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SYNTHESIS, CRYSTAL STRUCTURE DETERMINATION AND ANTIPROLIFERATIVE EVALUATION OF NOVEL BENZAZOYL BENZAMIDES

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Abstract – A series of benzazoyl-benzamides containing different substituents (**7**-**17**) were synthesized by condensation of 2-aminobenzazole derivatives (**3a**-**6**) with *p*-substituted benzoyl chlorides. All compounds were characterized by IR, ^{1}H and ^{13}C NMR, MS and elemental analysis. Crystal structure was determined for the compound (**9**). Some of the new synthesized compounds (**7**-**17**) were screened for antitumor activities. Based on presented *in vitro* screening results we may conclude that compounds (**10**, **15a**, **15b** and **16**) showed accentuated cell growth inhibitory activity.

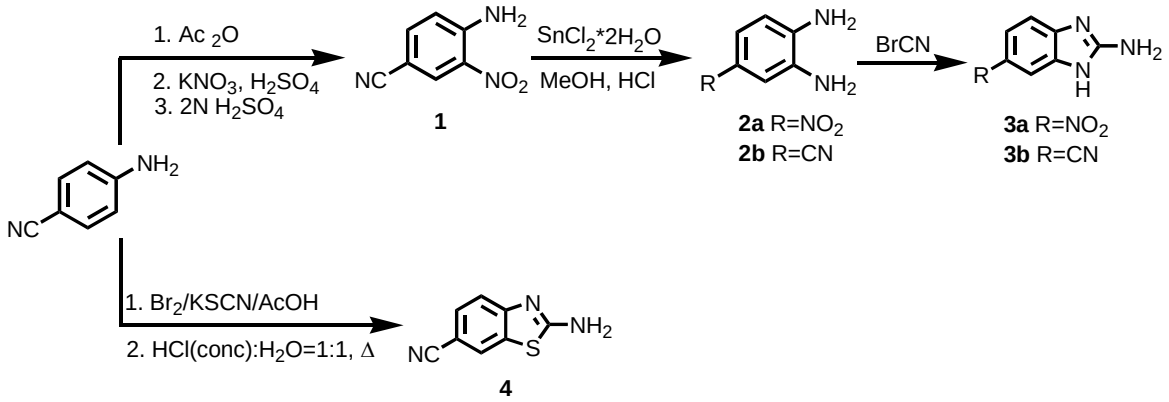
At the beginning of the 21st century cancer remains a major public health issue. Cancer is not a single disease but a broad group characterized malignant cells that are clearly distinguished from normal cells. Because of that there is a great medical need for new anticancer small molecule therapeutics.[1]

The amide bond is a building unit in many important natural or synthetic compounds[2,3] and this makes the amide group important to synthetic chemists. There is wide variety of conventional methods for

preparation of amides which in many cases needs harsh conditions (temperature, reaction periods) or the use of strong catalysts.

In the past few years benzimidazole and related benzothiazole analogs were studied extensively for their anti-bacterial[4], antitumor[5], antiviral[6] and antibiotic activities as a new non-nucleoside topoisomerase I poisons[7]. Apart from the above-mentioned activities some interesting efficacy of antiHIV[8] and kinase inhibition activities on benzazole derivatives[9] has been reported. Therefore various benzimidazole and benzothiazole derivatives are of considerable interest for their diverse pharmaceutical uses and play a vital role in the synthesis of fused heterocyclic systems.

Due to a broad spectrum of above mentioned activities, we have synthesized a series of new compounds, determined crystal structure of one of them and tested their antitumor activity *in vitro*.

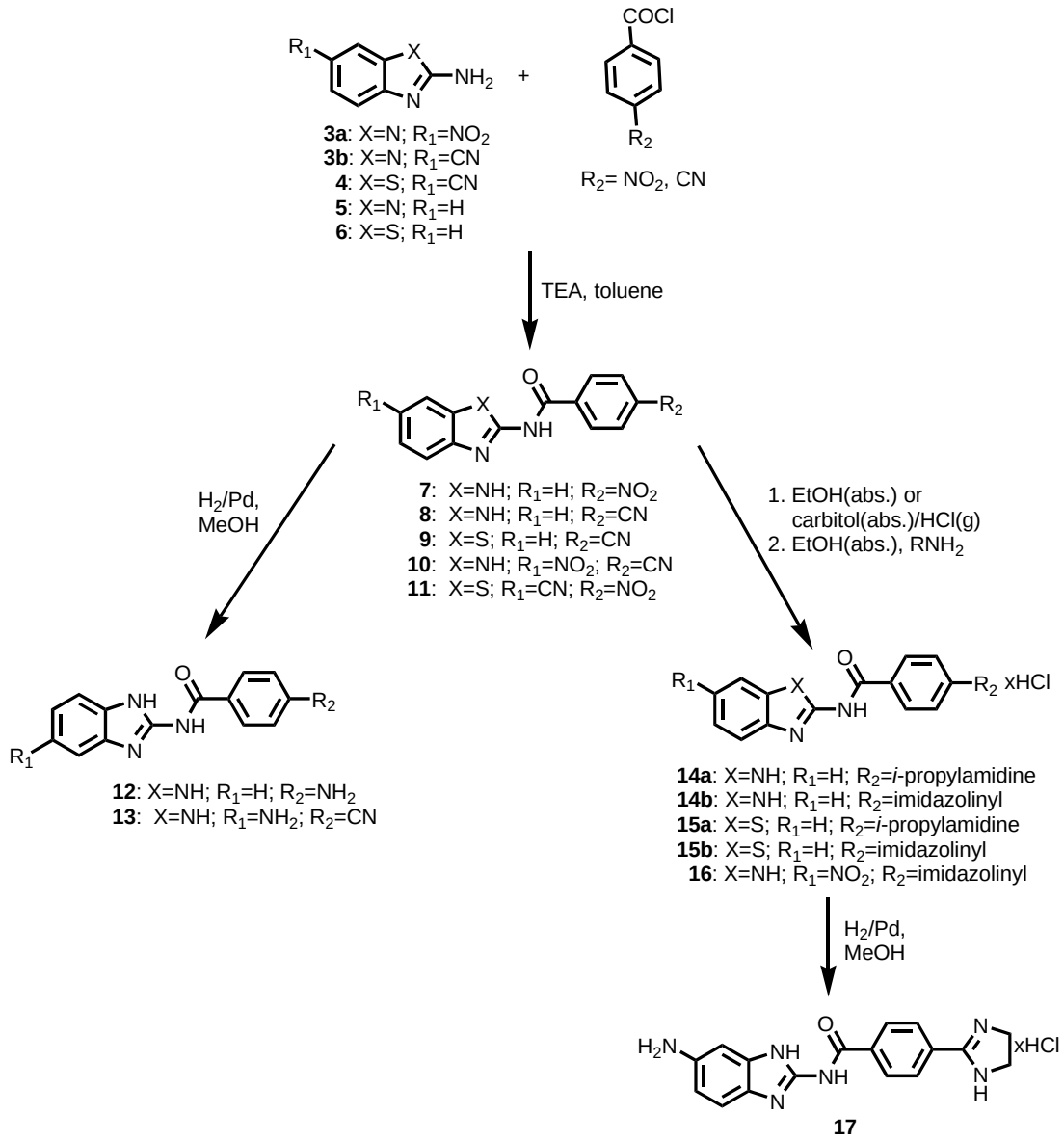


Scheme 1 Synthesis of 2-aminobenzazole derivatives

RESULTS AND DISCUSSION

For the synthesis of the requisite target compounds (**3b**) and (**4**) in the **Scheme 1**, we used commercially available 4-aminobenzonitrile and 4-nitro-1,2-phenylenediamine (**2a**) as starting material. In the first step it was necessary to conduct the reaction with acetic anhydride to protect the amino group of the *p*-aminobenzonitrile which was then nitrated to obtain 4-amino-3-nitrobenzonitrile (**1**). The compound (**1**) was reduced with $SnCl_2$ to give desired 3,4-diaminobenzonitrile (**2b**). The ring annulation of **2a** and **2b** was accomplished with cyanogen bromide in acetonitrile to afford 2-amino-6-nitrobenzimidazole (**3a**) and 2-amino-6-cyanobenzimidazole (**3b**). 2-Amino-6-cyanobenzothiazole (**4)** was obtained using a slightly changed conventional procedure[10] with potassium thiocyanate and HCl(concd)/H_2O in the two step synthesis.

Prepared compounds (**3a, 3b** and **4**) were used for the synthesis of series of novel amides (**Scheme 2**) by published method[11]. Substituted *N*-(benzazol-2-yl)benzamides (**7-11**) were prepared by reaction of the appropriate benzoyl chloride and 2-aminobenzazoles substituted in the position 6. We found that the benzothiazoles provide better yield then benzimidazoles in the amides synthesis.



Scheme 2 Synthesis of novel benzamide derivatives

Cyano substituted amides (**8**, **9** and **10**) were converted into appropriate amidines (**14a**, **14b**, **15a**, **15b** and **16**) by classical Pinner reaction[12]. The Pinner reaction was conducted in two different solvents, EtOH (abs. abs.) and carbitol (abs.), because of low ethanol solubility of cyano substituted benzothiazolyl-amides (**9** and **11**). Attempts to isolate amidino derivatives of compound (**11**) and *i*-propylamidino substituted compound (**10**) were not successful because they polymerized during the isolation procedure owing to theirs instability and sensitivity to air moisture. The reduction of the nitro group of compounds (**7**, **10** and **16**) is readily accomplished by catalytical hydrogenation (H_2/Pd-C), while reduction of compound (**11**) was not successful.

The structures of new compounds were confirmed by elemental analysis, MS, IR, ^{1}H, and ^{13}C NMR spectra, while structure of compound (**9**) was also additionally confirmed by X-Ray structure analysis on single crystals. The X-Ray molecular structure of compound (**9**) is shown in **Figures 1** and **2**.

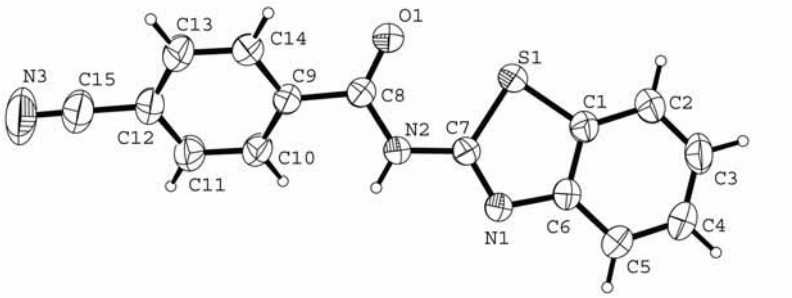


Figure 1 An ORTEP-3[13] drawing of molecular structure of **9**, showing the crystallographic numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

The molecule of 4-cyano-N-(benzothiazol-2-yl)benzamide (**9**) is not planar. The dihedral angle between least-squires planes calculated through benzothiazole and benzene moieties amounts 33.7°. The bond lengths and bond angles correspond to the geometry parameters expecting for atom types and the type of hybridization.[14] Both carbonyl and amide group are coplanar with benzothiazole fragment. Torsion angle N2-C8-C9-C10 defining twisting around single C8-C9 bond is 33.3(3)°. The molecules are connected in the cyclic dimmers by N-H…N hydrogen bonding (**Figure 2**) between the amide group and N atom of benzothiazole moiety [N2…N1i 2.985(2) Å (i: -x, y, 1/2+2-z); ∢D-H…A 155.6°]. The discrete dimmers link together into 3D hydrogen-bonded network by C-H…N and C-H…O hydrogen bonds with: C3…N3ii 3.596(3) Å (ii: x-1/2, y-1/2, z-1); C11…O1iii 3.159(3) Å (iii: x, 1-y, z+1/2).

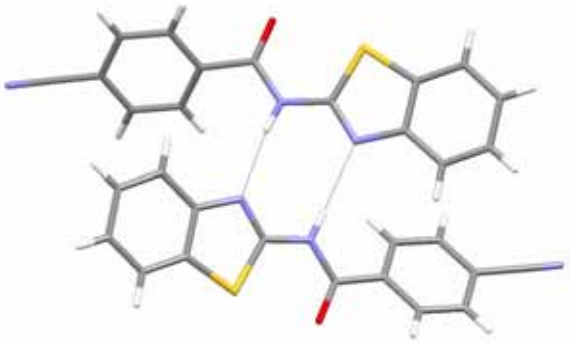


Figure 2 Projection of the dimmers of **9** viewed along the *b*-axes drawn by the program Mercury.[15]

Antiproliferative effect of compounds *in vitro*

The compounds (**7**–**17**) were tested for the potential antiproliferative effect on the panel of six human cell lines, five of which are derived from five cancer types: HeLa (cervical carcinoma), MCF–7 (breast carcinoma), SW 620 (colon carcinoma), MiaPaCa–2 (pancreatic carcinoma), (H 460) lung carcinoma and

WI 38 (diploid fibroblasts). All tested compounds showed prominent antiproliferative effect on the presented panel cell lines (**Table 1**).

Table 1 Growth inhibitory effects of different benzamide compounds (**7**, **8**, **10**, **12** and **13-16)** on the growth of malignant tumor cell lines in comparison with their effects on the growth of normal human diploid fibroblast (WI 38).

IC_{50} (μM)*						
Comp.	**Cell lines**					
	WI 38	**HeLa**	**MiaPaCa-2**	**SW 620**	**MCF-7**	**H 460**
7	2±0,2	27±2	6±2	4±6	11±8	>100
8	2±2	10±5	2±1	>100	74±42	>100
10	15±4	12±2	7±0,08	10±0,0	8±6	22±2
12	2±1	32±6	15±2	>100	24±14	80±20
14a	20±5	13±0,6	23±21	38±14	≥100	>100
15a	16±3	9±7	17±4	17±2	13±5	16±2
15b	2±1	2±1	5±0,6	3±0,4	3±2	3±1
16	5±0,6	2±0,2	2±0,4	2±1	18±15	7±5
17	24±16	36±16	32±5	>100	12±20	>100

*IC_{50}; the concentration that causes a 50% reduction of the cell growth.

The compounds (**12**, **14a** and **17**) showed moderate inhibitory effect on all cell lines, but mostly at the highest tested concentration. The compounds (**7** and **8**) have somewhat more pronounced activity; however, they are also very toxic to normal cells. On the other hand the compounds (**10**, **15a**, **15b** and **16**) were the most active one (IC_{50} concentrations are in low micromolar range) (**Figure 3**).

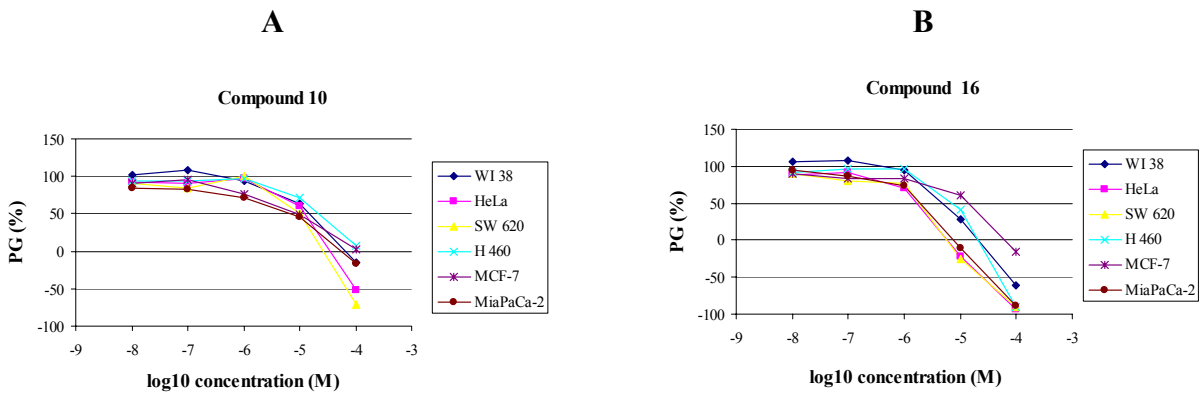


Figure 3 Dose-response profiles for compounds **10** (**A**) and **16** (**B**) tested *in vitro*. PG=percentage of growth.

Besides, the compounds (**10**, **15a**, **15b** and **16**) were cytotoxic to all cell lines. It should be also taken into consideration that compounds **7**, **8**, **14a** and **15b** precipitated when diluted with the cell-culture medium at the maximal tested concentration and they were used as the suspension. The compounds (**9**, **11**, **13** and **14b**) were not tested because of their very low solubility in DMSO.

In conclusion, we have prepared a series of benzazoyl-benzamides and tested them on their antiproliferative activity. Based on presented *in vitro* screening results we may conclude that compounds (**10**, **15a**, **15b** and **16**) showed accentuated cell growth inhibitory activity. We will continue our work in order to find new benzazoyl-benzamides with more selective antiproliferative effect.

EXPERIMENTAL

Chemistry

Melting points were obtained on an Original Kofler Mikroheitztisch apparatus (Reichert, Wien) and are uncorrected. IR spectra were recorded on a Nicolet Magna 760 spectrophotometer in KBr discs. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or a Bruker Avance DPX 300 and 500 spectrometer at 300, 500 and 75 MHz, respectively. All NMR spectra were measured in DMSO-d_6 solutions using TMS as an internal standard. Elemental analysis for carbon, hydrogen and nitrogen were performed on a Perkin-Elmer 2400 elemental analyzer. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates and HPLC-MS.

4-Amino-3-nitrobenzonitrile (**1**) was prepared by earlier described procedure[12] to give physical constants according with literature values, yield 82%, mp 159-161°C (lit.,[12] mp 160-161°C).

5-Nitro-1,2-phenylendiammine (**2a**) was commercially available and used without purification.

3,4-Diaminobenzonitrile (**2b**)

The amino compound (**2a**) was prepared by modified method of reduction[16]. Nitro derivative (**1)** (6.95g, 0.043mol) was refluxed with $SnCl_2$ x $2H_2O$ (76.4g, 0.338mol) in the mixture of concd HCl (113mL) and MeOH (113mL) for 30 mins. MeOH was evaporated and the residue poured on ice. The solution of NaOH was added to the cold mixture (until p*H* 10). The crude product was filtered off and recrystallized from EtOH; yield 3.62g (64%), mp 145–147°C (lit., [12] mp 144-146°C).

2-Amino-6-cyanobenzimidazole (**3a**)

3,4-diaminobenzonitrile (**2b**) (3.98g, 0.030mol) was added to a mixture of water (62,5mL), MeOH (62,5mL) and solution of cyanogen bromide (3.17g, 0.030mol) in acetonitrile (6mL) and left to stir overnight. The mixture was decolorized with charcoal and adjust to p*H*>9, with concd NH_4OH. White precipitate **(3a)** was obtained: yield 4.07g (86%), mp 213-215°C. IR (cm^{-1}): 3429, 3330, 3139, 2898,

2364, 2214, 1675, 1571, 1467, 1290 cm^{-1}; ^1H NMR (DMSO) σ/ppm: 11.00 (brs, 1H, NH), 7.39 (s, 1H), 7.20 (d, 1H, *J*=8.1Hz), 7.13 (d, 1H, *J*=8.1Hz) 6.62 (s, 2H, NH); *Anal.* Calcd for $C_8H_6N_4$: C 60.75; H 3.82; N 35.42. Found: C 60.95; H 3.97; N 35.12.

2-Amino-6-nitrobenzimidazole (**3b**)

Compound (**3b)** was prepared in a same manner as (**3a)**; 5-nitro-1,2-phenylendiamine (8.72g, 0.057mol) was added to a water (120mL), MeOH (120mL) and cyanogen bromide (6.04g, 0.057mol) in acetonitrile (11.3mL) afforded 0.120g (35.1%) of dark yellow powder; mp 190-192°C (mp 189-190°C[17]).

2-Amino-6-cyanobenzothiazole (**4**) was prepared by slightly changed conventional procedure[10] and recrystallized from EtOH to give physical constants in accord with literature values, yield: 60% mp 216-218°C (lit.,[10] mp 217-218°C).

General procedure for amide synthesis

Reaction mixture of appropriate benzoyl chloride, corresponding 2-aminobenzazole derivatives (**1-4**) and Et_3N in equimolar amounts in dry toluen was refluxed for 4-5h. After cooling, precipitate was filtered off, washed with water and recrystallized from MeOH.

4-Nitro-*N*-(1H-benzimidazol-2-yl)benzamide (**7**)

Yield 0.533g (32.7%), green-yellow powder; mp >300°C IR (KBr)/cm^{-1}): 3135, 1700, 1616, 1529, 1519; ^1H NMR (DMSO) σ/ppm: 12.80 (brs, 2H, NH), 8.28 (2H, d, *J*=8.52Hz), 8.18 (2H, d, *J*=8.52Hz), 7.46-7.43 (2H, m), 7.02-6.99 (2H, m); MS *m/z*: 282.8 (M+1); *Anal.* Calcd for $C_{14}H_{10}N_4O_3$: C 59.61; H 3.57; N 19.86. Found: C 59.57; H 3.57; N 19.85.

4-Cyano-*N*-(1H-benzimidazol-2-yl)benzamide (**8**)

Yield 0.458g (57.13%), pale yellow powder; mp 260-261°C; IR (KBr)/cm^{-1}): 3151, 2647, 2231, 1700; ^1H NMR (DMSO) σ/ppm: 12.51 (s, 2H, NH), 8.30 (d, 2H, *J*=8.50Hz), 7.98 (d, 2H, *J*=8.50Hz), 7.46-7.42 (m, 2H), 7.20-7.16 (m, 2H); ^{13}C NMR (DMSO) σ/ppm: 112.24, 112.31, 113.41, 118.58, 122.33 (2C), 129.06, 129.11 (2C), 131.65, 132.70 (2C), 140.83, 151.90, 170.43; *Anal.* Calcd for $C_{15}H_{10}N_4O$: C 68.68; H 3.84; N 21.38. Found: C 68.69; H 3.84; N 21.36.

4-Cyano-*N*-(benzothiazol-2-yl)benzamide (**9**)

Yield 1.27g (75.3%), white powder; mp 274-276°C. IR (KBr)/cm^{-1}): 2910, 2227, 1674; ^1H NMR (DMSO) σ/ppm: 13.13 (brs, 1H, NH), 8.27 (d, 2H, *J*=8.1Hz), 8.04-8.00 (m, 3H), 7.78 (d, 1H, *J*=7.83Hz), 7.48 (t, 1H, *J*=7.83Hz), 7.35 (t, 1H, *J*=7.83Hz); MS *m/z*: 280.1 (M+1) *Anal.* Calcd for $C_{15}H_9N_3OS$: 64.50 C; 3.25 H; 15.04 N. Found: 64.54 C; 3.37 H; 15.18 N.

4-Cyano-*N*-(6-nitro-1H-benzimidazol-2-yl)benzamide (**10**)

Yield 0.293g (15.8%), yellow powder; mp 272-274°C. IR (KBr)/cm^{-1}) 3278, 3099, 2232, 1887, 1687, 1643; ^1H NMR (DMSO) σ/ppm: 12.84 (brs, 2H, NH), 8.35 (s, 1H), 8.25 (d, 2H, *J*=8.25Hz), 8.05 (d, 1H,

J=8.82Hz), 8.03 (d, 2H, *J*=8.25Hz), 7.62 (d, 1H, *J*=8.82Hz); MS *m/z*: 307.8 (M+1); *Anal.* Calcd for $C_{15}H_9N_5O_3$: C 58.67; H 2.95; N 22.81. Found: C 58.53; H 2.85; N 22.79.

4-Nitro-*N*-(6-cyanobenzothiazol-2-yl)benzamide (11)

Yield 0.662g (71.6%), pale yellow powder; mp>300°C. IR (KBr)/cm^{-1}): 3110, 2223, 1676; ^{1}H NMR (DMSO) σ/ppm: 13.53 (brs, 1H, NH), 8.54 (s, 1H), 8.32 (d, 2H, *J*=8.7Hz), 8.17 (d, 2H, *J*=8.7Hz), 7.86 (d, 1H, *J*=8.4Hz), 7.81 (d, 1H, *J*=8.4Hz); MS *m/z*: 325.0 (M+1); *Anal.* Calcd for $C_{15}H_8N_4O_3S$: 55.55 C; 2.49 H; 17.28 N. Found: 55.48 C; 2.52 H; 17.20 N.

4-Amino-*N*-(1H-benzimidazol-2-yl)benzamide (12)

A solution of 4-nitro-*N*-(6-cyanobenzothiazol-2-yl)benzamide (**11**) (0.500g, 1.77mmol) in MeOH (150mL) and 0.050g of 10% Pd-C was hydrogenated until the required quantity of H_2 was taken up. The solution was filtered trough Celite to remove the catalyst and the MeOH was removed under reduced pressure. The resulting solid was triturated with a small amount of MeOH and collected by filtration to afford 0.354g (79.4%). IR (KBr)/cm^{-1}): 3287, 2878, 1690, 1598; ^{1}H NMR (DMSO) σ/ppm: 11.52 (brs, 2H, NH), 7.87 (d, 2H, *J*=8.5Hz) 7.44 (d, 2H, *J*=8.5Hz), 7.18-7.15 (m, 2H), 6.96-6.93 (m, 2H), 5.95 (brs, 2H, NH); ^{13}C NMR (DMSO) σ/ppm: 166.63, 154.77, 153.46, 148.49, 146.4, 136.47, 131.67, 130.55, 125.18, 122.54, 121.34, 120.56, 113.03, 111.82; MS *m/z*: 252.9 (M+1); *Anal.* Calcd for $C_{14}H_{12}N_4O$: C 66.65; H 4.79; N 22.21. Found: C 66.55; H 4.69; N 21.91.

4-Cyano-*N*-(6-amino-1H-benzimidazol-2-yl)benzamide (13)

Compound (**13**) was prepared in a same manner as (**12**). Yield 0.022g (5.5%), dark green powder; mp >300 °C. IR (KBr)/cm^{-1}): 3375, 3189, 2225, 1573; ^{1}H NMR (DMSO) σ/ppm: 12.18 (brs, 2H, NH), 8.27 (d, 2H, *J*=7.98Hz), 7.92 (d, 2H, *J*=7.98Hz), 7.09 (d, 1H, *J*=8.43Hz), 6.61 (s, 1H), 6.47 (d, 1H, *J*=8.43Hz); ^{13}C NMR (DMSO) σ/ppm: 171.15, 152.50, 145.79 (2C), 132.53 (2C), 130.30, 129.43 (2C), 119.34, 118. 84, 113.29, 112.65, 111.00; MS *m/z*: 277.8 (M+1); *Anal.* Calcd for $C_{15}H_{12}N_4O$: C 68.17; H 4.58; N 21.20. Found: C 68.05; H 4.60; N 21.09.

General procedure for amidine synthesis

Amidino-substituted *N*-(benzazol-2-yl)benzamide hydrochlorides (**14-16**) were prepared from the corresponding nitriles by a modified Pinner reaction.[12] A suspension of cyano substituted amides (0.4g) in absolute EtOH (20mL) or carbitol (5mL) was cooled to 0°C and saturated with dry HCl gas. After the mixture returned to rt, the flask was stoppered, and the contents stirred until IR spectra indicated the lack of the cyano peak (3-5ds). The imido ester hydrochloride (80-95%) intermediates were then precipitated from the solution by addition of dry diethyl ether, filtered off washed with dry ether and dried under reduced pressure over KOH. The appropriate amine (3 x equimolar) was added to the suspension of the crude product in dry EtOH (5mL) under nitrogen atmosphere. Isopropyl amidines: the reaction mixture

was stopper, the contents stirred at rt for 1 day and evaporated to dryness. Imidazolinyl amidines: the reaction mixture was refluxed for 4h under nitrogen atmosphere and stirred overnight at rt and evaporated to dryness.

4-(*N*-Isopropylamidino)-*N*-(1H-benzimidazol-2-yl)benzamide hydrochloride (14a)

Yield 0.108g (26.4%), white powder, mp>300°C. IR (KBr)/cm^{-1}) 2977, 1675, 1625, 1548.;^{1}H NMR (DMSO) σ/ppm: 12.48 (brs, 2H, NH), 9.63 (brs, 3H, NH), 8.32 (d, 2H, *J*=8.37Hz), 7.85 (d, 2H, *J*=8.31Hz), 7.47-7.44 (m, 2H), 7.20-7.17 (m, 2H), 4.09-4.05 (m, 1H, C<u>H</u>), 1.3 (d, 6H, C<u>H</u>$_3$); *Anal.* Calcd for $C_{18}H_{20}ClN_5O$: C 60.53; H 5.64; N 19.61. Found: C 60.42; H 5.63; N 19.57.

4-Imidazolinyl-*N*-(benzimidazol-2-yl)benzamide hydrochloride (14b)

Yield 0.189g (54%) pale green powder, mp >300°C. IR (cm^{-1}): 3313, 2865, 1600, 1523; ^{1}H NMR (DMSO) σ/ppm: 12.35 (brs, 2H, N<u>H</u>), 9.40 (brs, 2H, N<u>H</u>), 8.20 (d, 2H, *J*=8.14Hz), 7.97 (d, 2H, *J*=8.14Hz), 7.47-7.44 (m, 2H), 7.16-7.13 (m, 2H), 3.67 (s, 4H, C<u>H</u>$_2$); ^{13}C NMR (DMSO) σ/ppm: 168.84, 163.31, 149.70, 145.81, 136.77, 133.29, 132.83, 128.21 (2C), 127.08, 127.02 (2C), 121.79, 113.00, 112.95, 40.52; *Anal.* Calcd for $C_{17}H_{16}N_5$: C 59.74; H 4.72; N, 20,49. Found: C 59.61; H 4.92; N, 20,19.

4-(*N*-Isopropylamidino)-*N*-(benzothiazol-2-yl)benzamide hydrochloride (15a)

Yield 0.125g (41.9%), white powder, mp 288-289°C. IR (KBr)/cm^{-1}): 2975, 1668, 1537; ^{1}H NMR (DMSO) σ/ppm: 12.8 (brs, 1H, NH), 9.85 (s, 1H, NH), 9.69 (s,1H, NH), 9.44 (s, 1H, NH), 8.31 (d, 2H, *J*=8.25Hz), 8.04 (d, 1H, *J*=7.98Hz), 7.93 (d, 2H, *J*=8.25Hz), 7.80 (d, 1H, *J*=7.98Hz), 7.49 (t, 1H, *J*=7.83Hz), 7.36 (t, 1H, *J*=7.83Hz), 4.23-4.10 (m, 1H, C<u>H</u>), 1.29 (d, 6H, *J*=6.33Hz, C<u>H</u>$_3$); ^{13}C NMR (DMSO) σ/ppm: 161.56, 161.49, 136.58, 136.42, 136.09, 133.22, 131.63, 129.22, 129.01, 126.83, 124.37, 122.37, 120.56, 45.73, 21.66. *Anal.* Calcd for $C_{18}H_{19}ClN_4OS$: 57.67 C; 5.11 H; 14.94 N. Found: 57.60 C; 5.09 H; 14.72 N.

4-Imidazolinyl-*N*-(benzothiazol-2-yl)benzamide hydrochloride (15b)

Yield 0.101g (39.1%), white powder, mp >300°C white powder. IR (KBr)/cm^{-1}): 2883, 1667, 1562; ^{1}H NMR (DMSO) σ/ppm: 13.17 (brs, 1H, NH), 11.12 (s, 2H, NH), 8.32 (d, 2H, *J*=8.32Hz), 8.25 (d, 2H, *J*=8.32Hz), 8.03 (d, 1H, *J*=7.92Hz), 7.79 (d, 1H, *J*=7.92Hz), 7.48 (t, 1H, *J*=7.89Hz), 7.35 (t, 1H, *J*=7.89Hz), 4.03 (s, 4H, C<u>H</u>$_2$); ^{13}C NMR (DMSO) σ/ppm: 165.82, 164.42, 159.65, 148.10, 137.59, 131.66, 129.43, 126.84, 126.05, 124.39, 122.38, 120.58, 44.94. *Anal.* Calcd for $C_{17}H_{15}ClN_4OS$: 56.90 C; 4.22 H; 15.61 N. Found: 56.76 C; 4.27 H; 15.4 N.

4-Imidazolinyl-*N*-(6-nitro-1H-benzimidazol-2-yl)benzamide hydrochloride (16)

Yield 0.201g (80%), pale yellow powder, mp >300°C. IR (KBr)/cm^{-1}) 3368, 2913, 2056, 1673, 1600; ^{1}H NMR (DMSO) σ/ppm: 12.34 (brs, 2H, N<u>H</u>), 9.98 (brs, 2H, N<u>H</u>), 8.37 (s, 1H), 8.23 (d, 2H, *J*=8.1Hz), 8.09 (d, 1H, *J*=8.6Hz), 8.04 (d, 2H, *J*=8.1Hz), 7.64 (d, 2H, *J*=8.6Hz), 4.27 (s, 4H, C<u>H</u>$_2$); MS *m/z*: 350.9; *Anal.*Calcd for $C_{17}H_{15}ClN_6O_3$: C 52.88; H 4.01; N 21.76. Found: C 52.79; H 3.91; N 21.73.

4-Imidazolinyl-*N*-(6-amino-1H-benzimidazol-2-yl)benzamide hydrochloride (**17**)

A solution of 4-imidazolinyl-*N*-(6-nitro-1H-benzimidazol-2-yl)benzamide (**16**) (0.190g, 0.54mmol) in MeOH (150mL) and 0.025g of 10% Pd-C was hydrogenated until the required quantity of H_2 was taken up. The solution was filtered trough Celite to remove the catalyst and the MeOH was removed under reduced pressure. The resulting solid was triturated with a small amount of MeOH and collected by filtration to afford 0.090g (51.36%) dark red powder, mp>300°C. IR (KBr)/cm^{-1}): 3137, 1650, 1564; ^{1}H NMR (DMSO) σ/ppm: 12.15 (brs, 2H, NH), 10.42 (brs, 2H, NH), 8.33 (d, 2H, *J*=8.45Hz), 8.08 (d, 2H, *J*=8.45Hz), 7.12 (d, 1H, *J*=8.44Hz), 6.62 (s, 1H), 6.50 (d, 1H, *J*=8.44Hz), 5.03 (brs, 2H, NH), 4.02 (s, 4H, CH_2); MS *m/z*: 320.9; *Anal.* Calcd for $C_{17}H_{17}ClN_6O$: C 57.33; H 4.80; N 23.56. Found: C 57.22; H 4.80; N 23.55.

X-Ray crystal structure determination

Crystal data of **9**. $C_{15}H_9N_3OS$, M_r = 279.3, monoclinic, space group *C* 2/c (No. 15), a = 30.257(3), b = 7.9545(8), c = 11.3393(14) Å, β = 103.26(1)°, V = 2656.35(65) Å^3, Z = 8, D_c = 1,40 g cm^{-3}, $F(000)$ = 1152. Colourless prisms, dimensions 0.10 x 0.30 x 0.65 mm, μ(MoK_α) = 0.241 mm^{-1}.

Data collection, analysis and refinement. An Oxford Diffraction Xcaliburg Kappa CCD X-Ray diffractometer with graphite-monochromated MoK_α (λ = 0.71073 Å) radiation was used to collect the diffraction data.[18] The data set were collected using the ω scan mode over the 2θ range up to 54°. The structure was solved by the direct methods and refined using SHELXS and SHELXL programs.[19,20] The structural refinement was performed on F^2 using all data. The hydrogen atoms were placed geometrically with isotropic thermal parameters calculated as 1.2xU_{eq} of corresponding non-H atom. All calculations were performed and the drawings were prepared using WINGX crystallographic suite of programs.[21]

In the final cycles of refinement a total of 2877 data with $I > 2\sigma(I)$ yielded an R value of 0.048 for 182 parameters; the maximum and minimum electron density residues amount 0.200 and -0.266 e Å^{-3}, respectively; S = 1.079.

The lists of atomic coordinates and equivalent isotropic thermal parameters, bond distances, selected valence bond angles, torsion angles and hydrogen bonding geometry are given in **Tables 2-5** as supplementary material.

Antitumor Activity Assay

The HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW 620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), Hep-2 (laryngeal carcinoma) and WI 38 (diploid fibroblasts) cells were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10%

fetal bovine serum (FBS), 2mM Lglutamine, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO2 at 37°C. The growth inhibition activity was assessed according to the slightly modified procedure performed at the National Cancer Institute, Developmental Therapeutics Program [22,23]. The cells were inoculated onto standard 96-well microtiter plates on day 0. The cell concentrations were adjusted according to the cell population doubling time (PDT): $1x10^4$/mL for HeLa, H 460, MiaPaCa-2 and SW 620 cell lines (PDT= 20-24hs) $2x10^4$/mL for MCF-7 cell lines (PDT= 33 hs) and $3x10^4$/mL for WI 38 (PDT = 47hs). Test agents were then added in five, 10-fold dilutions (10-8 to 10-4 mol/L) and incubated for further 72 hs. Working dilutions were freshly prepared on the day of testing. The solvent (DMSO) was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in working concentrations. After 72hs of incubation, the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. The absorbance (OD, optical density) was measured on a microplate reader at 570nm. The percentage of growth (PG) of the cell lines was calcd according to one or the other of the following two expressions:

If $(\text{mean } OD_{test} - \text{mean } OD_{tzero}) \geq 0$ then

$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero}) / (\text{mean } OD_{ctrl} - \text{meanOD}_{tzero})$.

If $(\text{mean } OD_{test} - \text{mean } OD_{tzero}) < 0$ then:

$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero}) / OD_{tzero}$.

Where:

Mean OD_{tzero} = the average of optical density measurements before exposure of cells to the test compound.

Mean OD_{test} = the average of optical density measurements after the desired period of time.

Mean OD_{ctrl} = the average of optical density measurements after the desired period of time with no exposure of cells to the test compound.

Each test point was performed in quadruplicate in three individual experiments. The results are expressed as IC50, which is the concentration necessary for 50% of inhibition. The IC50 values for each compound are calcd 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%). If however, for a given cell line all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g. PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each result is a mean value from three separate experiments.

SUPPLEMENTARY MATERIAL

Crystallographic data for **9** have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.ac.uk or www: http://www.ccdc.cam.ac.uk). These data can be obtained free of charge from the Director upon request

quoting the CCDC deposition number CCDC 615624.

Table 2 Atomic coordinates and equivalent isotropic thermal displacement parameters ($x10^4$ Å^2) of non-hydrogen atoms for **9**.

Atom	*x*	*y*	*z*	U_{eq}
S1	-0.00005(2)	0.21833(7)	-0.08871(4)	480(2)
O1	0.08540(5)	0.29251(22)	0.00723(12)	607(6)
N1	-0.03531(5)	0.23373(20)	0.09993(13)	412(4)
N2	0.04223(5)	0.29552(20)	0.14527(13)	417(5)
N3	0.26899(8)	0.54337(49)	0.54588(27)	1262(14)
C1	-0.05739(6)	0.17369(24)	-0.10883(16)	426(5)
C2	-0.08836(7)	0.13034(29)	-0.21541(17)	545(7)
C3	-0.13270(7)	0.10312(30)	-0.21052(18)	586(7)
C4	-0.14574(6)	0.11550(29)	-0.10100(19)	567(8)
C5	-0.11515(6)	0.15566(28)	0.00517(18)	511(7)
C6	-0.07017(6)	0.18813(23)	0.00148(15)	401(5)
C7	0.00230(5)	0.25222(23)	0.06416(15)	384(5)
C8	0.08236(6)	0.31567(24)	0.11127(16)	425(6)
C9	0.12230(5)	0.36895(23)	0.20761(15)	401(5)
C10	0.11825(6)	0.47345(26)	0.30122(18)	495(6)
C11	0.15632(7)	0.52225(30)	0.38691(20)	583(7)
C12	0.19858(6)	0.46383(30)	0.37952(19)	558(7)
C13	0.20310(6)	0.35999(31)	0.28473(19)	591(7)
C14	0.16504(6)	0.31373(27)	0.19892(17)	499(6)
C15	0.23805(8)	0.50948(40)	0.47224(25)	802(11)

Table 3 Bond distances (Å) for **9**.

	d **/ Å**		***d*** **/ Å**
S1 - C1	1.7339(19)	C3 - C4	1.3902(32)
S1 - C7	1.7394(18)	C4 - C5	1.3773(26)
O1 - C8	1.2182(24)	C5 - C6	1.3953(27)
N1 - C6	1.3965(21)	C8 - C9	1.4926(22)
N1 - C7	1.3011(23)	C9 - C10	1.3757(28)

N2 - C7	1.3837(20)	C9 - C14	1.3905(25)
N2 - C8	1.3650(25)	C10 - C11	1.3814(26)
N3 - C15	1.1348(34)	C11 - C12	1.3813(30)
C1 - C2	1.3922(24)	C12 - C13	1.3869(33)
C1 - C6	1.3961(27)	C12 - C15	1.4446(29)
C2 - C3	1.3725(31)	C13 - C14	1.3765(25)

Table 4 Selected bond and torsion angles (°) for **9**.

	Angles / °		**Angles / °**
C1 - S1 - C7	88.54(8)	C7 - S1 - C1 - C2	179.63(19)
C6 - N1 - C7	109.57(15)	C1 - S1 - C7 - N1	-0.18(15)
C7 - N2 - C8	122.55(15)	C1 - S1 - C7 - N2	179.28(15)
S1 - C1 - C2	128.29(15)	C7 - N1 - C6 - C5	179.26(18)
S1 - C1 - C6	109.91(14)	C6 - N1 - C7 - S1	0.23(20)
C2 - C1 - C6	121.79 (16)	C6 - N1 - C7 - N2	-179.22(15)
C1 - C2 - C3	118.31(19)	C8 - N2 - C7 - N1	179.85(17)
C2 - C3 - C4	120.35(19)	C7 - N2 - C8 - O1	-1.77(28)
C3 - C4 - C5	121.74(19)	C7 - N2 - C8 - C9	177.72(15)
C4 - C5 - C6	118.73(17)	S1 - C1 - C2 - C3	-178.84(16)
N1 - C6 - C1	115.00(15)	C6 - C1 - C2 - C3	0.68(30)
N1 - C6 - C5	125.96(17)	S1 - C1 - C6 - C5	-179.42(15)
C1 - C6 - C5	119.04(17)	C2 - C1 - C6 - C5	0.97(28)
S1 - C7 - N1	116.98(13)	C1 - C2 - C3 - C4	-1.30(32)
S1 - C7 - N2	122.11(12)	C2 - C3 - C4 - C5	0.27(33)
N1 - C7 - N2	120.90(15)	C3 - C4 - C5 - C6	1.40(31)
O1 - C8 - N2	121.76(17)	C4 - C5 - C6 - N1	178.60(18)
O1 - C8 - C9	121.52(15)	O1 - C8 - C9 - C10	146.23(20)
N2 - C8 - C9	116.72(15)	O1 - C8 - C9 - C14	-31.82(26)
C8 - C9 - C10	122.33(16)	N2 - C8 - C9 - C10	-33.26(25)
C8 - C9 - C14	117.90(15)	N2 - C8 - C9 - C14	148.69(17)
C9 - C10 - C11	120.31(19)	C10 - C9 - C14 - C13	1.42(29)
C10 - C11 - C12	119.73(19)	C9 - C10 - C11 - C12	-0.93(32)
C12 - C13 - C14	119.47(20)	C15 - C12 - C13 - C14	178.17(21)

Table 5 Hydrogen bonding geometry (Å, °) for 9.

D–H···*A*	*D*–H	*D*···*A*	H···*A*	*D*–H···*A*
N2-H1N2···N1^{i}	0.860	2.985(2)	2.181	155.55
C3-H3···N3^{ii}	0.930	3.596(3)	2.691	164.63
C11-H11···O1^{iii}	0.930	3.159(3)	2.471	130.87

Symmetry codes: (i) -*x*, *y*, 1/2+2-*z*; (ii) *x*-1/2, *y*-1/2, *z*-1; (iii) *x*, 1-*y*, *z*+1/2.

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