Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 1218–1225
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of novel 3-amino-2-(4-chloro-2-mercaptobenzenesulfonyl)-guanidine derivatives as potential antitumor agents

Zdzisław Brzozowski, Franciszek Sączewski*, Jarosław Sławiński

Department of Chemical Technology of Drugs, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

Received 8 November 2006; received in revised form 12 January 2007; accepted 16 January 2007
Available online 27 January 2007

Abstract

Novel 3-amino-2-(4-chloro-2-mercaptobenzenesulfonyl)guanidine derivatives have been synthesized as potential anticancer agents. The in vitro antitumor activity of these compounds has been evaluated in the US National Cancer Institute (NCI), and relationships between structure and antitumor activity are discussed. The prominent compound was 1-allyl-2-[4-chloro-5-(4-chlorophenylcarbamoyl)-2-methylthiobenzenesulfonyl]-3-(5-nitrofurfurylideneamino)guanidine (**8**) with remarkable activity against 21 human tumor cell lines representing leukemia, lung, colon, melanoma, ovarian, renal, prostate and breast ($GI_{50} = 0.3–3.0$ μM), and selectivity toward leukemia RPMI-8226 cell line ($GI_{50} = 0.3$ μM, $TGI = 1.4$ μM).

Keywords: 3-Amino-2-(4-chloro-2-mercaptobenzenesulfonyl)guanidines; Synthesis; Antitumor effect

1. Introduction

Sulfonamides are among a growing list of compounds with desirable anticancer activity [1–27]. Although they have a common chemical motif of aromatic/heterocyclic sulfonamide, there are a variety of mechanisms for their anticancer action, such as disruption of microtubule assembly, cell cycle arrest in the G1 phase, functional suppression of the transcriptional activator NF-Y, angiogenesis inhibition as well as carbonic anhydrase inhibition [1]. Aryl sulfonamides incorporating disulfide grouping have recently been found to act as hypoxia-activatable prodrugs of 2-mercaptobenzenesulfonamides [28] or 4-(2-mercaptophenylcarboxamido)benzenesulfonamides [29] that target the tumor-associated isoform IX of carbonic anhydrase.

On the other hand, aminoguanidine and related derivatives are effective in inhibiting nitric oxide production in cells expressing inducible nitric oxide synthase (NOS2) [30,31], and therefore, aminoguanidines exhibit protective effect against nephrotoxicity [32] and ototoxicity [33] induced by chemotherapeutic agents.

In view of the above findings one may conclude that the compounds incorporating both the benzenesulfonyl and aminoguanidine moieties present potential use as safe antitumor drugs. Indeed, we and others have already found that 3-amino-2-(2-mercaptobenzenesulfonyl)guanidine derivatives (Fig. 1, structures **I** [6] and **II** [10]) and 3-amino-2-(5-indanylsulfonyl)-guanidine (Fig. 1, structure **III** [34]) display remarkable anticancer properties. These results encouraged us to design compounds of type **IV** (Fig. 1) resulting from the replacement of C=C of **I** by a C≡C group. Moreover, following the finding that various heterocyclic Schiff bases of aminoguanidine may also act as antitumor agents [35–37], we have also synthesized novel compounds of general structure **V** (Fig. 1).

2. Results and discussion

2.1. Chemistry

In general, we synthesized two series of 2-mercaptobenzenesulfonamide derivatives: series **A** (compounds **1–9**)

* Corresponding author. Tel.: +48 58 349 3250; fax: +48 58 349 3257.
E-mail address: saczew@amg.gda.pl (F. Sączewski).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.01.020

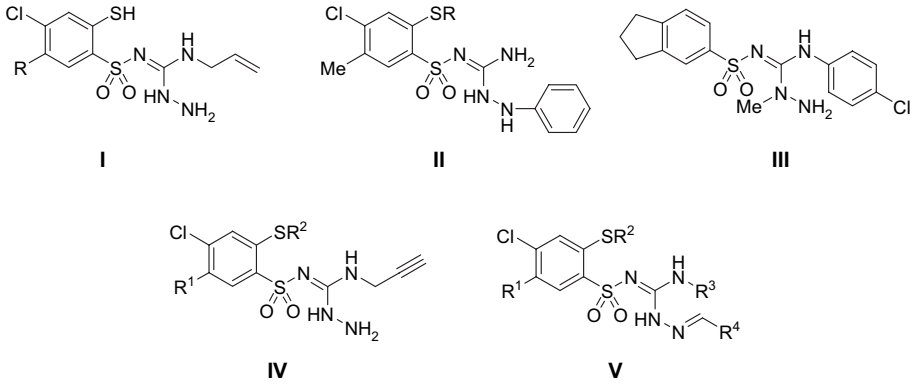


Fig. 1.

characterized by the presence of 1-allylguanidine group; series **B** (compounds **14**–**19**) containing 1-(2-propynyl)guanidine moiety.

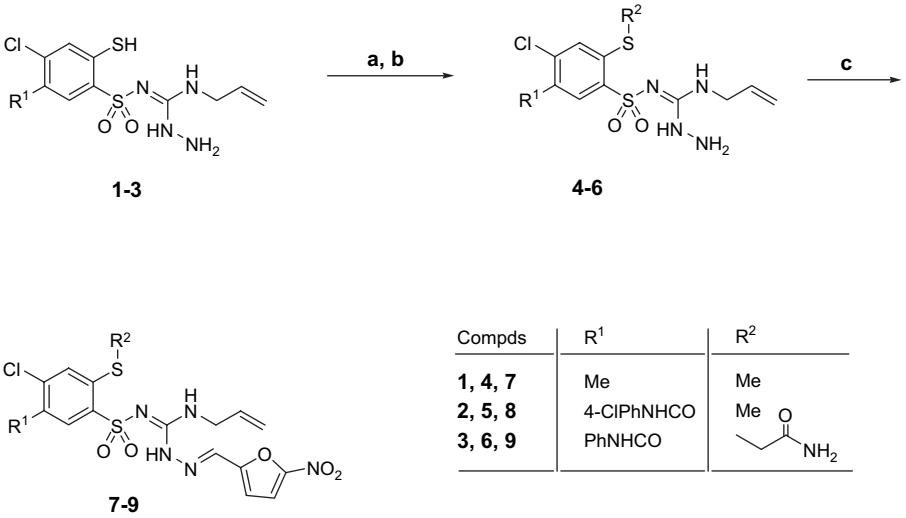
For the preparation of novel 3-amino-2-(4-chloro-2-mercaptobenzenesulfonyl)-1-allylguanidines **5**–**9** we made use of a two-step synthesis from the previously described aminoguanidines **2** and **3** [6] commencing with alkylation of the mercapto group with appropriate alkylating agents under alkaline conditions which gave the sulfides **5** and **6** in 94–96% yield. Subsequent treatment of **4** [6], **5** and **6** with 5-nitro-2-furaldehyde gave rise to the formation of the corresponding Schiff bases **7**–**9** in 83–92% yields (Scheme 1).

The syntheses of the compounds of series **B** were achieved by a convenient procedure depicted in Schemes 2 and 3. First, a readily available 3-methylthio-1,4,2-benzodithiazine 1,1-dioxides **10** [38] and **11** [39] were subjected to the reaction with 2-propynylamine in dry benzene to give the substitution products **12** and **13** in 89 and 96% yield, respectively. 3-(2-Propynylamino)-1,4,2-benzodithiazine 1,1-dioxides thus obtained were converted into the corresponding aminoguanidine derivatives **14** and **15** upon treatment with an excess of hydrazine hydrate in methanolic solution at room temperature (Scheme 2). Then, the reaction of **14** with appropriate alkylating agents under alkaline conditions furnished the expected sulfides **16**–**18** in 75–94% yields. The synthesis of the final Schiff base **19** was achieved by reacting aminoguanidine **16** with 5-nitro-2-furaldehyde in refluxing ethanol (Scheme 3).

The structures of all newly obtained compounds were confirmed by elemental analyses as well as by IR and NMR spectroscopy as shown in Section 4.

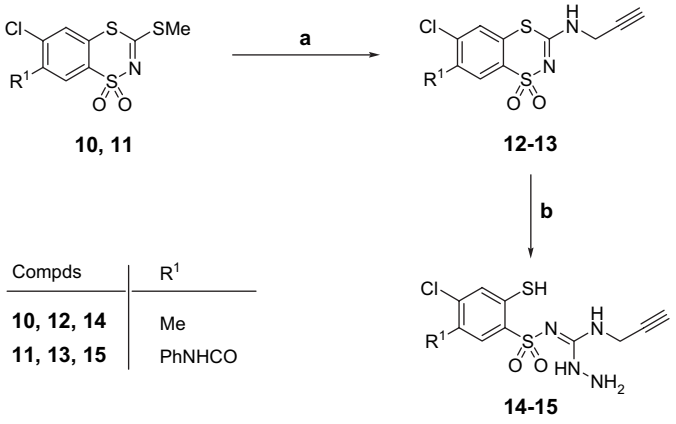
2.2. Biology

Three previously described compounds of series **A** (**1**–**3**) [6] and 11 newly prepared compounds of series **A** (**5**–**9**) and series **B** (**14**–**19**) were tested in the US National Cancer Institute (NCI, Bethesda, MD) for their in vitro anticancer activity. To facilitate the discussion of structure–activity relationships, the series **A** and **B** were further subdivided into three substructures according to substitution pattern: substructure **I** with $R^1 = H$ and unsubstituted NH_2 group; substructure **II** with $R^1 =$ alkyl and unsubstituted NH_2 group; substructure **III** with $R^1 =$ alkyl and NH_2 group involved in the formation of Schiff base (Table 1).



Compds	R^1	R^2
1, 4, 7	Me	Me
2, 5, 8	4-ClPhNHCO	Me
3, 6, 9	PhNHCO	

Scheme 1.



Scheme 2.

Table 1
Results of primary anticancer assay for compounds of series **A** and **B**

Substructure	Series **A**		Series **B**	
	Compound	Activity[a]	Compound	Activity[a]
I	**1**[b]	A	**14**	NA
	2[b]	A	**15**	NA
	3[b]	A		
II	**5**	NA	**16**	NA
	6	NA	**17**	NA
			18	NA
III	**7**	NA	**19**	A
	8	A		
	9	A		

[a] Activity denoted as: A = active; NA = not active.
[b] See Ref. [6].

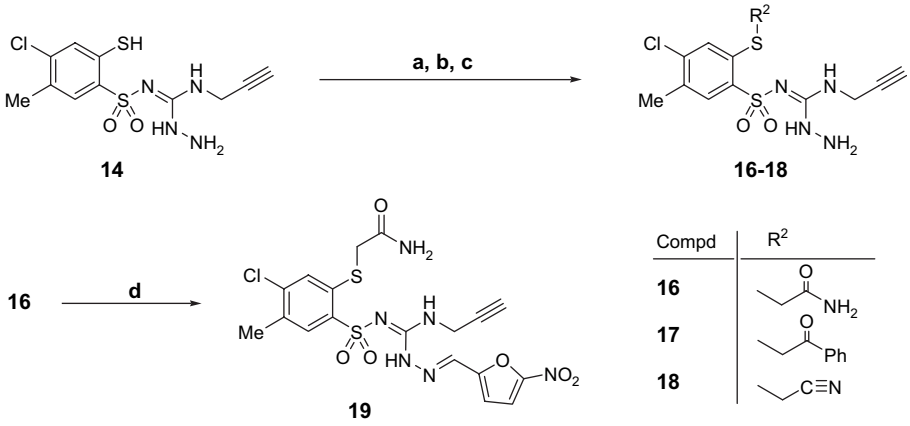
Primary anticancer assay at concentration of 100 μM in the 3-cell line panel, consisting of the MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) showed that compounds **5**, **6** (series **A-II**), **7** (series **A-III**) as well as **14**, **15** (series **B-I**) and **16–18** (series **B-II**) were essentially inactive (Table 1). Thus, it was interesting to find that replacement of the allyl group of the active 2-mercaptobenzenesulfonamides **1–3** [6] with 2-propynyl group (series **B-I** versus **A-I**) decreased cytotoxicity considerably, and the compounds with sulfide moiety and unsubstituted amino group (series **A-II** and **B-II**) were inactive.

On the other hand, the Schiff bases **8** and **9** (series **A-III**) as well as **19** (series **B-III**) exhibited some level of ability to inhibit the growth of human tumor cells in culture. Therefore, a secondary screening to determine their cytostatic activity was performed on a panel of approximately 60 human tumor cell lines, derived from nine different cancer types: leukemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast. The compounds were tested 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 cell growth. Details of this test system, and the information which is encoded by the activity pattern over all cell lines, have been published [40–42]. The anticancer activity of tested compounds is reported for each cell lines by GI_{50} value (GI_{50} = molar concentration of the compounds that inhibits 50% net cell growth) and TGI value (TGI = molar concentration of the compounds leading to total inhibition).

As shown in Table 2, the compound **8** (R^1 = 4-ClPhNHCO, R^2 = Me) was most active and exhibited a remarkable activity against 21 human tumor cell lines with GI_{50} values in the low micromolar range of 0.3–3.0 with selectivity toward leukemia RPMI-8226 cell line (GI_{50} = 0.3 μM, TGI = 1.4 μM). Moreover, compounds **9** (R^1 = PhNHCO, R^2 = CH_2CONH_2) and **19** (R^1 = Me, R^2 = CH_2CONH_2) showed modest cytotoxic effects indicating that incorporation of 5-nitrofurfurylidene group into the aminoguanidine moiety of both 1-allyl- and 1-(2-propynyl)-3-aminoguanidines may result in good potency. Table 2 also shows that a combination of *S*-alkyl in the phenyl ring with 5-nitrofurylideno group at the aminoguanidine moiety yielded derivatives of series **A-III** which were >10-fold more potent than the previously described [6] parent compounds of series **A-I**.

3. Conclusions

The main purpose of this study was to evaluate new types of benzenesulfonamide derivatives bearing variously substituted aminoguanidine group for their cytotoxic activity. Among the



Scheme 3.

Table 2
Inhibition of in vitro cancer cell lines growth by guanidine derivatives **1**–**3**, **8**, **9** and **19**[a]

Compound	Number of the cell lines					Most sensitive cell lines ($GI_{50} < 20.0$ μM)			
	Investigated	Giving positive GI_{50} and TGI				Panel	Cell line	GI_{50}[b] (μM)	TGI[c] (μM)
		GI_{50}[b] (μM)		TGI[c] (μM)					
		No	Range	No	Range				
1[d]	58	53	15.9–93.3	10	37.7–90.4				
2[d]	55	54	11.5–81.7	17	37.1–97.4				
3[d]	56	48	6.2–97.3	7	52.5–83.3				
8	56	49	0.3–82.4	25	1.4–63.5	Leukemia	CCRF-CEM	5.4	45.1
							HL-60(TB)	1.0	8.1
							K-562	1.2	4.5
							MOLT-4	1.7	15.6
							RPMI-8226	0.3	1.4
							SR	1.6	5.5
						Non-small cell	EKVX	2.6	6.5
						lung cancer	HOP-62	1.8	5.7
							NCI-H460	3.0	12.4
						Colon cancer	HCT-116	0.9	4.0
							HCT-15	2.9	13.6
							SW-620	0.8	2.5
						Melanoma	MALME-3M	5.0	63.4
							M14	2.1	4.6
							SK-MEL-2	2.1	5.9
							SK-MEL-5	2.0	4.8
							UACC-257	5.4	35.9
						Ovarian cancer	OVCAR-3	1.5	14.1
							OVCAR-4	2.9	11.6
							OVCAR-8	1.5	4.2
						Renal cancer	ACHN	3.0	25.1
						Prostate cancer	DU-145	1.8	12.4
						Breast cancer	MDA-MB-231/ATCC	2.3	6.1
							MDA-MB-435	2.8	7.0
							BT-549	4.1	63.5
							T-47D	9.9	26.5
9	56	46	5.4–6.8	27	18.3–95.8	Leukemia	CCRF-CEM	5.7	27.6
							K-562	9.1	49.8
							MOLT-4	13.4	43.9
						Non-small cell	HOP-92	13.6	52.2
						lung cancer	NCI-H322M	16.3	57.1
						Colon cancer	COLO-205	6.5	>100.0
							KM-12	17.1	32.5
							SW-620	5.9	37.9
						CNS cancer	SF-539	18.8	41.5
						Melanoma	LOX IMV1	17.0	42.1
							SK-MEL-2	16.1	45.3
							SK-MEL-5	5.4	18.3
							UACC-257	19.5	49.0
							UACC-62	17.5	33.3
						Ovarian cancer	OVCAR-3	14.7	39.8
						Breast cancer	MDA-MB-231/ATCC	14.0	50.8
							HS 578T	15.6	63.5
							MDA-MB-435	17.9	36.7
							BT-549	10.4	31.9
19	56	55	1.8–94.4	49	9.4–84.9	Leukemia	MOLT-4	13.2	31.9
							RPMI-8226	12.8	34.5
							SR	17.0	50.1
						Non-small cell	A549/ATCC	18.0	35.3
						lung cancer	EKVX	17.8	32.2
							HOP-62	12.9	25.7
							NCI-H23	18.5	33.1
							NCI-H460	12.5	29.9
							NCI-H522	10.0	23.3

(*continued on next page*)

Table 2 (*continued*)

Compound	Number of the cell lines				Most sensitive cell lines ($GI_{50} < 20.0$ μM)			
	Investigated	Giving positive GI_{50} and TGI			Panel	Cell line	GI_{50}[b] (μM)	TGI[c] (μM)
		GI_{50}[b] (μM)		TGI[c] (μM)				
		No	Range	No Range				
					Colon cancer	HCT-116	8.0	20.3
						KM12	18.8	38.9
						SW-620	4.4	17.1
					CNS cancer	SF-256	15.0	35.8
						SF-539	17.3	31.7
					Melanoma	LOX IMVI	8.1	35.2
						MALME-3M	13.6	27.7
						SK-MEL-2	4.9	18.3
						SK-MEL-28	14.5	32.7
						SK-MEL-5	4.1	15.2
						UACC-257	13.9	27.0
					Ovarian cancer	IGROV1	13.9	27.2
						OVCAR-4	16.5	33.3
						OVCAR-5	19.9	34.5
						OVCAR-8	13.8	29.6
					Prostate cancer	DU-145	11.6	24.5
					Breast cancer	MCF-7	6.5	22.5
						MDA-MB-231/ATCC	11.4	26.4
						HS 578T	19.8	39.5
						BT-549	1.7	9.4
						T-47D	18.8	34.9

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell lines screen [40–42].
[b] GI_{50}: molar concentration that inhibits 50% net cell growth.
[c] TGI: molar concentration giving total growth inhibition.
[d] See Ref. [6].

evaluated compounds, 1-allyl-2-[4-chloro-5-(4-chlorophenylcarbamoyl)-2-methylthiobenzenesulfonyl]3-(5-nitrofurfurylideneamino)guanidine (**8**) showed the best activity. This Schiff bases is therefore anticancer lead for further synthetic optimization.

4. Experimental protocols

Melting points: Büchi 535 apparatus; IR spectra: KBr pellets, 400–4000 cm^{-1} Perkin–Elmer 1600 FTIR spectrophotometer; ^1H NMR spectra: Varian Gemini 200 apparatus (chemical shifts are expressed at δ values relative to Me_4Si as standard). ^{13}C NMR spectra were taken on a Varian Unity 500 spectrometer. Analyses of C, H, N were within ±0.4% of theoretical values.

*4.1. 1-Allyl-3-amino-2-[4-chloro-5-(4-chlorophenylcarbamoyl)-2-methylthiobenzenesulfonyl]guanidine (**5**)*

1-Allyl-3-amino-2-[4-chloro-5-(4-chlorophenylcarbamoyl)-2-mercaptobenzenesulfonyl]guanidine **2** (2.37 g, 5 mmol) was dissolved in a solution of NaOH (0.28 g, 7 mmol) in water (45 ml). The resulting solution was cooled on an ice bath with stirring and then Me_2SO_4 (0.83 g, 6.6 mmol) was added dropwise. The reaction mixture was stirred at 0–1 °C for 1.5 h and then at room temperature for 3 h. The precipitate thus obtained was filtered off, washed thoroughly with water and methanol (4 × 3 ml), and dried to afford title compound **5** (2.4 g, 98%); m.p. 236–237 °C dec. IR (KBr): 3340, 3290, 3250 (NH_2, NH), 1680 (C=O), 1650 (C=N), 1355, 1130 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.54 (s, 3H, CH_3S), 3.79 (t, 2H, CH_2), 4.54 (s, 2H, NH_2), 4.97 (dd, 1H, CH=CH_A), 5.04 (dd, 1H, CH=CH_B), 5.74–5.82 (m, 1H, CH_2CH=CH_2), 7.42 (d, 2H, arom.), 7.55 (s, 1H, NH), 7.73 (d, 2H, arom.), 8.61 (s, 1H, H-3, $PhSO_2$), 8.10 (s, 1H, H-6, $PhSO_2$), 10.69 (s, 1H, NHCO) ppm. Anal. ($C_{18}H_{19}Cl_2N_5O_3S_2$) C, H, N.

*4.2. 1-Allyl-3-amino-2-[4-chloro-2-(carbamoylmethylthio)-5-(phenylcarbamoyl)benzenesulfonyl]guanidine (**6**)*

To a suspension of 1-allyl-3-amino-2-[4-chloro-2-mercapto-5-phenylcarbamoylbenzenesulfonyl]guanidine **3** (4.4 g, 10 mmol) in methanol (100 ml), triethylamine (1.1 g, 11 mmol) was added with stirring. The solution thus obtained was treated portionwise with chloroacetamide (1.03 g, 11 mmol) at 18–22 °C (water bath). Then the reaction mixture was stirred at room temperature for 2 h, followed by reflux for 1 h. After cooling to room temperature the precipitate of **6** was collected by filtration, washed with methanol (4 × 4 ml) and dried (4.7 g, 94%); m.p. 226–228 °C dec. IR (KBr) 3410, 3370, 3350, 3320, 3290 (NH_2, NH), 1660 (C=O), 1625 (C=N), 1355, 1130 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.30 and 3.81 (2s, 2 × 1H, $CONH_2$), 3.78 (s, 2H, SCH_2), 4.54 (s, 2H, N–NH_2), 4.97 (dd, 1H, CH=CH_A), 5.06 (dd, 1H, CH=CH_B), 5.77–5.84 (m, 1H, CH_2CH=CH_2), 7.11 (t, $J = 7.2$ Hz, 1H, NH–CH_2), 7.29 (s, 1H, NH–N), 7.36

(t, $J = 7.2$ Hz, 2H, N–CH_2), 7.56–7.70 (m, 5H, PhN), 7.99 (s, 1H, H-3, $PhSO_2$), 8.11 (s, 1H, H-6, $PhSO_2$), 10.57 (s, 1H, PhNHCO) ppm. Anal. ($C_{19}H_{21}ClN_6O_4S_2$) C, H, N.

4.3. Preparation of 1-allyl-2-(4-chlorobenzenesulfonyl)-3-(5-nitrofurfurylideneamino)guanidine derivatives (7–9)

To a suspension of the corresponding 1-allyl-3-amino-2-(4-chlorobenzenesulfonyl)guanidine **4**, **5** or **6** (3 mmol) in ethanol (25–30 ml), 5-nitro-2-furaldehyde (0.5 g, 3.5 mmol) was added. The reaction mixture was stirred at reflux for 7 h. After cooling to room temperature and standing overnight the precipitate of the adequate 3-(5-nitrofurfurylideneamino) guanidine was filtered off, washed with ethanol (4 × 3 ml), and dried.

4.3.1. 1-Allyl-2-(4-chloro-5-methyl-2-methylthiobenzenesulfonyl)-3-(5-nitrofurfurylideneamino)guanidine (7)

Starting from 1-allyl-3-amino-2-(4-chloro-5-methyl-2-methylthiobenzenesulfonyl)guanidine **4** (1.05 g), the title compound **7** was obtained (1.3 g, 92%); m.p. 200–201 °C dec. IR (KBr) 3390, 3240 (NH), 1645, 1620 (C=N), 1350, 1335, 1140 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.36 (s, 3H, CH_3Ph), 2.49 (s, 3H, CH_3S), 4.01 (s, 2H, CH_2), 5.15 (dd, 1H, CH=CH_A), 5.22 (dd, 1H, CH=CH_B), 5.80–5.90 (m, 1H, CH_2CH=CH_2), 6.57 (br s, 1H, NH–CH_2), 6.87 (s, 1H, N=CH), 7.24 (s, 1H, H-3, furyl), 7.37 (s, 1H, H-4, furyl), 7.96 (s, 2H, H-3 and H-6, $PhSO_2$), 10.90 (s, 1H, NH–N=C) ppm. ^{13}C NMR ($CDCl_3$) δ: 16.62, 19.64, 43.98, 113.25, 115.10, 117.59, 126.76, 130.93, 132.60, 132.99, 133.49, 138.30, 138.77, 150.51, 152.16 ppm. Anal. ($C_{17}H_{18}ClN_5O_5S_2$) C, H, N.

4.3.2. 1-Allyl-2-[4-chloro-5-(4-chlorophenylcarbamoyl)-2-methylthiobenzenesulfonyl]-3-(5-nitrofurfurylideneamino)guanidine (8)

Starting from 1-allyl-3-amino-2-[4-chloro-5-(4-chlorophenylcarbamoyl)-2-methylthiobenzenesulfonyl]guanidine **5** (1.46 g), the title compound **8** was obtained (1.5 g, 83%); m.p. 220–221 °C. IR (KBr) 3380, 3275, 3240 (NH), 1665 (C=O), 1630, 1610 (C=N), 1355, 1335, 1170 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.63 (s, 3H, CH_3S), 3.87 (s, 2H, CH_2), 5.02 (dd, 1H, CH=CH_A), 5.08 (dd, 1H, CH=CH_B), 5.77–5.82 (m, 1H, CH_2CH=CH_2), 7.38–7.83 (m, 7H, arom. and N=CH), 8.08 (s, 1H, H-3, $PhSO_2$), 8.20 (s, 1H, H-6, $PhSO_2$), 10.62 (s, 1H, HN–N=C), 11.01 (s, 1H, NHCO) ppm. Anal. ($C_{23}H_{20}Cl_2N_6O_6S_2$) C, H, N.

4.3.3. 1-Allyl-2-[4-chloro-2-(carbamoylmethylthio)-5-(phenylcarbamoyl)benzenesulfonyl]-3-(5-nitrofurfurylideneamino)guanidine (9)

Starting from 1-allyl-3-amino-2-[4-chloro-2-(carbamoylmethylthio)-5-phenylcarbamoyl)benzenesulfonyl]guanidine **6** (1.5 g), the title compound **9** was obtained (1.6 g, 86%); m.p. 193–195 °C dec. IR (KBr) 3390, 3280, 3250 (NH), 1660 (C=O), 1615, 1600 (C=N), 1350, 1330, 1165 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.82 (s, 2H, CH_2), 3.90 (s, 2H, CH_2), 5.03 (dd, 1H, CH=CH_A), 5.09 (dd, 1H, CH=CH_B), 5.83 (br s, 1H, $NHCH_2$), 7.10–7.14 (m, 1H, H-4, PhN), 7.30–7.40 (m, 2H, PhN), 7.64–7.72 (m, 2H, PhN), 7.83 (s, 1H, $CONH_a$), 8.08 (s, 1H, H-3, $PhSO_2$), 8.20 (s, 1H, $CONH_b$), 8.45 (s, 1H, H-6, $PhSO_2$), 10.60 (s, 1H, NH–N=C), 11.01 (s, 1H, NHCO) ppm. Anal. ($C_{24}H_{22}ClN_7O_7S_2$) C, H, N.

4.4. Preparation of 6-chloro-3-(2-propynylamino)-1,4,2-benzodithiazine 1,1-dioxides (12 and 13)

A solution of the corresponding methylbenzodithiazine **10** or **11** (0.02 mol) and 2-propynylamine (1.1 g, 0.02 mol) in dry benzene (150 ml) was stirred at room temperature for 3 h. The suspension obtained was refluxed until the evolution of CH_3SH had ceased (40–50 h). The precipitate was filtered off, washed successively with benzene (3 × 5 ml) and methanol (4 × 5 ml), and dried.

4.4.1. 6-Chloro-7-methyl-3-(2-propynylamino)-1,4,2-benzodithiazine 1,1-dioxide (12)

Starting from methylbenzodithiazine **10** (5.9 g), the title compound **12** was obtained (5.8 g, 96%); m.p. 213–215 °C. IR (KBr) 3285 (NH), 2125 (C≡C), 1560 (C=N), 1345, 1150 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.41 (s, 3H, CH_3), 3.16 (s, 1H, C≡CH), 4.18 (d, $J = 2.5$ Hz, CH_2), 7.89 (s, 1H, H-5), 7.98 (s, 1H, H-8), 10.13 (s, 1H, NH) ppm. ^{13}C NMR (DMSO-d_6) δ: 19.32, 32.21, 75.00, 78.62, 126.47, 127.29, 128.09, 131.10, 137.14, 137.36, 162.50 ppm. Anal. ($C_{11}H_9ClN_2O_2S_2$) C, H, N.

4.4.2. N-Phenyl-6-chloro-3-(2-propynylamino)-1,1-dioxo-1,4,2-benzodithiazine-7-carboxamide (13)

Starting from methylbenzodithiazine **11** (8.0 g), the title compound **13** was obtained (7.2 g, 89%); m.p. 148–150 °C. IR (KBr) 3295, 3280, 3215 (NH), 2125 (C≡C), 1655 (C=O), 1560 (C=N), 1325, 1160 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.38 (s, 1H, C≡CH), 4.21 (s, 2H, CH_2), 7.36–7.45 (m, 3H, arom.), 7.68–7.72 (m, 2H, arom.), 8.09–8.20 (m, 2H, arom.), 10.28 (s, 1H, NH), 10.82 (s, 1H, NH) ppm. Anal. ($C_{17}H_{12}ClN_3O_3S_2$) C, H, N.

4.5. Preparation of 3-amino-2-(4-chloro-2-mercaptobenzenesulfonyl)-1-(2-propynyl)-guanidines (14–15)

A solution of the corresponding benzodithiazine **12** or **13** (0.01 mol) and 99–100% hydrazine hydrate (1.3 g, 0.025 mol) in methanol (30 ml) was stirred at room temperature for 26 h. The solvent was evaporated under reduced pressure, and the solid residue was dissolved in water (350 ml) pH 9. After 1.5 h, a small amount of insoluble by-products was filtered out. The resulting filtrate was acidified with 1% hydrochloric acid to pH 3. The precipitate thus obtained was collected by filtration, washed successively with water (4 × 10 ml) and methanol (3 × 2 ml), and dried at temperatures gradually increasing to 100 °C.

*4.5.1. 3-Amino-2-(4-chloro-2-mercapto-5-methylbenzenesulfonyl)-1-(2-propynyl)guanidine (**14**)*

Starting from benzodithiazine **12** (3.0 g), the title compound **14** was obtained (2.6 g, 78%); m.p. 141–143 °C. IR (KBr) 3350, 3260 (NH_2, NH), 2550 (SH), 2115 (C≡C), 1650 (C=N), 1345, 1180 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.27 (s, 3H, CH_3), 3.10 (s, 1H, C≡CH), 3.95 (s, 2H, CH_2), 4.55 (s, 2H, NH_2, disappearing on deuteration), 6.00 (s, 1H, SH, disappearing on deuteration), 7.57 (s, 1H, H-3), 7.70 (s, 1H, NH, disappearing on deuteration), 7.81 (s, 1H, H-6), 8.16 (s, 1H, NH, disappearing on deuteration) ppm. ^{13}C NMR (DMSO-d_6) δ: 18.95, 29.76, 73.09, 81.29, 130.15, 130.39, 131.39, 132.25, 135.86, 138.80, 156.34 ppm. Anal. ($C_{11}H_{13}ClN_4O_2S_2$) C, H, N.

*4.5.2. 3-Amino-2-[4-chloro-5-(phenylcarbamoyl)-2-mercaptobenzenesulfonyl]-1-(2-propynyl)guanidine (**15**)*

Starting from benzodithiazine **13** (4.1 g), the title compound **15** was obtained (3.3 g, 75%); m.p. 208–210 °C. IR (KBr) 3330, 3300, 3245 (NH_2, NH), 2560 (SH), 2125 (C≡C), 1685 (C=O), 1575 (C=N), 1360, 1140 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.02 (s, 1H, C≡CH), 3.97 (s, 2H, $NCH_2C{\equiv}C$), 4.56 (s, 2H, NH_2), 5.98 (s, 1H, SH), 7.11 (m, 1H, Ph), 7.35 (m, 2H, Ph), 7.69 (m, 2H, Ph), 7.78 (s, 2H, HN–C–NH), 8.04 (s, 1H, H-3, $PhSO_2$), 8.21 (s, 1H, H-6, $PhSO_2$), 10.53 (s, 1H, NHCO) ppm. Anal. ($C_{17}H_{16}ClN_5O_3S_2$) C, H, N.

*4.6. 3-Amino-2-[4-chloro-2-(carbamoylmethylthio)-5-methylbenzenesulfonyl]-1-(2-propynyl)guanidine (**16**)*

To a solution of triethylamine (1.6 g, 0.016 mol) and 3-amino-2-(4-chloro-2-mercapto-5-methylbenzenesulfonyl)-1-(2-propynyl)guanidine **14** (5.0 g, 0.015 mol) in methanol (45 ml), chloroacetamide (1.5 g, 0.016 mol) was added. The reaction mixture was stirred at room temperature for 4 h, followed by reflux for 3 h. The resulting solution was poured into water (60 ml) and stirred at room temperature for 2 h. The precipitate thus obtained was collected by filtration, washed with water (6 × 10 ml) and 2-propanol (3 × 2 ml), and dried to give **16** (5.4 g, 92%); m.p. 188–190 °C dec. IR (KBr) 3350, 3345, 3300 (NH_2, NH), 2120 (C≡C), 1690 (C=O), 1645 (C=N), 1360, 1340, 1190 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.31 (s, 3H, CH_3), 3.09 (s, 1H, C≡CH), 3.68 (s, 2H, NCH_2), 3.96 (s, 2H, SCH_2), 4.56 (s, 2H, H_2N–N), 7.23 (s, 1H, $CONH_a$), 7.57 (s, 1H, H-3, Ph), 7.72 (s, 1H, $CONH_b$), 7.85 (s, 1H, H-6, Ph), 8.06 (s, 1H, HN–N) ppm. Anal. ($C_{13}H_{16}ClN_5O_3S_2$) C, H, N.

*4.7. 3-Amino-2-(4-chloro-5-methyl-2-phenacylthiobenzenesulfonyl)-1-(2-propynyl)guanidine (**17**)*

To an ice-cooled solution of triethylamine (0.6 g, 6 mmol) and 3-amino-2-(4-chloro-2-mercapto-5-methylbenzenesulfonyl)-1-(2-propynyl)guanidine **14** (1.7 g, 5 mmol) in CH_2Cl_2 (15 ml), phenacyl bromide (1.0 g, 5 mmol) was added with stirring. After 0.5 h, the ice bath was removed and the reaction mixture was stirred at room temperature for 3 h, followed by reflux for 5 h. After cooling to room temperature the resulting suspension was left to stand overnight. The precipitate was collected by filtration, washed with water, dried and recrystallized from 2-propanol (80 ml) to give **17** (1.7 g, 75%); m.p. 144–146 °C dec. IR (KBr): 3350, 3300, 3255 (NH_2, NH), 2120 (C≡C), 1685 (C=O), 1645 (C=N), 1355, 1135 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 3.05 (s, 1H, C≡CH), 3.84 (s, 2H, NCH_2), 4.53 (s, 2H, SCH_2), 4.75 (s, 2H, NH_2), 7.49 (s, 1H, NH), 7.53–7.56 (m, 2H, arom.), 7.65–7.68 (m, 2H, arom.), 7.84 (s, 1H, H-6, $PhSO_2$) 8.05–8.08 (m, 3H, HN–N and arom.) ppm. Anal. ($C_{19}H_{19}ClN_4O_3S_2$) C, H, N.

*4.8. 3-Amino-2-[4-chloro-2-(cyanomethylthio)-5-methylbenzenesulfonyl]-1-(2-propynyl)guanidine (**18**)*

To a solution of triethylamine (0.65 g, 6.4 mmol) and 3-amino-2-(4-chloro-2-mercapto-5-methylbenzenesulfonyl)-1-(2-propynyl)guanidine **14** (2.0 g, 6 mmol) in methanol (25 ml), bromoacetonitrile (0.77 g, 6.4 mmol) was added. The reaction mixture was stirred at room temperature for 4 h, followed by reflux for 2 h. The resulting solution was poured into water (150 ml) and stirred at room temperature for 6 h. The precipitate thus obtained was collected by filtration, washed successively with water (5 × 5 ml) and 2-propanol (2 × 2 ml), and dried to give **18** (3.1 g, 94%); m.p. 141–143 °C. IR (KBr): 3350, 3285 (NH_2, NH), 2245 (C≡N), 2125 (C≡C), 1645 (C=N), 1360, 1345, 1135 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.36 (s, 3H CH_3), 3.08 (s, 1H, C≡CH), 3.94 (s, 2H, $CH_2C{\equiv}C$), 4.35 (s, 2H, CH_2CN), 4.56 (s, 2H, NH_2), 7.57 (s, 1H, H-3, Ph), 7.75 (br s, 1H, NH), 7.91 (s, 1H, H-6, Ph), 8.08 (s, 1H, HN–N) ppm. Anal. ($C_{13}H_{14}ClN_5O_2S_2$) C, H, N.

*4.9. 2-[4-Chloro-2-(carbamolymethylthio)-5-methylbenzenesulfonyl]-3-(5-nitrofurfurylideneamino)-1-(2-propynyl)guanidine (**19**)*

A mixture of aminoguanidine **16** (1.2 g, 3 mmol) and 5-nitrofurane-2-carbaldehyde (0.46 g, 3.3 mmol) in ethanol (20 ml) was stirred at reflux for 7 h. After cooling to room temperature the resulting suspension was left overnight. The precipitate was collected by filtration, washed with ethanol (4 × 2 ml) and dried initially at room temperature and then at 90 °C to give **19** (1.3 g, 85%); m.p. 210–212 °C dec. IR (KBr): 3415, 3395, 3275 (NH_2, NH), 2125 (C≡C), 1685 (C=O), 1655, 1625 (N=C, N=CH), 1350, 1170 (SO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ: 2.32 (s, 3H, CH_3), 3.12 (s, 1H, C≡CH), 3.69 (s, 2H, SCH_2), 4.02 (d, $J = 5.1$ Hz, 2H, NCH_2), 7.22 (s, 1H, $CONH_a$), 7.38 (d, $J = 3.9$ Hz, 1H, H-3, furane), 7.55 (s, 1H, $CONH_b$), 7.60 (s, 1H, H-3, $PhSO_2$), 7.81 (d, $J = 3.9$ Hz, 1H, H-4, furane), 7.91 (s, 1H, H-6, $PhSO_2$), 8.33 (t, $J = 5.1$ Hz, 1H, HN–CH_2), 8.45 (s, 1H, N=CH), 11.21 (s, 1H, HN–N) ppm. Anal. ($C_{18}H_{17}ClN_6O_6S_2$) C, H, N.

Acknowledgements

This work was supported by the Polish State Committee for Scientific Research (grant no. 2 P05 035 27). The authors are very grateful to Dr. V.L. Narayanan, Chief of Drug Synthesis, Chemistry Branch, National Cancer Institute (Bethesda, MD) for the in vitro screening.

References

[1] A. Casini, A. Scozzafava, A. Mastrolorenzo, C.T. Supuran, Curr. Cancer Drug Targets 2 (2002) 55–75.
[2] A. Scozzafava, A. Casini, C.T. Supuran, Curr. Med. Chem. 9 (2002) 1167–1185.
[3] C.T. Supuran, A. Casini, A. Scozzafava, Med. Res. Rev. 23 (2003) 535–558.
[4] A. Scozzafava, T. Owa, A. Mastrolorenzo, C.T. Supuran, Curr. Med. Chem. 10 (2003) 925–953.
[5] Z. Brzozowski, F. Sączewski, J. Med. Chem. 45 (2002) 430–437.
[6] Z. Brzozowski, F. Sączewski, M. Gdaniec, Eur. J. Med. Chem. 37 (2002) 285–293.
[7] E. Pomarnacka, M. Gdaniec, Bioorg. Med. Chem. 11 (2003) 1259–1267.
[8] Z. Brzozowski, F. Sączewski, M. Gdaniec, Eur. J. Med. Chem. 38 (2003) 991–999.
[9] Z. Brzozowski, F. Sączewski, M. Gdaniec, Bioorg. Med. Chem. 11 (2003) 3673–3681.
[10] J. Sławiński, P. Bednarski, R. Grünert, P. Reszka, Pol. J. Chem. 77 (2003) 53–64.
[11] J. Sławiński, P. Bednarski, P. Reszka, Pol. J. Chem. 78 (2004) 369–379.
[12] K.L. Labb, P.A. Hipskind, J.A. Aikins, E. Alvarez, Y.Y. Cheung, L.E. Considine, A. DeDios, G.L. Durst, R. Ferritto, C.S. Grossman, D.D. Giera, B.H. Hollister, Z. Huang, P.W. Iversen, K.L. Law, T. Li, H.S. Lin, B. Lopez, J.E. Lopez, L.M. Martin Cabrejas, D.J. McCann, V. Molero, J.E. Reilly, M.E. Riched, C. Shih, B. Teicher, J.H. Wikel, W.T. Mader, J. Med. Chem. 47 (2004) 5367–5380.
[13] R. Villar, I. Encio, M. Migliaccio, M.J. Gil, V. Martinez-Merino, Bioorg. Med. Chem. 12 (2004) 963–968.
[14] N.S. Reddy, M.R. Mallireddigari, S. Cosenza, K. Gumireddy, S.C. Bell, E.P. Reddy, M.V.R. Reddy, Bioorg. Med. Chem. Lett. 14 (2004) 4093–4097.
[15] I. Encio, D.J. Morre, R. Villar, M.J. Gil, V. Martinez-Merino, Br. J. Cancer 92 (2005) 690–695.
[16] G. Laconde, P. Depreux, P. Berthelot, N. Pommery, J.P. Henichart, Eur. J. Med. Chem. 40 (2005) 167–172.
[17] J. Sławiński, M. Gdaniec, Eur. J. Med. Chem. 40 (2005) 377–389.
[18] J.M. Berry, T.D. Bradshaw, I. Fichtner, R. Ren, C.H. Schwalbe, G. Wells, E.H. Chew, M.F.G. Stevens, A.D. Westwell, J. Med. Chem. 48 (2005) 639–644.
[19] L. Buissane, S. El Kazzouli, S. Leonce, B. Pfeiffer, E.M. Rakib, M. Khouili, G. Guillaumet, Bioorg. Med. Chem. 14 (2006) 1078–1088.
[20] E. Pomarnacka, P.J. Bednarski, P. Reszka, E. Dziemidowicz-Borys, A. Bieńczak, W. Werel, R. Hałasa, Eur. J. Med. Chem. 41 (2006) 633–639.
[21] Z. Brzozowski, F. Sączewski, N. Neamati, Bioorg. Med. Chem. 14 (2006) 2985–2993.
[22] J. Sączewski, Z. Brzozowski, F. Sączewski, P.J. Bednarski, M. Liebeke, M. Gdaniec, Bioorg. Med. Chem. Lett. 16 (2006) 3663–3667.
[23] J. Sławiński, Z. Brzozowski, Eur. J. Med. Chem. 41 (2006) 1180–1189.
[24] Y.-H. Kim, K.-J. Shin, T.G. Lee, E. Kim, M.-S. Lee, S.H. Ryu, P.-G. Suh, Biochem. Pharmacol. 69 (2005) 1333–1341.
[25] R. Silvestri, G. Marfe, M. Artico, G. La Regina, A. Lavecchia, E. Novellino, M. Morgante, C. Di Stefano, G. Catalano, G. Filomeni, E. Abruzzese, M.R. Ciriolo, M.A. Russo, S. Amadori, R. Cirilli, F. La Torre, P.S. Salimei, J. Med. Chem. 49 (2006) 5840–5844.
[26] L. Hu, Z. Li, Y. Li, J. Qu, Y.-H. Ling, J. Jiang, D.W. Boykin, J. Med. Chem. 49 (2006) 6273–6282.
[27] S.M. Rubinstein, V. Baichwal, H. Beckmann, D.L. Clark, W. Frankmoelle, D. Roche, E. Santa, S. Schwender, M. Thoolen, Q. Ye, J.C. Jaen, J. Med. Chem. 44 (2001) 3599–3605.
[28] F. Sączewski, J. Sławiński, A. Kornicka, Z. Brzozowski, E. Pomarnacka, A. Innocenti, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett. 16 (2006) 4846–4851.
[29] G. De Simone, C. Pedone, A. Scozzafava, J.-L. Montero, J.-Y. Vinum, C.T. Supuran, J. Med. Chem. 49 (2006) 5544–5551.
[30] G.J. Southan, C. Szabo, M.P. O'Connor, A.L. Salman, Ch. Thiemermann, Eur. J. Pharmacol. 291 (1995) 311–318.
[31] B. Billach, D.E. Heck, D.M. Porterfield, R.P. Salchow, P.J.S. Smith, C.R. Gardnem, D.L. Laskin, J.D. Laskin, Biochem. Pharmacol. 61 (2001) 1581–1586.
[32] M.A. Mansour, A.M. Mustafa, M.N. Nagi, M.M. Khattab, O.A. Al-Shabanah, Comp. Biochem. Physiol. 132 (2002) 123–128.
[33] J.H. van den Berg, J.H. Beijnen, A.J.M. Balm, J.H.M. Schnellens, Cancer Treat. Rev. 32 (2006) 390–397.
[34] S. Matsumo, M. Myaguchi, A. Ito, N. Hara, N. Sakurada, Japan Patent No. 06 192215, 1994. Chem. Abstr. 121 (1994) 300598t.
[35] P.B. Konaru, E.J. Lien, V.I. Aramis, Pharm. Res. 10 (1993) 515–520.
[36] A.W. Tai, E.J. Lien, M.M.C. Lai, T.A. Khwaja, J. Med. Chem. 27 (1984) 236–238.
[37] A. T'ang, E.J. Lien, M.M.C. Lai, J. Med. Chem. 28 (1985) 1103–1106.
[38] Z. Brzozowski, J. Sławiński, Acta Pol. Pharm 41 (1984) 133–139.
[39] Z. Brzozowski, F. Gajewski, J. Sławiński, E. Pomarnacka, Acta Polon. Pharm. Drug Res. 50 (1993) 199–203.
[40] M.R. Boyd, Am. Assoc. Cancer Res. 30 (1989) 652–663.
[41] A.P. Monks, D.A. Scudiero, P. Skehan, K.D. Shoemaker, D. Poull, C. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Cambell, M. Mayo, M. Boyd, J. Natl. Cancer Inst. 83 (1991) 757–766.
[42] J.N. Weinstein, T.G. Myers, P.M. O'Connor, S.H. Friend, A.J. Fornance Jr., K.W. Kohn, T. Fojo, S.E. Bates, L.V. Rubinstein, N.L. Anderson, J.K. Buolamwini, W.W. van Osdol, A.P. Monks, D.A. Scudiero, E.A. Sausiville, D.W. Zaharevitz, B. Bunow, V.N. Viswanadhan, G.S. Johnson, R.E. Wittes, K.D. Paull, Science 275 (1997) 343–349.