Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 1519–1524
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of amide and urea derivatives of benzothiazole as Raf-1 inhibitor

Eun Young Song [a], Navneet Kaur [a], Mi-Young Park [a], Yinglan Jin [a], Kyeong Lee [a,*], Guncheol Kim [b], Ki Youn Lee [b], Jee Sun Yang [c], Jae Hong Shin [d], Ky-Youb Nam [d], Kyoung Tai No [c,d], Gyoonhee Han [c,**]

[a] *Korea Research Institute of Bioscience and Biotechnology (KRIBB), 52 Eoen-dong, Yuseong-gu, Daejeon 305-806, Republic of Korea*
[b] *Department of Chemistry, Chungnam National University, Yuseong-gu, Daejeon 305-764, Republic of Korea*
[c] *Department of Biotechnology, Yonsei University, Seodaemun-gu, Seoul 120-740, Republic of Korea*
[d] *Bioinformatics & Molecular Design Research Center (BMD), Yonsei Engineering Research Complex, Seodaemun-gu, Seoul 120-740, Republic of Korea*

Received 15 May 2007; received in revised form 28 September 2007; accepted 4 October 2007
Available online 11 October 2007

Abstract

A series of amide and urea derivatives of benzothiazole have been synthesized and evaluated for their antiproliferative profile in human SK-Hep-1 (liver), MDA-MB-231 (breast), and NUGC-3 (gastric) cell lines. Among them, compounds **1**–**2**, **16**–**18**, **23**, and **25**–**26** had potent to moderate inhibitory activities. Further these compounds were investigated for their ability to inhibit Raf-1 activity.

Keywords: Benzothiazole derivatives; Antiproliferative; Raf-1 activity

1. Introduction

The Ras–Raf–MEK–ERK pathway plays a critical role in many aspects of tumorigenesis. This Ras signal transduction pathway normally function to transmit signals from growth factors and cytokine receptors on cell surface to nucleus, resulting in regulation of cell differentiation and division [1]. The highest incidence of Ras alteration is seen in pancreatic cancer (90%), thyroid cancer (50%), colon cancer (50%), lung cancer (30%), and acute myeloid leukemia (30%) [2,3]. Raf was the first identified and most characterized downstream effector kinase of Ras. There are three members of the Raf family of kinases, Raf-1 (C-raf), A-raf, and B-raf. Drugs targeting the Ras signal transduction pathway at the level of Raf may be particularly useful, because Raf is the key activator of this pathway, whereas other upstream targets such as growth factor ligands, receptor tyrosine kinases or even Ras, have many other potential effectors. Furthermore, dominant-negative mutants of Raf can impair Ras transforming activity, confirming that inhibition of Raf is a viable theraupeutic approach [4].

The design of new chemical entity (NCE) must complement the size, shape, and electronic properties of the molecular targets. Benzothiazole-type compounds have attracted considerable attention to anticancer research [5–12], and modified benzothiazole derivatives having additional functional groups may enhance the biological activity. We herein report our efforts toward the synthesis of a series of benzothiazole derivatives and their ability to inhibit Raf-1 kinase.

Accordingly, a series of benzothiazole derivatives (Fig. 1) have been synthesized and investigated for their biological activity. The structure of this series comprises benzothiazole part, linker and phenyl ring. Depending upon the nature of linker group between benzothiazole and phenyl ring the whole

* Corresponding author. Tel.: +82 42 8604382; fax: +82 42 8604595.
** Corresponding author. Tel.: +82 2 21232882; fax: +82 2 3627265.
E-mail addresses: kaylee@kribb.re.kr (K. Lee), gyoonhee@yonsei.ac.kr (G. Han).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.10.008

work has been divided into two parts, that is, compounds bearing amide linkages (**1**–**18**) and the compounds bearing urea linkage (**19**–**26**). The role of various substitutions on benzothiazole and phenyl ring has been investigated.

2. Results and discussion

Scheme 1 summarizes the general synthetic approach that we employed for the synthesis of amide-linked compounds **1**–**18**. Under this synthetic strategy, a solution of 2-aminobenzothiazole derivatives in dry DMF was stirred with NaH followed by reaction with *para* derivative of benzoyl chloride to afford desired urea derivatives **19**–**26**.

All the compounds (**1**–**18**) were initially evaluated in SRB assay using three cancer cell lines' panel consisting of SK-Hep-1 (liver), MDA-MB-231 (breast), and NUGC-3 (gastric) and their antiproliferative activity data (GI_{50}) are provided in Table 1. Adriamycin was used as a reference compound. Compounds with 4-methoxy derivative of benzothiazole (**1**–**5**) showed variable activity but this activity depends upon the nature of *para* substitutent on phenyl ring. Among these compounds, the highest activity was observed when this *para* position bears methyl group, but moderate activity was observed when this *para* position has methoxy group. Absolutely no activity was observed if this position has cyano, trifluoromethyl or nitro group. Further to establish the role of methoxy group on benzothiazole ring, another set of compounds (**6**–**10**) was studied which otherwise is similar to compounds **1**–**5** but differs in position of methoxy group on benzothiazole ring. From the comparative studies of these two sets of compounds, we concluded that no activity was observed if compounds bear methoxy group at the 5th position of benzothiazole ring. Since among these compounds (**1**–**10**), the highest activity was observed only with **1**, comparison of **1** with other positional isomers was also necessary, and thus we studied compound **11**, which differs from compound **1** only in the position of methoxy group, that is, in former OMe group present at the 6th position while in later it is present at the 4th position. Comparison showed that only **1** has the antiproliferative activity, while **11** and **6** have no activity. Another compound **13** in this series with nitro group on 6-position of benzothiazole and trifluoromethyl at the *para* position of phenyl was synthesized utilizing the general approach as shown in Scheme 1 and was found to be moderately active with GI_{50} value of 1.65 μg/mL in NUGC-3 cell lines. However when the position of these two groups was interchanged, that is, **12**, the compound lost its activity.

In the final stage of these studies some di-substituted derivatives were planned and synthesized with methyl groups at the 5th and 7th positions of benzothiazole ring (**14**–**18**) and variable groups at *para* position of phenyl ring. In general, all these compounds were potent inhibitors. Compound **18** was the most cytotoxic in MDA-MD-231 cell lines. While amongst all these compounds **16** has the highest antiproliferative activity in NUGC-3 cell line.

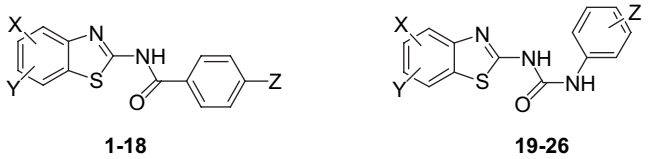


Fig. 1. General structures of the synthesized benzothiazole derivatives.

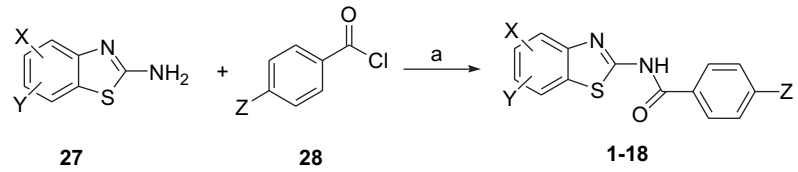
Table 1
In vitro cytotoxicity of amide derivatives of benzothiazole **1**–**18** {GI_{50} (μg/mL)}

Compds	X	Y	Z	SK-Hep-1	MDA-MB-231	NUGC-3
1	4-OMe	H	Me	2.01	0.86	>10
2	4-OMe	H	OMe	2.11	1.25	3.46
3	4-OMe	H	CN	>10	>10	>10
4	4-OMe	H	CF_3	>10	>10	>10
5	4-OMe	H	NO_2	>10	>10	>10
6	5-OMe	H	Me	>10	>10	>10
7	5-OMe	H	OMe	>10	>10	>10
8	5-OMe	H	CN	>10	>10	>10
9	5-OMe	H	CF_3	>10	>10	>10
10	5-OMe	H	NO_2	>10	>10	>10
11	6-OMe	H	Me	>10	>10	>10
12	6-CF_3	H	NO_2	>10	>10	>10
13	6-NO_2	H	CF_3	2.10	1.89	1.65
14	5-Me	7-Me	Me	2.17	0.44	2.37
15	5-Me	7-Me	OMe	>10	1.03	>10
16	5-Me	7-Me	CN	1.18	0.48	0.62
17	5-Me	7-Me	CF_3	1.36	0.45	1.40
18	5-Me	7-Me	NO_2	1.74	0.29	1.32
Adriamycin				0.07	0.39	0.11

The general synthetic approach for the synthesis of compounds **19**–**26** is shown in Scheme 2. The appropriate substituted 2-aminobenzothiazole and commercially available substituted phenyl isocyanate were reacted in the presence of 2,6-dimethylaminopyridine (5 mol%) to furnish urea derivatives of benzothiazole (**19**–**26**) (Table 2).

The antiproliferative activity (GI_{50}) for compounds **19**–**22** in SK-Hep-1 (liver), MDA-MB-231 (breast), and NUGC-3 (gastric) was not encouraging. The insertion of nitro group in phenyl ring (compound **23**) showed moderate antiproliferative activity in MDA-MA-231 and NUGC-3 cell lines. The replacement of fluoro group in **23** with methyl group at 6-position of benzothiazole ring in **24** led to loss in activity. The probable reason for this loss in activity is change in electronic parameter at 6-position of benzothiazole ring, particularly, electron withdrawing group (fluoro) in **23** while electron releasing group (methyl) in **24**. Further to validate the effect of electron withdrawing group at 6-position of benzothiazole with the synergistic effect of *m*-nitro group on phenyl ring, compound **25** was synthesized (6-chloro) and as expected it was found to have moderate antiproliferative activity compared to **23**. Another compound in this series, **26**, has been previously [13] known for its antiviral properties and in our studies it exhibited strong inhibition against growth of all cancer cell lines.

To investigate whether these benzothiazole compounds inhibit Raf kinase activity, compounds **1**–**2**, **16**–**17**, **23**, and **25**–**26** which exhibited potent antiproliferative activity in cancer cell lines were further tested with respect to Raf inhibition. GW4054 was used as a positive control [14]. Inhibitory



Scheme 1. Reagents and conditions: (a) NaH, DMF (anhydrous), rt, 5 h, yield: 60–70%.

activity of the tested compounds was determined using a reported assay protocol with modification [15]. The results shown in Table 3 are presented as percentage inhibitions at the tested concentrations. GW4054 inhibited Raf activity in a dose-dependent manner, and complete inhibition was observed at 1 μM. On the other hand, compounds **1–2**, **23**, and **26** showed moderate to poor inhibitory profiles ranging from 37 to 7.8 at% inhibition, and compounds **16–18** and **25** were inactive at 50 μM.

In summary, a series of amide and urea derivatives of benzothiazole have been synthesized, and initial antiproliferative activity screening using cancer cell lines showed that some analogues had potent to moderate inhibitory activities. Based on these results, further evaluations were conducted to investigate the abilities of the selected analogues to inhibit Raf-1 kinase. However, most of the tested compounds exhibited moderate to poor inhibition. The further mechanism study of these series will be the subject of future publications.

3. Experimental

All reactions were performed in oven-dried glassware with magnetic stirring. Commercial grade reagents were used without further purification. TLC analysis was performed using glass plate precoated with silica gel 60F254. Flash column chromatography was performed with 230–400 mesh silica gel. ^{1}H NMR spectra were obtained on 400 MHz spectrometers. ^{1}H NMR spectra were recorded in parts per million (ppm) relative to the peak for tetramethylsilane ($\delta = 0.00$) as an internal standard.

*3.1. General procedure for compounds **1–18***

To a solution of 2-amino-benzothiazole in DMF (0.15 M) NaH (2 equiv) was added slowly and the mixture was stirred vigorously for 5 min at room temperature. To the resulting solution, benzoyl chloride (1.3 equiv) in 2 mL of DMF was added, and the mixture was stirred for 5 h at room temperature. The reaction mixture was quenched by addition of water and diluted with ethyl acetate. The organic layer was washed with brine two times and dried over $MgSO_4$. After filtration and concentration, the crude product was purified by column chromatography (hexane:ethyl acetate) to afford the desired amide in 60–70% yield.

*3.1.1. N-(4-Methoxybenzo[d]thiazol-2-yl)-4-methylbenzamide (**1**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.44 (s, 3H), 3.98 (s, 3H), 7.09 (d, $J = 1.60$ Hz, 1H), 7.192 (dd, $J = 8.38$ and 1.60 Hz, 1H), 7.30 (d, $J = 7.58$ Hz, 2H), 7.78 (d, $J = 7.98$ Hz, 2H), 8.55 (s, 1H), 8.63 (d, $J = 8.78$ Hz, 1H); ESI-MS (*m/z*, %) 299 (MH^+, 100); HRMS (M^+) calcd. for $C_{16}H_{14}N_2O_2S$ 298.0776, found 298.0779.

*3.1.2. 4-Methoxy-N-(4-methoxybenzo[d]thiazol-2-yl)benzamide (**2**)*

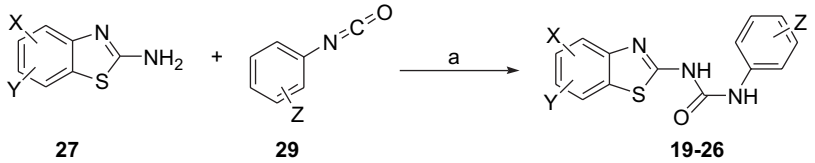
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H), 3.99 (s, 3H), 6.99 (d, $J = 8.78$ Hz, 2H), 7.09 (d, $J = 2.39$ Hz, 1H), 7.19 (dd, $J = 8.78$ and 2.39 Hz, 1H), 7.85 (d, $J = 8.78$ Hz, 2H), 8.51 (br s, 1H), 8.62 (d, $J = 8.78$ Hz, 1H); ESI-MS (*m/z*, %) 315 (MH^+, 100); HRMS (M^+) calcd. for $C_{16}H_{14}N_2O_3S$ 314.0725, found 314.0729.

*3.1.3. 4-Cyano-N-(4-methoxybenzo[d]thiazol-2-yl)benzamide (**3**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (s, 3H), 7.12 (d, $J = 2.00$ Hz, 1H), 7.21 (dd, $J = 8.38$ and 2.00 Hz, 1H), 7.82 (d, $J = 8.38$ Hz, 2H), 7.99 (d, $J = 8.38$ Hz, 2H), 8.55 (s, 1H), 8.59 (d, $J = 8.78$ Hz, 1H); ESI-MS (*m/z*, %) 310 (MH^+, 22).

*3.1.4. N-(4-Methoxybenzo[d]thiazol-2-yl)-4-(trifluoromethyl)benzamide (**4**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (s, 3H), 7.11 (d, $J = 1.60$ Hz, 1H), 7.21 (dd, $J = 8.78$ and 1.60 Hz, 1H), 7.78 (d, $J = 8.38$ Hz, 2H), 7.99 (d, $J = 7.98$ Hz, 2H), 8.57 (s, 1H), 8.61 (d, $J = 8.38$ Hz, 1H); ESI-MS (*m/z*, %) 353 (MH^+, 17); HRMS (M^+) calcd. for $C_{16}H_{11}N_2O_2SF_3$ 352.0493, found 352.0476.



Scheme 2. Reagents and conditions: (a) 2,6-dimethylaminopyridine (5 mol%), Et_3N, DMF, rt, 8 h, yield: 40–60%.

Table 2
In vitro cytotoxicity of urea derivatives of benzothiazole **19–26** {GI_{50} (μg/mL)}

Compds	X	Y	Z	SK-Hep-1	MDA-MB-231	NUGC-3
19	6-F	H	*o*-Me	>10	>10	>10
20	6-F	H	*p*-OMe	>10	>10	>10
21	6-F	H	H	>10	>10	>10
22	6-F	H	*o*-F	>10	>10	>10
23	6-F	H	*m*-NO_2	1.96	0.89	0.83
24	6-Me	H	*m*-NO_2	>10	>10	>10
25	6-Cl	H	*m*-NO_2	2.21	0.82	1.03
26	5-Me	6-Me	*o*-Me	0.18	0.10	0.18

3.1.5. N-(4-Methoxybenzo[d]thiazol-2-yl)-4-nitrobenzamide (**5**) [16]

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 (s, 3H), 7.12 (d, $J = 2.39$ Hz, 1H), 7.22 (dd, $J = 8.38$ and 2.00 Hz, 1H), 8.05 (d, $J = 8.78$ Hz, 2H), 8.37 (d, $J = 9.18$ Hz, 2H), 8.58 (s, 1H), 8.60 (d, $J = 8.38$ Hz, 1H); ESI-MS (*m*/*z*, %) 328 ($M^+ - 1$, 12).

3.1.6. N-(5-Methoxybenzo[d]thiazol-2-yl)-4-methylbenzamide (**6**)

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.38 (s, 3H), 3.90 (s, 3H), 7.34 (d, $J = 8.38$ Hz, 2H), 7.55 (s, 2H), 7.76 (s, 1H), 7.87 (d, $J = 8.38$ Hz, 2H), 10.41 (s, 1H); ESI-MS (*m*/*z*, %) 299 (MH^+, 100); HRMS (M^+) calcd. for $C_{16}H_{14}N_2O_2S$ 298.0776, found 298.0787.

3.1.7. 4-Methoxy-N-(5-methoxybenzo[d]thiazol-2-yl)benzamide (**7**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 3H), 3.92 (s, 3H), 6.96 (d, $J = 8.78$ Hz, 2H), 7.01 (dd, $J = 8.38$ and 1.60 Hz, 1H), 7.45 (d, $J = 7.98$ Hz, 1H), 7.81 (d, $J = 2.00$ Hz, 1H), 7.85 (d, $J = 8.78$ Hz, 2H), 8.19 (s, 1H); ESI-MS (*m*/*z*, %) 315 (MH^+, 100); HRMS (MH^+) calcd. for $C_{16}H_{15}N_2O_3S$ 315.0798, found 315.0805.

3.1.8. 4-Cyano-N-(5-methoxybenzo[d]thiazol-2-yl)benzamide (**8**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (s, 3H), 7.54 (d, $J = 1.20$ Hz, 1H), 7.57 (s, 1H), 7.73 (d, $J = 1.60$ Hz, 1H), 8.04 (d, $J = 7.98$ Hz, 2H), 8.10 (d, $J = 8.38$ Hz, 2H), 10.71 (s, 1H); EI-MS (*m*/*z*, %) 309 (M^+, 100). Anal. Calcd for $C_{16}H_{11}N_3O_2S$: C: 62.12; H: 3.58; N: 13.58. Found: C: 63.19; H: 3.49; N: 13.64.

Table 3
In vitro inhibition of Raf-1 by the representative thiazoles and GW4054

Compds	Concentration (μM)	Raf kinase inhibition (%)[a]
1	50	37
2	50	7.8
16	50	na
17	50	na
18	50	na
23	50	7.9
25	50	na
26	50	14.9
GW4054	0.003	4.5
	0.01	19.4
	0.03	85.3
	0.1	97.1

[a] Values are means of three experiments, na = not active.

3.1.9. N-(5-Methoxybenzo[d]thiazol-2-yl)-4-(trifluoromethyl)benzamide (**9**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 (s, 3H), 7.01 (dd, $J = 8.38$ and 2.00 Hz, 1H), 7.51 (d, $J = 8.38$ Hz, 1H), 7.78 (d, $J = 8.38$ Hz, 2H), 7.81 (d, $J = 2.00$ Hz, 1H), 7.99 (d, $J = 8.38$ Hz, 2H), 8.01 (br s, 1H); ESI-MS (*m*/*z*, %) 353 (MH^+, 42); HRMS (M^+) calcd. for $C_{16}H_{11}N_2O_2SF_3$ 352.0493, found 352.0475.

3.1.10. N-(5-Methoxybenzo[d]thiazol-2-yl)-4-nitrobenzamide (**10**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (s, 3H), 7.02 (dd, $J = 7.98$ and 2.00 Hz, 1H), 7.55 (d, $J = 8.38$ Hz, 1H), 7.80 (d, $J = 2.40$ Hz, 1H), 7.92 (s, 1H), 8.05 (d, $J = 8.78$, 2H), 8.38 (d, $J = 8.78$ Hz, 2H); ESI-MS (*m*/*z*, %) 330 (MH^+, 5).

3.1.11. N-(6-Methoxybenzo[d]thiazol-2-yl)-4-methylbenzamide (**11**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.99 (s, 3H), 3.86 (s, 3H), 6.82 (d, $J = 8.8$ Hz, 1H), 7.06 (d, $J = 8.8$ Hz, 1H), 7.20 (d, $J = 8.4$ Hz, 2H), 7.30 (s, 1H), 7.88 (d, $J = 8.4$ Hz, 2H), 11.7 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 164.8, 157.2, 146.2, 140.8, 132.2, 131.2, 129.4, 126.9, 118.3, 113.0, 105.2, 56.0, and 21.0; EI-MS (*m*/*z*, %) 298 (M^+, 100). Anal. Calcd for $C_{16}H_{14}N_2O_2S$: C: 64.41; H: 4.73; N: 9.39. Found: C: 64.59; H: 4.79; N: 9.44.

3.1.12. 4-Nitro-N-(6-(trifluoromethyl)benzo[d]thiazol-2-yl)benzamide (**12**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 2H), 8.15 (s, 1H), 8.23 (d, $J = 8.78$ Hz, 2H), 8.41 (d, $J = 8.78$ Hz, 2H), 10.64 (br s, 1H); ESI-MS (*m*/*z*, %) 368 (MH^+, 100); HRMS (MH^+) calcd. for $C_{15}H_9N_3O_3SF_3$ 368.0311, found 368.0298.

3.1.13. N-(6-Nitrobenzo[d]thiazol-2-yl)-4-(trifluoromethyl)benzamide (**13**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 2H), 7.88 (s, 2H), 8.00 (s, 1H), 8.48 (d, $J = 8.48$ Hz, 2H), 9.05 (s, 1H); ESI-MS (*m*/*z*, %) 368 (MH^+, 35); HRMS (MH^+) calcd. for $C_{15}H_9N_3O_3SF_3$ 368.0267, found 368.0264.

3.1.14. N-(5,7-Dimethylbenzo[d]thiazol-2-yl)-4-methylbenzamide (**14**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H), 2.58 (s, 6H), 7.28 (d, $J = 7.98$ Hz, 2H), 7.53 (s, 2H), 7.75 (d, $J = 8.38$ Hz, 2H), 7.90 (s, 1H), 7.99 (d, $J = 8.38$ Hz, 1H); ESI-MS (*m*/*z*, %) 297 (MH^+, 100); HRMS (M^+) calcd. for $C_{17}H_{16}N_2OS$ 296.0983, found 296.0999.

*3.1.15. N-(5,7-Dimethylbenzo[d]thiazol-2-yl)-4-methoxybenzamide (**15**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 (s, 6H), 3.87 (s, 3H), 6.96 (d, $J = 8.78$ Hz, 2H), 7.52 (s, 2H), 7.82 (d, $J = 8.78$ Hz, 2H), 7.91 (s, 1H); ESI-MS (*m/z*, %) 313 (MH^+, 100); HRMS (M^+) calcd. for $C_{17}H_{16}N_2O_2S$ 312.0932, found 312.0934.

*3.1.16. 4-Cyano-N-(5,7-dimethyl-benzothiazol-2-yl)-benzamide (**16**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.54 (s, 6H), 7.73 (s, 2H), 8.03 (d, $J = 7.9$ Hz, 2H), 8.10 (d, $J = 7.9$ Hz, 2H), 10.59 (s, 1H); EI-MS (*m/z*, %) 308 (MH^+, 100), 292 (40), 177 (100), 162 (30); HRMS (M^+) calcd. for $C_{17}H_{13}N_3OS$ 307.0779, found 307.0785.

*3.1.17. N-(5,7-Dimethylbenzo[d]thiazol-2-yl)-4-(trifluoromethyl)benzamide (**17**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.58 (s, 6H), 7.53 (s, 2H), 7.73 (d, $J = 8.38$ Hz, 2H), 7.96 (d, $J = 8.38$ Hz, 2H), 8.038 (s, 1H); ESI-MS (*m/z*, %) 351 (MH^+, 67); HRMS (M^+) calcd. for $C_{17}H_{13}N_2OSF_3$ 350.0701, found 350.0688.

*3.1.18. N-(5,7-Dimethylbenzo[d]thiazol-2-yl)-4-nitrobenzamide (**18**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.54 (s, 6H), 7.73 (s, 2H), 8.17 (d, $J = 8.78$ Hz, 2H), 8.37 (d, $J = 8.78$ Hz, 2H), 10.68 (s, 1H); ESI-MS (*m/z*, %) 328 (MH^+, 77); HRMS (M^+) calcd. for $C_{16}H_{13}N_3O_3S$ 327.0678, found 327.0680.

*3.2. General procedure for compounds **19–26***

To a solution of 2-amino-benzothiazole (1 equiv) in 10 mL of DMF (0.1 M) Et_3N (1.6 equiv) and 2,6-dimethyaminopyridine (5 mol%) were added. Isocyanate (1.6 equiv) was added, and the mixture was stirred for 8 h at room temperature. The reaction mixture was quenched by the addition of water diluted with ethyl acetate. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and concentration, the crude product was purified by column chromatography (hexane:ethyl acetate) to afford the desired urea in 40–60% yield.

*3.2.1. 1-(6-Fluorobenzo[d]thiazol-2-yl)-3-o-tolylurea (**19**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (s, 3H), 7.02 (t, $J = 7.18$ Hz, 1H), 7.20 (m, 3H), 7.67 (dd, $J = 8.78$ and 1.20 Hz, 1H), 7.83 (d, $J = 7.18$ Hz, 2H), 8.56 (s, 1H), 11.13 (br s, 1H); ESI-MS (*m/z*, %) 302 (MH^+, 100); HRMS (MH^+) calcd. for $C_{15}H_{13}N_3OSF$ 302.0763, found 302.0768.

*3.2.2. 1-(6-Fluorobenzo[d]thiazol-2-yl)-3-(4-methoxyphenyl)urea (**20**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.72 (s, 3H), 6.90 (d, $J = 7.98$ Hz, 2H), 7.21 (m, 1H), 7.40 (d, $J = 7.98$ Hz, 2H), 7.63 (s, 1H), 7.81 (d, $J = 7.58$ Hz, 1H), 8.94 (s, 1H), 10.77 (br s, 1H); ESI-MS (*m/z*, %) 318 (MH^+, 100); HRMS (M^+) calcd. for $C_{15}H_{12}N_3O_2SF$ 317.0634, found 317.0647.

*3.2.3. 1-(6-Fluorobenzo[d]thiazol-2-yl)-3-phenylurea (**21**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.05 (t, $J = 7.18$ Hz, 1H), 7.22 (td, $J = 9.18$ and 2.39 Hz, 1H), 7.33 (t, $J = 7.98$ Hz, 2H), 7.50 (d, $J = 7.98$ Hz, 2H), 7.65 (dd, $J = 8.38$ and 4.39 Hz, 1H), 7.82 (dd, $J = 8.78$ and 2.39 Hz, 1H), 9.12 (s, 1H), 10.81 (br s, 1H); ESI-MS (*m/z*, %) 288 (MH^+, 100); HRMS (M^+) calcd. for $C_{14}H_{10}N_3OSF_2$ 287.0529, found 287.0518.

*3.2.4. 1-(6-Fluorobenzo[d]thiazol-2-yl)-3-(2-fluorophenyl)urea (**22**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.09 (m, 1H), 7.23 (m, 3H), 7.67 (dd, $J = 8.78$ and 4.39 Hz, 1H), 7.84 (dd, $J = 8.38$ and 2.39 Hz, 1H), 8.10 (t, $J = 7.98$ Hz, 1H), 9.10 (s, 1H), 11.04 (br s, 1H); ESI-MS (*m/z*, %) 306 (MH^+, 100).

*3.2.5. 1-(6-Fluoro-benzothiazol-2-yl)-3-(3-nitro-phenyl)-urea (**23**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.09 (m, 1H), 7.23 (m, 3H), 7.66 (dd, $J = 8.78$ and 4.39 Hz, 1H), 7.83 (dd, $J = 8.38$ and 2.39 Hz, 1H), 8.10 (t, $J = 7.98$ Hz, 1H), 9.10 (br s, 1H), 11.03 (br s, 1H); ESI (*m/z*) 333 (MH^+); HRMS (MH^+) calcd. for $C_{14}H_{10}N_4O_3SF$ 333.0458, found 333.0467.

*3.2.6. 1-(6-Methylbenzo[d]thiazol-2-yl)-3-(3-nitrophenyl)urea (**24**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.37 (s, 3H), 7.19 (d, $J = 7.98$ Hz, 1H), 7.48 (d, $J = 7.98$ Hz, 1H), 7.58 (t, $J = 7.98$ Hz, 1H), 7.66 (s, 1H), 7.85 (t, $J = 9.58$ Hz, 2H), 8.60 (s, 1H), 9.71 (s, 1H), 11.37 (br s, 1H); ESI-MS (*m/z*, %) 329 (MH^+, 100); HRMS (MH^+) calcd. for $C_{15}H_{13}N_4O_3S$ 329.0708, found 329.0698.

*3.2.7. 1-(6-Chlorobenzo[d]thiazol-2-yl)-3-(3-nitrophenyl)urea (**25**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (dd, $J = 8.38$ and 2.00 Hz, 1H), 7.60 (t, $J = 8.38$ Hz, 2H), 7.83 (d, $J = 7.58$ Hz, 1H), 7.89 (d, $J = 7.98$ Hz, 1H), 8.05 (s, 1H), 9.77 (s, 1H), 11.36 (br s, 1H); ESI-MS (*m/z*, %) 349 (MH^+, 42).

*3.2.8. 1-(5,6-Dimethylbenzo[d]thiazol-2-yl)-3-o-tolylurea (**26**) [13]*

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.28 (s, 3H), 2.29 (s, 3H), 2.31 (s, 3H), 7.03 (td, $J = 7.58$ and 1.20 Hz, 1H), 7.22 (t, $J = 7.98$ Hz, 2H), 7.46 (s, 1H), 7.65 (s, 1H), 7.85 (d, $J = 7.58$ Hz, 1H), 8.73 (s, 1H), 11.11 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 153.1, 146.3, 122.0, 134.9, 134.0, 133.3, 131.5, 130.1, 127.2, 125.3, 121.5, 120.0, 19.0, 18.2, 15.1; EI-MS (*m/z*, %) 311 (M^+, 100).

3.3. Biology

3.3.1. For SRB assay (sulforhodamine B assay) for GI_{50}

Cells were harvested from exponential phase cultures by trypsinization, counted and plated in 96-well plates. Optimal seeding densities for the MDA-MB-231 cell lines were determined to ensure exponential growth during a 5-day assay. The SRB assay was performed according to the method of Skehan

et al. and Papazisis et al., with minor modifications [17,18]. The culture medium was aspirated prior to fixation of the cells by the addition of 200 μL 10% cold trichloroacetic acid. After 1-h incubation at 4 °C, cells were washed five times with deionized water. The cells were then stained with 200 μL 0.1% SRB (Sigma–Aldrich) dissolved in 1% acetic acid for at least 15 min and subsequently washed four times with 1% acetic acid to remove unbound stain. The plates were left to dry at room temperature, bound protein stain was solubilized with 200 μL 10 mM unbuffered Tris base, and optical density (OD) was read at 540 nm.

3.3.2. For Raf-1 assay

For Raf-1 assay, Raf-1 (Upstate, NY, USA) and MEK (Upstate, NY, USA) were diluted together with Assay dilution buffer (20 mM MOPS, pH 7.2, 25 mM β-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM DTT) to 5 and 50 μg/mL, respectively. For evaluation of inhibitors, test compounds were serially diluted from 30 mM stock solutions into 10% DMSO (1% final DMSO concentration). Serially diluted inhibitor (5 μL) was added to 20 μL the enzyme–substrate mixture. The kinase reaction was initiated by adding 25 μL [γ-^{33}P] ATP (1000–3000 dpm/pmol) in Assay dilution buffer. The reaction mixtures were incubated at 32 °C, usually for 22 min, and incorporation of ^{33}P into protein was assayed by harvesting the reaction onto phosphocellulose mats, washing away free counts with 1% phosphoric acid, and quantization of phosphorylation by liquid scintillation counting.

Acknowledgments

This research was supported by the Korea Science and Engineering Foundation (KOSEF) grant (M10601000155) and Seoul R&BD Program (10541), Korea.

References

[1] M.J. Robinson, M.H. Cobb, Curr. Opin. Cell Biol. 9 (1997) 180–186.
[2] J.L. Bos, Cancer Res. 49 (1989) 4682–4689.
[3] M. Malumbres, M. Barbacid, Nat. Rev. Cancer 3 (2003) 459–465.
[4] J.F. Lyons, S. Wilhelm, B. Hibner, G. Bollag, Endocr. Relat. Cancer 8 (2001) 219–225.
[5] K. Yamazaki, Y. Kaneko, K. Suwa, S. Ebara, K. Nakazawa, K. Yasuno, Bioorg. Med. Chem. 13 (2005) 2509–2522.
[6] E. Brantley, S. Antony, G. Kohlhagen, L.H. Meng, K. Agama, S.F. Stinson, E.A. Sausville, Y. Pommier, Cancer Chemother. Pharmacol. 58 (2006) 62–72.
[7] C.J. Lion, C.S. Matthews, G. Wells, T.D. Bradshaw, M.F.G. Stevens, A.D. Westwell, Bioorg. Med. Chem. Lett. 16 (2006) 5005–5008.
[8] S.-T. Huang, I.-J. Hsei, C. Chena, Bioorg. Med. Chem. 14 (2006) 6106–6119.
[9] M. Yoshida, I. Hayakawa, N. Hayashi, T. Agatsuma, Y. Oda, F. Tanzawa, S. Iwasaki, K. Koyama, H. Furukawa, S. Kurakata, Y. Sugano, Bioorg. Med. Chem. Lett. 15 (2005) 3328–3332.
[10] J. Das, R.V. Moquin, J. Lin, C. Liu, A.M. Doweyko, H.F. DeFex, Q. Fang, S. Pang, S. Pitt, D.R. Shen, G.L. Schieven, J.C. Barrish, J. Wityak, Bioorg. Med. Chem. Lett. 13 (2003) 2587–2590.
[11] S.E. O'Brien, H.L. Browne, T.D. Bradshaw, A.D. Westwell, M.F.G. Stevens, C.A. Laughton, Org. Biomol. Chem. 1 (2003) 493–497.
[12] I. Hutchinson, S.A. Jennings, B.R. Vishnuvajjala, A.D. Westwell, M.F.G. Stevens, J. Med. Chem. 45 (2002) 744–747.
[13] C.J. Paget, K. Kisner, R.L. Stone, D.C. DeLong, J. Med. Chem. 12 (1969) 1016–1018.
[14] K. Lackey, M. Cory, R. Davis, S.V. Frye, P.A. Harris, R.N. Hunter, D.K. Jung, O.B. McDonald, R.W. McNutt, M.R. Peel, R.D. Rutkowske, J.M. Veal, E.R. Wood, Bioorg. Med. Chem. Lett. 10 (2000) 223–226.
[15] R.A. Smith, J. Barbosa, C.L. Blum, M.A. Bobko, Y.V. Caringal, R. Dally, J.S. Johnson, M.E. Katz, N. Kennure, J. Kingery-Wood, W. Lee, T.B. Lowinger, J. Lyons, V. Marsh, D.H. Rogers, S. Swartz, T. Walling, H. Wild, Bioorg. Med. Chem. Lett. 11 (2001) 2775–2778.
[16] K. Tsuneaki, H. Hama, T. Yamanaka, Jpn. Kokai Tokkyo Koho (1993) (JP 05107575 A 19930430).
[17] K.T. Papazisis, G.D. Geromichalos, K.A. Dimitriadis, A.H. Kortsaris, J. Immunol. Methods 208 (1997) 151–158.
[18] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107–1112.