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# Syntheses of a New Series of *N*-Amino-*N′*-(benzenesulphonyl)guanidine Derivatives with Potential Antitumor Activity

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Syntheses of novel *N*-amino-*N′*-(benzenesulphonyl)guanidines (**4a–h, 5a–g**) from *N*-(benzenesulphonyl)cyanamide potassium salt and hydrazine derivatives are described. Compounds **4b**, **5a–c** and **5e** were evaluated by *in vitro* assays of growth inhibition against several human tumor cell lines. The highest *in vitro* cytotoxic activities were found for 3-phenylamino-1-(2-benzylthio-4-chloro-5-methylbenzenesulphonyl)guanidine (**5b**) ($IC_{50}$ = 2.35–8.14 $\mu$M) and 3-phenylamino-1-(4-chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulphonyl)guanidine (**5c**) ($IC_{50}$ = 2.74–10.6 $\mu$M), while other tested compounds (**5a**, **5e**) showed the moderate cytotoxic activities.The molecular orbital calculation of the possible tautomeric forms of the benzenesulphonylguanidine derivatives were also presented.

**Key words**: cyanamides, benzenesulphonylguanidines, tautomeric equilibria, cytotoxic activity, structure-activity relationship

Recently, a variety of arylsulphonylguanidines [1–3] and arylsulphonylaminoguanidines [4–6] have been shown to possess anticancer activity. As a result of our studies on the synthesis of 1,1-dioxo-1,4,2-benzodithiazine derivatives and their conversions into 2-mercaptobenzenesulphonamides, we previously reported the synthesis of 2-(4-chloro-2-mercaptobenzenesulphonyl)guanidine derivatives [7] (**I**, Figure 1) and 3-amino-2-(4-chloro-2-mercaptobenzenesulphonyl)guanidine derivatives [6, 8] (**II**, Figure 1) designed as anticancer agents. A remarkable antineoplastic activity against human cancer cell lines found for the 1-allyl-3-amino-2-(4-chloro-2-mercaptobenzenesulphonyl)guanidine derivatives [6] prompted us to extend these investigations to a series of novel *N*-amino-*N′*-(4-chloro-2-mercapto-5-methylbenzenesulphonyl)guanidine derivatives (**III**, Figure 1).

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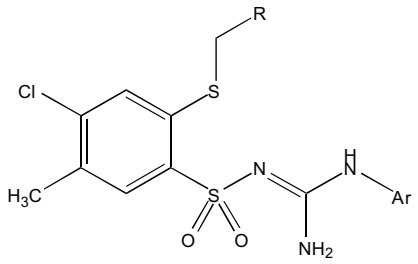
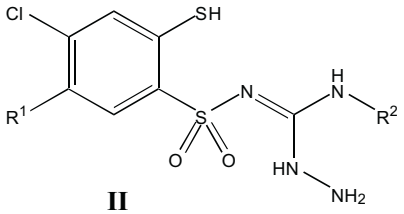


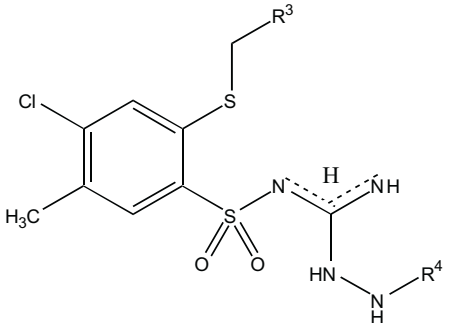
**I**

R = $CO_2Et$, Ph, 2-amino-6-dimethylamino-1,3,5-triazin-4-yl
Ar = Ph or substituted phenyl



**II**

$R^1$ = Me, $CO_2Me$, PhNHCO, 4-(Cl, F)PhNHCO
$R^2$ = Me, Pr, Bu, Bn, $PhCH_2CH_2$-, Ph and substituted phenyl



**III**

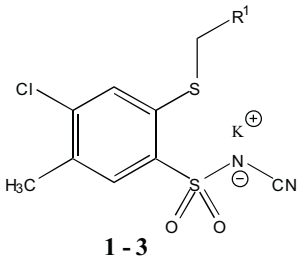
$R^3$ = Me, Ph, $CO_2Et$
$R^4$ = H, Me, $CONH_2$, Ph, 4-$NO_2$Ph, 4-$CH_3$Ph, 2-pyridyl

**Figure 1.**

## RESULTS AND DISCUSSION

In the present work, we wish to report on the efficient methods of the syntheses of novel *N*-amino-*N′*-(4-chloro-2-mercaptobenzenesulphonyl)guanidine derivatives of type **III** (Figure 1), which possess either two unsubstituted amino groups or a substituent at the *N*-terminal nitrogen atom of the hydrazine moiety.

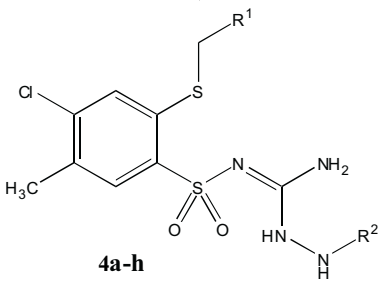
In general, the method is based on the reaction of hydrazine or hydrazine derivatives with appropriate *N*-(2-alkylthio-4-chloro-5-methylbenzenesulphonyl)cyanamide potassium salt **1**, **2** or **3** (Scheme 1). It was found, however, that depending on the substitution pattern of hydrazine derivative used, the two different tautomeric forms **4** and **5** of products were found.



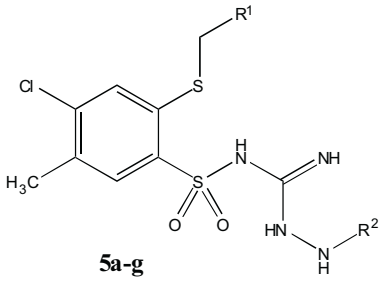
**1 - 3**

$R^1$= $CH_3$(**1**), Ph (**2**), COOEt (**3**)

**i, ii, iii** **i, iii**



**4a-h**



**5a-g**

| Compounds | Method | $R^1$ | $R^2$ |
|---|---|---|---|
| **1**, **4a** | i | $CH_3$ | H |
| **2**, **4b** | i | Ph | H |
| **1**, **4c** | i | $CH_3$ | $CH_3$ |
| **2**, **4d** | i | Ph | $CH_3$ |
| **3**, **4e** | i | $CO_2Et$ | $CH_3$ |
| **3**, **4f** | ii | $CO_2Et$ | H |
| **2**, **4g** | iii | Ph | $CONH_2$ |
| **3**, **4h** | iii | $CO_2Et$ | $CONH_2$ |

| Compounds | Method | $R^1$ | $R^2$ |
|---|---|---|---|
| **1**, **5a** | i | $CH_3$ | Ph |
| **2**, **5b** | i | Ph | Ph |
| **3**, **5c** | i | $CO_2Et$ | Ph |
| **2**, **5d** | i | Ph | 4-$NO_2$Ph |
| **2**, **5e** | i | Ph | 2-pyridyl |
| **2**, **5f** | iii | Ph | 4-$CH_3$Ph |
| **3**, **5g** | iii | $CO_2Et$ | 4-$CH_3$Ph |

**Scheme 1.** Reagents and conditions for methods: (**i**) $H_2N$-NH-$R^2$ (2 molar equiv.), $H_2SO_4$ (1 molar equiv.), $CCl_4$, reflux 24 h; (**ii**) $H_2N$-$NH_2 \cdot H_2O$ (2 molar equiv.), $H_2SO_4$ (1 molar equiv.), dioxane, reflux 48 h; (**iii**) $H_2N$-NH-$R^2 \cdot$HCl (1 molar equiv.), toluene, reflux 2 h.

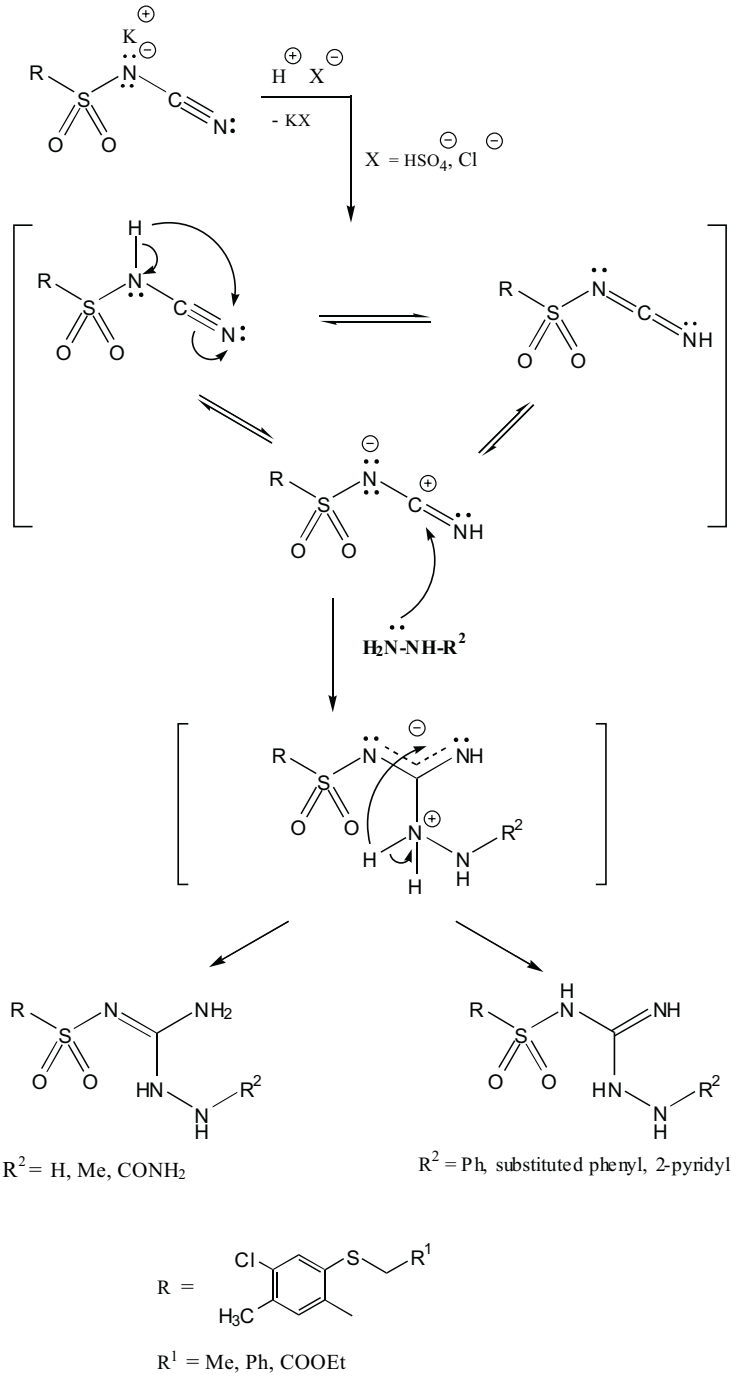
Thus, treatment of **1**, **2** or **3** with two molar equivalents of hydrazine hydrate or methylhydrazine in boiling carbon tetrachloride in the presence of one molar equivalent of conc. sulfuric acid leads to the formation of the desired 3-amino-2-(4-chloro-5-methylbenzenesulphonyl)guanidines **4a–e** in good yields (**i**, Scheme 1). On the other hand, treatment of **1**, **2** or **3** with phenylhydrazine, 4-nitrophenylhydrazine or

2-hydrazinopyridine under similar reaction conditions affords 3-arylamino-1-(4-chloro-5-methylbenzenesulphonyl)guanidines **5a–e** (**i**, Scheme 1).

It should be mentioned that treatment of **3** with hydrazine hydrate under these conditions leads to the mixture of products and the desired compound **4f** could be isolated in poor yield. We have overcome this difficulty by changing the reaction conditions. Thus, using dioxane as a solvent and a low reagents concentration, the product **4f** was isolated in 96% yield (**ii**, Scheme 1).

Furthermore, *N*-(2-alkylthio-4-chloro-5-methylbenzenesulphonyl)cyanamide potassium salts **2** and **3** were converted into ureidoguanidines **4g–h** and aminoguanidines **5f–g** (**iii**, Scheme 1) by the reaction with equimolar amount of hydrazine hydrochloride derivatives such as semicarbazide hydrochloride or p-tolylhydrazine hydrochloride in boiling toluene. The mechanism of this reaction could be explained by transient formation of the unstable free sulphonylcyanamide N–H acid upon mineral acid addition followed by the nucleophilic attack of the hydrazine nitrogen atom at the electron deficient cyanamide carbon atom (Scheme 2).

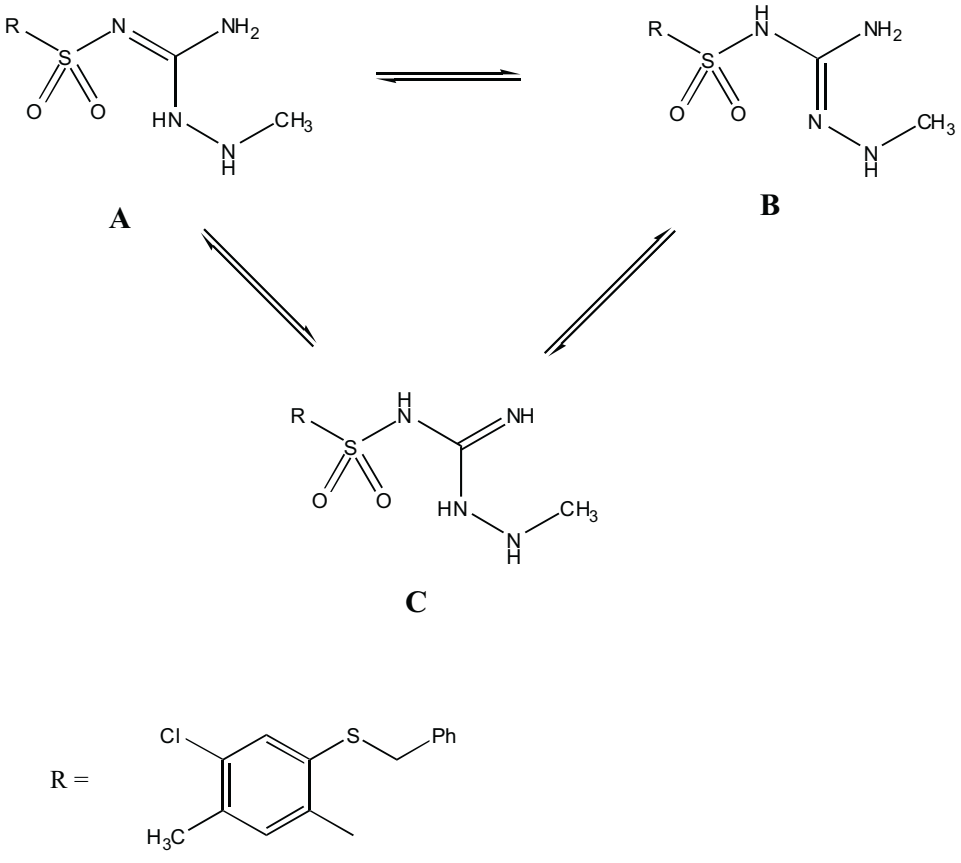
We have also studied the tautomerism of compounds **4a–h** and **5a–g** both in solution by NMR spectroscopy and in the gas phase by *ab initio* calculations for isolated molecules. Thus, inspection of the $^1$H NMR spectra revealed that in DMSO solution depending on the electronic nature of the substituent $R^2$ the two different tautomeric forms **4a–h** or **5a–g** exist, as shown in Scheme 1. The most typical iminosulphonyl tautomer of type **4** [6, 9–12] was observed in the case of compounds, which possess either unsubstituted nitrogen atom at the hydrazine moiety (**4a–b** and **4f**) or substituent $R^2$, such as methyl or carbamoyl group (**4c–e** and **4g–h**). The spectra of the compounds **4a–b** and **4f** exhibit two broad singlets attributable to the protons of $NH_2$ group in the region $\delta$ = 4.50–4.51 ppm and $\delta$ = 6.97–6.99 ppm and a sharp singlet of NH proton in the region $\delta$ = 8.40–8.43 ppm. Two broad singlets of exchangeable protons of *N*-methyl derivatives **4c–e** at $\delta$ = 4.85 ppm and at $\delta$ = 6.95–7.01 ppm are assigned to the $NH_2$ and -NH-NH- protons respectively. The spectra of **4g–h** exhibit two signals of $NH_2$ protons at $\delta$ = 6.05–6.06 ppm and $\delta$ = 7.11–7.55 ppm and two singlets of NH protons in the region $\delta$ = 7.95–7.96 and $\delta$ = 8.88–8.91 ppm. The presence of aryl substituents (**5a–g**) renders the aminosulphonyl tautomer of type **5** to be more favoured in DMSO solution as shown in Scheme 1. The four separate signals observed for the chemically non-equivalnet NH protons are assigned as follows: C=N<u>H</u> protons appear in the region $\delta$ = 6.85–7.12 ppm, N<u>H</u>-Ar at $\delta$ = 7.15–7.44 ppm, N<u>H</u>-N-Ar at $\delta$ = 7.56–8.43 and the signals of $SO_2$N<u>H</u> protons are found downfield at the region $\delta$ = 9.03–9.15 ppm, however, in the case of p-nitrophenyl substituent (**5d**) the signal of N<u>H</u>-Ar proton is observed at the $\delta$ = 9.34 ppm. The possible tautomers of **4d** were also examined by quantum chemical calculations in the gas phase. A recent comprehensive study for heterocyclic compounds incorporating the $SO_2$ moiety has shown that the density functional methods, that include electron correlation, provide a good geometric and electronic description [13,14]. Thus, our study was performed using Becke, Predew (BP) [15], BLYP (Becke, Lee, Yang, Parr) [16] and B3LYP models with 6-31G** polarization basis set [16,17]. For comparison a molecular *ab initio*




**Scheme 2.** Probable mechanism of *N*-amino-*N′*-(benzenesulphonyl)guanidine formation from *N*-(benzenesulphonyl)cyanamide potassium salt and hydrazines in the presence of one molar equivalent of mineral acids.

method at the Hartree-Fock level with the 6-31G** basis set was used [17]. In general, compound **4d** can exist in the form of three possible tautomers **A**, **B** and **C** as shown in Figure 2. The calculated total energies of isolated molecules are shown in Table 1. The results indicate that the iminosulphonyl tautomer **A** is more stable by 2.5–19.2 kcal/mol than the tautomer **B** and by 6.6–10.1 kcal/mol than the tautomer **C** (Table 1). Hence, this finding is in agreement with $^{1}$H NMR studies presented above for the compound **4d** in DMSO solution.

It is pertinent to note that analogous quantum chemical calculations performed for alternative tautomeric forms of **5a** did not confirm the results obtained from elucidation of NMR spectra. Thus, in the gas phase tautomer **A** was found to be more stable than tautomer **B** and **C** by 9.5–10.7 kcal/mol and 8.7–14.1 kcal/mol, respectively.




R =




**Figure 2.** Possible tautomeric forms of compound **4d**.

**Table 1.** Calculated energies (E, hartrees), relative energies (ΔE, kcal/mol) of tautomers **A**, **B** and **C**.

| Tautomer | Method | E (hartrees) | ΔE (kcal/mol) |
|---|---|---|---|
| **A** | RHF/6-31G** | –2238.91766 | 0 |
| **B** | RHF/6-31G** | –2238.88706 | 19.2 |
| **C** | RHF/6-31G** | –2238.90224 | 9.7 |
| **A** | PB/6-31G** | –2247.21011 | 0 |
| **B** | PB/6-31G** | –2247.20370 | 4.0 |
| **C** | PB/6-31G** | –2247.19605 | 8.8 |
| **A** | BLYP/6-31G** | –2246.64812 | 0 |
| **B** | BLYP/6-31G** | –2246.64414 | 2.5 |
| **C** | BLYP/6-31G** | –2246.63758 | 6.6 |
| **A** | B3LYP/6-31G** | –2247.11975 | 0 |
| **B** | B3LYP/6-31G** | –2247.11190 | 4.9 |
| **C** | B3LYP/6-31G** | –2247.10361 | 10.1 |

**Biological activity and discussion**. The *in vitro* cytotoxic activity of compounds **4b**, **5a–c** and **5e** 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. The corresponding values of 50% growth inhibitory concentration ($IC_{50}$) are listed in Table 2.

Among a series of aminoguanidine derivatives **4b**, **5a–c** and **5e** the highest *in vitro* cytotoxic activity was found for compound **5b** having both the phenyl substituent at the nitrogen atom and phenyl substituent at the methylthio side chain of benzene ring. Replacement of phenyl substituent at the nitrogen atom by hydrogen atom leads to total loss of cytotixic activity (**4b**).

**Table 2.** Inhibition of *in vitro* human cancer cell lines by compounds (**4b**, **5a–c** and **5e**)[a].

| Human cell lines | $IC_{50}$ [μM][b] | | | | |
|---|---|---|---|---|---|
| | Compd. No. | | | | |
| | **4b** | **5a** | **5b** | **5c** | **5e** |
| *Urinary bladder cancer* | | | | | |
| RT-4 | – | 17.46 | 3.45 | 4.84 | 8.11 |
| RT-112 | – | 22.49 | 5.30 | 9.55 | 13.54 |
| 5637 | – | 16.88 | 3.01 | 4.23 | 5.98 |
| *Esophagus cancer* | | | | | |
| KYSE-70 | – | 25.71 | 4.25 | 9.81 | 9.48 |
| KYSE-510 | NA[c] | 28.07 | 4.00 | 6.51 | 9.05 |
| KYSE-520 | – | 31.73 | 5.70 | 9.97 | 13.10 |
| *Pancreatic cancer* | | | | | |
| YAPC | – | 27.99 | 8.14 | 10.60 | 15.59 |
| DANG-G | – | 24.26 | 3.39 | 6.51 | 9.32 |
| *Cervix cancer* | | | | | |
| SISO | NA | 17.66 | 4.89 | 7.21 | 11.67 |
| *Non-small cell lung cancer* | | | | | |
| LCLC-103H | NA | 8.02 | 3.35 | 3.60 | 10.03 |
| *Breast cancer* | | | | | |
| MCF-7 | – | 11.09 | 2.35 | 2.74 | 4.41 |

[a]See Experimental; [b]$IC_{50}$ – concentration producing a 50% growth inhibitory effect; [c]not active.

The activity of the **5c**, bearing ethoxycarbonyl substituent at the methylthio side chain, was comparable with **5b**, but replacement of phenyl with 2-pyridyl substituent at the nitrogen atom caused a two-fold decrease of cytotoxic activity (**5e**). Similarly, the introduction of methyl group at the methylthio side chain caused a moderate decrease of cytotoxic activity (**5a**). In conclusion, the presence of electron withdrawing substituent, such as phenyl or 2-pyridyl at the nitrogen atom, was found to be essential for cytotoxic activity of this class of compounds.

## EXPERIMENTAL

$^1$H, $^{13}$C NMR spectra were recorded on a Gemini (200 MHz) and Varian Unity Plus (500 MHz) spectrometers or Tesla-Brno BS 587A (80 MHz) spectrometer, in DMSO-$d_6$ with TMS as internal standard. IR spectra were measured as KBr discs on a Perkin-Elmer FT IR 1600 spectrophotometer. Melting points were determined on a Büchi SMP 20 apparatus and were uncorrected. Thin-layer chromatography was performed on Merck Kieselgel 60$F_{254}$ plates and visualised with UV or with iodine vapour. The starting *N*-(4-chloro-5-methylbenzenesulphonyl)cyanamide potassium salt **1–3** were synthesized according to the procedure described in [18].

**3-Amino-2-(4-chloro-5-methylbenzenesulphonyl)guanidines (4a–e). General procedure**: To a stirred suspension of **1**, **2** or **3** (5 mmol) in dry $CCl_4$ (35 ml), hydrazine hydrate or methylhydrazine (10 mmol) and 96% sulfuric acid (0.14 ml, 2.5 mmol) were added. The reaction mixture was stirred at reflux for 24 h and left to stand at room temperature overnight. The resulting precipitate was filtered off, washed well with $CCl_4$ (3 × 1.5 ml) and dried, then treated with water (50 ml). After vigorously stirring for 20 minutes the precipitate was collected by filtration and dried to afford crude reaction product, which was purified by recrystallization from ethanol.

**3-Amino-2-(4-chloro-2-ethylthio-5-methylbenzenesulphonyl)guanidine (4a)**. Yield 1.13 g, 70%, (white amorphous powder), m.p. 235–238°C (dec.). IR, $\nu_{max}$ (KBr) cm$^{-1}$: 3466, 3351, 3267, 3226, 2972, 2929, 2860, 1662, 1615, 1570, 1341, 1131. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 1.24 (t, 3H, $J$ = 7.3 Hz, $CH_3$), 2.31 (s, 3H, $CH_3$), 2.98 (q, 2H, $J$ = 7.3 Hz, $SCH_2$), 4.51 (s, 2H, C-$NH_2$), 6.97 (s, 2H, N-$NH_2$), 7.35 (s, 1H, H-3), 7.83 (s, 1H, H-6), 8.40 (s, 1H, NH). $^{13}$C NMR (50 MHz, DMSO-$d_6$) $\delta$: 13.19 ($CH_3$), 18.96 ($CH_3$-Ph), 25.85 ($SCH_2$), 126.65, 130.43, 131.19, 135.93, 136.21, 140.15, (6C, aromatic), 158.83 (C, guanid.). Anal. Calcd. for $C_{10}H_{15}ClN_4O_2S_2$ (322.84): C, 37.20; H, 4.68; N, 17.35. Found: C, 37.06; H, 4.42; N, 17.51.

**3-Amino-2-(2-benzylthio-4-chloro-5-methylbenzenesulphonyl)guanidine (4b)**. Yield 1.13 g, 59%, (white plates), m.p. 164–167°C. IR, $\nu_{max}$ (KBr) cm$^{-1}$: 3454, 3336, 3224, 2919, 2848, 1654, 1604, 1572, 1340, 1131. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.29 (s, 3H, $CH_3$), 4.28 (s, 2H, $SCH_2$), 4.51 (br.s, 2H, N-$NH_2$), 6.98 (br.s, 2H, $NH_2$), 7.25–7.45 (m, 5H, aromatic), 7.41 (s, 1H, H-3), 7.82 (s, 1H, H-6), 8.43 (s, 1H, NH). $^{13}$C NMR (50 MHz, DMSO-$d_6$) $\delta$:18.93 ($CH_3$), 36.16 ($SCH_2$), 127.19, 128.44, 129.12, 130.33, 131.47, 135.55, 136.05, 136.24, 139.97 (9C, aromatic), 158.76 (C, guanid.). Anal. Calcd. for $C_{15}H_{17}ClN_4O_2S_2$ (384.91): C, 46.81; H, 4.45; N, 14.56. Found: C, 46.71; H, 4.19; N, 14.46.

**3-Methylamino-2-(4-chloro-2-ethylthio-5-methylbenzenesulphonyl)guanidine (4c)**. Yield 0.88 g, 53%, (white crystals), m.p. 229–234°C. IR, $\nu_{max}$ (KBr) cm$^{-1}$: 3463, 3352, 3325, 3247, 2976, 2963, 2927, 2869, 1646, 1580, 1539, 1311, 1134. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 1.24 (t, 3H, $J$ = 7.3 Hz, $CH_3$), 2.31 (s, 3H, $CH_3$), 3.01 (q, 2H, $J$ = 7.3 Hz, $SCH_2$), 3.10 (s, 3H, N-$CH_3$), 4.85 (s, 2H, C-$NH_2$), 6.95 (br.s, 2H, NH-NH), 7.37 (s, 1H, H-3), 7.83 (s, 1H, H-6). $^{13}$C NMR (50 MHz, DMSO-$d_6$) $\delta$:13.24 ($CH_3$), 18.95 ($CH_3$-Ph), 25.70 ($SCH_2$), 38.75 ($NCH_3$), 126.58, 130.43, 131.11, 135.91, 136.41, 139.59 (6C, aromatic), 157.30 (C, guanid.). Anal. Calcd. for $C_{11}H_{17}ClN_4O_2S_2$ (336.87): C, 39.22; H, 5.08; N, 16.63. Found: C, 39.34; H, 4.88; N, 16.99.

**3-Methylamino-2-(2-benzylthio-4-chloro-5-methylbenzenesulphonyl)guanidine (4d)**. Yield 1.19 g, 60%, (white bipyramid crystals), m.p. 188–189°C (dec.). IR, $\nu_{max}$ (KBr) cm$^{-1}$: 3430, 3330, 2919, 2848, 1651, 1604, 1587, 1340, 1131. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.28 (s, 3H, $CH_3$), 3.09 (s, 3H, $CH_3$-N), 4.30 (s, 2H, $SCH_2$), 4.85 (s, 2H, $NH_2$), 6.98 (br.s, 2H, NH-NH), 7.25–7.39 (m, 5H, aromatic),

7.43 (s, 1H, H-3), 7.80 (s, 1H, H-6). Anal. Calcd. for $C_{16}H_{19}ClN_4O_2S_2$ (398.93): C, 48.17; H, 4.8; N, 14.04. Found: C, 48.42; H, 4.2; N, 13.66.

**3-Methylamino-2-(4-chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulphonyl)guanidine (4e)**. Yield 1.71 g, 86%, (white prisms), m.p. 167–172°C. IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3474, 3354, 3236, 2969, 2943, 1725, 1654, 1584, 1299, 1134. $^1$H NMR (80 MHz, DMSO-$d_6$) $\delta$: 1.2 (t, 3H, $J$ = 7 Hz, $CH_3$), 2.33 (s, 3H, $CH_3$), 3.21 (s, 3H, N-$CH_3$), 3.98 (s, 2H, $SCH_2$), 4.12 (q, 2H, $J$ = 7 Hz, $OCH_2$), 4.85 (br.s, 2H, $NH_2$), 7.01 (br.s, 2H, NH-NH), 7.42 (s, 1H, H-3), 7.85 (s, 1H, H-6). Anal. Calcd. for $C_{13}H_{19}ClN_4O_2S_2$ (394.90): C, 39.54; H, 4.85; N, 14.18. Found: C, 39.25; H, 4.71; N, 14.01.

**3-Amino-2-(4-chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulphonyl)guanidine (4f)**. To a stirred suspension of **3** (1.93 g, 5 mmol) in dry dioxane (80 ml), hydrazine hydrate 99% (0.5 g, 10 mmol) and 96% sulfuric acid (0.14 ml, 2.5 mmol) were added. The reaction mixture was stirred at reflux for 48 h, then concentrated under diminished pressure to 1/3 volume and left to stand at room temperature overnight. The resulting precipitate was filtered off, washed well with dioxane (3 × 1.5 ml) and dried, then treated with water (50 ml). After vigorously stirring for 20 minutes the precipitate was collected by filtration and dried, then purified by recrystallization from ethanol to afford **4f** as a white crystals (1.83 g, 96%, m.p. 149–152°C. IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3476, 3361, 3214, 2995, 2948, 1728, 1653, 1607, 1308, 1133. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 1.18 (t, 3H, $J$ = 7 Hz, $CH_3$), 2.31 (s, 3H, $CH_3$), 3.93 (s, 2H, $SCH_2$), 4.02–4.17 (q, 2H, $J$ = 7 Hz, $OCH_2$), 4.51 (br.s, 2H, $NH_2$), 6.99 (br.s, 2H, $NH_2$), 7.39 (s, 1H, H-3), 7.83 (s, 1H, H-6), 8.43 (s, 1H, NH). $^{13}$C NMR (50 MHz, DMSO-$d_6$) $\delta$: 14.4 ($CH_3$), 18.95 ($CH_3$-Ph), 34.47 ($SCH_2$), 61.12 ($OCH_2$), 127.56, 130.41, 132.17, 134.28, 136.14, 140.29, (6C, aromatic), 158.75 (C-guanid.), 169.17 (C=O). Anal. Calcd. for $C_{12}H_{17}ClN_4O_2S_2$ (380.88): C, 37.84; H, 4.49; N, 14.71. Found: C, 37.55; H, 4.38; N, 15.05.

**2-(2-Benzylthio-4-chloro-5-methylbenzesulphonyl)-3-ureidoguanidine (4g)**. To a stirred suspension of **2** (1.95 g, 5 mmol) in dry toluene (40 ml), semicarbazide hydrochloride (0.56 g, 5 mmol) was added. The reaction mixture was stirred at reflux for 2 h and left to stand at room temperature overnight. The resulting precipitate was filtered off, and dried, then treated with water (40 ml). After vigorously stirring for 20 minutes the precipitate was collected by filtration under diminished pressure and dried to give **4i** as a white crystals (2.13 g, 75%, m.p. 125–130°C). IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3550, 3443, 3394, 3327, 3208, 1681, 1633,1552, 1396, 1136. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.30 (s, 3H, $CH_3$), 4.29 (s, 2H, $SCH_2$), 6.05 (s, 2H, $NH_2$), 7.01–7.50 (m, 8H, aromatic 6H and-$NH_2$), 7.86 (s, 1H, H-6), 7.96 (s, 1H, NH), 8.88 (s, 1H, NH). $^{13}$C NMR (50 MHz, DMSO-$d_6$) $\delta$: 19.20 ($CH_3$), 36.57 ($SCH_2$), 127.52, 127.81, 128.78, 129.47, 130.68, 132.07, 135.83, 136.52, 136.78, 139.98 (10C, aromatic), 158.64 (C=N), 159.12 (C=O). Anal. Calcd. for $C_{16}H_{18}ClN_5O_3S_2$ (426.93): C, 45.01; H, 4.25; N, 16.40. Found: C, 44.85; H, 4.45; N, 16.28.

**2-(4-Chloro-2-ethoxycarbonylmethylthio-5-methylbenzesulphonyl)-3-ureidoguanidine (4h)**. To a stirred suspension of **3** (1.93 g, 5 mmol) in dry toluene (40 ml), semicarbazide hydrochloride (0.56 g, 5 mmol) was added. The reaction mixture was stirred at reflux for 2.5 h and left to stand at room temperature overnight. The resulting precipitate was filtered off, and purified by crystallization form mixture DMF/$H_2O$ (50 ml/125 ml) and dried to give **4h** as a white needles (1.66 g, 79%, m.p. 228–232°C (dec.). IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3454, 3330, 3295, 3209, 1739, 1686, 1619, 1557, 1378, 1137. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 1.21 (t, 3H, $J$ = 7 Hz, $CH_3$), 2.35 (s, 3H, $CH_3$), 3.97 (s, 2H, $SCH_2$), 4.18 (q, 2H, $J$ = 7 Hz, $OCH_2$), 6.06 (s, 2H, $NH_2$), 7.11–7.20 (br.s, 2H, -$NH_2$), 7.47 (s, 1H, H-3), 7.90 (s, 1H, H-6), 7.95 (s, 1H, NH), 8.91 (s, 1H, NH). Anal. Calcd. for $C_{13}H_{18}ClN_5O_5S_2$ (423.90): C, 36.83; H, 4.28; N, 16.52. Found: C, 36.77; H, 4.15; N, 16.77.

**3-Arylamino-1-(4-chloro-5-methylbenzenesulphonyl)guanidines (5a–e)**. **General procedure**: To a stirred suspension of **1**, **2** or **3** (5 mmol) in dry $CCl_4$ (35 ml), phenylhydrazine, 4-nitrophenylhydrazine or 2-hydrazinopyridine (10 mmol) and 96% sulfuric acid (0.14 ml, 2.5 mmol) were added. The reaction mixture was stirred at reflux for 24 h and left to stand at room temperature overnight. The resulting precipitate was filtered off, washed well with $CCl_4$ (3×1.5 ml) and dried, then treated with water (50 ml). After vigorously stirring for 20 minutes the precipitate was collected by filtration and dried to afford crude reaction product, which was purified by recrystallization from ethanol.

**3-Phenylamino-1-(4-chloro-2-ethylthio-5-methylbenzenesulphonyl)guanidine (5a)**. Yield 1.36 g, 68%, (white crystals), m.p. 214–216°C (dec.). IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3455, 3331, 3293, 2964, 2925, 2870, 1612, 1571, 1560, 1302, 1131. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 1.27 (t, 3H, $J$ = 7.30 Hz, $CH_3$), 2.32 (s, 3H, $CH_3$), 3.05 (q, 2H, $J$ = 7.30 Hz, $SCH_2$), 6.69–6.83 (m, 3H, aromatic), 7.04 (s, 1H, C=NH), 7.18 (m, 2H,

aromatic), 7.38 (s, 1H, NH-Ph), 7.47 (s, 1H, H-3), 7.89 (s, 1H, H-6), 7.91 (s, 1H, NH-N-Ph), 9.07 (s, 1H, $SO_2NH$). $^{13}C$ NMR (50 MHz, DMSO-$d_6$) $\delta$:13.19 ($CH_3$), 18.96 ($CH_3$-Ph), 25.88 ($SCH_2$), 112.69, 119.75, 126.73, 128.80, 130.50, 131.39, 136.03, 136.51, 139.84, 148.00 (10C, aromatic), 159.01 (C, guanid.). Anal. Calcd. for $C_{16}H_{19}ClN_4O_2S_2$ (398.94): C, 48.17; H, 4.80; N, 14.04. Found: C, 47.96; H, 4.89; N, 13.96.

**3-Phenylamino-1-(2-benzylthio-4-chloro-5-methylbenzenesulphonyl)guanidine (5b)**. Yield 1.63 g, 71%, (white crystals), m.p. 192–194°C (dec.). IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3454, 3301, 2960, 1610, 1543, 1340, 1134. $^1H$ NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.31 (s, 3H, $CH_3$-Ph), 4.35 (s, 2H, $SCH_2$), 6.68 (m, 2H, aromatic), 6.78 (m, 1H, aromatic), 7.02 (br.s, 1H, C=NH), 7.15 (m, 2H, aromatic), 7.26–7.44 (m, 6H, aromatic 5H and NH-Ph), 7.46 (s, 1H, H-3), 7.89 (s, 2H, NH-N-Ph and H-6), 9.08 (s, 1H, $SO_2NH$). $^{13}C$ NMR (50 MHz, DMSO-$d_6$) $\delta$: 19.23 ($CH_3$), 36.47 ($SCH_2$), 112.95, 119.99, 127.54, 128.77, 129.09, 129.41, 130.74, 131.92, 136.02, 136.43, 136.64, 139.87, 148.19 (13C, aromatic), 159.20 (C, guanid.). Anal. Calcd. for $C_{21}H_{21}ClN_4O_2S_2$ (461,0): C, 54.71; H, 4.59; N, 12.15. Found: C, 54.45; H, 4.36; N, 12.12.

**3-Phenylamino-1-(4-chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulphonyl)guanidine (5c)**. Yield 1.48 g, 65%, (white prisms), m.p. 160–162°C. IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3430, 3307, 2978, 2929, 1739, 1604, 1540, 1340, 1134. $^1H$ NMR (80 MHz, DMSO-$d_6$) $\delta$: 1.18 (t, 3H, $J$ = 7 Hz, $CH_3$), 2.32 (s, 3H, $CH_3$), 3.98 (s, 2H, $SCH_2$), 4.12 (q, 2H, $J$ = 7 Hz, $OCH_2$), 6.65–6.87 (m, 3H, C=NH and 2H aromatic), 7.10–7.32 (m, 4H, NH-Ph and 3H aromatic), 7.50 (s, 1H, H-3), 7.85 (s, 1H, NH-N-Ph), 7.90 (s, 1H, H-6), 9.07 (s, 1H, $SO_2NH$). $^{13}C$ NMR (50 MHz, DMSO-$d_6$) $\delta$: 14.01 ($CH_3$), 18.96 ($CH_3$-Ph), 34.50 ($SCH_2$), 61.57 ($OCH_2$), 112.72, 119.75, 127.68, 128.85, 130.49, 132.34, 134.42, 136.42, 139.99, 147.90 (10C, aromatic), 158.95 (C, guanid.), 169.09 (C=O). Anal. Calcd. for $C_{18}H_{21}ClN_4O_2S_2$ (456.98): C, 47.31; H, 4.63; N, 12.26. Found: C, 47.81; H, 4.40; N, 12.21.

**3-(4-Nitrophenylamino)-1-(2-benzylthio-4-chloro-5-methylbenzenesulphonyl)guanidine (5d)**. Yield 1.82 g, 72%, (pale orange powder), m.p. 206–208°C (dec.). IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3445, 3342, 3266, 2919, 1637, 1595, 1328, 1309, 1133. $^1H$ NMR (200 MHz, DMSO-$d_6$,) $\delta$: 2.31 (s, 3H, $CH_3$), 4.36 (s, 2H, $CH_2$), 6.69–6.74 (2H, AA′XX′ system), 7.12 (br.s, 1H, C=NH), 7.21–7.36 (m, 3H, aromatic), 7.43–7.50 (m, 3H, aromatic), 7.63 (br.s, 1H, NH-N-$C_6H_4$–), 7.89 (s, 1H, H-6), 8.02–8.06 (2H, AA′XX′ system), 9.03 (1H, s, $SO_2NH$), 9.34 (br.s, 1H, NH-$C_6H_4$–). Anal. Calcd. for $C_{21}H_{20}ClN_5O_4S_2$ (506.01): C, 49.85; H, 3.98; N, 13.84. Found: C, 49.96; H, 4.17; N, 13.49.

**3-(2-Pyridylamino)-1-(2-benzylthio-4-chloro-5-methylbenzenesulphonyl)guanidine (5e)**. Yield 1.98 g, 86%, (cream-coloured needles), m.p. 170–172°C. IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3429, 3302, 2923, 2854, 1613, 1601, 1582, 1342, 1139. $^1H$ NMR (200 MHz, DMSO-$d_6$) $\delta$: 2.31 (s, 3H, $CH_3$), 4.33 (s, 2H, $SCH_2$), 6.52–6.56 (m, 1H, pyridine H-5), 6.76–6.81 (m, 1H, aromatic), 7.06 (br.s, 1H, C=NH), 7.21–7.55 (m, 7H, aromatic 6H and NH-$C_5H_4N$), 7.89 (s, 1H, H-6), 8.09–8.11 (m, 1H, pyridine H-6), 8.43 (s, 1H, NH-N-$C_5H_4N$), 9.15 (s, 1H, $SO_2NH$). $^{13}C$ NMR (50 MHz, DMSO-$d_6$) $\delta$: 18.95 ($CH_3$), 36.22 ($SCH_2$), 106.73, 115.66, 127.23, 127.35, 128.47, 129.15, 130.45, 131.66, 135.70, 136.16, 136.35, 137.67, 139.60, 147.64, 158.93 (15C, aromatic), 159.02 (C, guanid.). Anal. Calcd. for $C_{20}H_{20}ClN_5O_2S_2$ (461.99): C, 51.99; H, 4.36; N, 15.16. Found: C, 52.23; H, 4.18; N, 15.40.

**3-(4-Methylphenylamino)-1-(2-benzylthio-4-chloro-5-methylbenzenesulphonyl)guanidine (5f)**. To a stirred suspension of **2** (1.95 g, 5 mmol) in dry toluene (30 ml), p-tolylhydrazine hydrochloride (0.79 g, 5 mmol) was added. The reaction mixture was stirred at reflux for 2 h, then left to stand in a refrigerator overnight. The precipitate of KCl was filtered off, and the filtrate was evaporated do dryness under diminished pressure to afford gummy residue. The crude product thus obtained was fractionally recrystallized from ethanol to give **5f** as a white powder (0.71 g, 30%, m.p.185–189°C. IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3448, 3341, 3301, 3206, 2926, 2855, 1634, 1624, 1342, 1136. $^1H$ NMR (500 MHz, DMSO-$d_6$) $\delta$: 2.18 (s, 3H, $CH_3$-$C_6H_4$), 2.31 (s, 3H, $CH_3$-$C_6H_2$), 4.33 (s, 2H, $SCH_2$), 6.57–6.59 (2H, AA′XX′ system), 6.93–6.95 (2H, AA′XX′ system), 7.01 (br.s, 1H, C=NH), 7.25–7.28 (m, 2H, aromatic), 7.31–7.39 (m, 3H, aromatic), 7.44 (s, 1H, NH-$C_6H_4$–), 7.46 (s, 1H, H-3), 7.87 (s, 1H, NH-N-$C_6H_4$–), 7.94 (s, 1H, H-6), 9.05 (s, 1H, $SO_2NH$). Anal. Calcd. for $C_{22}H_{23}ClN_4O_2S_2$ (475,04): C, 55.30; H, 4.88; N, 11.79. Found: C, 55.03; H, 4.54; N, 11.94.

**3-(4-Methylphenylamino)-1-(4-chloro-2-ethoxycarbonylmethylthio-5-methylbenzenesulphonyl) guanidine (5g)**. To a stirred suspension of **3** (1.93 g, 5 mmol) in dry toluene (30 ml), p-tolylhydrazine hydrochloride (0.79 g, 5 mmol) was added. The reaction mixture was stirred at reflux for 2 h, then left to stand in a refrigerator overnight. The precipitate of desired product and KCl was filtered off and dried,

then treated with water (20 ml). After 20 minutes of stirring the precipitate was collected by suction and dried to give **5g** as a pale beige powder (0.7 g, 30%, m.p. 166–167°C). IR, $\nu_{max}$ (KBr) $cm^{-1}$: 3436, 3307, 3248, 2978, 2919, 2866, 1731, 1610, 1343, 1137. $^1$H NMR (200 MHz, DMSO-$d_6$) $\delta$: 1.19 (t, 3H, $J$ = 7 Hz, $CH_3$), 2.19 (s, 3H, $CH_3$-$C_6H_4$), 2.34 (s, 3H, $CH_3$-$C_6H_2$), 4.00 (s, 2H, $SCH_2$), 4.14 (q, 2H, $J$= 7 Hz, $OCH_2$), 6.59–6.63 (2H, AA′XX′ system), 6.98–7.00 (2H, AA′XX′ system), 7.01(s, 1H, C=NH), 7.37 (s, 1H, NH-$C_6H_4$–), 7.47 (s, 1H, H-3), 7.56 (s, 1H, NH-N-$C_6H_4$–), 7.90 (s, 1H, H-6), 9.09 (s, 1H, $SO_2$NH). Anal. Calcd. for $C_{19}H_{23}ClN_4O_4S_2$ (471.0): C, 48.45; H, 4.92; N, 11.89. Found: C, 48.27; H, 4.61; N, 12.00.

**Cytotoxicity studies**. All reagents were purchased from Sigma (Deisenhofen, Germany) and the cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) (Brauschweig, Germany). Cell lines used here were: human bladder cancer 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. The culture medium was RPMI-1640 medium containing 2 g/l $HCO_3$, and 10% FCS. Cells were grown in 75 $cm^2$ plastic culture flasks (Sarstedt, Nümbrecht, Germany) in a humid atmosphere of 5% $CO_2$ at 37°C and passaged shortly before becoming confluent.

For the cytoxicity studies, 100 $\mu$l of a cell suspension were seeded into 96-well microtiter plates (Sarstedt) at a density of 1000 cell/well, except for the LCLC-103H cell line, which was plated out at 250 cells/well. One day after plating cells were treated with test substance at five concentrations per compound: 1000 fold concentrated stock solutions in DMF were serially diluted by 50% in DMF to give the feed solutions, which were diluted 500 fold into culture medium. The controls received just DMF. Each concentration was test in 8 wells, with each well receiving 100 $\mu$l of the medium containing the test substance. The concentration ranges were chosen to bracket the expected $IC_{50}$ values as best as possible. Cells were then incubated 96 h, after which time the medium was removed and replaced with a 1% glutaraldehyde/PBS solution for 20 min. Cells were stored at 4°C under PBS. Staining with crystal violet was done as previously described [19]. O.D. was measured at $\lambda$ = 570 nm with an Anthos 2010 plate reader (Salzburg, Austria).

Corrected T/C values were calculated by the equation:

$$(T/C)_{corr}\ (\%) = (O.D._{\cdot T} - O.D._{\cdot c,0})/(O.D._{\cdot c} - O.D._{\cdot c,0}) \times 100$$

where $O.D._{\cdot T}$ is the mean absorbance of the treated cells, $O.D._{\cdot c}$ the mean absorbance of the controls and $O.D._{\cdot c,0}$ the mean absorbance at the time drug was added. The $IC_{50}$ values were estimated by a linear least-squares regression of the $T/C_{corr}$ values *versus* the logarithm of the substance concentration; only concentrations that yielded $T/C_{corr}$ values between 10 and 90% were used in the calculation. The reported $IC_{50}$ values are the average of 2–3 independent experiments and these varied less than 20% from the individual values.

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