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Synthesis and Antiproliferative Activity of New Aminoisoquinolinylurea Derivatives against Melanoma Cell Line

Hye Jung Cho, Mohammed I. El-Gamal,[†,‡] Chang-Hyun Oh,[†,‡] So Ha Lee, Garam Kim,[§] Jun Hee Hong,[§] Hong Seok Choi,[§] and Kyung Ho Yoo[*]

Chemical Kinomics Research Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea. [*]E-mail: khyoo@kist.re.kr
[†]Center for Biomaterials, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Korea
[‡]Department of Biomolecular Science, University of Science and Technology, Daejeon 305-333, Korea
[§]College of Pharmacy, Chosun University, Gwangju 501-759, Korea
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A series of new diarylureas possessing aminoisoquinoline scaffold was synthesized, and their *in vitro* antiproliferative activity against A375P human melanoma cell line was tested. Compounds **1d**, **1l**, **1n**, **1p**, **1q**, and **1t** showed superior potency against A375P to Sorafenib. The highest potency was shown by compound **1p** possessing 3,5-bis(trifluoromethyl)phenyl terminal ring with IC_{50} value of 0.41 μM.

Key Words : A375P, Aminoisoquinoline, Anticancer, Diarylurea, Melanoma

Introduction

Melanoma is the most aggressive form of skin cancer. It is a malignant tumor that arises from melanocytic cells and primarily involves the skin. The major risk factors for melanoma development include exposure to solar ultraviolet irradiation, fair skin, dysplastic nevi syndrome, and a family history of melanoma. Melanomas can metastasize either by the lymphatic or by the hematogenous route.[1] Metastatic melanoma is a particularly aggressive form of cancer that has a very poor prognosis, and is resistant to standard anticancer therapies. Early stage melanoma (stage I/II) primary tumors can be surgically resected with more than 95% success rate.[2] While late-stage (Stage IV) metastatic melanoma is one of the most deadly forms of cancer, with the median survival of patients with distant metastases being 7-9 months.[3] Due to the rapid incidence of melanoma in the United States and other developed countries, there is an urgent need to develop more efficient antiproliferative therapeutics.[4-6]

Sorafenib (Nexavar®) is a diarylurea derivative that has been extensively used in clinical trials.[7] In addition, a number of reports have recently highlighted diarylureas as potential antiproliferative agents against melanoma cell lines.[8-19] Encouraged by the interesting antiproliferative activity of diarylurea derivatives, we report a new series of diarylureas containing 5-aminoisoquinoline scaffold in the present investigation (Fig. 1). Their *in vitro* antiproliferative activity against A375P human melanoma cell line is reported.

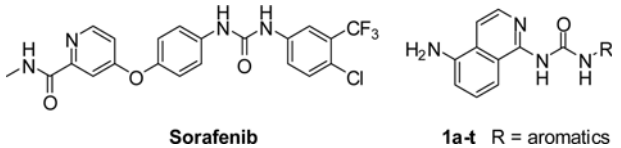


Figure 1. Structures of Sorafenib and the target compounds **1a-t**.

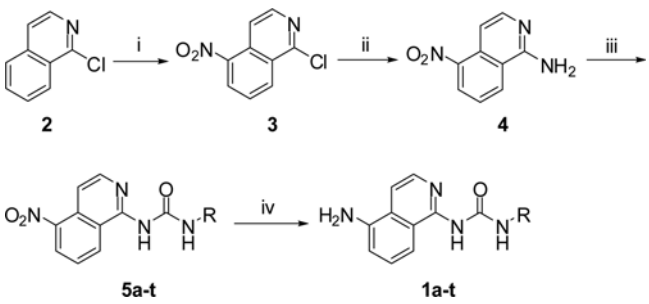
Results and Discussion

Chemistry. The target compounds **1a-t** were synthesized according to the sequence of reactions shown in Scheme 1. Nitration of 1-chloroisoquinoline (**2**) using a mixture of nitric and sulfuric acids gave 1-chloro-5-nitroisoquinoline (**3**).[20] Treatment of **3** with 7 N ammonia solution in methanol led to nucleophilic displacement and formation of 1-amino-5-nitroisoquinoline (**4**). Reaction of **4** with the appropriate aryl isocyanate derivatives afforded the corresponding diarylurea derivatives **5a-t**. Reduction of the nitro group of **5a-t** with palladium over carbon in hydrogen atmosphere produced the target aminoisoquinolinylureas **1a-t**.

Biological Evaluation.

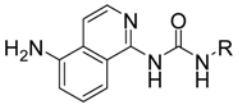
Antiproliferative Activity Against A375P Human Melanoma Cell Line: The antiproliferative activity of the newly synthesized compounds against A375P human melanoma cell line was examined. The ability of target diarylureas to inhibit the growth of A375P cell line is summarized in Table 1. Sorafenib was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

As shown in Table 1, compounds **1d**, **1l**, **1n**, **1p**, **1q**, and

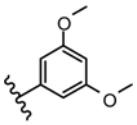
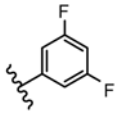
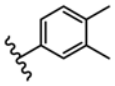
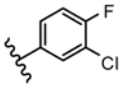
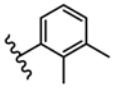
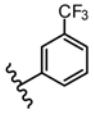
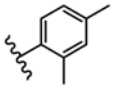
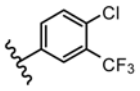
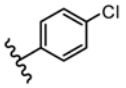
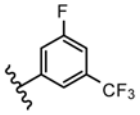
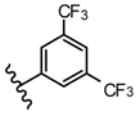
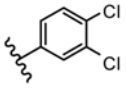
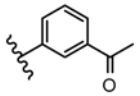
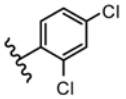
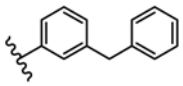
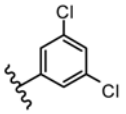
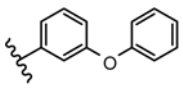
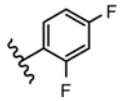
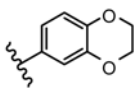


Scheme 1. Reagents and conditions: (i) HNO_3, H_2SO_4, rt, 3 h, 99%; (ii) NH_3 (7 N solution in MeOH), reflux, 40 h, 77%; (iii) R-NCO, CH_2Cl_2, rt, 20 h, 28-96%; (iv) Pd/C, H_2, MeOH, rt, 2 h, 27-87%.

Table 1. Antiproliferative activity of aminoisoquinolinylureas **1a-t** against A375P human melanoma cell line



1a-t

Compd. No.	R	IC_{50} (μM)	Compd. No.	R	IC_{50} (μM)
1a		7.4	**1k**		> 10
1b		7.1	**1l**		3.0
1c		> 10	**1m**		5.9
1d		4.3	**1n**		2.6
1e		6.2	**1o**		> 10
1f		5.7	**1p**		0.41
1g		6.6	**1q**		2.9
1h		> 10	**1r**		> 10
1i		> 10	**1s**		7.4
1j		> 10	**1t**		2.9
Sorafenib		5.1			

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

1t showed higher potencies than Sorafenib. In addition, compounds **1f** and **1m** demonstrated similar potency as Sorafenib against A375P. The IC_{50} values of compounds **1d**, **1l**, **1n**, **1q**, and **1t** were in micromolar scale. While that of compound **1p** was in sub-micromolar scale.

Compound **1g** with 3,4-dichlorophenyl terminal ring was more potent than its positional isomers **1h**, **i** with 2,4-dichlorophenyl and 3,5-dichlorophenyl, respectively. This may be rationalized that the orientation of groups at receptor site may affect the affinity and potency. Similarly, compound **1d** with 2,4-dimethylphenyl showed higher potency than its positional isomers **1b**, **c**.

The introduction of *para*-chloro or *meta*-trifluoromethyl groups on the 3'-trifluoromethylphenyl moiety enhanced the potency of **1n** and **1p**, respectively, compared with **1m**. On the other hand, *meta*-fluoro decreased the potency of compound **1o** compared with **1m**.

Compounds **1q** and **1t** bearing acetylphenyl and benzo [*b*][1,4]dioxine groups exhibited superior antiproliferative activities to Sorafenib.

Among all the target compounds, the best activity was encountered with compound **1p** possessing 3,5-bis(trifluoromethyl)phenyl terminal ring. This moiety is seemed to be the most optimal for activity of this series of compounds. This may be attributed to steric and/or electronic effect(s) of the two trifluoromethyl groups.

Conclusion

A new series of diarylurea derivatives with 5-aminoisoquinoline 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 **1d**, **1l**, **1n**, **1p**, **1q**, and **1t** demonstrated higher potencies against A375P human melanoma cell line than that of the reference compound, Sorafenib. The IC_{50} values of **1d**, **1l**, **1n**, **1q**, and **1t** were in micromolar scale. But that of **1p** was in sub-micromolar range (0.41 μM). Compound **1p** with 3,5-bis(trifluoromethyl)phenyl terminal ring was 12.4 times more potent than Sorafenib. It can be considered as a promising lead for further development of potent antiproliferative agents for melanoma.

Experimental

General. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using tetramethylsilane as an internal standard. LC-Mass spectra were determined on a Waters Quattro Micro system. Column chromatography was carried out using silica gel (230-400 mesh). Solvents and liquid reagents were transferred using hypodermic syringes. Purity (%) of all the target compounds were determined by LC-MS and found to be > 95%. All solvents and reagents were commercially available and used without further purification.

1-Chloro-5-nitroisoquinoline (3). To a solution of 1-chloroisoquinoline (**2**) (1.0 g, 6.11 mmol) in sulfuric acid (5.0 mL, 84.4 mmol) was added nitric acid (0.9 mL, 21.4 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 3 h. Upon completion, the reaction mixture was cooled to 0 °C and neutralized with 10% $NaHCO_3$ aqueous solution. The resulting precipitate was collected by filtration and dried to give **3** (1.26 g, 99%). ^{1}H NMR (DMSO-d_6) δ 8.65-8.77 (m, 2H), 8.53 (d, J = 6.1 Hz, 1H), 8.29 (d, J = 6.1 Hz, 1H), 8.00 (t, J = 8.1 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 152.6, 145.3, 144.8, 133.3, 130.3, 128.9, 127.5, 127.0, 115.8.

5-Nitroisoquinolin-1-amine (4). 1-Chloro-5-nitroisoquinoline (**3**) (500 mg, 2.40 mmol) was added to ammonia (7 N solution in MeOH) (10 mL), and the mixture was refluxed for 40 h. After completion of the reaction, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, ethyl acetate/hexane 2:1 v/v) to give **4** (350 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 7.8 Hz, 1H), 8.08-8.19 (m, 2H), 7.76 (d, J = 6.3 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 5.32 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 167.4, 157.8, 146.1, 144.4, 129.3, 127.6, 118.0, 104.1, 102.2.

General Procedure for the Synthesis of 1-Substituted 3-(5-nitroisoquinolin-1-yl)ureas 5a-t. To a solution of 5-nitroisoquinolin-1-amine (**4**) in CH_2Cl_2 was added the appropriate aryl isocyanate derivative, and the mixture was stirred at room temperature for 20 h. Upon completion, the reaction mixture was filtered to give **5a-t**.

1-(3,5-Dimethoxyphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5a). Yield 66.1%; ^{1}H NMR (DMSO-d_6) δ 12.10 (s, 1H), 9.04 (d, J = 8.5 Hz, 1H), 8.59 (d, J = 7.6 Hz, 1H), 8.48 (d, J = 6.1 Hz, 1H), 7.81-7.89 (m, 2H), 7.00 (s, 1H), 6.90 (s, 2H), 6.25 (s, 1H), 3.76 (s, 6H).

1-(3,4-Dimethylphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5b). Yield 59.5%; ^{1}H NMR (DMSO-d_6) δ 12.03 (s, 1H), 10.16 (s, 1H), 9.06 (d, J = 8.5 Hz, 1H), 8.58 (d, J = 7.7 Hz, 1H), 8.44 (d, J = 6.3 Hz, 1H), 7.79-7.87 (m, 2H), 7.38-7.44 (m, 2H), 7.10 (d, J = 7.8 Hz, 1H), 2.38 (s, 3H), 2.26 (s, 3H).

1-(2,3-Dimethylphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5c). Yield 60.6%; ^{1}H NMR (DMSO-d_6) δ 12.18 (s, 1H), 10.28 (s, 1H), 9.12 (d, J = 8.5 Hz, 1H), 8.59 (d, J = 7.7 Hz, 1H), 8.40 (d, J = 6.3 Hz, 1H), 7.82-7.39 (m, 4H), 6.97 (d, J = 7.4 Hz, 1H), 2.31 (s, 6H).

1-(2,4-Dimethylphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5d). Yield 60.6%; ^{1}H NMR (DMSO-d_6) δ 12.20 (s, 1H), 10.28 (s, 1H), 9.12 (d, J = 8.6 Hz, 1H), 8.59 (d, J = 7.7 Hz, 1H), 8.40 (d, J = 6.3 Hz, 1H), 7.96 (d, J = 8.2 Hz, 1H), 7.81-7.89 (m, 2H), 7.01-7.07 (m, 2H), 2.37 (s, 3H), 2.26 (s, 3H).

1-(4-Chlorophenyl)-3-(5-nitroisoquinolin-1-yl)urea (5e). Yield 53.9%; ^{1}H NMR (DMSO-d_6) δ 12.22 (s, 1H), 9.82 (s, 1H), 9.04 (d, J = 7.3 Hz, 1H), 8.57 (d, J = 7.5 Hz, 1H), 8.45 (brs, 1H), 7.79-7.87 (m, 2H), 7.72 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 7.2 Hz, 2H).

1-(2-Chlorophenyl)-3-(5-nitroisoquinolin-1-yl)urea (5f). Yield 68.7%; ^{1}H NMR (DMSO-d_6) δ 13.09 (s, 1H), 10.49 (s, 1H), 9.13 (d, J = 8.6 Hz, 1H), 8.60 (d, J = 7.2 Hz, 1H), 8.40-8.46 (m, 2H), 7.83-7.80 (m, 2H), 7.56 (dd, J = 1.3, 1.4 Hz, 1H), 7.35-7.40 (m, 2H).

1-(3,4-Dichlorophenyl)-3-(5-nitroisoquinolin-1-yl)urea (5g). Yield 54.0%; ^{1}H NMR (DMSO-d_6) δ 12.37 (s, 1H), 9.95 (s, 1H), 9.04 (d, J = 8.8 Hz, 1H), 8.59 (d, J = 7.8 Hz, 1H), 8.48 (d, J = 6.3 Hz, 1H), 8.09 (d, J = 2.0 Hz, 1H), 7.88 (t, J = 6.5 Hz, 1H), 7.70 (d, J = 4.2 Hz, 1H), 7.53-7.61 (m, 2H).

1-(2,4-Dichlorophenyl)-3-(5-nitroisoquinolin-1-yl)urea (5h). Yield 62.9%; ^{1}H NMR (DMSO-d_6) δ 13.23 (s, 1H), 9.34 (s, 1H), 9.13 (d, J = 8.6 Hz, 1H), 8.60 (d, J = 7.6 Hz, 1H), 8.43-8.49 (m, 2H), 7.83-7.91 (m, 2H), 7.73 (d, J = 2.4 Hz, 1H), 7.47 (dd, J = 2.4, 2.6 Hz, 1H).

1-(3,5-Dichlorophenyl)-3-(5-nitroisoquinolin-1-yl)urea (5i). Yield 34.8%; ^{1}H NMR (DMSO-d_6) δ 12.48 (s, 1H), 10.41 (s, 1H), 9.04 (d, J = 9.6 Hz, 2H), 8.50 (d, J = 6.8 Hz, 1H), 7.80-7.86 (m, 5H).

1-(2,4-Difluorophenyl)-3-(5-nitroisoquinolin-1-yl)urea (5j). Yield 35.8%; ^{1}H NMR (DMSO-d_6) δ 12.49 (s, 1H), 9.09 (d, J = 8.5 Hz, 1H), 8.60 (d, J = 7.5 Hz, 1H), 8.38 (d, J = 6.2 Hz, 1H), 8.22 (dd, J = 8.7, 8.8 Hz, 1H), 7.83-7.89 (m, 2H), 7.36-7.44 (m, 1H), 7.03-7.10 (m, 2H).

1-(3,5-Difluorophenyl)-3-(5-nitroisoquinolin-1-yl)urea (5k). Yield 50.3%; ^{1}H NMR (DMSO-d_6) δ 12.94 (s, 1H), 9.43 (s, 1H), 9.04 (d, J = 8.4 Hz, 1H), 8.58 (d, J = 6.2 Hz, 1H), 7.88-7.97 (m, 2H), 7.48 (d, J = 8.5 Hz, 2H), 6.83-6.90 (m, 2H).

1-(3-Chloro-4-fluorophenyl)-3-(5-nitroisoquinolin-1-yl) urea (5l). Yield 53.3%; ^{1}H NMR (DMSO-d_6) δ 12.31 (s, 1H), 9.04 (d, J = 8.5 Hz, 1H), 8.57 (d, J = 6.3 Hz, 1H), 8.47 (s, 1H), 8.02 (d, J = 6.2 Hz, 1H), 7.77-7.86 (m, 2H), 7.64 (d, J = 7.8 Hz, 1H), 7.36-7.43 (m, 2H).

1-(3-Trifluoromethylphenyl)-3-(5-nitroisoquinolin-1-yl) urea (5m). Yield 43.2%; ^{1}H NMR (DMSO-d_6) δ 12.13 (s, 1H), 9.97 (s, 1H), 9.04 (d, J = 8.3 Hz, 1H), 8.58-8.65 (m, 3H), 7.80-7.88 (m, 3H), 7.53-7.60 (m, 2H).

1-(4-Chloro-3-trifluoromethylphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5n). Yield 78.1%; ^{1}H NMR (DMSO-d_6) δ 12.67 (s, 1H), 9.78 (s, 1H), 9.03 (d, J = 8.5 Hz, 1H), 8.57 (d, J = 7.6 Hz, 1H), 8.38 (brs, 1H), 7.89 (brs, 1H), 7.78-7.87 (m, 2H), 7.67 (d, J = 8.5 Hz, 2H).

1-(3-Fluoro-5-(trifluoromethyl)phenyl)-3-(5-nitroisoquinolin-1-yl)urea (5o). Yield 39.6%; ^{1}H NMR (DMSO-d_6) δ 12.96 (s, 1H), 9.64 (s, 1H), 9.03 (d, J = 8.4 Hz, 1H), 8.57 (d, J = 7.8 Hz, 1H), 8.02 (s, 1H), 7.79-7.87 (m, 3H), 7.25-7.34 (m, 2H).

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(5-nitroisoquinolin-1-yl)urea (5p). Yield 28.9%; ^{1}H NMR (DMSO-d_6) δ 13.67 (s, 1H), 10.31 (s, 1H), 9.04 (d, J = 8.2 Hz, 1H), 8.57 (d, J = 7.7 Hz, 1H), 8.40-8.47 (m, 3H), 7.83 (t, J = 8.0 Hz, 1H), 7.69 (s, 1H), 7.39 (brs, 1H).

1-(3-Acetylphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5q). Yield 44.6%; ^{1}H NMR (DMSO-d_6) δ 12.25 (s, 1H), 9.05 (d, J = 8.4 Hz, 1H), 8.59 (d, J = 7.6 Hz, 1H), 8.44-8.49 (m, 2H), 8.23 (s, 1H), 7.93 (d, J = 8.2 Hz, 1H), 7.87 (t, J = 6.1 Hz, 2H), 7.70 (d, J = 7.3 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 2.61 (s, 3H).

1-(3-Benzylphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5r). Yield 61.8%; ^{1}H NMR (DMSO-d_6) δ 12.06 (s, 1H), 9.32 (s, 1H), 9.04 (d, J = 8.5 Hz, 1H), 8.58 (d, J = 7.6 Hz, 1H), 8.44 (d, J = 6.3 Hz, 1H), 7.81-7.87 (m, 2H), 7.49-7.57 (m, 2H), 7.25-7.43 (m, 6H), 6.94 (d, J = 7.4 Hz, 1H), 3.95 (s, 2H).

1-(3-Phenoxyphenyl)-3-(5-nitroisoquinolin-1-yl)urea (5s). Yield 96.0%; ^{1}H NMR (DMSO-d_6) δ 12.53 (s, 1H), 8.86 (s, 1H), 7.33-7.47 (m, 4H), 7.26 (t, J = 8.1 Hz, 1H), 7.20 (t, J = 2.1 Hz, 1H), 7.12-7.19 (m, 2H), 7.06-7.14 (m, 2H), 7.00-7.06 (m, 3H), 6.61 (d, J = 1.7 Hz, 1H).

1-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(5-nitroisoquinolin-1-yl)urea (5t). Yield 63.0%; ^{1}H NMR (DMSO-d_6)

δ 12.01 (s, 1H), 9.35 (s, 1H), 9.05 (d, *J* = 9.0 Hz, 1H), 8.58 (d, *J* = 7.8 Hz, 1H), 8.43 (d, *J* = 6.1 Hz, 1H), 7.80-7.86 (m, 2H), 7.30 (s, 1H), 7.04 (d, *J* = 8.7 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 4.23 (s, 4H).

General Procedure for the Synthesis of 1-Substituted 3-(5-aminoisoquinolin-1-yl)ureas 1a-t. To a solution of the appropriate 1-substituted 3-(5-aminoisoquinolin-1-yl)urea **5** in MeOH was added 5% Pd/C, and the mixture was hydrogenated for 2 h. Upon completion, the reaction mixture was filtered through celite. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by column chromatography (silica gel, ethyl acetate/hexane 1:1 v/v) to give the title compound **1**.

1-(3,5-Dimethoxyphenyl)-3-(5-aminoisoquinolin-1-yl) urea (1a). Yield 35.1%; ^{1}H NMR (DMSO-d_6) δ 12.68 (s, 1H), 9.55 (s, 1H), 8.10 (d, *J* = 6.1 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.61 (d, *J* = 6.2 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 2.1 Hz, 2H), 6.23 (t, *J* = 2.0 Hz, 1H), 5.95 (s, 2H), 3.76 (s, 6H); MS *m/z* 339 $(M + 1)^+$.

1-(3,4-Dimethylphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1b). Yield 39.3%; ^{1}H NMR (DMSO-d_6) δ 12.56 (s, 1H), 9.49 (s, 1H), 8.07 (d, *J* = 6.1 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 6.2 Hz, 1H), 7.36-7.43 (m, 2H), 7.32 (t, *J* = 8.1 Hz, 1H), 7.09 (d, *J* = 7.7 Hz, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 5.94 (s, 2H), 2.36 (s, 3H), 2.26 (s, 3H); MS *m/z* 307 $(M + 1)^+$.

1-(2,3-Dimethylphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1c). Yield 45.7%; ^{1}H NMR (DMSO-d_6) δ 12.63 (s, 1H), 9.60 (s, 1H), 8.03 (d, *J* = 5.8 Hz, 1H), 7.90 (d, *J* = 7.9 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.61 (d, *J* = 6.6 Hz, 1H), 7.33 (t, *J* = 8.1 Hz, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 6.88-6.97 (m, 2H), 5.96 (s, 2H), 2.30 (s, 6H); MS *m/z* 307 $(M + 1)^+$.

1-(2,4-Dimethylphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1d). Yield 32.6%; ^{1}H NMR (DMSO-d_6) δ 12.63 (s, 1H), 9.59 (s, 1H), 7.97-8.04 (m, 2H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 4.9 Hz, 1H), 7.32 (t, *J* = 7.1 Hz, 1H), 7.06 (s, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 6.91 (d, *J* = 7.4 Hz, 1H), 5.95 (s, 2H), 2.37 (s, 3H), 2.25 (s, 3H); MS *m/z* 307 $(M + 1)^+$.

1-(4-Chlorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1e). Yield 42.9%; ^{1}H NMR (DMSO-d_6) δ 12.85 (s, 1H), 9.67 (s, 1H), 8.08 (d, *J* = 6.0 Hz, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.70 (d, *J* = 8.3 Hz, 2H), 7.62 (d, *J* = 5.5 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.33 (t, *J* = 8.1 Hz, 1H), 6.91 (d, *J* = 7.5 Hz, 1H), 5.96 (s, 2H); MS *m/z* 313 $(M + 1)^+$.

1-(2-Chlorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1f). Yield 38.4%; ^{1}H NMR (DMSO-d_6) δ 13.51 (s, 1H), 9.83 (s, 1H), 8.45 (d, *J* = 7.8 Hz, 1H), 8.06 (d, *J* = 6.0 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 5.4 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.31-7.39 (m, 2H), 7.08 (t, *J* = 7.7 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 5.98 (s, 2H); MS *m/z* 312 $(M + 1)^+$.

1-(3,4-Dichlorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1g). Yield 43.5%; ^{1}H NMR (DMSO-d_6) δ 13.02 (s, 1H), 9.77 (s, 1H), 8.09 (s, 2H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.53-7.70 (m, 3H), 7.34 (t, *J* = 8.1 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 5.98 (s, 2H); MS *m/z* 347 $(M + 1)^+$.

1-(2,4-Dichlorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1h). Yield 37.2%; ^{1}H NMR (DMSO-d_6) δ 13.67 (s, 1H), 9.90 (s, 1H), 8.49 (d, *J* = 9.1 Hz, 1H), 8.08 (d, *J* = 6.0 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.70 (d, *J* = 2.3 Hz, 1H), 7.65 (d, *J* = 6.0 Hz, 1H), 7.45 (dd, *J* = 2.4, 2.5 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 6.92 (d, *J* = 7.7 Hz, 1H), 5.99 (s, 2H); MS *m/z* 347 $(M + 1)^+$.

1-(3,5-Dichlorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1i). Yield 27.2%; ^{1}H NMR (DMSO-d_6) δ 13.13 (s, 1H), 9.81 (s, 1H), 8.13 (d, *J* = 6.1, 1H), 7.79-7.84 (m, 2H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.64 (d, *J* = 6.1 Hz, 1H), 7.35 (t, *J* = 7.4 Hz, 1H), 7.25-7.32 (m, 1H), 6.92 (d, *J* = 7.9 Hz, 1H), 5.97 (s, 2H); MS *m/z* 347 $(M + 1)^+$.

1-(2,4-Difluorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1j). Yield 41.4%; ^{1}H NMR (DMSO-d_6) δ 13.02 (s, 1H), 9.81 (s, 1H), 8.22-8.27 (m, 1H), 8.00 (d, *J* = 6.1 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 6.1 Hz, 1H), 7.33-7.40 (m, 2H), 7.10 (t, *J* = 7.8 Hz, 1H), 6.91 (t, *J* = 7.7 Hz, 1H), 5.97 (s, 2H); MS *m/z* 365 $(M + 1)^+$.

1-(3,5-Difluorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1k). Yield 34.5%; ^{1}H NMR (DMSO-d_6) δ 13.12 (s, 1H), 9.78 (s, 1H), 8.12 (d, *J* = 6.1 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 6.1 Hz, 1H), 7.43-7.48 (m, 2H), 7.33 (t, *J* = 8.1 Hz, 1H), 6.85-6.92 (m, 2H), 5.97 (s, 2H); MS *m/z* 315 $(M + 1)^+$.

1-(3-Chloro-4-fluorophenyl)-3-(5-aminoisoquinolin-1-yl)urea (1l). Yield 30.2%; ^{1}H NMR (DMSO-d_6) δ 12.91 (s, 1H), 9.74 (s, 1H), 8.10 (d, *J* = 6.1 Hz, 1H), 8.01 (dd, *J* = 2.6, 2.7 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.57-7.65 (m, 2H), 7.40 (t, *J* = 9.1 Hz, 1H), 7.33 (t, *J* = 7.9 Hz, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 5.96 (s, 2H); MS *m/z* 331 $(M + 1)^+$.

1-(3-Trifluoromethylphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1m). Yield 43.7%; ^{1}H NMR (DMSO-d_6) δ 13.01 (s, 1H), 9.75 (s, 1H), 8.17 (s, 1H), 8.12 (d, *J* = 6.1 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 6.1 Hz, 1H), 7.59 (t, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 5.97 (s, 2H); MS *m/z* 347 $(M + 1)^+$.

1-(4-Chloro-3-trifluoromethylphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1n). Yield 62.1%; ^{1}H NMR (DMSO-d_6) δ 12.77 (s, 1H), 8.13 (d, *J* = 3.6 Hz, 1H), 7.88-7.95 (m, 2H), 7.67 (s, 1H), 7.43-7.50 (m, 2H), 7.30-7.36 (m, 2H), 7.02 (d, *J* = 7.1 Hz, 1H), 4.27 (s, 2H); MS *m/z* 381 $(M + 1)^+$.

1-(3-Fluoro-5-(trifluoromethyl)phenyl)-3-(5-aminoisoquinolin-1-yl)urea (1o). Yield 53.8%; ^{1}H NMR (DMSO-d_6) δ 13.20 (s, 1H), 9.86 (s, 1H), 8.15 (d, *J* = 6.2 Hz, 1H), 7.95 (s, 1H), 7.77 (d, *J* = 8.1 Hz, 1H), 7.66 (d, *J* = 5.8 Hz, 1H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.15 (s, 1H), 6.94 (d, *J* = 5.7 Hz, 1H), 5.98 (s, 2H); MS *m/z* 337 $(M + 1)^+$.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(5-aminoisoquinolin-1-yl)urea (1p). Yield 86.8%; ^{1}H NMR (DMSO-d_6) δ 13.27 (s, 1H), 10.15 (s, 1H), 8.42 (s, 2H), 8.32 (s, 1H), 7.58-7.74 (m, 3H), 7.31 (t, *J* = 7.5 Hz, 1H), 6.92 (d, *J* = 7.0 Hz, 1H), 5.92 (s, 2H); MS *m/z* 415 $(M + 1)^+$.

1-(3-Acetylphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1q). Yield 49.3%; ^{1}H NMR (DMSO-d_6) δ 12.85 (s, 1H), 9.67 (s, 1H), 8.21 (s, 1H), 8.12 (d, *J* = 6.1 Hz, 1H), 7.92 (d, *J* = 7.4 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.59-7.74 (m, 3H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 6.92 (d, *J* = 7.6 Hz, 1H), 5.97 (s, 2H), 2.61 (s, 3H); MS *m/z* 321 $(M + 1)^+$.

1-(3-Benzylphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1r). Yield 27.1%; ^{1}H NMR (DMSO-d_6) δ 12.63 (s, 1H), 9.53 (s, 1H), 8.06 (d, J = 6.0 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 6.1 Hz, 1H), 7.47-7.54 (m, 2H), 7.18-7.37 (m, 6H), 7.19 (d, J = 7.0 Hz, 1H), 6.90 (d, J = 7.5 Hz, 2H), 5.94 (s, 2H), 3.94 (s, 2H); MS *m/z* 315 $(M + 1)^+$.

1-(3-Phenoxyphenyl)-3-(5-aminoisoquinolin-1-yl)urea (1s). Yield 30.0%; ^{1}H NMR (DMSO-d_6) δ 12.80 (s, 1H), 9.60 (s, 1H), 8.08 (s, 1H), 7.75 (d, J = 5.9 Hz, 1H), 7.55-7.60 (m, 2H), 7.33-7.41 (m, 5H), 7.06-7.12 (m, 3H), 6.92 (s, 1H), 6.68 (s, 1H), 5.96 (s, 2H); MS *m/z* 371 $(M + 1)^+$.

1-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(5-aminoisoquinolin-1-yl)urea (1t). Yield 29.7%; ^{1}H NMR (DMSO-d_6) δ 12.54 (s, 1H), 9.51 (s, 1H), 8.06 (d, J = 6.1 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 6.1 Hz, 1H), 7.27-7.34 (m, 2H), 6.99 (dd, J = 2.5, 2.6 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 6.82 (d, J = 8.7 Hz, 1H), 5.94 (s, 2H), 4.23 (s, 4H); MS *m/z* 369 $(M + 1)^+$.

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.

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References

1. Garbe, C.; Hauschild, A.; Volkenandt, M.; Schadendorf, D.; Stolz, W.; Reinhold, U.; Kortmann, R. D.; Kettelhack, C.; Frerich, B.; Keilholz, U.; Dummer, R.; Sebastian, G.; Tilgen, W.; Schuler, G.; Mackensen, A.; Kaufmann, R. *Melanoma Res.* **2007**, *17*, 393.
2. Balch, C. M.; Buzaid, A. C.; Soong, S. J.; Atkins, M. B.; Cascinelli, N.; Coit, D. G.; Fleming, I. D.; Gershenwald, J. E.; Houghton, A., Jr.; Kirkwood, J. M.; McMasters, K. M.; Mihm, M. F.; Morton, D. L.; Reintgen, D. S.; Ross, M. I.; Sober, A.; Thompson, J. A.; Thompson, J. F. *J. Clin. Oncol.* **2001**, *19*, 3635.
3. Lee, M. L.; Tomsu, K.; Von Eschen, K. B. *Melanoma Res.* **2000**, *10*, 81.
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. *Expert Rev. Anticancer Ther.* **2007**, *7*, 79.
7. Wilhelm, S. M.; Adnane, L.; Newell, P.; Villanueva, A.; Llovet, J. M.; Lynch, M. *Mol. Cancer Ther.* **2008**, *7*, 3129.
8. 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.
9. 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.
10. Choi, W.-K.; Oh, C.-H. *Bull. Korean Chem. Soc.* **2009**, *30*, 2027.
11. 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.
12. 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.
13. 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.
14. Lee, J.; Nam, B. S.; Kim, H.; Oh, C.-H.; Lee, S. H.; Cho, S. J.; Sim, T. B.; Hah, J.-M.; Kim, D. J.; Tae, J.; Yoo, K. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5722.
15. 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.
16. Choi, W.-K.; El-Gamal, M. I.; Choi, H. S.; Baek, D.; Oh, C.-H. *Eur. J. Med. Chem.* **2011**, *46*, 5754.
17. 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.
18. 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.
19. El-Gamal, M. I.; Oh, C.-H. *Bull. Korean Chem. Soc.* **2012**, *33*, 1571.
20. Gamage, S. A.; Spicer, J. A.; Rewcastle, G. W.; Milton, J.; Sohal, S.; Dangerfield, W.; Mistry, P.; Vicker, N.; Charlton, P. A.; Denny, W. A. *J. Med. Chem.* **2002**, *45*, 740.