DOI 10.5012/bkcs.2011.32.4.1209

Synthesis and Antiproliferative Activity of Pyridinylcarbonylpyrimidines Against Melanoma Cell Line

Hyemi Ahn, Jun A Lee, Hwan Kim, Chang-Hyun Oh, So Ha Lee, Taebo Sim, Jung-Mi Hah,† Dong Jin Kim, and Kyung Ho Yoo*

Life/Health Division, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, Korea
**E-mail: khyoo@kist.re.kr*
†*Department of Pharmacy, College of Pharmacy, Hanyang University, Gyunggi-do 426-791, Korea*
Received January 3, 2011, Accepted February 7, 2011

The synthesis of the series of pyrimidinylamines **1a-d** and pyrimidinylureas **1e-u** bearing a novel pyridinylcarbonylpyrimidine scaffold and their antiproliferative activities against A375 human melanoma cell line were described. Among them, three compounds **1e**, **1h**, and **1o** showed superior antiproliferative activities to Sorafenib (IC_{50} = 5.5 μM) as a reference compound. In our series, urea compound **1o** having 4-chloro-3-trifluoromethyl moiety on the benzene nucleus exhibited very good antiproliferative activity with IC_{50} value of 1.4 μM.

Key Words : Pyridinylcarbonylpyrimidines, Antiproliferative activity, Melanoma cell line

Introduction

Melanoma is a malignant tumor that arises from melanocytic cells and primarily involves the skin. Incidence of melanoma has tripled in the last 40 years, and more than 80% of skin cancer deaths are due melanoma. Generally, two major risk factors for melanoma development are an individual's family history and an environmental factor. The most relevant environmental factor is exposure to solar ultraviolet irradiation that causes damage to the DNA of cells.[1]

In early-stage melanoma without metastasis, treatment for localized melanoma normally involves surgery to remove the lesion. Melanomas can metastasize either by the lymphatic or by the hematogenous route.[2] The 5-year survival rate for patients with metastatic melanoma below 15% and median survival of about 6-8 months.[3-6]

Decarbazine (DTIC)[7,8] is the only cytotoxic agent formally approved for the treatment of melanoma and Temozolomide (Temodar)[9] is an imidazotetrazine with a mechanism of action similar to DTIC. Both of them are used most frequency for stage IV melanoma patients as a chemotherapy. The current treatments involve surgical removal of the tumor, immunotherapy, radiotherapy, chemotherapy, various combinations. However, due to the development of metastatic disease which is highly resistant to conventional chemotherapeutics and radiation,[10] the intensive research and effort into new drugs and treatments[11-19] for new targeted therapy and advanced melanoma have not afforded the effective response rates yet.

In this paper, based on the structural features of Sorafenib (Nexavar)[20] that has been used extensively in clinical trials, a novel scaffold having pyridinylcarbonylpyrimidine group by the introduction of pyridinyl and pyrimidine moieties as hinge and linker was designed as shown in Figure 1. We report here the synthesis of pyrimidinylamine derivatives **1a-d** and pyrimidinylurea derivatives **1e-u**, and their antiproliferative activities against A375 human melanoma cell line compared with Sorafenib.

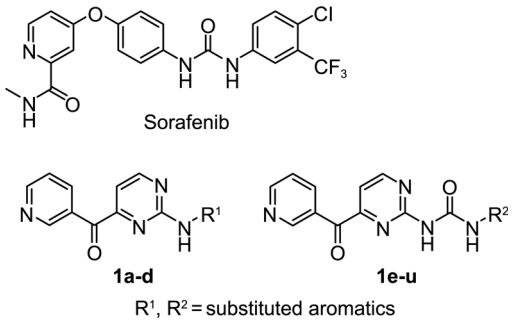
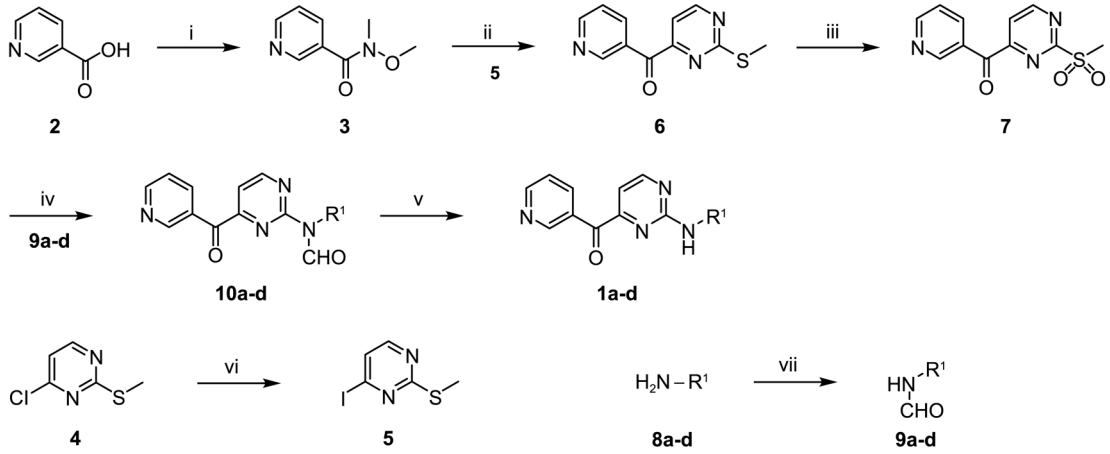


Figure 1. Structures of Sorafenib and target compounds **1a-d** and **1e-u**.

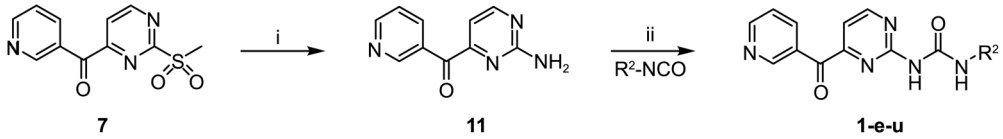
Results and Discussion

Chemistry. Pyridinylcarbonylpyrimidine derivatives **1a-d** with amine moiety were prepared by the sequence outlined in Scheme 1.

Amidation of carboxylic acid group of nicotinic acid (**2**) as a starting material with *N,O*-dimethylhydroxylamine hydrochloride in the presence of HOBt, EDCI, and triethylamine gave *N*-methoxy-*N*-methylamide (Weinreb amide) **3**, which was then coupled with 2-iodo-4-methylthiopyrimidine (**5**) using *i*-PrMgCl to give methylthiopyrimidine **6**.[21] Conversion of chloro group of 2-chloro-4-methylthiopyrimidine (**4**) to iodo group with HI provided the desired compound **5** in good yield.[22] Oxidation of **6** with oxone in MeOH[23] and subsequent amination of the resulting **7** with the appropriate formamides **9a-d** in the presence of NaH afforded the corresponding pyrimidinylformamides **10a-d**, respectively.



Scheme 1. *Reagents and reaction conditions:* (i) *N,O*-dimethylhydroxylamine hydrochloride, HOBt, EDCI, triethylamine, DMF, 90 °C, 18 h; (ii) *i*-PrMgCl, toluene, rt, 10 h; (iii) oxone, MeOH, rt, 5 h; (iv) NaH, THF, reflux, 5 h; (v) 3 M NaOH, EtOH-THF (2:1), rt, 1 h; (vi) HI, 0 °C, 18 h; (vii) formic acid, ZnO, 70 °C, 10 h.



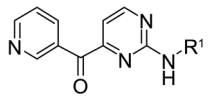
Scheme 2. *Reagents and reaction conditions:* (i) NH_3 (2 M solution in IPA), 70 °C, 10 h; (ii) pyridine, reflux, 10 h.

Formamides **9a-d** were obtained from the corresponding amines **8a-d** by formylation using formic acid and ZnO, respectively.[23] The title compounds **1a-d** were obtained by hydrolysis of formyl group using 3 M NaOH.[24]

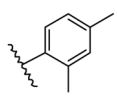
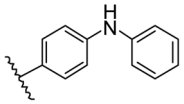
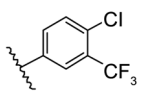
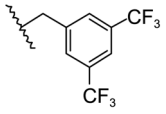
The synthesis of pyridinylcarbonylpyrimidine derivatives **1e-u** having urea moiety was outlined in Scheme 2.

Amidation of methylsulfonylpyrimidine **7** with ammonia solution in isopropyl alcohol using a sealed tube gave aminopyrimidine **11**,[25] which was reacted with the appropriate isocyanates in pyridine to afford the corresponding title compounds **1e-u**, respectively.

Table 1. Antiproliferative activity of pyrimidinylamine derivatives **1a-d**



1a-d

Compds	R^1	A375 (IC_{50}, μM)	Compds	R^1	A375 (IC_{50}, μM)
1a		> 30[a]	**1c**		> 30
1b		> 30	**1d**		> 30
			Sorafenib		5.5

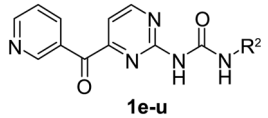
[a]Drug concentration of 30 μM did not inhibit growth of cell by 50%.

Biological Evaluation. Table 1 and 2 show the *in vitro* antiproliferative activities (IC_{50} values) of pyrimidinylamines **1a-d** and pyrimidinylureas **1e-u** against A375 human melanoma cell line together with that of Sorafenib as a reference compound.

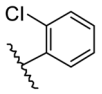
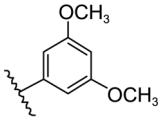
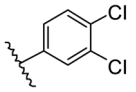
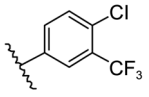
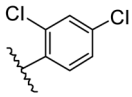
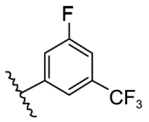
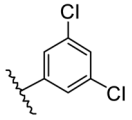
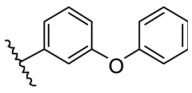
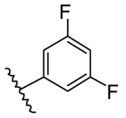
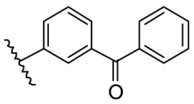
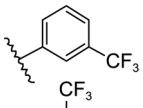
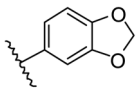
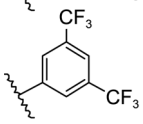
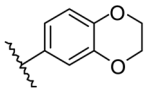
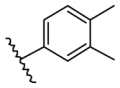
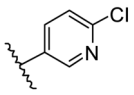
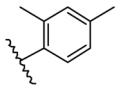
All the synthesized compounds were evaluated by MTT assays using CellTiter 96® (Promega) and the results are summarized in Table 1 and 2. All the amine compounds did not show any significant activities. Generally, urea compounds **1e-u** possessed more potent activities as compared to amine compounds **1a-d**. Urea compound **1o** with 4-chloro-3-trifluoromethyl moiety showed better antiproliferative activity than the corresponding amine compound **1b**. As shown in Table 2, three compounds **1e**, **1h**, and **1o** showed superior antiproliferative activity against A375 human melanoma cell line to Sorafenib. Among them, compound **1o** bearing 4-chloro-3-trifluoromethyl moiety exhibited very good antiproliferative activity with IC_{50} value of 1.4 μM. In general, compounds **1e**, **f**, **h**, **j**, **o**, **p** having electron-withdrawing groups on the benzene nucleus possessed better activities compared to compounds **1l-n**, **q** with electron-donating groups. The bulkier substituents in compounds **1q-t** did not give the positive effect to antiproliferative activity.

There are identified mutations in the RasRaf/MAPK pathway in over 80% of cases of melanoma. The commonest of these somatic mutations is the V600E mutation in b-Raf. The representative compound **1o** was screened against V600E-b-Raf enzyme to identify Ras/Raf/MAPK pathway using HotSpot kinase assay by Reaction Biology Corp.. Compound **1o** showed the marginal inhibitory activity with IC_{50} value of 61.9 μM against mutant b-Raf enzyme. Veri-

Table 2. Antiproliferative activity of pyrimidinylurea derivatives **1e-u**



1e-u

Compds	R^2	A375 (IC_{50}, μM)	Compds	R^2	A375 (IC_{50}, μM)
1e		5.0	**1n**		> 30
1f		6.4	**1o**[a]		1.4
1g		> 30	**1p**		12.4
1h		1.6	**1q**		> 30
1i		> 30	**1r**		> 30
1j		26.6	**1s**		> 30
1k		> 30	**1t**		> 30
1l		> 30	**1u**		> 30
1m		> 30	Sorafenib		5.5

[a]Enzymatic assay against V600E-b-Raf: IC_{50} = 61.9 μM.

fication of mode of action is under way.

In conclusion, a novel scaffold having pyridinylcarbonylpyrimidine group based on the structural features of Sorafenib was designed. In our series, pyrimidinylurea compounds **1e**, **1h**, and **1o** exhibited superior antiproliferative activity against A375 human melanoma cell line to Sorafenib. These results suggest that pyridinylcarbonylpyrimidine group has potentials as a scaffold for treatment of melanoma.

Experimental Section

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. All solvents and reagents were commercially available and used without further purification.

***N*-Methoxy-*N*-methylnicotinamide (3).** To a solution of nicotinic acid (**2**) (500 mg, 3.62 mmol), *N,O*-dimethylhydroxylamine hydrochloride (458 mg, 4.7 mmol), HOBt (364 mg, 4.7 mmol), and EDCI (1.04 g, 5.43 mmol) in DMF was added triethylamine (2.5 mL, 18.1 mmol). The mixture was stirred at 90 °C for 18 h. Upon completion, the reaction mixture was treated with 10% $NaHCO_3$. The resulting solution was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/*n*-hexane, 2/1, v/v) to give the compound **3** (528 mg, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (d, *J* = 1.64 Hz, 1H), 8.71 (dd, *J* = 1.65, 4.85 Hz, 1H), 8.03 (dd, *J* = 5.95, 7.90 Hz, 1H), 7.51 (m, 1H), 3.58 (s, 3H), 3.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.4, 151.5, 148.7, 135.9, 130.7, 123.4, 61.4, 33.2.

(2-Methylthiopyrimidin-4-yl)(pyridin-3-yl)methanone (6). To a solution of 2-iodo-4-methylthiopyrimidine (**5**) (1.82 g, 7.22 mmol) in dry toluene at 0 °C was added isopropyl magnesium chloride (2 M in THF) (4.15 mL, 9.03 mmol) dropwise. The mixture was stirred at the same temperature for 1 h, and then compound **3** was added and the resulting mixture was stirred at room temperature for 10 h. Upon completion, the reaction was quenched with NH_4Cl solution and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/*n*-hexane, 2/1, v/v) to give the compound **6** (552 mg, 38% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (t, *J* = 0.73 Hz, 1H), 8.83 (m, 2H), 8.45 (dt, *J* = 7.98, 1.83 Hz, 1H), 7.61 (d, *J* = 4.91 Hz, 1H), 7.47 (m, 1H), 2.57 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.0, 171.5, 159.7, 158.3, 154.9, 152.1, 134.7, 130.2, 125.4, 111.9, 13.8.

(2-Methylsulfonylpyrimidin-4-yl)(pyridin-3-yl)methanone (7). To a solution of compound **6** (400 mg, 1.72 mmol) in MeOH at 0 °C was added oxone (1.06 g, 1.72 mmol) and water. The mixture was stirred at room temperature for 5 h. Upon completion, the reaction mixture was extracted with chloroform. The organic layer was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure to give the compound **7** (209 mg, 46% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (d, *J* = 1.63 Hz, 1H), 9.28 (d, *J* = 4.98 Hz, 1H), 8.87 (dd, *J* = 1.65, 4.86 Hz, 1H), 8.55 (d, *J* = 8.08 Hz, 1H), 7.53 (m, 1H), 3.40 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.7, 168.4, 159.9, 159.7, 155.2, 151.8, 137.7, 130.6, 124.2, 122.1, 38.4.

***N*-(2,4-Dimethylphenyl)-*N*-(4-nicotinoylpyrimidin-2-yl)-formamide (10a).** To a stirred solution of sodium hydride (60% dispersion in mineral oil) (20 mg, 0.86 mmol) in dry THF was added *N*-(2,4-dimethylphenyl)formamide (**9a**) (101 mg, 0.68 mmol). The mixture was refluxed for 30 min, and then compound **7** was added and the resulting mixture

was stirred at the same temperature for 5 h. Upon completion, the reaction was quenched with water and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/*n*-hexane, 1/2, v/v) to give the compound **10a** (91 mg, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.02 (s, 1H), 9.19 (s, 1H), 8.88 (d, *J* = 4.86 Hz, 1H), 8.76 (d, *J* = 3.45 Hz, 1H), 8.26 (d, *J* = 7.92 Hz, 1H), 7.77 (d, *J* = 4.86 Hz, 1H), 7.23 (m, 2H), 7.05 (d, *J* = 7.71 Hz, 1H), 2.41 (s, 3H), 2.07 (s, 3H).

General Procedure for the Synthesis of (2-substituted aminopyrimidin-4-yl)(pyridin-3-yl)-methanones 1a-d. To a solution of the appropriate formamide compound **10** (0.03 mmol) in EtOH and THF (2:1 mixture solution) was added 3 M NaOH solution (0.04 mmol), and the reaction mixture was stirred at room temperature for 1 h. When the reaction was completed, the reaction mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure to give the title compound **1**.

(2-(2,4-Dimethylphenylamino)pyrimidin-4-yl)(pyridin-3-yl)methanone (1a). Yield 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (d, *J* =1.54 Hz, 1H), 8.82 (dd, *J* = 1.67, 4.84 Hz, 1H), 8.65 (d, *J* = 4.88 Hz, 1H), 8.44 (dt, *J* = 7.99, 1.98 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H) 7.41 (dd, *J* = 4.87, 7.93 Hz, 1H), 7.29 (d, *J* = 4.88 Hz, 1H), 7.01 (d, *J* = 8.18 Hz,1H), 2.31 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.2, 170.7, 160.4, 157.0, 152.8, 152.5, 140.4, 139.2, 137.5, 132.1, 130.0, 129.1, 127.5, 125.1, 115.3, 105.9, 20.6, 18.1.

(2-(4-Chloro-3-trifluoromethylphenylamino)pyrimidin-4-yl)(pyridin-3-yl)methanone (1b). Yield 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (d, *J* = 1.89 Hz, 1H), 8.85 (dd, *J* = 1.64, 4.82 Hz, 1H), 8.76 (d, *J* = 4.86 Hz, 1H), 8.38 (dt, *J* = 1.84, 7.94 Hz, 1H), 8.04 (d, *J* = 2.57 Hz, 1H), 7.66 (dd, *J* = 2.50, 8.66 Hz, 1H), 7.52 (s, 1H), 7.45 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.1, 170.0, 158.7, 155.3, 152.8, 152.7, 141.3, 137.4, 130.2, 129.7, 129.2, 127.4, 123.4, 122.3, 121.9, 112.5, 107.8.

(2-(4-Phenylaminophenylamino)pyrimidin-4-yl)(pyridin-3-yl)methanone (1c). Yield 96.1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (s. 1H), 9.07 (d, *J* = 12.21 Hz, 1H) , 9.03 (d, *J* = 4.74 Hz, 1H), 8.43 (m, 2H), 8.29 (t, *J* = 8.08 Hz, 1H), 7.82 (d, *J* = 4.88 Hz, 1H), 7.41 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.8, 171.0, 157.4, 154.1, 151.4, 150.3, 141.4, 136.9, 131.3, 130.7, 129.9, 129.1, 128.7, 124.4, 121.9, 120.9, 120.8, 119.9, 119.8, 118.4, 118.2, 106.4.

(2-(3,5-Bis(trifluoromethyl)benzylamino)pyrimidin-4-yl)(pyridin-3-yl)methanone (1d). Yield 28%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.30 (s, 1H), 8.78 (dd, *J* = 1.45, 4.81 Hz, 1H), 8.58 (d, *J* = 4.89 Hz, 1H), 8.26 (m, 1H), 7.76 (m, 3H), 7.35 (m, 1H), 7.24 (d, *J* = 4.91 Hz, 1H), 6.25 (s, 1H), 4.74 (d, *J* = 5.73 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.8, 164.1, 158.8, 155.0, 152.8, 152.7, 141.2, 137.4, 131.3, 131.1, 130.2, 126.9, 126.7, 124.5, 124.1, 124.0, 121.8, 104.9, 45.7.

2-Iodo-4-methylthiopyrimidine (5). 2-Chloro-4-methylthiopyrimidine (**4**) (1.0 g, 6.2 mmol) was added to hydriodic acid (47%) (10 mL) at 0 °C, and the mixture was stirred at room temperature for 18 h. Upon completion, the reaction mixture was neutralized with NaOH solution and extracted with methylene chloride. The organic layer was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure to give the compound **5** (1.2 g, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, *J* = 5.28 Hz, 1H), 7.40 (d, *J* = 5.28 Hz, 1H), 2.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.7 159.6 129.2 124.7 13.9.

***N*-(2,4-Dimethylphenyl)formamide (9a).** A mixture of 2,4-dimethylaniline (**8a**) (1.0 g, 8.25 mmol), zinc oxide (335 mg, 4.12 mmol), and formic acid (1 mL, 25 mmol) was stirred at 70 °C for 10 h. Upon completion, the reaction mixture was diluted with ethyl acetate and filtered to remove the zinc oxide. The filtrate was washed with 10% $NaHCO_3$. The resulting solution was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure to give the compound **9a** (870 mg, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.25 (s, 1H), 7.56 (d, *J* = 8.08 Hz, 1H), 7.01 (m, 2H), 2.24 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 142.7, 135.1, 131.6, 131.0, 125.4, 112.9, 21.0, 17.5.

(2-Aminopyrimidin-4-yl)(pyridin-3-yl)methanone (11). Compound **7** (196 mg, 0.75 mmol) was added to ammonia (2 M solution in isopropyl alcohol) (1.9 mL, 3.75 mmol), and the mixture was stirred at 70 °C for 10 h. After the reaction completed, the solvent was removed under reduced pressure. The reaction mixture was treated with water and methylene chloride. The organic layer was dried over anhydrous $MgSO_4$, and then evaporated under reduced pressure to give the compound **11** (1.2 g, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (dd, *J* = 0.78, 2.15 Hz, 1H), 8.82 (dd, *J* = 4.85, 1.74 Hz, 1H), 8.58 (d, *J* = 4.92 Hz, 1H), 8.40 (dt, *J* = 1.99, 7.96 Hz, 1H), 7.45 (m, 1H), 7.22 (d, *J* = 4.89, 1H), 5.21 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.1, 156.4, 155.2, 151.6, 147.1, 135.7, 129.3, 125.4, 106.7.

General Procedure for the Synthesis of 1-substituted 3-(4-nicotinoylpyrimidin-2-yl)ureas 1e-u. To a solution of compound **11** (0.175 mmol) in pyridine was added the appropriate isocyanate compound (1.75 mmol), and the mixture was refluxed for 10 h. Upon completion, the reaction mixture was treated with 10% $NaHCO_3$. After the reaction completed, pyridine was distilled off. The residue was purified by silica gel column chromatography (ethyl acetate/*n*-hexane, 2/1, v/v) to give the title compound **1**.

1-(2-Chlorophenyl)-3-(4-nicotinoylpyrimidin-2-yl)urea (1e). Yield 21%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1H), 10.51 (s, 1H), 9,10 (s, 1H), 9.02 (dd, *J* = 0.78, 4.95 Hz, 1H), 8.71 (d, *J* = 4.57 Hz, 1H), 8.32 (d, *J* = 7.82 Hz, 1H), 7,63 (s, 1H), 7.54 (dd, *J* = 0.92, 4.87 Hz, 1H), 7.49 (dd, *J* = 4.71, 7.75 Hz, 1H), 7.45 (t, *J* = 7.81 Hz, 1H), 7.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.3, 162.9, 157.6, 155.2, 151.8, 151.7, 148.6, 135.7, 134.7, 132.0, 130.1, 130.0, 129.2, 124.3, 124.4, 124.2, 106.8; MS *m/z* 354 $(M+H)^+$.

1-(3,4-Dichlorophenyl)-3-(4-nicotinoylpyrimidin-2-yl)-

urea (1f). Yield 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 10.69 (s, 1H), 9.22 (d, J = 2.01 Hz, 1H), 9.01 (d, J = 4.94 Hz, 1H), 8.85 (dd, J = 1.52, 4.85 Hz, 1H), 8.46 (dt, J = 1.72. 7.95 Hz, 1H), 7.61 (m, 2H), 7.50 (d, J = 8.82 Hz, 1H), 7.41 (d, J = 2.36 Hz, 1H), 7.18 (dd, J = 2.36, 8.78 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.8, 161.0, 158.7, 155.4, 151.5, 151.4, 148.1, 135.9, 135.6, 131.1, 130.7, 130.4, 129.1, 124.1, 124.0, 121.7, 105.8; MS *m/z* 389 $(M+H)^+$.

1-(2,4-Dichlorophenyl)-3-(4-nicotinoylpyrimidin-2-yl)-urea (1g). Yield 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.66 (s, 1H), 10.84 (s, 1H), 9.23 (s, 1H), 9.02 (dd, J = 1.06, 4.95 Hz, 1H), 8.85 (d, J = 4.76 Hz, 1H), 8.48 (d, J = 6.32 Hz, 1H), 8.33 (dd, J = 0.89, 8.95 Hz, 1H), 7.63 (m, 3H), 7.41 (t, J = 9.85 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.7, 160.4, 157.6, 155.3, 151.8, 151.5, 147.2, 135.6, 132.3, 130.9, 130.4, 129.6, 128.5, 127.8, 124.1, 121.8, 106.1; MS *m/z* 389 $(M+H)^+$.

1-(3,5-Dichlorophenyl)-3-(4-nicotinoylpyrimidin-2-yl)-urea (1h). Yield 35%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (s, 1H), 10.74 (s, 1H), 9.20 (d, J = 1.64 Hz, 1H), 9.00 (d, J = 4.94 Hz, 1H), 8.83 (dd, J = 1.51, 4.79 Hz, 1H), 8.44 (dt, J = 1.86, 7.99 Hz, 1H), 7.60 (m, 2H), 7.52 (d, J = 1.83 Hz, 1H), 7.20 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.8, 161.0, 158.3, 155.7, 152.1, 151.9, 148.2, 138.1, 136.5, 130.7, 129.3, 129.0, 125.2, 124.9, 120.4, 120.2, 106.6; MS *m/z* 389 $(M+H)^+$.

1-(3,5-Difluorophenyl)-3-(4-nicotinoylpyrimidin-2-yl)-urea (1i). Yield 22%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (s, 1H), 10.68 (s, 1H), 9.17 (d, J = 1.69 Hz, 1H), 8.99 (d, J = 4.89 Hz, 1H), 8.82 (dd, J = 1.53, 4.78 Hz, 1H), 8.42 (dt, J = 1.84, 7.98 Hz, 1H), 7.54 (m, 2H), 7.49 (d, J = 1.81 Hz, 1H), 7.20 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.2, 164.4, 160.9, 160.3, 158.6, 155, 151.9, 151.7, 148.6, 136.7, 130, 124.8, 124.4, 114.7, 111.3, 106.8, 104.9; MS *m/z* 356 $(M+H)^+$.

1-(4-Nicotinoylpyrimidin-2-yl)-3-(3-trifluoromethylphenyl)urea (1j). Yield 25%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 10.66 (s, 1H), 9,22 (s, 1H), 9.01 (dd, J = 0.86, 4.98 Hz, 1H), 8.81 (d, J = 4.67 Hz, 1H), 8.46 (d, J = 7.72 Hz, 1H), 7,72 (s, 1H), 7.61 (dd, J = 0.92, 4.92 Hz, 1H), 7.58 (dd, J = 4.69, 7.68 Hz, 1H), 7.45 (t, J = 7.92 Hz, 1H), 7.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.7, 161.1, 159.3, 155.7, 152.1, 151.9, 149.2, 136.5, 136.0, 130.8, 130.1, 129.4, 124.4, 123.9, 123.4, 123.1, 120.5, 105.9; MS *m/z* 388 $(M+H)^+$.

1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-nicotinoylpyrimidin-2-yl)urea (1k). Yield 27%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 1H), 10.74 (s, 1H), 9.27 (d, J = 2.35 Hz, 1H), 8.99 (d, J = 4.87 Hz, 1H), 8.75 (dd, J = 1.48, 4.91 Hz, H), 8.36 (dt, J = 1.83, 7.99 Hz, 1H), 8.02 (m, 1H), 7.78 (d, J = 4.88 Hz, 1H), 7.53 (m, 2H), 7.42 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.9, 159.5, 158.2, 155.7, 152.2, 151.8, 147.3, 135.9, 135.5, 131.4, 131.2, 130.1, 129.8, 129.7, 125.2, 125.1, 124.9, 117.6, 105.7; MS *m/z* 456 $(M+H)^+$.

1-(3,4-Dimethylphenyl)-3-(4-nicotinoylpyrimidin-2-yl)-urea (1l). Yield 21%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 10.45 (s, 1H), 9.20 (d, J = 1.81 Hz, 1H), 8.98 (d, J = 4.94 Hz, 1H), 8.84 (dd, J = 1.55, 4.83 Hz, 1H), 8.45 (dt, J = 1.86, 8.12 Hz, 1H), 7.59 (m, 2H), 6.99 (m, 2H), 6.62 (s, 1H), 2.12 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.1, 160.4, 158.6, 155.7, 151.8, 151.5, 147.2, 136.1, 135.7, 132.5, 132.2, 130.7, 128.8, 124.2, 121.8, 118.4, 107.3, 19.2, 19.0; MS *m/z* 348 $(M+H)^+$.

1-(2,4-Dimethylphenyl)-3-(4-nicotinoylpyrimidin-2-yl)-urea (1m). Yield 31%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 10.51 (s, 1H), 9.21 (d, J = 1.72 Hz, 1H), 8.97 (d, J = 4.99 Hz, 1H), 8.83 (dd, J = 1.57, 4.76 Hz, 1H), 8.48 (dt, J = 1.91, 8.12 Hz, 1H), 7.76 (d, J = 8.21 Hz, 1H), 7.56 (m 2H), 6.97 (d, J = 7.54 Hz, 2H), 2.23 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.7, 160.8, 158.4, 155.1, 151.9, 151.5, 148.2, 143.1 136.0, 134.7, 131.4, 131.6, 130.2, 126.1, 124.7, 114.3, 106.5, 21.9, 17.8; MS *m/z* 348 $(M+H)^+$.

1-(3,5-Dimethoxyphenyl)-3-(4-nicotinoylpyrimidin-2-yl)-urea (1n). Yield 27%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 10.81 (s, 1H), 9.18 (d, J = 1.61 Hz, 1H), 8.99 (d, J = 4.90 Hz, 1H), 8.79 (dd, J = 1.53, 4.74 Hz, 1H), 8.43 (dt, J = 1.83, 7.94 Hz, 1H), 7.58 (m, 2H), 7.54 (d, J = 1.82 Hz, 1H), 7.17 (m, 4H), 3.86 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.2, 160.9, 160.3, 160.3, 158.6, 155.7, 151.9, 151.7, 148.6, 137.9, 136.7, 130.0, 124.4, 106.8, 102.5, 102.5, 96, 55.0, 55.0; MS *m/z* 380 $(M+H)^+$.

1-(4-Chloro-3-trifluoromethylphenyl)-3-(4-nicotinoylpyrimidin-2-yl)urea (1o). Yield 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 10.74 (s, 1H), 9.23 (d, J = 1.63 Hz, 1H), 9.01 (d, J = 4.96 Hz, 1H), 8.81 (dd, J = 4.82, 1.6 Hz, 1H), 8.47 (dt, J = 1.84, 8.01 Hz, 1H), 7.80 (d, J = 2.41 Hz, 1H), 7.63 (d, J = 4.97 Hz, 1H), 7.59 (m, 2H), 7.45 (dd, J = 2.38, 8.78 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.0, 161.7, 159.2, 155.0, 152.3, 152.1, 147.6, 135.9, 135.1, 130.8, 129.7, 129.2, 129.1, 128.7, 123.4, 123.0, 117.6, 105.7; MS *m/z* 422 $(M+H)^+$.

1-(3-Fluoro-5-trifluoromethylphenyl)-3-(4-nicotinoylpyrimidin-2-yl)urea (1p). Yield 28%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.44 (s, 1H), 10.81 (s, 1H), 9.23 (d, J = 2.40 Hz, 1H), 9.03 (d, J = 4.96 Hz, 1H), 8.81 (dd, J = 1.59, 4.89 Hz, 1H), 8.47 (dt, J = 1.80, 8.05 Hz, 1H), 7.64 (d, J = 4.95 Hz, 1H), 7.59 (m, 1H), 7.42 (s, 1H), 7.34 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.2, 162.4, 160.8, 157.9, 155.2, 151.9, 151.7, 146.3, 137.6, 136.2, 132.9, 130.1, 124.5, 124.0, 121.3, 118.8, 107.6, 106.4; MS *m/z* 406 $(M+H)^+$.

1-(4-Nicotinoylpyrimidin-2-yl)-3-(3-phenoxyphenyl)urea (1q). Yield 29%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H), 10.53 (s, 1H), 9.20 (d, J = 2.15 Hz, 1H), 8.98 (d, J = 5.00 Hz, 1H), 8.78 (dd, J = 1.52 , 4.79 Hz, 1H), 8.45 (dt, J = 1.71, 7.96 Hz, 1H), 7.59 (d, J = 4.91 Hz, 1H), 7.56 (dd, J = 4.86, 7.97 Hz, 1H), 7.40 (m, 2H), 7.19 (m, 3H), 7.01 (m, 2H), 6.74 (dd, J = 1.15, 8.10 Hz, 1H), 6.63 (dd, J = 2.35, 8.14 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.3, 160.7, 158.2, 157.1, 155.9, 151.8, 151.6, 148.4, 136.1, 135.7, 130.2, 128.9, 128.7, 128.3, 124.1, 121.9, 118.1, 118.0, 114.4, 114.1, 107.0, 106.5; MS *m/z* 412 $(M+H)^+$.

1-(3-Benzoylphenyl)-3-(4-nicotinoylpyrimidin-2-yl)urea (1r). Yield 35%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H), 10.68 (s, 1H), 9.21 (d, J = 1.76 Hz, 1H), 9.01 (d, J =

4.96 Hz, 1H), 8.84 (dd, J = 1.56, 4.82 Hz, 1H), 8.46 (dt, J = 1.90, 8.01 Hz, 1H), 7.61 (m, 9H), 7.30 (d, J = 8.68 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.7, 189.7, 161.1, 157.6, 155.3, 151.4, 151.1, 148.2, 145.1, 142.5, 137.4, 135.1, 132.4, 132.3, 130.2, 130.1, 129.8, 128.9, 128.7, 124.7, 124.6, 122.1, 117.9, 105.8; MS *m/z* 424 $(M+H)^+$.

1-(Benzo[*d*][1,3]dioxol-5-yl)-3-(4-nicotinoylpyrimidin-2-yl)urea (1s). Yield 32%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 10.47 (s, 1H), 9.20 (d, J = 1.91 Hz, 1H), 8.98 (d, J = 4.92 Hz, 1H), 8.84 (dd, J = 0.76, 4.86 Hz, 1H), 8.44 (dt, J = 1.86, 8.15 Hz, 1H), 7.58 (d, J = 4.96 Hz, 1H), 6.90 (d, J = 2.05 Hz, 1H), 6.76 (d, J = 8.35 Hz, 1H), 6.45 (dd, J = 2.09, 8.31 Hz, 1H), 5.97 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.5, 160.9, 158.2, 155.3, 151.9, 151.8, 147.6, 147.2, 144.4, 137.8, 130.6, 129.2, 124.1, 115.9, 112.0, 106.9, 105.3, 101.7; MS *m/z* 364 $(M+H)^+$.

1-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-3-(4-nicotinoylpyrimidin-2-yl)urea (1t). Yield 27%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 10.52 (s, 1H), 9.18 (d, J = 1.85, 1H), 8.92 (d, J = 4.83 Hz, 1H), 8.75 (dd, J = 0.81, 4.91 Hz, 1H), 8.50 (dt, J = 1.79, 8.21 Hz, 1H), 7.54 (d, J = 4.87 Hz, 1H), 6.97 (d, J = 8.27 Hz, 1H), 6.81 (d, J = 2.01, 8.29 Hz, 1H), 4.20 (dd, J = 3.72, 11.90 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.4, 160.7, 157.6, 155.2, 151.8, 151.5, 148.2, 143.1, 135.7, 130.9, 128.2, 123.4, 114.8, 110.9, 106.2, 105.3, 64.3, 64.2; MS *m/z* 378 $(M+H)^+$.

1-(6-Chloropyridin-3-yl)-3-(4-nicotinoylpyrimidin-2-yl)-urea (1u). Yield 25%; ^{1}H NMR (100 MHz, DMSO-d_6) δ 11.27 (s, 1H), 10.68 (s, 1H), 9.24 (d, J = 1.60 Hz, 1H), 9.01 (d, J = 4.71 Hz, 1H), 8.79 (dd, J = 4.78, 1.61 Hz, 1H), 8.35 (dt, J = 1.79, 7.97 Hz, 1H), 7.75 (d, J = 2.38 Hz, 1H), 7.67 (d, J = 4.94 Hz, 1H), 7.46 (m, 2H), 7.31 (dd, J = 2.17, 8.69 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.7, 161.4, 160.5, 157.6, 155.2, 151.1, 151.0, 147.6, 140.1, 136.3, 136.1, 130.1, 125.0, 124.1, 123.4, 104.8; MS *m/z* 355 $(M+H)^+$.

A375P Cell Culture and Anti-proliferative Activity of Tested Compound on A375P. 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 ºC. 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 ºC for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment of various concentration (3-fold 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.

Acknowledgments. This research was supported by a grant (K00060-282) from the Fundamental R&D Program for Core Technology of Materials funded by the Ministry of Knowledge Economy, Republic of Korea.

References

1. Gruijl, F. R. *Pigment Cell Res.* **2003**, *16*, 591.
2. 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.
3. Carlson, J. A.; Ross, J. S.; Slominski, A.; Linette, G.; Mysliborski, J.; Hill, J.; Mihm, M., Jr. *J. Am. Acad. Dermatol.* **2005**, *52*, 743.
4. Buzaid, A. C.; Anderson, C. M. *Curr. Oncol. Rep*. **2000**, *2*, 322.
5. Middleton, M. R.; Grob, J. J.; Aaronson, N.; Fierlbeck, G.; Tilgen, W.; Seiter, S.; Gore, M.; Aamdal, S.; Cebon, J.; Coates, A.; Dreno, B.; Henz, M.; Schadendorf, D.; Kapp, A.; Weiss, J.; Fraass, U.; Statkevich, P.; Muller, M.; Thatcher, N. *J. Clin. Oncol*. **2000**, *18*, 158.
6. Bedikian, A. Y.; Millward, M.; Pehamberger, H.; Conry, R.; Gore, M.; Trefzer, U.; Pavlick, A. C.; DeConti, R.; Hersh, E. M.; Hersey, P.; Kirkwood, J. M.; Haluska, F. G. *J. Clin. Oncol*. **2006**, *24*, 4738.
7. Serrone, L.; Zeuli, M.; Sega, F. M.; Cognetti, F. *J. Exp. Clin. Cancer Res*. **2000**, *19*, 21.
8. Fletcher, W. S.; Daniels, D. S.; Sondak, V. K.; Dana, B.; Townsend, R.; Hynes, H. E.; Hutchins, L. F.; Pancoast, J. R. *Am. J. Clin. Oncol*. **1993**, *16*, 359.
9. Anderson, C. M.; Buzaid, A. C.; Legha, S. S. *Oncology* (Williston Park) **1995**, *9*, 1149.
10. Grossman, D.; Altieri, D. C. *Cancer Metastasis Rev.* **2001**, *20*, 3.
11. Hayes, M. T.; Bartley, J.; Parsons, P. G.; Eaglesham, G. K.; Prakash, A. S. *Biochemistry* **1997**, *36*, 10646.
12. Franciosi, V.; Cocconi, G.; Michiara, M.; Di Costanzo, F.; Fosser, V.; Tonato, M.; Carlini, P.; Boni, C.; Di Sarra, S. *Cancer* **1999**, *85*, 1599.
13. Middleton, M. R.; Lorigan, P.; Owen, J.; Ashcroft, L.; Lee, S. M.; Harper, P.; Thatcher, N. *Br. J. Cancer* **2000**, *82*, 1158.
14. Gray-Schopfer, V.; Wellbrock, C.; Marais, R. *Nature* **2007**, *445*, 851.
15. Eton, O.; Legha, S. S.; Bedikian, A. Y.; Lee, J. J.; Buzaid, A. C.; Hodges, C.; Ring, S. E.; Papadopoulos, N. E.; Plager, C.; East, M. J.; Zhan, F.; Benjamin, R. S. *J. Clin. Oncol*. **2002**, *20*, 2045.
16. 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.
17. 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.
18. 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.
19. Aquila, B.; Cook, D. J.; Johnstone, C.; Lee, S.; Lyne, P.; Rudge, D. A.; Vasbinder, M.; Wang, H. WO 2008/020203; WO 2008/068507.
20. Wilhelm, S. M.; Adnane, L.; Newell, P.; Villanueva, A.; Llovet, J. M.; Lynch, M. *Mol. Cancer Ther.* **2008**, *7*, 3129.
21. Remuinan, M.; Gonzalez, J. J.; Del Pozo, C.; Francesch, A.; Cuevas, C.; Munt, S.; Manzanares, I. WO 2003/006457.
22. Johns, B. A.; Gudmundsson, K. S.; Turner, E. M.; Allen, S. H.; Samano, V. A.; Ray, J. A.; Freeman, G. A.; Boyd, F. L.; Sexton, C. J.; Selleseth, D. W.; Creech, K. L.; Moniri, K. R. *Bioorg. Med. Chem.* **2005**, *13*, 2397.
23. Hosseini-Sarvari, M.; Sharghi, H. *J. Org. Chem*. **2006**, *71*, 6652.
24. Nagata, T.; Masuda, K.; Maeno, S.; Miura, I. *Pest Manag. Sci.* **2004**, *60*, 399.
25. Van Rossom, W.; Maes, W.; Kishore, L.; Ovaere, M.; Van Meervelt, L.; Dehaen, W. *Org. Lett.* **2008**, *10*, 585.