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Synthesis, crystal structure and antiproliferative evaluation of some new substituted benzothiazoles and styrylbenzothiazoles

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Abstract

The multistep synthesis of a series of new substituted-benzothiazoles as hydrochloride or quaternary salts is described. 6-Amidino substituted 2-aminobenzothiazoles (**5**, **6**), N-methyl-2-(4-cyanostyryl)benzothiazolium iodide (**8**), cyano-substituted-2-styrylbenzothiazoles (**9-11**) and amidino and bis-amidino-substituted 2-styrylbenzothiazoles (**12-17**) were prepared. The crystal structure of amidino derivative (**6**) was determined by single crystal X-ray analysis. All new prepared compounds were tested on the cytostatic activities against malignant cell lines: (SW620, colon carcinoma; Hep2, laryngeal carcinoma; HBL, melanoma; HeLa, cervical carcinoma and WI38, human normal fibroblasts). The compounds exerted a different inhibitory effect, depended on concentration and type of the cells. The best inhibitory effect was achieved with compounds (**12-15**), with slight differences among them. All of them inhibited the growth of examined tumor cell lines and also normal fibroblasts. Other examined compounds exhibited a moderate inhibitory effect, depending on type of the cells. Majority of them inhibited the growth of HeLa cells and WI38.

Keywords: Amidinobenzothiazoles; Amidinostyrylbenzothiazoles; X-ray crystal structure analysis; Antitumor activity; Cell lines

1. Introduction

Benzothiazoles comprise a class of therapeutic compounds shown to exert a wide range of antitumor activity, specially 2-phenylsubstituted benzothiazoles. A series of potent and selective antitumor agents mostly from substituted 2-(4-aminophenyl)benzothiazoles was developed and examined, *in vitro*, their antitumor activity to ovarian, brest, lung, renal and colon carcinoma human cell lines [1-11]. Pyrimido[2,1-b]benzothiazole and benzothiazolo[2,3-b]quinazoline derivatives [12], imidazo[2,1-b]benzothiazoles [13,14], as well as, polymerised benzothiazoles [15] showed antitumor activity too.

On the other hand amidino-substituted aromatic and heteroaromatic compounds are widely investigated on their antitumor activity[16-19].

2. Results and discussion

2.1. Chemistry

In connection with our studies on the synthesis of heterocyclicaly substituted benzothiazoles [20-22], we turned our attention on the synthesis and antitumor activity of novel 2-amino- or 2-methyl-6-amidinobenzothiazoles, as well as, on amidino- or diamidino-substituted 2-(2-styryl)benzothiazoles prepared as hydrochloride or dihydrochloride salts. The purpose of the current study was to identify novel agents endowed with significant antiproliferative activity to be exploited for the development of potential anticancer drugs.

The chemical synthesis and data regarding biological activity of 17 substituted benzothiazoles, mostly as hydrochloride salts, against colon adenocarcinoma, (CaCo-2), human cervical adenocarcinoma (HeLa), human laryngeal carcinoma (Hep-2), human mammary adenocarcinoma (MCF7), and normal lung fibroblast (WI-38) are here reported and

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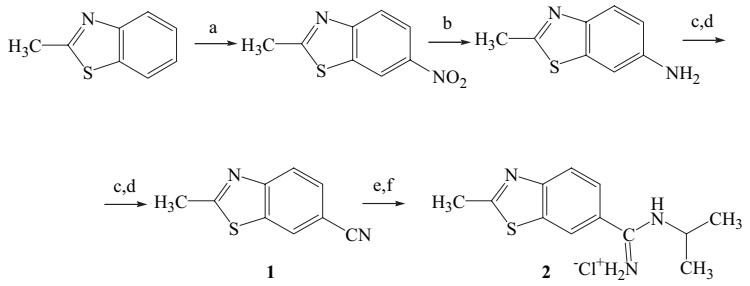
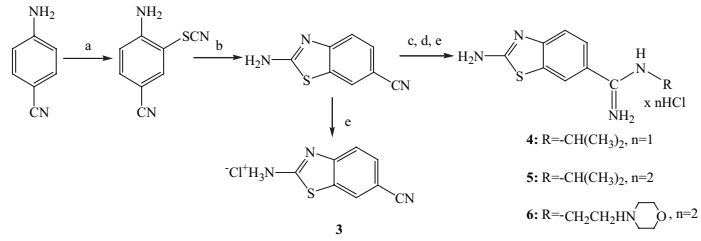


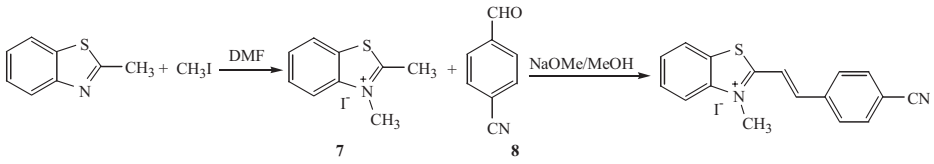
Reaction conditions: (a) HNO_3, H_2SO_4; (b) $SnCl_2x2H_2O$, HCl, MeOH, reflux, 30 minutes;
(c) $NaNO_2$, HCl, H_2O, 0°C; (d) $CuSO_4x5H_2O$, KCN, H_2O, 50°C, 30 minutes
(e) $HCl_{(g)}$, $C_2H_5OH_{(abs.)}$, 5°C; (f) $(CH_3)_2CHNH_2$, $C_2H_5OH_{(abs.)}$

Scheme 1.

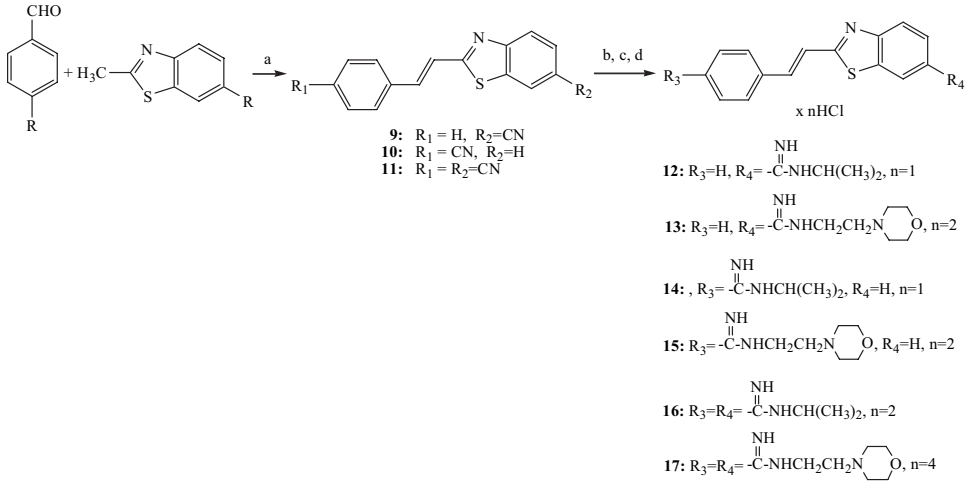


Reaction conditions: (a) $KSCN/Br_2/H_2O$; (b) HCl/H_2O; (c) $HCl_{(g)}$, $C_2H_5OH_{(abs.)}$; (d)$RNH_2/C_2H_5OH_{(abs.)}$; (e) $HCl_{(g)}$, $C_2H_5OH_{(abs.)}$

Scheme 2.



Scheme 3.



Reaction conditions: (a) NaOMe/MeOH or t-BuOK/ t-BuOH; (b) $HCl_{(g)}$, $EtOH_{(abs.)}$; (c) RNH_2, $EtOH_{(abs.)}$; (d) $HCl_{(g)}$, $EtOH_{(abs.)}$

Scheme 4.

discussed. The preparation of the target compounds is outlined in the Schemes 1-4.

All amidino compounds (**2**), (**3**), (**4-6**) and (**12-17**) were prepared in the several steps. The synthesis of the compound (**2**) started from 2-methylbenzothiazole in which was introduced the nitro substituent in the position 6 [23], reduced into the amino derivative[23-25] which was converted in the cyano compound (**1**) [26]. Cyano compound (**1**) in the Pinner

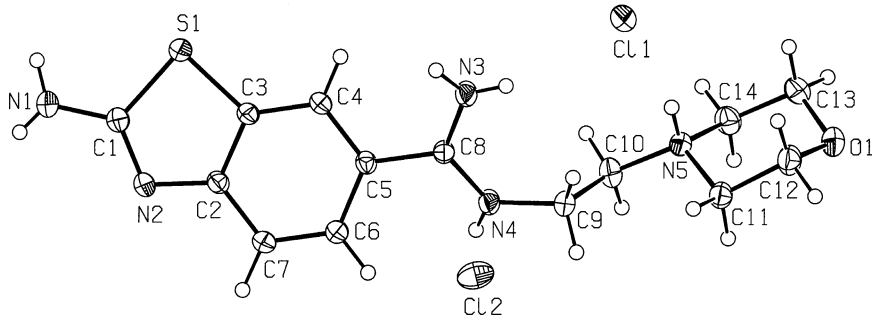


Fig. 1. Perspective view and atom labelling of the compound (**6**). Displacement ellipsoids are drawn at the 40% probability level.

reaction [27,28] was converted into the corresponding amidino compound (**2**).(Scheme 1).

Compound (**3**) was prepared on the similar way from 6-cyano-2-aminobenzothiazole [24]. Amidino compounds (**4-6**) were prepared according to the Scheme 2.

N-methyl iodide salts of the 2-methylbenzothiazole (**7**) and of 2-(4-cyanostyryl)benzothiazole (**8**) were prepared by quaternization of the corresponding benzothiazole and subsequent condensation according the Scheme 3 [29,30].

Amidino substituted styrylbenzothiazoles (**12-17**) were prepared from corresponding cyano substituted 2-methylbenzothiazole by the condensation with corresponding benzaldehyde and subsequent Pinner reaction [27]. On this way were prepared first amidino substituted 2-styrylbenzothiazoles according to the Scheme 4.

2.2. Crystal structure of (*6*)

The molecular structure of (**6**) with the atom numbering scheme is shown in Fig. 1. Selected bond lengths and bond angles are given in Table 1.

Table 1
Selected bond lengths (Å) and angles (°) for (**6**)

S1–C3	1.742(1)	C2–C7	1.407(2)
S1–C1	1.765(2)	C2–C3	1.411(2)
N1–C1	1.345(2)	C3–C4	1.381(2)
N2–C1	1.313(2)	C4–C5	1.403(2)
N2–C2	1.384(2)	C5–C6	1.412(2)
N3–C8	1.320(2)	C5–C8	1.476(2)
N4–C8	1.327(2)	C6–C7	1.388(2)
N4–C9	1.452(2)		
Bond angles			
C3–S1–C1	88.2(1)	C2–C3–S1	109.8(1)
C1–N2–C2	109.9(1)	C3–C4–C5	118.9(1)
C8–N4–C9	124.9(1)	C4–C5–C6	119.8(1)
C14–N5–C11	109.6(1)	C4–C5–C8	119.0(1)
N2–C1–N1	124.4(1)	C6–C5–C8	121.2(1)
N2–C1–S1	116.5(1)	C7–C6–C5	121.0(1)
N1–C1–S1	119.1(1)	C6–C7–C2	119.6(1)
N2–C2–C7	125.6(1)	N3–C8–N4	121.4(1)
N2–C2–C3	115.6(1)	N3–C8–C5	119.2(1)
C7–C2–C3	118.7(1)	N4–C8–C5	119.3(1)
C4–C3–C2	122.1(1)	N4–C9–C10	111.6(1)
C4–C3–S1	128.2(1)		

The structure comprises 2-amino-6-(*N*-(2-morpholin-4-yl-ethyl))amidinobenzothiazole cation and two chloride anions. The morpholine ring adopts a chair conformation, in which O1 and N5 atoms lie 0.673(1) and -0.665(1) Å from the mean plane of the other ring atoms (C11, C12, C13 and C14). The benzothiazole moiety is planar, with the largest deviation of the ring atoms from the mean plane of 0.011(1) Å for the atom C4. The five-membered heterocyclic ring is almost coplanar with its fused benzene ring; the dihedral angle between the mean planes of the rings is only 0.3(1)°.

The bond lengths and angles in the benzothiazole and carboxamidinium moiety agree very well with the equivalent ones in 6-amidinobenzothiazoles [28,31]. The bond length C5–C8 is shortened and exhibits partial double-bond character, what is a result of the π-electron interactions between amidino and benzothiazole moiety. The amidino moiety is inclined with respect to the benzothiazole moiety due to the steric interactions between the C4 and C6 hydrogen atoms and neighbouring N3 and N4 hydrogen atoms. The dihedral angle between the planes defined by the atoms N3/C8/N4 and the mean plane of the benzene ring amounts to 20.6(2)°. It's interesting to note that the value of this angle is much lower than those found in the similar structures (42.3(2), 37.2(2)° [28]; 36.9(2)° [31]). Such orientation of the amidino moiety with respect to the benzene ring could be explained by the influence of the hydrogen bonds. In the hydrogen bonding are involved all N–H donor atoms and each of chloride anions are acceptors for three hydrogen bonds (Fig. 2, Table 2). The cations and anions interconnected in the hydrogen bonding pattern like this, form three-dimensional network.

2.3. Biological activity

Antiproliferative evaluation was tested on different cell lines (SW620, colon carcinoma; Hep2, laryngeal carcinoma; HBL, melanoma; HeLa, cervical carcinoma and WI38, human normal fibroblasts). The results are summarized in Table 3 and they are expressed as IC_{50} (μM). This value represents a concentration of compound that inhibit the cell proliferation for 50 %. The compounds exterted a different inhibitory effect, depending on the concentration and type of the cells (as illustrated in Fig. 3) for compound (**12**). The best inhibitory effect was achived with the compounds (**12-15**),

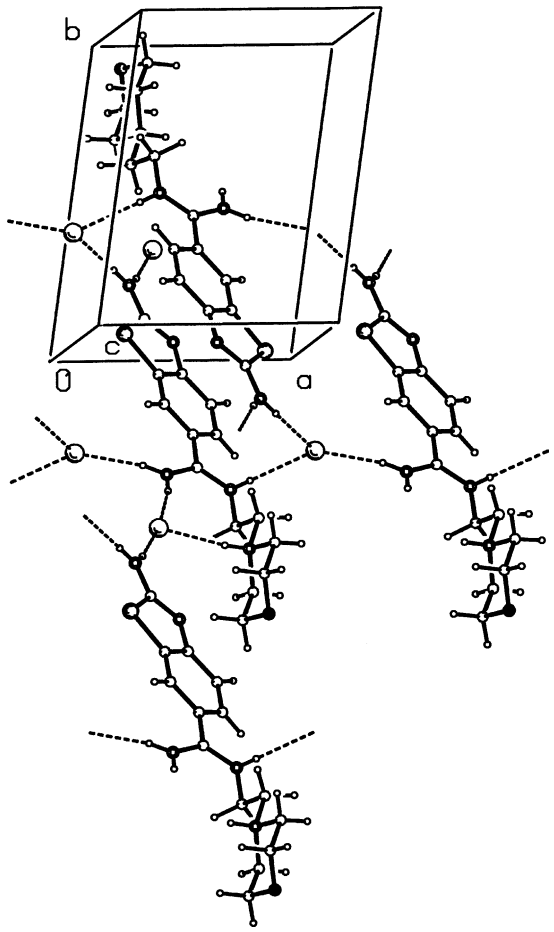


Fig. 2. Crystal packing diagram of the compound (**6**).

Table 3
Biological activity of the compounds **1-17**

Compound	IC50 (μM)				
	SW620	Hep2	HBL	HeLa	WI38
1	>1000	549	416	52.4	18.6
2	>1000	>1000	>1000	>1000	>1000
3	>1000	112	>1000	15.8	>1000
4	>1000	>1000	>1000	>1000	250
5	>1000	>1000	>1000	>1000	>1000
6	>1000	>1000	>1000	>1000	117
7	>1000	>1000	>1000	>1000	>1000
8	12.6	316	31.6	45.7	47.8
9	>1000	>1000	53.7	6.16	40.7
10	245	6.16	>1000	2.5	38.9
11	>1000	>1000	>1000	39.8	48.9
12	4.47	11.2	16.6	10.2	19.5
13	7.76	7.4	15.1	10	16.6
14	5.37	10	12.6	7.24	13.2
15	6.46	12.6	15.8	4.47	16.2
16	>1000	199	39.8	42.6	>1000
17	>1000	>1000	>1000	120	>1000

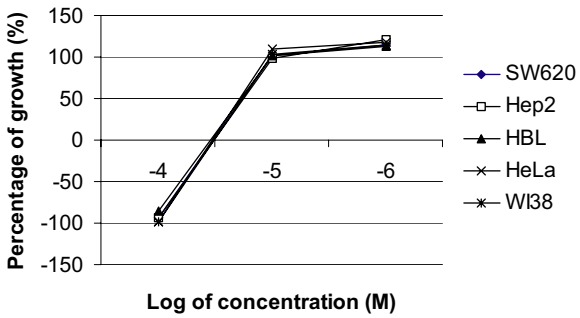


Fig. 3. The effect of compound (**12**) on the growth of tumor (SW620, Hep2, HBL, HeLa) and normal (WI38) cell lines.

with slight differences among them. The best inhibition was achived on SW620 cells (IC_{50} = 4.47 - 7.76 μM). A very good inhibition was also observed on other examined cell lines (Hep 2, IC_{50} = 7.4 - 12.6 μM; HBL, (IC_{50} = 12.6 - 16.6 μM; HeLa, IC_{50} = 4.47 - 10.2 μM).), including a normal human fibroblasts (WI38) (IC_{50} = 13.2 - 19.5 μM). Other examined compounds exibited a moderate inhibitory effect, depending on type of the cells. According results, the inhibitory effect depends also on the substituent and its positions. Amidino substituted styrylbenzothiazoles showed much better inhibitory effect, compared with amidino benzothiazoles. None of the compounds exhibited a selective inhibitory effect between tumor and normal cell lines.

3. Experimental

3.1. General

Melting points were determined on a Kofler block apparatus and are uncorrected. IR spectra were determined with a Nicolet Magna 760 infrared spectrophotometer in KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectral data (Table 5.) were determined using Brucker Avance DPX 300 MHz NMR or Varian- Gemini 300 MHz spectrometers with tetramethylsi-

Table 2
Hydrogen-bonding geometry (Å, º)

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (º)	Symmetry code
N1–H11···Cl1	0.75(2)	2.52(2)	3.264(2)	174(2)	x, +y+1, +z-1
N1–H12···Cl2	0.84(3)	2.40(3)	3.233(2)	172(3)	-x+1, -y, -z+1
N3–H31···Cl2	0.86(2)	2.28(3)	3.131(1)	169(2)	x-1, +y, +z
N3–H32···Cl1	0.77(3)	2.54(3)	3.279(1)	161(3)	
N4–H4N···Cl2	0.80(2)	2.39(3)	3.113(1)	151(2)	
N5–H5N···Cl1	0.85(3)	2.26(3)	3.092(1)	167(2)	

Table 4
Chemical and physical data of the compounds (**5-17**)

Comp.	Yield%	m.p. °C (rec.solv.)	ELEMENTAL ANALYSIS		
			Formulae	calcd. (%) C; H; N	found (%) C; H; N
5	95.0	>300	$C_{11}H_{16}Cl_2N_4S$	42.99; 5.25; 18.24	43.06; 5.28; 18.20
6	70.0	279-281	$C_{14}H_{21}Cl_2N_5OS$	44.44; 5.59; 18.52	44.51; 5.45; 18.46
8	45.0	234-236	$C_{17}H_{13}IN_2S$	50.50; 3.24; 6.93	50.24; 3.51;6.98
9	40.4	195-197	$C_{16}H_{10}N_2S$	73.25; 3.84; 10.68	73.29; 3.96; 10.82
10	38.5	189-192	$C_{16}H_{10}N_2S$	73.25; 3.84; 10.68	73.18; 3.92; 10.89
11	39.35	280-283	$C_{17}H_9N_3S$	71.05; 3.16; 14.63	71.20; 3.19; 14.55
12	60.3	285-288	$C_{19}H_{20}ClN_3S$	63.76; 5.63; 11.74	63.79; 5.67; 11.77
13	50.8	264-266	$C_{22}H_{26}Cl_2N_4OS$	56.77; 5.63; 12.04	56.67; 5.88; 12.14
14	46.5	>300	$C_{19}H_{20}ClN_3S$	63.76; 5.63; 11.74	64.04; 5.45; 11.69
15	32.4	270-273	$C_{22}H_{26}Cl_2N_4OS$	56.77; 5.63; 12.04	56.89; 5.80; 12.05
16	59.1	>300	$C_{23}H_{29}Cl_2N_5S$	57.73; 6.11; 14.64	57.69; 6.38; 14.38
17	44.66	258-260	$C_{29}H_{41}Cl_4N_7O_2S$	50.22; 5.96; 14.14	50.51; 6.01; 14.26

lane as an internal standard. Elemental analyses (Table 4 .) were carried out in the Microanalitical laboratory at the "Rugjer Boskovic" Institute.

*3.2. General procedure for preparation of compounds (**1**) and (**2**)*

2-Methylbenzothiazole (23.5 g, 0.157 mol) was dissolved in sulphuric acid (36 ml, d 1.84) below 5°C by portiowise addition and with vigorous stirring. Nitric acid (19 ml, d=1.5) was added dropwise so that temperature was maintained at 20°C. Reaction mixture was stirred over night. The mixture was than poured on ice (250 g) with stirring, and aqueous ammonia (d=0.88) added until the solids became slightly orange (pH=10). The solids were filtered, washed with water and dried. The crude product was recrystallized from ethanol (550 ml) and 17.75 g of 2-methyl-6-nitrobenzothiazole was obtained (58% yield), (m.p. 164-166°C; literature [23] m.p.165°C).

6-amino-2-methylbenzothiazole was prepared by modified method of reduction [23]. Nitro derivative (8 g, 0.041 mol) was refluxed with $SnCl_2{\cdot}2H_2O$ (76.2 g, 0.338 mol) in the mixture of concentrated HCl (112 ml) and methanol (112 ml) for 30 minutes. Methanol was evaporated and the residue poured on ice. The 20% solution of NaOH was added to the cold mixture (until pH=10). The crude product was filtered off and recrystallized from ethanol. The amino compound was obtained in the yield of 5.17g (76%) (m.p. 120-123°C; literature [23] m.p. 122°C). The ^{1}H- and ^{13}C-NMR spectra of nitro- and amino-compounds are in accordance with literature data [24,25].

6-Cyano-2-methylbenzothiazole (**1**) was prepared by modified procedure [26]. The amino-derivative (5 g, 0.03 mol) was dissolved in a mixture of concentrated HCl (10 ml) and water (25 ml), and diazotised with the solution of $NaNO_2$ (2.5 g, 0.036 mol) in water (15 ml) at 0°C. The clear diazo-solution was added portionwise to the solution of $CuSO_4{\cdot}5H_2O$ (9.25 g, 0.0367 mol) in water (63 ml) and KCN (10.9 g, 0.168 mol) at 50°C. Reaction was kept at 50°C for 30 minutes and then a solution of KCN (4.2 g, 0.064 mol) in water (50 ml) was added to the reaction mixture. The reaction was cooled and extracted with diethylether (5x100 ml). Ether was evaporated to dryness and the crude product purified with filtration through an alumina column (3.5 cm x 10 cm) with toluene as solvent. Toluene was evaporated to dryness and the residue recrystallized from ethanol. It was obtained 3.15g of compound (**1**) (59.5%) (m.p. 142-144°C; literature [26] m.p. 144°C).

6-(*N*-isopropyl)amidino-2-methylbenzothiazole hydrochloride (**2**) was prepared from the corresponding nitrile by modified Pinner reaction [27,28]. A suspension of compound (**1**) (1 g, 0.0057 mol) in abs. ethanol (125 ml) was cooled to 0°C and saturated with dry HCl gas. After returning to room temperature the flask was stoppered, and the content was stirred until IR indicated the disappearance of the nitrile group (seven days). The imido ester hydrochloride intermediate was then precipitated from the solution by addition of dry diethylether, filtered off, washed with dry ether, and dried under reduced pressure over KOH. Isopropylamine (2.5 ml, 0.028 mol) was added to the suspension of the crude product (1.44 g, 0,0056 mol) in dry ethanol (50 ml) under nitrogen atmosphere. The reaction mixture was stoppered, the content stirred at room temperature for 7 days and evaporated to dryness.

The crude product was recrystallized from mixture of water (1 ml) and acetone (10 ml). It was obtained 1.05 g of compound (**2**) (69%) (m.p. 198-201°C; literature [28] m.p. 198-201°C).

*3.3. General procedure for preparation of compounds (**3-6**)*

A solution of *p*-aminobenzonitrile (9 g, 0.085 mol) in 95% acetic acid (50 ml) was added to a solution of KSCN (30 g, 0.308 mol) in 95% acetic acid (100 ml). The mixture was cooled to 0°C, and a solution of Br_2 (7.5 ml) in acetic acid (30 ml) was added slowly with vigorous stirring so that the temperature remained between 0 and 10°C. After addition was complete, the stirring was continued for 1h at 5°C and

Table 5
Spectroscopic data of the compounds (**5-17**)

Com.	IR (KBr)/ (cm^{-1})	^{1}H-NMR (DMSO-d_6, TMS), δ (ppm)	^{13}C-NMR (DMSO-d_6, TMS), δ (ppm)
5[*]	1600, 1650 (C=N)	9.65 (s, 1H, amidino-NH,), 9.49 (s, 1H, amidino-NH), 9.23 (s, 1H, amidino-NH), 8.25 (s, 1H, H_7), 7.71 (d, 1H, J=8.4 Hz, H_5), 7.61 (d, 1H, J=8.4 Hz, H_4), 5.34 (s, 3H, amino-NH_3^+), 4.18-4.07 (m, 1H, -CH), 1.27 (d, 6H, J=6.3 Hz, -CH_3)	169.8(s), 161.3(s), 147.7(s), 127.3(s), 127.1(s), 123.35(s), 122.97(s), 115.1(s), 45.15(s), 21.35(s)
6[#]	1615, 1675 (C=N)	11.50 (s, 1H, morpholino-NH^+,), 9.90 (s, 1H, amidino-NH), 9.65 (s, 1H, amidino-NH), 9.37 (s, 1H, amidino-NH), 8.55 (s, 2H, -NH_2), 8.29 (s, 1H, H_7), 7.75 (d, 1H, J=8.4 Hz, H_5), 7.5 (d, 1H, J=8.4 Hz, H_4), 4.00-3.17 (m, 12H, -CH_2-)	169.7(s), 162.75(s), 149.8(s), 127.9(s), 127.1(s), 122.7(s), 122.1(s), 115.4(s), 63.0(s), 53.5(s), 50.99(s), 37.05(s)
8[*]	1610(C=C)	8.49 (d, 1H, J=8.1 Hz, H_7), 8.31 (d, 1H, J=8.1 Hz, H_4), 8.26-8.193 (m, 4H, -CH=CH-, H-arom.), 8.06 (d, 2H, J=8.4 Hz, H-arom.), 7.95-7.82 (m, 2H, H_5, H_6), 4.41 (s, 3H, -CH_3)	171.8(s), 146.1(s), 142.6(s), 138.7(s), 133.4(s), 130.5(s), 130.2(s), 129.3(s), 128.8(s), 124.9(s), 118.9(s), 117.8(s), 117.7(s), 113.98(s), 37.3(s)
9[#]	2210(C≡N), 1600(C=C)	8.64 (s, 1H, H_7.), 8.04 (d, 1H, J=8.4 Hz, H_4), 7.84 (d, 1H, J=8.4 Hz, H_5), 7.75-7.72 (m, 3H, H-arom., -CH=CH-), 7.62 (d, 1H, J=16.2 Hz, -CH=CH-), 7.40-7.33 (m, 3H, H-arom.)	170.94(s), 155.83(s), 139.3(s), 134.83(s), 134.7(s), 129.9(s), 129.7(s), 128.9(s), 127.95(s), 127.4(s) 123.2(s), 121.1(s), 118.8(s), 107.25(s)
10[#]	2200(C≡N), 1600(C=C)	8.06 (d, 1H, J=7.8 Hz, H_7), 7.94 (d, 1H, J=7.8 Hz, H_4), 7.92 (d, 2H, J=8.4 Hz, H-arom.), 7.83 (d, 2H, J=8.4 Hz, H-arom.) 7.76 (d, 1H, J=16.2 Hz, -CH=CH-), 7.68 (d, 1H, J=16.2 Hz, -CH=CH-), 7.49-7.39 (m, 2H, H_5, H_6).	165.6(s), 153.3(s), 139.8(s), 135.2(s), 134.8(s), 132.6(s), 128.2(s), 126.6(s), 125.7(s), 125.1(s), 122.7(s), 122.2(s) 118.65(s), 109.5(s)
11[*]	2205(C≡N), 1620(C=C)	8.70 (s, 1H, H_7), 8.11 (d, 1H, J=8.7 Hz, H_5), 7.96 (d, 1H, J=8.4 Hz, H_4), 7.89-7.83 (m, 6H, H-arom., -CH=CH-)	170.7 (s), 156.25(s), 139.9 (s) 137.6(s), 135.5(s), 133.2(s), 130.3(s), 129.1(s), 128.1(s), 124.95(s), 123.99(s), 119.2(s), 119.1(s), 112.2(s), 108.2(s),
12[*]	1675(C=N), 1620(C=C)	9.78 (s, 1H, amidino-NH), 9.71 (s, 1H, amidino-NH), 9.35 (s, 1H, amidino-NH), 8.58 (s, 1H, H_7), 8.14 (d, 1H, J=8.4 Hz, H_4), 7.85-7.78 (m, 4H, H-arom. and -CH=CH-), 7.70 (d, 1H, J=16.2 Hz, -CH=CH-), 7.5-7.38 (m, 3H, H-arom.), 4.2-4.13 (m, 1H, -CH), 1.30 (6H, d, J=6.3 Hz, -CH_3)	170.2(s), 161.3(s), 156.0(s), 139.05(s), 134.9(s), 134.0(s), 129.7(s), 128.9(s), 127.9(s), 126.5(s), 125.8(s), 123.2(s), 122.2(s), 121.3(s), 45.15(s), 21.2(s)
13[#]	1670(C=N), 1618(C=C)	11.57 (s, 1H, morpholino-NH^+), 10.18 (s, 1H, amidino-NH), 9.89 (s, 1H, amidino-NH), 9.68 (s, 1H, amidino-NH), 8.68 (s, 1H, H_7), 8.09 (d, 1H, J=8.4 Hz, H_4), 7.92 (d, 1H, J=8.4 Hz, H_5), 7.76-7.74 (m, 3H, H-arom., -CH=CH-), 7.65 (d, 1H, J=16.2 Hz, -CH=CH-), 7.42-7.36 (m, 3H, H-arom.), 3.95-3.78 (m, 6H, -CH_2), 3.53-3.13 (m, 6H, -CH_2)	170.4(s), 163.0(s), 156.3(s), 139.2(s), 134.9(s), 134.1(s), 129.95(s), 128.9(s), 127.97(s), 126.7 (s), 125.35(s), 123.5(s), 122.35(s), 121.35 (s), 63.1(s), 53.6(s), 51.1(s), 37.3(s)
14[#]	1680(C=N), 1615(C=C)	9.60 (s, 1H, amidino-NH), 9.47 (s, 1H, amidino-NH), 9.18 (s, 1H, amidino-NH), 8.07 (d, 1H, J=7.8 Hz, H_7), 7.95 (d, 3H, J=8.4 Hz, 2H-arom., H_4), 7.79 (d, 1H, J=16.2 Hz, -CH=CH-), 7.74 (d, 2H, J=8.4 Hz, H-arom.), 7.71 (d, 1H, J=16.2 Hz, -CH=CH-), 7.49-7.39 (m, 2H, H_5, H_6), 4.08-4.03 (m, 1H, -CH), 1.22 (d, 6H, J=6.4 Hz, -CH_3)	165.7(s), 161.2(s), 153.4(s), 139.7(s), 135.5(s), 134.2(s), 129.3(s), 128.9(s), 127.6(s), 126.6(s), 125.7(s), 124.5(s), 122.7(s), 122.25(s), 45.1(s), 21.2(s)
15[#]	1675(C=N), 1610(C=C)	11.55 (s, 1H, morpholino-NH^+), 10.09 (s, 1H, amidino-NH), 9.79 (s, 1H, amidino-NH), 9.56 (s, 1H, amidino-NH), 8.07 (d, 1H, J=7.8 Hz, H_7), 8.98-7.88 (m, 5H, H-arom., H_4), 7.79 (d, 1H, J=16.2 Hz, -CH=CH-), 7.70 (d, 1H, J=16.2 Hz, -CH=CH-), 7.49-7.39 (m, 2H, H_5, H_6), 3.94-3.80 (m, 6H, -CH_2), 3.52-3.13 (m, 6H, -CH_2)	165.7(s), 162.7(s), 153.4(s), 140.0(s), 135.45(s), 134.2(s), 129.0 (s), 128.7(s), 127.65(s), 126.6(s), 125.7(s), 124.65(s), 122.7(s), 122.25(s), 63.1(s), 53.5(s), 51.1(s), 37.1(s)
16[*]	1670(C=N), 1610(C=C)	9.8-9.2 (s, 6H, amidino-NH), 8.55 (s, 1H, H_7), 8.10 (d, 1H, J=7.8 Hz, H_4), 7.97 (d, 2H, J=8.4 Hz, H-arom.), 7.84-7.77 (m, 5H, H-arom., H_5, -CH=CH-), 4.14-4.07 (m, 2H, -CH), 1.24 (d, 6H, J=6.4 Hz, -CH_3), 1.23 (d, 6H, J=6.4 Hz, -CH_3)	169.6(s), 161.3 (s), 161.1(s), 155.9(s), 139.4(s), 137.1(s), 134.2(s), 129.6(s), 128.9(s), 127.8(s), 126.6(s), 126.1(s), 123.99(s), 123.4(s), 122.5(s), 45.2(s), 45.1(s), 21.15(s), 21.2(s)
17[*]	1675(C=N), 1615(C=C)	11.59 (s, 2H, morpholino-NH^+), 10.26 (s, 1H, amidino-NH), 10.22 (s, 1H, amidino-NH), 9.97 (s, 1H, amidino-NH), 9.92 (s, 1H, amidino-NH), 9.50 (s, 1H, amidino -NH), 9.69 (s, 1H, amidino-NH,), 8.78 (s, 1H, H_7), 8.19 (d, 1H, J= 8.4 Hz, H_4), 8.07-7.92 (m, 7H, H-arom., H_5, -CH=CH-), 3.99-3.89 (m, 12H, -CH_2-), 3.57-3.30 (m, 12H, -CH_2-)	169.7(s), 162.8(s), 162.5(s), 156.1(s), 139.6(s), 137.2(s), 134.2(s), 129.1(s), 128.99(s), 127.9(s), 126.8(s), 125.55(s), 124.1(s), 123.6(s), 122.5(s), 63.0(s), 53.5(s), 51.0(s), 37.1(s)

[*]At 300.13 MHz; [#]At 600.13 MHz

then the mixture was poured into 1 l of water. The solid was collected and recrystallized from ethanol to yield 42.3% of 4-cyano-2-thiocyanatoaniline. The product (6.3 g, 0.036 mol), concentrated HCl (27 ml) and water (54 ml) were refluxed for 2 h. The solution was cooled, and the product was filtered off, washed with water, and recrystallized from ethanol to yield 5.89 g (39,7%) of 2-amino-6-cyanobenzothiazole (m.p. 216-218°C; literature[24] m.p. 217-218°C). The ^{1}H- and ^{13}C-NMR spectra are in accordance with literature data [24].

2-Amino-6-cyanobenzothiazole hydrochloride salt (**3**) was prepared from a suspension of 2-amino-6-cyanobenzothiazole (0,25g) in dry toluene (25 ml). Suspension was cooled to 0°C and saturated with dry HCl gas. After 5h white solid precipitate and was collected by vacuum filtration and washed with ethanol. It was obtained 0,288 g of compound (**3**) (95,4%).

Amidino derivative (**4**), (**5**) and (**6**) were prepared from the corresponding nitrile by modified Pinner reaction [27,28]. A suspension of 2-amino-6-cyanobenzothiazole (1 g, 0.0057 mol) in abs. ethanol (125 ml) was cooled to 0°C and saturated with dry HCl gas. After returning to room temperature the flask was stoppered, and the content was stirred until IR indicated the disappearance of the nitrile group (3 days). The imido ester hydrochloride intermediate was then precipitated from the solution by addition of dry diethylether, filtered off, washed with dry ether, and dried under reduced pressure over KOH. Corresponding amine (5 ml, 0.058 mol) was added to the suspension of the crude product (1.52 g, 0,0055 mol) in dry ethanol (50 ml) under nitrogen atmosphere. The reaction mixture was stoppered, the content stirred at room temperature and after two days filtered off and washed with dry ether. The crude product was recrystallized from mixture of water (1 ml) and acetone (10 ml). The corresponding amidine was filtered off and dried. Compound (**4**) was obtained in the yield of 1.35g (91.4%) (m.p.>300°C; literature [28] m.p.>300°C).

Dry amidine was suspended in abs. ethanol, cooled to 0°C and saturated with dry HCl gas. The pure compounds (**5**) (1.455g, 95%) and (**6**) (1.468g, 70.0%) were obtained by filtration.

*3.4. General procedure for preparation of compounds (**7**) and (**8**)*

A solution of 2-methylbenzothiazole (3.35g, 2.245×10^{-2} mol) and iodomethane (12.5g, 8.81×10^{-2} mol) in DMF (10 ml) was heated under reflux for 24 hours. After cooling desired quaternary salt (**7**) was collected by filtration under reduced pressure and recrystallized from ethanol [29]. The yield is 5.9g (90.2%).

Quaternary salt 7 (2g, 6.87×10^{-3} mol) was added in a solution of sodium metoxide (0.37g, 6.87×10^{-3}mol) in methanol. The mixture was stirred for 10 minutes and then 4-cyanobenzaldehyde (0.9g, 6.87×10^{-3} mol) was added. Stirred mixture become red and yellow-orange solid precipitated. The product (**8**) was recrystallized from methanol and dried [30]. The yield is 1.25g (45%).

*3.5. General procedure for preparation of compounds (**9-17**)*

A solution of the 2-methylbenzothiazole (1g, 6.7×10^{-3} mol) or 6-cyano-2-methylbenzothiazole (1g, 5.74×10^{-3} mol) and appropriate base (potassium t-butoxide (0.752g, 6.7×10^{-3} mol) or sodium metoxide (0.61g, 5.74×10^{-3} mol)) in abs. alcohol (t-buthanol (80 ml) or methanol (100 ml)) was stirred for 30 minutes and then appropriate aldehyde was added (4-cyanobenzaldehyde or benzaldehyde) in eqvimolar amounts. The solution was then left at room temperature for 24h and the reaction mixture were collected by vacuum filtration [30]. Compounds (**9**) (0.711g, 40.4%), (**10**) (0.58g, 38.5%), (**11**) (0.65g, 39.3%) (m.p. 280-283°C; literature [26] 281-283°C) were obtained by purification through column chromatography on silica gel.

Amidino derivative (**12-17**) were prepared from the corresponding nitrile by modified Pinner reaction [28]. A suspension of compounds (**9-11**) (0.5 g,) in abs. ethanol (125 ml) was cooled to 0°C and saturated with dry HCl gas. After returning to room temperature the flask was stoppered, and the content was stirred until IR indicated the disappearance of the nitrile group (3 days). The imido ester hydrochloride intermediate was then precipitated from the solution by addition of dry diethylether, filtered off, washed with dry ether, and dried under reduced pressure over KOH. Corresponding amine (5 ml) was added to the suspension of the crude product in dry ethanol (50 ml) under nitrogen atmosphere. The reaction mixture was stoppered, the content stirred at room temperature and after 44 days filtered off and washed with dry ether. Crude product was suspended in abs. ethanol, cooled to 0°C and saturated with dry HCl gas. The pure compounds (**12**) (0.324g, 60.3%), (**13**) (0.39g, 50.8%), (**14**) (0.244g, 46.5%), (**15**) (0.221g, 32.4%), (**16**) (0.25g, 59.1%), (**17**) (0.28g, 44.66%) were obtained by filtration.

3.6. X-ray crystal structure analysis

The single crystal suitable for X-ray crystal structure analysis was obtained by growth under slow evaporation at room temperature from a water/acetone mixture (1:10 *v/v*).

The intensities were collected at 293 K on a Oxford Diffraction Xcalibur 2 diffractometer with graphite-monochromated MoK_α radiation (λ =0.71073 Å). The data collection and reduction were carried out with the CrysAlis programs [32]. The intensities were corrected for Lorentz and polarization effects. The crystal structure was solved by direct methods. All nonhydrogen atoms were refined anisotropically by full-matrix least-squares calculations based on F^2. Hydrogen atoms were located in a difference Fourier map and their coordinates and isotropic thermal parameters were refined freely. Programs used for structure solution, refinement, analysis and drawings include *SIR*92 [33], *SHELXL*97 [34], *PARST*95 [35], and *PLATON* [36]. Crystal data, data collection and refinement parameters are summarized in Table 6.

Crystallographic data excluding structure factors for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-224459. Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Table 6
Crystal data and summary of data collection and refinement for (**6**)

Empirical formula	$C_{14}H_{21}Cl_2N_5OS$
Formula weight	378.32
Temperature [K]	293(2)
Wavelength [Å]	0.71073
Crystal size [mm]	0.75x0.65x0.45
Crystal colour	colourless
Crystal system	triclinic
Space group	$P\ \bar{1}$
a [Å]	7.9412(9)
b [Å]	10.4470(10)
c [Å]	10.8730(10)
α [°]	81.183(8)
β [°]	85.433(9)
γ [°]	81.890(9)
V [Å^3]	880.92(15)
Z	2
$D_{calc.}$ [gcm^{-3}]	1.426
μ [mm^{-1}]	0.498
F(000)	396
scan-mode	ω
θ range for data collection [°]	4.56 - 32.90
Index ranges	$-11 \le h \le 11$
	$-15 \le k \le 15$
	$-16 \le l \le 16$
Collected reflections	17932
Independent reflections / $R_{int.}$	5347 / 0.0286
Data / restrains / parameters	5347 / 0 / 292
Weighting parameters $a;b$[a]	0.0855; 0.3155
Goodness-of-fit on F^2	1.073
R [$I \ge 2\sigma(I)$] / R [all data]	0.0485 / 0.0536
wR [$I \ge 2\sigma(I)$] / wR [all data]	0.1331 / 0.1403
Max. / min. electron density [eÅ^{-3}]	0.835 / -0.343

[a] $w = 1/[\sigma^2(F_o^2) + (aP)^2 + bP]$, where $P = (F_o^2 + 2F_c^2)/3$

3.7. Biological assays

Biological evaluation of the compounds was preformed to test a potential antitumor activity. In this study, the influence of a different concentration of each compound on proliferation of tumor and normal cell lines was examined. The cells (SW620, colon carcinoma; Hep2, laryngeal carcinoma; HBL, melanoma; HeLa, cervical carcinoma and WI38, human normal fibroblasts) were seeded in 96-well plates at a concentration of 3x10^4/ml in D-MEM, supplemented with 10% FBS and glutamine (2mM) and grown in humified atmosphere with 5% CO_2. A 24 hrs later, the test compounds were added in final concentration of 10^{-6}, 10^{-5} and 10^{-4} M. After 72 hrs of treatmant, the number of the cells was determined by MTT test [37]. The compounds (**1-7**) were desolved in dH_2O as 10^{-2} M solution and diluted with a medium. Control cells were grown in D-MEM without any addition. The compounds (**8-12**) were desolved in DMSO as 10^{-1} M solution and diluted with a medium to appropriate concentration. A final concentration of DMSO was less than 0.1% and at that concentration it does not influence on cell growth. The results are expressed as IC_{50}, a concentration necessary for 50% of inhibition. Each result is a mean value from three separate experiments, performed in triplicate.

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