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Synthesis and Antiproliferative Activity of Cyano and Amidino Substituted 2-Phenylbenzothiazoles

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Summary. Series of cyano, dicyano, amidino, and diamidino substituted 2-phenylbenzothiazoles were prepared. Mono- and dicyano substituted benzothiazoles were obtained by condensation of appropriate substituted benzaldehydes with 2-aminothiophenol or 4-amino-3-mercaptobenzonitrile. The appropriate amidines or diamidines were prepared by *Pinner* reaction. The compounds were tested against breast, prostate, and lung cancer cell lines in a 72 h cytotoxicity assay. Many of the compounds had at 10 μM activity equivalent to 2-(4-aminophenyl)benzothiazole, while four compounds had significantly better activity, particularly in the breast cancer model.

Keywords. Heterocycles; Benzothiazoles; Amidines; Antitumor agents; Structure-activity relationship.

Introduction

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 have been studied as antitumor [1], antimicrobial [2], antifungal agents [3], and as imaging agents for β-amyloid [4]. On the other hand, the function of the amidinic group present in a variety of antimicrobial and antiparasitic agents is also well known [5].

Series of potent and selective antitumor agents derived from 2-(4-aminophenyl) benzothiazole have been extensively examined and developed during recent years. The fluorinated analogue, 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole is a

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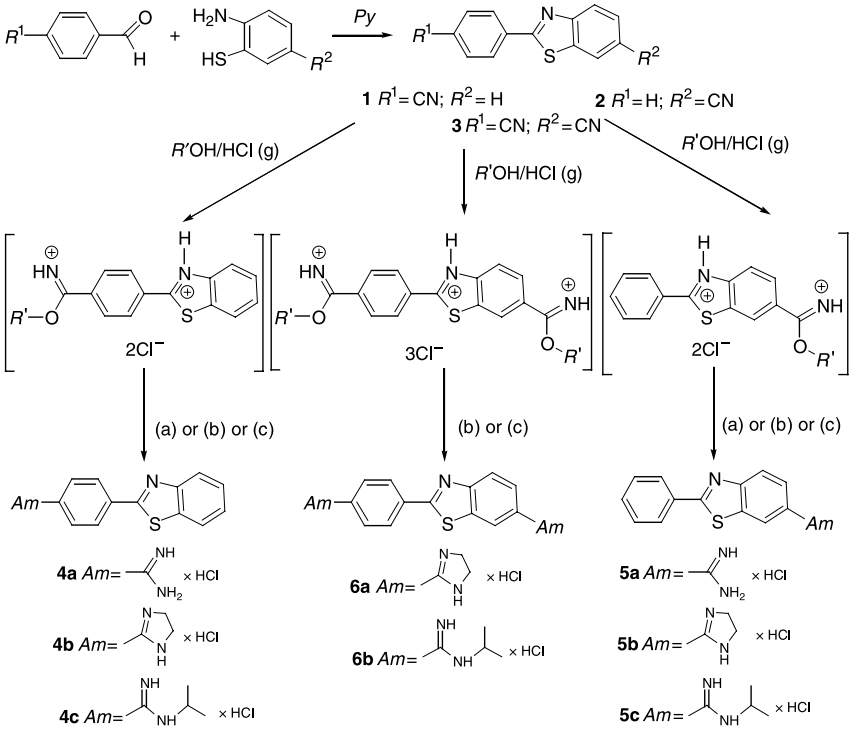
novel agent with potent and selective antitumor properties which in the form of its L-lysylamide prodrug, Phortress, is in an early phase of clinical studies [6]. Besides, two substituted 2-phenylbenzothiazole compounds enhanced reduction of B16 melanoma pulmonary metastases mediated by A-NK cells adoptive immunotherapy and can augment the anti-metastatic therapy [7].

In connection with our studies on synthesis and antitumor activity of amidino substituted heterocyclic molecules [8], we describe the synthesis and biological activity of a number of new cyano, dicyano, amidino, and diamidino substituted 2-phenylbenzothiazoles, structurally similar to the previously described 2-(4-aminophenyl)benzothiazole [9].

Results and Discussion

Synthesis

The synthesis route to nitriles **1**–**3**, monosubstituted amidines **4a**–**4c**, **5a**–**5c**, as well as disubstituted amidines **6a** and **6b** of 2-phenylbenzothiazoles is outlined in Scheme 1.



4a, **5a**; (a): i) K_2CO_3, NH_4Cl, *Et*OH/H_2O, reflux, 5 h; ii) HCl (aq)

4b, **5b**, **6a**; (b): i) $NH_2(CH_2)_2NH_2$, *Et*OH, reflux 24 h; ii) HCl (aq)

4c, **5c**, **6b**; (c): i) isopropylamine, *Et*OH, reflux 24 h

Scheme 1

The mononitriles 2-(4-cyanophenyl)benzothiazole (**1**) [10] and 6-cyano-2-phenylbenzothiazole (**2**) and the dinitrile, 6-cyano-2-(4-cyanophenyl)benzothiazole (**3**) [11], were synthesized from benzaldehyde or 4-cyanobenzaldehyde by condensation with 4-amino-3-mercaptobenzonitrile [12] or 2-aminothiophenol in pyridine followed by oxidation with alcoholic ferric chloride solution [13]. As previously developed [8] a *Pinner* reaction was used to transform the cyano into an amidino group. The imidate ester hydrochloride generated as an intermediate product was very hygroscopic and tended to hydrolyse fairly rapidly if left exposed to the atmosphere. Thus, it was immediately converted into imidazolinyl and isopropyl substituted amidines.

For preparing 2-(4-amidinophenyl)benzothiazole hydrochloride (**4a**) [14] and 6-amidino-2-phenylbenzothiazole hydrochloride (**5a**) the corresponding imidate ester hydrochloride was converted into the free base. Treating the solution of imidate ester with NH_4Cl in 75% ethanol the corresponding amidine was obtained. The hydrochloride salt was obtained from the amidine by treatment of the ethanol-xylene solution of the corresponding free base with HCl. In spite of an overall yield for the monoamidines **4a** and **5a** of about 60%, the same approach to prepare 6-amidino(4-amidinophenyl)benzothiazole [11] was unsuccessful. The attempted reaction of the corresponding imidate ester hydrochloride with ethylenediamine in ethanol gave the free bases 2-[(4-(imidazolin-2-yl)phenyl)]benzothiazole, 6-(imidazolin-2-yl)-2-phenylbenzothiazole, and 2-[(4-(imidazolin-2-yl)phenyl)]-6-(imidazolin-2-yl)benzothiazole. They were converted into their water-soluble hydrochlorides **4b**, **5b**, and **6a** with HCl in overall moderate to good yield. Analogous treatment of the corresponding imidate ester hydrochloride with isopropylamine gave 2-[4-(*N*-isopropylamidino)phenyl]benzothiazole hydrochloride (**4c**), 6-(*N*-isopropylamidino)-2-phenylbenzothiazole hydrochloride (**5c**), and 6-(*N*-isopropylamidino)-2-[4-(*N*-isopropylamidino)phenyl]benzothiazole dihydrochloride (**6b**). In spite of the excess of isopropylamine the hydrochlorides were isolated in a yield of about 50%.

The structures of the new compounds were confirmed by elemental analysis, IR, ^{1}H NMR, and ^{13}C NMR spectra.

Cytotoxicity

The compounds were tested against DU-145 (human metastatic prostate carcinoma), HT-1080 (human fibrosarcoma), MDA-MB-231 (human metastatic breast adenocarcinoma, and A549-Met2 (a metastatic variant of the human A549 lung carcinoma [*Brunson et al.*, manuscript in preparation]) at 10 μM for 72 h and analyzed for their effect on cell proliferation using an *MTT* assay. The results are shown in Table 1. A two-way ANOVA using a *Bonferroni* post-test was used to compare each drug to 2-(4-aminophenyl)benzothiazole (**7**), which was prepared as described by *Shi et al.* [9].

Most of the compounds inhibited cell proliferation to a similar extent as 2-(4-aminophenyl)benzothiazole. However, **4a**, **4b**, **5b**, and **6a** were more effective against the HT-1080 and MDA-MB-231 cell lines than 2-(4-aminophenyl)benzothiazole ($p < 0.001$). It can be seen that the antitumor activity depends on the kind and position of an amidino substituent on the 2-phenylbenzothiazole skeleton. It is interesting that the activity of imidazoline derivatives is fairly better than that of isopropylamidino derivatives, the best being the activity of **6a**. Further analysis

Table 1. Effect of novel benzothiazoles on cell number (Percent of Control Cell Number ± *SD*) after 72 h incubation

Comp.	Cell Line			
	A549-Met2	DU-145	HT-1080	MDA-MB-231
1	47 ± 6	47 ± 7	48 ± 5	36 ± 3
2	49 ± 5	45 ± 11	48 ± 7	39 ± 6
3	43 ± 8	33 ± 4	42 ± 9	35 ± 14
4a	42 ± 8	61 ± 10[a]	18 ± 18[a]	8 ± 6[a]
4b	43 ± 5	57 ± 6[a]	12 ± 9[a]	15 ± 6[a]
4c	50 ± 6	68 ± 6[a]	43 ± 6	28 ± 21
5a	44 ± 4	98 ± 15[a]	64 ± 22	23 ± 11[a]
5b	39 ± 5	64 ± 5[a]	16 ± 16[a]	11 ± 8[a]
5c	49 ± 4	67 ± 8[a]	36 ± 14	53 ± 10[a]
6a	4 ± 3[a]	56 ± 14[a]	4 ± 2[a]	2 ± 2[a]
6b	64 ± 17[a]	58 ± 10[a]	68 ± 4[a]	42 ± 10
7	42 ± 5	40 ± 4	44 ± 2	35 ± 4

[a] Significantly different ($p < 0.001$) than treatment of the cell line with 2-(4-aminophenyl)benzothiazole (**7**)

of other cell lines at various concentrations will provide a more complete picture of the possible anti-cancer activity of these novel compounds.

Experimental

Melting points were determined on a *Kofler* block apparatus. ^{1}H and ^{13}C NMR spectral data were determined with a Brucker Avance DPX 300 MHz NMR spectrometer with *TMS* as an internal standard. IR spectra were determined with a Nicolet Magna 760 infrared spectrophotometer in KBr pellets. Elemental analyses (C, H, N) were carried out in the Microanalitical Laboratory at the ≪Rudjer Boskovic Institute≫, Zagreb, and the results agreed with the calculated values within experimental errors.

2-(4-Cyanophenyl)benzothiazole (**1**) [10], 6-cyano-2-(4-cyanophenyl)benzothiazole (**3**) [11], and 2-(4-aminophenyl)benzothiazole (**7**) [9] were prepared according to established procedures.

Cell lines (HT-1080, DU-145, and MDA-MB-231) were obtained from the American Type Culture Collection (Manassas, VA). The A549-Met2 cell line was a generous gift of Dr. *K.W. Brunson* (UNT Health Science Center). Cell lines were cultured in RPMI 1640 (HT-1080), L-15 (MDA-MB-231), F-12K (A549-Met2), or DMEM (DU-145) medium containing 10% fetal bovine serum, 100 U/cm^3 penicillin, and 100 μg/cm^3 streptomycin sulfate (complete medium). All cell culture reagents were from Gibco BRL (Rockville, MD).

6-Cyano-2-phenylbenzothiazole (**2**, $C_{14}H_8N_2S$)

To 0.940 g benzaldehyde (10.0 mmol) dissolved in 25 cm^3 pyridine 1.65 g 4-amino-3-mercaptobenzonitrile (11.0 mmol) were added, and the stirred reaction mixture was refluxed for 4 h. The mixture was then poured into 200 cm^3 2 *M* HCl, and after cooling overnight the obtained crystalline product was filtered off, washed with water and dried. The crude product was dissolved in *Et*OH, gently boiled for 5 min with an *Et*OH solution of 2.7 g $FeCl_3 \cdot 6H_2O$, and poured in 200 cm^3 H_2O, and filtered off. Recrystallization from *Et*OH afforded 1.13 g (48%) **2**. Mp 240–242°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 8.79$ (d, $J = 1.4$ Hz, 1H, H-Bt), 8.23 (d, $J = 8.5$ Hz, 1H, H-Bt), 8.16 (dd, $J = 1.8$, 7.9 Hz, 2H, H-Ph), 7.97 (dd, $J = 1.6$, 8.5 Hz, 1H, H-Ph), 7.66–6.61 (m, 3H, H-Ph) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 107.5$ (s), 118.7 (s), 123.6 (d), 127.5 (d, 2C), 127.7 (d), 128.5 (s), 129.5 (d, 2C), 129.8 (d), 132.1 (s), 132.3 (d), 155.8 (s), 171.9 (s) ppm; IR (KBr): $\bar{\nu} = 2228$ (CN), 1508 (C=N) cm^{-1}.

2-(4-Amidinophenyl)benzothiazole hydrochloride hydrate (**4a**)
A suspension of 2.36 g **1** (10 mmol) in 100 cm^3 2-(2-ethoxyethoxy)ethanol was saturated with HCl gas at 5°C. The flask was then stoppered, and the content was stirred at room temp. for 5 d (until IR spectra indicated the disappearance of the cyano peak). The excess HCl was removed from the suspension by a stream of N_2. The reaction mixture was poured into 500 cm^3 dry ether and the crystals of imidate ester dihydrochloride were filtered off, washed with dry ether, and dried under reduced pressure over KOH. The corresponding crude imidate ester hydrochloride was poured into 200 cm^3 cold H_2O containing 30 cm^3 20% K_2CO_3 and the free base was extracted with $CHCl_3$. The solvent was evaporated and the residual oil was dissolved in 70 cm^3 *Et*OH and 0.588 g NH_4Cl (11 mmol) dissolved in 10 cm^3 H_2O were added. The mixture was heated under reflux for 5 h. After filtration (charcoal) the solvent was evaporated and the residue was dissolved in H_2O. The cold solution was made alkaline with 2 *M* NaOH and the crystals were filtered off, washed with H_2O, and dried. Free base was dissolved in 100 cm^3 *Et*OH-xylene mixture (*v*/*v*, 1/1) and to the obtained solution 1 cm^3 HCl was added by stirring. The stirring was continued overnight and after cooling for 2 d at 5°C the product was collected. Recrystallization from *Et*OH afforded 1.85 g (60%) **4a**. Mp >300°C (Ref. [14] 305°C); ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 9.67$ (brs, 2H, H-Am disappeared with D_2O), 9.47 (brs, 2H, H-Am disappeared with D_2O), 8.32 (d, $J = 8.5$ Hz, 2H, H-Ph), 8.23 (d, $J = 8.0$ Hz, 1H, H-Bt), 8.14 (d, $J = 8.0$ Hz, 1H, H-Bt), 8.06 (d, $J = 8.5$ Hz, 2H, H-Ph), 7.64–7.51 (m, 2H, H-Bt) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 122.5$ (d), 123.2 (d), 126.1 (d), 126.9 (d), 127.3 (d, 2C), 129.2 (d, 2C), 130.1 (s), 134.8 (s), 137.1 (s), 153.4 (s), 164.9 (s), 165.5 (s) ppm; IR (KBr): $\bar{\nu} = 3358$ and 3056 (NH), 1679, 1657, and 1611 (C=N) cm^{-1}.

2-[4-(Imidazolin-2-yl)phenyl]benzothiazole hydrochloride hydrate (**4b**, $C_{16}H_{13}N_3S \cdot HCl \cdot H_2O$)
A suspension of 2.36 g **1** (10 mmol) in 100 cm^3 2-(2-ethoxyethoxy)ethanol was saturated with HCl gas at 5°C. The flask was then stoppered, and the content was stirred at room temp. for 5 d (until IR spectra indicated the disappearance of the cyano peak). The excess HCl was removed from the suspension by a stream of N_2. The reaction mixture was poured into 500 cm^3 dry ether and the crystals of imidate ester dihydrochloride were filtered off, washed with dry ether, and dried under reduced pressure over KOH. To the suspension of crude imidate ester hydrochloride in 40 cm^3 dry *Et*OH, a solution of 1.2 g freshly distilled ethylenediamine (20.0 mmol) dissolved in 10 cm^3 dry *Et*OH was added. The reaction mixture was refluxed for 20 h. After evaporation of solvent the residue was suspended in H_2O, and basified with 2 *M* NaOH. The crude product was filtered off, washed with H_2O, and dried in vacuum at 80°C. The free base was dissolved in 100 cm^3 *Et*OH-xylene mixture (*v*/*v*, 1/1) and to the hot solution 1 cm^3 HCl was added by stirring. The stirring was continued overnight and after cooling for 2 d at 5°C the product was collected. Recrystallization from *Et*OH afforded 0.85 g (64%) **4b**. Mp >300°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 11.01$ (brs, 2H, H-Am disappeared with D_2O), 8.36 (d, $J = 8.3$ Hz, 2H, H-Ph), 8.26–8.22 (m, 3H, 1H-Bt + 2H-Ph), 8.14 (d, $J = 8.1$ Hz, 1H, H-Bt), 7.64–7.51 (m, 2H, H-Bt), 4.04 (s, 4H, H-CH_2) ppm; ^{13}C NMR (*DMSO*-d_6): $\delta = 45.0$ (t, 2C), 123.2 (d), 123.9 (d), 124.8 (s), 126.8 (d), 127.6 (d), 128.2 (d, 2C), 130.2 (d, 2C), 135.5 (s), 138.2 (s), 154.0 (s), 164.5 (s), 166.0 (s) ppm; IR (KBr): $\bar{\nu} = 3065$ and 2965 (NH), 1623 and 1606 (C=N) cm^{-1}.

2-[4-(N-Isopropylamidino)phenyl]benzothiazole hydrochloride hydrate
(**4c**, $C_{17}H_{17}N_3S \cdot HCl \cdot H_2O$)
A suspension of 0.94 g **1** (4 mmol) in 40 cm^3 2-(2-ethoxyethoxy)ethanol was saturated with HCl gas at 5°C. The flask was then stoppered, and the content was stirred at room temp for 5 d (until IR spectra indicated the disappearance of the cyano peak). The excess HCl was removed from the suspension by a stream of N_2. The reaction mixture was poured into 300 cm^3 dry ether and the crystals of imidate ester dihydrochloride were filtered off, washed with dry ether, and dried under reduced pressure over KOH. To the suspension of crude imidate ester hydrochloride in 20 cm^3 dry *Et*OH 0.59 g freshly distilled isopropylamine (10.0 mmol) dissoved in 5 cm^3 dry *Et*OH were added. The reaction mixture was refluxed for 24 h, solvent was evaporated, and the obtained crude product filtered off. Recrystallization from H_2O-acetone afforded 0.670 g (48%) **4c**. Mp >300°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 9.70$ (brs, 3H, H-Am disappeared with D_2O), 8.29 (d, $J = 7.5$ Hz, 2H, H-Ph), 8.23 (d, $J = 7.9$ Hz,

1H, H-Bt), 8.14 (d, $J = 8.0$ Hz, 1H, H-Bt), 7.94 (d, $J = 7.4$ Hz, 2H, H-Ph), 7.64–7.51 (m, 2H, H-Bt), 4.22 (m, 1H, H-CH), 1.31 (d, $J = 5.7$ Hz, 6H, H-CH_3) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 21.8$ (q, 2C), 45.8 (d), 123.1 (d), 123.8 (d), 126.7 (d), 127.5 (d), 127.7 (d, 2C), 130.2 (d, 2C), 131.9 (s), 135.3 (s), 137.1 (s), 154.0 (s), 161.5 (s), 166.6 (s) ppm; IR (KBr): $\bar{\nu} = 3204$ and 3046 (NH), 1671 and 1618 (C=N) cm^{-1}.

6-Amidino-2-phenylbenzothiazole hydrochloride hydrate (**5a**, $C_{14}H_{11}N_3S \cdot HCl \cdot H_2O$)
Compound **5a** was prepared by the method described for **4a** from 2.36 g **2** (10 mmol) and 0.588 g NH_4Cl (11 mmol). Recrystallization from *Et*OH afforded 1.94 g (63%) **5a**. Mp 274–278°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 9.68$ (brs, 2H, H-Am disappeared with D_2O), 9.49 (brs, 2H, H-Am disappeared with D_2O), 8.77 (d, $J = 1.6$ Hz, 1H, H-Bt), 8.27 (d, $J = 8.7$ Hz, 1H, H-Bt), 8.18 (dd, $J = 1.5$, 7.3 Hz, 2H, H-Ph), 8.00 (dd, $J = 1.8$, 8.6 Hz, 1H, H-Bt), 7.66–7.60 (m, 3H, H-Ph) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 122.9$ (d), 123.4 (d), 124.7 (s), 126.3 (d), 127.5 (d, 2C), 129.5 (d, 2C), 132.1 (s), 132.2 (d), 134.6 (s), 156.5 (s), 165.4 (s), 171.4 (s) ppm; IR (KBr): $\bar{\nu} = 3237$ and 2962 (NH), 1672 and 1594 (C=N) cm^{-1}.

6-(Imidazolin-2-yl)-2-phenylbenzothiazole hydrochloride hydrate (**5b**, $C_{16}H_{13}N_3S \cdot HCl \cdot H_2O$)
Compound **5b** was prepared by the method described for **4b** from 0.94 g **2** (4 mmol) and 1.2 g ethylenediamine (20.0 mmol). Recrystallization from H_2O afforded 0.867 g (65%) **5b**. Mp >300°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 11.09$ (brs, 2H, H-Am disappeared with D_2O), 8.99 (s, 1H, H-Bt), 8.28 (d, $J = 8.6$ Hz, 1H, H-Bt), 8.21 (d, $J = 8.7$ Hz, 1H, H-Bt), 8.15 (d, $J = 8.0$ Hz, 2H, H-Ph), 7.69–7.59 (m, 3H, H-Ph), 4.05 (s, 4H, H-CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 44.3$ (t, 2C), 118.8 (s), 123.2 (d), 123.8 (d), 126.7 (d), 127.6 (d, 2C), 129.5 (d, 2C), 132.0 (s), 132.3 (d), 134.7 (s), 156.7 (s), 164.2 (s), 171.9 (s) ppm; IR (KBr): $\bar{\nu} = 3068$ and 2969 (NH), 1613 (C=N) cm^{-1}.

6-(N-Isopropylamidino)-2-phenylbenzothiazole hydrochloride hydrate (**5c**, $C_{17}H_{17}N_3S \cdot HCl \cdot H_2O$)
Compound **5c** was prepared by the method described for **4c** from 0.94 g **2** (4 mmol) and 1.18 g isopropylamine (20.0 mmol). Recrystallization from H_2O afforded 0.812 g (58%) **5c**. Mp 292–294°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 9.63$ (brs, 3H, H-Am disappeared with D_2O), 8.65 (s, 1H, H-Bt), 8.26 (d, $J = 8.5$ Hz, 1H, H-Bt), 8.17 (d, $J = 7.8$ Hz, 2H, H-Ph), 7.88 (d, $J = 8.5$ Hz, 1H, H-Bt), 7.64–7.60 (m, 3H, H-Ph), 4.19 (m, 1H, H-CH), 1.31 (d, $J = 6.3$ Hz, 6H, H-CH_3) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 21.2$ (q, 2C), 45.2 (d), 122.7 (d), 123.5 (d), 126.0 (s), 126.6 (d), 127.5 (d, 2C), 129.5 (d, 2C), 132.1 (d), 132.2 (s), 134.4 (s), 156.1 (s), 161.3 (s), 171.1 (s) ppm; IR (KBr): $\bar{\nu} = 3192$ and 3056 (NH), 1665 and 1617 (C=N) cm^{-1}.

2-[4-(Imidazolin-2-yl)phenyl]-6-(imidazolin-2-yl)benzothiazole dihydrochloride dihydrate (**6a**, $C_{19}H_{17}N_5S \cdot 2HCl \cdot 2H_2O$)
Compound **6a** was prepared by the method described for **4b** from 1.045 g **3** (4 mmol) and 2.4 g ethylenediamine (40.0 mmol). Recrystallization from *Et*OH afforded 0.754 g (41%) **6a**. Mp >300°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 11.98$ (brs, 4H, H-Am disappeared with D_2O), 9.02 (s, 1H, H-Bt), 8.40 (d, $J = 8.4$ Hz, 2H, H-Ph), 8.33 (d, $J = 8.6$ Hz, 1H, H-Bt), 8.29 (d, $J = 8.4$ Hz, 2H, H-Ph), 8.22 (d, $J = 8.6$ Hz, 1H, H-Bt), 4.06 (s, 4H, H-CH_2), 4.05 (s, 4H, H-CH_2) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 45.1$ (t, 4C), 120.2 (s), 124.3 (d), 124.8 (d), 125.7 (s), 127.5 (d), 128.7 (d, 2C), 130.4 (d, 2C), 135.9 (s), 137.6 (s), 157.2 (s), 164.8 (s), 165.2 (s), 170.7 (s) ppm; IR (KBr): $\bar{\nu} = 3081$ and 2962 (NH), 1618 (C=N) cm^{-1}.

6-(N-Isopropylamidino)-2-[4-(N-isopropylamidino)phenyl]benzothiazole dihydrochloride dihydrate (**6b**, $C_{21}H_{25}N_5S \cdot 2HCl \cdot 2H_2O$)
Compound **6b** was prepared by the method described for **4c** from 1.045 g **3** (4 mmol) and 2.36 g isopropylamine (40.0 mmol). Recrystallization from H_2O-acetone afforded 0.742 g (38%) **6b**. Mp >300°C; ^{1}H NMR (300 MHz, *DMSO*-d_6): $\delta = 9.62$ (brs, 6H, H-Am disappeared with D_2O), 8.71 (d,

$J = 1.4$ Hz, 1H, H-Bt), 8.36 (d, $J = 8.4$ Hz, 2H, H-Ph), 8.31 (d, $J = 8.5$ Hz, 1H, H-Bt), 7.98 (d, $J = 8.4$ Hz, 2H, H-Ph), 7.91 (dd, $J = 1.4$, 8.6 Hz, 1H, H-Bt), 4.16 (m, 2H, H-CH), 1.31 (d, $J = 6.2$ Hz, 12H, H-CH_3) ppm; ^{13}C NMR (75 MHz, *DMSO*-d_6): $\delta = 21.1$ (q, 2C), 21.2 (q, 2C), 45.2 (d, 2C), 123.1 (d), 123.7 (d), 126.5 (s), 126.9 (d), 127.5 (d, 2C), 129.6 (d, 2C), 132.0 (s), 134.7 (s), 135.9 (s), 155.9 (s), 160.9 (s), 161.3 (s), 169.5 (s) ppm; IR (KBr): $\bar{\nu} = 3222$ and 3081 (NH), 1668 and 1619 (C=N) cm^{-1}.

Cytotoxicity Assay

A 10 *mM* stock solution of each compound was prepared in *DMSO*. A single cell suspension was prepared from each cell line and diluted to a concentration of 25000 cells/cm^3 in their respective complete medium. To aliquots of this suspension, compounds were added to a final concentration of 10 μM. Samples (200 mm^3) of each aliquot were placed in 96 well plates in replicates of five. Cells with no drug, but containing solvent (*DMSO*) were run as a control. The plates were incubated for 72 h at 37°C. Cell viability after incubation with the compounds was measured using *MTT* (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma, St. Louis, MO). Results were expressed as percent of control cell number and represent an average of 10 readings (2 sets of 5 replicates).

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