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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of benzothiazole derivatives and their biological evaluation as anticancer agents

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Abstract This article describes the synthesis and the biological evaluation of two sets of benzothiazole derivatives bearing at C-2 an arylamide (**1a–e**, **2a–e**) or an arylurea (**3a–d**, **4a–d**) moiety. Five compounds (**3d** and **4a–d**) were selected and screened by the National Cancer Institute for the in vitro primary anticancer assay against a panel of 60 human tumor cell lines. Compounds **4a** and **4c** showed interesting anticancer activities, more marked for compound **4c**. All compounds were also submitted to a preliminary in vitro assay as potential inhibitors of the ubiquitin-activating enzyme (E1), but they lacked significant activity.

Keywords Anticancer activity · Benzothiazole derivatives · Synthesis

Introduction

Nowadays, cancer is a disease of striking impact and the global research efforts in this field are focused both on the development of new potent antineoplastic agents and on the discovery of novel biological targets.

Benzothiazole derivatives have been extensively studied as potential drug candidates; a considerable attention has been directed to their anticancer properties (Wells *et al.*, 2000; Yoshida *et al.*, 2005; Brantley *et al.*, 2006; Lion *et al.*, 2006; Mortimer *et al.*, 2006; Song *et al.*, 2008; Aiello *et al.*, 2008). Therefore, compounds bearing the benzothiazole core in a more complex structure may be used to deepen the relationship between their structure and the antineoplastic activity. Besides the benzothiazole core, most of these compounds provided with an anticancer activity possess a second aromatic region directly linked to C-2 or through an amide or an urea moiety (Yoshida *et al.*, 2005; Song *et al.*, 2008; Das *et al.*, 2003). Other selected regions of interest for SAR evaluation and for improving the in vitro activity and metabolic stability, are usually obtained by introducing substituents both at the benzothiazole benzene ring and at the side aromatic portion. Moreover, a recent patent claimed to have identified a family of benzothiazoles as inhibitors of the ubiquitin-activating enzyme (E1) (Parlati *et al.*, 2005). Compounds which showed the most promising E1 inhibitory activity generally possess a methoxy group at C-6 of the benzothiazole and different substituents at the second phenyl ring (Parlati *et al.*, 2005; Guédat and Colland, 2007). On this basis, we started a new research program aimed at the identification of antitumor agents. For such a purpose, we planned the synthesis of two sets of benzothiazole derivatives endowed with a second benzene ring linked to the C-2 by means of an amide (**1**–**2**) or an urea (**3**–**4**) moiety. The methoxy and the trifluoromethoxy groups have been chosen as the substituents of the benzothiazole benzene ring (in both cases attached at C-6), whereas the substituents of the second benzene ring, attached at position 2′ and/or 4′, were chosen on the basis of their different steric and electronic properties. All synthesized compounds were submitted to the National Cancer Institute (NCI, Bethesda, MD) to evaluate their cytotoxic and/or growth inhibitory effects on 60 human cancer cell lines. Moreover, these compounds were tested in vitro as E1 inhibitors due to their structural similarity with some

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benzothiazole derivatives reported in the above-mentioned patent (Parlati *et al.*, 2005).

Results and discussion

Chemistry

New compounds **1a–e**, **2a–e**, **3a–d**, and **4a–d** were synthesized through the straightforward steps depicted in Scheme 1. The amide-linked compounds **1a–e** and **2a–e** were obtained by conjugating the 2-amino-6-substituted benzothiazole scaffold **7** or **8** with various aroyl chlorides in basic conditions. The urea-linked compounds **3a–d** and **4a–d** were obtained by reacting **5** or **6** with various aryl-isocyanates in dry dichloromethane at room temperature. The 2-amino-6-substituted benzothiazoles **7** and **8** were, in turn, obtained starting from the commercially available *p*-methoxy- or *p*-trifluoromethoxy-aniline following a reported procedure (Jimonet *et al.*, 1999; Sankaranarayanan *et al.*, 2009).

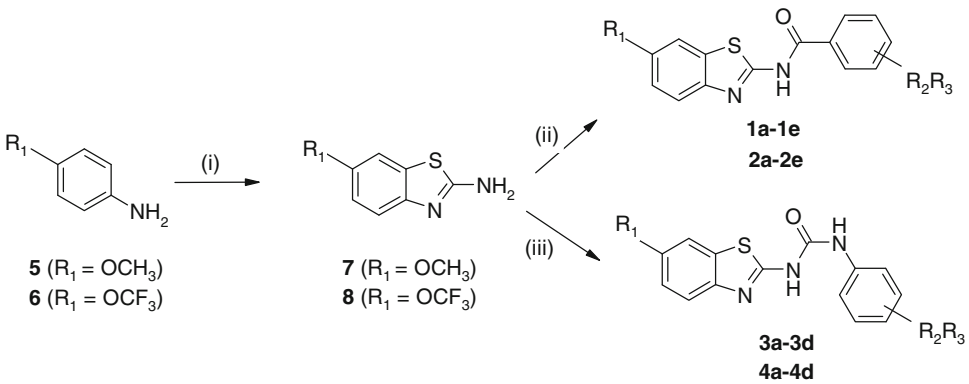
Biological activity

Anticancer activity assay

To evaluate the cytotoxic and/or growth inhibitory effects, all synthesized compounds were submitted for testing at the NCI. Five benzothiazole derivatives bearing an urea moiety at C-2 (**3d** and **4a–d**) were selected for a primary in vitro antitumor assay. Each compound was routinely tested, at 10^{-5} molar (M) concentration, against 60 human tumor cell lines derived from nine neoplastic diseases namely Leukemia (L, 6 cell lines), Non-Small Cell Lung (NSCLC, 9 cell lines), Colon Cancer (CC, 7 cell lines), Central Nervous System Cancer (CNSC, 6 cell lines), Melanoma (M, 9 cell lines), Ovarian Cancer (OC, 7 cell lines), Renal Cancer (RC, 8 cell lines), Prostate Cancer (PC, 2 cell lines), and Breast Cancer (BC, 6 cell lines) (Monks *et al.*, 1991; Boyd and Paull, 1995; Boyd, 1997).

The obtained results, expressed as % growth mean values against each subpanel, are reported in Table 1. Within the one dose, compounds **3d**, **4b**, and **4d** turned out to be essentially inactive, while remarkable low growth percent values were obtained for compounds **4a** and **4c**. Although limited, these data indicate that the presence of an electron withdrawing substituent at *para*-position of the phenyl ring positively influences the anticancer activity of these benzothiazol-2-ylurea derivatives.

As required by the pre-screening test, the compounds showing a percent growth lower than 75% were approved for the further screening test. In this step, the selected compounds **4a** and **4c** were additional evaluated at tenfold dilutions of five different concentrations (from 10^{-4} to 10^{-8} M) on the 60 human tumor cell line panels. The antitumor activity of the test compounds is expressed by three different dose–response parameters for each of the 60 cell lines considered: GI_{50} (molar concentration required



Scheme 1 (*i*) KSCN, CH_3COOH, Br_2; (*ii*) aroyl chloride, pyridine or NaH, DMF, N_2; (*iii*) aryl-isocyanate, CH_2Cl_2

Comp.	R_1	R_2	R_3	Comp.	R_1	R_2	R_3
1a, 3a	OCH_3	4-F	H	**2a, 4a**	OCF_3	4-F	H
1b, 3b	OCH_3	4-OCH_3	H	**2b, 4b**	OCF_3	4-OCH_3	H
1c, 3c	OCH_3	4-CN	H	**2c, 4c**	OCF_3	4-CN	H
1d, 3d	OCH_3	2-F	6-F	**2d, 4d**	OCF_3	2-F	6-F
1e	OCH_3	4-$NHCOCH_3$	H	**2e**	OCF_3	4-$NHCOCH_3$	H

Table 1 Anticancer activity of compounds **3d** and **4a–d** as % growth at 10^{-5} M

	3d	4a	4b	4c	4d
L	91.25	17.30	87.05	−17.36	64.44
NSCLC	99.19	5.63	95.83	−6.18	77.60
CC	106.93	0.24	101.11	−11.22	91.78
CNSC	104.44	21.07	102.95	2.60	96.05
M	102.11	12.01	104.21	−9.77	89.64
OC	108.27	12.93	97.47	8.95	90.21
RC	97.93	15.75	99.37	−7.46	89.37
PC	100.20	12.43	95.34	12.99	83.81
BC	106.79	19.22	97.84	−5.18	89.51

for half-growth inhibition), TGI (molar concentration leading to total growth inhibition), and LC_{50} (molar concentration required for 50% cell death). Furthermore, a mean graph midpoint (MG_MID) is calculated for each of the above-mentioned parameters, which displays an averaged activity parameter over all cell lines, as well as the Delta (Δ) parameter that is the difference between the highest and the average values (Boyd, 1997).

A general overview of the main parameters that characterize the anticancer activity of compounds **4a** and **4c** on the most sensitive tumor cell lines is reported in Table 2. It can be noticed that the highest activity is associated to compound **4c** (MG_MID: log GI_{50} −5.88, log TGI −5.08, log LC_{50} −4.3). Both tested compounds showed no selectivity on all 60 cell lines: the values of Δlog GI_{50} are equal to 0.18 (**4a**) and 0.61 (**4c**) (Δ < 1 is considered low).

The log GI_{50} values against the sixty human tumor cell lines for derivatives **4a** and **4c** are reported in Table 3 and compared with those of 5-fluorouracil (5-FU), the NCI standard anticancer agent. Both compounds possess log GI_{50} values lower than −5, showing a notable activity level; furthermore compound **4c** showed the log GI_{50} values lower than −6 for several cell lines, as Leukemia (K-562, RPMI-8226, SR with log GI_{50} −6.27, −6.00, −6.13, respectively), Non-Small Cell Lung Cancer (HOP-92, NCI-H23, NCI-H460, NCI-H522 with log GI_{50} −6.49, −6.24, −6.22, −6.37, respectively), Colon Cancer (HCT-116 with log GI_{50} −6.26), Melanoma (MALME-3 M, UACC-62 with log GI_{50} −6.07, −6.13, respectively), Ovarian Cancer (OVCAR-3 with log GI_{50} −6.24), Renal Cancer (A498, ACHN with log GI_{50} −6.33, −6.14, respectively), Breast Cancer (MCF7, MDA-MB-231/ATCC, T-47D, MDA-MB-468 with log GI_{50} −6.00, −6.03, −6.37, −6.34, respectively).

A comparison of the present data with those reported for 5-FU revealed the following: growth inhibition of all cell lines of 5-FU (MG_MID −6.05) is higher than that displayed by derivatives **4a** and **4c** (MG_MID −5.5 and −5.88, respectively). However, compounds **4a** and **4c** demonstrate higher activity than standard 5-FU against the following cell lines: CCRF-CEM, K-562 (Leukemia), SK-MEL-2, UACC-257 (Melanoma), EKVX, HOP-92, NCI-H226, NCI-H522 (Non-Small Cell Lung Cancer), SNB-19, SNB-75 (CNS Cancer), OVCAR-4, OVCAR-5, SK-OV-3 (Ovarian Cancer), RXF 393 (Renal Cancer), PC-3 (Prostate Cancer), as well as MDA-MB-231/ATCC, HS 578T, BT-549, T-47D (Breast Cancer).

Table 2 Overview of the results of the in vitro anticancer activity screening for compounds **4a** and **4c**

Comp	log GI_{50}[a]		log TGI[b]		log LC_{50}[c]		MG_MID[d] and Δ[e]			Most sensitive cell lines
	N[f]	Range[g]	N[f]	Range[g]	N[f]	Range[g]	log GI_{50}	log TGI	log LC_{50}	
4a	60	−5.68 to −5.35	48	−5.39 to −4.07	3	−5.10 to −4.14	−5.50, 0.18	−4.56, 0.83	−4.03, 1.07	Colon COLO 205, Breast MCF7, CNS SF-539, Ovarian OVCAR-3
4c	60	−6.49 to −5.37	59	−6.00 to −4.36	49	−5.25 to −4.07	−5.88, 0.61	−5.08, 0.92	−4.30, 0.95	Lung HOP-92, NCI-H522, Breast T-47D, MDA-MB-468, Renal A498

Data obtained from the NCI's in vitro disease-oriented human tumor cells

[a] The log of the molar concentration that inhibits 50% net cell growth

[b] The log of the molar concentration leading to total growth inhibition

[c] The log of the molar concentration leading to 50% net cell death

[d] MG_MID = mean graph midpoint = arithmetical mean value for all tested cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation

[e] The reported data represent the logarithmic difference between the parameter value referred to the most sensible cell line and the same mean parameter. Delta is considered low if <1, moderate if >1 and <3, high if >3

[f] Number of sensitive cell lines

[g] The values >−4.00 were excluded

Table 3 In vitro anticancer activity of compounds **4a** and **4c** against 60 human cancer cell lines

Panel/cell line	log GI_{50}		
	4a	**4c**	**5-FU**[a]
Leukemia			
CCRF-CEM	−5.40	−5.82	−5.01
HL-60(TB)	−5.52	−5.61	−5.64
K-562	−5.45	−6.27	−5.45
MOLT-4	−5.51	−5.73	−6.45
RPMI-8226	−5.60	−6.00	−7.35
SR	−5.43	−6.13	−7.62
Mean	−5.48	−5.93	−6.25
Non-Small Cell Lung Cancer			
A549/ATCC	−5.46	−5.92	−6.72
EKVX	−5.58	−5.87	−4.21
HOP-62	−5.42	−5.60	−6.40
HOP-92	−5.41	−6.49	−4.11
NCI-H226	−5.55	−5.70	−4.26
NCI-H23	−5.54	−6.24	−6.48
NCI-H322 M	−5.35	−5.62	−6.75
NCI-H460	−5.52	−6.22	−7.25
NCI-H522	−5.50	−6.37	−5.14
Mean	−5.48	−6.00	−5.70
Colon Cancer			
COLO 205	−5.68	−5.77	−6.80
HCC-2998	−5.41	−5.89	−7.28
HCT-116	−5.53	−6.26	−6.64
HCT-15	−5.52	−5.86	−6.96
HT29	−5.41	−5.68	−6.75
KM12	−5.57	−5.99	−6.67
SW-620	−5.43	−5.79	−6.03
Mean	−5.51	−5.89	−6.73
CNS Cancer			
SF-268	−5.43	−5.51	−5.80
SF-295	−5.59	−5.93	−6.64
SF-539	−5.65	−5.54	−7.20
SNB-19	−5.43	−5.82	−5.42
SNB-75	−5.46	−5.74	−4.10
U251	−5.40	−5.85	−6.04
Mean	−5.49	−5.73	−5.87
Melanoma			
LOX IMVI	−5.49	−5.83	−6.61
MALME-3 M	−5.53	−6.07	−7.29
M14	−5.36	−5.62	−6.01
MDA-MB-435	−5.53	−5.85	–
SK-MEL-2	−5.51	−5.75	−4.25
SK-MEL-28	−5.44	−5.64	−5.99
SK-MEL-5	−5.61	−5.94	−6.33
UACC-257	−5.45	−5.87	−5.45
UACC-62	−5.45	−6.13	−6.28
Mean	−5.48	−5.85	−6.03
Ovarian Cancer			
IGROV1	−5.41	−5.70	−5.91
OVCAR-3	−5.65	−6.24	−7.80
OVCAR-4	−5.53	−5.79	−5.35
OVCAR-5	−5.44	−5.37	−4.96
OVCAR-8	−5.37	−5.70	−5.76
NCI/ADR-RES	−5.47	−5.81	–
SK-OV-3	−5.59	−5.58	−4.66
Mean	−5.49	−5.74	−5.74
Renal Cancer			
786-0	−5.46	−5.70	−6.14
A498	−5.53	−6.33	−6.40
ACHN	−5.62	−6.14	−6.53
CAKI-1	−5.64	−5.80	−7.14
RXF 393	−5.57	−5.73	−5.58
SN12C	−5.59	−5.80	−6.30
TK-10	−5.39	−5.91	−5.95
UO-31	−5.46	−5.78	−5.85
Mean	−5.53	−5.90	−6.24
Prostate Cancer			
PC-3	−5.55	−5.87	−5.63
DU-145	−5.46	−5.57	−6.44
Mean	−5.50	−5.72	−6.03
Breast Cancer			
MCF7	−5.67	−6.00	−7.10
MDA-MB-231/ATCC	−5.48	−6.03	−5.18
HS 578T	−5.51	−5.75	−5.01
BT-549	−5.42	−5.56	−4.97
T-47D	−5.58	−6.37	−5.09
MDA-MB-468	−5.63	−6.34	–
Mean	−5.55	−6.01	−5.47
MG_MID[b]	−5.50	−5.88	−6.05

[a] NCI's data for 5-fluorouracil NSC 19893 (NCI standard compound)

[b] MG_MID = mean graph midpoint = arithmetical mean value for all tested cell lines

In vitro E1-assay

All compounds were tested in vitro for E1 inhibitory activity by evaluating their ability to block the ubiquitin displacement on the E2 enzyme, from E1. As showed in Fig. 1, all the compounds, tested for the first screening at 10 μM, did not show any considerable E1 inhibition. Thus, they were not elected for further assays on E1 inhibition.

Conclusion

To sum up, we synthesized two sets of benzothiazole-type compounds bearing a functionalized amide or urea moiety

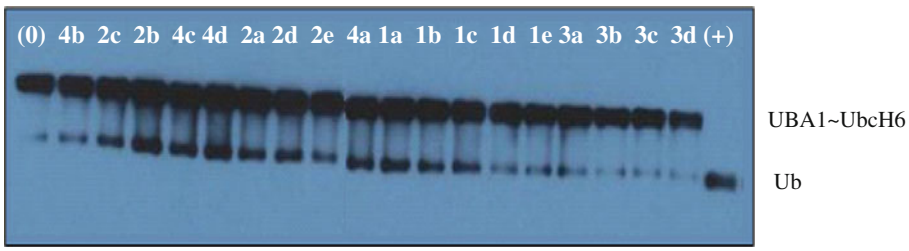


Fig. 1 In vitro activity of ubiquitin-activating enzyme UBA1 with and without test compounds. A 10 µl assay mix containing E1 (His_6-UBA1), E2 (His_6-UbcH6), Ub in the assay buffer was incubated with 10 µM compounds at room temperature for 1.5 h followed by Western Blot analysis using His_6 specific antibody. An analog of the Nedd8-activating enzyme inhibitor MLN4924 from the Millennium Pharmaceuticals was employed as positive control; this compound was a gift from OICR medical chemistry platform, Toronto, Canada. (0) UBA1 activity without test compounds. (+) UBA1 activity of positive control

at C-2 of the benzothiazole core and tested them in vitro for cytotoxic and/or growth inhibitory effects as well as inhibitors of the E1 activity. Among the selected compounds, urea-linked benzothiazoles **4a** and **4c**, both endowed with an electron withdrawing substituent at the *para*-position of the phenyl ring, showed significant growth inhibitory activity on several human tumor cell lines, particularly evident for compound **4c**. Moreover, none of the tested compounds showed inhibitory properties against the E1 enzyme.

Experimental

Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were carried out on a C. Erba Model 1106 (Elemental Analyzer for C, H, and N) and the results are within ±0.4% of the theoretical values. Merck Silica Gel 60 F_{254} plates were used as analytical TLC; flash column chromatography was performed on Merck Silica Gel (200–400 mesh) or was conducted using prepacked cartridges on a MP-LC BUCHI system. ^{1}H-NMR spectra were recorded in $CDCl_3$, acetone-d_6 or DMSO-d_6 on a Varian Gemini 300 spectrometer at 300 MHz; chemical shifts are expressed in δ [ppm] relative to TMS as internal standard and coupling constants J in Hz. All exchangeable protons were confirmed by addition of D_2O. Compounds **7** and **8** were prepared according to the procedures reported by Jimonet et al. (1999) and Sankaranarayanan et al. (2009).

*General procedures for the synthesis of compounds **1a–e***

2-Amino-6-methoxybenzothiazole **5** (100 mg, 0.55 mmol) was dissolved in pyridine (5 ml) and reacted with the appropriate benzoyl chlorides (0.83 mmol, 1.5 equiv) at 60°C for 12 h under stirring. Then, the mixture was diluted with ethyl acetate (30 ml) and washed with water (3 × 40 ml). The organic layer was dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash chromatography (*c*-hexane/EtOAc 5:5), to afford the corresponding amide-linked benzothiazoles as a white powder.

4-Fluoro-N-(6-methoxybenzothiazol-2-yl)benzamide ***(1a)*** Yield: 34%. Mp 178–190°C. $R_f = 0.60$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 3.81 (s, 3H), 7.05 (dd, 1H, $J_o = 8.5$ Hz, $J_m = 2.5$ Hz, H-5), 7.32 (d, 1H, $J_m = 2.7$, H-7), 7.56 (d, 1H, $J_o = 8.8$ Hz, H-4), 7.82 (d, 2H, $J = 8.3$ Hz, H-3′,5′), 8.11 (d, 2H, $J = 8.5$ Hz, H-2′,6′), 9.65 (br s, 1H, NH). Anal. Calcd. for $C_{15}H_{11}FN_2O_2S$: C, 59.59; H, 3.67; N, 9.27. Found: C, 59.71; H, 3.43; N, 9.41.

4-Methoxy-N-(6-methoxybenzothiazol-2-yl)benzamide ***(1b)*** Yield: 46%. Mp 202–203°C. $R_f = 0.67$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 3.81 (s, 3H), 3.85 (s, 3H), 6.98 (m, 3H, H-5, H-3′,5′), 7.28 (d, 1H, $J_m = 1.1$ Hz, H-7), 7.57 (d, 1H, $J_o = 8.8$ Hz, H-4), 8.10 (d, 2H, $J = 6.4$ Hz, H-2′,6′), 11.35 (br s, 1H, NH). Anal. Calcd. for $C_{16}H_{14}N_2O_3S$: C, 61.13; H, 4.49; N, 8.91. Found: C, 61.34; H, 4.77; N, 9.13.

4-Cyano-N-(6-methoxybenzothiazol-2-yl)benzamide ***(1c)*** Yield: 70%. Mp 243–245°C. $R_f = 0.82$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 3.81 (s, 3H), 7.05 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2.5$ Hz, H-5), 7.33 (d, $J_m = 2.5$ Hz, H-7), 7.57 (d, 1H, $J_o = 8.8$ Hz, H-4), 7.82 (d, 2H, $J_o = 8.3$ Hz, H-3′,5′), 8.11 (d, 2H, $J = 8.3$ Hz, H-2′,6′), 9.13 (br s, 1H, NH). Anal. Calcd. for $C_{16}H_{11}N_3O_2S$: C, 62.12; H, 3.58; N, 13.58. Found: C, 62.35; H, 3.29; N, 13.77.

2,6-Difluoro-N-(6-methoxybenzothiazol-2-yl)benzamide ***(1d)*** Yield: 43%. Mp 209–211°C. $R_f = 0.57$, (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 3.85 (s, 3H), 6.85 (m, 3H, H-5, H-3′,5′), 7.02 (d, 1H, $J_o = 8.8$, H-4), 7.30 (m, 2H, H-4′, H-7), 11.15 (br s, 1H, NH)). Anal. Calcd. for $C_{15}H_{10}F_2N_2O_2S$: C, 56.25; H, 3.15; N, 8.75. Found: C, 56.38; H, 2.97; N, 8.89.

*4-Acetylamino-N-(6-methoxybenzothiazol-2-yl)benzamide (**1e**)* Yield: 37%. Mp 225–228°C. $R_f = 0.26$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 2.23 (s, 3H), 3.82 (s, 3H), 7.05 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 2.5$ Hz, H-5), 7.32 (d, 1H, $J_m = 2.5$ Hz, H-7), 7.58 (d, 1H, $J_o = 8.8$ Hz, H-4), 7.82 (d, 2H, $J_o = 8.8$ Hz, H-3′,5′), 8.17 (d, 2H, $J_o = 8.8$ Hz, H-2′,6′), 10.28 (br s, 1H, NH). Anal. Calcd. for $C_{17}H_{15}N_3O_3S$: C, 59.81; H, 4.43; N, 12.31. Found: C, 60.08; H, 4.21; N, 12.51.

*General procedures for the synthesis of compounds **2a–e***

To a solution of 2-amino-6-trifluoromethoxybenzothiazole **6** (100 mg, 0.42 mmol) in DMF (4 ml), NaH (13 mg, 0.42 mmol) was slowly added and the reaction mixture was stirred vigorously for further 10 min at room temperature. To the resulting suspension, the appropriate benzoyl chloride (0.64 mmol, 1.5 equiv) in DMF (0.5 ml) was added, and the mixture was stirred for 6 h at room temperature. The reaction mixture was quenched by addition of water and diluted with ethyl acetate (40 ml). The organic layer was washed with water (3 × 40 ml) and dried over $MgSO_4$. After filtration and concentration, the crude product was purified by flash chromatography (*c*-hexane/EtOAc 5:5) to afford the desired amide in 35–70% yield.

*4-Fluoro-N-(6-trifluoromethoxybenzothiazol-2-yl)benzamide (**2a**)* Yield: 35%. Mp 199–200°C. $R_f = 0.77$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 7.23 (dd, 2H, $J_{H-H} = 8.8$ Hz, $J_{H-F} = 8.2$ Hz, H-2′,6′), 7.28 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 1.1$ Hz, H-5), 7.56 (d, 1H, $J_o = 8.8$ Hz, H-4), 7.74 (d, 1H, $J_m = 1.1$ Hz, H-7), 8.20 (dd, 2H, $J_{H-H} = 9.1$ Hz, $J_{H-F} = 5.1$ Hz, H-3′,5′), 10.15 (br s, 1H, NH). Anal. Calcd for $C_{15}H_8F_4N_2O_2S$: C, 50.57; H, 2.26; N, 7.86. Found C, 50.67; H, 2.04; N, 7.98.

*4-Methoxy-N-(6-trifluoromethoxybenzothiazol-2-yl)benzamide (**2b**)* Yield: 51%. Mp 270–271°C. $R_f = 0.66$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 3.81 (s, 1H) 6.85 (d, 2H, $J = 8.2$ Hz, H-3′,5′), 7.30 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 1.1$ Hz, H-5), 7.65 (d, 1H, $J_o = 8.8$ Hz, H-4), 7.54 (d, 1H, $J_m = 1.1$ Hz, H-7), 7.95 (d, 2H, $J = 8.22$ Hz, H-2′,6′), 11.35 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{11}F_3N_2O_3S$: C, 52.17; H, 3.01; N, 7.61. Found C, 52.39; H, 2.83; N, 7.85.

*4-Cyano-N-(6-trifluoromethoxybenzothiazol-2-yl)benzamide (**2c**)* Yield: 58%. Mp 259–260°C. $R_f = 0.72$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 7.24 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 1.1$ Hz, H-5), 7.30 (d, 1H, $J_o = 8.8$ Hz, H-4), 7.78 (m, 3H, H-7, 3′,5′), 8.13 (d, 2H, $J = 8.22$ Hz, H-2′,6′), 11.35 (br s, 1H, NH). Anal. Calcd for $C_{16}H_8F_3N_3O_2S$: C, 52.90; H, 2.22; N, 11.57. Found: C, 52.73; H, 2.55; N, 11.31.

*2,6-Difluoro-N-(6-trifluoromethoxybenzothiazol-2-yl)benzamide (**2d**)* Yield: 43%. Mp 250–251°C. $R_f = 0.78$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR ($CDCl_3$): 7.16 (m, H-4′), 7.38 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 1.1$ Hz, H-5), 7.48 (d, 1H, $J_o = 8.8$ Hz, H-4), 7.52 (d, 1H, $J_m = 1.1$ Hz, H-7), 7.75 (m, H-3′,5′), 11.15 (br s, 1H, NH). Anal. Calcd for $C_{15}H_7F_5N_2O_2S$: C, 48.14; H, 1.89; N, 7.48. Found: C, 48.37; H, 1.76; N, 7.66.

*4-Acetylamino-N-(6-trifluoromethoxybenzothiazol-2-yl)benzamide (**2e**)* Yield 41%. Mp 297–298°C. $R_f = 0.17$ (*c*-hexane/EtOAc 5:5). ^{1}H NMR (DMSO-d_6): 2.23 (s, 3H), 6.63 (br s, 1H, NH), 7.58 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 1.1$ Hz, H-5), 7.65 (d, 2H, $J = 8.22$ Hz, H-2′,6′), 7.93 (d, 1H, $J_o = 8.8$ Hz, H-4), 8.25 (m, H-7, 3′,5′), 10.28 (br s, 1H, NH); Anal. Calcd for $C_{17}H_{12}F_3N_3O_3S$: C, 51.65, H, 3.06, N, 10.63. Found: C, 51.88, H, 2.93, N, 10.86.

*General procedures for the synthesis of compounds **3a–d***

2-Amino-6-methoxybenzothiazole **5** (100 mg, 0.55 mmol) was dissolved in dry CH_2Cl_2 (5 ml) and reacted with the appropriate aryl-isocyanates (0.83 mmol, 1.5 equiv) at room temperature under stirring. After 3 h, the desired product was obtained as a precipitate which was collected by filtration under *vacuum*, purified by washing with ethyl ether, and air-dried to give a white powder.

*1-(4-Fluorophenyl)-3-(6-methoxybenzothiazol-2-yl)urea (**3a**)* Yield: 60%. Mp > 300°C. $R_f = 0.41$ (Et_2O). ^{1}H NMR (DMSO-d_6): 7.15 (d, 1H, $J_m = 1.1$ Hz, H-7), 7.33 (m, 2H, H-3′,5′), 7.68 (m, 4H, H-4, H-5, H-2′,6′), 9.32 (br s, 1H, NH), 10.80 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{12}FN_3O_2S$: C, 56.77; H, 3.81; N, 13.24. Found: C, 57.09; H, 3.68; N, 13.42.

*1-(4-Methoxyphenyl)-3-(6-methoxybenzothiazol-2-yl)urea (**3b**)* Yield: 73%. Mp >300°C. $R_f = 0.32$ (Et_2O). ^{1}H NMR (DMSO-d_6): 3.80 (s, 3H), 3.85 (s, 3H), 7.10 (d, 2H, $J = 6.75$, H-3′,5′), 7.54 (dd, 1H, $J_o = 8.24$ Hz, $J_m = 1.1$ Hz, H-5), 7.71 (d, 2H, $J = 6.75$, H-2′,6′), 7.98 (d, 1H, $J = 8.25$, H-4), 8.05 (d, 1H, $J_m = 1.1$ Hz, H-7), 9.25 (br s, 1H, NH), 10.90 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.35; H, 4.59; N, 12.76. Found: C, 58.61; H, 4.32; N, 12.98.

*1-(4-Cyanophenyl)-3-(6-methoxybenzothiazol-2-yl)urea (**3c**)* Yield: 85%. Mp 275–276°C. $R_f = 0.48$ (Et_2O). ^{1}H NMR (DMSO-d_6): 3.80 (s, 3H), 7.45 (dd, 1H, $J_o = 8.24$ Hz, $J_m = 1.1$ Hz, H-5) 8.0 (m, 5-H, H-3′,5′, H-2′,6′, H-4), 8.15 (d, 1H $J_m = 1.1$ Hz, H-7), 9.59 (br s, 1H, NH), 10.20 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{12}N_4O_2S$: C, 59.25; H, 3.73; N, 17.27. Found: C, 59.49; H, 3.59; N, 17.42.

*1-(2,6-Difluorophenyl)-3-(6-methoxybenzothiazol-2-yl)urea (**3d**)* Yield: 78%. Mp >300°C. $R_f = 0.59$ (Et_2O). ^1H NMR (DMSO-d_6): 3.80 (s, 3H), 7.15 (m, 2H, H-5, H-4′), 7.44 (m, 2H, H-3′,5′), 7.71 (d, 1H, $J_o = 8.24$, H-4), 8.05 (d, 1H, $J_m = 1.1$ Hz, H-7), 8.65 (br s, 1H, NH), 10.70 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{11}F_2N_3O_2S$: C, 53.73; H, 3.31; N, 12.53. Found: C, 53.96; H, 3.49; N, 12.25.

*General procedures for the synthesis of compounds **4a–d***

To a solution of 2-amino-6-trifluoromethoxybenzothiazole **6** (100 mg, 0.42 mmol) in anhydrous CH_2Cl_2 (6 ml) was added the appropriate aryl-isocyanate (0.64 mmol, 1.5 equiv) and stirred at room temperature for 5 h. The precipitate was filtered off, washed with dichloromethane, and air-dried to give the corresponding ureido compounds, as a white powder.

*1-(4-Fluorophenyl)-3-(6-trifluoromethoxybenzothiazol-2-yl) urea (**4a**)* Yield: 91%. Mp 252–254°C. $R_f = 0.43$; (*c*-hexane/EtOAc 5:5). ^1H NMR (acetone-d_6): 7.30 (dd, 2H, $J_{H-H} = 9.1$ Hz, $J_{H-F} = 8.8$ Hz, H-2′,6′), 7.53 (dd, 1H, $J_o = 8.8$ Hz, $J_m = 1.1$ Hz, H-5), 7.80 (dd, 2H, $J_{H-H} = 9.1$ Hz, $J_{H-F} = 4.9$ Hz, H-3′,5′), 7.92 (d, 1H, $J_o = 8.8$ Hz, H-4), 8.12 (d, 1H, $J_m = 1.1$ Hz, H-7), 9.15 (br s, 1H, NH), 10.40 (br s, 1H, NH). Anal. Calcd for $C_{15}H_9F_4N_3O_2S$: C, 48.52; H, 2.44; N, 11.32. Found: C, 48.75; H, 2.25; N, 11.52.

*1-(4-Methoxy)-3-(6-trifluoromethoxybenzothiazol-2-yl)urea (**4b**)* Yield: 84%. Mp 220–221°C. $R_f = 0.45$ (*c*-hexane/EtOAc 5/5). ^1H NMR (acetone-d_6): 3.96 (s, 3H), 7.10 (d, 2H, $J = 6.69$, H-3′,5′), 7.54 (dd, 1H, $J_o = 8.24$ Hz, $J_m = 1.1$ Hz, H-5), 7.66 (d, 2H, $J = 6.69$, H-2′,6′), 7.91 (d, 1H, $J = 8.24$, H-4), 8.11 (d, 1H, $J_m = 1.1$ Hz, H-7), 8.90 (br s, 1H, NH), 10.28 (br s, 1H, NH). Anal. Calcd for $C_{16}H_{12}F_3N_3O_3S$: C, 50.13; H, 3.16; N, 10.96. Found: C, 50.53; H, 3.04; N, 11.14.

*1-(4-Cyanophenyl)-3-(6-trifluoromethoxybenzothiazol 2-yl) urea (**4c**)* Yield: 93%. Mp 265–266°C. $R_f = 0.27$ (*c*-hexane/EtOAc 5:5). ^1H NMR (acetone-d_6): 7.56 (dd, 1H, $J_o = 8.24$ Hz, $J_m = 1.1$ Hz, H-5) 8.0 (m, H-5, H-3′,5′, H-2′,6′, H-4), 8.15 (d, 1H $J_m = 1.1$ Hz, H-7), 9.59 (br s, 1H, NH), 10.20 (br s, 1H, NH). Anal. Calcd for $C_{16}H_9F_3N_4O_2S$: C, 50.80; H, 2.40; N, 14.81. Found: C, 50.98; H, 2.18; N, 15.23.

*1-(2,6-Difluorophenyl)-3-(6-trifluoromethoxybenzothiazol-2-yl)urea (**4d**)* Yield: 81%. Mp 276–280°C. $R_f = 0.62$ (*c*-hexane/EtOAc 5:5). ^1H NMR (acetone-d_6): 7.30 (m, 2H, H-5, H-4′), 7.56 (m, 2H, H-2′,6′), 7.92 (d, 1H, $J = 8.24$, H-4) 8.65 (br s, 1H, NH), 10.74 (br s, 1H, NH). Anal. Calcd for $C_{15}H_8F_5N_3O_2S$: C, 46.28; H, 2.07; N, 10.79. Found: C, 46.44; H, 1.88; N, 10.95.

Pharmacology

In vitro antitumor screening

The evaluation of cytotoxic and/or growth inhibitory effects was performed at the National Cancer Institute (NCI) of Bethesda, USA, following an in vitro screening program, based upon the use of multiple panels of 60 human tumor cell lines derived from nine neoplastic diseases. The synthesized benzothiazoles were preliminarily tested at single 10^{-5} M concentration. Results for each test agent are expressed as percent growth of the treated cells (compared to untreated control cells). Compounds having percent growth lower than 75%, were further evaluated in the full panel of 60 cell lines at five different concentrations ranging from 10^{-4} to 10^{-8} M. The percent growth was evaluated spectrophotometrically versus controls (not treated with test agents). A 48 h continuous drug exposure protocol was followed and a sulforhodamine B (SRB) protein assay was used to estimate cell viability of growth (Monks *et al.*, 1991; Boyd and Paull, 1995; Boyd, 1997).

In vitro E1 assays

The in vitro assays to demonstrate E1-inhibition were performed at the Laboratory of Princess Margaret Hospital, Toronto, Canada. His_6-tagged E1, His_6-tagged E2, and ubiquitin were incubated with compounds **1a–e**, **2a–e**, **3a–d**, and **4a–d** at a concentration of 10 μM at room temperature for 1.5 h to evaluate their effect on E2 loading of ubiquitin by E1. The products were resolved on nonreducing 4 to 20% gradient SDS-PAGE, followed by immunoblotting with anti-His antibody, then fluorescent dye–labeled secondary antibody. Fluorescent bands were detected by a gel imager (LI-COR) (Xu *et al.*, 2010).

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References

Aiello S, Wells G, Stone EL, Kadri H, Bazzi R, Bell DR, Stevens MFG, Matthews CS, Bradshaw TD, Westwell AD (2008) Synthesis and biological properties of benzothiazole, benzoxazole, and chromen-4-one analogues of the potent antitumor agent 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610, NSC 721648). J Med Chem 51:5135–5139

Boyd MR (1997) Preclinical screening, clinical trials, and approval. Humana Press, Totowa, NJ

Boyd MR, Paull KD (1995) Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen. Drug Dev Res 34:91–109

Brantley E, Antony S, Kohlhagen G, Meng LH, Agama K, Stinson SF, Sausville EA, Pommier Y (2006) Anti-tumor drug candidate 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole induces single-strand breaks and DNA-protein cross-links in sensitive MCF-7 breast cancer cells. Cancer Chemother Pharmacol 58:62–72

Das J, Moquin RV, Lin J, Liu C, Doweyko AM, DeFex HF, Fang Q, Pang S, Pitt S, Shen DR, Schieven GL, Barrish JC, Wityak J (2003) Discovery of 2-amino-heteroaryl-benzothiazole-6-anilides as potent $p56^{lck}$ inhibitors. Bioorg Med Chem Lett 13: 2587–2590

Guédat P, Colland F (2007) Patented small molecule inhibitors in the ubiquitin proteasome system. BMC Biochemistry 8(Suppl 1): S14

Jimonet P, Audiau F, Barreau M, Blanchard JC, Boireau A, Bour Y, Coléno MA, Doble A, Doerflinger G, Huu GD, Donat MH, Duchesne JM, Ganil P, Guérémy C, Honoré H, Just B, Kerphirique R, Gontier S, Hubert P, Laduron PM, Le Blevec J, Meunier M, Miquet JM, Nemecek C, Pasquet M, Piot O, Pratt J, Rataud J, Reibaud J, Stutzmann JM, Magnani S (1999) Riluzole series. Synthesis and in vivo "antiglutamate" activity of 6-substituted-2-benzothiazolamines and 3-substituted-2-iminobenzothiazolines. J Med Chem 42:2828–2843

Lion CJ, Matthews CS, Wells G, Bradshaw TD, Stevens MFG, Westwell AD (2006) Antitumour properties of fluorinated benzothiazole-substituted hydroxycyclohexa-2,5-dienones ("quinols"). Bioorg Med Chem Lett 16:5005–5008

Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M (1991) Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst 83:757–766

Mortimer CG, Wells G, Crochard JP, Stone EL, Bradshaw TD, Stevens MFG, Westwell AD (2006) Antitumor benzothiazoles. 26. 2-(3,4-Dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, NSC 721648), a simple fluorinated 2-arylbenzothiazole, shows potent and selective inhibitory activity against lung, colon, and breast cancer cell lines. J Med Chem 49:179–185

Parlati F, Ramesh UV, Singh R, Payan DG, Lowe R, Look GC Benzothiazole and thiazole[5,5-b] pyridine compositions and their use as ubiquitin ligase inhibitors Publication No. WO 2005/037845

Sankaranarayanan A, Raman G, Busch C, Schultz T, Zimin PI, Hoyer J, Kohler R, Wulff H (2009) Naphtho[1, 2-d] thiazol-2-ylamine (SKA-31), a new activator of KCa2 and KCa3.1 potassium channels, potentiates the endothelium-derived hyperpolarizing factor response and lowers blood pressure. Mol Pharmacol 75:281–295

Song EY, Kaur N, Park MY, Jin Y, Lee K, Kim G, Lee KY, Yang YS, Shin JH, Nam KY, No KT, Ha G (2008) Synthesis of amide and urea derivatives of benzothiazole as Raf-1 inhibitor. Eur J Med Chem 43:1519–1524

Wells G, Bradshaw TD, Diana P, Seaton A, Shi D, Westwell AD, Stevens MFG (2000) Antitumour benzothiazoles. Part 10: The synthesis and antitumour activity of benzothiazole substituted quinol derivatives. Bioorg Med Chem Lett 10:513–515

Xu GW, Ali M, Wood TE, Wong D, Maclean N, Wang X, Gronda M, Skrtic M, Li X, Hurren R, Mao X, Venkatesan M, Beheshti Zavareh R, Ketela T, Reed JC, Rose D, Moffat J, Batey RA, Dhe-Paganon S, Schimmer AD (2010) The ubiquitin-activating enzyme E1 as a therapeutic target for the treatment of leukemia and multiple myeloma. Blood 115:2251–2259

Yoshida M, Hayakawa I, Hayashi N, Agatsuma T, Oda Y, Tanzawa F, Iwasaki S, Koyama K, Furukawa H, Kurakata S, Sugano Y (2005) Synthesis and biological evaluation of benzothiazole derivatives as potent antitumor agents. Bioorg Med Chem Lett 15:3328–3332