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Synthesis of New Pyrimidinylaminobenzene Derivatives and Their Antiproliferative Activities against Melanoma Cell Line

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A series of new diarylamide and diarylurea derivatives possessing pyrimidinylaminobenzene scaffold was synthesized. Their *in vitro* antiproliferative activities were tested against A375P human melanoma cell line. Among them, compounds **1a-c**, **k** and **2b-d**, **f**, **g**, **j**, **l** showed superior potencies against A375P human melanoma cell line to Sorafenib. In particular, compound **2f** possessing 3-fluoro-5-trifluoromethyl moiety exhibited the highest potency with IC_{50} value in nanomolar scale.

Key Words : Antiproliferative activity, Pyrimidinylaminobenzene, Diarylamide, Diarylurea, Melanoma

Introduction

Melanoma is a malignant tumor that arises from melanocytic cells and primarily involves the skin. Exposure to solar ultraviolet irradiation, fair skin, dysplastic nevi syndrome, and a family history of melanoma are major risk factors for melanoma development. 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] With the rapid incidence of melanoma in the United States and other developed countries, there is an urgent need to develop more effective drugs.[4-6]

Sorafenib (Nexavar®) is a diarylurea derivative that has been extensively used in clinical trials.[7] A number of reports have recently highlighted diarylureas as potential antiproliferative agents against melanoma cell lines.[8-16] Encouraged by the interesting antiproliferative activity of diarylurea and diarylamide derivatives, we synthesized a series of new diarylureas and diarylamides containing pyrimidinylaminobenzene scaffold (Figure 1). Their *in vitro* antiproliferative activities against A375P human melanoma cell line are reported.

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.

2-Amino-6-(2-methyl-5-nitrophenylamino)pyrimidin-4(3*H*)-one (4). 6-Amino-4-chloropyrimidin-2(1*H*)-one (**3**, 200 mg, 0.84 mmol) and 2-methyl-5-nitroaniline (627 mg, 2.52 mmol) were heated at 170 °C for 3 h. The mixture was then cooled to rt and diethyl ether was added. The mixture was sonicated for 5 min. The suspension was filtered, and the filter cake was dissolved in MeOH and purified by column chromatography (silica gel, dichloromethane/methanol 9:1 v/v) to afford **4** (128 mg, 60%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.29 (s, 1H), 8.16 (d, J = 2.4 Hz, 1H), 7.85 (d, J = 2.4 Hz, 1H), 7.46 (d, J = 8.6 Hz, 1H), 6.35 (s, 2H), 4.71 (s, 1H), 2.30 (s, 3H).

2-Amino-6-(5-amino-2-methylphenylamino)pyrimidin-4(3*H*)-one (5). A mixture of compound **4** (522 mg, 2.0 mmol) and 5% Pd/C in MeOH was stirred in hydrogen atmosphere at room temperature for 2 h. Upon completion,

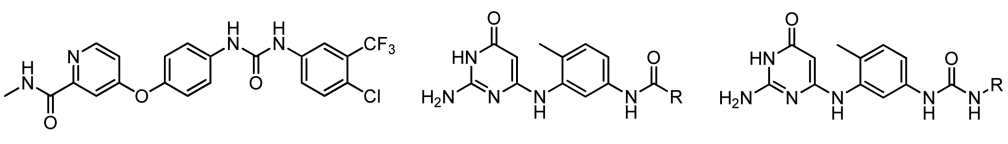


Figure 1. Structures of Sorafenib and target compounds.

the reaction mixture was filtered through celite. The filtrate was evaporated under reduced pressure, and the resulting residue was filtered to afford **5** (310 mg, 67%) as a brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 7.68 (s, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.40 (s, 1H), 6.30 (d, J = 10.6 Hz, 1H), 6.23 (s, 2H), 4.86 (s, 2H), 4.32 (s, 1H), 1.97 (s, 3H).

General Procedure for the Synthesis of Pyrimidinyl-aminobenzeneamides 1a-l. A mixture of compound **5** (0.13 mmol), the appropriate acid (0.17 mmol), HOBt (0.17 mmol), and EDCI (0.19 mmol) in dry DMF was cooled to 0 °C under nitrogen atmosphere. To the reaction mixture, triethylamine (0.16 mmol) was added at 0 °C. The mixture was then stirred at 90 °C for 12 h. The reaction mixture was cooled and then partitioned between water and ethyl acetate and the organic layer was separated. The aqueous layer was then extracted with ethyl acetate and the combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography to afford the corresponding compounds **1a-l**.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-4-chloro-3-(trifluoromethyl)benzamide (1a).** Pale yellow solid; yield 37%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.47 (s, 1H), 10.40 (s, 1H), 9.60 (s, 1H), 8.38 (s, 1H), 8.25 (d, J = 8.2 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 9.4 Hz, 2H), 7.16 (d, J = 8.2 Hz, 1H), 6.20 (s, 2H), 4.50 (s, 1H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 164.7, 160.2, 153.1, 143.7, 133.8, 133.6, 132.6, 132.2, 129.2, 129.0, 125.2, 124.8, 118.7, 110.9, 109.7, 82.3, 18.9; MS *m/z* 438 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-4-nitro-3-(trifluoromethyl)benzamide (1b).** Brown solid; yield 53%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25(s, 1H), 9.65 (s, 1H), 8.40 (s, 1H), 8.30 (d, J = 6.9 Hz, 1H), 8.11 (d, J = 5.6 Hz, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.49 (d, J = 3.9 Hz, 2H), 4.49 (s, 1H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 164.7, 161.8, 154.3, 148.8, 143.5, 134.7, 131.7, 129.8, 124.7, 124.6, 124.3, 123.5, 119.2, 113.5, 106.8, 82.3, 18.7; MS *m/z* 449 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3,5-bis(trifluoromethyl)benzamide (1c).** Brown solid; yield 55%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 10.24 (s, 1H), 9.26 (s, 1H), 8.26 (d, J = 7.3 Hz, 1H), 8.14 (d, J = 9.4 Hz, 2H), 7.88 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.72 (s, 1H), 4.49 (s, 1H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 164.7, 161.2, 154.3, 144.3, 134.8, 133.8, 131.4, 130.3, 127.1, 124.6, 124.1, 107.9, 82.1, 19.8; MS *m/z* 472 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-2,3-dihydrobenzo[1,4]dioxine-6-carboxamide (1d).** Brown solid; yield 45%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 10.11 (s, 1H), 9.50 (s, 1H), 7.91 (d, J = 7.3 Hz, 1H), 7.61 (s, 1H), 7.49 (d, J = 2.5 Hz, 2H), 7.23-7.20 (m, 1H), 6.99 (d, J = 2.3 Hz, 1H), 4.50 (s, 1H), 4.34-4.26 (m, 4H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.9, 164.7, 159.8, 155.1, 150.1, 146.8, 142.5, 132.7, 131.7, 126.4, 124.7, 119.7, 115.1, 112.3, 111.1, 108.7, 81.0, 64.2, 18.9; MS *m/z* 394 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-4-oxo-4*H*-chromene-2-carboxamide (1e).** Pale yellow solid; yield 40%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 10.69 (s, 1H), 9.95 (s, 1H), 8.05 (t, J = 7.8 Hz, 1H), 7.92 (t, J = 9.4 Hz, 1H), 7.90 (d, J = 7.1 Hz, 1H), 7.72 (s, 1H), 7.58-7.53 (m, 2H), 7.23 (d, J = 8.3 Hz, 1H), 6.95 (s, 2H), 6.27 (s, 2H), 4.48 (s, 1H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 165.9, 163.8, 161.2, 157.2, 153.3, 144.7, 135.3, 134.3, 131.2, 124.6, 123.9, 123.5, 118.8, 117.7, 111.0, 107.9, 81.3, 19.6; MS *m/z* 404 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-2,5-dimethylfuran-3-carboxamide (1f).** Brown solid; yield 47%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1H), 9.59 (s, 1H), 9.12 (s, 1H), 7.88 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.23 (d, J = 7.8 Hz, 1H), 6.13 (s, 1H), 2.17 (s, 3H), 2.15 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3, 164.7, 161.3, 158.0, 154.3, 152.6, 143.1, 132.7, 130.3, 117.6, 111.5, 107.8, 104.7, 81.2, 19.5, 13.7, 10.9; MS *m/z* 354 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-5-bromothiophene-2-carboxamide (1g).** Pale yellow solid; yield 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.18 (s, 1H), 9.75 (s, 1H), 8.75 (s, 1H), 8.34 (d, J = 3.7 Hz, 1H), 8.01 (d, J = 4.2 Hz, 1H), 7.88 (d, J = 8.6 Hz, 1H), 7.79 (s, 1H), 7.72 (d, J = 8.2 Hz, 1H), 4.51 (s, 1H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 161.8, 160.3, 153.4, 144.5, 140.1, 138.9, 133.6, 132.2, 130.3, 124.6, 122.0, 107.7, 81.5, 19.4; MS *m/z* 421 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-5-methylisoxazole-3-carboxamide (1h).** Brown solid; yield 41%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 9.36 (s, 1H), 7.84 (s, 1H), 7.46 (d, J = 7.4 Hz, 1H), 7.15 (d, J = 7.2 Hz, 1H), 6.63 (s, 1H), 4.50 (s, 1H), 2.35 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 166.4, 162.6, 160.2, 152.3, 150.0, 143.5, 133.8, 130.5, 123.7, 112.7, 107.8, 100.5, 81.3, 19.8, 12.4; MS *m/z* 341 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)thiazole-4-carboxamide (1i).** Brown solid; yield 41%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 9.84 (s, 1H), 9.21 (s, 1H), 9.14 (s, 1H), 8.20 (s, 1H), 7.86 (d, J = 10.1 Hz, 1H), 7.41 (s, 1H), 7.11 (d, J = 2.3 Hz, 1H), 4.48 (s, 1H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 162.6, 160.8, 154.2, 152.5, 146.8, 143.0, 133.8, 129.9, 124.7, 119.0, 112.3, 81.5, 18.9; MS *m/z* 343 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)benzo[*b*]thiophene-2-carboxamide (1j).** Brown solid; yield 54%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H), 9.43 (s, 1H), 9.03 (s, 1H), 8.94 (s, 1H), 8.79 (d, J = 7.3 Hz, 2H), 8.49 (d, J = 3.8 Hz, 1H), 8.32 (s, 1H), 7.75 (d, J = 2.3 Hz, 1H), 7.59 (s, 1H), 7.23 (d, J = 8.6 Hz, 1H), 4.51 (s, 1H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 161.8, 160.3, 153.7, 150.5, 146.3, 143.5, 141.7, 134.7, 131.2, 124.6, 124.4, 124.3, 123.2, 122.8,

120.5, 107.7, 81.5, 19.5; MS *m/z* 392 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-chloroisonicotinamide (1k).** Brown solid; yield 56%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.89 (s, 1H), 9.26 (s, 1H), 9.15 (d, *J* = 4.2 Hz, 1H), 8.15 (s, 1H), 7.69 (d, *J* = 2.3 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 4.48 (s, 1H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.7, 164.7, 160.4, 152.2, 148.6, 148.3, 143.1, 133.6, 131.0, 129.8, 124.6, 119.7, 111.2, 107.9, 82.3, 18.9; MS *m/z* 371 $[M + H]^+$.

***N*-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)pyridazine-4-carboxamide (1l).** Brown solid; yield 39%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.24 (s, 1H), 10.25 (s, 1H), 9.82 (s, 1H), 9.49 (d, *J* = 3.5 Hz, 1H), 8.12 (s, 1H), 6.90-6.86 (m, 2H), 6.79 (s, 1H), 4.48 (s, 1H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 164.7, 161.8, 154.8, 151.9, 150.9, 144.6, 133.4, 132.5, 130.1, 125.0, 124.8, 112.3, 108.7, 81.2, 18.8; MS *m/z* 338 $[M + H]^+$.

General Procedure for the Synthesis of Pyrimidinylaminobenzeneureas 2a-l. To a solution of compound **5** (0.11 mmol) in anhydrous THF, the appropriate isocyanate (0.12 mmol) was added. The mixture was stirred at room temperature for 4 h. After evaporation of the organic solvent, the residue was purified by column chromatography to afford the corresponding compounds **2a-l**.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(3,4-dimethylphenyl)urea (2a). Brown solid; yield 42%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.62 (s, 1H), 8.48 (s, 1H), 8.03 (s, 1H), 7.32 (d, *J* = 2.1 Hz, 1H), 7.21 (d, *J* = 1.9 Hz, 1H), 7.17-7.12 (m, 2H), 7.08 (d, *J* = 8.3 Hz, 1H), 6.99 (d, *J* = 8.2 Hz, 1H), 6.23 (s, 2H), 4.39 (s, 1H), 2.16 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 160.2, 153.2, 151.9, 144.9, 137.0, 133.8, 132.7, 132.4, 131.3, 129.1, 125.6, 121.2, 118.5, 112.3, 106.9, 81.2, 21.8, 19.6; MS *m/z* 379 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(2-chlorophenyl)urea (2b). Pale brown solid; yield 48%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.43 (s, 1H), 8.27 (s, 1H), 8.17 (d, *J* = 11.0 Hz, 1H), 7.98 (d, *J* = 9.1 Hz, 2H), 7.61-7.57 (m, 2H), 7.45 (d, *J* = 1.2 Hz, 1H), 7.04-7.01 (m, 1H), 4.50 (s, 1H), 2.13 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 161.2, 153.8, 151.9, 144.7, 134.8, 133.6, 130.5, 129.9, 129.0, 127.1, 125.8, 124.6, 123.0, 111.5, 107.9, 81.7, 19.0; MS *m/z* 385 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(3,4-dichlorophenyl)urea (2c). Brown solid; yield 53%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 8.99 (s, 1H), 8.80 (s, 1H), 7.93 (s, 1H), 7.85 (d, *J* = 4.5 Hz, 1H), 7.49 (d, *J* = 8.8 Hz, 1H), 7.34-7.30 (m, 2H), 7.16 (d, *J* = 1.9 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 6.22 (s, 2H), 4.40 (s, 1H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.4, 159.2, 153.2, 151.0, 142.1, 135.3, 133.5, 132.9, 130.4, 130.0, 128.9, 125.4, 123.4, 121.1, 110.5, 106.9, 82.1, 19.8; MS *m/z* 420 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(3,5-dichlorophenyl)urea (2d). Pale brown solid; yield 46%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.07 (s, 1H), 8.89 (s, 1H), 7.96 (s, 1H), 7.51 (d, *J* = 1.8 Hz, 2H), 7.33 (d, *J* = 2.0 Hz, 1H), 7.17 (d, *J* = 2.0 Hz, 1H), 7.14-7.09 (m, 2H), 6.22 (s, 2H), 4.40 (s, 1H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 159.2, 152.3, 151.2, 144.5, 138.6, 135.9, 132.7, 129.8, 126.0, 123.3, 120.1, 112.7, 106.8, 82.0, 18.8; MS *m/z* 420 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(4-chloro-3-(trifluoromethyl)phenyl)-urea (2e). Brown solid; yield 71%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.55 (s, 1H), 9.21 (s, 1H), 8.08 (d, *J* = 2.0 Hz, 1H), 7.93 (s, 1H), 7.60 (d, *J* = 2.1 Hz, 1H), 7.34 (d, *J* = 1.8 Hz, 1H), 7.18 (d, *J* = 1.9 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 6.22 (s, 2H), 4.54 (s 1H), 2.10 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 160.2, 153.2, 151.9, 143.3, 134.1, 133.7, 129.3, 129.0, 126.3, 126.0, 124.8, 119.3, 117.7, 112.3, 108.9, 81.3, 19.5; MS *m/z* 453 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(3-fluoro-5-(trifluoromethyl)phenyl)-urea (2f). Brown solid; yield 53%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.21 (s, 1H), 8.94 (s, 1H), 7.99 (s, 1H), 7.98 (s, 1H), 7.71 (s, 1H), 7.24-7.18 (m, 3H), 4.50 (s, 1H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 163.4, 160.1, 152.3, 151.8, 144.3, 137.7, 133.4, 132.8, 130.2, 124.6, 124.2, 113.4, 113.0, 111.2, 107.9, 107.7, 81.2, 19.6; MS *m/z* 437 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(3,5-bis(trifluoromethyl)phenyl)urea (2g). Pale brown solid; yield 46%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.56 (s, 1H), 9.07 (s, 1H), 8.21 (s, 2H), 7.94 (s, 1H), 7.61 (s, 1H), 7.36 (d, *J* = 1.6 Hz, 1H), 7.21 (d, *J* = 1.7 Hz, 1H), 7.10 (d, *J* = 13.2 Hz, 1H), 6.23 (s, 2H), 4.54 (s, 1H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 160.2, 153.3, 151.9, 143.4, 136.4, 133.6, 131.5, 130.0, 124.5, 120.7, 118.7, 111.5, 107.9, 81.2, 19.6; MS *m/z* 487 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(benzo[1,3]dioxol-5-yl)urea (2h). Brown solid; yield 53%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.62 (s, 1H), 8.52 (s, 1H), 7.95 (d, *J* = 7.0 Hz, 2H), 7.54-7.48 (m, 3H), 6.39-6.36 (m, 1H), 4.52 (s, 1H), 3.61-3.56 (m, 2H), 2.15 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.7, 160.8, 154.2, 152.9, 149.0, 144.4, 142.3, 132.7, 129.9, 129.1, 124.6, 115.2, 114.9, 110.9, 107.7, 105.7, 101.2, 82.6, 19.5; MS *m/z* 395 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-urea (2i). Brown solid; yield 52%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 9.33 (s, 1H), 7.81 (s, 1H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.49 (d, *J* = 2.1 Hz, 1H0, 7.47 (d, *J* = 2.1 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 4.49 (s, 1H), 4.33-4.25 (m, 4H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 160.2, 153.3, 151.8, 146.7, 143.4, 142.3, 132.6, 130.0, 128.1, 124.7, 115.1, 113.7, 111.4, 106.9, 105.4, 81.2, 64.0, 19.7; MS *m/z* 409 $[M + H]^+$.

1-(3-Acetylphenyl)-3-(3-(2-amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)urea (2j). Brown solid;

yield 45%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.53 (s, 1H), 9.13 (s, 1H), 8.12 (s, 2H), 7.83 (d, J = 2.3 Hz, 1H), 7.60 (s, 1H), 7.47 (d, J = 1.6 Hz, 1H), 7.31 (d, J = 1.9 Hz, 1H), 7.13 (d, J = 9.8 Hz, 1H), 6.20 (s, 2H), 4.50 (s, 1H), 2.30 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.5, 160.3, 153.4, 152.0, 143.6, 138.6, 133.5, 132.5, 130.0, 124.7, 124.5, 113.6, 113.2, 111.3, 107.8, 107.6, 19.5; MS m/z 393 $[M + H]^+$.

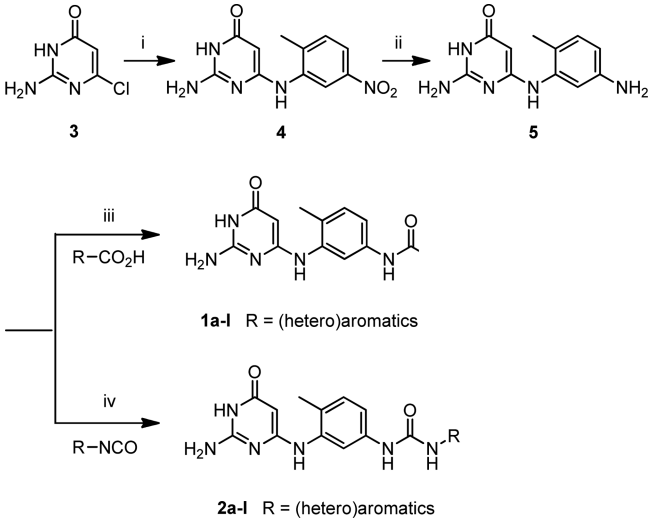
1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(3-phenoxyphenyl)urea (2k). Pale brown solid; yield 40%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 8.78 (s, 1H), 8.76 (s, 1H), 7.97 (d, J = 9.3 Hz, 2H), 7.62-7.57 (m, 2H), 7.51 (t, J = 2.6 Hz, 2H), 7.39 (t, J = 10.5 Hz, 2H), 7.20 (d, J = 2.8 Hz, 1H), 7.02 (d, J = 11.0 Hz, 2H), 6.60 (d, J = 2.7 Hz, 1H), 4.49 (s, 1H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 160.1, 157.2, 157.0, 153.2, 150.9, 143.7, 135.5, 133.9, 129.9, 128.7, 128.5, 124.6, 121.9, 117.5, 114.7, 113.1, 111.4, 107.9, 82.0, 19.3; MS m/z 443 $[M + H]^+$.

1-(3-(2-Amino-6-oxo-1,6-dihydropyrimidin-4-ylamino)-4-methylphenyl)-3-(6-chloropyridin-3-yl)urea (2l). Pale brown solid; yield 54%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.48 (s, 1H), 8.47 (s, 1H), 8.47 (d, J = 3.6 Hz, 1H), 8.45 (d, J = 3.5 Hz, 1H), 8.01-7.96 (m, 2H), 7.56 (s, 1H), 7.44-7.40 (m, 2H), 7.19 (d, J = 2.6 Hz, 1H), 7.08 (d, J = 10.9 Hz, 1H), 4.51 (s, 1H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.4, 162.3, 160.1, 152.3, 151.5, 142.5, 137.3, 136.8, 132.6, 131.1, 125.2, 124.6, 123.8, 112.5, 108.5, 82.0, 18.9; MS m/z 385 $[M + H]^+$.

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.

Results and Discussion

Chemistry. The target compounds **1a-1** and **2a-l** were synthesized according to the sequence of reactions illustrated in Scheme 1. Treatment of 6-amino-4-chloropyrimidin-2(1*H*)-one (**3**) with 2-methyl-5-nitroaniline led to nucleophilic displacement of chloro group by aromatic amine group to give the aminated compound **4**.[17] Reduction of nitro group of **4** using palladium over carbon in hydrogen atmosphere produced the key intermediate **5**. Synthesis of pyrimidinylamonobenzeneamides **1a-l** was carried out by condensation of amino group of **5** with the appropriate carboxylic acid derivatives in the presence of HOBt, EDCI, and triethylamine. Reaction of **5** with the appropriate aryl isocyanate derivatives afforded the corresponding pyrimidinylaminobenzeneureas **2a-l**.



Scheme 1. Reagents and reaction conditions: (i) 2-methyl-5-nitroaniline, 170 °C, 3 h; (ii) 5% Pd/C, H_2, MeOH, rt, 2 h; (iii) HOBt, EDCI, Et_3N, DMF, 90 °C, 12 h; (iv) THF, rt, 4 h.

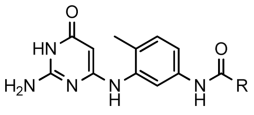
Biological Evaluation.

Antiproliferative Activity Against A375P Human Melanoma Cell Line: The antiproliferative activity of the newly synthesized compounds against A375P human melanoma cell line was tested. The ability of pyrimidinylaminobenzeneamides **1a-l** and pyrimidinylaminobenzeneureas **2a-l** to inhibit the growth of A375P cell line is summarized in Tables 1 and 2. Sorafenib was selected as a reference standard because it has been extensively used in clinical trials for treatment of melanoma.[4,18]

In general, urea compounds **2a-l** demonstrated higher potencies than compounds **1a-l** with amide linker. This may be attributed to that the longer spacer, urea moiety, may geometrically permit appropriate fitting of the molecule at the receptor site. Or the terminal NH group of the urea moiety may form additional hydrogen bond(s) at the receptor site. Any or both of these effects would enable optimal drug-receptor interaction, and hence higher antiproliferative activity. Compounds bearing terminal bicyclic or heteroaromatic moiety in comparison with terminal aromatic compounds did not show the meaningful activity, except compound **2l**. Compounds **1a-c**, **k** and **2b-d**, **f**, **g**, **j**, **l** exhibited superior activities against A375P to Sorafenib.

In Table 2, compounds **2d**, **f**, **g** with 3,5-disubstituted

Table 1. Antiproliferative activity of pyrimidinylaminobenzeneamides **1a-l** against A375P human melanoma cell line



1a-l

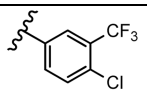
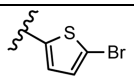
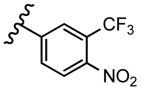
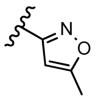
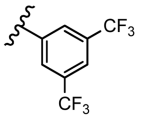
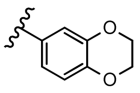
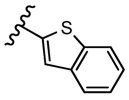
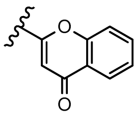
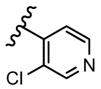
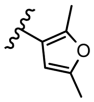
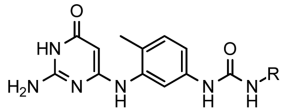
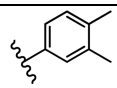
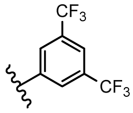
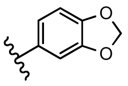
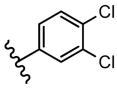
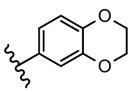
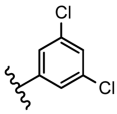
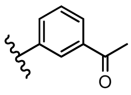
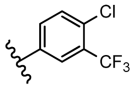
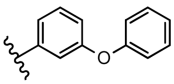
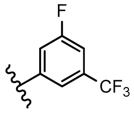
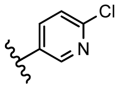
Compd. No.	R	IC_{50} (μM)	Compd. No.	R	IC_{50} (μM)
1a		2.5	**1g**		> 20
1b		1.8	**1h**		> 20
1c		2.0	**1i**		> 20
1d		> 20	**1j**		> 20
1e		> 20	**1k**		1.3
1f		> 20	**1l**		> 20
Sorafenib		3.5			

Table 2. Antiproliferative activity of pyrimidinylaminobenzeneureas **2a-l** against A375P human melanoma cell line



2a-l

Compd. No.	R	IC_{50} (μM)	Compd. No.	R	IC_{50} (μM)
2a		> 20	**2g**		1.2
2b		1.3	**2h**		> 20
2c		1.3	**2i**		> 20
2d		1.0	**2j**		1.2
2e		13.0	**2k**		> 20
2f		0.19	**2l**		1.5
Sorafenib		3.5			

phenyl ring were more potent than compounds **2c**, **e** with 3,4-disubstituted phenyl ring. This can be attributed to the influence of the substituents orientation at the receptor site on the activity.

Among all of these derivatives, compound **2f** possessing 3-fluoro-5-trifluoromethyl moiety exhibited the most potent antiproliferative activity against A375P human melanoma cell line with IC_{50} value in nanomolar scale.

Conclusion

As a continuation of our ongoing anticancer development program, a series of new diarylamide and diarylurea derivatives possessing pyrimidinylaminobenzene scaffold was synthesized based on our previous literature studies. Compounds **1a-c**, **k** and **2b-d**, **f**, **g**, **j**, **l** demonstrated higher potencies against A375P human melanoma cell line than that of Sorafenib. Among all of these derivatives, compound **2f** possessing 3-fluoro-5-trifluoromethyl moiety exhibited the highest antiproliferative activity with IC_{50} value in nanomolar scale. It can be considered as a promising lead for further development of potent antiproliferative agents for melanoma.

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