

Full Paper

Synthesis and Antitumor Evaluation of Novel Derivatives of 6-Amino-2-phenylbenzothiazoles

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Abstract: Novel derivatives of 6-amino-2-phenylbenzothiazole bearing different substituents (amino, dimethylamino or fluoro) on the phenyl ring were prepared as the corresponding hydrochloride salts. 6-Nitro-2-(substituted-phenyl)benzothiazoles (**1-6**) were synthesized by condensation reactions of substituted benzaldehydes with 2-amino-5-nitrothiophenol. Nitro derivatives were reduced to the amino derivatives with SnCl₂/HCl. Water soluble hydrochloride salts of 6-amino-2-(substituted-phenyl)benzothiazole (**13-19**) were prepared using concentrated or gaseous HCl. Compounds **13-19** were found to exert cytostatic activities against malignant human cell lines: cervical (HeLa), breast (MCF-7), colon (CaCo-2), laryngeal carcinoma (Hep-2), and normal human fibroblast cell lines (WI-38).

Keywords: Benzothiazole synthesis, antitumor activity.

Introduction

The study of benzothiazole derivatives is of considerable current interest as a result of their important biological and biophysical properties. 2-Aryl or 2-heteroaryl substituted benzothiazoles are studied as antitumor [1], antimicrobial [2], antifungal [3] agents, as well as imaging agents for β-

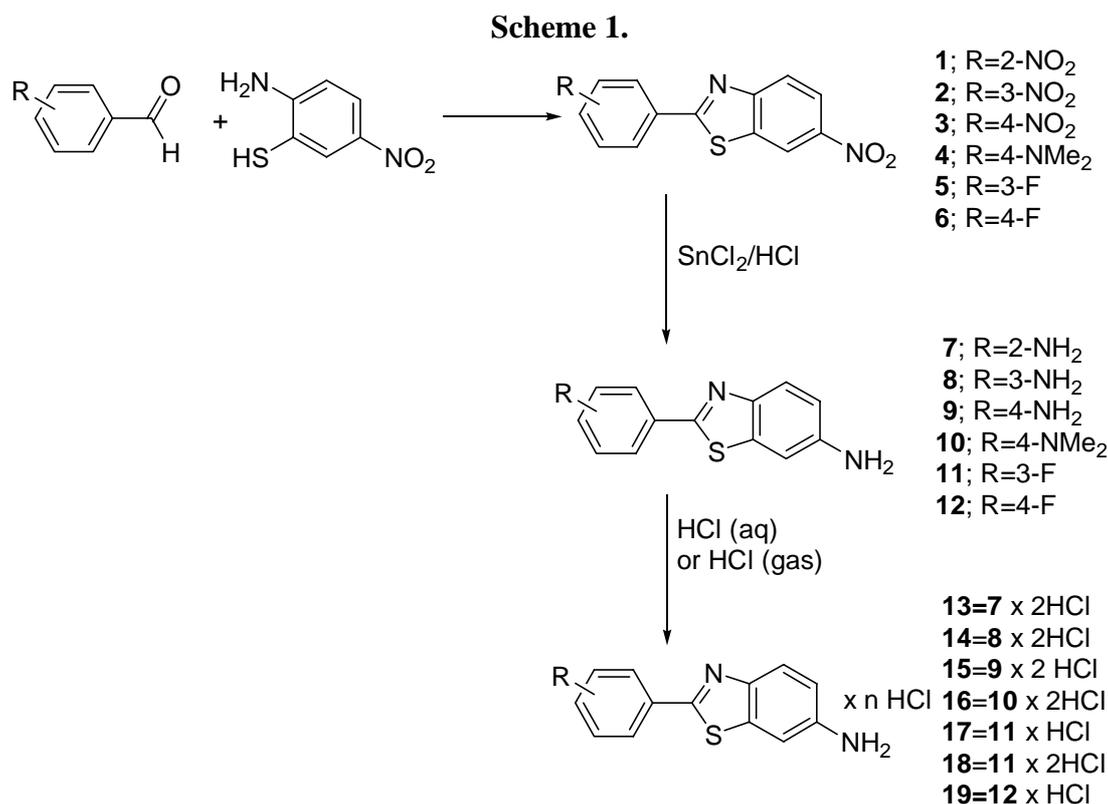
amyloid [4]. A series of potent and selective antitumor agents derived from 2-(4-aminophenyl)benzothiazole was extensively examined and developed during recent years and the fluorinated analogue, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole is a novel agent with potent and selective antitumor properties which in the form of its L-lysylamide prodrug, *Phortress*, is in early phase clinical studies [5].

In connection with our studies on the synthesis and biological properties of substituted benzothiazoles [6-8] we turned our attention to the synthesis and antitumor evaluation of novel 2-(substituted-phenyl)-6-aminobenzothiazole as analogous of 2-(4-aminophenyl)benzothiazole prepared as hydrochloride or dihydrochloride salts.

Results and Discussion

Synthesis

An efficient synthesis of 2-phenylbenzothiazole derivatives was carried out by the reactions outlined in Scheme 1.



Starting from 2-amino-5-nitrothiophenol (freshly prepared by basic hydrolysis of 6-nitrobenzothiazole) and the corresponding aromatic aldehyde 6-nitro-2-(monosubstituted phenyl)-benzothiazoles **1-6** were obtained in moderate yield (40-70%) by condensation in pyridine (procedure **A**). The reduction of nitro compounds was accomplished with SnCl₂ in methanol-HCl mixtures in a short time at reflux giving the desired 6-amino-2-phenylbenzothiazole derivatives **7-12** in good yields of 70-80% (procedure **B**). Treating the solutions of amines **10-11** or diamines **7-9**, dissolved in a mixture of abs. ethanol and xylene (1:1, v/v) with concd. hydrochloric acid (procedure **C**) the corresponding amines were protonated and isolated as pure hydrochlorides **13-17**, **19** in excellent

yields (91-99%) without recrystallization. The same products can be obtained in similar yields by treating the amines with gaseous HCl (procedure **D**). Only for 6-amino-2-(3-fluorophenyl)-benzothiazole (**11**) was selective preparation of the monohydrochloride salt **17** (procedure **C**) and the dihydrochloride salt **18** (procedure **D**), in which protonation occurs on both the amino and benzothiazole nitrogens possible. The structures of new compounds **1-19** were confirmed by elemental analysis and spectral data (IR, NMR). Due to a very low solubility of nitro compounds **2** and **3**, only elemental analysis, and IR spectra were recorded.

Biological Activity

The effects of the seven water soluble compounds **13-19** were tested on the human malignant cell lines: cervical (HeLa), breast (MCF-7), colon (CaCo-2), laryngeal carcinoma (Hep-2) and normal human fibroblast cell lines (WI-38). Inhibitory effects are shown in Table 1.

Table 1.

Comp. No.	IC ₅₀ (M)				
	HeLa	CaCo-2	HEP-2	MCF7	WI-38
13	6×10 ⁻⁴	4×10 ⁻³	9×10 ⁻⁵	6×10 ⁻⁴	4×10 ⁻³
14	3×10 ⁻³	8×10 ⁻⁴	4×10 ⁻⁵	7×10 ⁻⁵	4×10 ⁻⁵
15	9×10 ⁻⁵	1×10 ⁻⁴	2×10 ⁻⁵	6×10 ⁻⁵	7×10 ⁻³
16	4×10 ⁻³	8×10 ⁻⁴	5×10 ⁻⁵	2×10 ⁻⁴	7×10 ⁻⁵
17	8×10 ⁻⁵	9×10 ⁻⁵	2×10 ⁻⁵	4×10 ⁻⁵	4×10 ⁻⁴
18	9×10 ⁻⁵	1×10 ⁻⁴	3×10 ⁻⁵	3×10 ⁻⁵	7×10 ⁻⁵
19	5×10 ⁻⁵	3×10 ⁻⁴	9×10 ⁻⁶	4×10 ⁻⁵	8×10 ⁻⁵

All benzothiazoles tested exhibited moderate antitumour activity with IC₅₀ values ranging from 9×10⁻⁶ to 4×10⁻³ M. Most sensitive cell lines were HEP-2 and MCF-7. Our results showed up that activity of the compounds investigated decreasing from *ortho*- *meta*- to *para*- position ($p < 0.05$) the fluoro substituted benzothiazoles **17**, **18** and **19** being more active.

Experimental

General

Melting points were determined on a Kofler hot stage microscope and are uncorrected. IR spectra were recorded on a Nicolet Magna 760 spectrophotometer in KBr discs. ¹H- and ¹³C-NMR spectra were recorded on either a Varian Gemini 300 or a Bruker Avance DPX 300 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, and coupling constants (*J*) are given in hertz (Hz). Elemental analyses were carried out in the Microanalytical laboratory at the Rugjer Boskovic Institute. All compounds were routinely checked by

TLC with Merck silica gel 60F-254 glass plates. 2-Amino-5-nitrothiophenole was prepared according to the procedure described for preparation of 2-amino-5-cyanothiophenole [9] from 6-nitrobenzothiazole.

Synthesis of 6-nitro-2-(substituted-phenyl)benzothiazoles 1-6

Procedure A: To a boiling solution of the appropriate substituted benzaldehyde (0.02 mol) in pyridine (20 mL), a solution of 2-amino-5-nitrothiophenole (0.02 mol) in pyridine (20 mL) was added dropwise, and the stirred reaction mixture was refluxed for 20 h. The mixture was then poured into 300 ml of 2 M hydrochloric acid and after cooling overnight the obtained crystalline product was filtered off and crystallized from appropriate solvent. Using this procedure the following compounds were prepared.

6-Nitro-2-(2-nitrophenyl)benzothiazole (1). Yield: 40.0% after crystallisation from xylene; mp 164-165 °C; IR cm^{-1} : 1569, 1517, 1348, 842, 756, 749, 721; $^1\text{H-NMR}$ (DMSO- d_6) δ : 9.33 (d, 1H, $J = 2.0$ Hz), 8.40 (dd, 1H, $J = 2.0$ Hz, $J = 8.9$ Hz), 8.26 (d, 1H, $J = 8.9$ Hz), 8.16 (d, 1H, $J = 8.3$ Hz), 8.06 (d, 1H, $J = 8.1$ Hz), 7.96-7.87 (m, 2H); Anal. Calcd. for $\text{C}_{13}\text{H}_7\text{N}_3\text{O}_4\text{S}$: C, 51.83; H, 2.34; N, 13.95; Found: C, 51.68; H, 2.40; N, 14.03.

6-Nitro-2-(3-nitrophenyl)benzothiazole (2). Yield: 70.9% (from xylene); mp 214-215 °C; IR cm^{-1} : 1601, 1538, 1514, 1340, 829, 752, 739, 720; Anal. Calcd. for $\text{C}_{13}\text{H}_7\text{N}_3\text{O}_4\text{S}$: C, 51.83; H, 2.34; N, 13.95; Found: C, 51.99; H, 2.11; N, 13.90.

6-Nitro-2-(4-nitrophenyl)benzothiazole (3) Yield: 67.1% (from xylene); mp 256-257 °C; IR cm^{-1} : 1606, 1517, 1346, 854, 754, 719; Anal. Calcd. for $\text{C}_{13}\text{H}_7\text{N}_3\text{O}_4\text{S}$: C, 51.83; H, 2.34; N, 13.95; Found: C, 51.92; H, 2.29; N, 13.88.

6-Nitro-2-(4-*N,N*-dimethylaminophenyl)benzothiazole (4). Yield: 66.1% (from xylene); mp 262-263 °C; IR cm^{-1} : 1610, 1594, 1474, 1434, 1330, 810, 752; $^1\text{H-NMR}$ (DMSO- d_6) δ : 9.09 (s, 1H), 8.30 (d, 1H, $J = 9.0$ Hz), 8.05 (d, 1H, $J = 9.0$ Hz), 7.96 (d, 2H, $J = 8.8$ Hz), 6.85 (d, 2H, $J = 8.8$ Hz), 3.05 (s, 12H); Anal. Calcd for $\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$: C, 60.18; H, 4.38; N, 14.04; Found: C, 60.41; H, 4.44; N, 14.30.

6-Nitro-2-(3-fluorophenyl)benzothiazole (5). Yield: 45.7% (from toluene); mp 199-200 °C; IR cm^{-1} : 1588, 1515, 1448, 1343, 809, 785, 753; $^1\text{H-NMR}$ (DMSO- d_6) δ : 9.24 (d, 1H, $J = 2.1$ Hz), 8.35 (dd, 1H, $J = 8.9$ Hz, $J = 2.3$ Hz), 8.24 (d, 1H, $J = 8.9$ Hz), 7.99-7.93 (m, 2H), 7.65 (m, 1H), 7.49 (m, 1H); Anal. Calcd. for $\text{C}_{13}\text{H}_7\text{FN}_2\text{O}_2\text{S}$: C, 56.93; H, 2.57; N, 10.21; Found: C, 57.20; H, 2.63; N, 10.00.

6-Nitro-2-(4-fluorophenyl)benzothiazole (6). Yield: 58.2% (from xylene); mp 183-185 °C; IR cm^{-1} : 1592, 1510, 1474, 1334, 839, 751; $^1\text{H-NMR}$ (DMSO- d_6) δ : 9.23 (d, 1H, $J = 2.4$ Hz), 8.36 (dd, 1H, $J = 8.9$ Hz, $J = 2.4$ Hz), 8.25-8.20 (m, 3H), 7.46 (dd, 2H, $J = 8.8$ Hz, $J = 10.8$ Hz); $^{13}\text{C-NMR}$ (DMSO- d_6) δ : 172.6, 164.6 (d, $J = 251$ Hz), 157.4, 144.6, 135.4, 130.4 (d, $J = 9.3$ Hz, 2C), 129.0, 123.3, 122.1,

119.7, 116.8 (d, $J = 22.4$ Hz, 2C); Anal. Calcd for $C_{13}H_7FN_2O_2S$: C, 56.93; H, 2.57; N, 10.21; Found: C, 56.74; H, 2.65; N, 10.02.

Synthesis of 6-amino-2-(substituted-phenyl) benzothiazoles 7-12

Procedure B: A solution of tin(II) chloride dihydrate (9 g, 0.04 mol), concd. HCl (18 mL) and methanol (18 mL) was added to the appropriate 6-nitro-2-(nitrophenyl)benzothiazole **1-3** (0.005 mol), or 0.01 mol of the compounds **4-6**. The mixture was stirred and refluxed for 15 min. Methanol was removed by vacuum evaporation and the residue was dissolved in water. After basification to pH > 9 with 20% NaOH the product was extracted with ether and dried ($MgSO_4$). The solvent was removed under reduced pressure and the white solid obtained was crystallised from the appropriate solvent. The following compounds were prepared by thus method:

6-Amino-2-(2-aminophenyl)benzothiazole (7). Yield: 74.5% (after crystallisation from toluene); mp 185-187 °C; IR cm^{-1} : 3438, 3351, 3299, 1611, 1487, 817, 754; 1H -NMR (DMSO- d_6) δ : 7.62 (d, 1H, $J = 8.6$ Hz), 7.47 (d, 1H, $J = 7.6$ Hz), 7.13 (s, 2H), 7.10 (d, 1H, $J = 8.0$ Hz), 7.04 (s, 1H), 6.79 (d, 1H, $J = 8.3$ Hz), 6.72 (m, 1H), 6.58 (m, 1H, $J = 7.5$ Hz), 5.42 (s, 2H); Anal. Calcd for $C_{13}H_{11}N_3S$: C, 64.70; H, 4.59; N, 17.41; Found: C, 64.83; H, 4.63; N, 17.29.

6-Amino-2-(3-aminophenyl)benzothiazole (8). Yield: 68.2% (from xylene); mp 202-205 °C; IR cm^{-1} : 3406, 3300, 3200, 1621, 1602, 1485, 815, 788; 1H -NMR (DMSO- d_6) δ : 7.62 (d, 1H, $J = 8.6$ Hz), 7.19 (s, 1H), 7.13-7.02 (m, 3H), 6.74 (d, 1H, $J = 8.6$ Hz), 6.62 (d, 1H, $J = 7.6$ Hz), 5.44 (s, 2H), 5.35 (s, 2H); ^{13}C -NMR (DMSO- d_6) δ : 161.4, 149.2, 147.2, 145.1, 136.0, 134.0, 129.5, 122.9, 115.7, 114.9, 114.0, 111.3, 103.7; Anal. Calcd for $C_{13}H_{11}N_3S$: C, 64.70; H, 4.59; N, 17.41; Found: C, 64.75; H, 4.42; N, 17.38.

6-Amino-2-(4-aminophenyl)benzothiazole (9). Yield: 68.8% (toluene); mp 263-265 °C; IR cm^{-1} : 3456, 3365, 3304, 3193, 1621, 1606, 1488, 822; 1H -NMR (DMSO- d_6) δ : 7.74 (d, 2H, $J = 8.3$ Hz), 7.67 (d, 1H, $J = 8.6$ Hz), 7.14 (s, 1H), 6.83 (d, 2H, $J = 8.3$ Hz), 5.84 (s, 2H), 5.43 (s, 2H); ^{13}C -NMR (DMSO- d_6) δ : 161.6, 151.0, 146.4, 145.4, 135.3, 127.8 (2C), 122.0, 120.9, 114.4, 113.5 (2C), 104.0; Anal. Calcd for $C_{13}H_{11}N_3S$: C, 64.70; H, 4.59; N, 17.41; Found: C, 64.58; H, 4.50; N, 17.62.

6-Amino-2-(4-*N,N*-dimethylaminophenyl)benzothiazole (10). Yield: 68.7% (from xylene); mp 230-233 °C; IR cm^{-1} : 3459, 3306, 3188, 1610, 1489, 813; 1H -NMR (DMSO- d_6) δ : 7.78 (d, 2H, $J = 8.9$ Hz), 7.60 (d, 1H, $J = 8.6$ Hz), 7.07 (d, 1H, $J = 2.1$ Hz), 6.81 (d, 2H, $J = 8.9$ Hz), 6.75 (dd, 1H, $J = 2.1$ Hz, $J = 8.6$ Hz), 5.36 (s, 2H), 3.02 (s, 6H); ^{13}C -NMR (DMSO- d_6) δ : 161.4, 151.4, 146.5, 145.4, 135.4, 127.5 (2C), 122.2, 120.9, 114.5, 111.8 (2C), 104.0, 39.7 (2C); Anal. Calcd for $C_{15}H_{15}N_3S$: C, 66.88; H, 5.61; N, 15.60; Found: C, 66.95; H, 5.72; N, 15.52.

6-Amino-2-(3-fluorophenyl)benzothiazole (11). Yield: 74.6% (toluene-petroleum ether, 1:1, v/v); mp 127-128 °C; IR cm^{-1} : 3408, 3312, 3218, 1602, 1442, 852, 827, 793; 1H -NMR (DMSO- d_6) δ : 7.76-7.68 (m, 3H), 7.54 (m, 1H), 7.31 (m, 1H), 7.08 (s, 1H), 6.79 (d, 1H, $J = 8.6$ Hz), 5.57 (s, 2H); ^{13}C -NMR

(DMSO- d_6) δ : 162.4 (d, $J = 244$ Hz), 158.7, 147.8, 144.9, 136.5, 135.6 (d, $J = 8.2$ Hz), 131.3 (d, $J = 8.4$ Hz), 123.4, 122.6 (d, $J = 2.6$ Hz), 116.8 (d, $J = 21.0$ Hz), 115.3, 112.5 (d, $J = 23.0$ Hz), 103.5. Anal. Calcd for $C_{13}H_9FN_2S$: C, 63.92; H, 3.71; N, 11.47; Found: C, 63.73; H, 3.80; N, 11.52.

6-Amino-2-(4-fluorophenyl)benzothiazole (12). Yield: 79.3% (from toluene-petroleum ether, 1:1, v/v); mp 156–157 °C; IR cm^{-1} : 3407, 3314, 3218, 1606, 1487, 839, 827, 801; 1H -NMR (DMSO- d_6) δ : 7.97 (dd, 2H, $J = 8.6$ Hz, $J = 5.3$ Hz), 7.66 (d, 1H, $J = 8.6$ Hz), 7.33 (dd, 2H, $J = 8.6$ Hz, $J = 10.3$ Hz), 7.07 (s, 1H), 6.77 (d, 1H, $J = 8.6$ Hz), 5.50 (s, 2H); ^{13}C -NMR (DMSO- d_6) δ : 163.2 (d, $J = 247$ Hz), 159.3, 147.6, 145.2, 136.5, 130.2, 128.7 (d, $J = 7.7$ Hz), 123.3, 116.3 (d, $J = 21.8$ Hz), 115.3, 103.8; Anal. Calcd for $C_{13}H_9FN_2S$: C, 63.92; H, 3.71; N, 11.47; Found: C, 63.78; H, 3.85; N, 11.55.

Synthesis of amine hydrochloride salts 13–19.

Procedure C: The amines **7–12** (0.005 mol) were dissolved in a warm mixture of ethanol and xylene (1:1, v/v) followed by the addition of concd. HCl (1.0 mL). The contents were stirred in a stoppered flask for 4 h. The precipitated pure crystals were collected by filtration, washed with dry ether, and dried *in vacuo*. The following compounds were prepared by this method:

6-Amino-2-(2-aminophenyl)benzothiazole dihydrochloride (13). Yield: 96.6%; mp 224–227 °C; IR cm^{-1} : 2816, 2662, 2524, 1486, 1463, 980, 767; 1H -NMR (DMSO- d_6) δ : 8.11 (s, 1H), 8.07 (d, 1H, $J = 8.6$ Hz), 7.64 (d, 1H, $J = 8.0$ Hz), 7.49 (d, 1H, $J = 8.6$ Hz), 7.24 (dd, 1H, $J = 8.0$ Hz, $J = 7.3$ Hz), 6.29 (d, 1H, $J = 8.3$ Hz), 6.69 (dd, 1H, $J = 7.7$ Hz, $J = 7.3$), 4.78 (br s); ^{13}C -NMR (DMSO- d_6) δ : 169.6, 152.7, 144.2, 133.7, 132.3, 130.4, 129.2 (2C), 123.2, 122.3, 118.8, 117.1, 115.7; Anal. Calcd for $C_{13}H_{13}Cl_2N_3S$: C, 49.69; H, 4.18; N, 13.37; Cl, 22.56; Found: C, 49.55; H, 4.15; N, 13.37; Cl, 22.38.

6-Amino-2-(3-aminophenyl)benzothiazole dihydrochloride (14). Yield: 97.4%; mp 253–255 °C; IR cm^{-1} : 2896, 2605, 1595, 1521, 1456, 818, 786; 1H -NMR (DMSO- d_6) δ : 8.05 (d, 1H, $J = 8.6$ Hz), 7.97 (s, 1H), 7.88 (s, 1H), 7.80 (d, 1H, $J = 7.7$ Hz), 7.53 (dd, 1H, $J = 8.0$ Hz, $J = 7.7$ Hz), 7.40 (d, 1H, $J = 8.8$ Hz), 7.32 (d, 1H, $J = 7.7$ Hz); ^{13}C -NMR (DMSO- d_6) δ : 167.2, 152.4, 135.5, 134.3, 133.8, 131.0, 130.5, 126.1, 125.8, 124.0, 122.2, 121.1, 116.9; Anal. Calcd for $C_{13}H_{13}Cl_2N_3S$: C, 49.69; H, 4.18; N, 13.37; Cl, 22.56; Found: C, 49.70; H, 4.10; N, 13.35; Cl, 22.63.

6-Amino-2-(4-aminophenyl)benzothiazole dihydrochloride (15). Yield: 99.3%; mp 247–248 °C; IR cm^{-1} : 2852, 2554, 1605, 1507, 1485, 974, 822; 1H -NMR (DMSO- d_6) δ : 7.95–7.92 (m, 2H), 7.79 (d, 2H, $J = 8.7$ Hz), 7.36 (d, 1H, $J = 8.3$ Hz), 6.76 (d, 2H, $J = 8.7$ Hz), 4.41 (br s); ^{13}C -NMR (DMSO- d_6) δ : 168.5, 152.7, 143.5, 135.1 (2C), 129.4 (2C), 128.9, 126.1, 123.2, 122.0, 119.4, 116.9; Anal. Calcd for $C_{13}H_{13}Cl_2N_3S$: C, 49.69; H, 4.18; N, 13.37; Cl, 22.56; Found: C, 49.68; H, 4.06; N, 13.37; Cl, 22.64.

6-Amino-2-(4-N,N-dimethylaminophenyl)benzothiazole dihydrochloride (16). Yield: 99.5%; mp 240–242 °C; IR cm^{-1} : 2796, 2568, 2453, 1607, 1519, 841; 1H -NMR (DMSO- d_6) δ : 8.06 (s, 1H), 7.99 (d, 1H, $J = 8.6$ Hz), 7.89 (d, 2H, $J = 8.9$ Hz), 7.44 (d, 1H, $J = 8.6$ Hz), 6.85 (d, 2H, $J = 8.9$ Hz), 3.01 (s, 6H); ^{13}C -NMR (DMSO- d_6) δ : 169.8, 152.0, 150.2, 134.5, 128.9 (2C), 127.6, 122.6, 122.4, 121.6, 116.5,

114.7 (2C), 41.2 (2C); Anal. Calcd for C₁₅H₁₇Cl₂N₃S: C, 52.63; H, 5.01; N, 12.27; Cl, 20.71; Found: C, 52.60; H, 4.97; N, 12.23; Cl, 20.80.

6-Amino-2-(3-fluorophenyl)benzothiazole monohydrochloride (17). Yield: 96.1%; mp 206-207 °C; IR cm⁻¹: 2806, 2589, 1585, 1514, 1265, 848, 787; ¹H-NMR (DMSO-*d*₆) δ: 8.06 (d, 1H, *J* = 8.6 Hz), 7.99 (s, 1H), 7.89-7.83 (m, 2H), 7.60 (dd, 1H, *J* = 7.3 Hz, *J* = 7.0 Hz), 7.44-7.38 (m, 2H), 3.95 (br s, 3H); ¹³C-NMR (DMSO-*d*₆) δ: 166.1, 162.4 (d, *J* = 245 Hz), 151.5, 135.5, 134.7 (d, *J* = 8.2 Hz), 132.0, 131.6 (d, *J* = 8.3 Hz), 123.8, 123.4 (d, *J* = 2.6 Hz), 121.4, 118.2 (d, *J* = 21.0 Hz), 115.4, 113.4 (d, *J* = 22.4 Hz); Anal. Calcd for C₁₃H₁₀ClFN₂S: C, 55.62; H, 3.60; N, 9.98; Cl, 12.63; Found: C, 55.43; H, 3.56; N, 9.79; Cl, 12.81.

6-Amino-2-(4-fluorophenyl)benzothiazole monohydrochloride (19). Yield: 91.3%; mp 245-247 °C; IR cm⁻¹: 3058, 2854, 2586, 1602, 1488, 1237, 839, 816; ¹H-NMR (DMSO-*d*₆) δ: 8.13 (dd, 2H, *J* = 5.6 Hz, *J* = 8.3 Hz), 8.06 (d, 1H, *J* = 8.6 Hz), 7.97 (s, 1H), 7.45-7.37 (m, 3H), 3.63 (br s); ¹³C-NMR (DMSO-*d*₆) δ: 166.7, 163.9 (d, *J* = 245 Hz), 151.8, 135.5, 131.0, 129.5 (d, *J* = 8.9 Hz, 2C), 129.1, 123.6, 121.1, 116.5 (d, *J* = 22.2 Hz, 2C), 115.3; Anal. Calcd for C₁₃H₁₀ClFN₂S: C, 55.62; H, 3.60; N, 9.98; Cl, 12.63; Found: C, 55.45; H, 3.56; N, 9.87; Cl, 12.78.

Procedure D: The amines **7-12** (0.005 mol) were dissolved in a warm mixture of ethanol and xylene (1:1, v/v). A stream of dry hydrogen chloride was passed through the solution for 1 h while cooling to room temperature. The contents were then stirred in a stoppered flask for 2 h. The precipitated pure crystals were collected by filtration, washed with dry ether, and dried *in vacuo*. The following compound was prepared by this method:

6-Amino-2-(3-fluorophenyl)benzothiazole dihydrochloride (18). Yield: 97.2%; mp 209-210 °C; IR cm⁻¹: 3045, 2798, 2590, 2541, 1588, 1486, 861, 795; ¹H-NMR (DMSO-*d*₆) δ: 8.17-8.14 (m, 2H), 7.94-7.88 (m, 2H), 7.64 (dd, 1H, *J* = 7.6 Hz, *J* = 6.3 Hz), 7.53 (d, 1H, *J* = 8.6 Hz), 7.46 (m, 1H), 4.45 (br s); ¹³C-NMR (DMSO-*d*₆) δ: 167.2, 162.4 (d, *J* = 244 Hz), 152.4, 135.3, 134.6 (d, *J* = 8.2 Hz), 131.6 (d, *J* = 8.4 Hz), 129.7, 123.8, 123.6, 122.3, 118.4 (d, *J* = 20.9 Hz), 117.2, 113.5 (d, *J* = 21.4 Hz); Anal. Calcd for C₁₃H₁₁Cl₂FN₂S: C, 49.22; H, 3.50; N, 8.83; Cl, 22.35; Found: C, 49.45; H, 3.50; N, 8.98; Cl, 22.30.

Cell culture

Five different human cell lines were used for cytotoxicity screening: colon adenocarcinoma (CaCo-2), cervical adenocarcinoma (HeLa), laryngeal carcinoma (Hep-2), mammary adenocarcinoma (MCF7), lung fibroblast (WI-38). Cells were seeded in tissue culture flasks (Sarstedt, Numbrecht, Germany) in Dulbecco's Modified Eagle medium (DMEM) (Gibco, Paisley, Scotland) supplemented with 10% foetal bovine serum (FBS; Gibco), 2 mM glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere with 5% CO₂ at 37 °C. The cultures were passaged at preconfluent densities with the use of 0.025% trypsin (Institute of Immunology, Zagreb, Croatia).

Cytotoxicity assays

For the experiments the cells were seeded onto 96-well microtiter plates at a density of 2×10^3 cells per well and allowed to adhere for 24 h before the benzothiazoles were added over a concentration range of 1-100 μM . Stock solutions of benzothiazoles were dissolved in DMSO (Sigma, Deisenhofen, Germany) at the concentration of 0.1 M and were diluted with medium without bovine calf serum. The DMSO concentration was adjusted to be the same in test and control plates (1%). All drug solutions were freshly prepared on the day of testing. After incubation with benzothiazoles for 72 h, cell growth was established according to the results of MTT assay [10] - Sigma, Deisenhofen, Germany. Each experiment was made in quadruplicate and repeated three times. The absorbency was measured on a microplate reader at 570 nm. (Labsystems, Finland) The percentage of growth (PG) of the cell lines was calculated according to the following expression:

$$[(\text{Ti} - \text{Tz}) / (\text{C} - \text{Tz})] \times 100 \text{ for concentrations for which } \text{Ti} > / = \text{Tz} \%$$

where mean Tz = the average of optical density measurements before exposure of cells to the test compound, mean Ti = the average of optical density measurements after the desired period of time, and mean C = the average of optical density measurements after the desired period of time with no exposure of cells to the test compound. The results are expressed as IC₅₀, which is the concentration necessary for 50% inhibition. The IC₅₀ values for each compound are calculated from dose response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (i.e. 50%). Each result is a mean of IC₅₀ \pm SD value from three separate experiments.

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Sample availability: Available from the author.

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