs, 1H, NH), 7.81 (s, 1H, H-6 arom.), 11.21 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-

d_6): δ 19.4, 19.6, 35.9, 79.9, 103.4, 120.7, 124.8, 126.6, 128.3, 129.2, 129.6, 130.6, 130.9, 131.0, 131.3, 132.9, 133.0, 134.3, 136.5, 137.5, 137.8, 140.3, 154.7 ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{23}ClN_4O_2S_2$ [M + H^+] 511.1024, found 511.1036. Anal. ($C_{25}H_{23}ClN_4O_2S_2$) C, H, N.

*4.1.3.4. 2-{4-Chloro-5-methyl-2-[(2-methylbenzyl)thio]benzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**29**).* Starting from **15** (0.199 g) and 4-phenylbut-3-yn-2-one with stirring for 8 h, the title compound **29** was obtained (0.181 g, 69%): m.p. 190–193 °C; IR (KBr): 3451, 3276 (NH), 2920 (CH), 2179 (C≡C), 1636 (NH), 1567, 1491, 1445 (C=N, C=C), 1342, 1173 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.12 (s, 3H, CH_3), 2.26 (s, 6H, 2 × CH_3), 4.24 (s, 2H, SCH_2), 7.06–7.12 (m, 2H, arom.), 7.15–7.17 (m, 1H, arom.), 7.22 (d, *J* = 7.3 Hz, 1H, arom.), 7.42–7.45 (m, 3H, arom.), 7.48–7.50 (m, 2H, NH, H-3 arom.), 7.66–7.68 (m, 2H arom.), 7.71 (brs, 1H, NH), 7.85 (s, 1H, H-6 arom.), 10.91 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{25}ClN_4O_2S_2$ [M + H^+] 525.1180, found 525.1170. Anal. ($C_{26}H_{25}ClN_4O_2S_2$) C, H, N.

*4.1.3.5. 2-{4-Chloro-5-methyl-2-[(3-methylbenzyl)thio]benzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**30**).* Starting from **16** (0.199 g) and phenylpropiolaldehyde diethyl acetal with stirring for 7 h, the title compound **30** was obtained (0.143 g, 56%): m.p. 185–187 °C; IR (KBr): 3376, 3308, 3225 (NH), 2914 (CH), 2192 (C≡C), 1644 (NH), 1568, 1512, 1489, 1450 (C=N, C=C), 1329, 1154 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.22 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.25 (s, 2H, SCH_2), 7.02–7.04 (m, 1H, arom.), 7.12–7.14 (m, 4H, N=CH, arom.), 7.44–7.47 (m, 2H, arom.), 7.49–7.52 (m, 2H, H-3, arom.), 7.69–7.72 (m, 3H, NH, arom.), 7.82 (brs, 1H, NH), 7.85 (s, 1H, H-6 arom.), 11.28 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{23}ClN_4O_2S_2$ [M + H^+] 511.1024, found 511.1011. Anal. ($C_{25}H_{23}ClN_4O_2S_2$) C, H, N.

*4.1.3.6. 2-{4-Chloro-5-methyl-2-[(3-methylbenzyl)thio]benzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**31**).* Starting from **16** (0.199 g) and 4-phenylbut-3-yn-2-one with stirring for 4 h, the title compound **31** was obtained (0.218 g, 83%): m.p. 194–196 °C; IR (KBr): 3451, 3277 (NH), 3108, 2920 (CH), 2180 (C≡C), 1636 (NH), 1567, 1491, 1445 (C=N, C=C), 1342, 1174 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.12 (s, 3H, CH_3), 2.26 (s, 6H, 2 × CH_3), 4.24 (s, 2H, SCH_2), 7.06–7.18 (m, 3H, arom.), 7.22 (d, *J* = 7.4 Hz, 1H, arom.), 7.42–7.45 (m, 2H, arom.), 7.48–7.49 (m, 2H, NH, arom.), 7.51 (s, 1H, H-3 arom.), 7.67–7.68 (m, 2H arom.), 7.74 (brs, 1H, NH), 7.85 (s, 1H, H-6 arom.), 10.93 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 19.4, 19.6, 22.9, 35.9, 80.9, 102.4, 120.6, 126.6, 128.3, 129.0, 129.5, 130.7, 130.8, 130.9, 131.2, 132.8, 132.9, 133.4, 134.2, 136.7, 137.4, 137.8, 140.4, 154.5 ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{25}ClN_4O_2S_2$ [M + H^+] 525.1180, found 525.1173. Anal. ($C_{26}H_{25}ClN_4O_2S_2$) C, H, N.

*4.1.3.7. 2-{4-Chloro-5-methyl-2-[(4-methylbenzyl)thio]benzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**32**).* Starting from **17** (0.199 g) and phenylpropiolaldehyde diethyl acetal with stirring for 18 h, the title compound **30** was obtained (0.143 g, 56%): m.p. 177–180 °C; IR (KBr): 3390, 3303, 3229 (NH), 2192 (C≡C), 1638 (NH), 1568, 1505, 1452 (C=N, C=C), 1331, 1154 (SO_2) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.21 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 4.25 (s, 2H, SCH_2), 7.01–7.04 (m, 2H, arom.), 7.14–7.24 (m, 3H, N=CH, arom.), 7.42–7.52 (m, 4H, NH, H-3, arom.), 7.69–7.84 (m, 5H, H-6, NH, arom.), 11.29 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{23}ClN_4O_2S_2$ [M + H^+] 511.1024, found 511.1046. Anal. ($C_{25}H_{23}ClN_4O_2S_2$) C, H, N.

*4.1.3.8. 2-{4-Chloro-5-methyl-2-[(4-methylbenzyl)thio]benzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**33**).* Starting from **17** (0.199 g) and 4-phenylbut-3-yn-2-one with stirring for 2 h, the title compound **33** was obtained (0.218 g, 83%): m.p. 176–178 °C; IR (KBr): 3440, 3251 (NH), 2919 (CH), 2179 (C≡C), 1635 (NH), 1556, 1516, 1491, 1445 (C=N, C=C), 1341, 1173 (SO_2) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.18 (s, 3H, CH_3), 2.23 (s, 6H, 2 × CH_3), 4.24 (s, 2H, SCH_2), 7.04 (d, *J* = 8.0 Hz, 2H, arom.), 7.22 (d, *J* = 8.0 Hz, 2H, arom.), 7.45–7.51 (m, 5H, NH, H-3, arom.), 7.69–7.73 (m, 3H, NH, arom.), 7.83 (s, 1H, H-6 arom.), 10.98 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{25}ClN_4O_2S_2$ [M + H^+] 525.1180, found 525.1202. Anal. ($C_{26}H_{25}ClN_4O_2S_2$) C, H, N.

*4.1.3.9. 2-{4-Chloro-5-methyl-2-[(2-trifluoromethylbenzyl)thio]benzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**34**).* Starting from **18** (0.226 g) and phenylpropiolaldehyde diethyl acetal with stirring for 9 h, the title compound **34** was obtained (0.244 g, 86%): m.p. 203–206 °C; IR (KBr): 3379, 3297, 3215 (NH), 2190 (C≡C), 1637 (NH), 1561, 1519, 1490, 1447 (C=N, C=C), 1315, 1159 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 4.39 (s, 2H, SCH_2), 7.05 (s, 1H, N=CH), 7.42–7.45 (m, 4H, arom.), 7.49 (d, *J* = 7.4 Hz, 1H, arom.), 7.52–7.57 (m, 2H, H-3, arom.), 7.66–7.69 (m, 4H, NH, arom.), 7.82 (brs, 1H, NH), 7.89 (s, 1H, H-6 arom.), 11.22 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 19.6, 34.6, 79.9, 103.4, 110.0, 120.7, 124.9, 126.7, 128.0, 128.2, 128.7, 129.5, 131.1, 131.3, 132.6, 132.8, 133.4, 133.8, 135.0, 135.3, 137.5, 140.7, 154.7 ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{20}ClF_3N_4O_2S_2$ [M + H^+] 565.0741, found 565.0737. Anal. ($C_{25}H_{20}ClF_3N_4O_2S_2$) C, H, N.

*4.1.3.10. 2-{4-Chloro-5-methyl-2-[(2-trifluoromethylbenzyl)thio]benzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**35**).* Starting from **18** (0.226 g) and 4-phenylbut-3-yn-2-one with stirring for 5 h, the title compound **35** was obtained (0.246 g, 85%): m.p. 178–180 °C; IR (KBr): 3396, 3281, 3228 (NH), 2926 (CH), 2181 (C≡C), 1633 (NH), 1560, 1490, 1454 (C=N, C=C), 1314, 1163 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.13 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.38 (s, 2H, SCH_2), 7.42–7.49 (m, 6H, NH, arom.), 7.51–7.56 (m, 2H, H-3, arom.), 7.66–7.68 (m, 3H arom.), 7.74 (brs, 1H, NH), 7.86 (s, 1H, H-6 arom.), 10.91 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{22}ClF_3N_4O_2S_2$ [M + H^+] 579.0898, found 579.0899. Anal. ($C_{26}H_{22}ClF_3N_4O_2S_2$) C, H, N.

*4.1.3.11. 2-{4-Chloro-5-methyl-2-[(3-trifluoromethylbenzyl)thio]benzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**36**).* Starting from **19** (0.226 g) and phenylpropiolaldehyde diethyl acetal with stirring for 10 h, the title compound **36** was obtained (0.224 g, 79%): m.p. 178–181 °C; IR (KBr): 3409, 3306, 3222 (NH), 3085, 2923, 2852 (CH), 2193 (C≡C), 1641 (NH), 1568, 1503, 1452 (C=N, C=C), 1330, 1165 (SO_2) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 4.44 (s, 2H, SCH_2), 7.14 (s, 1H, N=CH), 7.47–7.57 (m, 6H, NH, H-3, arom.), 7.63–7.73 (m, 5H, arom.), 7.78–7.87 (m, 2H, NH, H-6 arom.), 11.30 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{20}ClF_3N_4O_2S_2$ [M + H^+] 565.0741, found 565.0749. Anal. ($C_{25}H_{20}ClF_3N_4O_2S_2$) C, H, N.

*4.1.3.12. 2-{4-Chloro-5-methyl-2-[(3-trifluoromethylbenzyl)thio]benzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**37**).* Starting from **19** (0.226 g) and 4-phenylbut-3-yn-2-one with stirring for 4 h, the title compound **37** was obtained (0.220 g, 76%): m.p. 171–174 °C; IR (KBr): 3450, 3255, 3200 (NH), 2923 (CH), 2182 (C≡C), 1631 (NH), 1556, 1511, 1491, 1447 (C=N, C=C), 1332, 1167 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.16 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 4.41 (s, 2H, SCH_2), 7.43–7.46 (m, 3H, arom.), 7.48–7.51 (m, 2H, H-3, arom.), 7.53 (brs, 1H, NH), 7.58 (d, *J* = 7.3 Hz, 1H arom.), 7.62 (d, *J* = 7.8 Hz, 1H, arom.), 7.67–7.70 (m,

3H, arom.), 7.78 (brs, 1H, NH), 7.82 (s, 1H, H-6 arom.), 10.96 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{22}ClF_3N_4O_2S_2$ [M + H$^+$] 579.0898, found 579.0922. Anal. ($C_{26}H_{22}ClF_3N_4O_2S_2$) C, H, N.

*4.1.3.13. 2-{4-Chloro-5-methyl-2-[(4-trifluoromethylbenzyl)thio] benzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**38**).* Starting from **20** (0.226 g) and phenylpropiolaldehyde diethyl acetal with stirring for 2 h, the title compound **38** was obtained (0.218 g, 77%): m.p. 176–179 °C; IR (KBr): 3398, 3292, 3248 (NH), 2190 (C≡C), 1637 (NH), 1560, 1514, 1489, 1446 (C=N, C=C), 1323, 1177 (SO_2) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 4.47 (s, 2H, SCH_2), 7.17 (s, 1H, N=CH), 7.47–7.54 (m, 3H, H-3, arom.), 7.58 (m, 5H, arom.), 7.70–7.74 (m, 2H, arom.), 7.84–7.89 (m, 3H, 2 × NH, H-6 arom.), 11.35 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{20}ClF_3N_4O_2S_2$ [M + H$^+$] 565.0741, found 565.0727. Anal. ($C_{25}H_{20}ClF_3N_4O_2S_2$) C, H, N.

*4.1.3.14. 2-{4-Chloro-5-methyl-2-[(4-trifluoromethylbenzyl)thio] benzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**39**).* Starting from **20** (0.226 g) and 4-phenylbut-3-yn-2-one with stirring for 4 h, the title compound **39** was obtained (0.270 g, 94%): m.p. 187–190 °C; IR (KBr): 3455, 3280, 3249 (NH), 2921 (CH), 2184 (C≡C), 1636 (NH), 1558, 1513, 1492, 1441 (C=N, C=C), 1324, 1162 (SO_2) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.18 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 4.45 (s, 2H, SCH_2), 7.47–7.54 (m, 3H, H-3, arom.), 7.57–7.70 (m, 6H, NH, arom.), 7.71–7.74 (m, 2H, arom.), 7.83–7.85 (m, 2H, NH, H-6 arom.), 11.03 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{22}ClF_3N_4O_2S_2$ [M + H$^+$] 579.0898, found 579.0915. Anal. ($C_{26}H_{22}ClF_3N_4O_2S_2$) C, H, N.

*4.1.3.15. 2-{4-Chloro-2-[(2-chlorobenzyl)thio]-5-methylbenzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**40**).* Starting from **21** (0.210 g) and phenylpropiolaldehyde diethyl acetal with stirring for 3 h, the title compound **40** was obtained (0.231 g, 87%): m.p. 190–193 °C; IR (KBr): 3373, 3265 (NH), 2193 (C≡C), 1633 (NH), 1566, 1511, 1489, 1474 (C=N, C=C), 1330, 1168 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 4.34 (s, 2H, SCH_2), 7.08 (s, 1H, N=CH), 7.20–7.27 (m, 2H, arom.), 7.38–7.40 (m, 2H, arom.), 7.43–7.46 (m, 3H, NH, H-3, arom.), 7.48–7.51 (m, 1H, arom.), 7.67–7.68 (m, 3H, arom.), 7.80 (brs, 1H, NH), 7.87 (s, 1H, H-6 arom.), 11.23 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{20}Cl_2N_4O_2S_2$ [M + H$^+$] 531.0477, found 531.0483. Anal. ($C_{24}H_{20}Cl_2N_4O_2S_2$) C, H, N.

*4.1.3.16. 2-{4-Chloro-2-[(2-chlorobenzyl)thio]-5-methylbenzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**41**).* Starting from **21** (0.210 g) and 4-phenylbut-3-yn-2-one with stirring for 6 h, the title compound **41** was obtained (0.186 g, 68%): m.p. 177–179 °C; IR (KBr): 3396, 3291, 3244 (NH), 2181 (C≡C), 1633 (NH), 1567, 1508, 1491, 1474 (C=N, C=C), 1340, 1169 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.16 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 4.45 (s, 2H, SCH_2), 7.20–7.23 (m, 1H, arom.), 7.26–7.29 (m, 1H, arom.), 7.38–7.40 (m, 2H, arom.), 7.42–7.51 (m, 5H, NH, H-3, arom.), 7.67–7.69 (m, 2H, arom.), 7.73 (brs, 1H, NH), 7.85 (s, 1H, H-6 arom.), 10.93 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{22}Cl_2N_4O_2S_2$ [M + H$^+$] 545.0634, found 545.0627. Anal. ($C_{25}H_{22}Cl_2N_4O_2S_2$) C, H, N.

*4.1.3.17. 2-{4-Chloro-2-[(3-chlorobenzyl)thio]-5-methylbenzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**42**).* Starting from **22** (0.210 g) and phenylpropiolaldehyde diethyl acetal with stirring for 3 h, the title compound **42** was obtained (0.247 g, 93%): m.p. 195–197 °C; IR (KBr): 3373, 3306 (NH), 2192 (C≡C), 1643 (NH), 1568, 1511, 1451 (C=N, C=C), 1331, 1154 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 4.33 (s, 2H, SCH_2), 7.13 (s, 1H, N=CH), 7.26–7.29 (m, 3H, arom.), 7.39 (s, 1H, arom.), 7.44–7.51 (m, 4H, H-3, arom.), 7.69–7.70 (m, 2H, arom.), 7.72 (brs, 1H, NH), 7.83 (brs, 1H, NH), 7.85 (s, 1H, H-6 arom.), 11.26 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{20}Cl_2N_4O_2S_2$ [M + H$^+$] 531.0477, found 531.0506. Anal. ($C_{24}H_{20}Cl_2N_4O_2S_2$) C, H, N.

*4.1.3.18. 2-{4-Chloro-2-[(3-chlorobenzyl)thio]-5-methylbenzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**43**).* Starting from **22** (0.210 g) and 4-phenylbut-3-yn-2-one with stirring for 10 h, the title compound **43** was obtained (0.202 g, 76%): m.p. 175–178 °C; IR (KBr): 3447, 3257, 3200 (NH), 2182 (C≡C), 1631 (NH), 1555, 1511, 1491, 1477 (C=N, C=C), 1341, 1173 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.18 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 4.32 (s, 2H, SCH_2), 7.25–7.31 (m, 3H, arom.), 7.39 (s, 1H, arom.), 7.44–7.46 (m, 2H, arom.), 7.49–7.51 (m, 2H, H-3, arom.), 7.55 (brs, 1H, NH), 7.70–7.72 (m, 2H, arom.), 7.79 (brs, 1H, NH), 7.83 (s, 1H, H-6 arom.), 10.98 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 19.6, 22.9, 36.1, 80.9, 102.6, 120.6, 127.9, 128.4, 129.2, 129.5, 129.6, 130.8, 130.9, 131.3, 132.9, 133.2, 133.5, 133.6, 135.5, 137.4, 139.4, 140.6, 154.5 ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{22}Cl_2N_4O_2S_2$ [M + H$^+$] 545.0634, found 545.0620. Anal. ($C_{25}H_{22}Cl_2N_4O_2S_2$) C, H, N.

*4.1.3.19. 2-{4-Chloro-2-[(4-chlorobenzyl)thio]-5-methylbenzenesulfonyl}-3-(3-phenylprop-2-ynylideneamino)guanidine (**44**).* Starting from **23** (0.210 g) and phenylpropiolaldehyde diethyl acetal with stirring for 12 h, the title compound **44** was obtained (0.237 g, 89%): m.p. 175–177 °C; IR (KBr): 3386, 3302, 3220 (NH), 2191 (C≡C), 1639 (NH), 1567, 1507, 1491 (C=N, C=C), 1330, 1162 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.23 (s, 3H, CH_3), 4.31 (s, 2H, SCH_2), 7.14 (s, 1H, N=CH), 7.25 (d, J = 8.8 Hz, 2H, arom.), 7.34 (d, J = 8.3 Hz, 2H, arom.), 7.43–7.49 (m, 2H, arom.), 7.50–7.52 (m, 1H, arom.), 7.53 (s, 1H, H-3 arom.), 7.68–7.70 (m, 2H, arom.), 7.77 (brs, 1H, NH), 7.82–7.83 (m, 2H, NH, H-6 arom.), 11.29 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{24}H_{20}Cl_2N_4O_2S_2$ [M + H$^+$] 531.0477, found 531.0508. Anal. ($C_{24}H_{20}Cl_2N_4O_2S_2$) C, H, N.

*4.1.3.20. 2-{4-Chloro-2-[(4-chlorobenzyl)thio]-5-methylbenzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**45**).* Starting from **23** (0.210 g) and 4-phenylbut-3-yn-2-one with stirring for 5 h, the title compound **45** was obtained (0.223 g, 84%): m.p. 202–205 °C; IR (KBr): 3451, 3261 (NH), 2179 (C≡C), 1632 (NH), 1564, 1491, 1444 (C=N, C=C), 1342, 1175 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.17 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 4.30 (s, 2H, SCH_2), 7.27 (d, J = 8.8 Hz, 2H, arom.), 7.33 (d, J = 8.3 Hz, 2H, arom.), 7.43–7.46 (m, 2H, H-3, arom.), 7.48–7.51 (m, 2H, arom.), 7.55 (brs, 1H, NH), 7.69–7.70 (m, 2H, arom.), 7.77 (brs, 1H, NH), 7.81 (s, 1H, H-6 arom.), 10.97 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{22}Cl_2N_4O_2S_2$ [M + H$^+$] 545.0634, found 545.0637. Anal. ($C_{25}H_{22}Cl_2N_4O_2S_2$) C, H, N.

*4.1.3.21. 2-[4-Chloro-5-methyl-2-(naphthalene-1-ylmethylthio)benzenesulfonyl]-3-(3-phenylprop-2-ynylideneamino)guanidine (**46**).* Starting from **24** (0.218 g) and phenylpropiolaldehyde diethyl acetal with stirring for 20 h, the title compound **46** was obtained (0.213 g, 78%): m.p. 191–194 °C; IR (KBr): 3446, 3274 (NH), 3060, 2913, 2856 (CH), 2188 (C≡C), 1636 (NH), 1563, 1510, 1490, 1443 (C=N, C=C), 1343, 1168 (SO_2) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 4.76 (s, 2H, SCH_2), 6.97 (s, 1H, N=CH), 7.37–7.42 (m, 3H, arom.), 7.46–7.51 (m, 4H, NH, arom.), 7.58 (s, 1H, H-3 arom.), 7.61–7.63 (m, 3H, arom.), 7.78 (brs, 1H, NH), 7.83–7.84 (d, J = 8.0 Hz, 1H, arom.), 7.88 (s, 1H, H-6 arom.), 7.91–7.92 (m, 1H, arom.),

8.16–8.18 (m, 1H, arom.), 11.18 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{28}H_{23}ClN_4O_2S_2$ [M + H^+] 547.1024, found 545.1029. Anal. ($C_{28}H_{23}ClN_4O_2S_2$) C, H, N.

*4.1.3.22. 2-[4-Chloro-5-methyl-2-(naphthalene-1-ylmethylthio)benzenesulfonyl}-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**47**).* Starting from **24** (0.218 g) and 4-phenylbut-3-yn-2-one with stirring for 8 h, the title compound **47** was obtained (0.199 g, 71%): m.p. 177–180 °C; IR (KBr): 3448, 3265 (NH), 2182 (C≡C), 1633 (NH), 1566, 1491, 1444 (C=N, C=C), 1342, 1171 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.05 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.75 (s, 2H, SCH_2), 7.37–7.41 (m, 4H, arom.), 7.45–7.51 (m, 4H, arom.), 7.59–7.63 (m, 3H, NH, H-3, arom.), 7.71 (brs, 1H, NH), 7.84–7.86 (m, 2H, H-6, arom.), 7.91–7.93 (m, 1H, arom.), 8.16 (d, J = 8.3 Hz, 1H, arom.), 10.87 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{29}H_{25}ClN_4O_2S_2$ [M + H^+] 561.1180, found 561.1193. Anal. ($C_{29}H_{25}ClN_4O_2S_2$) C, H, N.

*4.1.3.23. 2-[4-Chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]-3-(3-phenylprop-2-ynylideneamino)guanidine (**48**).* Starting from **25** (0.232 g) and phenylpropiolaldehyde diethyl acetal with stirring for 15 h, the title compound **48** was obtained (0.101 g, 35%): m.p. 192–195 °C; IR (KBr): 3430, 3329, 3236 (NH), 3062, 2902 (CH), 2190 (C≡C), 1633 (NH), 1567, 1502, 1487 (C=N, C=C), 1346, 1171 (SO_2) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 4.23 (s, 2H, SCH_2), 6.02 (s, 2H, O-CH_2-O), 6.91 (s, 1H, N=CH), 6.97 (s, 1H, arom.), 7.09 (s, 1H, arom.), 7.42–7.50 (m, 4H, H-3, arom.), 7.66–7.68 (m, 3H, NH, arom.), 7.79 (brs, 1H, NH), 7.86 (s, 1H, H-6 arom.), 11.21 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{25}H_{20}Cl_2N_4O_4S_2$ [M + H^+] 575.0376, found 575.0391. Anal. ($C_{25}H_{20}Cl_2N_4O_4S_2$) C, H, N.

*4.1.3.24. 2-[4-Chloro-2-(6-chlorobenzo[d][1,3]dioxol-5-ylmethylthio)-5-methylbenzenesulfonyl]-3-(1-methyl-3-phenylprop-2-ynylideneamino)guanidine (**49**).* Starting from **25** (0.232 g) and 4-phenylbut-3-yn-2-one with stirring for 14 h, the title compound **49** was obtained (0.092 g, 31%): m.p. 178–181 °C; IR (KBr): 3454, 3258 (NH), 2180 (C≡C), 1639 (NH), 1552, 1471 (C=N, C=C), 1342, 1176 (SO_2) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.18 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 4.23 (s, 2H, SCH_2), 6.04 (s, 2H, O-CH_2-O), 6.92 (s, 1H, arom.), 7.01 (s, 1H, arom.), 7.44–7.50 (m, 5H, NH, H-3, arom.), 7.66–7.71 (m, 3H, NH, arom.), 7.85 (s, 1H, H-6 arom.), 10.94 (s, 1H, NH) ppm; HRMS (ESI-TOF) *m/z* calcd for $C_{26}H_{22}Cl_2N_4O_4S_2$ [M + H^+] 589.0532, found 589.0554. Anal. ($C_{26}H_{22}Cl_2N_4O_4S_2$) C, H, N.

4.2. X-ray structure determination

Diffraction intensity data were collected on an IPDS 2T dual-beam diffractometer (STOE & Cie GmbH, Darmstadt, Germany) at 120.0(2) K with Mo-Kα (**35**, **42** and **43**) or Cu-Kα (**28**) radiation of a microfocus x-ray source (GeniX 3D Mo High Flux, 50 kV, 1.0 mA, λ = 71.073 p.m. or GeniX 3D Cu High Flux, 0.6 mA, λ = 154.186 p.m., Xenocs, Sassenage, France). The crystal was thermostated in nitrogen stream at 120 K using CryoStream-800 device (Oxford CryoSystem, UK) during the entire experiment. Data collection and data reduction were controlled by X-Area 1.75 program [39]. An absorption correction was performed on the integrated reflections by a combination of frame scaling, reflection scaling and a spherical absorption correction. Outliers have been rejected according to Blessing's method [40]. Numerical absorption correction was performed after the optimization of the crystal-shape description by Herrendorf's method [41].

The structure was solved using direct methods with SHELXS-13 program and refined by SHELXL-2013 [42] program run under control of WinGx [43]. All C-H type hydrogen atoms were attached at their geometrically expected positions and refined as riding on heavier atoms with the usual constraints. The N-H hydrogen atoms were found in the differential Fourier electron density map and were refined with N-H bond distances constrained to 0.88 Å.

Crystallographic data for structures of **28**, **35**, **42** and **43** reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publications No. CCDC1496366 (**35**), CCDC1496367 (**28**), CCDC1496368 (**42**) and CCDC1496369 (**43**). Copies of tha data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: (+44) 1223-336-033; Email: deposit@ccdc.cam.ac.uk).

4.3. Cell culture and cell viability assay

All chemicals, if not stated otherwise, were obtained from Sigma-Aldrich (St. Louis, MO, USA). The MCF-7 and HeLa cell lines were purchased from Cell Lines Services (Eppelheim, Germany), the HCT-116 cell line was purchased from ATCC (ATCC-No: CCL-247). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C in an incubator (HeraCell, Heraeus, Langenselbold, Germany).

Cell viability was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide) assay. Stock solutions of the studied compounds were prepared in 100% DMSO. Working solutions were prepared by diluting the stock solutions with DMEM medium, the final concentration of DMSO did not exceed 0.5% in the treated samples. Cells were seeded in 96-well plates at a density of 5 × 10^3 cells/well and treated for 72 h with the examined compounds in the concentration range 1–100 μM (1, 10, 25, 50 and 100 μM). Following treatment, MTT (0.5 mg/mL) was added to the medium and cells were further incubated for 2 h at 37 °C. Cells were lysed with DMSO and the absorbance of the formazan solution was measured at 550 nm with a plate reader (1420 multilabel counter, Victor, Jügesheim, Germany). The optical density of the formazan solution was measured at 550 nm with a plate reader (Victor 1420 multilabel counter). The experiment was performed in triplicate. Values are expressed as the mean ± SD of at least three independent experiments.

4.4. QSAR study

4.4.1. Numerical representation of compounds optimized 3D structures

Three dimensional structures of the studied derivatives were obtained using *in silico* approach. 2D models, after being created in ACD ChemSketch (Advanced Chemistry Development, Inc., Toronto, Canada), were subjected to geometrical optimization. To shorten the calculation time, method called Semi-empirical AM1 force field was applied using HyperChem (v 8.0.8, HyperCube, Gainesville, FL, USA) was used for pre-optimization step. After this part, pre-optimized structured were subjected to Density Functional Theory (DFT) calculations. DFT calculations were conducted using Gaussian software [44] at the B3LYP/6–311 G(d) level of theory.

Using DRAGON 6.0 Software (Talete, Milano, Italy), a set of 4885 descriptors was calculated from optimized structures. After exclusion of descriptors that had constant value or no standard deviation value, 2762 descriptors, (divided in blocks, such as edge adjacency indices, 2D autocorrelation and geometrical descriptors to name a few) were imported into STATISTICA 10.0 software (Statsoft, Tulsa, OK, USA) to perform further statistic and chemometric calculations.

4.4.2. Statistic and chemometric approaches

Statistical analysis was performed using STATISTICA package.

Dataset, consisting of 24 compounds, was divided into two datasets – training set containing 18 compounds, and test set comprising 6 compounds, chosen randomly. All of the activity values were transformed using y = log(x) function to provide data distribution closer to normal. Data matrix was also subjected to standardization procedure. Using data mining and feature selection function of Statistica software, 5 most relevant descriptors were chosen among the whole training dataset. Feature selection is a tool used to develop a subset of most statistically important and relevant features. A theory behind this technique, is that large datasets contains features that are irrelevant or redundant. Feature selection excludes irrelevant features, and limits dataset to only the most relevant features, simplifying the model that is created afterwards and making it easier to interpret.

Support vector machines (SVM) method is a model that has been developed by Vapnik [45], and the theory behind Support Vector Regression was extensively described by Basak et al. [46]. The basic concept behind SVM is to map the studied data into higher dimensional feature space using kernel function, K (x, x_i). To calculate best possible values and kernel type for SVM model, optimization option of winSVM program was used. After performing 100 optimization runs with the training set on different parameter values and kernel types, mean square errors were compared. The combination of kernel type, capacity and epsilon values that produced the lowest mean square error were chosen and imported into Statistica software. Regression is then performed, providing a model for predicting values. The values for our regression was type 1 regression, with capacity of 10 and epsilon = 0.1 Our model used Radial Basis Factor kernel, with gamma = 0.2. Both training and test dataset were randomly chosen for each of 100 iterations of this model, meaning that presented model was created 100 times, each time using different, randomly chosen training and test sets.

4.5. Metabolic stability

Stock solutions of studied compounds were prepared at concentration of 100 mM in DMSO, then thinned down do 100 μM using phosphate buffer. Incubation mixes consisted of 5 μM of studied compound, 100 μM of NADPH in phosphate buffer and 1 mg/mL of pooled human liver microsomes (HLM) (Sigma-Aldrich, St. Louis, MO, USA) in potassium phosphate buffer (0.1 M, pH 7.4). Incubations were carried out in 96-well plates at 37 °C. Incubation mixtures (excluding compound solution) were subjected to 5 min pre-incubation, and started by addition of 20 μl of compound stock solution. After 0, 5, 10, 15, and 30 min 25 μl samples of incubation reaction were added to the equal volume of ice-cold acetonitrile containing 1 μM of internal standard (which was one of the formerly studied amidines, JBS 134). Control incubations were performed without NADPH to assess possible chemical instability. All samples were immediately centrifuged (10 min, 7500 g) and resulted supernatant was directly subjected to LC-MS analysis.

LC-MS analysis was performed on an Agilent 1260 system coupled to SingleQuad 6120 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Poroshell C18 EC120 column (3.0 × 100 mm, 2.7 μm, Agilent Technologies, Santa Clara, CA, USA) was used in reversed-phase mode with gradient elution starting with 60% of phase A (0.1% formic acid in deionised water) and 40% of phase B (0.1% formic acid in acetonitrile). Gradient elution program was: 0.00–10.00 min - 40%–100% B; 10.01 min-11.00 min - 100%–40% B; 11.00–15.00 min - 40%B. Total analysis time was 15 min at 40 °C, flow rate was 1.0 mL/min and the injection volume was 5 μL. The mass spectrometer was equipped with electrospray ionization source and ionization mode was positive. Mass analyzer was set individually to each derivative to detect pseudomolecular ions [M + H^+]. MSD parameters of the ESI source were as follows: nebulizer pressure 35 psig (N_2), drying gas 12 L/min (N_2), drying gas temperature 350 °C, capillary voltage 3.0 kV, fragmentor voltage 150 V.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.06.059.

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