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Syntheses and *In Vitro* Antitumor Activity of 3-Amino-*N*-(4-chlorobenzenesulfonyl)guanidine Derivatives Containing *N′*-Arylidene Moiety

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The syntheses of new 3-arylideneamino-1-(2-alkythio-4-chloro-5-methylbenzenesulfonyl)guanidines **2**–**8** and 1-arylidene-2-(2-alkylthio-4-chloro-5-R^3-benzenesulfonyl)-3-methylaminoguanidines **9**–**16** are described. The *in vitro* antitumor screening of compounds **2, 3, 9** and **10** was evaluated at the Institute of Pharmacy, University of Greifswald. The remaining compounds **5**, **11**–**14** and **16** were screened at the National Cancer Institute (NCI) for their activities against a panel of 55 human tumor cell lines, and relationships between structure and anticancer activity *in vitro* are discussed. The highest anticancer activity was found for 2-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrothienylidene)guanidine (**12**) (GI_{50} in the range 0.3–0.6 μM), while other compounds exhibit reasonable (**16**) or moderate (**9, 13**) anticancer activities.

Key words: 3-amino-*N*-(benzenesulfonyl)guanidines, synthesis, anticancer activity

Numerous of structurally novel sulfonamide derivatives have been reported to show substantial antitumor [1–4] or/and anti-HIV [2–7] properties. Although they have a common chemical motif of aromatic/heterocyclic sulfonamide, there are a variety of mechanisms of their antitumor action, such as cell cycle perturbation in the G1 phase, distribution of microtubule assembly, angiogenesis inhibition, functional suppression of the transcriptional activator NF-Y and carbonic anhydrase inhibition. On the other hand, many aminoguanidine derivatives [8–11] or arylsulfonylaminoguanidine [12] act as anticancer agents. Recently, we reported the syntheses of new series of arylsulfonylaminoguanidine derivatives possessing electron withdrawing substituents, either at the *N*-terminal nitrogen atom of the hydrazine moiety of type **I** [13] or at the opposite nitrogen atom of guanidine moiety of type **II** [14] with pronounced anticancer activity (Figure 1). This prompted us to investigate further the chemistry and biological activity of the related aminoguanidine derivatives.

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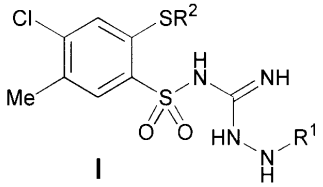
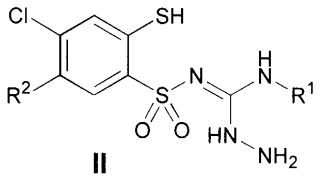


I

R^1= Ph, 2-pyridyl

R^2= Et, CH_2Ph, CH_2COOEt



II

R^1= $CH_2CH{=}CH_2$

R^2= Me, COOMe, PhNHCO,

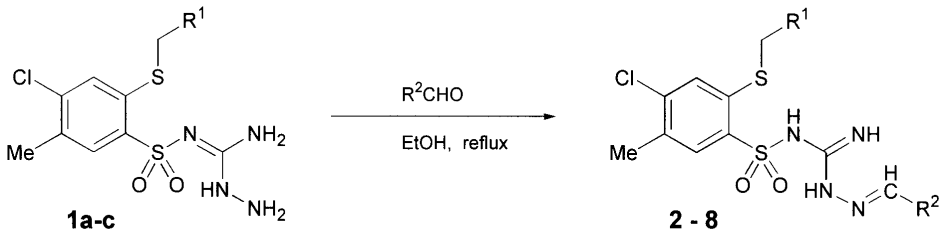
4-(Cl or F)-PhNHCO

Figure 1.

RESULTS AND DISCUSSION

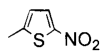
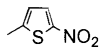
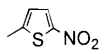
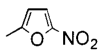
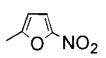
We wish to report on an efficient method of the syntheses of novel 3-arylideneamino-1-benzenesulfonylguanidines (**2**–**8**) (Scheme 1) and related 1-arylidene-2-benzenesulfonyl-3-methylaminoguanidines (**9**–**16**) (Scheme 2), as structural analogues of the reported compounds of type **I** and **II.** A series of 3-arylideneamino-1-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)guanidines (**2**–**8**) were synthesized

Scheme 1

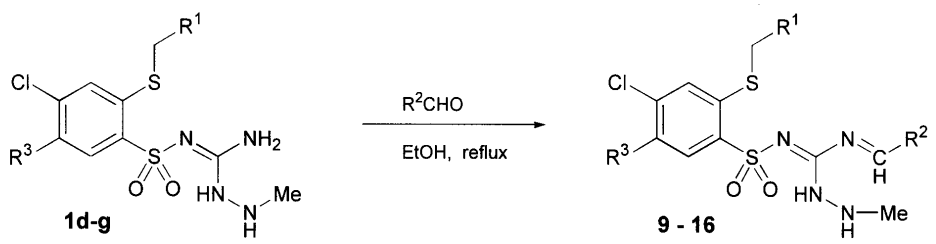


1a-c **2 - 8**

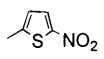
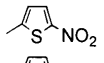
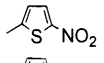
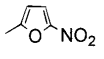
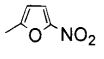
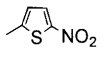
1a: R^1= Me
1b: R^1= Ph
1c: R^1= COOEt

Compd.	R^1	R^2
1a, 2	Me	Ph
1b, 3	Ph	Ph
1a, 4	Me	
1b, 5	Ph	
1c, 6	COOEt	
1b, 7	Ph	
1c, 8	COOEt	

Scheme 2



Compd.	R^1	R^3
1d	Me	Me
1e	Ph	Me
1f	COOEt	Me
1g	Ph	4-Cl-PhNHCO

Compd.	R^1	R^2	R^3
1d, 9	Me	Ph	Me
1e, 10	Ph	Ph	Me
1d, 11	Me		Me
1e, 12	Ph		Me
1f, 13	COOEt		Me
1e, 14	Ph		Me
1f, 15	COOEt		Me
1g, 16	Ph		4-Cl-PhNHCO

from 3-amino-2-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)guanidines (**1a**–**c**) by condensation with an appropriate aromatic aldehyde such as benzaldehyde, 5-nitrothiophene-2-carbaldehyde or 5-nitrofurane-2-carbaldehyde in ethanol at reflux as shown in Scheme 1. The condensation product desired was separated in a good to high yields. Two amino groups of the aminoguanidine **1a**–**c** exhibited different reactivity in reaction with aldehydes. This phenomenon should be considered on the ground of both typical guanidine tautomerism and nucleophilicity of the nitrogen atom. It seemed, that the relatively higher nucleophilicity of NH_2 group at N^3 position could be explained by possible amino-imino tautomeric equilibrium, due to migration of the proton from N^1 to the N^2 atom, to favour the opposite NH_2 group to the condensation with formyl group.

In order to introduce arylidene substituent into the nitrogen atom at the position 1 of guanidine moiety, the opposite primary amino group at the position 3 should be blocked. Therefore, 3-methylaminoguanidine derivatives were used for further reactions (Scheme 2). Thus, treatment of 2-(2-alkylthio-4-chloro-5-R^3-benzenesulfonyl)-3-methylaminoguanidines (**1d**–**g**) with such aldehydes under similar reaction conditions furnished the corresponding 1-arylidene-2-(2-alkylthio-4-chloro-5-R^3-benzenesulfonyl)-3-methylaminoguanidines (**9**–**16**) in high yields. The structure of the new compounds was evidenced by IR, ^1H and ^{13}C NMR spectra as well as elemental

analyses. Inspection of the ^{1}H NMR spectrum revealed, that in DMSO solution one stable tautomeric form of the compounds **2**–**8** exists. These spectra provided evidence for the confirmation of the sulfonylamino tautomeric form (**2**–**8**), since three separate signals of the chemically non-equivalent protons of N-H groups were found. Two broad singlet signals of exchangeable protons in the region δ = 7.23–7.40 ppm and δ = 7.72–7.86 ppm are assigned to the C=NH and -NH-N=C protons, respectively. The sharp singlet of the proton attributable to the SO_2NH group was found downfield in the region δ = 11.39–11.84 ppm. The ^{1}H NMR spectra of the compounds **9**–**16** exhibit two singlet signals of chemically non-equivalent protons of -N<u>H</u>-N<u>H</u>-CH_3 moiety. Two broad singlets of exchangeable protons in the region δ = 7.18–7.46 ppm and δ = 7.95–8.30 ppm are assigned to the NH-N<u>H</u>-CH_3 and N<u>H</u>-NH-CH_3 protons, respectively.

The IR spectra of all compounds showed absorption bands of the N-H valence vibrations in the wide region 3483–3166 cm^{-1} (two to four bands). The spectra of **6, 8, 13, 15** and **16** showed absorption band resulted from the C=O stretching vibrations of ester and amide in the region 1738–1731 cm^{-1} and 1684 cm^{-1}, respectively. IR spectra of **4**–**8** and **11**–**16** exhibited strong absorption band of the NO_2 antisymmetric vibrations in the 1569–1492 cm^{-1} region. Overlapped absorption bands resulted from the NO_2 symmetric and SO_2 antisymmetric stretching vibrations between 1352–1307 cm^{-1} and an absorption band of the SO_2 symmetric vibrations between 1143–1129 cm^{-1}.

The ^{1}H NMR spectra of **4**–**6, 11**–**13** and **16**, having 5-nitrothienylidene moiety, showed two separate doublet signals with equal coupling constant in the range J = 3.9–4.4 Hz of the H-3 and H-4 protons of thiophene ring in the region δ = 7.50–7.59 ppm and δ = 8.08–8.13 ppm, respectively. The spectra of **7, 8, 14** and **15**, bearing 5-nitrofurfurylidene moiety, revealed similarly two doublets of the H-3 and H-4 protons of furane ring at δ = 7.36–7.51 ppm and δ = 7.80–7.83 ppm with coupling constant J = 3.8–3.9 Hz. The ^{1}H MNR spectra of all compounds showed a singlet of the –N=C<u>H</u>-Ar proton in the downfield region δ = 7.86–8.11 ppm and characteristic singlet signals of the benzenesulfonamide H-3 and H-6 protons in the region δ = 7.40–7.54 ppm and δ = 7.94–8.27 ppm, respectively.

Biological activity and discussion. The following is to be noted regarding the strategy, which was applied in the biological evaluation process. Firstly, our effort tended toward the selection of anticancer active skeleton among of benzylidene derivatives **2, 3** and **9, 10**. Secondly, on the basis of these findings, the syntheses of the lead structure analogues were carried out for further anticancer screening. The *in vitro* anticancer screening of the selected compounds **2, 3, 9** and **10** was evaluated, using the human bladder cancer cell lines RT-4, RT-112 and 5637, human esophagus cancer KYSE-70, KYSE-510 and KYSE- 520, human pancreatic cancer YAPC and DAN-G, human cervix cancer SISO, human non-small cell lung cancer LCLC-103H and human breast cancer MCF-7 [15, 16]. The corresponding values of 50% growth inhibitory concentration (GI_{50}) are listed in Table 1.

Table 1. Inhibition of *in vitro* human cancer cell lines by compounds **2, 3, 9** and **10**[a].

Human cell lines	GI_{50} [μM][b]
	Compd. **9**
Urinary bladder cancer	
RT-4	*
RT-112	*
5637	20.38
Esophagus cancer	
KYSE-70	19.05
KYSE-510	29.27
KYSE-520	17.97
Pancreatic cancer	
YAPC	34.45
DAN-G	32.13
Cervix cancer	
SISO	29.58
Non-small cell lung cancer	
LCLC-103H	8.73
Breast cancer	
MCF-7	19.87

[a] Compounds **2**, **3** and **10** were inactive; [b] GI_{50} – molar concentration producing 50% growth inhibitory effect [15,16]; * – not active.

Among a series of benzylidene derivatives **2, 3, 9** and **10**, the anticancer activity was found for **9**, whereas the other compounds were inactive. Hence, the skeleton **9** was chosen as a leading structure. In consequence, a series of 2-(2-alkylthio-4-chloro-5-R^3-benzenesulfonyl)-3-methylamino-1-(5-nitrothienylidene or 5-nitrofurfurylidene)guanidines **11**–**14, 16**, as analogues of compound **9** and related compound **5**, were submitted to the US National Cancer Institute (Bethesda, MD), for *in vitro* testing against a panel approximately 55 tumor cell lines. Cell lines, derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. The compounds were at five concentrations at 10-fold dilution. A 48 h continuous drug exposure protocol was used and sulforhodamine B (SRB) protein assay was used to estimate the cell growth. Details of this system and the information, which is encoded by the activity pattern over all cell lines, have been published [17–19]. The antitumor activity of tested compounds is expressed by GI_{50}– molar concentration of the compound, that inhibits 50% net cell growth.

Compounds **5, 11** and **14** were inactive (GI_{50} > 100 μM), whereas the other compounds **12, 13** and **16** exhibited reasonable (**12, 16**) or moderate (**13**) *in vitro* anticancer activity against human cancer cell lines (Table 2). Relatively highest sensitivity (GI_{50} in the range 0.3–0.6μM) to the compounds described here was found for cell lines of leukemia (RPMI-8226), colon cancer (COLO 205, HCC-2998, HCT-116 and HCT-15) and melanoma (MALME-3M, SK-MEL-5).

Table 2. Inhibition of *in vitro* human cancer cell lines by compounds **5**, **11**–**14** and **16**[a, b].

Tumor cell lines	GI_{50} [μM][c]		
	12	**13**	**16**
Leukemia			
CCRF-CEM	NT	30.3	*
K-562	44.7	NT	10.5
HL-60(TB)	NT	21.7	*
MOLT-4	*	16.7	*
RPMI-8226	0.3	36.7	NT
SR	17.6	NT	*
Non-small cell lung cancer			
A549/ATCC	40.3	32.7	6.6
HOP-62	*	13.3	*
HOP-92	7.6	13.2	4.3
NCI-H226	*	33.1	*
NCI-H332M	*	26.0	*
NCI-H460	41.1	35.1	2.5
NCI-H552	*	30.2	*
NCI-H23	*	14.5	*
Colon cancer			
COLO 205	0.3	16.0	5.9
HCC-2998	0.3	NT	1.4
HCT-116	0.6	13.5	1.3
HCT-15	0.5	18.8	9.1
HT29	30.9	15.0	7.9
KM12	2.0	18.5	*
SW-620	40.2	31.1	4.4
CNS cancer			
SF-268	*	29.8	*
SF-295	*	16.1	*
SNB-19	*	28.8	*
U251	*	17.0	*
Melanoma			
LOX IMVI	*	17.8	*
MALME-3M	0.4	19.9	*
M14	9.5	23.1	15.4
SK-MEL-2	*	31.0	NT
SK-MEL-28	*	16.1	*
SK-MEL-5	0.4	15.2	9.9
UACC-257	*	28.7	*
UACC-62	31.2	14.1	*
Ovarian cancer			
IGROV1	*	42.7	*
OVCAR-3	*	10.3	*
OVCAR-4	*	20.7	*
OVCAR-5	*	6.0	*
OVCAR-8	*	32.6	*
SK-OV-3	*	24.3	*

Table 2. (Continued)

Renal cancer			
786-0	*	20.2	*
ACHN	*	17.0	*
CAKI-1	*	17.6	*
RXF-393	*	22.0	*
SN12C	NT	NT	3.3
TK-10	*	23.0	*
UO-31	*	36.6	*
Prostate cancer			
PC-3	*	25.0	*
DU-145	*	7.4	*
Breast cancer			
MCF7	95.8	32.6	*
NCI/ADR-RES	*	12.7	*
MDA-MB-231/ATCC	32.2	11.7	10.3
HS-578T	*	28.0	38.1
MDA-MB-435	18.9	16.3	9.4
BT-549	*	20.5	*
T-47D	NT	12.5	6.4

[a] Data obtained from the NCI`s *in vitro* disease-oriented human tumor cell screen [17–19]; [b] Compounds **5**, **11** and **14** were inactive; [c] Molar concentration of the compound that inhibits 50% net cell growth; NT – not tested; * – not active.

Interestingly, compounds **12** and **16** (R^1 = Ph) act selectively against one or more of the cell lines of leukemia, lung cancer, colon cancer, melanoma and breast cancer, whereas compound **13** ($R^1 = CO_2Et$) exhibited moderate activity against all of the fifty one human cancer cell lines tested. However, it is noteworthy, that replacement of thiophene ring in **12** (R^2 = 5-nitrothenyl) by furane ring (**14**, R^2 = 5-nitrofurfuryl) resulted in the total loss of activity. The same situation has been evidenced after replacement of phenyl group in **12** (R^1 = Ph) by methyl group (**11**, $R^1 = CH_3$). At the present stage, we may infer that the anticancer activity of this class of compounds depends on the electronic nature of all substituents.

EXPERIMENTAL

Melting points were determined on a Bűchi SMP 20 apparatus and were uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer FT IR 1600 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini (200 MHz) and Varian Unity Plus (500 MHz) spectrometers using TMS as internal standard (δ values in ppm). Thin-layer chromatography was performed on Merck Kieselgel $60F_{254}$ plates and visualised with UV or with iodine vapour. The starting guanidine derivatives **1a**–**f** were obtained according to the procedure described in [13]. The starting material for the synthesis of **1g**, *i.e.* *N*-[2-benzylthio-4-chloro-5-(4-chlorophenylcarbamoyl)benzenesulfonyl]cyanamide potassium salt was prepared in a similar manner as described in [20]: 78% yield, m.p. 152–154°C (dec.). IR (KBr) cm^{-1}: 3404, 3354, 3189, 2174, 1668, 1309, 1139. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.38 (s, 2H, SCH_2), 7.26–7.29 (m, 1H, aromat.), 7.34–7.37 (m, 2H, aromat.), 7.41 (d, J = 8.79 Hz, 2H, AA`XX` system),

7.48–7.49 (m, 2H, aromat.), 7.54 (s, 1H, H-3), 7.71 (d, J = 8.79 Hz, 2H, AA`XX` system), 7.87 (s, 1H, H-6), 10.74 (s, 1H, CONH). Anal. Calcd. for $C_{21}H_{14}Cl_2KN_3O_3S_2$: C, 47.5; H, 2.7; N, 7.9. Found: C, 47.3; H, 2.6; N, 8.1.

2-[2-Benzylthio-4-chloro-5-(4-chlorophenylcarbamoyl)benzenesulfonyl]-3-methylaminoguanidine (1g). To a suspension of *N*-[2-benzylthio-4-chloro-5-(4-chlorophenylcarbamoyl)benzenesulfonyl] cyanamide potassium salt (5.3 g, 10 mmol) in dry dioxane (40 ml) methylhydrazine (0.5 g, 11 mmol) and 96% sulfuric acid (0.28 ml, 5 mmol) were added. The reaction mixture was stirred at reflux for 4 h, then left to stand at room temperature overnight. The precipitate thus obtained was filtered off and dried, then treated with water (50 ml). After vigorously stirring for 20 minutes the precipitate was collected by filtration and dried to afford **1g** as a white crystals (3.5 g, 65%, m.p. 254–257°C dec.). IR (KBr) cm^{-1}: 3464, 3420, 3252, 3184, 1674, 1582, 1308, 1137. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.11 (s, 3H, NCH_3), 4.42 (s, 2H, SCH_2), 4.88 (s, 2H, NH_2), 6.92–7.13 (br.s, 2H, NH-NH), 7.26–7.29 (m, 1H, aromat.), 7.34–7.37 (m, 2H, aromat.), 7.41 (d, J = 8.79 Hz, 2H, AA`XX` system), 7.45–7.47 (m, 2H, aromat.), 7.59 (s, 1H, H-3), 7.71 (d, J = 8.79 Hz, 2H, AA`XX` system), 7.97 (s, 1H, H-6), 10.71 (s, 1H, NH). Anal. Calcd. for $C_{22}H_{21}Cl_2N_5O_3S_2$: C, 49.1; H, 3.9; N, 13.0. Found: C, 48.9; H, 3.7; N, 12.8.

3-Benzylideneamino-1-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)guanidines (2–3). To a suspension of **1a** or **1b** (4 mmol) in ethanol (25 ml) benzaldehyde (0.47 g, 4.4 mmol) was added. The reaction mixture was stirred at reflux for 4 h and left to stand in a refrigerator overnight. The resulting precipitate was filtered off, washed with ethanol (2×2 ml) and dried, then purified by recrystallization from ethanol.

3-Benzylideneamino-1-(4-chloro-2-ethylthio-5-methylbenzenesulfonyl)guanidine (2). Yield 1.3 g, 78%, (white crystals), m.p. 179–180°C (dec.). IR (KBr) cm^{-1}: 3446, 3325, 3248, 1604, 1340, 1115. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.22 (t, J = 7.3 Hz, 3H, CH_3), 2.33 (s, 3H, CH_3-Ar), 3.01 (q, J = 7.3 Hz, 2H, SCH_2), 7.25 (s, 1H, C=NH), 7.39–7.42 (m, 4H, aromat. H-3), 7.78–7.80 (m, 3H, NH, aromat.), 7.94 (s, 1H, N=CH), 8.04 (s, 1H, H-6), 11.39 (s, 1H, SO_2NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 13.24 (CH_3), 18.92 (CH_3-Ar), 26.02 (SCH_2), 127.03, 127.24, 128.63, 130,00, 130.58, 131.61, 133.85, 135.96, 136.70, 139.55, 144.48, 154.89 (C-guanid.). Anal. Calcd. for $C_{17}H_{19}ClN_4O_2S_2$: C, 49.7; H, 4.7; N, 13.7. Found: C, 49.8; H, 4.5; N 13.8.

3-Benzylideneamino-1-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)guanidine (3). Yield 1.6 g, 82%, (white crystals), m.p. 162–163°C. IR (KBr) cm^{-1}: 3465, 3357, 3226, 1606, 1577, 1343, 1132 . ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.31 (s, 3H, CH_3-Ar), 4.32 (s, 2H, SCH_2), 7.18–7.30 (m, 4H, C=NH, aromat.), 7.38–7.46 (m, 5H, aromat.), 7.50 (s, 1H, H-3), 7.78–7.86 (m, 3H, NH, aromat.), 7.93 (s, 1H, N=CH), 8.07 (s, 1H, H-6), 11.43 (s, 1H, SO_2NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 18.93 (CH_3-Ar), 36.21 (SCH_2), 127.21, 127.27, 127.66, 128.38, 128.67, 129.11, 130.04, 130.50, 131.91, 133.88, 135.55, 136.19, 136.56, 139.49, 144.94, 154.90 (C-guanid.). Anal. Calcd. for $C_{22}H_{21}ClN_4O_2S_2$: C, 55.9; H, 4.5; N, 11.8. Found: C, 55.6; H, 4.7; N, 12.0.

1-(2-Alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-(5-nitrothienylideneamino)guanidines (4–6). To a suspension of appropriate **1a**–**c** (4 mmol) in ethanol (20 ml) 5-nitrothiophene-2-carbaldehyde (0.69 g, 4.4 mmol) was added. The reaction mixture was stirred at reflux for 4 h and left to stand overnight. The resulting precipitate was filtered off washed with hot ethanol (3×2 ml) and dried to afford pure title product.

1-(4-Chloro-2-ethylthio-5-methylbenzenesulfonyl)-3-(5-nitrothienylideneamino)guanidine (4). Yield 1.8 g, 97%, (yellow powder), m.p. 223–225°C (dec.). IR (KBr) cm^{-1}: 3469, 3358, 3271, 1645, 1615, 1533, 1331, 1130. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.22 (t, J = 7.3 Hz, 3H, CH_3), 3.01 (q, J = 7.3 Hz, 2H, SCH_2), 7.29 (br.s, 1H, C=NH), 7.43 (s, 1H, H-3), 7.52 (d, J = 3.9 Hz, 1H, *H*-thiophene), 7.84 (br.s, 1H, NH), 7.94 (s, 1H, N=CH), 8.08 (d, J = 3.9 Hz, 1H, *H*-thiophene), 8.20 (s, 1H, H-6), 11.77 (s, 1H, SO_2NH). Anal. Calcd. for $C_{15}H_{15}ClN_5O_4S_3$: C, 39.1; H, 3.3; N, 15.2. Found: C, 39.2; H, 3.1; N, 15.4.

1-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(5-nitrothienylideneamino)guanidine (5). Yield 1.9 g, 90%, (yellow crystals), m.p. 183–185°C. IR (KBr) cm^{-1}: 3447, 3227, 1619, 1597, 1567, 1333, 1135. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3-Ar), 4.33 (s, 2H, SCH_2), 7.21–7.34 (m, 4H, C=NH, aromat.), 7.38–7.14 (m, 2H, aromat.), 7.52 (s, 1H, H-3), 7.56 (d, J = 4.2 Hz, 1H, *H*-thiophene),

7.82 (br.s, 1H, NH), 7.93 (s, 1H, N=CH), 8.08 (d, J = 4.2 Hz, 1H, *H*-thiophene), 8.23 (s, 1H, H-6), 11.82 (s, 1H, SO_2NH). Anal. Calcd. for $C_{20}H_{18}ClN_5O_4S_3$: C, 45.8; H, 3.5; N, 13.4. Found: C, 45.7; H, 3.2; N, 13.1.

1-(4-Chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulfonyl)-3-(5-nitrothienylideneamino)guanidine (6). Yield 1.8 g, 85%, (yellow crystals), m.p. 172–174°C. IR (KBr) cm^{-1}: 3448, 3330, 3242, 3166, 1731, 1622, 1569, 1331, 1134. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.14 (t, J = 7.1 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3-Ar), 4.00 (s, 2H, SCH_2), 4.09 (q, J = 7.1 Hz, 2H, OCH_2), 7.31 (br.s, 1H, C=NH), 7.46 (s, 1H, H-3), 7.54 (d, J = 4.3 Hz, 1H, *H*-thiophene), 7.85 (br.s, 1H, NH), 7.86 (s, 1H, N=CH), 8.08 (d, J = 4.3 Hz, 1H, *H*-thiophene), 8.19 (s, 1H, H-6), 11.81 (s, 1H, SO_2NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 13.96 (CH_3), 18.89 (CH_3-Ar), 34.31 (SCH_2), 61.12 (OCH_2), 127.64, 129.38, 130.34, 130.63, 132.48, 134.49, 136.81, 137.81, 139.09, 151.04, 145.96, 154.38 (C-guanid.), 168.94 (C=O). Anal. Calcd. for $C_{17}H_{18}ClN_5O_6S_3$: C, 39.3; H, 3.5; N, 13.5. Found: C, 39.2; H, 3.5; N, 13.6.

1-(2-Alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-(5-nitrofurfurylideneamino)guanidines (7–8). To a suspension of **1b** or **1c** (4 mmol) in ethanol (20 ml) 5-nitrofurane-2-carbaldehyde (0.62 g, 4.4 mmol) was added. The reaction mixture was stirred at reflux for 4 h and left to stand overnight. The resulting precipitate was filtered off, washed with hot ethanol (3×2 ml) and dried to give pure **7**. The crude product **8** was triturated with chloroform and recrystallized from methanol to afford pure compound **8.**

1-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-(5-nitrofurfurylideneamino)guanidine (7). Yield 1.6 g, 78%, (yellow powder), m.p. 207–209°C. IR (KBr) cm^{-1}: 3459, 3353, 3264 , 1632, 1606, 1565, 1350, 1326, 1134. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3-Ar), 4.33 (s, 2H, SCH_2), 7.27–7.34 (m, 4H, C=NH, aromat.), 7.36–7.41 (m, 3H, aromat.), 7.51 (s, 1H, H-3), 7.75 (br.s, 1H, NH), 7.80 (d, J = 3.8 Hz, 1H, *H*-furane), 7.92 (s, 1H, N=CH), 7.99 (s, 1H, H-6), 11.84 (s, 1H, SO_2NH). Anal. Calcd. for $C_{20}H_{18}ClN_5O_5S_2$: C, 47.3; H, 3.6; N, 13.8. Found: C, 47.4; H, 3.3; N, 13.8.

1-(4-Chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulfonyl)-3-(5-nitrofurfurylideneamino)guanidine (8). Yield 1.1 g, 53%, (yellow powder), m.p. 154–156°C. IR (KBr) cm^{-1}: 3462, 3351, 3217, 1733 , 1635, 1615, 1560, 1352, 1323, 1134. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.16 (t, J = 7.1 Hz, 3H, CH_3), 2.35 (s, 3H, CH_3-Ar), 4.01 (s, 2H, SCH_2), 4.10 (q, J = 7.1 Hz, 2H, OCH_2), 7.36 (d, J = 3.9 Hz, 1H, *H*-furane), 7.46 (br.s, 2H, C=NH, H-3), 7.74 (br.s, 1H, NH), 7.80 (d, J = 3.9 Hz, 1H, *H*-furane), 7.97 (s, 2H, N=CH, H-6), 11.84 (s, 1H, SO_2NH). Anal. Calcd. for $C_{17}H_{18}ClN_5O_7S_2$: C, 40.5; H, 3.6; N, 13.9. Found: C, 40.3 H, 3.3; N, 13.7.

1-Benzylidene-2-(2-alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-methylaminoguanidines (9–10). To a suspension of **1d** or **1e** (4 mmol) in ethanol (25 ml) benzaldehyde (0.47 g, 4.4 mmol) was added. The reaction mixture was stirred at reflux for 4 h and left to stand in a refrigerator overnight. The resulting precipitate was filtered off, washed with ethanol (2×2 ml) and dried, then purified by recrystallization from ethanol.

1-Benzylidene-2-(4-chloro-2-ethylthio-5-methylbenzenesulfonyl)-3-methylaminoguanidine (9). Yield 1.4 g, 83%, (white crystals), m.p. 164–166°C. IR (KBr) cm^{-1}: 3470, 3347, 1595, 1344, 1136. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.21 (t, J = 7.3 Hz, 3H, CH_3), 2.33 (s, 3H, CH_3-Ar), 3.03 (q, J = 7.3 Hz, 2H, SCH_2), 3.40 (s, 3H, NCH_3), 7.28 (br.s, 1H, NH), 7.36–7.46 (m, 4H, aromat.), 7.86–8.02 (m, 4H, N=CH, aromat.), 8.04 (br.s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 13.30 (CH_3), 18.90 (CH_3-Ar), 25.93 (SCH_2), 29.88 (N-CH_3),127.10, 127.65, 128.58, 129.94, 130.53, 131.59, 134.09, 135.93, 136.95, 138.98, 142.40, 154.77 (C-guanid.). Anal. Calcd. for $C_{18}H_{21}ClN_4O_2S_2$: C, 50.9; H, 4.9; N, 13.2. Found: C, 50.9; H, 5.1; N, 13.2.

1-Benzylidene-2-(2-benzylthio-4-chloro-5-methylbenzenesulfonyl)- 3-methylaminoguanidine (10). Yield 1.6 g, 81%, (white powder), m.p. 140–142°C. IR (KBr) cm^{-1}: 3477, 3354, 1594, 1342, 1139. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.33 (s, 3H, CH_3-Ar), 3.41 (s, 2H, SCH_2), 4.35 (s, 3H, N-CH_3), 7.18–7.34 (m, 4H, NH, aromat.), 7.34–7.50 (m, 5H, aromat.), 7.54 (s, 1H, H-3), 7.89–7.99 (m, 3H, aromat., N=CH), 8.03 (br.s, 2H, NH, H-6). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 18.89 (CH_3-Ar), 29.91 (N-CH_3), 35.99 (SCH_2), 127.20, 127.50, 127.67, 128.39, 128.59, 128.94, 129.94, 130.43, 131.82, 134.12, 135.65, 136.29, 136.80, 138.73 (14C, aromatic), 142.48 (N=CH), 154.77 (C-guanid.). Anal. Calcd. for $C_{23}H_{23}ClN_4O_2S_2$: C, 56.7; H, 4.8; N, 11.5. Found: C, 56.5; H, 4.5; N, 11.4.

2-(2-Alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrothienylidene)guanidines (11–13). To a suspension of appropriate **1d**–**f** (4 mmol) in ethanol (20 ml) 5-nitrothiophene-2-carbaldehyde (0.69 g, 4.4 mmol) was added. The reaction mixture was stirred at reflux for 4 h and left to stand overnight. The resulting precipitate was filtered off, washed with hot ethanol (3×2 ml) and dried to afford pure title product.

2-(4-Chloro-2-ethylthio-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrothienylidene)guanidine (11). Yield 1.8 g, 92%, (yellow crystals), m.p. 190–192°C. IR (KBr) cm^{-1}: 3462, 3344, 1616, 1587, 1492, 1330, 1130. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.22 (t, J = 7.2 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3-Ar), 3.04 (q, J = 7.2 Hz, 2H, SCH_2), 3.39 (s, 3H, NCH_3), 7.37 (br.s, 1H, NH), 7.45 (s, 1H, H-3), 7.50 (d, J= 4.3 Hz, 1H, *H*-thiophene), 7.94 (s, 2H, NH, N=CH), 8.10 (d, J = 4.3 Hz, 1H, *H*-thiophene), 8.22 (s, 1H, H-6). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 13.33 (CH_3), 18.92 (CH_3-Ar), 25.95 (SCH_2), 30.64 (NCH_3), 127.19, 129.27, 130.39, 130.59, 131.71, 135.85, 136.00, 137.15, 138.63, 146.29, 150.98, 154.32 (C-guanid.). Anal. Calcd. for $C_{16}H_{18}ClN_5O_4S_3$: C, 40.4; H, 3.8; N, 14.7. Found: C, 40.1; H, 3.6; N, 14.5.

2-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrothienylidene) guanidine (12). Yield 1.7 g, 80%, (yellow crystals), m.p. 188–190°C. IR (KBr) cm^{-1}: 3471, 3438, 3333, 1616, 1587, 1493, 1335, 1129. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3-Ar), 3.37 (s, 3H, NCH_3), 4.35 (s, 2H, SCH_2), 7.20–7.31 (m, 3H, NH, aromat.), 7.32–7.39 (m, 2H, aromat.), 7.54 (s, 1H, H-3), 7.57 (d, J = 4.3 Hz, 1H, *H*-thiophene), 7.92 (s, 1H, N=CH), 7.97 (br.s, 1H, NH), 8.11 (d, J = 4.3 Hz, 1H, *H*-thiophene), 8.25 (s, 1H, H-6). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 18.91 (CH_3-Ar), 30.65 (NCH_3), 36.02 (SCH_2), 127.25, 127.68, 128.43, 128.94, 129.29, 130.42, 130.49, 131.95, 135.73, 135.89, 136.31, 137.00, 138.41, 146.30, 151.01, 154.33 (C-guanid.). Anal. Calcd. for $C_{21}H_{20}ClN_5O_4S_3$: C, 46.9; H, 3.8; N, 13.0. Found: C, 46.9; H, 3.6; N, 13.2.

2-(4-Chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrotheinylidene)guanidine (13). Yield 1.8 g, 87%, (yellow crystals), m.p. 121–123°C. IR (KBr) cm^{-1}: 3463, 3339, 3288, 1734, 1617, 1585, 1506, 1333, 1143. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.13 (t, J = 7.1 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3-Ar), 3.39 (s, 3H, NCH_3), 4.05 (s, 2H, SCH_2), 4.09 (q, J = 7.1 Hz, 2H, OCH_2), 7.36 (br.s, 1H, NH), 7.48 (s, 1H, H-3), 7.56 (d, J= 4.4 Hz, 1H, *H*-thiophene), 7.95 (s, 1H, N=CH), 7.99 (br.s, 1H, NH), 8.11 (d, J = 4.4 Hz, 1H, *H*-thiophene), 8.24 (s, 1H, H-6). Anal. Calcd. for $C_{18}H_{20}ClN_5O_6S_3$: C, 40.5; H, 3.8; N, 13.1. Found: C, 40.2; H, 3.5; N, 13.4.

2-(2-Alkylthio-4-chloro-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrofurfurylidene)guanidines (14–15). To a suspension of **1e** or **1f** (4 mmol) in ethanol (20 ml) 5-nitrofurane-2-carbaldehyde (0.62 g, 4.4 mmol) was added. The reaction mixture was stirred at reflux for 4 h and left to stand overnight. The resulting precipitate was filtered off, washed with hot ethanol (3×2 ml) and dried to give pure **15**. The crude reaction product **14** was extracted with ethanol (50 ml) at reflux, then filtered out to afford pure title product **14.**

2-(2-Benzylthio-4-chloro-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrofurfurylidene) guanidine (14). Yield 1.4 g, 68%, (yellow powder), m.p. 225–228°C (dec.). IR (KBr) cm^{-1}: 3481, 3372, 1617, 1597, 1507, 1349, 1314, 1134. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3-Ar), 3.37 (s, 3H, NCH_3), 4.35 (s, 2H, SCH_2), 7.21–7.39 (m, 5H, aromat.), 7.45 (br.s, 1H, NH), 7.51–7.53 (m, 2H, H-3, *H*-furane), 7.83 (d, J = 3.8 Hz, 1H, *H*-furane), 7.94 (s, 1H, N=CH), 7.98 (s, 1H, H-6), 8.35 (br.s, 1H, NH). Anal. Calcd. for $C_{21}H_{20}ClN_5O_5S_2$: C, 48.3; H, 3.9; N, 13.4. Found: C, 48.0; H, 3.7; N, 13.2.

2-(4-Chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulfonyl)-3-methylamino-1-(5-nitrofurfurylidene)guanidine (15). Yield 1.9 g, 92%, (yellow crystals), m.p. 180–182°C. IR (KBr) cm^{-1}: 3482, 3373, 1738, 1617, 1584, 1506, 1348, 1314, 1130. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.12 (t, J = 7.2 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3-Ar), 3.39 (s, 3H, NCH_3), 4.02–4.13 (m, 4H, SCH_2, OCH_2)**,** 7.48 (s, 2H, H-3, NH), 7.51 (d, J = 3.9 Hz, 1H, *H*-furane), 7.82 (d, J = 3.9 Hz, 1H, *H*-furane), 7.94 (s, 2H, N=CH, H-6), 8.06 (br.s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 13.95 (CH_3), 18.89 (CH_3-Ar), 30.45 (NCH_3), 34.09 (SCH_2), 61.08 (OCH_2), 113.24, 115.05, 127.63, 130.36, 130.61, 132.38, 134.62, 137.06, 138.45, 151.56, 152.78, 154.33 (C-guanid), 168.88 (C=O). Anal. Calcd. for $C_{18}H_{20}ClN_5O_7S_2$: C, 41.7; H, 3.9; N, 13.5. Found: C, 41.9; H, 3.9; N, 13.6.

2-[2-Benzylthio-4-chloro-5-(4-chlorophenylcarbamoyl)benzenesulfonyl]-3-methylamino-1-(5-nitrothienylidene)guanidine (16). To a suspension of **1g** (2.15 g, 4 mmol) in ethanol (70 ml) 5-nitrofurane-2-carboxaldehyde (0.62 g, 4.4 mmol) was added. The reaction mixture was stirred at reflux for 8 h and left to stand in a refrigerator overnight. The resulting precipitate was filtered off, washed with ethanol (3×2 ml) and dried. The crude reaction product was dissolved in DMSO (7 ml) at 35–40°C, then precipitated after addition of dry methanol (25 ml) to give **16** as a yellow crystals (1.9 g, 71%, m.p. 265–266°C dec.). IR (KBr) cm^{-1}: 3483, 3354, 3301, 3271, 1684, 1616, 1592, 1507, 1331, 1307, 1131. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.39 (s, 3H, NCH_3), 4.47 (s, 2H, SCH_2), 7.23–7.26 (m, 1H, aromat.), 7.29–7.33 (m, 2H, aromat.), 7.41–7.46 (m, 5H, aromat. H-3, NH), 7.59 (d, J = 3.9 Hz, 1H, *H*-thiophene), 7.70–7.73 (m, 3H, aromat.), 8.03 (br.s, 1H, NH), 8.11 (s, 1H, N=CH), 8.13 (d, J = 3.9 Hz, 1H, *H*-thiophene), 8.27 (s, 1H, H-6), 10.74 (s, 1H, NHCO). Anal. Calcd. for $C_{27}H_{22}Cl_2N_6O_5S_3$: C, 47.9; H, 3.9; N, 12.4. Found: C, 47.6; H, 3.8; N, 12.2.

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