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# Synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives and their antiproliferative activity against melanoma cell line

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ABSTRACT

Synthesis of a new series of diarylureas and amides having pyrrolo[2,3-*d*]pyrimidine scaffold is described. Their in vitro antiproliferative activities against A375 human melanoma cell line and HS 27 fibroblast cell line were tested and the effect of substituents on pyrrolo[2,3-*d*]pyrimidine was investigated. The newly synthesized compounds, except *N*-acetyl derivatives (**Id**, **Ie**, and **Im**), generally showed superior or similar activity against A375 to Sorafenib. Among all of these derivatives, compounds **Iq** and **Ir** having imidazole and morpholine moieties, respectively, showed the most potent antiproliferative activity against A375.



Melanoma is the most aggressive form of skin cancer and is the fastest growing cancer in the United States.[1,2] Early stage melanoma can be cured surgically. However, melanoma metastasizing to major organs (stage IV) is virtually incurable.[2] Patients with advanced melanoma have a median survival time of less than one year, and the estimated 5-years survival rate is less than 15%.[2,3] With the incidence of melanoma rapidly rising in the United States and other developed countries, there is an urgent need to develop more effective drugs.[4–6]

The current treatments involve surgical removal of the tumor, immunotherapy, radiotherapy, chemotherapy, various combinations, or the use of new treatments in clinical trials. As for immunotherapy, interferon alfa-2b (Intron-A)[7] is approved by both the FDA and EMEA for adjuvant treatment of melanoma patients, and aldesleukin (proleukin)[8,9] is also approved for the treatment of metastatic melanoma in the USA.

It is recently reported that diarylurea derivatives, such as Sorafenib, are promising, potent, and selective antiproliferative agents for treatment of melanoma.[10–15] There is a considerable number and variety of diarylureas identified as anticancer agent.[16–21]

Encouraged by the interesting antiproliferative activity of diarylurea derivatives, a new series of diarylureas and amides containing pyrrolo[2,3-*d*]pyrimidine moiety was synthesized. We now report the synthesis and antiproliferative activity against A375 human melanoma cell line and HS 27 fibroblast cell line of these compounds (Fig. 1).

The general method for synthesis of pyrrolo[2,3-*d*]pyrimidine ureas and amides is shown in Schemes 2–4. The geminal dicyano intermediate **2** was prepared from **1**[22] with malononitrile by heating in DMF in the presence of anhydrous $K_2CO_3$. Synthesis of compound **3** can be achieved by refluxing **2** with thiourea in the presence of potassium *tert*-butoxide. Cyclization to **4** can be achieved by neutralization of the thiol potassium salt **3** using 5 N aqueous HCl followed by heating with 10 N aqueous NaOH. Reduction of the thiol compound **4** using Raney nickel afforded 7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (**5**) (Scheme 1).

The *N*-benzoyl protected compound **6** was prepared by reacting **5** with benzoyl chloride in pyridine. N-Arylation of **6** using 1-iodo-4-nitrobenzene in the presence of potassium carbonate, copper iodide, and L-proline afforded compound **7**. The 4-benzoylamino substituted *p*-nitrophenyl compound **7** was reduced and deprotected using tin(II)chloride to provide the diamino compound **8**, which was subsequently treated with the appropriate isocyanates to provide the corresponding urea derivatives (**Ia–c**). The amide derivatives (**Ik–l**) were obtained by condensation of **8** with the corresponding carboxylic acid derivatives using EDCI/HOBt (Scheme 2).

The *N*-acetyl compound **9** was obtained by condensation of the amino compound **5** with acetic acid in the presence of EDCI/HOBt.

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0960-894X/$ - see front matter © 2009 Published by Elsevier Ltd.
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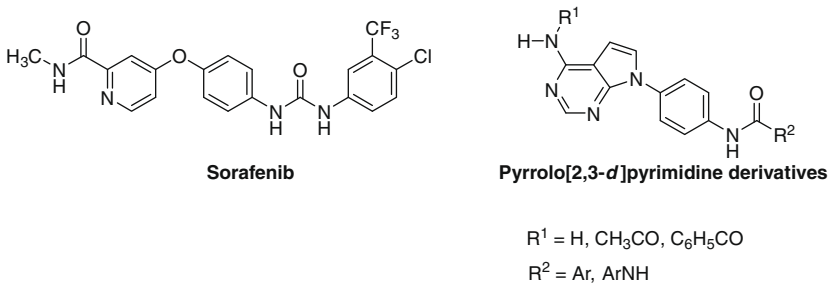
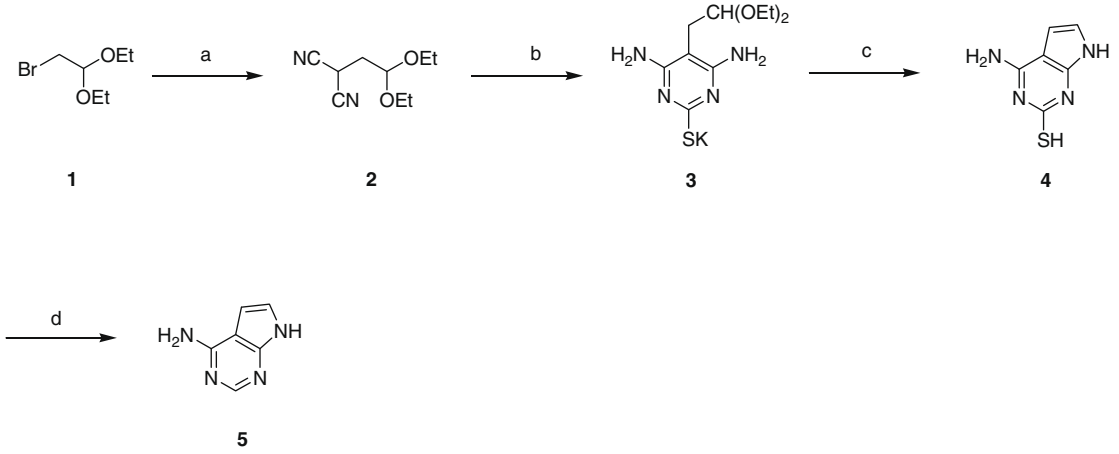
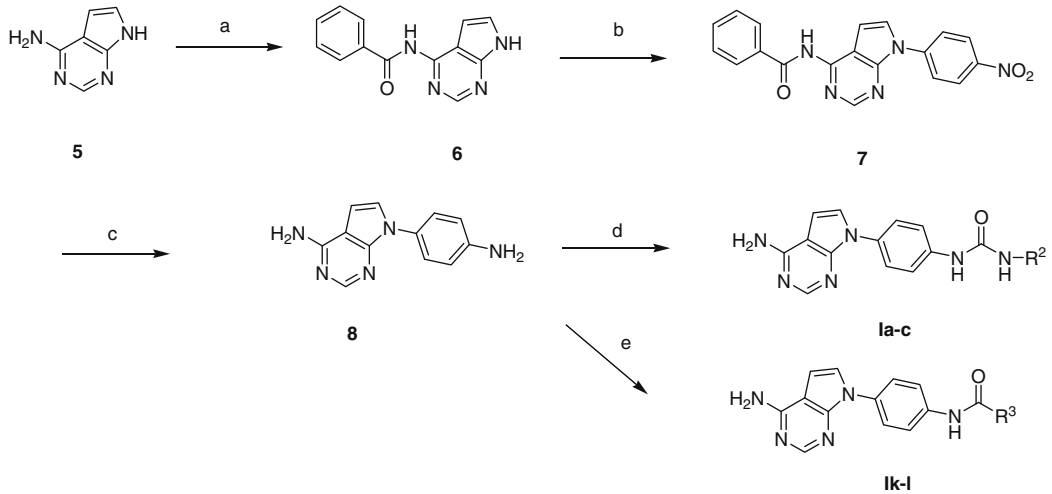



**Figure 1.** Structures of Sorafenib and pyrrolo[2,3-*d*]pyrimidine derivatives.




**Scheme 1.** Reagents and conditions: (a) malononitrile, $K_2CO_3$, DMF, 50 °C, 54%; (b) thiourea, potassium *tert*-butoxide, EtOH, reflux, 61%; (c) 5 N HCl, $H_2O$, 10 N NaOH, 50 °C, 88%; (d) Raney-Ni, $H_2O$, reflux, 87%.

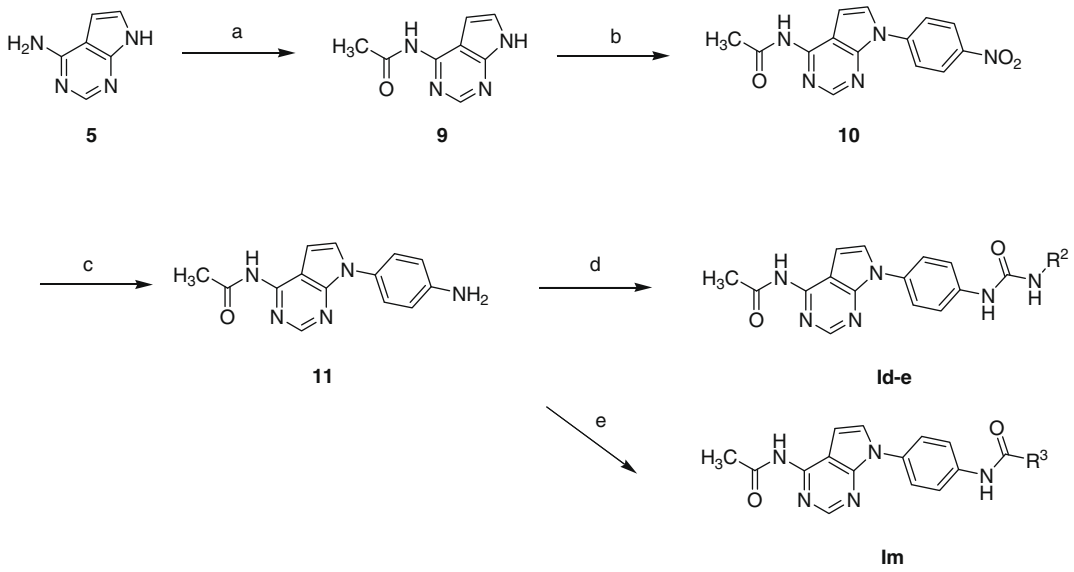



**Scheme 2.** Reagents and conditions: (a) benzoyl chloride, pyridine, 50 °C, 33%; (b) 1-iodo-4-nitrobenzene, $K_2CO_3$, CuI, L-proline, DMSO, 90 °C, 67%; (c) $Sn_2Cl \cdot H_2O$, EtOH, reflux, 80%; (d) aryl isocyanate, THF, **Ia**: 27%, **Ib**: 58%, **Ic**: 35%; (e) benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C, **Ik**: 86%, **Il**: 47%.

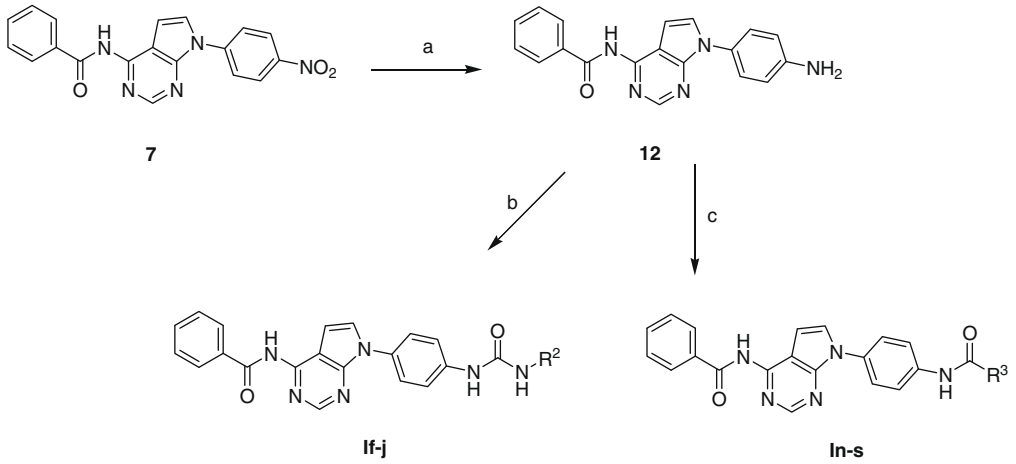
Preparation of the 4-acetylamino substituted *p*-nitrophenyl compound **10** was accomplished by the same procedure described for the preparation of **7**. Reduction of the nitro compound **10** using Pd–C/$H_2$ afforded the corresponding amino compound **11**, which was subsequently treated with the appropriate isocyanates to provide the corresponding urea derivatives (**Id–e**). The amide derivative (**Im**) was obtained by condensation of **11** with 4-chloro-3-(trifluoromethyl)benzoic acid using EDCI/HOBt (Scheme 3).

The 4-benzoylamino substituted *p*-nitrophenyl compound **7** was reduced with Pd–C/$H_2$ to the corresponding amino compound **12**. Compounds **If–j** and **In–s** were also prepared as described for the preparation of compounds **Ia–e** and **Ik–m**, respectively (Scheme 4).

The antiproliferative activity of the newly synthesized compounds against A375 human melanoma cell line and HS 27 fibroblasts was tested.[23] The ability of pyrrolo[2,3-*d*]pyrimidine




**Scheme 3.** Reagents and conditions: (a) acetic acid, HOBt, EDCI, TEA, DMF, 80 °C, 45%; (b) 1-iodo-4-nitrobenzene, $K_2CO_3$, CuI, L-proline, DMSO, 90 °C, 47%; (c) Pd/C, $H_2$, THF, 25%; (d) aryl isocyanate, THF, **Id**: 70%, **Ie**: 31%; (e) 4-chloro-3-(trifluoromethyl)benzoic acid, HOBt, EDCI, TEA, DMF, 80 °C, **Im**: 47%.




**Scheme 4.** Reagents and conditions: (a) Pd/C, $H_2$, THF, 85%; (b) aryl isocyanate, THF, If: 22%, **Ig**: 25%, **Ih**: 54%, Ii: 82%, **Ij**: 56%; (c) benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C, In: 26%, **Io**: 33%, **Ip**: 36%, **Iq**: 17%, **Ir**: 17%, **Is**: 96%.

derivatives to inhibit the growth of A375 and HS 27 cell lines is summarized in Tables 1 and 2. Sorafenib was selected as the reference standard, because it has been extensively used in clinical trials for melanoma.[4,24]

The newly synthesized compounds, except *N*-acetyl derivatives (**Id**, **Ie**, and **Im**), generally showed superior or similar activity against A375 to Sorafenib. Among these compounds, **Ia**, **Ic**, **If–h**, **Ik**, and **In–s** showed similar or slightly improved activity against A375 compared with Sorafenib.

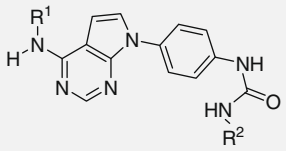
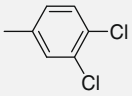
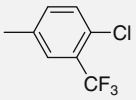
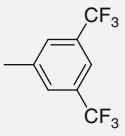
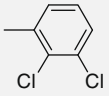
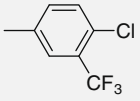
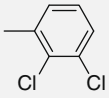
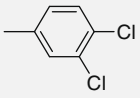
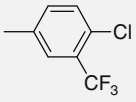
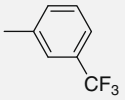
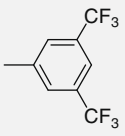
Regarding the substituents on the 4-position of pyrrolo[2,3-*d*]pyrimidine, compounds **Ig**, **Ih**, **Io**, and **Ip** having benzoylamino moiety were generally more potent than compounds **Ia**, **Ib**, **Ik**, and **Il** having an unsubstituted amino group against A375. This suggests that the aromatic amide substituent at this position is more favorable.

The effect of substituents on the phenyl ring of the tail was also investigated. The introduction of chloro group at *para* position in 3′-trifluoromethylphenyl ring of tail (**Ih, Ik**, and **Io**) significantly enhanced the antiproliferative activity compared with compounds (**Ii, Il**, and **Ip**). This can be attributed to the enhanced affinity produced by this chloro group to the target protein.

By comparing the activity of derivatives substituted with amide and urea moieties at pyrrolo[2,3-*d*]pyrimidine side chain as a linker, it was found that the derivatives with amide moieties (**Ik, In, Io**, **Ip**, and **Is**) were generally more potent than that with urea moieties (**Ib, Ig, Ih**, **Ii**, and **Ij**). In addition, most of the newly synthesized amide derivatives (**Ik** and **In–s**) demonstrated higher antiproliferative activity against A375 than that of Sorafenib with urea moiety. These results were seemed to indicate the effect of the linker on the activity.

Upon comparing the antiproliferative activity of the newly synthesized compounds with that of Sorafenib against HS 27 fibroblast cell line, it was found that the new pyrrolo[2,3-*d*]pyrimidine derivatives generally showed superior or similar potency against HS 27 to Sorafenib. This assumes little selectivity of the newly synthesized compounds towards A375. Among all of

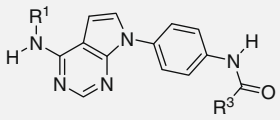
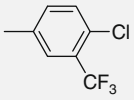
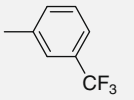
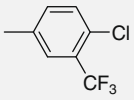
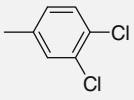
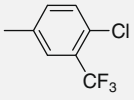
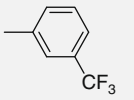
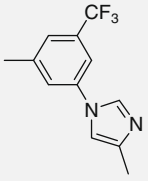
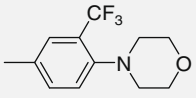
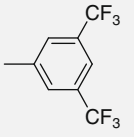
**Table 1**
Antiproliferative activity of pyrrolo[2,3-*d*]pyrimidine ureas (**Ia**–**j**).

| Structure | Compd no. | $R^1$ | $R^2$ | $IC_{50}$ (μM) | |
|---|---|---|---|---|---|
| | | | | A375P | HS 27 |
|  | **Ia** | H |  | 5.4 | 2.9 |
| | **Ib** | H |  | 8.1 | >10 |
| | **Ic** | H |  | 3.0 | 9.5 |
| | **Id** | $CH_3CO$ |  | >10 | >10 |
| | **Ie** | $CH_3CO$ |  | >10 | >10 |
| | **If** | $C_6H_5CO$ |  | 4.1 | 7.8 |
| | **Ig** | $C_6H_5CO$ |  | 4.0 | 4.5 |
| | **Ih** | $C_6H_5CO$ |  | 4.6 | 6.0 |
| | **Ii** | $C_6H_5CO$ |  | 9.5 | 4.5 |
| | **Ij** | $C_6H_5CO$ |  | 9.8 | >10 |
| Sorafenib | | | | 5.6 | 7.8 |

these derivatives, compound **Iq** having 4-benzoylamino substituted 4′-amide and imidazole moieties demonstrated superior activity against A375 and lower potency against HS 27 compared with Sorafenib.

In conclusion, a new series of pyrrolo[2,3-*d*]pyrimidine derivatives was synthesized based on our previous literature studies, by focusing on the structure–activity relationship studies of this class of compounds. Among all of these derivatives, compounds

**Table 2**
Antiproliferative activity of pyrrolo[2,3-*d*]pyrimidine amides (**Ik**–**s**).

| Structure | Compd no. | $R^1$ | $R^3$ | $IC_{50}$ (μM) | |
|---|---|---|---|---|---|
| | | | | A375P | HS 27 |
|  | **Ik** | H |  | 2.9 | 5.6 |
| | **Il** | H |  | 9.7 | 7.1 |
| | **Im** | $CH_3CO$ |  | >10 | >10 |
| | **In** | $C_6H_5CO$ |  | 2.0 | 5.0 |
| | **Io** | $C_6H_5CO$ |  | 2.3 | 5.5 |
| | **Ip** | $C_6H_5CO$ |  | 4.7 | 5.9 |
| | **Iq** | $C_6H_5CO$ |  | 0.8 | 2.8 |
| | **Ir** | $C_6H_5CO$ |  | 0.9 | 1.6 |
| | **Is** | $C_6H_5CO$ |  | 2.0 | 6.6 |
| Sorafenib | | | | 5.6 | 7.8 |

**Iq**[25] and **Ir**[25] having 4-benzoylamino substituted 4′-amide moieties, and imidazole and morpholine moieties, respectively, showed the most potent antiproliferative activity against A375 human melanoma cell line.

Further modification of these compounds in order to improve their potency is currently in progress. Our ultimate goal is to identify several compounds that are highly potent and highly selective against melanoma cells.

## Acknowledgment

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## References and notes

1. Atallah, E.; Flaherty, L. *Curr. Treat. Opt. Oncol.* **2005**, *6*, 185.
2. Barth, A.; Wanek, L. A.; Morton, D. L. *J. Am. Coll. Surg.* **1995**, *181*, 193.
3. Anderson, C. M.; Buzaid, A. C.; Legha, S. S. *Oncology* **1995**, *9*, 1149.
4. Gray-Schopfer, V.; Wellbrock, C.; Marais, R. *Nature* **2007**, *445*, 851.
5. Garbe, C.; Eigentler, T. K. *Melanoma Res.* **2007**, *17*, 117.
6. Koon, H. B.; Atkins, M. B. *Exp. Rev. Anticancer Ther.* **2007**, *7*, 79.
7. Lawson, D. H. *Cancer Control* **2005**, *12*, 236.
8. Rosenburg, S. A.; Lotze, M. T.; Yang, J. C.; Aebersold, P. M.; Linehan, W. M.; Seipp, C. A.; White, D. E. *Ann. Surg.* **1989**, *210*, 474.
9. Atkins, M. B.; Lotze, M. T.; Dutcher, J. P.; Fisher, R. I.; Weiss, G.; Margolin, K.; Abrams, J.; Sznol, M.; Parkinson, D.; Hawkins, M.; Paradise, C.; Kunkel, L.; Rosenberg, S. A. *J. Clin. Oncol.* **1999**, *17*, 2105.
10. Wilhelm, S. M.; Carter, C.; Tang, L.; Wilkie, D.; Mcnabola, A.; Rong, H. *Cancer Res.* **2004**, *64*, 7099.

11. Strumberg, D.; Richly, H.; Hilger, R. A.; Schleucher, N.; Korfee, S. *J. Clin. Oncol.* **2005**, *23*, 965.
12. Clark, J. W.; Eder, J. P.; Ryan, D.; Lathia, C.; Lenz, H. J. *Clin. Cancer Res.* **2005**, *11*, 5472.
13. Hirte, H. W.; Moore, M.; Siu, L.; Oza, A.; Hotte, S. J. *Ann. Oncol.* **2005**, *16*, 1688.
14. Strumberg, D.; Voliotis, D.; Moller, J. G.; Hilger, R. A.; Richly, H.; Kredtke, S. *J. Clin. Pharmacol. Ther.* **2002**, *40*, 580.
15. Richly, H.; Kupsh, P.; Passage, K.; Grubert, M.; Voigtmann, R.; Schwartz, B. *Int. J. Clin. Pharmacol. Ther.* **2004**, *42*, 650.
16. Wood, J. E.; Wild, H; Rogers, D. H.; Lyons, J.; Katz, M. E.; Caringal, Y. V. PCT Pat. Appl. WO 98052559, May 23, 1997.
17. Bankston, D.; Dumas, J.; Natero, R.; Riedl, D. R.; Monahan, M.-K. *Org. Proc. Res. Dev.* **2002**, *6*, 777.
18. Khire, U. R.; Bankston, D.; Barbosa, J.; Brittelli, D. R.; Caringal, Y.; Carlson, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 783.
19. Wan, P. T. C.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescudunaz, D. *Cell* **2004**, *116*, 855.
20. 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.
21. Li, H.-F.; Lu, T.; Zhu, T.; Jiang, Y.-J.; Rao, S.-S.; Hu, L.-Y.; Xin, B.-T.; Chen, Y.-D. *Eur. J. Med. Chem.* **2009**, *44*, 1240.
22. Matthew, M. B.; Feng, X.; Marjorie, W.; Robert, A. R.; Edward, J. I. G. *J. Org. Chem.* **2004**, *69*, 6257.
23. A375P cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, US) and maintained in DMEM medium (Welgene, Daegu, Korea) supplemented with 10% FBS (Welgene) and 1% penicillin/streptomycin (Welgene) 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 $5 \times 10^3$ 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 of various concentration (threefold serial dilution, 12 points) of 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, US). The $IC_{50}$ was calculated using GraphPad Prism 4.0 software.
24. Eisen, T.; Ahmad, T.; Flaherty, K. T.; Gore, M.; Kaye, S.; Marais, R.; Gibbens, I.; Hackett, S.; James, M.; Schuchter, L. M.; Nathanson, K. L.; Xia, C.; Simantov, R.; Schwartz, B.; Poulin-Costello, M.; O'Dwyer, P. J.; Ratain, M. J. *Br. J. Cancer* **2006**, *95*, 581.
25. Compound **Iq**: IR (KBr): 3417, 1504, 1273, 1128, 752 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.27 (br s, 1H), 10.73 (br s, 1H), 8.68 (s, 1H), 8.50–8.44 (m, 2H), 8.28 (br s, 1H), 8.21 (br s, 1H), 8.10 (d, 2H, $J$ = 7.2 Hz), 8.00–7.89 (m, 5H), 7.75 (br s, 1H), 7.66–7.64 (m, 1H),7.59–7.54 (m, 2H), 6.84 (d, 1H, $J$ = 3.6 Hz). MS (ESI) $m/z$ 582.1 $(M+H)^+$. Compound **Ir**: IR (KBr): 3380, 1523, 1314, 1130, 931, 743 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.20 (br s, 1H), 10.57 (br s, 1H), 8.67 (s, 1H), 8.30 (s, 1H), 8.16–8.07 (m, 5H), 7.98–7.95 (m, 1H), 7.89–7.88 (m, 1H), 7.67–7.51 (m, 4H), 7.37–7.34 (m, 1H), 6.84 (d, 1H, $J$ = 3.6 Hz), 3.76–3.72 (m, 4H), 2.99–2.98 (m, 4H). MS (ESI) $m/z$ 587.2 $(M+H)^+$.