http://dx.doi.org/10.5012/bkcs.2012.33.9.2991

Design, Synthesis, and Preliminary Cytotoxicity Evaluation of New Diarylureas and Diarylamides Possessing 1,3,4-Triarylpyrazole Scaffold

Won-Kyoung Choi,[†,#] Mohammed I. El-Gamal,[†,‡,$] Hong Seok Choi,[§] Jun Hee Hong,[§] Daejin Baek,[¶] Kihang Choi,[#] and Chang-Hyun Oh[†,‡,*]

[†]*Center for Biomaterials, Korea Institute of Science and Technology, P.O. Box 131,Cheongryang, Seoul 130-650, Korea*
[*]*E-mail: choh@kist.re.kr*
[‡]*Department of Biomolecular Science, University of Science and Technology, Daejeon 305-333, Korea*
[§]*College of Pharmacy, Chosun University, Gwangju 501-759, Korea*
[#]*Department of Chemistry, Korea University, Seoul 136-701, Korea*
[¶]*Department of Chemistry, Hanseo University, Seosan 356-706, Korea*
[$]*Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt*
Received March 15, 2012, Accepted June 18, 2012

A series of new diarylureas and diarylamides possessing 1,3,4-triarylpyrazole scaffold was synthesized and their *in vitro* antiproliferative activities against A375P human melanoma cell line and NCI-60 cell line panel were tested. Compounds **9**, **11**, **12**, **14**, and **17-21** showed superior potency against A375P to Sorafenib. Over the NCI-60 cancer cell line panel, compound **14** possessing a methoxy group, amide linker, and 4-chloro-3-(trifluoromethyl)phenyl terminal ring showed the highest potency and broad-spectrum anticancer activity. Compound **13** showed high selectivity towards leukemia subpanel over other cancer types.

Key Words : A375P, Anticancer, Diarylamide, Diarylurea, 1,3,4-Triarylpyrazole

Introduction

Cancer is a major leading cause of death worldwide, and it accounted for 7.6 million deaths (around 13% of all deaths) in 2008 according to WHO reports. 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 11 million in 2030.[1] Despite 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.

Much interest has been recently focused on diarylureas and diarylamides as potential antiproliferative agents against a variety of cancer cell lines.[2-14] Sorafenib (Nexavar®) is an anticancer diarylurea derivative that has been approved by the U. S. Food and Drug Administration (FDA) for treatment of advanced renal cancer.[15] It has also been approved in Europe for treatment of hepatocellular carcinoma (HCC).[16] 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 minimal side effects.[17]

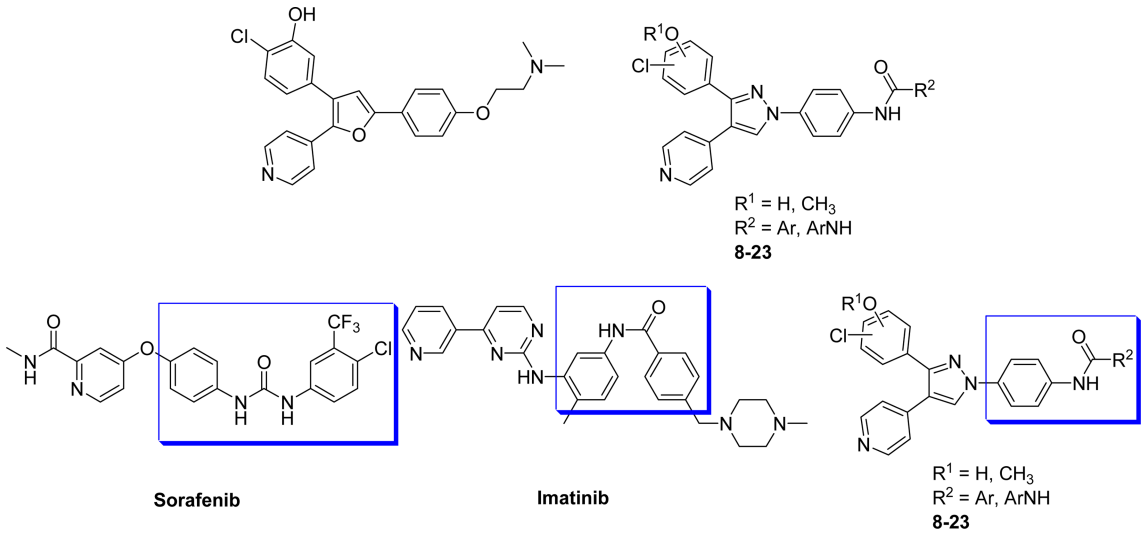


Figure 1. Rational design of the target compounds **8-23**.

In the present investigation, the target 1,3,4-triarylpyrazole compounds **8-23** were designed with similarity to the lead triarylfuran compound[18] (Fig. 1). The central furan ring was isosterically replaced with pyrazole ring. The phenolic hydroxyl group was retained in half of the compounds, and was replaced with methoxy group in the other eight compounds. The eastern part of the lead compound, dimethylaminoethoxy, was replaced with arylurea and arylamide moieties. In addition, the diarylurea and diarylamide scaffolds of the target compounds are similar to highly efficient and marketed anticancer agents such as Sorafenib and Imatinib, respectively (Fig. 1). The *in vitro* antiproliferative activities of the target compounds against 61 human cancer cell lines of nine different cancer types are reported.

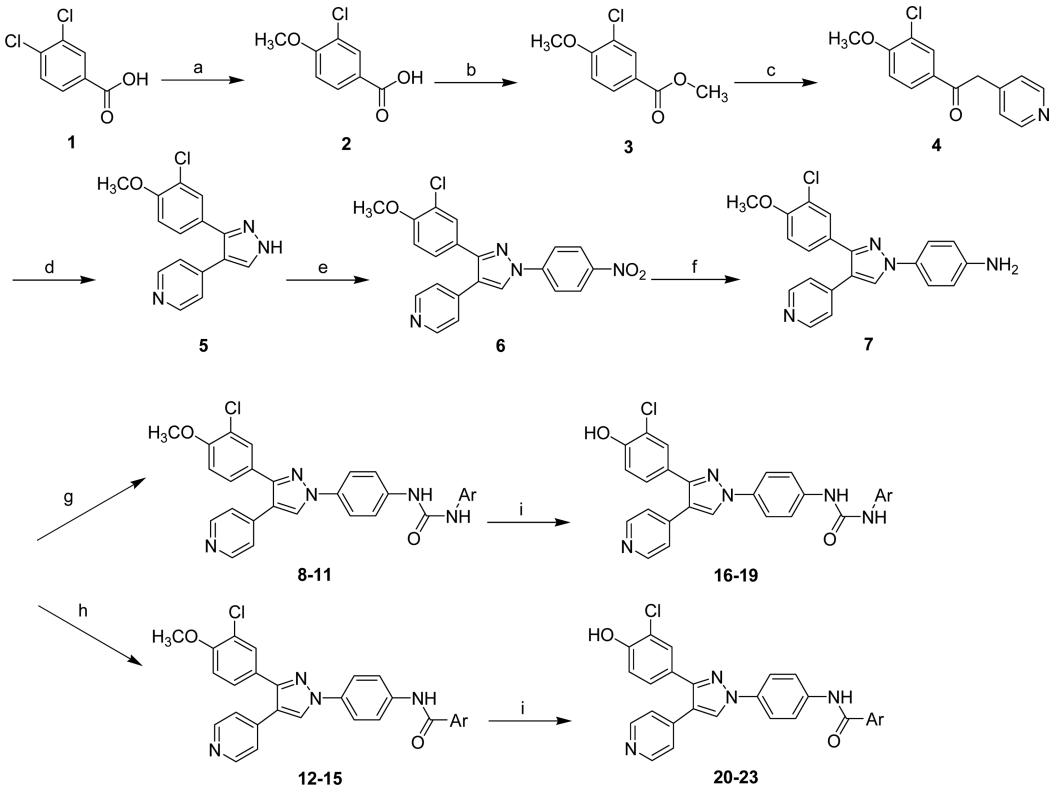
Results and Discussion

Chemistry. The target compounds **8-23** were prepared according to the sequence of reactions shown in Scheme 1. Heating 3,4-dichlorobenzoic acid (**1**) with three molar equivalents of sodium methoxide in hexamethylphosphoramide (HMPA) followed by acidification with hydrochloric acid gave 3-chloro-4-methoxybenzoic acid (**2**). Esterification of **2** with methanol in the presence of acetyl chloride afforded the corresponding methyl ester **3**. The pyridyl derivative **4** was obtained by treatment of **3** with 4-picoline in the presence of lithium bis(trimethylsilyl)amide (LHMDS). Cyclization to pyrazole compound **5** was carried out by treatment of **4** with dimethylformamide dimethyl acetal (DMF-DMA), and subsequent treatment with hydrazine monohydrate.[19] 1,3,4-Triarylpyrazole derivative **7** with amino group was prepared through *N*-arylation of **5** using 1-iodo-4-nitrobenzene in the presence of anhydrous K_2CO_3, CuI, and L-proline to give the nitro compound **6**, and subsequent reduction of the nitro group of **6** using palladium over carbon in hydrogen atmosphere. Reaction of the amino group of **7** with the appropriate aryl isocyanates afforded the corresponding urea derivatives **8-11**. Condensation of the amino compound **7** with the appropriate benzoic acid derivatives in the presence of HOBt, EDCI, and triethylamine produced the target products **12-15** with an amide linker. Demethylation of the methoxy group of **8-15** using boron tribromide yielded the corresponding hydroxyl derivatives **16-23**.

Biological Evaluation.

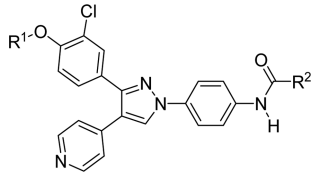
Antiproliferative Activity Against A375P Human Melanoma Cell Line. The antiproliferative activity of the newly synthesized compounds against A375P human melanoma cell line was examined. The ability of target diarylureas and diarylamides to inhibit the growth of A375P cell line is summarized in Table 1. Sorafenib was utilized as the reference standard.

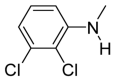
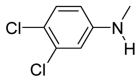
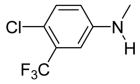
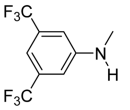
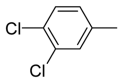
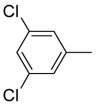
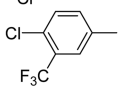
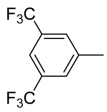
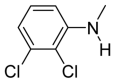
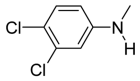
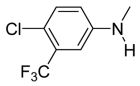
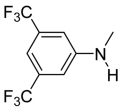
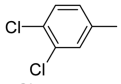
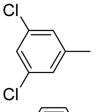
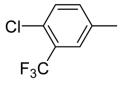
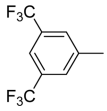
As shown in Table 1, compounds **9**, **11**, **12**, **14**, and **17-21** showed higher potency, compared with Sorafenib. Compound **22** demonstrated similar potency as Sorafenib against A375P.



Scheme 1. Reagents and conditions: (a) sodium methoxide, HMPA, 115-120 °C, 15 h; (b) acetyl chloride, CH_3OH, rt, 15 h; (c) 4-picoline, LHMDS, THF, rt, overnight; (d) (i) DMF-DMA, rt, 18 h; (ii) hydrazine monohydrate, C_2H_5OH, rt, overnight; (e) 1-iodo-4-nitrobenzene, K_2CO_3, CuI, L-proline, DMSO, 90 °C, 8 h; (f) H_2, 10% Pd/C, THF, rt, 2 h; (g) appropriate aryl isocyanate, THF, rt, 12 h; (h) appropriate benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C, 12 h; (i) BBr_3, CH_2Cl_2, −78 °C, 30 min; rt, 1 h.

Table 1. Antiproliferative activity of the target compounds **8-23** against A375P cell line



Compound No.	R^1	R^2	IC_{50} (μM)[a]
8	CH_3		>10
9	CH_3		3.5
10	CH_3		>10
11	CH_3		6.7
12	CH_3		3.3
13	CH_3		9.4
14	CH_3		5.3
15	CH_3		>10
16	H		>10
17	H		6.5
18	H		4.3
19	H		2.2
20	H		4.5
21	H		4.5
22	H		7.4
23	H		8.9
Sorafenib			7.4

[a]IC_{50} values are expressed as means of triplicate experiments.

The highest potency was found in compounds **9**, **12**, and **19** (IC_{50} = 3.5, 3.3, and 2.2 μM, respectively). These three compounds were 2.11, 2.24, and 3.36 times more potent than Sorafenib, respectively.

Compounds **18**, **19**, **21**, and **23** with hydroxyl group were more potent than the corresponding methoxy derivatives **10**, **11**, **13**, and **15**. On the contrary, methoxy compounds **9**, **12**, and **14** were more potent than the corresponding hydroxyl analogues **17**, **20**, and **22**.

The effects of the amide and urea moieties as linkers on the activity were also investigated. Compounds **12**, **14**, and **20** with amide moiety were more potent than the corresponding urea derivatives **9**, **10**, and **17**. But urea compounds **11**, **18**, and **19** showed higher potency than the corresponding derivatives **15**, **22**, and **23** with amide linker.

The target compounds may possess antiproliferative activity due to RAF kinase inhibition similar to the triarylfuran lead compound[18] and Sorafenib, and/or due to inhibition of other kinases similar to the diarylurea and diarylamide compounds, Sorafenib and Imatinib, respectively.

Antiproliferative Activities Against 60 Cell Line Panel at the NCI. Structures of the synthesized compounds were submitted to National Cancer Institute (NCI), Bethesda, Maryland, USA,[20] and the 15 compounds shown in Table 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 of all of the tested compounds over the full panel of cell lines are illustrated in Table 2. From these data, it was found that the urea derivatives **9-11** and **18** showed higher activities than the corresponding amide derivatives **12-15** and **22**. Compound **9** with 3,4-dichlorophenyl terminal ring was more active than **8** with 2,3-dichlorophenyl moiety. Similarly, compound **12** with 3,4-dichlorophenyl terminal moiety showed higher mean % inhibition than the corresponding compound **13** with 3,5-dichlorophenyl ring. This may be due to different affinities to the receptor site, which may be affected by the substituent orientation. Among all the terminal rings, 4-chloro-3-(trifluoromethyl)phenyl ring was the most promising. Compounds **10**, **14**, **18**, and **22** possessing that terminal moiety were more active than the corresponding derivatives with other terminal rings. For example, compound **10** demonstrated higher mean % inhibition over the NCI-60 cell line panel than compounds **8**, **9**, and **11** with 2,3-dichlorophenyl, 3,4-dichlorophenyl, and 3,5-bis(trifluoromethyl)phenyl moieties, respectively. This may be attributed to different steric and/or electronic effect(s) of 4-chloro-3-(trifluoromethyl)phenyl ring compared with those of other terminal rings, which may affect the drug-receptor interaction and hence the anticancer activity.

Table 2. Mean inhibition percentages observed with the final compounds in single dose (10 μM) 60-cancer cell line screening

Compound No.	Mean % inhibition[a]
8	30.11
9	91.67
10	103.16
11	90.01
12	70.19
13	47.29
14	88.49
15	37.71
16	45.04
18	90.06
19	41.98
20	72.30
21	70.68
22	80.16
23	43.34

[a]Mean % inhibition represents the mean inhibition percentages over the 60 cell lines. The inhibition percentages are calculated by subtracting the growth percentages from 100.

No further testing was done for compounds **8** and **15** with the lowest mean % inhibition over the 60-cell line panel. The other thirteen compounds 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 3.

As shown in Table 3, most of the compounds exhibited high potency (in micromolar scale) over all the nine cancer types. Most of the mean IC_{50} data were less than 10 μM. Of special interest, compound **14** with methoxy group, amide linker, and 4-chloro-3-(trifluoromethyl) terminal ring showed the highest potencies. Its mean IC_{50} values were in sub-micromolar range over leukemia, colon, CNS, and renal cancers. The mean IC_{50} values of **14** were less than 2.45 μM over the other types of cancer. Compound **13** demonstrated the merit of high selectivity towards leukemia cell lines over other cancer types. It was 13.65 times more selective against leukemia subpanel as compared with the second most susceptible cancer type, renal cancer.

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 4. From these data, we find that RPMI-8226 leukemia cell line and RXF 393 renal cancer cell line were the most susceptible cell lines to the target compounds. At RPMI-8226 cell line, eight tested compounds demonstrated IC_{50} values in sub-micromolar scale. In addition, nine compounds showed sub-micromolar IC_{50} values over RXF 393. On the other hand, OVCAR-5 ovarian cancer cell line was generally the least sensitive towards the target compounds. Among all the target compounds, compound **14** showed the highest potency and it has broad-spectrum anticancer activity. Its IC_{50} data were in sub-micromolar range over eight of the selected cell lines, and was 1.33 μM over OVCAR-5 ovarian cancer cell line. Compound **20** showed sub-micromolar IC_{50} values over seven cell lines (Table 4) but it was generally less potent than compound **14**. This may be attributed to increased bulkiness and/or electronic properties differences of compound **14** with methoxy and trifluoromethyl groups, compared with compound **20** with hydroxyl and chloro groups.

Conclusion

A new series of diarylurea and diarylamide derivatives with 1,3,4-triarylpyrazole scaffold was synthesized based on our previous literature studies, and as a continuation of our ongoing anticancer development program. Among all of these derivatives, compounds **9**, **11**, **12**, **14**, and **17-21** demonstrated higher potencies against A375P human

Table 3. 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.	**9**	1.80	2.45	2.33	2.52	2.34	2.58	1.85	2.63	2.03
	10	1.46	1.78	1.55	1.69	1.61	2.02	1.50	1.93	1.58
	11	2.32	2.75	2.49	2.62	2.44	2.90	2.11	2.99	2.42
	12	1.93	9.16	1.73	2.18	9.62	13.65	8.72	26.07	11.92
	13	2.26	59.90	45.05	40.91	40.22	>100	30.86	52.69	41.94
	14	0.37	1.26	0.70	0.60	1.22	1.61	0.58	2.44	1.59
	16	8.43	11.61	8.92	8.44	6.74	16.42	12.60	7.27	5.28
	18	3.13	5.59	5.10	3.35	3.37	4.19	4.61	3.74	4.05
	19	4.05	4.63	4.71	3.29	3.65	3.64	3.20	4.45	3.08
	20	1.08	11.53	5.87	6.67	8.27	12.26	6.12	2.63	1.99
	21	1.30	11.48	7.35	1.92	3.10	12.17	6.87	2.07	5.34
	22	1.25	3.23	2.71	1.79	2.24	5.71	7.60	2.36	1.97
	23	4.94	10.69	7.96	6.30	6.28	10.05	7.47	7.36	7.15

[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.

Table 4. IC_{50} values (μM) of the tested compounds over the most sensitive cell line of each subpanel

		Cancer cell lines[a]								
		RPMI-8226[a]	NCI-H522[b]	HCT-15[c]	U251[d]	LOX IMVI[e]	OVCAR-5[f]	RXF 393[g]	PC-3[h]	MDA-MB-231/ATCC[i]
Compound No.	**9**	0.54	1.39	1.70	1.36	2.24	2.80	0.86	1.29	1.50
	10	0.39	1.27	1.12	0.93	1.44	1.88	0.74	0.79	1.20
	11	1.23	1.90	2.88	1.84	3.31	2.64	1.69	1.73	1.81
	12	0.95	1.79	0.91	1.32	0.94	2.47	0.80	2.14	0.90
	13	0.49	5.11	1.77	1.84	3.20	NA[j]	0.36	5.38	1.39
	14	0.29	0.53	0.29	0.35	0.30	1.33	0.17	0.61	0.26
	16	0.91	4.03	5.33	2.54	3.45	>32.50	0.97	1.73	1.52
	18	1.99	2.53	2.95	2.53	2.14	5.29	1.67	2.49	3.20
	19	2.81	3.79	3.36	2.98	3.73	3.17	2.54	2.92	3.61
	20	0.54	0.80	0.71	0.69	0.78	>32.5	0.49	1.35	0.51
	21	1.07	1.03	0.91	1.32	0.85	>32.5	0.58	1.96	0.65
	22	0.65	1.00	1.21	0.87	1.12	10.10	0.72	1.37	0.73
	23	2.34	4.60	4.84	4.55	5.59	9.18	3.10	5.02	2.92

[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.

melanoma cell line than that of the reference compound, Sorafenib. Fifteen derivatives were also tested at a single dose of 10 μM at the NCI over 60 cell line panel and thirteen of them were subsequently tested in 5-dose testing mode. Compound **13** was highly selective for leukemia subpanel, compared with other cancer types. Compound **14** showed the highest potencies among all the tested compounds. It demonstrated broad-spectrum anticancer activity against all the tested cancer types. The possible mechanism of cytotoxicity of the target compounds is inhibition of ERK pathway and/or other kinases. Further modification of these compounds in order to improve their potency is currently in progress.

Experimental

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 Detector (Waters, Milford, MA, USA). Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker ARX-300, 300 MHz (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Purities of the target compounds **8-23** (> 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-(3-(3-Chloro-4-methoxyphenyl)-1*H*-pyrazol-4-yl)pyridine (5): It was prepared by the 4-step procedure as described in the literature.[19]

4-(3-(3-Chloro-4-methoxyphenyl)-1-(4-nitrophenyl)-1*H*-pyrazol-4-yl)pyridine (6): A mixture of compound **5** (0.5 g, 1.7 mmol), 1-iodo-4-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 8 h. The cooled solution was partitioned between water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was 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 1:5 v/v) to yield compound **6** (0.8 g, 86%). ^{1}H NMR (DMSO-d_6) δ 9.23 (s, 1H), 8.58 (d, 2H, J = 4.3 Hz), 8.43 (d, 2H, J = 8.6 Hz), 8.24 (d, 2H, J = 8.6 Hz), 7.62 (brs, 1H), 7.41-7.37 (m, 3H), 7.23 (d, 1H, J = 8.6 Hz), 3.91 (s, 3H).

4-(3-(3-Chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl)benzenamine (7): A mixture of compound **6** (0.5 g, 1.2 mmol) and 10% 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 **7** (0.4 g, 86.4%). ^{1}H NMR ($CDCl_3$) δ 8.54 (d, 2H, J = 4.5 Hz), 7.98 (s, 1H), 7.67 (d, 1H, J = 2.1 Hz), 7.53 (d, 2H, J = 8.0 Hz), 7.33 (d, 1H, J = 8.5 Hz), 7.26-7.24 (m, 2H), 6.90 (d, 1H, J = 8.6 Hz), 6.78 (d, 2H, J = 9.4 Hz), 3.93 (s, 3H).

General Procedure for Synthesis of Compounds 8-11. To a solution of compound **7** (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 nitrogen atmosphere. 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 compound.

1-{4-[3-(3-Chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(2,3-dichlorophenyl)urea (8): Yield: 61%; mp 133-135 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.57 (d, 1H, *J* = 5.9 Hz), 8.48 (d, 1H, *J* = 4.6 Hz), 8.21-8.09 (m, 2H), 7.97 (brs, 1H), 7.69 (d, 1H, *J* = 6.5 Hz), 7.62 (d, 1H, *J* = 5.9 Hz), 7.52 (brs, 2H), 7.42-7.34 (m, 3H), 7.11 (brs, 1H), 7.02 (brs, 1H), 6.92 (brs, 1H), 3.94 (s, 3H); ESI-MS: 565.1 $[M + 1]^+$.

1-{4-[3-(3-Chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(3,4-dichlorophenyl)urea (9): Yield: 49%; mp 196-198 °C; ^{1}H NMR ($CDCl_3$) δ 8.57 (d, 1H, *J* = 5.3 Hz), 8.54 (d, 1H, *J* = 4.5 Hz), 8.03 (s, 1H), 7.74 (d, 1H, *J* = 8.9 Hz), 7.67 (d, 1H, *J* = 2.1 Hz), 7.59 (d, 2H, *J* = 9.5 Hz), 7.49 (d, 2H, *J* = 8.9 Hz), 7.20 (d, 1H, *J* = 6.2 Hz), 7.12 (d, 1H, *J* = 6.1 Hz), 7.04 (d, 1H, *J* = 9.5 Hz), 6.92 (d, 1H, *J* = 8.5 Hz), 6.72 (t, 2H, *J* = 9.1 Hz), 3.94 (s, 3H); ESI-MS : 565.1 $[M + 1]^+$.

1-{4-[3-(3-Chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(4-chloro-3-(trifluoromethyl)-phenyl)urea (10): Yield: 31%; mp 196-197 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.56 (d, 2H, *J* = 5.9 Hz), 8.02 (s, 1H), 7.70-7.64 (m, 4H), 7.54 (d, 1H, *J* = 8.8 Hz), 7.44 (brs, 1H), 7.41 (d, 1H, *J* = 3.6 Hz), 7.37 (brs, 1H), 7.32 (d, 1H, *J* = 2.0 Hz), 7.29 (d, 1H, *J* = 2.0 Hz), 7.24 (brs, 1H), 3.44 (d, 1H, *J* = 8.5 Hz), 3.92 (s, 3H); ESI-MS: 599.0 $[M + 1]^+$.

1-{4-[3-(3-Chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(3,5-bis(trifluoromethyl)phenyl)urea (11): Yield: 47%; mp 200-201 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.57 (d, 1H, *J* = 5.9 Hz), 8.50 (d, 1H, *J* = 5.9 Hz), 8.04 (brs, 1H), 7.90 (d, 2H, *J* = 9.3 Hz), 7.74 (d, 1H, *J* = 8.9 Hz), 7.67 (d, 1H, *J* = 2.0 Hz), 7.55-7.49 (m, 2H), 7.33 (d, 1H, *J* = 8.4 Hz), 7.20 (d, 1H, *J* = 8.2 Hz), 7.13 (t, 2H, *J* = 7.5 Hz), 7.04 (d, 1H, *J* = 8.5 Hz), 6.90 (d, 1H, *J* = 8.5 Hz), 3.93 (s, 3H); ESI-MS: 633.1 $[M + 1]^+$.

General Procedure for Synthesis of Compounds 12-15. A mixture of compound **7** (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 water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 5 mL). The combined organic layer was 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 desired compound.

3,4-Dichloro-*N*-{4-[3-(3-chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl] phenyl}benzamide (12): Yield: 66%; mp 199-201 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.57 (d, 2H, *J* = 4.5 Hz), 8.11 (s, 1H), 8.00 (d, 1H, *J* = 2.1 Hz), 7.91 (brs, 1H), 7.80 (s, 5H), 7.73 (d, 1H, *J* = 8.4 Hz), 7.69 (d, 1H, *J* = 2.1 Hz), 7.60 (d, 1H, *J* = 8.3 Hz), 7.34 (d, 1H, *J* = 8.6 Hz), 6.92 (d, 1H, *J* = 8.6 Hz), 3.94 (s, 3H); ESI-MS: 550.1 $[M + 1]^+$.

3,5-Dichloro-*N*-{4-[3-(3-chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl] phenyl}benzamide (13): Yield: 55%; mp 216-218 °C; ^{1}H NMR ($CDCl_3$) δ 8.57 (d, 1H, *J* = 6.0 Hz), 8.49 (d, 1H, *J* = 6.1 Hz), 8.11 (s. 1H), 7.86 (brs, 1H), 7.80 (d, 2H, *J* = 3.2 Hz), 7.77 (d, 2H, *J* = 1.8 Hz), 7.73 (d, 1H, *J* = 1.8 Hz), 7.69 (d, 1H, *J* = 2.0 Hz), 7.62 (d, 1H, *J* = 8.8 Hz), 7.58-7.55 (m, 1H), 7.34 (d, 1H, *J* = 8.7 Hz), 7.11 (d, 1H, *J* = 6.1 Hz), 6.91 (d, 1H, *J* = 8.6 Hz), 3.94 (s, 3H); ESI-MS: 550.1 $[M + 1]^+$.

4-Chloro-3-trifluoromethyl-*N*-{4-[3-(3-chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}benzamide (14): Yield: 57%; mp 188-190 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.57 (d, 2H, *J* = 4.5 Hz), 8.22 (brs, 1H), 8.12 (s, 1H), 8.04-7.99 (m, 2H), 7.81 (s, 3H), 7.69 (s, 1H), 7.66 (brs, 1H), 7.34 (d, 1H, *J* = 8.5 Hz), 7.27 (brs, 2H), 6.92 (d, 1H, *J* = 8.6 Hz), 3.94 (s, 3H); ESI-MS: 584.1 $[M + 1]^+$.

3,5-Bis(trifluoromethyl)-*N*-{4-[3-(3-chloro-4-methoxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}benzamide (15): Yield: 73%; mp 205-208 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 8.57 (d, 2H, *J* = 4.5 Hz), 8.36 (brs, 2H), 8.13 (s, 1H), 8.09 (brs, 1H), 8.02 (brs, 1H), 7.84 (s, 4H), 7.69 (d, 1H, *J* = 2.1 Hz), 7.34 (d, 1H, *J* = 8.1 Hz), 7.28 (s, 1H), 6.92 (d, 1H, *J* = 8.5 Hz), 3.95 (s, 3H); ESI-MS: 618.1 $[M + 1]^+$.

General Procedure for Synthesis of Compounds 16-23. To a solution of compound **8-15** (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 $NaHCO_3$ until effervescence ceased. Ethyl acetate (5 mL) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 × 3 mL). The combined organic layer was washed with brine and 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 hydroxyl compound.

1-{4-[3-(3-Chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(2,3-dichlorophenyl)urea (16): Yield: 75%; mp 143-146 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.49 (s, 1H), 9.68 (s, 1H), 8.91 (s, 1H), 8.55-8.53 (m, 3H), 8.18 (d, 1H, *J* = 7.6 Hz), 7.88 (d, 2H, *J* = 9.0 Hz), 7.63 (d, 2H, *J* = 8.9 Hz), 7.47 (d, 1H, *J* = 2.1 Hz), 7.35-7.30 (m, 4H), 7.23 (d, 1H, *J* = 8.4 Hz), 7.01 (d, 1H, *J* = 8.4 Hz); ESI-MS: 551.0 $[M + 1]^+$.

1-{4-[3-(3-Chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(3,4-dichlorophenyl)urea (17): Yield: 82%; mp 191-193 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.50 (brs, 1H), 9.11 (brs, 1H), 9.06 (brs, 1H), 8.90 (s, 1H), 8.53 (d, 2H, *J* = 4.5 Hz), 7.91 (d, 1H, *J* = 2.4 Hz), 7.85 (d, 2H, *J* = 9.1 Hz), 7.61 (d, 2H, *J* = 9.0 Hz), 7.53 (d, 1H, *J* = 8.8

Hz), 7.46 (s, 1H), 7.37-7.33 (m, 2H), 7.24-7.19 (m, 2H), 7.00 (d, 1H, J = 8.5 Hz); ESI-MS: 551.0 $[M + 1]^+$.

1-{4-[3-(3-Chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(4-chloro-3-(trifluoromethyl)-phenyl)urea (18): Yield: 38%; mp 127-129 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.53 (s, 1H), 9.25 (s, 1H), 9.07 (s, 1H), 8.91 (s, 1H), 8.54 (d, 2H, J = 4.6 Hz), 8.14 (brs, 1H), 7.86 (d, 2H, J = 9.0 Hz), 7.69-7.62 (m, 4H), 7.47 (d, 1H, J = 2.0 Hz), 7.35 (d, 2H, J = 4.6 Hz), 7.23 (d, 1H, J = 8.4 Hz), 7.01 (d, 1H, J = 8.4 Hz); ESI-MS: 585.1 $[M + 1]^+$.

1-{4-[3-(3-Chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}-3-(3,5-bis(trifluoromethyl)phenyl)urea (19): Yield: 53%; mp 212-215 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.48 (s, 1H), 9.46 (s, 1H), 9.20 (s, 1H), 8.91 (s, 1H), 8.53 (d, 2H, J = 5.3 Hz), 8.16 (s, 2H), 7.87 (d, 2H, J = 8.8 Hz), 7.65 (d, 3H, J = 8.6 Hz), 7.47 (s, 1H), 7.35 (d, 2H, J = 5.2 Hz), 7.23 (d, 1H, J = 8.3 Hz), 7.01 (d, 1H, J = 8.3 Hz); ESI-MS: 618.1 $[M + 1]^+$.

3,4-Dichloro-*N*-{4-[3-(3-chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}benzamide (20): Yield: 63%; mp 228-231 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.57 (brs, 1H), 10.52 (brs, 1H), 8.95 (s, 1H), 8.54 (d, 2H, J = 4.5 Hz), 8.25 (d, 1H, J = 2.0 Hz), 7.94 (s, 5H), 7.85 (d, 1H, J = 8.4 Hz), 7.48 (d, 1H, J = 2.1 Hz), 7.35 (d, 2H, J = 4.6 Hz), 7.23 (d, 1H, J = 8.4 Hz), 7.01 (d, 1H, J = 8.4 Hz); ESI-MS: 536.0 $[M + 1]^+$.

3,5-Dichloro-*N*-{4-[3-(3-chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl] phenyl}benzamide (21): Yield: 76%; mp 250-252 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.60 (s, 1H), 10.51 (brs, 1H), 8.95 (s, 1H), 8.54 (d, 2H, J = 5.3 Hz), 4.01 (d, 2H, J = 1.9 Hz), 7.94 (s, 4H), 7.90 (brs, 1H), 7.40 (d, 1H, J = 2.0 Hz), 7.35 (d, 2H, J = 4.6 Hz), 7.23 (d, 1H, J = 8.4 Hz), 7.01 (d, 1H, J = 8.4 Hz); ESI-MS: 536.0 $[M + 1]^+$.

4-Chloro-3-trifluoromethyl-*N*-{4-[3-(3-chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}benzamide (22): Yield: 62%; mp 154-157 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.70 (s, 1H), 10.51 (brs, 1H), 8.96 (s, 1H), 8.54 (d, 2H, J = 5.3 Hz), 8.42 (brs, 1H), 8.29 (d, 1H, J = 8.3 Hz), 7.97 (s, 1H), 7.95 (s, 4H), 7.48 (d, 1H, J = 2.0 Hz), 7.35 (d, 2H, J = 5.3 Hz), 7.23 (d, 1H, J = 8.4 Hz), 7.01 (d, 1H, J = 8.4 Hz); ESI-MS: 570.1 $[M + 1]^+$.

3,5-Bis(trifluoromethyl)-*N*-{4-[3-(3-chloro-4-hydroxyphenyl)-4-(pyridin-4-yl)-1*H*-pyrazol-1-yl]phenyl}benzamide (23): Yield: 48%; mp 285-288 °C; ^{1}H NMR (DMSO-d_6) δ 10.83 (s, 1H), 10.51 (s, 1H), 8.98 (s, 1H), 8.64 (s, 2H), 8.54 (d, 2H, J = 4.5 Hz), 8.41 (brs, 1H), 7.96 (s, 4H), 7.49 (d, 1H, J = 2.0 Hz), 7.36 (d, 2H, J = 4.5 Hz), 7.25 (d, 1H, J = 9.2 Hz), 7.01 (d, 1H, J = 8.4 Hz); ESI-MS: 604.1 $[M + 1]^+$.

Evaluation of the Antiproliferative Activity Against A375P Human Melanoma Cell Line. A375P cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained in Dulbecco's modified eagle medium (DMEM, Welgene, Daegu, Republic of Korea) supplemented with 10% fetal bovine serum (FBS, Welgene, Daegu, Republic of Korea) and 1% penicillin/streptomycin (Welgene, Daegu, Republic of Korea) in a humidified atmosphere with 5% CO_2 at 37 °C. A375P cells were taken from culture substrate with 0.05% trypsin-0.02% EDTA and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37 °C for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment with various concentrations (3-fold serial dilution, 12 points) of the tested compounds. The cells were incubated for 48 h after treatment with the test compounds. The A357P cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. MTT assays were carried out with CellTiter 96® (Promega) according to the manufacturer's instructions. The absorbance at 590 nm was recorded using EnVision 2103 (Perkin Elmer; Boston, MA, USA). The IC_{50} values were calculated using GraphPad Prism 4.0 software. Triplicate testing was performed for each test compound.

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,[18] 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. We are also grateful to Korea Institute of Science and Technology (KIST) for financial support.

References

1. WHO website: http://www.who.int/mediacentre/factsheets/fs297/en/.
2. Jung, M.-H.; Oh, C.-H. *Bull. Korean Chem. Soc.* **2008**, *29*, 2231.
3. Jung, M.-H.; Kim, H.; Choi, W.-K.; El-Gamal, M. I.; Park, J.-H.; Yoo, K. H.; Sim, T. B.; Lee, S. H.; Baek, D.; Hah, J.-M.; Cho, J.-H.; Oh, C.-H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6538.
4. Nam, B. S.; Kim, H.; Oh, C.-H.; Lee, S. H.; Cho, S. J.; Sim, T. B.; Hah, J.-M.; Kim, D. J.; Choi, J. H.; Yoo, K. H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3517.
5. Song, D.-Q.; Du, N.-N.; Wang, Y.-M.; He, W.-Y.; Jiang, E.-Z.; Cheng, S.-X.; Wang, Y.-X.; Li, Y.-H.; Wang, Y.-P.; Li, X.; Jiang, J.-D. *Bioorg. Med. Chem.* **2009**, *17*, 3873.
6. Kim, H. J.; Jung, M.-H.; Kim, H.; El-Gamal, M. I.; Sim, T. B.; Lee, S. H.; Hong, J. H.; Hah, J.-M.; Cho, J.-H.; Choi, J. H.; Yoo, K. H.; Oh, C.-H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 413.
7. Yu, H.; Jung, Y.; Kim, H.; Lee, J.; Oh, C.-H.; Yoo, K. H.; Sim, T.; Hah, J.-M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3805.
8. Lee, J.; Kim, H.; Yu, H.; Chung, J. Y.; Oh, C.-H.; Yoo, K. H.; Sim, T.; Hah, J.-M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1573.
9. El-Deeb, I. M.; Lee, S. H. *Bioorg. Med. Chem.* **2010**, *18*, 3860.
10. Yao, P.; Zhai, X.; Liu, D.; Qi, B. H.; Tan, H. L.; Jin, Y. C.; Gong, P. *Arch. Pharm. Chem. Life Sci.* **2010**, *343*, 17.
11. El-Gamal, M. I.; Jung, M.-H.; Lee, W. S.; Sim, T.; Yoo, K. H.; Oh, C.-H. *Eur. J. Med. Chem.* **2011**, *46*, 3218.
12. Kim, H. J.; Cho, H. J.; Kim, H.; El-Gamal, M. I.; Oh, C.-H.; Lee, S. H.; Sim, T.; Hah, J.-M.; Yoo, K. H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3269.
13. El-Gamal, M. I.; Oh, C.-H. *Bull. Korean Chem. Soc.* **2012**, *33*, 1571.
14. Jung, M.-H.; El-Gamal, M. I.; Abdel-Maksoud, M. S.; Sim, T.; Yoo, K. H.; Oh, C.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4362.
15. Wilhelm, S.; Carter, C.; Lynch, M.; Lowinger, T.; Dumas, J.; Smith, R. A.; Schwartz, B.; Simantov, R.; Kelley, S. *Nat. Rev. Drug Discov.* **2006**, *5*, 835.
16. DGNews website: http://www.pslgroup.com/news/content.nsf/medicalnews/852571020057CCF685257384005A45B1?OpenDocument&id=&count=10.
17. Capdeville, R.; Buchdunger, E.; Zimmermann, J.; Matter, A. *Nat. Rev. Drug Disc.* **2002**, *1*, 493.
18. Dean, D. K.; Takle, A. K.; Wilson, D. M. WO 2003022832 A1.
19. El-Gamal, M. I.; Oh, C.-H. *Bull. Korean Chem. Soc.* **2011**, *32*, 821.
20. NCI website: www.dtp.nci.nih.gov.