ELSEVIER

Eur. J. Med. Chem. 37 (2002) 285–293

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

www.elsevier.com/locate/ejmech

Original article

Synthesis, molecular structure and anticancer activity of 1-allyl-3-amino-2-(4-chloro-2-mercaptobenzenesulphonyl)guanidine derivatives

Z. Brzozowski [a], F. Sączewski [a,*], M. Gdaniec [b]

[a] *Department of Chemical Technology of Drugs, Medical University of Gdańsk, Gdańsk, Poland*
[b] *Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznań, Poland*

Received 27 August 2001; accepted 23 January 2002

Abstract

A series of 3-allylamino-6-chloro-7-*R*-1,1-dioxo-1,4,2-benzodithiazines (**2a**–**e**) was obtained by the reaction of 6-chloro-3-methylthio-1,4,2-benzodithiazine-1,1-dioxides (**1a**–**e**) with allylamine. Selective hydrazinolysis of the allylaminobenzodithiazines (**2a**–**e**) gave the appropriate 1-allyl-3-amino-2-(4-chloro-2-mercaptobenzenesulphonyl)guanidines (**3a**–**e**) in good yields. The reaction of **3a** with dimethylsulphate under alkaline conditions provided the methylthio derivative **4**. The structures of these compounds were confirmed on the basis of elemental analysis, spectral data (IR, ^{1}H- and ^{13}C-NMR) and X-ray analysis. Screening data indicated that the compounds **3a** and **3d** exhibited significant in vitro activities against numerous human tumour cell lines, whereas compounds **3b** and **3c** showed a moderate activity.

Keywords: 1-Allyl-3-amino-2-(2-mercaptobenzenesulphonyl)guanidines; X-ray structure analysis; Antitumour effect

1. Introduction

Recently, arylsulphonamides have attracted attention as anticancer [1–3] and anti-HIV [4–6] agents. We have also described the syntheses of various 4-chloro-2-mercaptobenzenesulphonamide derivatives with the nitrogen atom of the sulphonamide moiety attached to a variety of heterocyclic ring systems. These compounds, depending on structure, exhibited either anticancer [7–13] or anti-HIV activities [7,9,12,14–16] and have been described by Neamati et al. [16] as a novel class of potent HIV-1 integrase inhibitors (**MBSAs**, Fig. 1). On the other hand, aminoguanidine derivatives possess various biological activities but many of them are still with unexplored pharmacological properties. Recent reports on the syntheses of arylsulphonylaminoguanidines [17] as well as pyrimidinyl pyrazole derivatives incorporating in their structure both aminoguanidine and allylamino moieties [18] with pronounced anticancer activity prompted us to develop a method for the synthesis of novel 1-allyl-3-amino-2-(4-chloro-2-mercaptobenzenesulphonyl)guanidines (**3a**–**e**) for biological screenings.

2. Results

2.1. Synthesis

The syntheses of the target compounds **3a**–**e** were achieved by a convenient two step procedure starting from methylthiobenzodithiazines **1a**–**e** as shown in Fig. 2.

First, the reactions of **1a**–**e** with allylamine carried out in dry benzene at elevated temperature led to the formation of 3-allylamino-1,4,2-benzodithiazines **2a**–**e** which could be separated either in high yields (91–98%) for (**2a**–**d**) or in good yield (74%) for (**2e**). Then, upon treatment of **2a**–**e** with an excess of hydrazine hydrate in methanolic solution at room temperature, the desired 1-allyl-3-amino-2-(4-chloro-2-mercaptobenzenesulpho-

* Correspondence and reprints.
E-mail address: saczew@amg.gda.pl (F. Sączewski).

0223-5234/02/$ - see front matter
PII: S0223-5234(02)01341-7

nyl)guanidines (**3a**–**e**) were obtained in high yields (82–92%).

The mechanism of this reaction could be explained by a regioselective nucleophilic attack of the hydrazine nitrogen atom at the C-3 carbon atom of the benzodithiazine **2** with simultaneous heterocyclic ring opening. In this procedure an excess of hydrazine hydrate was used in order to avoid a plausible oxidation of the mercapto group formed to the corresponding disulphide.

The structures of the compounds **2a**–**e** and the final products **3a**–**e** were confirmed by elemental analyses as well as by IR, ^{1}H-NMR, ^{13}C-NMR spectrophotometry. For example, in ^{1}H-NMR spectrum, the existence of an intact *N*-allyl group in **3a** was identified from the signals at δ 3.94 (N–CH_2, d, $J_{CH-NH} = 5.6$ Hz), 5.11 (dd, N–CH_2–CH_C=CH_A, $J_{cis} = 6.1$ Hz, $J_{gem} = 1.3$ Hz), 5.18 (N–CH_2–CH_C=CH_B, dd, $J_{trans} = 13.5$ Hz, $J_{gem} =$ 1.3 Hz) and 5.84 (m, N–CH_2–CH_C=CH_2). The ^{13}C-NMR spectrum exhibited the presence of three carbons attributed to *N*-allyl group at δ 43.92 (N–*C*H_2–CH=CH_2), 117.43 (N–CH_2–CH=*C*H_2) and 138.92 (N–CH_2–*C*H=CH_2). The presence of SH group in **3a** was confirmed in IR spectrum by the absorption at 2543 cm^{-1}.

We were not able to obtain crystals of **3** suitable for X-ray analysis. Therefore, in order to investigate in detail a tautomeric structure of the sulphonylaminoguanidines obtained, we prepared the methylthio derivative **4** by reacting **3a** with dimethylsulphate under alkaline conditions (Fig. 2).

2.2. Crystal and molecular structure of *4*

Previously, crystal structures were determined for a number of guanidine derivatives containing substituents such as cyano [19], nitro [20] and sulphonyl [21,22]. All these showed the electron withdrawing group on imino nitrogen. However, some acyl derivatives are firmly in the amino form [23] while others are so close to the borderline that both imino and amino forms have been isolated [24].

The tautomeric form assumed by the sulphonylguanidine **4** in the crystal (Fig. 3) is that observed for sulphaguanidine in the solid state, i.e. with the guanidine imino nitrogen joined to the sulphone S atom [21,22]. This form has been confirmed by localisation of the H atoms from the Fourier difference map and by geometrical parameters characteristic of the sulphaguanidine molecule in this tautomeric form [21], i.e. S1–N1 1.573(2) Å, O1–S1–N1 114.7(1)°, O2–S1–N1 106.8(1)°. The three guanidine C–N bonds are of similar length [C9–N1 1.344(3), C9–N2 1.331(3), C9–N3 1.329(3) Å], however, like in sulphaguanidine [19,20], the longest bond is formed to the N atom connected to the sulphone group.

The overall molecular conformation can be described as nearly perpendicular arrangement of the phenyl substituent and the allyl group relative to the guanidine moiety with the best plane of the phenyl ring bisecting the O2–S1–N1 angle. The hydrazine moiety is oriented with the N2 hydrogen directed to N1 and N4 atom directed to N3. This allows the intramolecular hydrogen bond to be formed between N2–H and O1 of the sulphone group [N2–H···O1 2.807(3) Å, H···O1 2.20(3) Å, $<$N2–H···O1 135(3)°]. The N2–H group participates in the three-center hydrogen bond acting also as a donor in a weak intermolecular hydrogen bond to O1 of the molecule related by a symmetry center [N2–H···O1^i 3.004(3) Å, H···O1^i 2.43(3) Å, $<$N2–H···O1^i 132(3)°; symmetry code (i): $-x, -y, -z$].

The lone pair of the sp^3 hybridised N4 atom is pointing towards the hydrogen atom bonded to N3 giving rise to another weak intramolecular hydrogen bond [N3–H···N4 2.646(4) Å, H···N(4) 2.27(3) Å, $<$N3–H···N4 112(3)°]. Only one hydrogen atom of the NH_2 group takes part in the intermolecular hydrogen

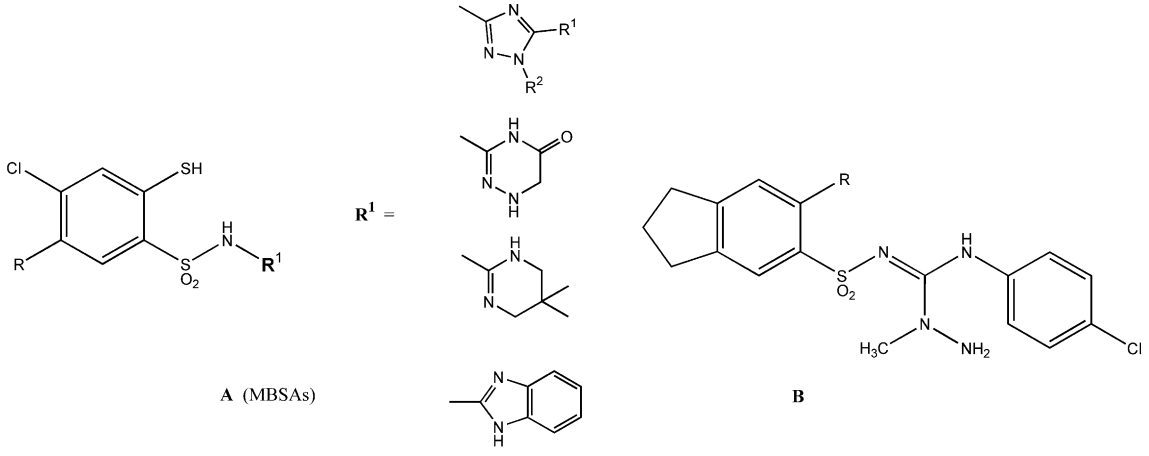

Fig. 1. Benzenesulphonylguanidines with anticancer and anti-HIV activities.

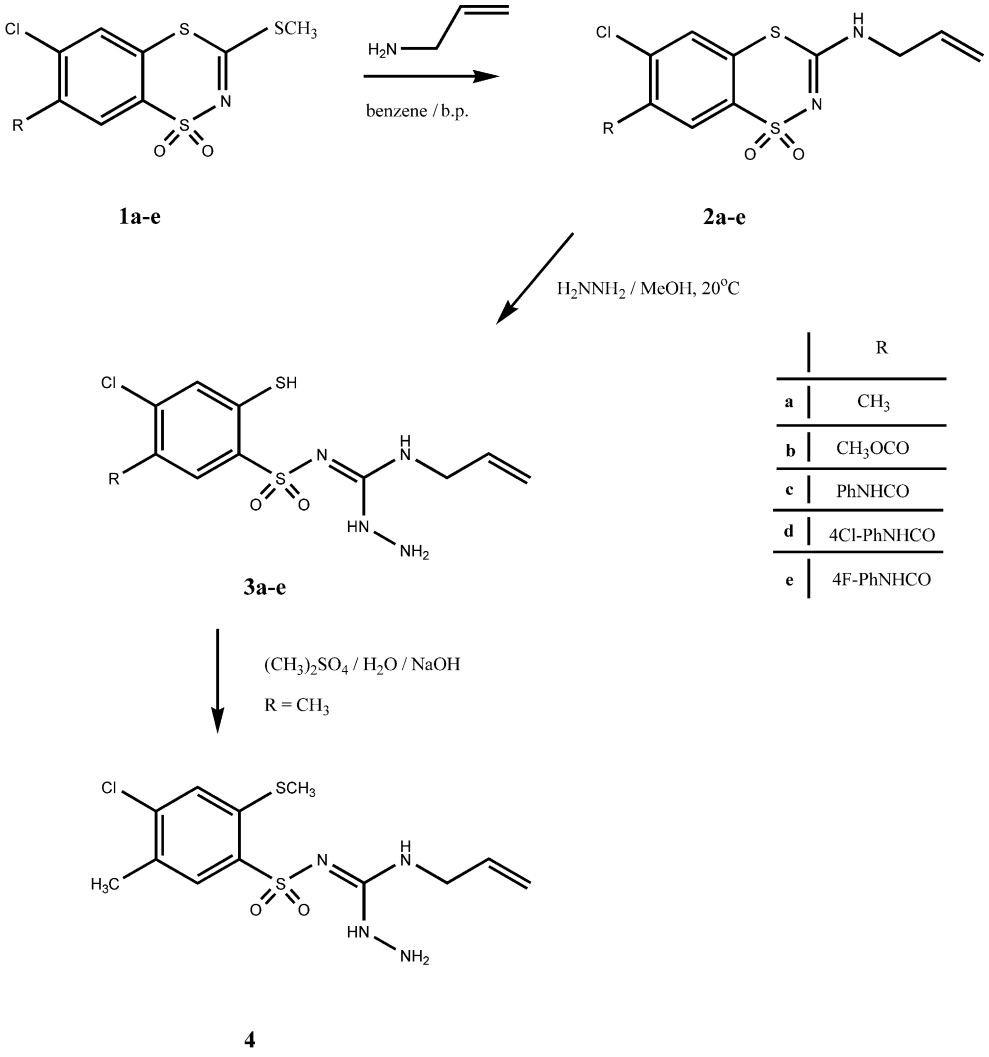

Fig. 2. Synthesis of arylsulphonylaminoguanidines from methylthiobenzodithiazines.

bond and is joined to the sulphone group of the molecule related by a glide plane [N4–H···O2^{ii} 3.127(3) Å, H···O2^{ii} 2.37(3) Å, $<$N4–H···O2^{ii} 135(3)°; symmetry code (ii): $x-0.5, -y+0.5, z-0.5$].

We have also examined tautomers of **4** by quantum chemical calculations using B3LYP hybrid density functional model with 6-31G* polarisation basis set [25]. The structures and relative energies obtained for tautomers **4A**, **4B** and **4C** are shown in Fig. 4. The sulphonylimino tautomer **4A** was calculated to be substantially lower in energy than sulphonylamino tautomers **4B** and **4C**. According to the following equation [26]:

$$\frac{N^1}{N^2} = \exp - 1060(E^1 - E^2)$$

N^1, N^2 = number of molecules;

E^1, E^2 = energy in a.u.

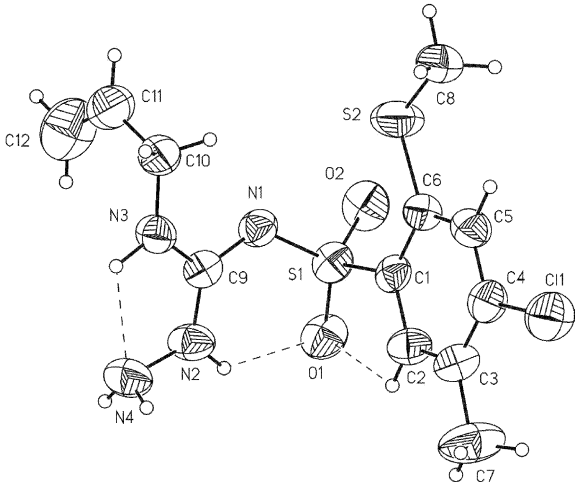

Fig. 3. ORTEP drawing of **4** with the atom labelling.

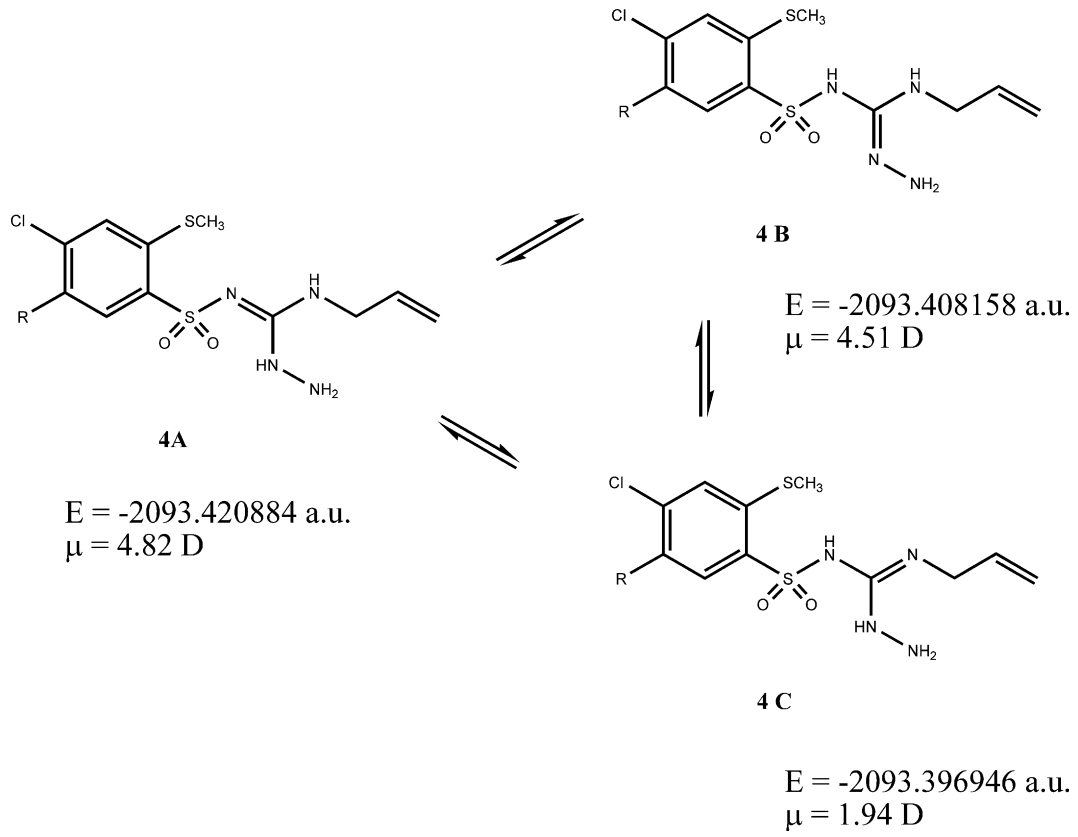

Fig. 4. Tautomeric forms of benzenesulphonylguanidine **4**.

at the temperature 296 K **4A** is identified as the overwhelmingly predominant tautomer by a factor of 7.22×10^5 over the less stable **4B** which in turn is favoured by a factor of 1.45×10^5 over **4C**. Moreover, based upon their calculated dipole moments, **4A** (4.82 D) would be predicted to predominate over **4B** (4.51 D) and **4C** (1.93 D) in polar solvents.

2.3. Biology

All the 2-mercaptobenzenesulphonamides **3a**–**e** synthesised were tested in US National Cancer Institute (Bethesda) for their in vitro anticancer activity.

The antitumour activities of the compounds were evaluated by using total 61 human tumour. Cell Lines, derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. The compounds were tested at five concentration at 10-fold dilution. A 48 h continuous drug exposure protocol was used and sulphorhodamine B (SRB) protein assay was used to estimate cell growth. Details of this test system and the information which is encoded by the activity pattern over all cell lines, have been published [27–29]. The antitumour activity of a test compound is reported for each cell line by three parameters: GI_{50}, molar concentration of the compound that inhibits 50% net cell growth; TGI, molar concentration of the compound leading to total inhibition; and LC_{50}, molar concentration of the compound leading to 50% net cell death.

Compounds **3a**–**e** exhibited reasonable antineoplastic activity against most (72–93%) of the 61 human cancer cell lines. Relatively highest sensitivity to the compounds described here was found for lung cancer (NCI-HS22), renal cancer (A 498) and melanoma (COX IMVI, MALME-3M, SF-MEL-2 and SK-MEL-5). The data of the most sensitive cell lines recorded in Table 1 indicate the following rank order of activity: **3e** (R = *p*-F–PhNHCO) < **3b** (R = CH_3OCO) < **3c** (R = PHNHCO), < **3a** (R = CH_3) ≤ **3d** (R = *p*-Cl–PHNHCO).

It is noteworthy that the activity of the compound **3a** containing a small, neutral, lipophilic CH_3 substituent was similar to that of **3d** with bulky, conjugative electron-withdrawing *p*-Cl–PhNHCO group, while the *p*-F–PhNHCO-derivative **3e** was the least active in this series.

These results are rather surprising from structure–activity relationships point of view, and may suggest that mechanisms by which compounds **3a** and **3d** exert their antineoplastic effects are different.

Table 1
Inhibition of in vitro cancer cell lines by compounds **3a–e**

Panel cell line	Response parameters [a] (μM) GI_{50} (A), TGI (B) and LC_{50} (C)														
	Compound **3a**			Compound **3b**			Compound **3c**			Compound **3d**			Compound **3e**		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Leukemia															
K-562	27.0	80.9	*	47.1	*	*	35.2	*	*	31.1	*	*	32.8	*	*
RPMI-8226	20.1	61.8	*	31.2	*	*	31.4	*	*	28.9	70.8	*	34.9	*	*
SR	37.1	90.4	*	37.5	*	*	41.8	*	*	27.2	*	*	43.4	*	*
Non-small cell lung cancer															
NCI-H522	22.3	47.0	99.0	19.5	47.5	*	23.4	76.1	*	28.2	64.1	*	33.4	94.2	*
Colon cancer															
HCC-2998	3.73	*	*	42.2	*	*	46.1	*	*	19.1	49.1	*	40.5	*	*
KM-12	61.8	*	*	40.4	*	*	33.3	*	*	28.0	97.0	*	49.3	*	*
CNS cancer															
SF-268	15.9	73.0	*	13.5	60.7	*	26.1	*	*	11.5	52.1	*	50.4	*	*
SF-539	43.8	*	*	40.5	*	*	38.4	*	*	30.7	81.8	*	39.5	*	*
SNB-75	93.5	*	*	24.7	75.3	*	*	*	*	NT	NT	NT	NT	NT	NT
Melanoma															
LOX IMVI	18.4	37.7	77.4	37.5	*	*	21.0	*	*	27.4	*	*	54.5	*	*
MALME-3M	16.7	69.3	*	24.0	97.5	*	31.2	77.0	*	17.9	39.1	85.3	29.1	70.1	*
M 14	27.8	*	*	31.5	*	*	26.5	*	*	21.7	69.5	*	42.3	*	*
SK-MEL-2	21.0	84.3	*	26.0	84.5	*	22.2	71.6	*	28.9	71.2	*	28.3	76.7	*
SK-MEL-28	41.6	*	*	36.7	*	*	45.3	*	*	22.3	83.9	*	56.2	*	*
SK-MEL-5	35.0	*	*	26.4	82.3	*	23.2	*	*	14.0	28.8	59.2	26.3	*	*
UACC-62	51.2	*	*	47.0	*	*	43.1	*	*	29.2	84.1	*	57.3	*	*
Ovarian cancer															
IGRV 1	23.9	53.3	*	29.6	*	*	6.2	83.3	*	36.2	*	*	71.0	*	*
OVCAR-8	32.1	*	*	34.8	*	*	30.8	*	*	25.4	59.3	*	73.8	*	*
Renal cancer															
A 498	21.6	69.8	*	34.5	72.8	*	17.7	57.4	*	18.1	37.1	75.8	17.2	64.1	*
Prostate cancer															
PC-3	63.6	*	*	42.8	*	*	29.0	*	*	30.0	94.0	*	30.3	*	*
Breast cancer															
MDA-MB-435	32.2	*	*	40.3	*	*	17.7	52.5	*	23.4	73.1	*	35.7	*	*
MDA-N	39.7	*	*	35.3	*	*	24.9	71.5	*	27.3	*	*	54.7	*	*
Percent of the cell lines giving positive GI50, TGI and LC50 testing result	91.4	17.2	5.2	77.6	12.1	0	81.0	12.1	0	93.1	29.3	5.2	72.4	6.9	0

*The values TGI or $LC_{50} > 100$ μM. NT, not tested.
[a] GI_{50}, concentration giving 50% inhibition; TGI, concentration giving total growth inhibition; LC_{50}, concentration having 50% lethal effect.

3. Experimental

3.1. Synthesis

M.p.s were taken on a Büchi 535 apparatus and are reported uncorrected. IR spectra in KBr were recorded on a Perkin–Elmer 1600 FTIR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL 200 spectrometer 200 MHz PMR using TMS as internal standard (δ values in ppm). The results of elemental analyses for C, H and N were within $\pm 0.4\%$ of the theoretical values. The starting 6-chloro-3-metylthio-1,4,2-benzodithiazine-1,1-dioxide derivatives were obtained according to methods described previously: **1a** [30]; **1b** [31] and **1c**–**e** [32].

3.2. Preparation of 3-allylamino-6-chloro-1,4-2-benzodithiazine-1,1-dioxides (**2a**–**e**)

A solution of the corresponding methylthiobenzodithiazine (**1a**), (**1b**), (**1c**), (**1d**) or (**1e**) (0.05 mol) and 2.9 g (0.05 mol) of allylamine in dry C_6H_6 (120–180 mL) was stirred for 3 h at room temperature (r.t.). The suspension obtained was refluxed until the evolution of CH_3SH had ceased (20–28 h). The precipitate was filtered off, washed successively with C_6H_6 (3×10 mL) and CH_3OH (3×5 mL). Experimental data: see Table 2.

In this manner the following products were obtained.

3.2.1. 3-Allylamino-6-chloro-7-methyl-1,4,2-benzodithiazine-1,1-dioxide (**2a**)

IR: 3260 (NH), 1642; 1565 (C=N and aromatic ring); 1345, 1305, 1152 (SO_2). ^{1}H-NMR ($CDCl_3$): 2.45 (s, 3H, CH_3); 4.12 (d, 2H, N–C*H*$_2$–CH=CH$_2$); 5.18–5.30 (m, 2H, NCH$_2$CH=C*H*$_2$); 5.76–5.95 (m, 1H, NCH$_2$–C*H*=CH$_2$); 6.76 (br.s, 1H, NH); 7.34 (s, 1H, aromat.); 7.97 (s, 1H, aromat.). ^{13}C-NMR ($CDCl_3$): 20.62 (CH_3); 47.01 (N *C*H$_2$–CH=CH$_2$); 119.54 (N–CH$_2$–CH=*C*H$_2$); 127.98, 128.18, 131.63, 132.02, 138.51, 139.00 (aromatic carbons); 144.62 (N–CH$_2$–*C*H=CH$_2$); 163.67 (C=N).

3.2.2. 3-Allylamino-6-chloro-7-methoxycarbonyl-1,4,2-benzodithiazine-1,1-dioxide (**2b**)

IR: 3330 (NH), 1730 (C=O); 1642, 1587, 1555 (C=N and aromatic ring); 1360, 1305, 1160, 1140, (SO_2). ^{1}H-NMR (DMSO-d_6): 3.89 (s, 3H, CH_3OCO); 4.01 (d, 2H, N–C*H*$_2$CH=CH$_2$); 5.15–5.27 (m, 2H, CH$_2$CH=C*H*$_2$); 5.79–5.92 (m, 1H, N–CH$_2$–C*H*=CH$_2$); 8.12 (s, 1H, aromat.); 8.33 (s, 1H, aromat.).

3.2.3. 3-Allylamino-6-chloro-7-phenylcarbamoyl-1,4,2-benzodithiazine-1,1-dioxide (**2c**)

IR: 3300, 3215 (NH); 1660 (C=O); 1615, 1595, 1555; (C=N and aromatic ring), 1320, 1140 (SO_2). ^{1}H-NMR (DMSO-d_6): 4.03 (d, 2H, N–C*H*$_2$CH=CH$_2$); 5.16–5.27 (m, 2H, N–CH$_2$CH=C*H*$_2$); 5.77–5.96 (m, 1H, N–CH$_2$C*H*=CH$_2$); 7.10–7.71 (m, 5H, aromat.); 8.09 (s, 1H, aromat.); 8.12 (s, 1H, aromat.); 10.02 (br.s, 1H, NH); 10.69 (s, 1H, NHCO).

3.2.4. 3-Allylamino-6-chloro-7-(4-chlorophenyl-carbamoyl)-1,4,2-benzodithiazine-1,1-dioxide (**2d**)

IR: 3310, 3285 (NH); 1640 (C=O), 1625, 1598, 1540 (C=N and aromatic ring), 1345, 1310, 1160 (SO_2). ^{1}H-NMR (DMSO-d_6): 4.02 (d, 2H, N–C*H*$_2$–CH=CH$_2$); 5.16–5.27 (m, 2H, N–CH$_2$CH=C*H*$_2$); 5.71–5.96 (m, 1H, N–CH$_2$–C*H*=CH$_2$); 7.43 (d, 2H, aromat.); 7.72 (d, 2H, aromat.); 8.12 (s, 1H, aromat.); 8.13 (s, 1H, aromat.); 10.02 (br.s, 1H, NH); 10.83 (s, 1H, NHCO).

3.2.5. 3-Allylamino-6-chloro-7-(4-fluorophenyl-carbamoyl)-1,4,2-benzodithiazine-1,1-dioxide (**2e**)

IR: 3300, 3235, (NH); 1655 (C=O), 1615, 1570, 1555 (C=N and aromatic ring), 1320, 1155 (SO_2). ^{1}H-NMR (DMSO-d_6): 4.03 (d, 2H, N–C*H*$_2$–CH=CH$_2$); 5.16–5.27 (d, 2H, N–CH$_2$CH=C*H*$_2$); 5.80–5.94 (m, 1H, N–CH$_2$–C*H*=CH$_2$); 7.22 (t, 2H, aromat.); 7.67–7.75 (m, 2H, aromat.); 8.11 (s, 2H, aromat.); 10.03 (s, NH); 10.75 (s, 1H, NHCO).

Table 2
Experimental data of compounds **2a**–**e**, **3a**–**e** and **4**

Compound	R	Yield (%)	m.p. (°C)	Molecular formula (molecular weight)
2a	CH_3	95	161–163	$C_{11}H_{11}ClN_2O_2S_2$(302.8)
2b	CH_3OCO	96	154–156	$C_{12}H_{11}ClN_2O_4S$ (346.8)
2c	C_6H_5NHCO	98	169–171	$C_{17}H_{14}ClN_3O_3S_2$ (407.9)
2d	4-Cl–C_6H_4NHCO	91	185–187	$C_{17}H_{13}Cl_2N_3O_3S_2$ (442.3)
2e	4-F–C_6H_4NHCO	74	188–189	$C_{17}H_{13}ClFN_3O_3S_2$ (425.9)
3a	CH_3	92	130–131	$C_{11}H_{15}ClN_4O_2S_2$ (334.8)
3b	CH_3OCO	84	129–131	$C_{12}H_{15}ClN_4O_4S_2$ (378.8)
3c	C_6H_5NHCO	88	142–144	$C_{17}H_{18}ClN_5O_3S_2$ (439.9)
3d	4-Cl–C_6H_4NHCO	82	145–147	$C_{17}H_{17}Cl_2N_5O_3S_2$ (474.4)
3e	4-F–C_6H_4NHCO	83	171–173	$C_{17}H_{17}ClFN_5O_3S_2$ (457.9)
4	CH_3	83	154–155	$C_{12}H_{17}ClN_4O_2S_2$ (348.87)

3.3. Preparation of 1-allyl-3-amino-2-(4-chloro-2-mercaptobenzenesulphonyl)guanidines (**3a–e**)

A mixture of the corresponding allylaminobenzodithiazine **2a–e** (0.01 mol) and 1.3 g (0.025 mol) of hydrazine hydrate (99–100%) in 15 mL of CH_3OH was stirred at r.t. for 20–24 h. The solvent was removed under vacuum (rotary evaporator) to give a dry residue, which was further purified by dissolving in water (350 mL, pH 8.5–9). After stirring for 0.5 h, a small amount of insoluble side-products (0.1–0.3 g) was filtered off and the filtrate was acidified with 1% HCl to pH 3. The precipitate thus obtained was collected by filtration, washed with water and dried initially at r.t. and then at 100 °C. Experimental data: see Table 1.

In this manner the following products were obtained.

3.3.1. 1-Allyl-3-amino-2-(4-chloro-2-mercapto-5-methylbenzenesulphonyl)guanidine (**3a**)

IR: 3345, 3320, 3245 (NH); 2540 (SH); 1650 (C=N); 1350, 1330 (SO_2). ^{1}H-NMR ($CDCl_3$): 2.34 (s, 3H, CH_3Ph); 3.91 (s, 2H, NH_2); 3.94 (d, 2H, N–CH_2–CH=CH_2); 5.04 (br.s, 1H, NH); 5.11 (dd, 1H, N–CH_2–CH=CH_A); 5.18 (dd, 1H, N–CH_2–CH=CH_B); 5.84 (m, 1H, N–CH_2–CH=CH_2); 6.70 (br.s, 1H, NH); 7.34 (s, 1H, aromat.); 7.87 (s, 1H, aromat.); 8.28 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$): 20.08 (CH_3–Ph); 43.92 (N–CH$_2$–CH=CH_2); 117.75 (N–CH_2–CH=CH$_2$); 131.1, 131.4, 133.4, 134.3, 138.1 (aromatic carbons); 138.93 (N–CH_2–CH=CH_2); 157.34 (C=N).

3.3.2. 1-Allyl-3-amino-2-(4-chloro-2-mercapto-5-methoxycarbonylbenzenesulphonyl)guanidine (**3b**)

IR: 3375, 3345, 3325, 3271 (NH); 2550 (SH); 1720 (C=O); 1640 (C=N); 1360, 1140 (SO_2). ^{1}H-NMR (DMSO-d_6): 3.76 (s, 1H, SH); 3.82 (d, 2H, N–CH_2–CH=CH_2); 3.86 (s, 3H, CH_3O); 5.02 (s, 1H, NH); 4.97–5.10 (m, 2H, N–CH_2–CH=CH_2); 5.8 (m, 1H, NH–CH_2–CH=CH_2); 7.63 (br.s, 1H, NH); 7.81 (s, 1H, aromat.); 8.05 (s, 1H, NH); 8.30 (s, 1H, aromat.). ^{13}C-NMR (DMSO-d_6): 42.41 (N–CH$_2$–CH=CH_2); 52.55 (CH_3O);115.22 (NH–CH_2–C=CH$_2$); 130.78, 130.81, 132.12, 132.19, 134.54, 135.24 (aromatic carbons); 139.03 (N–CH_2–CH=CH_2); 156.59 (C=N); 163.98 (C=O).

3.3.3. 1-Allyl-3-amino-2-(4-chloro-2-mercapto-5-phenylcarbamoylbenzenesulphonyl)guanidine (**3c**)

IR: 3340, 3250 (NH); 2548 (SH); 1670 (NC=O); 1645 (C=N); 1350, 1130 (SO_2). ^{1}H-NMR (DMSO-d_6): 3.48 (s, 1H, SH); 3.83 (d, 2H, N–CH_2–CH=CH_2); 4.60 (br.s, 1H, NH); 4.97–5.13 (m, 2H, N–CH_2–CH=CH_2); 5.72–5.91 (m, 1H, N–CH_2–CH=CH_2); 7.07–7.14, 7.31–7.38, 7.68–7.72 (m, 5H, aromat.); 7.57 (br.s, 1H, NH); 7.77(s, 1H, aromat.); 8.02 (s, 1H, aromat.); 8.14 (s, 1H, NH); 10.52 (s, 1H, CONH). ^{13}C-NMR (DMSO-d_6): 42.41 (N–CH$_2$–CH=CH_2); 78.93 (N–CH_2–CH=CH$_2$); 115.28, 119.56, 119.75, 123.88, 128.40, 128.74, 130.76, 131.87, 132.50, 135.25 (aromatic carbons); 138.76 (N–CH_2–CH=CH_2); 156.52 (C=N); 163.56 (C=O).

3.3.4. 1-Allyl-3-amino-2-[4-chloro-2-mercapto-5-(4-chlorophenylcarbamoyl)-benzenesulphonyl]guanidine (**3d**)

IR: 3340, 3290 (NH); 2552 (SH); 1670 (NC=O); 1650 (C=N); 1350, 1130 (SO_2). ^{1}H-NMR (DMSO-d_6): 3.83 (d, 2H, N–CH_2–CH=CH_2); 4.30–4.80 (br.s, 2H, SH and NH); 4.97–5.13 (m, 2H, N–CH_2–CH=CH$_2$); 5.73–5.92 (m, 1H, (N–CH_2–CH=CH_2); 7.43 (d, 2H, aromat.); 7.74 (d, 2H, aromat.); 7.62 (s, 1H, NH); 7.80 (s, 1H, aromat.); 8.03 (s, 1H, aromat.); 8.15 (s, 1H, SO_2NH); 10.69 (s, 1H, HNCO).

3.3.5. 1-Allyl-3-amino-2-[(4-chloro-2-mercapto-5-(4-fluorophenylcarbamoyl)-benzenesulphonyl]guanidine (**3e**)

IR: 3340, 3260 (NH); 2550 (SH); 1665 (NC=O); 1640 (C=N); 1350, 1130 (SO_2). ^{1}H-NMR (DMSO-d_6): 3.82 (d, 2H, N–CH_2–CH=CH_2); 4.4–4.6 (br.s, 2H, NH and SH); 4.97–5.13 (m, 2H, N–CH_2–CH=CH$_2$); 5.73–5.92 (m, 1H, N–CH_2–CH=CH_3); 7.14–7.28 (m, 2H, aromat.) and 7.69–7.76 (m, 2H, aromat.); 7.61 (s, 1H, NH); 7.79 (s, 1H, aromat.); 8.02 (s, 1H, aromat.); 8.15 (s, 1H, NH); 10.60 (s, 1H, HNCO).

3.4. Preparation of 1-allyl-3-amino-2-(4-chloro-5-methyl-2-methylthiobenzenesulphonyl)guanidine (**4**)

Compound **3a** (1.7 g, 5 mmol) was dissolved in a solution of NaOH (0.32 g, 8 mmol) in water (30 mL). The resulting solution was stirred and cooled on an ice bath, and then treated dropwise with Me_2SO_4 (0.76 g, 6 mmol). Stirring was continued at 0–3 °C for 1 h and at r.t. for 2 h. Then, the reaction mixture was acidified with 1% HCl to pH 6. The precipitate thus obtained was filtered off, washed thoroughly with water, dried (1.7 g, m.p. 152–154 °C) and purified by recrystallisation from 2-propanol. Yield: 1.4 g (83%), m.p. 154–155 °C. IR (KBr): 3385, 3340, 3280, 3245 (NH_2, NH); 2950, 2915, 2850 (CH_3, CH_2); 1635, 1570 (C=N, C=C); 1360, 1135 (SO_2). ^{1}H-NMR (DMSO-d_6): 2.31 (s, 3H, PhCH_3); 2.46 (s, 3H, SCH_3); 3.79 (t, 2H, CH_2); 4.57 (s, 2H, NH_2); 4.98–5.11 (m, 2H, C=CH_2); 5.71–5.87 (m, 1H, CH=C); 7.29 (s, 1H, H-3); 7.48 (t, 1H, NH–CH_2); 7.81 (s, 1H, H-6); 7.99 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6): 15.48 (CH$_3$–S); 19.16 (PhCH$_3$); 42.60 (N–CH$_2$–CH=CH_2); 115.39 (N–CH_2–CH=CH$_2$); 125.69, 130.47, 130.94, 135.75, 136.74, 137.65 (aromatic carbons); 139.40 (N–CH_2–CH=CH_2); 157.00 (C=N).

Table 3
Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for **4**

	x	y	z	U_{eq}
S(1)	895(1)	1748(1)	1794(1)	50(1)
S(2)	1648(1)	4137(1)	3392(1)	85(1)
Cl(1)	−3072(1)	4405(1)	2683(1)	81(1)
O(1)	273(2)	677(1)	1193(2)	65(1)
O(2)	1964(2)	1592(2)	2891(2)	67(1)
N(1)	1271(2)	2611(2)	1063(2)	50(1)
N(2)	−173(2)	1955(2)	−813(2)	59(1)
N(3)	792(2)	3734(2)	−549(2)	55(1)
N(4)	−723(3)	2151(3)	−2015(2)	66(1)
C(1)	−191(2)	2563(2)	2062(2)	44(1)
C(2)	−1400(2)	2176(2)	1519(2)	54(1)
C(3)	−2330(2)	2725(2)	1668(3)	57(1)
C(4)	−1977(2)	3684(2)	2407(2)	55(1)
C(5)	−788(2)	4116(3)	2939(2)	56(1)
C(6)	134(2)	3578(2)	2769(2)	51(1)
C(7)	−3637(4)	2287(5)	1040(6)	97(1)
C(8)	1563(4)	5480(4)	4065(4)	76(1)
C(9)	620(2)	2744(2)	−91(2)	44(1)
C(10)	1630(3)	4669(2)	98(3)	58(1)
C(11)	2851(3)	4577(4)	148(3)	75(1)
C(12)	3210(5)	3760(5)	−304(5)	106(1)

U_{eq} is defined as one-third of the trace of the orthogonalised U^{ij} tensor.

4. X-Ray structure analysis

The data were collected on a KumaCCD diffractometer using graphite monochromatised Mo Kα radiation with detector distance of 6 cm. More than hemisphere of reciprocal space was covered by combination of four sets of exposures; each set had a different ϕ-angle (0, 90, 180, 270) and each exposure of 30 s covered 0.75° in ω. Coverage of the unique set is over 99% complete. Out of 8871 reflections measured up to $(\sin\theta/\lambda)_{max} = 0.625$, 3257 were symmetry independent ($R_{int} = 0.0263$). The collected data were reduced using the program CRYSALIS RED [33]. The structure was solved by direct methods with the program SHELXS-97 [34] and refined by full-matrix least-squares method on F^2 with SHELXL-97 [35]. Hydrogen atoms have been located on ΔF maps and refined with isotropic displacement parameters.

Crystal data for **4**: $C_{12}H_{17}ClN_4O_2S_2$, monoclinic, $P2_1/n$, $a = 12.2808(9)$, $b = 11.2825(7)$, $c = 13.0269(9)$ Å, $\beta = 117.668(7)°$, $V = 1598.6(2)$ Å^3, $Z = 4$, $T = 293$ K, $D_x = 1.450$ g cm^{-3}, $\mu = 0.509$ mm^{-1}. The structure was refined on 3257 reflections; 258 refined parameters; $R_1 = 0.0500$, $wR_2 = 0.1221$, Goodness-of-fit = 1.087 for 2575 reflections with $F > 4\sigma(F)$ [$R_1 = 0.0686$, $wR_2 = 0.1366$ for all 3257 independent reflections]. Final atomic coordinates, bond lengths and angles are listed in Tables 3 and 4, respectively. Atom labelling is shown in Fig. 3.

Table 4
Bond lengths (Å) and angles (°) for **4**

Bond lengths	
S(1)–O(2)	1.434(2)
S(1)–O(1)	1.4498(18)
S(1)–N(1)	1.573(2)
S(1)–C(1)	1.783(2)
S(2)–C(6)	1.764(3)
S(2)–C(8)	1.778(4)
Cl(1)–C(4)	1.746(3)
N(1)–C(9)	1.344(3)
N(2)–C(9)	1.331(3)
N(2)–N(4)	1.406(3)
N(3)–C(9)	1.329(3)
N(3)–C(10)	1.441(3)
C(1)–C(2)	1.385(3)
C(1)–C(6)	1.406(3)
C(2)–C(3)	1.391(4)
C(3)–C(4)	1.377(4)
C(3)–C(7)	1.506(4)
C(4)–C(5)	1.381(4)
C(5)–C(6)	1.392(4)
C(10)–C(11)	1.474(5)
C(11)–C(12)	1.278(6)
Bond angles	
O(2)–S(1)–O(1)	116.39(12)
O(2)–S(1)–N(1)	106.80(12)
O(1)–S(1)–N(1)	114.73(11)
O(2)–S(1)–C(1)	106.87(12)
O(1)–S(1)–C(1)	106.33(11)
N(1)–S(1)–C(1)	104.85(11)
C(6)–S(2)–C(8)	103.94(17)
C(9)–N(1)–S(1)	123.81(17)
C(9)–N(2)–N(4)	119.8(2)
C(9)–N(3)–C(10)	124.9(2)
C(2)–C(1)–C(6)	120.2(2)
C(2)–C(1)–S(1)	117.33(19)
C(6)–C(1)–S(1)	122.48(19)
C(1)–C(2)–C(3)	122.9(2)
C(4)–C(3)–C(2)	115.8(2)
C(4)–C(3)–C(7)	123.2(3)
C(2)–C(3)–C(7)	121.0(3)
C(3)–C(4)–C(5)	123.0(2)
C(3)–C(4)–Cl(1)	119.1(2)
C(5)–C(4)–Cl(1)	117.9(2)
C(4)–C(5)–C(6)	121.0(2)
C(5)–C(6)–C(1)	117.1(2)
C(5)–C(6)–S(2)	122.2(2)
C(1)–C(6)–S(2)	120.7(2)
N(3)–C(9)–N(2)	117.5(2)
N(3)–C(9)–N(1)	117.4(2)
N(2)–C(9)–N(1)	125.1(2)
N(3)–C(10)–C(11)	114.1(3)
C(12)–C(11)–C(10)	126.4(4)

5. Supplementary data

Further details of the crystal structure investigation may be obtained from The Director of the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, in quoting the full journal citation.

Acknowledgements

The authors are very grateful to Dr V.L. Narayanan, Chief Drug Synthesis, Chemistry Branch, National Cancer Institute, Bethesda, Maryland 20892 USA., for evaluation of the in vitro antitumour tests.

References

[1] J. Howbert, C.S Grossman, T.A. Crowell, B.J. Rieder, R.W. Harper, K.E. Kromer, E.V. Tao, J. Aikinns, G.A. Poore, S.M. Rinzel, G.B. Grindey, W.N. Shaw, G.C. Tood, J. Med. Chem. 33 (1990) 2393–2407.
[2] T. Owa, H. Yoshino, T. Okauchi, K. Yoshimatsu, Y. Ozawa, N.H. Sugi, T. Nagasu, N. Kayanagi, K. Kitoh, J. Med. Chem. 42 (1999) 3789–3799.
[3] P.M. O'Brien, D.F. Ortwine, A.G. Pavlovsky, J.A. Picard, D.R. Sliskovic, B.D. Roth, R.D. Dyer, C.F. Johnson, C.F. Man, H. Hallak, J. Med. Chem. 43 (2000) 156–166.
[4] M. Artico, Farmaco 51 (1996) 305–331 Review paper.
[5] A.K. Debnath, S. Radigan, S. Jiang, J. Med. Chem. 42 (1999) 3203–3209.
[6] D. Leung, G. Abbenante, D.P.J. Fairlie, J. Med. Chem. 43 (2000) 305–341.
[7] (a) Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 52 (1995) 91–101;
(b) Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 52 (1955) 287–292;
(c) Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 53 (1996) 269–276.
[8] E. Pomarnacka, Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 54 (1997) 215–221.
[9] Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 54 (1997) 293–298.
[10] Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 55 (1998) 233–238.
[11] E. Pomarnacka, A.. Kornicka, Acta Polon. Pharm.—Drug Res. 55 (1998) 297–304.
[12] (a) Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 55 (1998) 375–379;
(b) Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 55 (1998) 473–480.
[13] Z. Brzozowski, A. Kornicka, Acta Polon. Pharm.—Drug Res. 56 (1999) 135–142.
[14] Z. Brzozowski, Acta Polon. Pharm.—Drug Res. 55 (1998) 49–56.
[15] E. Pomarnacka, Acta Polon. Pharm.—Drug Res. 55 (1998) 481–486.
[16] N. Neamati, A. Mazumder, S. Sunder, J.M. Owen, R.J. Schultz, Y. Pommier, Antiviral Chem. Chemother. 8 (1997) 485–495.
[17] S. Matsumo, M. Myaguchi, A. Ito, N. Hara, N. Sakurada, Japan Patent No. 06, 192,215, 1994 (Chem. Abstr. 121 (1994) 300598t).
[18] S. Naito, M. Sugimori, I. Mitsui, Y. Nakamura, M. Iwahana, M. Ishi, K. Hirotani, E. Kunazawa, A. Ejima, Chem. Pharm. Bull. 47 (1999) 1679–1684.
[19] E. Hadicke, F. Frickel, A. Francke, Chem. Ber. 111 (1978) 3222–3232.
[20] J.H. Bryden, L.A. Burkhand, E.W. Hughes, J. Donohue, Acta Crystallogr. 9 (1956) 573–578.
[21] A. Kálmán, M. Czugler, G. Argay, Acta Crystallogr. B37 (1981) 868.
[22] M. Alléaume, A. Gulko, F.H. Herbstein, M. Kapon, R.E. Marsh, Acta Crystallogr. B32 (1976) 669.
[23] G.J. Kruger, G. Gofner, Acta Crystallogr. B28 (1972) 272–283.
[24] E. Costakis, P. Conone, G. Tsatsas, Can. J. Chem. 7 (1969) 4483–4488.
[25] Molecular modelling studies were performed using B3LYP/6-31G* density functional model as implemented into TITAN program, Version 1.1 (1999), Wavefunction Inc–Schrödinger Inc.
[26] W.J. Hehre, A.J. Shusterman, J.E. Nelson, The Molecular Modeling Workbook for Organic Chemistry, Wavefunction, Inc, USA, 1998.
[27] M.R. Boyd, Am. Assoc. Cancer Res. 30 (1989) 652–653.
[28] A.P. Monks, D.A. Scudiero, P. Skehan, R. Shoemaker, K.D. Poull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, J. Natl. Cancer Inst. 83 (1991) 757–776.
[29] J.N. Weinstein, T.G. Myers, P.M. O'Connor, S.H. Friend, A.J. Fornance Jr., K.W. Kohn, J.K. Buolamwini, W.W. van Osdol, A.P. Monks, D.A. Scudeiro, E.A. Sansville, D.W. Zaharevitz, R.E. Bunow, K.D. Paull, Science 275 (1997) 343–349.
[30] Z. Brzozowski, J. Sławiński, Acta Polon. Pharm. 41 (1984) 133–139.
[31] Z. Brzozowski, J. Sławiński, Acta Polon. Pharm. 41 (1984) 5–13.
[32] Z. Brzozowski, F. Gajewski, J. Sławiński, E. Pomarnacka, Acta Polon. Pharm.—Drug Res. 50 (1993) 199–203.
[33] KUMA Diffraction, CRYSALIS RED, Version 1.168, Wrocław, Poland, 2001.
[34] G.M. Sheldrick, SHELXS-97, Program for the solution of crystal structures, University of Göttingen, Germany, 1997.
[35] G.M. Sheldrick, SHELXL-97, Program for the refinement of crystal structures, University of Göttingen, Germany, 1997.