European Journal of Medicinal Chemistry 95 (2015) 453–463

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis, *in vitro* antiproliferative evaluation, and kinase inhibitory effects of a new series of imidazo[2,1-*b*]thiazole derivatives

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ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form
26 March 2015
Accepted 28 March 2015
Available online 30 March 2015

Keywords:
Antiproliferative activity
Arylsulfonamido
ERK pathway
Imidazo[2,1-*b*]thiazole
V600E-B-RAF
C-RAF

ABSTRACT

Design and synthesis of a new series of 5,6-diarylimidazo[2,1-*b*]thiazole derivatives possessing terminal aryl sulfonamide moiety are described. Their *in vitro* antiproliferative activities against a panel of 57 human cancer cell lines of nine different cancer types were tested at the NCI. Compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u** showed the highest mean % inhibition values over the 57 cell line panel at 10 μM, and they were further tested in 5-dose testing mode to determine their IC_{50} values. Among the six compounds, compound **8u** possessing terminal *para*-hydroxybenzenesulfonamido moiety and ethylene linker showed the highest potency. It demonstrated superior potency than Sorafenib against eight different cell lines, and was equipotent to Sorafenib against COLO 205 colon cancer cell line. Its IC_{50} values over NCI-H460 non-small cell lung cancer cell line and MCF7 breast cancer cell line were 0.845 μM and 0.476 μM, respectively. Compounds **8a**, **8b**, **8q**, **8t**, and **8u** showed high selectivity indices towards cancer cells over L132 normal lung cell line. Compound **8u** showed potential inhibitory effects over the components of ERK pathway. Its IC_{50} value over V600E-B-RAF and C-RAF kinases were 39.9 nM and 19.0 nM, respectively.

1. Introduction

Imidazo[2,1-*b*]thiazole analogs exhibited potential antiproliferative activities against a variety of human cancer cell lines [1–11]. Some pyrimidinyl substituted imidazo[2,1-*b*]thiazole derivatives were reported as RAF kinases inhibitors (Fig. 1) [12].

The RAS-RAF-MEK-ERK signaling pathway (ERK pathway) plays an important role in tumorigenesis and cancer progression [13]. Sorafenib (Nexavar®, Fig. 1), a diarylurea derivative, is an example of kinase inhibitors targeting ERK pathway. Dysregulated signaling through RAF kinase isoforms has been detected in ~30% of human cancers [14]. Constitutive B-RAF activity can be caused by activating oncogenic mutations, such as B-RAF V600E mutation, which is associated with a variety of cancer types including melanoma, non-Hodgkin's lymphoma, colorectal adenocarcinoma, thyroid carcinoma, non-small cell lung carcinoma, renal cell carcinoma, hepatocellular carcinoma, ovarian cancer, gastrointestinal stromal tumors, and hairy cell leukemia [15]. Wild-type RAF1 (C-RAF) has been reported to prolong cell survival, independent of MAPK signaling, by direct interaction with anti-apoptotic and apoptotic regulatory proteins [14,16]. C-RAF kinase is over-expressed in cases

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http://dx.doi.org/10.1016/j.ejmech.2015.03.065
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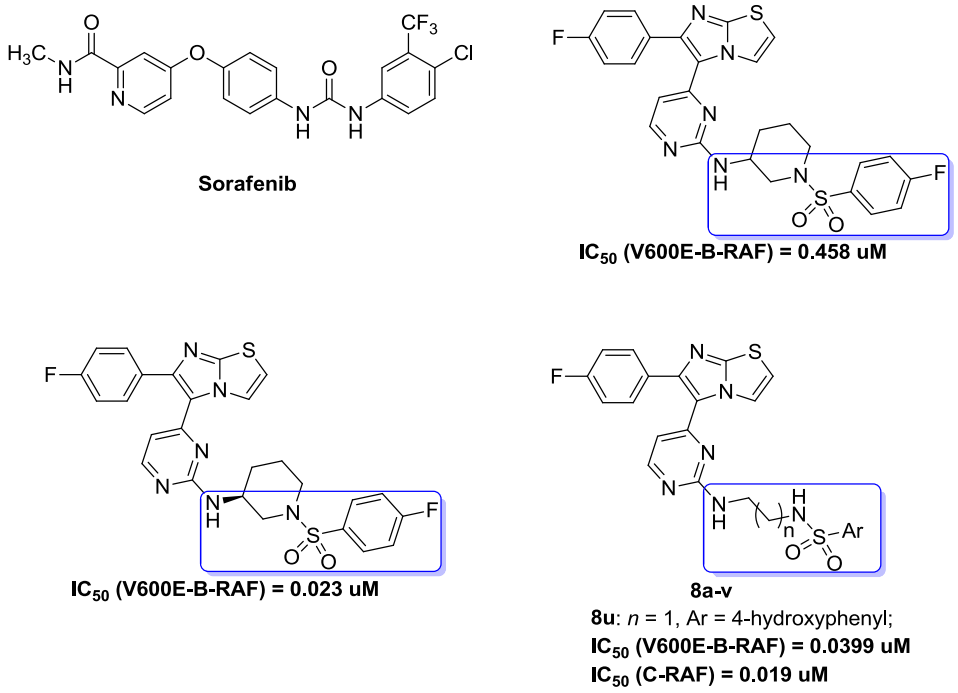


Fig. 1. Structures of Sorafenib, previously reported pyrimidinyl imidazo[2,1-*b*]thiazole derivatives [12], and the target compounds **8a**–**v**.

of renal cell carcinoma [17], hepatocellular carcinoma [18], and is associated with poor prognosis in ovarian [19] and androgen-insensitive prostate cancer [20]. There is also a relationship between C-RAF and disease progression and cell proliferation in melanomas [21].

Our target compounds in the present investigation were designed as open chain analogs to the previously reported RAF kinase inhibitors imidazo[2,1-*b*]thiazole derivatives (Fig. 1) [12]. Homologation of the spacer, ethylene or propylene, was carried out in order to study the effect of linker length on the activity. The target compounds were tested for *in vitro* antiproliferative activities against 57 human cancer cell lines of nine different cancer types, and B-RAF/V600E-B-RAF/C-RAF kinase inhibitory effect of the most potent compound was also examined in order to test the mechanism of action at molecular level.

2. Results and discussion

2.1. Chemistry

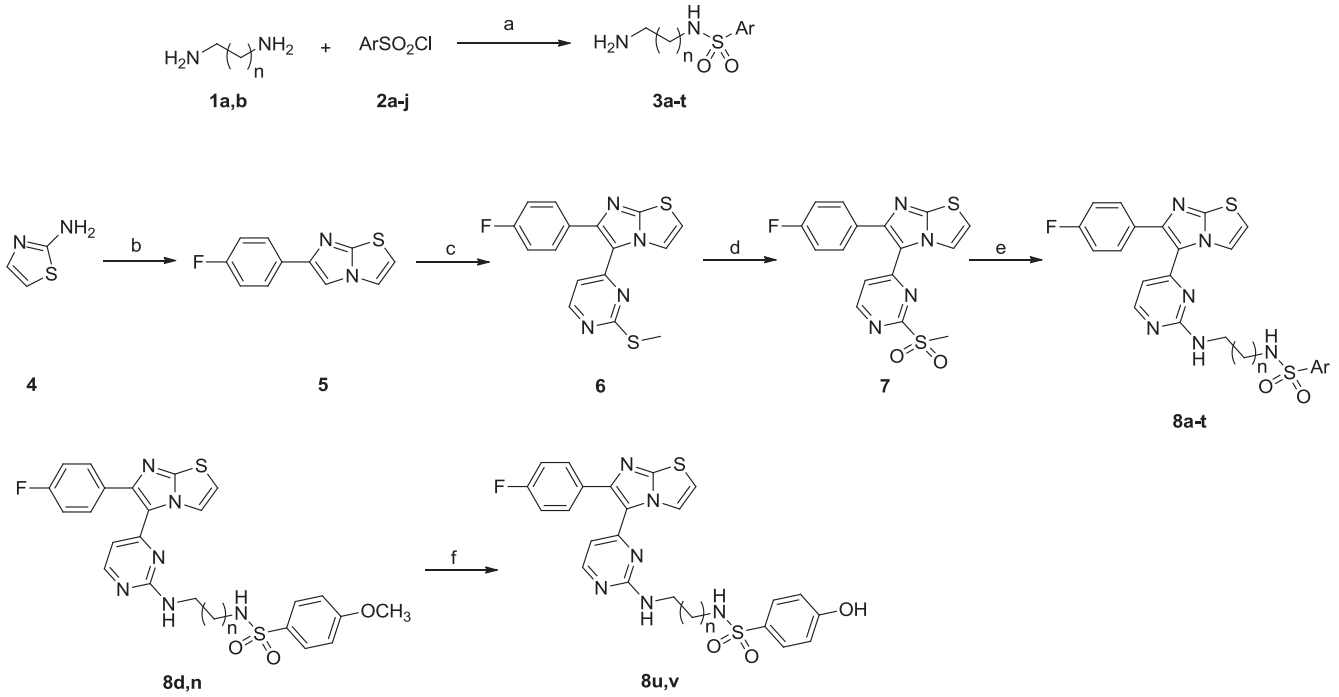
The target compounds **8a**–**v** were synthesized through the pathway illustrated in Scheme 1. It was important to synthesize the key mesyl intermediate compound **7** and the amino tail reagents **3a**–**t**. 1,2-Ethylenediamine (**1a**) or 1,3-propylenediamine (**1b**) were treated with the appropriate arylsulfonyl chloride derivatives **2a**–**j** in the presence of triethylamine as a base to afford the amino tail reagents **3a**–**t** [22,23]. Refluxing 2-aminothiazole (**4**) with α-bromo-4-fluoroacetophenone in ethanol led to cyclization to 6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole (**5**) [24]. Coupling compound **5** with 4-iodo-2-(methylthio)pyrimidine in presence of palladium acetate, cesium carbonate, and triphenylphosphine produced compound **6**. Oxidation of the methylsulfide moiety of compound **6** using oxone produced the corresponding methylsulfonyl compound **7** [7]. This was confirmed through deshielding of the methyl signal in ^{1}H NMR from 2.64 ppm to 3.38 ppm. Heating compound **7** with the amino reagents **3a**–**t** in the presence of diisopropylethylamine led to displacement of the mesyl group of compound **7** by amino group and formation of the target compounds **8a**–**t**. This was confirmed by disappearance of the mesyl signal at 3.38 ppm in ^{1}H NMR analysis, and appearance of the aliphatic and aromatic signals related to the ethylene/propylene linker and terminal aromatic ring, respectively, in both ^{1}H NMR and ^{13}C NMR analyses. The methoxy compounds **8d,n** were demethylated using boron tribromide to obtain the corresponding hydroxyl compounds **8u,v**. The disappearance of methoxy signals at 3.76 and 3.79 ppm in ^{1}H NMR analysis confirms demethylation reaction. LC-MS analysis was another tool to confirm identity of the analyzed compounds. Table 1 illustrates the target compound structures and their yield percentages.

2.2. Biological evaluation

2.2.1. In vitro antiproliferative activity over NCI-57 cancer cell line panel

2.2.1.1. One-dose results. Structures of the target compounds were submitted to National Cancer Institute (NCI), Bethesda, Maryland, USA [25], and the fifteen derivatives shown in Fig. 2 were selected on the basis of degree of structural variation and computer modeling techniques for testing their antiproliferative activity. The selected compounds were subjected to *in vitro* anticancer assay against tumor cells in a full panel of 57 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast). The compounds were tested at a single-dose concentration of 10 μM, and the percentages of growth inhibition over the 57 tested cell lines were determined. The mean inhibition percentages for each of the tested analogs over the full panel of cell lines are illustrated in Fig. 3.

Upon investigating the effect of linker length on antiproliferative activity, it was found that compounds **8a**, **8b**, **8e**, and **8u** with ethylene linker were more active the corresponding propylene derivatives **8k**, **8l**, **8o**, and **8v**. On the other hand, some propylene analogs such as **8s** and **8t** showed higher activity than



Scheme 1. Reagents and conditions: a) triethylamine, CH_2Cl_2, 0 °C; rt, overnight, 50–70%; b) α-bromo-4-fluoroacetophenone, EtOH, reflux, 16 h, 86%; c) 4-iodo-2-(methylthio) pyrimidine, $Pd(OAc)_2$, Cs_2CO_3, PPh_3, DMF, 80 °C, 12 h, 26%; d) oxone, MeOH, H_2O, rt, 16 h, 81%; e) **3a**–**t**, diisopropylethylamine, DMSO, 80 °C, 16 h; f) BBr_3, CH_2Cl_2, −78 °C, 1 h; rt, overnight.

the corresponding ethylene compounds **8i** and **8j**.

The effect of terminal aryl substituents on activity was also studied. Compound **8q** with *para*-fluorophenyl ring was more active than compounds **8k**, **8l**, **8n**, **8o**, **8s**, and **8v** possessing phenyl, *para*-tosyl, *para*-methoxyphenyl, 3,4-dimethoxyphenyl, *para*-(trifluoromethyl)phenyl, and *para*-hydroxyphenyl, respectively. So in case of propylene derivatives, *para*-fluorobenzenesulfonamido moiety was optimum for activity. Among ethylene analogs, compound **8u** possessing *para*-hydroxyphenyl terminal ring showed superior activity compared with analogs **8a**, **8b**, **8e**, **8h**, and **8i** containing phenyl, *para*-tosyl, 3,4-dimethoxyphenyl, *para*-iodophenyl, and *para*-(trifluoromethyl)phenyl, respectively.

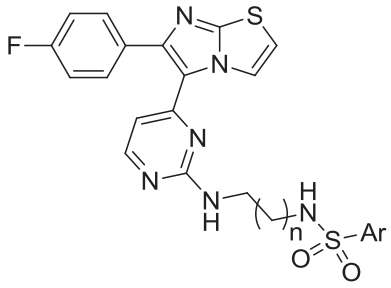
Among all the tested compounds, compounds **8q**, **8t**, and **8u** showed the highest mean inhibitions. The %inhibitions of these three compounds over each cell line of the 57 cancer cell line panel are illustrated in Fig. 3. At 10 μM concentration, the three compounds showed strong antiproliferative activities (>80% inhibition) over eight, seven, and six cell lines, respectively. Compounds **8q**, **8t**, and **8u** demonstrated broad-spectrum cytotoxicities over all the nine tested cancer types. Of special interest, compound **8q** with propylene linker and *para*-fluorophenyl terminal ring showed lethal effect over T-47D breast cancer cell line with 102.52% inhibition. It also inhibited the growth of HT29 colon cancer cell line and UO-31 renal cancer cell line by 96.72% and 96.86%, respectively. Compound **8t** exerted lethal effect against HT29 colon cancer cell line (107.72% inhibition). It also showed high inhibitory effect against K-562 leukemia cell line, COLO 205, and HCC-2998 colon cancer cell lines (96.25%, 89.90%, and 92.78%, respectively).

2.2.1.2. Five-dose results. Compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u** with promising results in single-dose testing were further tested in a five-dose testing mode in order to determine their IC_{50} values over the 57 cancer cell lines. The IC_{50} values of those five compounds over the most sensitive cell line(s) of each subpanel are summarized in Table 2. The results were compared with those of Sorafenib as a reference standard compound. The results of Sorafenib were obtained from NCI datawarehouse index [26], and are inserted in Table 2. The six tested compounds exerted high potency with one-digit micromolar IC_{50} values against most of the cell lines. Interestingly, compound **8u** demonstrated submicromolar IC_{50} values against COLO 205 colon cancer cell line and MCF7 breast cancer cell line (0.845 and 0.476 μM, respectively). Compound **8a** also showed submicromolar IC_{50} value of 0.722 μM against MCF7 breast cancer cell line. Compound **8a** with phenyl terminal ring was generally more potent than compound **8b** possessing *para*-tosyl ring. Similarly, compound **8n** with *para*-methoxyphenyl was more potent than compound **8q** containing *para*-fluorophenyl terminal ring against ten different cell lines. This may be rationalized that hydrogen bond-forming group on the terminal ring could be favorable for affinity at the receptor site(s), and hence potency. This assumption can be reinforced by the superior potencies expressed by compound **8u** with *para*-hydroxyphenyl terminal ring, compared with the other five compounds tested in 5-dose testing mode. Compound **8u** showed superior potencies than Sorafenib also against eight cell lines. And it was equipotent to Sorafenib over COLO 205 colon cancer cell lines.

In order to study the selectivity of the most potent compounds towards cancer cells over normal cells, the cytotoxicity of compounds **8a**, **8b**, **8q**, **8t**, and **8u** were tested against L132 human lung normal cell line (Table 2). The five compounds showed high IC_{50} values over L132 cell line, which indicates high selectivity towards cancer cells than normal cells. The high selectivity indices of those potent compounds could indicate high safety and diminished toxicity.

The mean IC_{50}, TGI, and LC_{50} for compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u** over the tested NCI-57 cancer cell line panel have been calculated and summarized in Table 3. Compound **8u** with ethylene spacer and *p*-hydroxyphenyl terminal ring showed the highest potency and efficacy, compared with the other five compounds. Compound **8n** possessing *p*-methoxyphenyl terminal ring was

Table 1
Structures of the target compounds and their yield percentages.



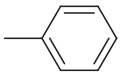
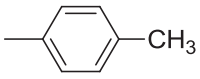
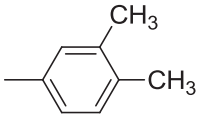
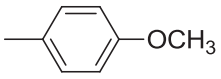
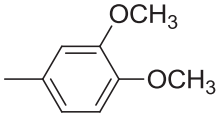
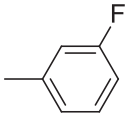
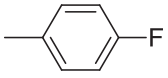
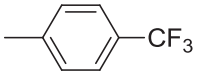
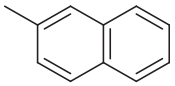
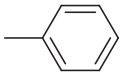
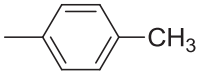
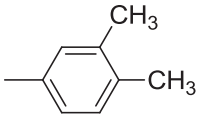
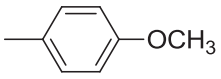
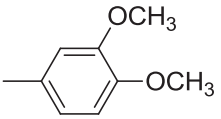
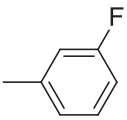
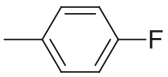
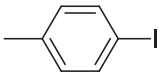
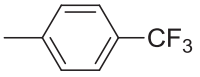
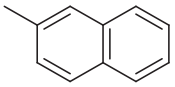
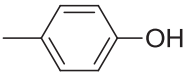
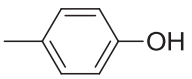
Compound no.	Ar	n	Yield%
8a		1	58%
8b		1	73%
8c		1	71%
8d		1	67%
8e		1	75%
8f		1	63%
8g		1	70%
8h		1	75%
8i		1	72%
8j		1	72%
8k		2	68%
8l		2	71%
8m		2	77%
8n		2	65%

Table 1 (*continued*)

Compound no.	Ar	n	Yield%
8o		2	79%
8p		2	65%
8q		2	63%
8r		2	70%
8s		2	71%
8t		2	74%
8u		1	59%
8v		2	62%

more potent than compound **8q** with *p*-fluorophenyl terminal moiety.

Some physicochemical parameters such as total polar surface area (TPSA), cLogP, and CMR were calculated for compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u** using ChemOffice 2012 software, and are summarized in Table 4. The results showed that polar group such as hydroxyl is required on the terminal arylsulfonamido moiety, and this is well correlated with the results obtained by compound **8u**. Increased bulkiness and lipophilicity of that terminal moiety is unfavorable for antiproliferative activity of this series of compounds. This is obvious upon comparing the cLogP and CMR values of compound **8u** with those of the other five compounds.

2.2.1.3. In vitro kinase screening. In order to investigate the mechanism of action of this series of compounds at molecular level, the most potent compound **8u** was selected as a representative example to be tested at a single-dose concentration of 10 μM over B-RAF (wild-type), V600E-B-RAF, and C-RAF kinases. As illustrated in Table 5, compound **8u** showed strong inhibitory effect over the three kinases at 10 μM. It was further tested in a 10-dose testing mode in order to determine its IC_{50} values over wild-type B-RAF, V600E mutated B-RAF, and C-RAF. The IC_{50} values were 428 nM, 39.9 nM, and 19 nM, respectively. So the affinity of compound **8u** was found to be 10.73 times more towards V600E-B-RAF than wild-type B-RAF. It was also 22.53 times more selective towards C-RAF than wild type B-RAF. It was almost equipotent to Sorafenib over V600E-B-RAF. Compound **8u** exerted high potency against cell lines with over-expressed V600E-B-RAF such as COLO 205, HT29 colon cancer cell lines, and SK-MEL-5 melanoma cell line. It has been also reported that C-RAF overexpression increases the resistance of MCF-7 breast cancer cells to standard chemotherapeutic agents [27]. So C-RAF inhibition by compound **8u** could play a role in its

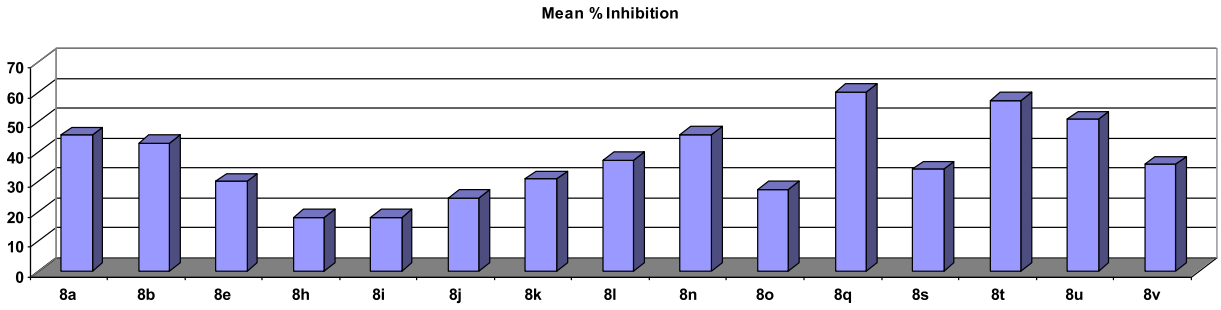


Fig. 2. Mean inhibition percentages observed with the target compounds in single-dose (10 μM) 60-cancer cell line screening. Mean % inhibition represents the mean inhibition percentages over the 60 cell lines. The inhibition percentages were calculated by subtracting the growth percentages from 100.

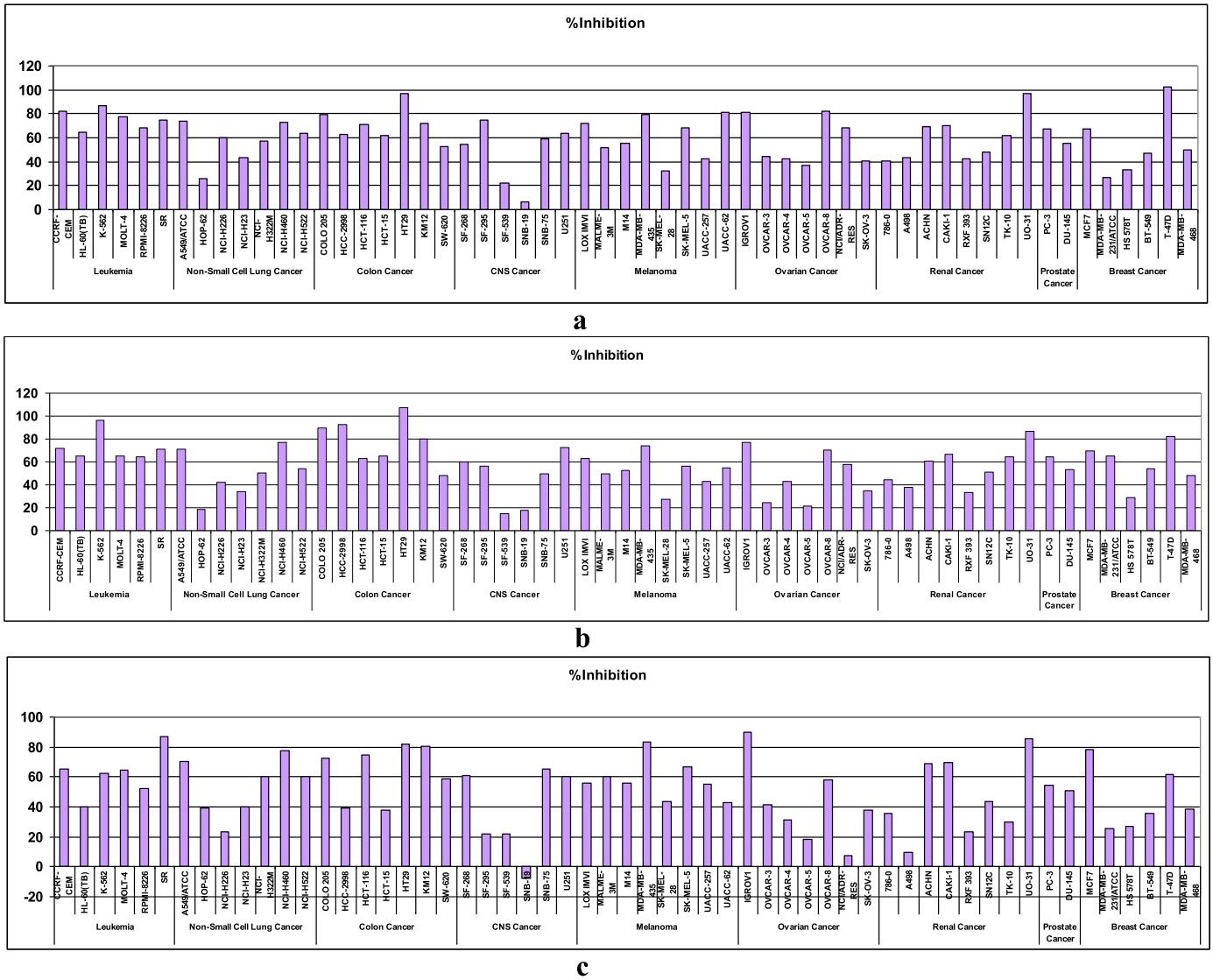


Fig. 3. % Inhibition expressed by compounds **8q** (a), **8t** (b), and **8u** (c) at a single-dose concentration of 10 μM over all cell lines of the NCI cancer cell line panel of nine different cancer types.

sensitivity towards the compound, and hence the high potency of **8u** against it. As explained in the introduction section, V600E-B-RAF and C-RAF are over-expressed in a variety of cancer types. So we can conclude that the inhibitory effect of compound **8u** on RAF kinases is, at least in part, a potential mechanism of its antiproliferative effect.

Moreover, compound **8u** was also tested for inhibitory effect over MEK and ERK kinases, other components of the ERK pathway, by Western Blot Assay (Fig. 4). The MEK/ERK-containing A375P cell lysate was treated with three different concentrations of the test

Table 2
IC_{50} values (μM) of Sorafenib and the tested compounds over the most sensitive cell line(s) of each subpanel and L132 human lung normal cell line.

		Compound no.						
		8a	**8b**	**8n**	**8q**	**8t**	**8u**	Sorafenib
Cancer cell line	SR[a]	3.46	NT[j]	3.69	8.26	4.01	1.82	3.16
	NCI-H460[b]	2.71	3.50	4.33	5.62	3.74	**0.845**	2.51
	COLO 205[c]	2.23	NT[j]	5.58	6.23	2.22	**2.00**	2.00
	HT29[c]	2.31	NT[j]	2.84	3.52	2.40	2.09	2.00
	SNB-75[d]	**1.61**	**2.65**	**1.56**	**2.16**	5.22	**1.68**	3.16
	MDA-MB-435[e]	2.56	NT[j]	3.28	3.69	3.67	**1.07**	1.58
	SK-MEL-5[e]	2.45	3.52	5.49	4.63	4.17	**1.39**	1.58
	IGROV1[f]	2.99	3.09	5.59	8.85	5.37	**1.70**	2.51
	ACHN[g]	**1.69**	**2.30**	2.73	4.61	3.42	**2.03**	2.51
	PC-3[h]	4.00	NT[j]	8.74	9.54	4.36	4.51	2.00
	MCF7[i]	**0.722**	**1.48**	2.74	3.24	3.71	**0.476**	2.51
L132 human lung normal cell line		310.10	70.62	NT[j]	84.35	384.14	66.56	NT[j]

Bold figures indicate higher potencies than Sorafenib.
[a] Leukemia cell line.
[b] Non-small cell lung cancer cell line.
[c] Colon cancer cell line.
[d] CNS cancer cell line.
[e] Melanoma cell line.
[f] Ovarian cancer cell line.
[g] Renal cancer cell line.
[h] Prostate cancer cell line.
[i] Breast cancer cell line.
[j] Not tested.

compound **8u** (1, 3, and 10 μM) and its inhibitory activities were compared with that of Sorafenib. The results showed that compound **8u** and Sorafenib significantly suppressed MEK1/2 and ERK1/2 phosphorylation in a dose-dependent manner. Due to the relationship between upregulation of RAF/MEK/ERK kinases and uncontrolled cell proliferation, increased cell survival, and tumor progression, herein it can be concluded that the inhibitory effect of compound **8u** can be its potential mechanism of antiproliferative activity at molecular level.

Table 3
Mean TGI and LC_{50} values of compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u** over the tested NCI-57 cancer cell line panel.

Compound no.	**8a**	**8b**	**8n**	**8q**	**8t**	**8u**
Mean TGI	70.79	72.44	81.28	81.28	83.18	30.90
Mean LC_{50}	97.72	100	100	100	97.72	87.10

Table 4
Calculated physicochemical parameters of compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u**.

Compound no.	TPSA[a]	cLogP[b]	CMR[c]
8a	98.52	4.49	13.07
8b	98.52	4.99	13.53
8n	107.75	4.77	13.69
8q	98.52	4.93	13.08
8t	98.52	5.66	14.76
8u	118.75	4.19	13.22

[a] Total Polar Surface Area ($Å^2$).
[b] Partition coefficient, a measure of lipophilicity.
[c] Molar refractivity, a measure of bulkiness or steric factor.

Table 5
Kinase inhibition results of compound **8u**, Sorafenib, and GW5074.

Kinase	Compound **8u**		Sorafenib IC_{50} (μM)	GW5074 IC_{50} (μM)
	%Inhibition at 10 μM	IC_{50} (μM)		
B-RAF	97.12%	0.428	0.025	–
V600E-B-RAF	97.28%	0.0399	0.038	–
C-RAF	97.51%	0.019	–	0.006

3. Conclusion

In this study, a new series of imidazo[2,1-*b*]thiazole derivatives possessing terminal aryl sulfonamide moiety was designed and synthesized. The target compounds were tested for *in vitro* antiproliferative activities over 57 cancer cell line panel of nine different cancer types at the NCI. Among them, compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u** showed the most promising results at a single-dose concentration of 10 μM. They were further tested in 5-dose testing mode in order to determine their IC_{50} values, and the results were compared with those of a reference anticancer drug, Sorafenib. Compounds **8u** with *para*-hydroxybenzenesulfonamido terminal moiety and ethylene spacer showed higher potencies than

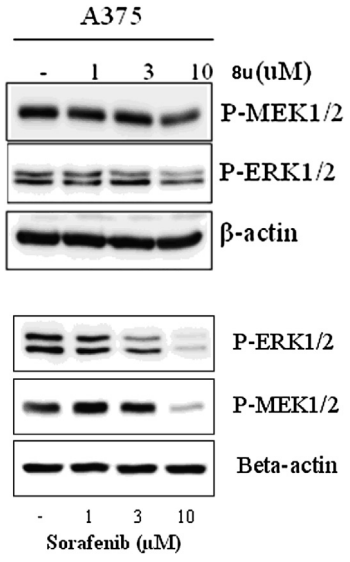


Fig. 4. Inhibitory effect of compound **8u** and Sorafenib on p-MEK1/2 and p-ERK1/2.

Sorafenib. It was also the most potent among the six compounds testing in 5-dose testing mode. It demonstrated submicromolar IC_{50} values over NCI-H460 NSCLC cell line and MCF7 breast cancer cell line (0.845 μM and 0.476 μM, respectively). Compounds **8a**, **8b**, **8q**, **8t**, and **8u** were further tested for cytotoxicity towards L132 normal lung cell line, and showed high selectivity indices against cancer cells over normal cells. These results could give an indication about high safety of those compounds. Compound **8u** showed potential inhibitory effects over V600E-B-RAF, wild-type B-RAF, C-RAF, MEK, and ERK kinases. It was almost equipotent to Sorafenib against V600E-B-RAF kinase. Compound **8u** exerted 2-digit nanomolar IC_{50} against both V600E-B-RAF and C-RAF (39.9 and 19.0 nM, respectively). So it can be concluded that inhibition of ERK pathway could be a mechanism of antiproliferative activity of this compound at molecular level. Hydrogen bond-forming group, such as the hydroxyl group of compound **8u**, on the terminal ring seems to be essential for activity. The physicochemical parameter calculation reinforced this postulation that the terminal arylsulfonamido moiety should carry a polar group and should not be bulky or much hydrophobic. Further structural modifications of this series of compounds in order to improve their potencies are currently in progress.

4. Experimental

4.1. General

All solvents and reagents were commercially available and used without further purification. The target compounds and intermediates were purified by column chromatography using silica gel (0.040–0.063 mm, 230–400 mesh) and technical grade solvents. Analytical thin layer chromatography (TLC) was adopting on silica gel 60 F_{254} plates from Merck. IR spectra (KBr disks) were recorded with a Bruker FT-IR instrument (Bruker Bioscience, Billerica, MA, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or 300 spectrometer using tetramethylsilane as an internal standard and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), brs (broad singlet), or dd (doublet of doublets). LC-MS analysis was carried out using the following system: Waters 2998 photodiode array detector, Waters 3100 mass detector, Waters SFO system fluidics organizer, Waters 2545 binary gradient module, Waters reagent manager, Waters 2767 sample manager, Sunfire™ C18 column (4.6 × 50 mm, 5 μm particle size); Solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) in water; solvent B: 0.035% TFA in CH_3OH; flow rate = 3.0 mL/min; the AUC was calculated using Waters MassLynx 4.1 software. Solvents and liquid reagents were transferred using hypodermic syringes. Purity % of all the target compounds were determined by HPLC and found to be >95%.

4.2. General procedure for preparation of ethylenediamine and propylenediamine sulfonamides *3a–t*

To a solution of proper diamine **1a,b** (10 mmol) in dichloromethane (20 mL), triethylamine (300 mg, 0.416 mL, 30 mmol) was added with stirring. The mixture was cooled to 0 °C, and a solution of the appropriate sulfonyl chloride **2a–j** (5 mmol) in dichloromethane (2 mL) was added dropwise. The mixture stirred at room temperature overnight. The reaction mixture was washed with 10% Na_2CO_3 (20 mL) and then with distilled water (3 × 15 mL). The organic layer separated and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified flash column chromatography (silica gel, appropriate ratio of hexanes-ethyl acetate) to give the purified semisolid compounds **3a–t**.

4.2.1. N-(2-Aminoethyl)benzenesulfonamide (*3a*)

White solid; yield 69%; mp 80–81 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.77 (d, 2H, *J* = 9.0 Hz), 7.46–7.38 (m, 3H), 3.99 (bs, 3H, exchangeable), 3.86 (t, 2H, *J* = 3.0 Hz), 2.67 (t, 2H, *J* = 3.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 140.0, 132.4, 129.1, 126.8, 45.2, 40.9. IR (KBr) cm^{-1}: 3563, 3272, 3065, 1586, 1479, 1446, 1323, 1157.

4.2.2. N-(2-Aminoethyl)-4-methylbenzenesulfonamide (*3b*)

White solid; yield 77%; mp.120–121 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (d, 2H, *J* = 9.0 Hz), 7.39 (d, 2H, *J* = 9.0 Hz), 3.45 (s, 2H, exchangeable), 2.89 (t, 2H, *J* = 6.0 Hz), 2.66 (t, 1H, *J* = 6.0 Hz), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.3, 137.0, 129.7, 127.0, 45.5, 41.0, 21.5. IR (KBr) cm^{-1}: 3585, 3360, 3290, 2585, 1926, 1311, LC-MS: *m*/*z* 215 (M+1).

4.2.3. N-(2-Aminoethyl)-3,4-dimethylbenzenesulfonamide (*3c*)

White solid; yield 86%; mp.93–94 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (s, 1H), 7.59 (dd, 1H, *J* = 3.0, *J* = 9.0 Hz), 7.27 (s, 1H), 2.95 (t, 2H, *J* = 6.0 Hz), 2.79 (t, 2H, *J* = 6.0 Hz), 2.32 (s, 6H).

4.2.4. N-(2-Aminoethyl)-4-methoxybenzenesulfonamide (*3d*)

White solid, yield 76%; mp.90–91 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, 2H, *J* = 9.0 Hz), 6.96 (d, 2H, *J* = 6.0 Hz), 3.85 (s, 3H), 3.10 (bs, 2H), 2.93 (t, 2H, *J* = 6.0 Hz), 2.76 (t, 2H, *J* = 6.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.3, 132.2, 129.6, 114.8, 56.2, 46.0, 41.6. LC-MS: *m*/*z* 231 (M+1).

4.2.5. N-(2-Aminoethyl)-3,4-dimethoxybenzenesulfonamide (*3e*)

White solid; yield 89%; mp.142–143 °C, ^{1}H NMR (CD_3OD, 300 MHz) δ 7.47 (dd, 1H, *J* = 3.0, 9.0 Hz), 7.38 (d, 1H, *J* = 3.0 Hz), 7.11 (d, 1H, *J* = 9.0 Hz), 3.91 (s, 3H), 3.90 (s, 3H), 3.34–3.31 (m, 2H, exchangeable), 2.90 (t, 2H, *J* = 6.0 Hz), 2.68 (t, 2H, *J* = 6.0 Hz).

4.2.6. N-(2-Aminoethyl)-3-fluorobenzenesulfonamide (*3f*)

Viscous light brown oil; yield 71%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.65–7.44 (m, 3H), 7.24–7.19 (m, 1H), 4.29 (bs, 2H, exchangeable), 2.59 (t, 2H, *J* = 6.0 Hz), 2.76 (t, 2H, *J* = 6.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.5, 161.0, 142.1, 131.1, 122.7, 114.3, 45.3, 41.0.

4.2.7. N-(2-Aminoethyl)-4-fluorobenzenesulfonamide (*3g*)

White solid; yield 76%; mp. 108–109 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.84–7.79 (m, 2H), 7.11 (t, 2H, *J* = 6.0 Hz), 2.89 (t, 2H, *J* = 6.0 Hz), 2.71 (t, 2H, *J* = 6.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.7, 136.0, 129.7 (*J* = 8.3 Hz), 116.3 (*J* = 22.5 Hz), 45.2, 40.9; IR (KBr) cm^{-1}: 3582, 3350, 3299, 3107, 3068, 1903, 1317, 838. LC-MS: *m*/*z* 219 (M+1).

4.2.8. N-(2-Aminoethyl)-4-iodobenzenesulfonamide (*3h*)

Yield 84%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88 (d, 2H, J = 8.3 Hz), 7.59 (d, 2H, *J* = 9.0 Hz), 2.97 (t, 2H, *J* = 6.0 Hz), 2.81 (t, 2H, *J* = 6.0 Hz).

4.2.9. N-(2-Aminoethyl)-4-(trifluoromethyl)benzenesulfonamide (*3i*)

Viscous oil; yield 72%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.99 (d, 1H, *J* = 6.0 Hz), 7.64 (d, 1H, *J* = 9.0 Hz), 7.52 (t, 2H, *J* = 6.0, *J* = 9.0 Hz), 2.91 (t, 2H, *J* = 6.0 Hz), 2.67 (t, 2H, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.7, 132.6, 130.6, 128.2, 128.1, 44.9, 40.8; IR (KBr) cm^{-1}: 3581, 3307, 3086, 2874, 2874, 1659, 1595, 1441, 1311, 1164, 560.

4.2.10. N-(2-Aminoethyl)naphthalene-2-sulfonamide (*3j*)

White solid; yield 85%; mp 132–133 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (s, 1H), 8.15 (t, 2H, *J* = 6.0 Hz), 8.04 (d, 1H, *J* = 9.0 Hz), 7.82 (d, 1H, *J* = 9.0 Hz), 7.70 (d, 2H, *J* = 6.0 Hz), 3.32 (s,

2H, exchangeable), 2.75 (t, 2H, J = 6.0 Hz), 2.50 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.8, 134.7, 132.1, 129.5, 129.2, 128.7, 128.3, 127.8, 127.5, 122.3, 45.5, 41.0; IR (KBr) cm^{-1}: 3576, 3364, 3306, 3055, 2563, 1927, 1819, 1592, 1312; LC-MS: m/z 251 (M+1).

*4.2.11. N-(3-Aminopropyl)benzenesulfonamide (**3k**)*

Buff solid; yield 67%; mp. 71–72 °C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.90 (d, 2H, J = 6.0 Hz), 7.60–7.51 (m, 3H), 3.11 (t, 2H, J = 6.0 Hz), 2.82 (t, 2H, J = 6.0 Hz), 1.62 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 139.8, 132.4, 129.1, 126.9, 41.1, 38.7, 30.2; IR (KBr) cm^{-1}: 3572, 3341, 3040, 2949, 1991, 1592, 1318.

*4.2.12. N-(3-Aminopropyl)-4-methylbenzenesulfonamide (**3l**)*

White solid; yield 82%; mp 119–120 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (d, 2H, J = 7.6 Hz), 7.19 (d, 2H, J = 8.0 Hz), 3.27 (bs, 3H, exchangeable), 2.89 (t, 2H, J = 6.0 Hz), 2.64 (t, 2H, J = 6.0 Hz), 2.31 (s, 3H), 1.48 (p, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.1, 137.1, 129.6, 127.0, 42.1, 40.1, 31.4, 21.4. IR (KBr) cm^{-1}: 3581, 3360, 3300, 3047, 1917, 1599, 1494, 1317. LC-MS: m/z 229 (M+1).

*4.2.13. N-(3-Aminopropyl)-3,4-dimethylbenzenesulfonamide (**3m**)*

White solid; yield 84%; mp 96–97 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (s, 1H), 7.58 (dd, 1H, J = 3.0, J = 9.0 Hz), 7.25 (d, 1H, J = 9.0 Hz), 3.06 (t, 2H, J = 6.0 Hz), 2.80 (t, 2H, J = 6.0 Hz), 2.32 (s, 3H), 2.29 (s, 3H), 1.60 (p, 2H, J = 6.0 Hz).

*4.2.14. N-(3-Aminopropyl)-4-methoxybenzenesulfonamide (**3n**)*

White solid; yield 88%; mp76-77 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (d, 2H, J = 9.0 Hz), 7.08 (d, 2H, J = 9.0 Hz), 3.88 (s, 3H), 2.88 (t, 2H, J = 6.0 Hz), 2.66 (t, 2H, J = 6.0 Hz), 1.60 (p, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.6, 131.6, 129.0, 114.2, 55.6, 41.6, 39.7, 31.1; IR (KBr) cm^{-1}: 3600, 3355, 3302, 2042, 1908, 1592, 1487, 1487, 1317, 1167.

*4.2.15. N-(3-Aminopropyl)-3,4-dimethoxybenzenesulfonamide (**3o**)*

White solid; yield 87%; mp 142–143; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.47 (dd, 1H, J = 3.0, J = 9.0 Hz), 7.37 (d, 1H, J = 3.0 Hz), 7.10 (d, 1H, J = 9.0 Hz), 3.91 (s, 3H), 3.90 (s, 3H), 2.92 (t, 2H, J = 6.0 Hz), 2.83 (t, 2H, J = 6.0 Hz), 1.72 (p, 2H, J = 6.0 Hz).

*4.2.16. N-(3-Aminopropyl)-3-fluorobenzenesulfonamide (**3p**)*

Viscous oil; yield 72%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (brs, 3H), 7.27 (s, 1H), 3.28 (s, 2H), 3.08 (s, 2H), 2.79 (s, 2H), 1.62 (s, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.7, 142.3, 130.8, 122.6, 119.5, 114.3, 42.7, 40.5, 31.0; IR (KBr) cm^{-1}: 3251, 3071, 2931, 1592, 1471, 1435, 1361, 1323, 1226.

*4.2.17. N-(3-Aminopropyl)-4-fluorobenzenesulfonamide (**3q**)*

White solid; yield 79%; mp.104–105 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.90 (dd, 2H, J = 9.0 Hz, J = 6.0 Hz), 7.32 (t, 2H, J = 9.0 Hz), 2.92 (t, 2H, J = 6.0 Hz), 2.67 (t, 2H, J = 6.0 Hz), 1.61 (p, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.9, 136.2, 129.4, 116.1, 41.1, 39.2, 31.5; IR (KBr) cm^{-1}: 3366, 3306, 3254, 3047, 2696, 2048, 1912, 1317.

*4.2.18. N-(3-Aminopropyl)-4-iodobenzenesulfonamide (**3r**)*

Yield 79%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87 (d, 2H, J = 9.0 Hz), 7.58 (d, 2H, J = 9.0 Hz), 3.10 (t, 2H, J = 6.0 Hz), 2.82 (t, 2H, J = 6.0 Hz), 1.60 (p, 2H, J = 6.0 Hz).

*4.2.19. N-(3-Aminopropyl)-4-(trifluoromethyl)benzenesulfonamide (**3s**)*

Yield 78%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (d, 2H, J = 8.2 Hz), 7.78 (d, 2H, J = 9.0 Hz), 3.13 (t, 2H, J = 6.0 Hz), 2.84 (t, 2H, J = 6.0 Hz), 1.62 (p, 2H, J = 6.0 Hz).

*4.2.20. N-(3-Aminopropyl)naphthalene-2-sulfonamide (**3t**)*

White solid; yield 80%; mp 114–115 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.41 (d, 1H, J = 9.0 Hz), 7.94–7.83 (m, 4H), 7.58 (d, 2H, J = 9.0 Hz), 3.12 (bs, 1H), 3.06 (t, 2H, J = 6.0 Hz), 2.73 (t, 2H, J = 6.0 Hz), 1.56 (m, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 136.9, 134.7, 132.1, 129.4, 129.1, 128.5, 128.4, 128.1, 127.8, 127.4, 122.3, 42.5, 40.4, 31.2. LC-MS: m/z 265 (M+1).

*4.3. Synthesis of the mesyl intermediate compound **7***

It was synthesized through a 3-step pathway as previously reported [7].

*4.4. General procedure for preparation of the target compounds **8a–t***

A mixture of mesyl compound **7** (0.19 g, 0.5 mmol), amino compound **3a–t** (0.55 mmol), and diisopropylethylamine (0.29 mL, 1.7 mmol) in DMSO (5 mL) was stirred at 80 °C for 16 h. The mixture was cooled to room temperature, quenched with water (20 mL), then extracted with ethyl acetate (3 × 20 mL). The combined organic layer extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, appropriate ratio of hexanes-ethyl acetate) to obtain the pure target compounds.

*4.4.1. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)benzenesulfonamide (**8a**)*

Colorless viscous oil; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.80 (brs, 1H, exchangeable), 8.42 (brs, 1H, exchangeable), 8.06 (d, 1H, J = 5.3 Hz), 7.78 (d, 2H, J = 7.8 Hz), 7.65–7.55 (m, 6H), 7.46 (d, 1H, J = 3.9 Hz), 7.30 (t, 2H, J = 8.8 Hz), 6.27 (d, 1H, J = 5.1 Hz), 2.85 (t, 2H, J = 6.0 Hz), 2.53 (t, 2H, J = 6.0 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.2, 162.5, 160.9, 158.2, 156.2, 151.6, 148.0, 140.9, 132.7, 131.7, 129.6, 126.8, 121.0, 116.1, 115.8, 114.6, 105.8, 38.8, 29.6; LC-MS: m/z 495 (M+1).

*4.4.2. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)-4-methylbenzenesulfonamide (**8b**)*

Semi-solid; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.82 (brs, 1H, exchangeable), 8.56 (brs, 1H, exchangeable), 8.06 (d, 1H, J = 6.0 Hz), 7.66 (d, 2H, J = 6.0 Hz), 7.60 (d, 2H, J = 6.0 Hz), 7.45 (s, 2H), 7.33 (d, 2H, J = 9.0 Hz), 7.28 (d, 2H, J = 9.0 Hz), 6.29 (d, 1H, J = 3.0 Hz), 3.51 (brs, 2H), 2.94 (brs, 2H), 2.31 (s, 3H); LC-MS: m/z 509 (M+1).

*4.4.3. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)-3,4-dimethylbenzenesulfonamide (**8c**)*

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.82 (brs, 1H, exchangeable), 8.51 (brs, 1H, exchangeable), 8.06 (d, 1H, J = 5.3 Hz), 7.64 (s, 1H), 7.61 (d, 1H, J = 3.0 Hz), 7.59 (s, 1H), 7.54 (s, 1H), 7.50 (d, 1H, J = 3.0 Hz) 7.48 (s, 1H), 7.33 (s, 1H), 7.29 (d, 1H, J = 3.0 Hz), 7.26 (d, 1H, J = 3.0 Hz), 6.28 (d, 1H, J = 5.2 Hz), 2.94 (t, 2H, J = 6.0 Hz), 2.50 (t, 2H, J = 6.0 Hz), 2.23 (s, 3H), 2.22 (s, 3H); LC-MS: m/z 523 (M+1).

*4.4.4. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)-4-methoxybenzenesulfonamide (**8d**)*

White solid; mp 185–186 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.82 (brs, 1H, exchangeable), 8.48 (brs, 1H, exchangeable), 8.06 (d, 1H, J = 3.0 Hz), 7.85 (t, 2H, J = 6.0 Hz), 7.62 (t, 2H, J = 6.0 Hz), 7.41–7.30 (m, 6H), 6.29 (s, 1H), 3.76 (s, 3H), 3.34 (brs, 2H), 2.92 (brs, 2H); LC-MS: m/z 525 (M+1).

*4.4.5. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)-3,4-dimethoxybenzenesulfonamide (**8e**)*

mp 197–199 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.85 (brs, 1H, exchangeable), 8.50 (brs, 1H, exchangeable), 8.05 (d, 1H, J = 6.0 Hz), 7.62 (t, 2H, J = 7.5 Hz), 7.46 (s, 1H), 7.37 (s, 1H), 7.33 (d, 1H, J = 3.0 Hz), 7.30 (s, 2H), 7.27 (s, 1H), 7.04 (d, 1H, J = 6.0 Hz), 6.28 (d, 1H, J = 6.0 Hz), 3.77 (s, 3H), 3.76 (s, 3H), 2.94 (t, 2H, J = 6.0 Hz), 2.51 (d, 2H, J = 6.0 Hz). LC-MS: m/z 555 (M+1).

*4.4.6. 3-Fluoro-N-(2-(4-(6-(4-fluorophenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-ylamino)ethyl)benzenesulfonamide (**8f**)*

mp 192–193 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.83 (brs, 1H, exchangeable), 8.48 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 6.0 Hz), 7.94 (brs, 1H), 7.69 (s, 1H), 7.65 (t, 4H, J = 6.0 Hz), 7.55–7.47 (m, 3H), 7.30 (t, 1H, J = 9.0 Hz), 6.30 (d, 1H, J = 6.0 Hz), 3.41 (s, 2H), 3.02 (s, 2H), 2.79 (s, 2H); LC-MS: m/z 513 (M+1).

*4.4.7. 4-Fluoro-N-(2-(4-(6-(4-fluorophenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-ylamino)ethyl)benzenesulfonamide (**8g**)*

mp 235–236 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.82 (brs, 1H, exchangeable), 8.48 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 3.4 Hz), 7.85 (t, 3H, J = 5.9 Hz), 7.62 (t, 2H, J = 6.0 Hz), 7.45–7.26 (m, 6H), 6.29 (d, 1H, J = 3.8 Hz), 2.96 (s, 2H); LC-MS: m/z 513 (M+1).

*4.4.8. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)-4-iodobenzenesulfonamide (**8h**)*

mp 213–214 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.80 (brs, 1H, exchangeable), 8.47 (brs, 1H, exchangeable), 8.06 (d, 1H, J = 5.2 Hz), 7.89 (d, 2H, J = 8.3 Hz), 7.63 (q, 3H, J = 4.8 Hz), 7.53 (d, 2H, J = 8.4 Hz), 7.46 (s, 1H), 7.45 (d, 2H, J = 8.8 Hz), 6.29 (d, 1H, J = 5.1 Hz), 2.98 (t, 2H, J = 6.4 Hz), 2.50 (s, 2H). LC-MS: m/z 621 (M+1).

*4.4.9. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)-4-(trifluoromethyl) benzenesulfonamide (**8i**)*

mp 164–165 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (brs, 1H, exchangeable), 8.45 (brs, 1H, exchangeable), 8.03 (q, 4H, J = 6.5 Hz), 7.92 (d, 2H, J = 8.3 Hz), 7.61 (t, 2H, J = 7.1 Hz), 7.46 (s, 1H), 7.29 (t, 2H, J = 8.8 Hz), 6.28 (d, 1H, J = 4.9 Hz), 3.40 (s, 2H), 3.01 (s, 2H). LC-MS: m/z 563 (M+1).

*4.4.10. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)naphthalene-2-sulfonamide (**8j**)*

mp 158–159 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.74 (brs, 2H, exchangeable), 8.40 (s, 1H), 8.03 (t, 4H, J = 6.1 Hz), 7.91 (s, 1H), 7.81 (d, 2H, J = 6.9 Hz), 7.59 (s, 5H), 7.43 (s, 1H), 7.28 (s, 2H), 6.21 (s, 1H), 3.02 (brs, 2H), 2.49 (brs, 2H).). LC-MS: m/z 545 (M+1).

*4.4.11. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)benzenesulfonamide (**8k**)*

mp 188–189 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.82 (brs, 1H, exchangeable), 8.48 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 5.3 Hz), 7.78 (d, 2H, J = 7.8 Hz), 7.65–7.55 (m, 6H), 7.46 (d, 1H, J = 3.9 Hz), 7.30 (t, 2H, J = 8.8 Hz), 6.28 (d, 1H, J = 5.1 Hz), 3.31 (s, 2H), 2.86 (t, 2H, J = 6.3 Hz), 1.69–1.66 (m, 2H).

*4.4.12. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)-4-methylbenzenesulfonamide (**8l**)*

mp 210–211 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (brs, 1H, exchangeable), 8.49 (brs, 1H, exchangeable), 8.06 (d, 1H, J = 5.1 Hz), 7.63 (d, 4H, J = 7.3 Hz), 7.54 (s, 1H), 7.45 (s, 2H), 7.31 (t, 4H, J = 6.5 Hz), 6.28 (s, 1H), 3.30 (s, 2H), 2.82 (brs, 2H), 2.32 (brs, 3H), 1.69–1.67 (m, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.8, 162.5, 161.4, 158.1, 156.2, 151.6, 148.1, 142.9, 138.0, 131.7, 130.5, 130.1, 129.9, 127.0, 126.9, 121.0, 116.1, 116.0, 115.8, 114.4, 105.8, 39.7, 29.5, 21.3.

*4.4.13. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)-3,4-dimethylbenzenesulfonamide (**8m**)*

mp 220–221 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.89 (brs, 1H, exchangeable), 8.57 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 6.0 Hz), 7.64 (d, 1H, J = 6.0 Hz), 7.61 (d, 1H, J = 6.0 Hz), 7.54 (s, 1H), 7.49 (s, 1H), 7.45 (d, 1H, J = 6.0 Hz), 7.30 (t, 4H, J = 9.0 Hz), 6.28 (d, 1H, J = 6.0 Hz), 2.82 (t, 2H, J = 6.0 Hz), 2.51 (t, 2H, J = 6.0 Hz), 2.25 (s, 3H), 2.23 (s, 3H), 1.68 (p, 2H, J = 6.0 Hz); LC-MS: m/z 537 (M+1).

*4.4.14. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)-4-methoxybenzenesulfonamide (**8n**)*

mp 180–181 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.83 (brs, 1H, exchangeable), 8.50 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 6.0 Hz), 7.66 (dt, 4H, J = 9.0, J = 6.0 Hz), 7.46 (s, 2H), 7.30 (t, 2H, J = 9.0 Hz), 7.05 (d, 2H, J = 6.0 Hz), 6.28 (d, 1H, J = 6.0 Hz), 3.79 (s, 3H), 2.81 (brs, 2H), 2.50 (brs, 2H), 1.68 (brs, 2H); LC-MS: m/z 539 (M+1).

*4.4.15. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)-3,4-dimethoxybenzenesulfonamide (**8o**)*

Colorless viscous oil; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (brs, 1H, exchangeable), 8.55 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 6.0 Hz), 7.65–7.61 (m, 3H), 7.45 (d, 1H, J = 6.0 Hz), 7.34 (dd, 2H, J = 3.0, J = 6.0 Hz), 7.29 (d, 1H, J = 3.0 Hz), 7.28 (d, 1H, J = 3.0 Hz), 7.07 (d, 1H, J = 6.0 Hz), 6.28 (d, 1H, J = 6.0 Hz), 3.79 (s, 3H), 3.78 (s, 3H), 2.83 (t, 2H, J = 6.0 Hz), 2.51 (t, 2H, J = 6.0 Hz), 1.65 (p, 2H, J = 6.0 Hz); LC-MS: m/z 569 (M+1).

*4.4.16. 3-Fluoro-N-(3-(4-(6-(4-fluorophenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-ylamino)propyl)benzenesulfonamide (**8p**)*

mp 209–210 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (brs, 1H, exchangeable), 8.54 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 6.0 Hz), 7.80 (s, 1H), 7.62–7.56 (m, 5H), 7.45 (d, 2H, J = 3.0 Hz), 7.30 (t, 2H, J = 9.0 Hz), 6.28 (d, 1H, J = 3.0 Hz), 3.35 (brs, 2H), 2.89 (brs, 2H), 1.69 (brs, 2H).

*4.4.17. 4-Fluoro-N-(3-(4-(6-(4-fluorophenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-ylamino)propyl)benzenesulfonamide (**8q**)*

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (brs, 1H, exchangeable), 8.54 (brs, 1H, exchangeable), 8.03 (d, 1H, J = 5.3 Hz), 7.65 (d, 4H, J = 7.7 Hz), 7.53 (s, 1H), 7.45 (s, 1H), 7.32 (t, 4H, J = 7.0 Hz), 6.28 (d, 1H, J = 4.8 Hz), 2.82 (s, 2H), 2.54 (s, 2H), 1.70–1.66 (m, 2H).

*4.4.18. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)-4-iodobenzenesulfonamide (**8r**)*

mp 206–207 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.84 (brs, 1H, exchangeable), 8.49 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 6.0 Hz), 7.91 (d, 2H, J = 9.0 Hz), 7.72 (s, 1H), 7.64 (dd, 2H, J = 3.0, J = 6.0 Hz), 7.52 (t, 2H, J = 9.0 Hz), 7.45 (s, 1H), 7.30 (t, 2H, J = 9.0 Hz), 6.29 (d, 1H, J = 6.0 Hz), 2.85 (brs, 2H), 2.50 (brs, 2H), 1.67 (t, 2H, J = 6.0 Hz); LC-MS: m/z 635 (M+1).

*4.4.19. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)-4-(trifluoromethyl) benzenesulfonamide (**8s**)*

mp 118–120 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.82 (brs, 1H, exchangeable), 8.45 (brs, 1H, exchangeable), 8.07 (d, 1H, J = 5.3 Hz), 7.99 (t, 4H, J = 7.6 Hz), 7.80 (brd, 1H, J = 8.1 Hz), 7.62 (t, 2H, J = 7.1 Hz), 7.46 (d, 1H, J = 3.8 Hz), 7.29 (t, 2H, J = 8.8 Hz), 6.28 (d, 1H, J = 4.3 Hz), 2.92–2.88 (t, 2H, J = 6.9 Hz), 2.50 (s, 2H), 1.70 (brs, 2H). LC-MS: m/z 577 (M+1).

*4.4.20. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)naphthalene-2-sulfonamide (**8t**)*

mp 198–199 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.79 (brs, 2H, exchangeable), 8.42 (d, 1H, J = 8.6 Hz), 8.06 (q, 3H, J = 8.4 Hz), 7.97 (d, 1H, J = 7.8 Hz), 7.80 (d, 2H, J = 8.6 Hz), 7.71–7.57 (m, 5H), 7.41 (s, 1H), 7.30 (t, 2H, J = 8.7 Hz), 6.25 (d, 1H, J = 4.6 Hz), 3.29 (s, 2H), 2.91 (t, 2H, J = 6.6 Hz), 1.70 (p, 2H, J = 6.0 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.8, 162.5, 161.4, 158.0, 156.2, 151.6, 148.1, 137.9, 134.5, 132.2, 131.8, 131.7, 129.8, 129.5, 129.1, 128.2, 127.9, 127.7, 122.7, 121.0, 116.2, 116.0, 115.8, 105.7, 41.1, 38.7, 30.0.

*4.5. Preparation of N-(2-(4-(6-(4-fluorophenyl)imidazo[2,1-b] thiazol-5-yl)pyrimidin-2-ylamino)ethyl)-4-hydroxybenzenesulfonamide (**8u**) and N-(3-(4-(6-(4-fluorophenyl) imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-ylamino)propyl)-4-hydroxybenzenesulfonamide (**8v**)*

A solution of the corresponding methoxy compound (**8d** or **8n**, 0.2 mmol) in anhydrous dichloromethane (3 mL) was cooled to −78 °C, and BBr_3 (17% in DCM solution, 1.2 mL, 2.1 mmol) was added dropwise thereto under nitrogen over a period of 10 min. The reaction mixture was stirred at the same temperature for 1 h, then the temperature was allowed to reach room temperature and kept overnight at same temperature. 10% Na_2CO_3 solution (15 mL) and ethyl acetate (15 mL) were added, and the alkaline aqueous layer was extracted with ethyl acetate (3 × 20). The combined organic layer extracts were washed with brine, separated, and dried over anhydrous Na_2SO_4. The organic solvent was evaporated under reduced pressure, and the residue was purified by column chromatography to obtain the purified title compounds.

*4.5.1. N-(2-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)ethyl)-4-hydroxybenzenesulfonamide (**8u**)*

White solid; mp 194–195 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.34 (s, 1H), 8.85 (brs, 1H, exchangeable), 8.21 (brs, exchangeable), 8.07 (d, 1H, J = 5.2 Hz), 7.61 (d, 4H, J = 8.4 Hz), 7.45 (s, 2H), 7.30 (t, 2H, J = 8.7 Hz), 6.87 (d, 2H, J = 8.5 Hz), 6.29 (d, 1H, J = 5.0 Hz), 2.90 (t, 2H, J = 6.0 Hz), 2.52 (brs, 2H). LC-MS: m/z 511 (M+1).

*4.5.2. N-(3-(4-(6-(4-Fluorophenyl)imidazo[2,1-b]thiazol-5-yl) pyrimidin-2-ylamino)propyl)-4-hydroxybenzenesulfonamide (**8v**)*

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.08 (d, 1H, J = 5.3 Hz), 7.65–7.57 (m, 4H), 7.46 (d, 1H, J = 4.5 Hz), 7.30 (t, 3H, J = 8.8 Hz), 6.86 (d, 2H, J = 7.6 Hz), 6.29 (d, 1H, J = 6 Hz), 5.75 (s, 1H), 3.17 (brs, 2H), 2.79 (t, 2H, J = 6.0 Hz), 1.68 (p, 2H, J = 6.0 Hz).

4.6. Cancer cell line screening at the NCI

Screening against the cancer cell lines was carried out at the National Cancer Institute (NCI), Bethesda, Maryland, USA [25] applying the standard protocol of the NCI [28].

4.7. B-RAF, V600E-B-RAF, and C-RAF kinase profiling

Reaction Biology Corp. Kinase HotSpot^{SM} service [29] was used for screening of compound **1g**. Assay protocol: In a final reaction volume of 25 μL, kinase (5–10 mU) is incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.66 mg/mL myelin basic protein, 10 mM magnesium acetate and [γ^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the Mg-ATP mix. After incubation for 40 min at room temperature, the reaction is stopped by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

4.8. MEK/ERK kinase screening

4.8.1. Protein immunoblotting and immunoprecipitation

For immunoblotting, A375 melanoma cells grown to 70%–80% confluence were harvested in RIPA lysis buffer and disrupted by sonication and centrifuged at 12,000 rpm for 10 min. The quantity of protein was determined with DC protein assay kit (Bio-Rad Lab., Hercules, CA). Protein samples were subjected to SDS-PAGE and immunoblotting.

4.8.2. Suppression of RAF-1/MEK/ERK signaling pathway in A375 cells

To assess the effect of compound **1u** on the MEK/ERK kinases, A375 cells were treated with compound **1u** and Sorafenib (1, 3, and 10 μM) for 24 h and immunoblotted with antibodies against phosphor-MEK1/2, phospho-ERK1/2 and β-actin, respectively.

4.9. Cytotoxicity screening over L132 normal cells

4.9.1. Cell culture

The human lung normal cell line (L132; human embryonic pulmonary epithelial cells) were obtained from the Korean cell line bank (KCLB, Seoul, Korea). Cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, penicillin (100 units/ml) and streptomycin sulfate (100 μg/mL). Cells were cultured at 37 °C in an atmosphere of 5% CO_2.

4.9.2. Quantification of cytotoxicity

MTT assay was used to determine sample cytotoxicity. Cells (5×10^4) were seeded in each well containing 100 μL of the medium supplemented with 10% FBS in a 96-well plate. After 24 h, various concentrations of sample were added. After 48 h, 20 μL of MTT (5 mg/mL stock solution, in phosphate buffered saline (PBS)) was added, and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue, which was formed in the cells, was dissolved with 200 μL DMSO. The optical density was measured at 540 nm using microplate readers (Molecular Devices, CA, USA).

Acknowledgments

This work was supported by Korea Institute of Science and Technology (KIST), Seoul, Republic of Korea, KIST Project (2E24680). We would like to thank the National Cancer Institute (NCI), Bethesda, Maryland, USA, for performing the *in vitro* anticancer testing over the cell lines.

Appendix A. Supplementary data

Supplementary data related to this article containing charts of the NCI-57 cell line screening results, and NMR and mass spectra can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.065.

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