European Journal of Medicinal Chemistry 65 (2013) 315–322

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

New triarylpyrazoles as broad-spectrum anticancer agents: Design, synthesis, and biological evaluation

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ARTICLE INFO

Article history:
Received 25 January 2013
Received in revised form
29 April 2013
Accepted 30 April 2013
Available online 13 May 2013

Keywords:
Anticancer
Cytotoxicity
Diarylurea
Diarylamide
1,3,4-Triarylpyrazole

ABSTRACT

A new series of diarylureas and diarylamides possessing 1,3,4-triarylpyrazole scaffold was designed and synthesized. Their *in vitro* antiproliferative activities against NCI-60 cell line panel were tested. Most of the compounds showed strong and broad-spectrum antiproliferative activities. Compound **18** exerted sub-micromolar IC_{50} values over all the subpanels of nine different cancer types. Its IC_{50} value over MDA-MB-435 melanoma cell line was 27 nM. Compounds **10**–**13**, **22**, and **23** possessing urea spacer exerted lethal effect over the NCI-60 panel with mean %inhibitions more than 100% in single-dose testing. Compounds **13** and **23** with urea linker and 3′,5′-*bis*(trifluoromethyl)phenyl terminal ring showed the highest mean %inhibition over the NCI-60 panel in single-dose testing, and showed high potencies and broad-spectrum anticancer activities in five-dose testing.

1. Introduction

Cancer is a generic term for a large group of diseases that can affect any part of the body. Cancer is a major leading cause of death worldwide, and it accounted for 7.6 million death cases (around 13% of all deaths) in 2008 according to WHO reports. Lung, stomach, liver, colon, and breast cancer cause the most cancer deaths each year. About 30% of cancer deaths are due to the five leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol intake. More than 70% of all cancer deaths occurred in low- and middle-income countries. Deaths from cancer worldwide are projected to continue to rise to over 13 million in 2030 [1]. Inspite of the extensive efforts and investment in research, the management of human malignancies still constitutes a major challenge for contemporary medicinal chemistry. There has been an urgent need for development of more efficient anticancer agents with minimal side effects.

Diarylureas and diarylamides have been highlighted as potential antiproliferative agents against a variety of cancer cell lines [2–17]. Sorafenib (Nexavar®) is an example of anticancer diarylureas that has been approved by the U.S. Food and Drug Administration (FDA) for treatment of advanced renal cancer [18]. It has also been approved in Europe for treatment of hepatocellular carcinoma (HCC) [19]. Sorafenib is currently subjected to clinical trials for other types of cancer. Imatinib (Gleevec®) is an example of diarylamides which is used for treatment of chronic myeloid leukemia (CML) with diminished side effects [20]. We have previously reported a series of diarylureas and diarylamides possessing 1,3,4-triarylpyrazole scaffold as potential antiproliferative agents against human melanoma cell lines [12]. In the present investigation, we present positional isomers of the previously reported compounds (Fig. 1) in order to compare their potencies and to optimize the scaffold. The cytotoxicities of the new analogs were tested against NCI-60 cancer cell line panel of nine different cancer types.

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http://dx.doi.org/10.1016/j.ejmech.2013.04.067

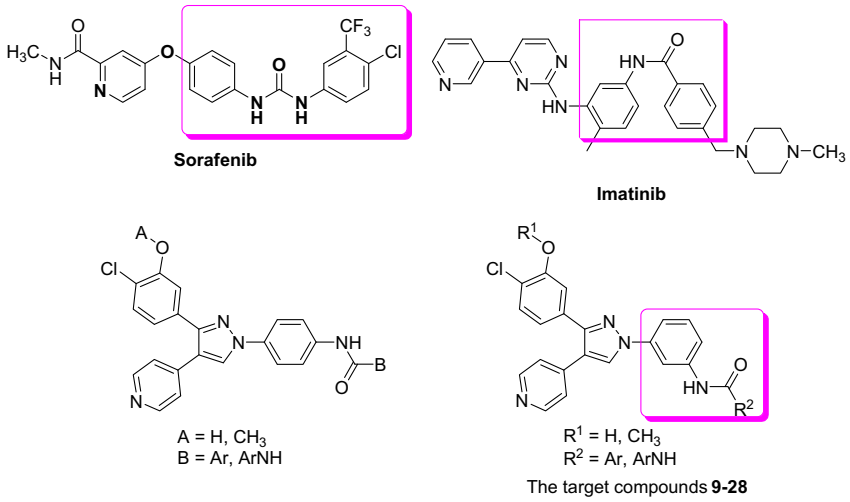
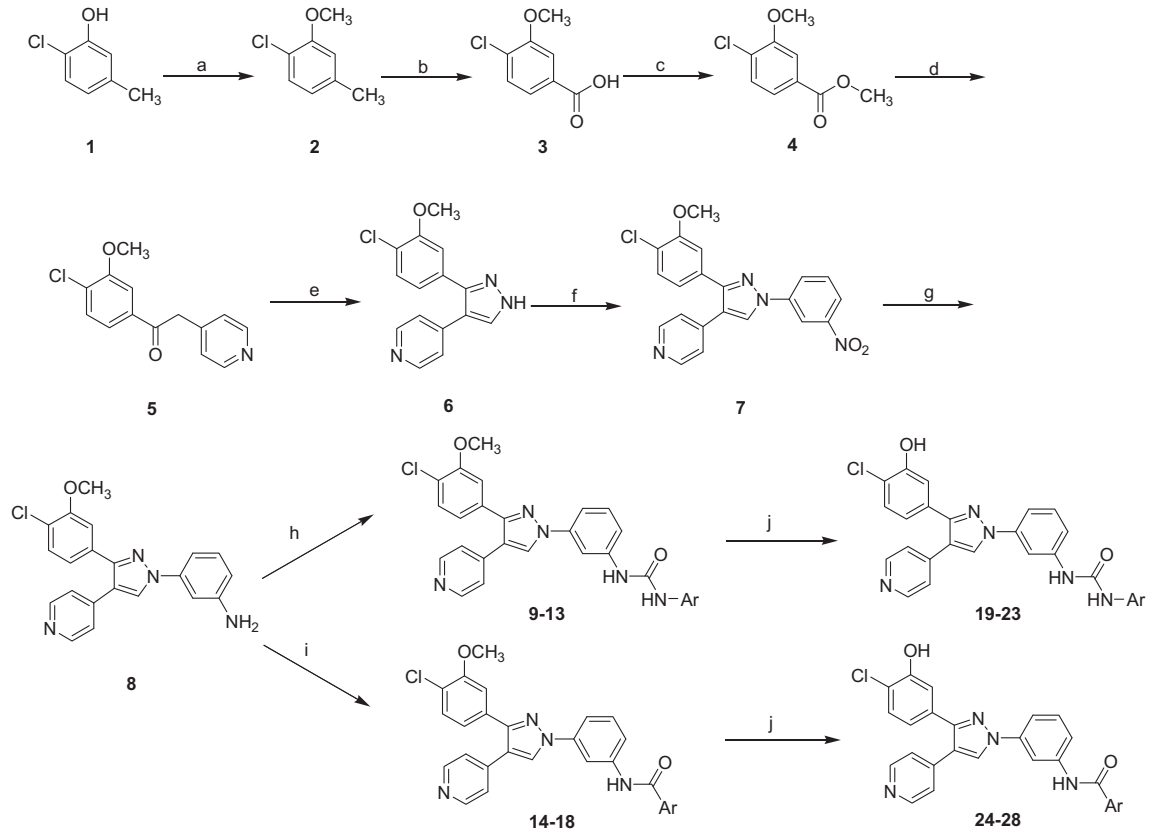


Fig. 1. Structures of Sorafenib, Imatinib, reported 1,3,4-triarylpyrazole derivatives [12], and the target compounds **9**–**28**.

2. Results and discussion

2.1. Chemistry

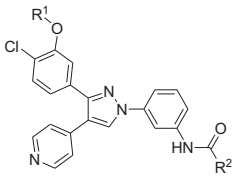
The target triarylpyrazole compounds **9**–**28** were synthesized as illustrated in Scheme 1. Methylation of the phenolic hydroxyl group of 2-chloro-5-methylphenol (**1**) using dimethyl sulfate gave 1-chloro-2-methoxy-4-methylbenzene (**2**) [21]. Oxidation of the methyl group of **2** using $KMnO_4$ gave the corresponding carboxylic acid **3** [22], which was further esterified with methanol in the presence of acetyl chloride afforded the corresponding methyl ester **4**. The pyridyl derivative **5** was obtained by treatment of **4** with 4-picoline in the presence of lithium hexamethyldisilazide (LiHMDS). Cyclization to the pyrazole compound **6** was carried out by treatment of **5** with dimethylformamide dimethyl acetal (DMF-DMA), and subsequent treatment with hydrazine monohydrate [12,23].



Scheme 1. Reagents and conditions: (a) $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux, 1 h; (b) $KMnO_4$, C_5H_5N, H_2O, 50 °C, 24 h, then rt, 13 h, 90%; (c) acetyl chloride, CH_3OH, rt, 15 h, 85%; (d) 4-picoline, LiHMDS, THF, rt, overnight, 45%; (e) (i) DMF-DMA, rt, 18 h, (ii) hydrazine monohydrate, C_2H_5OH, rt, overnight, 81%; f) 1-iodo-3-nitrobenzene, K_2CO_3, CuI, L-proline, DMSO, 90 °C, 8 h, 91.4%; g) H_2, Pd/C, THF, rt, 2 h, 64.8%; h) appropriate aryl isocyanate, THF, rt, 12 h, 32–85%; i) appropriate benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 35–84%; j) BBr_3, CH_2Cl_2, −78 °C, 30 min; then rt, 1 h, 40–88% (**19**–**23**), 36–72% (**24**–**28**).

1,3,4-Triarylpyrazole derivative **8** with amino group was prepared through *N*-arylation of **6** using 1-iodo-3-nitrobenzene in the presence of anhydrous K_2CO_3, CuI, and L-proline, and subsequent reduction of the nitro group of **7** using Pd/C in hydrogen atmosphere. Reaction of the amino group of **8** with the appropriate aryl isocyanate derivatives afforded the corresponding urea derivatives **9**–**13**. Synthesis of the methoxy compounds **14**–**18** with amide linker was carried out by condensation of the amino compound **8** with the appropriate benzoic acid derivatives in the presence of HOBt, EDCI, and triethylamine. Demethylation of the methoxy group of **9**–**18** using BBr_3 afforded the corresponding hydroxyl derivatives **19**–**28**. Table 1 illustrates structures of the final compounds, their yield percentages, and their melting points.

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



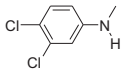
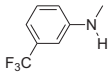
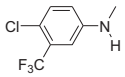
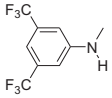
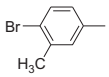
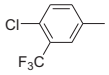
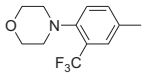
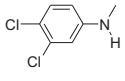
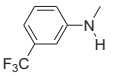
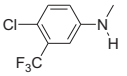
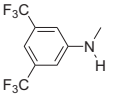
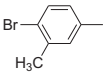
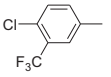
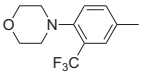
Compound no.	R^1	R^2	Yield%	Melting point (°C)
9	CH_3		32	194–197
10	CH_3		57	218–221
11	CH_3		66	195–198
12	CH_3		68	226–228
13	CH_3		85	244–247
14	CH_3		35	214–215
15	CH_3		60	218–221
16	CH_3		69	171–174
17	CH_3		41	232–235
18	CH_3		84	167–168
19	H		40	201–204
20	H		88	184–187

Table 1 (*continued*)

Compound no.	R^1	R^2	Yield%	Melting point (°C)
21	H		57	197–200
22	H		50	238–241
23	H		87	194–196
24	H		36	>280
25	H		39	242–245 (dec.)
26	H		29	156–159
27	H		70	190–192
28	H		72	148–151

2.2. Antiproliferative activities against 60 cell line panel at the NCI

2.2.1. Single-dose testing

Structures of the synthesized compounds were submitted to National Cancer Institute (NCI), Bethesda, Maryland, USA [24], and the 18 compounds shown in Fig. 2 were selected on the basis of degree of structural variation and computer modeling techniques for evaluation of their antineoplastic activity. The selected compounds were subjected to *in vitro* anticancer assay against tumor cells in a full panel of 60 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 60 tested cell lines were determined. The mean inhibition percentages for each of the tested compounds over the full panel of cell lines are illustrated in Fig. 2.

The results showed that the methoxy derivatives **10**, **11**, **14**, **15**, **17**, and **18** were more active than the corresponding hydroxyl derivatives **20**, **21**, **24**, **25**, **27**, and **28**. But the hydroxyl compounds **22** and **23** were exceptionally more active than the corresponding methoxy derivatives **12** and **13**. In addition, the urea derivatives **11**, **12**, and **22** exerted higher activities than the corresponding analogs with amide spacer **16**, **17**, and **27**. This may be attributed to that the longer linker, urea moiety, may geometrically permit appropriate fitting of the molecule at the receptor site. Or the terminal NH group of the urea moiety may form additional hydrogen bond(s) at the receptor site. Any or both of these effects would enable optimal drug–receptor interaction, and hence higher antiproliferative activity. Compounds **10**–**13**, **22**, and **23** containing urea spacer exerted lethal effect over the NCI-60 panel with mean percentage inhibitions more than 100%.

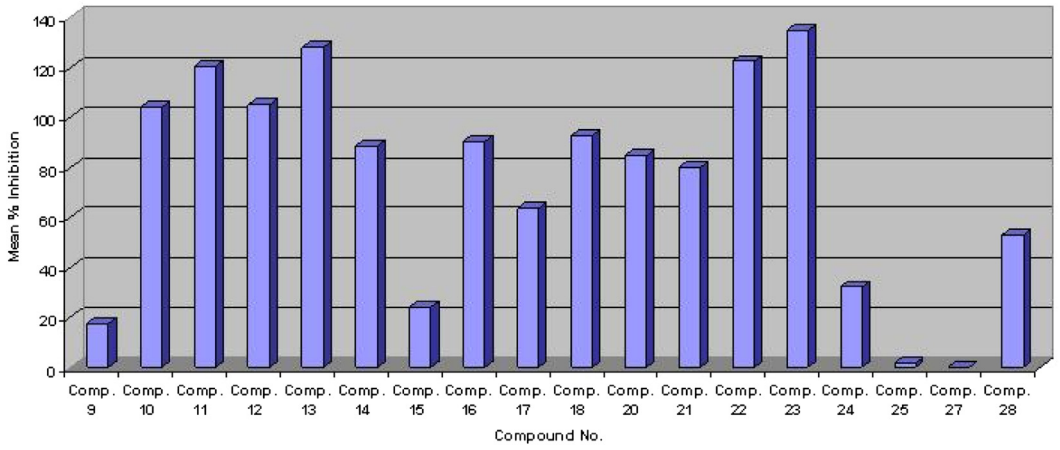


Fig. 2. Mean inhibition percentages observed with the final compounds in single-dose (10 μM) 60 cancer cell line screening. Mean %inhibition represents the mean inhibition percentages over the 60 cell lines. The inhibition percentages were calculated by subtracting the growth percentages from 100.

The effects of substituents on the terminal phenyl ring were also studied. Compounds **10**–**13** showed higher mean %inhibitions than compound **9**. So it can be concluded that chloro and/or trifluoromethyl substituents on the terminal phenyl ring of urea derivatives enhance the cytotoxic activity. Compound **16** with *m*-(trifluoromethyl)phenyl terminal ring was more active than compound **14** with terminal phenyl ring. Also compounds **18** and **28** with terminal 4′-morpholino-3′-(trifluoromethyl)phenyl moiety were more active than **14** and **24** with terminal phenyl ring. So 3′-(trifluoromethyl)phenyl and 4′-morpholino-3′-(trifluoromethyl) phenyl terminal moieties are favorable for activity. On the other hand, compounds **15** and **17** with 4′-bromo-3′-methylphenyl and 4′-chloro-3′-(trifluoromethyl)phenyl terminal rings, respectively, were less active than compound **14**. Similarly, compounds **25** and **27** showed lower activities than compound **24** with terminal phenyl ring. So 4′-bromo-3′-methylphenyl and 4′-chloro-3′-(trifluoromethyl)phenyl terminal moieties are unfavorable for anticancer activity.

Among all the target compounds, compounds **13** and **23** with urea spacer and 3′,5′-*bis*(trifluoromethyl)phenyl terminal ring showed the highest mean %inhibitions. So these moieties are favorable for anticancer activity of this series of compounds. The steric and/or electronic effect(s) of two trifluoromethyl groups could contribute to affinities of these compounds to the receptor site. The %inhibitions of these two compounds over each cell line of the NCI-60 panel are illustrated in Fig. 3. At 10 μM concentration, both compounds showed lethal effects (>100% inhibition) over 45 cell lines. Both compounds demonstrated broad-spectrum cytotoxicities over all the nine tested cancer types.

2.2.2. Five-dose testing

Compounds **10**–**14**, **16**–**18**, **20**–**23**, and **28** 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 60 cancer cell lines. The mean IC_{50} values of these 13 compounds over the nine cancer types are shown in Table 2.

As shown in Table 2, most of the compounds exhibited high potency (in sub-micromolar and micromolar scale) over all the nine cancer types. Most of the mean IC_{50} data were less than 10 μM. Of special interest, compound **18** with methoxy group, amide linker, and 4′-morpholino-3′-(trifluoromethyl) terminal ring showed the highest potencies over all the nine subpanels of nine different cancer types. All its mean IC_{50} values were in sub-micromolar range. Compounds **13** and **23** with urea spacer and 3′,5′-*bis*(trifluoromethyl)phenyl terminal ring exerted sub-micromolar IC_{50} values over the nine subpanels of nine cancer types.

The IC_{50} values of the 13 compounds tested in five-dose mode over the most sensitive cell line of each subpanel are summarized in Table 3. From these data, we find that HOP-92 non-small cell lung cancer was the most susceptible cell line to the target compounds.

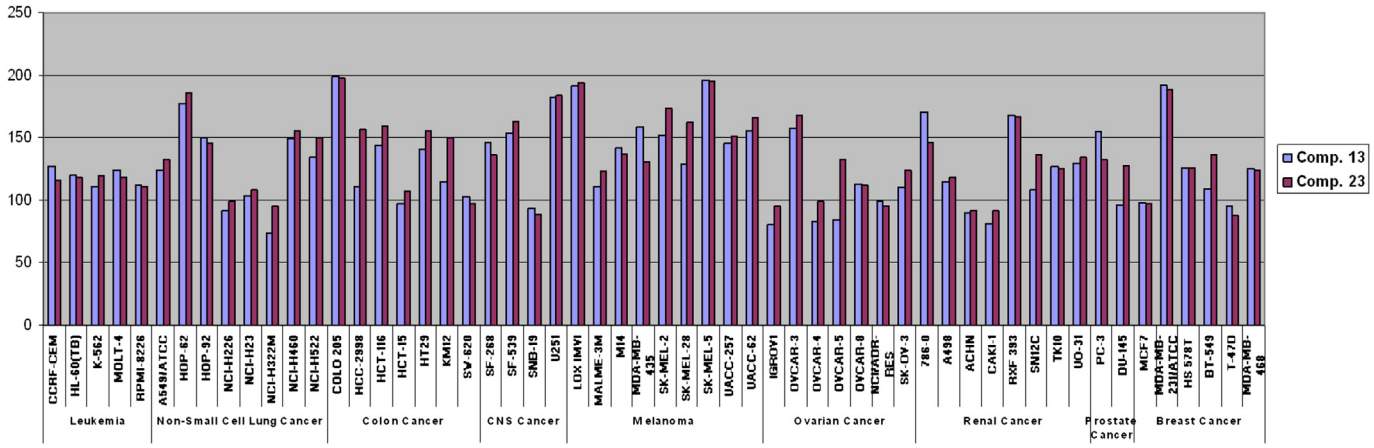


Fig. 3. %inhibition expressed by compounds **13** and **23** at a single-dose concentration of 10 μM over the NCI-60 cancer cell lines.

Table 2
Mean IC_{50} values (μM) of the tested compounds over *in vitro* subpanel cancer cell lines.[a]

		Subpanel cancer cell lines[b]								
		I	II	III	IV	V	VI	VII	VIII	IX
Compound No.	**10**	1.00	1.09	1.23	1.13	0.98	1.31	1.15	1.04	1.12
	11	0.84	0.99	0.92	0.89	0.88	1.08	1.01	1.02	1.05
	12	1.02	1.19	1.17	1.24	0.97	1.43	1.16	1.10	1.24
	13	0.75	0.77	0.71	0.73	0.83	0.85	0.79	0.78	0.95
	14	0.53	2.37	0.79	0.61	0.89	2.79	1.88	0.60	1.03
	16	1.55	2.71	1.78	1.54	4.50	2.10	1.92	1.50	5.24
	17	2.76	5.60	4.28	4.06	2.46	8.43	6.54	3.84	3.15
	18	0.27	0.42	0.42	0.35	0.44	0.59	0.63	0.32	0.52
	20	1.08	1.45	2.38	1.38	1.06	1.72	1.97	1.17	1.17
	21	1.56	3.38	4.72	2.27	2.29	4.04	2.37	1.35	3.03
	22	0.87	0.96	1.25	0.82	0.71	1.04	1.04	0.86	0.81
	23	0.88	0.79	0.76	0.76	0.84	0.86	0.90	0.78	0.93
	28	2.15	15.16	11.20	2.65	2.55	18.49	8.69	3.18	11.33

[a] Mean IC_{50} values were calculated by dividing the summation of IC_{50} values of the compound over cell lines of the same cancer type by the number of cell lines in the subpanel.
[b] I: Leukemia; II: Non-Small Cell Lung Cancer; III: Colon Cancer; IV: CNS Cancer; V: Melanoma; VI: Ovarian Cancer; VII: Renal Cancer; VIII: Prostate Cancer; IX: Breast Cancer.

At this cell line, all the thirteen tested compounds demonstrated IC_{50} values in sub-micromolar scale. In addition, compound **18** showed 2-digit nanomolar IC_{50} value, 27 nM, over MDA-MB-435 melanoma cell line. Among all the target compounds, compounds **11**, **13**, **14**, **18**, and **23** showed the highest potencies with sub-micromolar IC_{50} values over the nine different cell lines.

It is noteworthy that compounds **10**, **12**, **13**, **17**, **22**, and **23** were more potent against MDA-MB-435 melanoma cell line than the corresponding positional isomers with *para*-disubstituted central phenyl ring [12]. The IC_{50} values of these six compounds with *meta*-disubstituted central phenyl ring were in sub-micromolar scale, while IC_{50} values of the *para*-disubstituted phenyl isomers were in micromolar range.

3. Conclusions

A new series of diarylurea and diarylamide derivatives possessing 1,3,4-triarylpyrazole scaffold was synthesized based on our previous literature studies, and as a continuation of our ongoing anticancer development program. Eighteen final compounds were tested at a single dose of 10 μM at the NCI over 60 cell line panel, and thirteen of them were subsequently tested in five-dose testing mode. Compounds **10**–**13**, **22**, and **23** with urea spacer exerted lethal effect over the NCI-60 panel with mean %inhibitions more than 100% in single-dose testing. Among them, compounds **13** and **23** possessing 3′,5′-*bis*(trifluoromethyl)phenyl terminal ring also showed the highest mean %inhibitions in single-dose testing (128.13% and 134.64%, respectively). Both compounds and compound **18** demonstrated highly potent and broad-spectrum anticancer activities over all the tested nine cancer types. Further modifications of these compounds in order to optimize the scaffold and to improve their potencies are currently in progress.

4. Experimental

4.1. General

All melting points were obtained on a Walden Precision Apparatus Electrothermal 9300 apparatus and are uncorrected. Mass spectra (MS) were taken in ESI mode on a Waters 3100 Mass

Table 3
IC_{50} values (μM) of the tested compounds over the most sensitive cell line of each subpanel.

		Cancer cell lines								
		RPMI-8226[a]	HOP-92[b]	KM12[c]	SF-295[d]	MDA-MB-435[e]	OVCAR-3[f]	A498[g]	PC-3[h]	MDA-MB-468[i]
Compound No.	**10**	0.39	0.15	0.81	0.69	0.94	0.70	1.14	0.55	0.57
	11	0.34	0.56	0.57	0.31	0.87	0.58	0.96	0.37	0.60
	12	0.87	0.50	1.06	0.84	0.68	0.89	0.84	0.69	0.83
	13	0.31	0.14	0.53	0.40	0.89	0.41	0.61	0.25	0.31
	14	0.85	0.32	0.56	0.34	0.19	0.33	0.36	0.43	0.35
	16	1.57	0.56	1.66	1.02	0.71	1.20	0.69	1.23	1.32
	17	1.62	0.67	2.06	1.38	0.66	2.42	2.70	1.29	3.69
	18	0.34	0.12	0.25	0.15	0.027	0.22	0.23	0.25	0.18
	20	1.03	0.68	1.01	Not tested	1.10	1.05	0.94	0.83	0.73
	21	1.34	0.51	1.33	0.96	1.49	1.06	2.24	0.95	0.98
	22	0.58	0.38	1.00	0.74	0.66	0.83	1.40	0.59	0.54
	23	0.48	0.34	0.51	0.62	0.91	0.40	0.79	0.32	0.27
	28	1.40	0.56	1.94	1.71	2.04	4.81	15.50	2.15	7.04

[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.

Detector (Waters, Milford, MA, USA). Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker ARX-300, 300 MHz (Bruker Bioscience, Billerica, MA, USA) and a Bruker ARX-400, 400 MHz (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Purities of the target compounds **9**–**28** (>95%) were determined by LC-MS analysis 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 area under curve (AUC) was calculated using Waters MassLynx 4.1 software. Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

4.2. Synthesis of 4-(3-(4-chloro-3-methoxyphenyl)-1H-pyrazol-4-yl) pyridine (**6**)

It was synthesized by the previously reported 5-step method [12,23]. mp: 248–251 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.39 (brs, 1H), 8.48 (d, 2H, J = 6.0 Hz), 8.14 (brs, 1H), 7.45 (d, 1H, J = 8.0 Hz), 7.28 (d, 2H, J = 5.9 Hz), 7.18 (s, 1H), 6.97 (dd, 1H, J = 1.5 Hz, J = 8.2 Hz), 3.77 (s, 3H); MS m/z: 287.0 $[M + H]^+$.

4.3. 4-[3-(4-Chloro-3-methoxyphenyl)-1-(3-nitrophenyl)-1H-pyrazol-4-yl]pyridine (**7**)

A mixture of compound **6** (0.5 g, 1.7 mmol), 1-iodo-3-nitrobenzene (0.9 g, 3.5 mmol), K_2CO_3 (0.7 g, 5.2 mmol), CuI (0.033 g, 0.2 mmol), and L-proline (0.04 g, 0.2 mmol) in DMSO (7 mL) was heated at 90 °C under nitrogen atmosphere for 12 h. The cooled reaction mixture was partitioned between water and ethyl acetate. The organic phase was washed with brine (3 times) and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexane–ethyl acetate 1:5 v/v) to yield compound **7** (0.85 g, 91.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34–9.29 (m, 1H), 8.82–8.79 (m, 1H), 8.63 (s, 2H), 8.48 (d, 1H, J = 7.6 Hz), 8.25 (d, 1H, J = 6.8 Hz), 7.94–7.89 (m, 1H), 7.58–7.51 (m, 1H), 7.44–7.41 (m, 2H), 7.30 (t, 1H, J = 8.0 Hz), 7.12 (d, 1H, J = 6.8 Hz), 3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.3, 154.9, 150.4, 149.1, 140.2, 139.9, 132.6, 131.7, 130.6, 130.4, 124.9, 123.0, 122.0, 121.8, 121.7, 120.8, 113.4, 113.0, 56.5.

4.4. 4-[3-(4-Chloro-3-methoxyphenyl)-1-(3-aminophenyl)-1H-pyrazol-4-yl]pyridine (**8**)

A mixture of compound **7** (0.5 g, 1.2 mmol) and Pd/C (0.5 g) in THF (5 mL) was stirred at room temperature under hydrogen atmosphere for 2 h. The mixture was filtered through celite and the filtrate was evaporated under reduced pressure to give compound **8** (0.3 g, 64.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, 2H, J = 5.6 Hz), 8.08 (s, 1H), 7.34 (d, 1H, J = 8.0 Hz), 7.27–7.22 (m, 4H), 7.17–7.14 (m, 2H), 7.06 (d, 2H, J = 8.0 Hz), 6.65–6.63 (m, 2H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.0, 150.1, 149.6, 147.8, 140.7, 140.5, 132.4, 130.4, 130.3, 127.4, 123.0, 122.6, 121.5, 120.0, 113.8, 112.2, 108.8, 105.9, 56.1.

4.5. General procedure for synthesis of diarylurea derivatives **9**–**13**

To a solution of compound **8** (50 mg, 0.1 mmol) in anhydrous THF (1 mL), a solution of the appropriate aryl isocyanate (0.1 mmol) in THF (1 mL) was added dropwise at room temperature under N_2. The reaction mixture was stirred at room temperature for 12 h. The mixture was evaporated under reduced pressure, and the residue was purified by column chromatography (silica gel, hexane–ethyl acetate 1:5 v/v) to yield the target compounds **9**–**13**.

4.5.1. 1-(3-(3-(4-Chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-phenylurea (**9**)

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (brs, 1H), 8.98 (brs, 1H), 8.56 (s, 2H), 8.14 (s, 1H), 7.64–7.57 (m, 2H), 7.48 (dd, 1H, J = 1.4 Hz, 8.3 Hz), 7.43–7.40 (m, 2H), 7.36–7.25 (m, 6H), 7.16–7.13 (m, 2H), 7.07–7.03 (m, 1H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.0, 153.2, 153.0, 150.1, 140.0, 139.0, 137.0, 136.2, 130.8, 130.3, 130.2, 129.8, 129.2, 127.5, 125.1, 123.0, 121.6, 121.1, 120.8, 118.6, 114.8, 112.2, 110.7, 56.2; MS m/z: 497.15 $[M + H]^+$.

4.5.2. 1-(3-(3-(4-Chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3,4-dichlorophenyl)urea (**10**)

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.15 (brs, 1H), 9.08 (brs, 1H), 8.99 (s, 1H), 8.55 (d, 2H, J = 4.4 Hz), 8.16 (s, 1H), 7.92 (s, 1H), 7.58–7.47 (m, 3H), 7.44 (d, 1H, J = 5.5 Hz), 7.38–7.35 (m, 4H), 7.23 (s, 1H), 7.07 (d, 1H, J = 8.0 Hz), 3.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.4, 152.2, 149.9, 140.5, 139.7, 139.5, 132.7, 131.0, 130.5, 130.1, 130.0, 129.1, 122.6, 121.3, 119.6, 119.4, 118.5, 116.9, 112.5, 112.3, 108.9, 55.9; MS m/z: 565.13 $[M + H]^+$.

4.5.3. 1-(3-(3-(4-Chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (**11**)

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (s, 2H), 8.98 (s, 1H), 8.56 (d, 2H, J = 5.3 Hz), 8.17 (s, 1H), 8.05 (s, 1H), 7.60 (d, 1H, J = 4.7 Hz), 7.56 (d, 1H, J = 1.8 Hz), 7.52 (t, 2H, J = 3.7 Hz), 7.48 (d, 1H, J = 3.3 Hz), 7.45 (s, 1H), 7.39 (d, 2H, J = 5.9 Hz), 7.33 (d, 1H, J = 7.5 Hz), 7.24 (d, 1H, J = 1.6 Hz), 7.08 (dd, 1H, J = 1.7 Hz, 8.1 Hz), 3.78 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.9, 153.0, 150.3, 149.5, 141.1, 140.9, 140.3, 140.0, 133.2, 130.6, 130.4, 129.8, 129.6, 123.1, 122.9, 122.5, 121.8, 121.7, 120.1, 117.4, 114.8, 113.1, 112.8, 109.4, 56.4; MS m/z: 565.07 $[M + H]^+$.

4.5.4. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(3-(3-(4-chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)urea (**12**)

^{1}H NMR (300 MHz, CD_3OD) δ 8.62 (s, 1H), 8.39 (d, 2H, J = 4.5 Hz), 8.08 (s, 1H), 7.95 (s, 1H), 7.56 (d, 1H, J = 8.6 Hz), 7.45–7.35 (m, 6H), 7.33–7.30 (m, 2H), 7.15 (d, 1H, J = 5.7 Hz), 6.98 (dd, 1H, J = 1.8 Hz, 8.0 Hz), 3.72 (s, 3H); MS m/z: 599.0 $[M + H]^+$.

4.5.5. 1-(3,5-bis(trifluoromethyl)phenyl)-3-(3-(3-(4-chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)urea (**13**)

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.51 (brs, 1H), 9.37 (brs, 1H), 9.03 (s, 1H), 8.59 (d, 2H, J = 4.4 Hz), 8.21 (brs, 3H), 7.70–7.63 (m, 2H), 7.56–7.51 (m, 2H), 7.42 (d, 3H, J = 4.6 Hz), 7.28 (s, 1H), 7.11 (d, 1H, J = 8.1 Hz), 3.82 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.9, 152.9, 150.3, 149.5, 142.2, 140.8, 140.3, 140.0, 133.2, 131.4, 131.0, 130.6, 129.7, 123.1, 121.8, 120.1, 118.6, 117.8, 115.2, 113.0, 109.8, 56.4; MS m/z: 633.1 $[M + H]^+$.

4.6. General procedure for synthesis of diarylamide derivatives **14**–**18**

A mixture of compound **8** (50 mg, 0.1 mmol), the appropriate benzoic acid derivative (0.2 mmol), HOBt (36 mg, 0.3 mmol), and EDCI (38 mg, 0.2 mmol) in DMF (1.0 mL) was cooled to 0 °C under nitrogen atmosphere. Triethylamine (0.03 mL, 0.2 mmol) was added thereto at the same temperature. The mixture was then stirred at 80 °C for 12 h. The reaction mixture was cooled and then partitioned between H_2O and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 5 mL). The combined organic layer extracts were washed with

brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexane–ethyl acetate 1:1 *v*/*v*) to yield the target compounds **14**–**18**.

*4.6.1. N-(3-(3-(4-chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)benzamide (**14**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.45 (d, 2H, J = 4.6 Hz), 8.25 (s, 1H), 8.06 (s, 1H), 7.81 (d, 2H, J = 7.6 Hz), 7.49–7.44 (m, 3H), 7.40–7.32 (m, 3H), 7.24 (d, 1H, J = 8.0 Hz), 7.15 (d, 2H, J = 4.7 Hz), 7.04 (s, 1H), 6.96 (d, 1H, J = 8.1 Hz), 3.69 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 155.0, 150.0, 140.5, 140.0, 139.5, 134.5, 132.2, 130.3, 130.2, 128.9, 127.5, 127.2, 123.0, 122.8, 121.5, 120.3, 118.5, 114.9, 112.2, 110.9, 56.1; MS *m*/*z*: 482.11 $[M + H]^+$.

*4.6.2. 4-Bromo-N-(3-(3-(4-chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl) phenyl)-3-methylbenzamide (**15**)*

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (brs, 2H), 8.30 (s, 2H), 8.17 (s, 1H), 7.76 (s, 1H), 7.63 (d, 1H, J = 8.1 Hz), 7.58–7.53 (m, 3H), 7.47 (t, 1H, J = 7.9 Hz), 7.34 (d, 1H, J = 8.0 Hz), 7.25 (d, 2H, J = 3.8 Hz), 7.13 (s, 1H), 7.07 (d, 1H, J = 8.1 Hz), 3.79 (s, 3H), 2.46 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3, 162.3, 155.0, 150.2, 140.4, 140.0, 139.2, 139.0, 133.5, 132.9, 132.2, 130.4, 129.5, 127.4, 125.7, 123.0, 121.5, 120.4, 118.4, 115.0, 112.2, 110.8, 56.1, 23.1; MS *m*/*z*: 575.06 $[M + H]^+$.

*4.6.3. N-(3-(3-(4-Chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(trifluoromethyl)benzamide (**16**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, 2H, J = 4.7 Hz), 8.31 (s, 1H), 8.23 (s, 1H), 8.18 (d, 2H, J = 7.7 Hz), 8.10 (d, 1H, J = 7.7 Hz), 7.85 (d, 1H, J = 7.7 Hz), 7.69–7.60 (m, 4H), 7.52 (d, 1H, J = 8.1 Hz), 7.36 (d, 1H, J = 8.1 Hz), 7.27 (d, 1H, J = 1.4 Hz), 7.14 (s, 1H), 7.08 (dd, 1H, J = 1.7 Hz, 8.1 Hz), 3.81 (s, 3H); MS *m*/*z*: 550.1 $[M + H]^+$.

*4.6.4. 4-Chloro-N-(3-(3-(4-chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(trifluoromethyl)benzamide (**17**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, 2H, J = 4.7 Hz), 8.27–8.16 (m, 4H), 8.03 (d, 1H, J = 8.1 Hz), 7.68–7.61 (m, 3H), 7.51 (t, 1H, J = 7.8 Hz), 7.36 (d, 1H, J = 8.2 Hz), 7.27–7.24 (m, 2H), 7.13 (s, 1H), 7.07 (d, 1H, J = 8.0 Hz), 3.81 (s, 3H); MS *m*/*z*: 584.1 $[M + H]^+$.

*4.6.5. N-(3-(3-(4-Chloro-3-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-4-morpholino-3-(trifluoromethyl)benzamide (**18**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (brs, 1H), 8.56 (s, 2H) 8.31 (s, 1H), 8.18 (d, 1H, J = 6.8 Hz), 8.11 (s, 1H), 7.59 (d, 1H, J = 7.2 Hz), 7.48 (d, 1H, J = 7.8 Hz), 7.35 (t, 2H, J = 6.2 Hz), 7.25 (t, 2H, J = 3.6 Hz), 7.18 (s, 1H), 7.13 (d, 1H, J = 7.1 Hz), 7.07 (brs, 2H), 3.93–3.78 (m, 7H), 3.02 (t, 4H, J = 2.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 154.9, 150.1, 147.8, 140.7, 140.4, 140.0, 139.3, 132.3, 132.1, 132.0, 130.4, 130.3, 130.1, 127.4, 123.3, 123.0, 121.5, 119.9, 118.6, 115.0, 113.8, 112.2, 111.0, 108.8, 105.9, 67.1, 56.1, 53.4; MS *m*/*z*: 635.17 $[M + H]^+$.

*4.7. General procedure for demethylation to the target hydroxyl derivatives **19**–**28***

To a solution of compounds **9**–**18** (0.1 mmol) in methylene chloride (1 mL), BBr_3 (0.08 mL of a 1 M solution in methylene chloride, 1.2 mmol) was added dropwise at −78 °C under N_2, and the reaction mixture was stirred at the same temperature for 30 min. The mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was quenched with saturated aqueous $NaHCO_3$. Ethyl acetate was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate. The combined organic layer extracts were washed with brine, and then dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by short column chromatography (silica gel, hexane–ethyl acetate 1:5 *v*/*v*) to yield the target compounds **19**–**28**.

*4.7.1. 1-(3-(3-(4-Chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-phenylurea (**19**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (brs, 1H), 10.34 (brs, 1H), 8.90 (s, 1H), 8.72 (s, 2H), 8.13 (s, 1H), 7.65–7.59 (m, 2H), 7.48–7.40 (m, 3H), 7.33–7.24 (m, 6H), 7.22–7.19 (m, 2H), 7.04–6.94 (m, 1H); MS *m*/*z*: 483.1 $[M + H]^+$.

*4.7.2. 1-(3-(3-(4-Chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3,4-dichlorophenyl)urea (**20**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (brs, 1H), 9.27 (s, 1H), 9.19 (s, 1H), 8.63 (s, 1H), 8.55 (s, 2H), 7.98 (s, 1H), 7.89 (s, 1H), 7.78 (d, 1H, J = 7.4 Hz), 7.54 (d, 1H, J = 7.3 Hz), 7.45 (d, 1H, J = 7.1 Hz), 7.35 (brs, 5H), 7.16 (s, 1H), 6.91 (d, 1H, J = 8.0 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 153.6, 152.7, 150.4, 149.2, 140.3, 140.0, 139.4, 134.2, 133.8, 132.6, 131.5, 131.1, 130.5, 124.0, 123.1, 120.7, 120.4, 120.3, 120.1, 119.1, 118.7, 118.2, 116.7, 110.0; MS *m*/*z*: 551.03 $[M + H]^+$.

*4.7.3. 1-(3-(3-(4-Chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (**21**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (brs, 1H), 9.24–9.20 (m, 2H), 8.63 (s, 1H), 8.55–8.54 (m, 2H), 8.03–7.99 (m, 2H), 7.77 (d, 1H, J = 8.8 Hz), 7.61–7.51 (m, 4H), 7.48–7.39 (m, 4H), 7.15 (d, 1H, J = 1.8 Hz), 6.91 (dd, 1H, J = 1.9 Hz, 8.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 153.6, 152.8, 150.2, 149.8, 141.1, 140.7, 39.5, 134.2, 132.7, 132.6, 132.1, 132.0, 130.6, 123.5, 123.2, 121.0, 120.9, 120.8, 120.5, 119.1, 118.6, 118.2, 116.7, 109.8; MS *m*/*z*: 551.0 $[M + H]^+$.

*4.7.4. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(3-(3-(4-chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)urea (**22**)*

^{1}H NMR (400 MHz, CD_3OD) δ 8.56 (s, 1H), 8.37 (d, 2H, J = 5.5 Hz), 8.05 (d, 1H, J = 1.9 Hz), 7.92 (d, 1H, J = 2.4 Hz), 7.55 (dd, 1H, J = 2.4 Hz, 8.6 Hz), 7.45–7.40 (m, 2H), 7.35–7.32 (m, 3H), 7.26–7.23 (m, 2H), 6.99 (d, 1H, J = 2.0 Hz), 6.88 (dd, 1H, J = 1.9 Hz, 8.2 Hz); MS *m*/*z*: 585.1 $[M + H]^+$.

*4.7.5. 1-(3,5-bis(Trifluoromethyl)phenyl)-3-(3-(3-(4-chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)urea (**23**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (brs, 1H), 9.59 (s, 1H), 9.46 (s, 1H), 8.63 (s, 1H), 8.54 (d, 2H, J = 6.0 Hz), 8.16 (s, 1H), 7.99 (d, 1H, J = 2.5 Hz), 7.79 (d, 1H, J = 8.8 Hz), 7.68 (s, 1H), 7.52 (dd, 1H, J = 2.5 Hz, 8.8 Hz), 7.39–7.35(m, 3H), 7.15 (d, 1H, J = 1.9 Hz), 6.91 (dd, 1H, J = 1.9 Hz, 8.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 153.6, 152.8, 150.4, 149.2, 142.0, 140.3, 140.1, 139.4, 134.2, 133.8, 132.6, 131.9, 131.4, 131.0, 130.5, 125.5, 123.1, 121.1, 120.4, 120.3, 118.8, 118.6, 116.7, 110.5; MS *m*/*z*: 618.1 $[M + H]^+$.

*4.7.6. N-(3-(3-(4-Chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl) benzamide (**24**)*

^{1}H NMR (300 MHz, DMSO-d_6) δ 10.52 (s, 1H), 10.39 (s, 1H), 8.98 (s, 1H), 8.56 (d, 2H, J = 5.9 Hz), 8.50 (s, 1H), 8.01 (d, 2H, J = 6.8 Hz), 7.80 (d, 1H, J = 8.1 Hz), 7.67–7.51 (m, 5H), 7.42–7.37 (m, 3H), 7.16 (d, 1H, J = 1.8 Hz), 6.93 (dd, 1H, J = 1.8 Hz, 8.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.1, 153.1, 149.9, 140.4, 139.8, 139.3, 134.6, 132.3, 130.0, 128.4, 127.7, 126.9, 123.3, 122.5, 121.9, 120.0, 118.6, 114.9, 111.9, 110.7; MS *m*/*z*: 468.1 $[M + H]^+$.

*4.7.7. 4-Bromo-N-(3-(3-(4-chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-methylbenzamide (**25**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (brs, 1H), 10.38 (brs, 1H), 8.99 (s, 1H), 8.56 (brs, 2H), 8.47 (s, 1H), 8.00 (s, 1H), 7.82–7.77 (m, 3H), 7.68 (d, 1H, J = 7.2 Hz), 7.55 (t, 1H, J = 8.0 Hz), 7.42–7.37 (m, 3H), 7.16 (s, 1H), 6.94 (d, 1H, J = 7.8 Hz), 2.47 (s, 3H); MS *m*/*z*: 561.05 $[M + H]^+$.

*4.7.8. N-(3-(3-(4-Chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(trifluoromethyl)benzamide (**26**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, 2H, J = 4.7 Hz), 8.34–8.30 (m, 2H), 8.19 (d, 2H, J = 7.8 Hz), 8.14 (d, 1H, J = 7.9 Hz), 7.89 (d, 1H, J = 7.7 Hz), 7.68–7.61 (m, 4H), 7.58 (d, 1H, J = 8.0 Hz), 7.37 (d, 1H, J = 8.1 Hz), 7.36 (d, 1H, J = 7.9 Hz), 7.14 (s, 1H), 7.09 (dd, 1H, J = 1.7 Hz, 8.1 Hz); MS m/z: 536.1 $[M + H]^+$.

*4.7.9. 4-Chloro-N-(3-(3-(4-chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-3-(trifluoromethyl)benzamide (**27**)*

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (brs, 1H), 8.99 (s, 1H), 8.55–8.54 (m, 2H), 8.44 (d, 2H, J = 7.3 Hz), 8.31 (d, 1H, J = 7.9 Hz), 7.95 (d, 1H, J = 8.5 Hz), 7.82 (d, 1H, J = 7.9 Hz), 7.69 (d, 1H, J = 7.4 Hz), 7.56 (t, 1H, J = 8.1 Hz), 7.39–7.36 (m, 4H), 7.13 (s, 1H), 6.86 (d, 1H, J = 7.8 Hz); MS m/z: 570.1 $[M + H]^+$.

*4.7.10. N-(3-(3-(4-Chloro-3-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazol-1-yl)phenyl)-4-morpholino-3-(trifluoromethyl)benzamide (**28**)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (brs, 1H), 8.34 (d, 2H, J = 4.8 Hz), 8.27 (s, 1H), 8.15 (d, 3H, J = 6.9 Hz), 8.06 (d, 1H, J = 4.0 Hz), 7.59 (t, 2H, J = 6.9 Hz), 7.49 (d, 1H, J = 7.7 Hz), 7.43–7.37 (m, 3H), 7.29 (s, 1H), 7.10–7.03 (m, 2H), 3.86 (t, 4H, J = 4.3 Hz), 3.03 (t, 4H, J = 3.9 Hz); MS m/z: 620.16 $[M + H]^+$.

4.8. 60 Cancer cell line screening at the NCI

Screening against a panel of 60 cancer cell lines was applied at the National Cancer Institute (NCI), Bethesda, Maryland, USA [24], applying the following procedure. The human cell lines are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or 1/2 log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are kept for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

- $[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$
- $[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Growth inhibition of 50% (IC_{50}) is calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

Acknowledgments

This work was supported by Korea Institute of Science and Technology (KIST), KIST Project (2E22360). We would like to thank the National Cancer Institute (NCI), Bethesda, Maryland, USA, for performing the anticancer testing of the target compounds over 60 cancer cell lines.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.04.067.

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