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RESEARCH ARTICLE

Synthesis of quinolinylaminopyrimidines and quinazolinylmethylaminopyrimidines with antiproliferative activity against melanoma cell line

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Abstract

Synthesis of a new series of quinolinylaminopyrimidines **1a**–**k** and quinazolinylmethylaminopyrimidines **2a**–**i** containing aminoquinoline and aminoquinazoline as hinge regions is described. Their *in vitro* antiproliferative activities against A375P human melanoma cell line were tested. Among them, compounds **1h** and **1k** exhibited the highest antiproliferative activities against A375P cell line with IC_{50} values in sub-micromolar scale. Compounds **1i, 2b** and **2g** showed similar potency against A375P to Sorafenib as a reference compound. The representative compound **1h** showed high, dose-dependent inhibition of MEK and ERK kinases.

Keywords

Antiproliferative, MEK/ERK kinase inhibition, melanoma cell line, quinazoline, quinoline

History

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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) can be cured surgically with more than 95% success rate. But melanoma metastasizing to major organs (stage IV) is virtually incurable[2]. Patients with advanced melanoma have a median survival time of less than one year, and the estimated five-year survival rate is less than 15%[3,4]. With the rapid incidence of melanoma in the United States and other developed countries, there is an urgent need to develop more effective drugs[5–7]. The RAS-RAF-MEK-ERK signaling pathway (ERK pathway) plays an important role in tumorigenesis and cancer progression[8]. Sorafenib (Nexavar®, Figure 1) targets ERK pathway. It inhibits basal phosphorylation of ERK (p-ERK) in numerous cancer cell lines *in vitro*, including melanoma cell lines, independent of their K-RAS and b-RAF mutational status[9]. Vemurafenib (PLX4032, Zelboraf®, Figure 1) is another drug which targets ERK pathway through inhibition of V600E-B-RAF kinase. In 2011, it was approved by the U.S. Food and Drug Administration (FDA) for treatment of late-stage melanoma[10]. So, inhibition of ERK signaling pathway is a very potential avenue for treatment of melanoma.

Numerous compounds with new scaffolds consisting of hinge, spacer, and tail regions have recently reported as potential antiproliferative agents against melanoma cell lines[11–22]. In this work, a new series of quinolinylaminopyrimidines **1a**–**k** and quinazolinylmethylaminopyrimidines **2a**–**i** containing aminoquinoline and aminoquinazoline as hinge regions was designed and synthesized (Figure 1). Their *in vitro* antiproliferative activities were tested over A375P human melanoma cell line. And MEK and ERK kinases inhibitory activity for the representative compound **1h** is reported.

Experimental

Chemistry

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer using DMSO-d_6 containing tetramethylsilane as an internal standard. LC-MS spectra were determined on a Waters Quattro Micro System. The liquid chromatography high-resolution mass spectra (LC-HRMS, electron spray ionization) experiments were performed with Synapt G2 (Waters MS Technology, Manchester, Q-TOF MS, UK) mass analyzer. Data were acquired in the positive ion mode. Calibration was performed with an external calibration mixture immediately prior to analysis. Column chromatography was carried out using silica gel (230–400 mesh). Solvents and liquid reagents were

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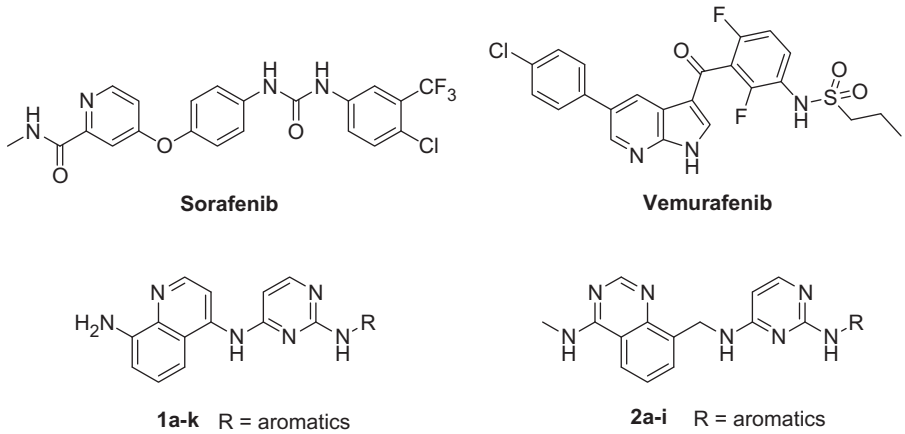
Figure 1. Structures of Sorafenib, Vemurafenib, and the target compounds.



transferred using hypodermic syringes. Purity % of all the target compounds were determined by LC-MS and found to be >95%.

*4-Chloro-8-nitroquinoline (**3**)*

To a solution of 4-chloroquinoline (**2**) (5.0 g, 0.031 mmol) in sulfuric acid (23 mL, 0.42 mol), nitric acid (4.5 mL, 0.11 mol) was added dropwise at 0 °C. The mixture was stirred at room temperature for 4 h. Upon completion, the reaction mixture was cooled to 0 °C and neutralized with 1 M NH_4OH. The resulting precipitate was collected by filtration, washed with water, and dried to give the title compound **3** (3.6 g, 56%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.93 (d, J = 4.59 Hz, 1H), 8.47 (d, J = 8.44 Hz, 1H), 8.08 (d, J = 7.33 Hz, 1H), 7.74 (t, J = 8.02 Hz, 1H), 7.59 (d, J = 6.76 Hz, 1H).

General procedure for the synthesis of pyrimidin-2,4-diamines 5

To a mixture of 2-chloropyrimidin-4-amine (**4**) (2.0 mmol), (±)-2,2-bis(diphenylphosphino)-1,1'-binaphinyl [(±)-BINAP] (0.20 mmol), palladium (II) acetate (0.20 mmol), and cesium carbonate (0.30 mmol) in toluene, the appropriate aminobenzene (2.1 mmol) was added. The mixture was refluxed under N_2 atmosphere for 6 h. Upon completion, the reaction mixture was filtered through a pad of celite. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by column chromatography to afford the corresponding compound **5** (21–60%).

General procedure for preparation of nitroquinolinylaminopyrimidines 6a–k

To a solution of 4-chloro-8-nitroquinoline (**3**) (0.50 mmol) in DMF, the appropriate pyrimidin-2,4-diamines **5** (0.55 mmol) and NaO^tBu (0.75 mmol) were added. After stirring at room temperature for 4 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography to afford the corresponding compounds **6a**–**k**.

*3,4-Dimethylphenyl-N4-(8-nitroquinolin-4-yl)pyrimidine-2,4-diamine (**6a**)*

White solid, yield 15%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.82 (s, 1H), 8.65 (d, J = 6.19 Hz, 1H), 8.25 (d, J = 7.88 Hz, 1H), 8.20 (s, 1H), 7.85 (s, 1H), 7.52 (s, 1H), 7.28–7.22 (m, 5H), 6.22 (s, 1H), 2.24 (s, 3H).

*2-Methyl-5-(4-(8-nitroquinolin-4-ylamino)pyrimidin-2-ylamino)-benzonitrile (**6b**)*

Brown solid, yield 15%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.51 (s, 1H), 8.85 (d, J = 4.32 Hz, 1H), 8.35–8.32 (m, 2H), 8.15–8.11 (m, 2H), 7.82 (s, 1H), 7.64 (d, J = 9.68 Hz, 1H), 7.22 (d, J = 8.64 Hz, 1H), 6.5 (s, 1H), 2.33 (s, 3H).

*8-Nitroquinolin-4-yl-N2-(4-trifluoromethylphenyl)pyrimidine-2,4-diamine (**6c**)*

White solid, yield 20%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.90 (s, 1H), 9.68 (s, 1H), 8.90 (d, J = 4.82 Hz, 1H), 8.38 (s, 2H), 8.23 (d, J = 5.69 Hz, 1H), 7.91 (d, J = 4.77 Hz, 1H), 7.83 (d, J = 8.45 Hz, 2H), 7.46 (d, J = 8.44 Hz, 1H), 6.55 (d, J = 5.69 Hz, 1H).

*8-Nitroquinolin-4-yl-N2-(3-trifluoromethylphenyl)pyrimidine-2,4-diamine (**6d**)*

White solid, yield 15%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.85 (d, J = 4.66 Hz, 1H), 8.54 (d, J = 9.44 Hz, 1H), 8.32 (d, J = 9.44 Hz, 1H), 8.26 (d, J = 5.66 Hz, 1H), 7.96 (s, 1H), 7.85 (s, 1H), 7.63 (d, J = 8.63 Hz, 1H), 7.55 (d, J = 4.71 Hz, 1H), 7.41 (t, J = 7.79 Hz, 1H), 7.29 (d, J = 7.47 Hz, 1H), 6.33 (d, J = 5.64 Hz, 1H).

*2-Chloro-4-trifluoromethylphenyl-N4-(8-nitroquinolin-4-yl) pyrimidine-2,4-diamine (**6e**)*

Brown solid, yield 135%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.86 (d, J = 4.82 Hz, 1H), 8.62 (d, J = 8.74 Hz, 1H), 8.45 (d, J = 9.40 Hz, 1H), 8.35 (d, J = 9.40 Hz, 1H), 8.30 (d, J = 5.60 Hz, 1H), 7.85 (s, 1H), 7.66 (s, 2H), 7.57 (d, J = 4.67 Hz, 1H), 7.44 (d, J = 8.74 Hz, 1H), 6.42 (d, J = 5.60 Hz, 1H).

*4-Chloro-3-trifluoromethylphenyl-N4-(8-nitroquinolin-4-yl) pyrimidine-2,4-diamine (**6f**)*

White solid, yield 12%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.85 (d, J = 4.68 Hz, 1H), 8.46 (d, J = 9.44 Hz, 1H), 8.32 (d, J = 9.44 Hz, 1H), 8.26 (d, J = 5.64 Hz, 1H), 8.0 (d, J = 2.47 Hz, 1H), 7.82 (s, 1H), 7.63 (d, J = 2.59 Hz, 1H), 7.56 (d, J = 4.72 Hz, 1H), 7.40 (d, J = 8.64 Hz, 1H), 7.16 (s, 1H), 6.34 (d, J = 5.67 Hz, 1H).

*4-Fluoro-3-trifluoromethylphenyl)-N4-(8-nitroquinolin-4-yl) pyrimidine-2,4-diamine (**6g**)*

Brown solid, yield 13%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.85 (d, J = 4.75 Hz, 1H), 8.47 (d, J = 9.45 Hz, 1H), 8.30

(d, $J = 9.42$ Hz, 1H), 8.24 (d, $J = 5.66$ Hz, 1H), 7.90–7.88 (m, 1H), 7.82 (s, 1H), 7.62–7.59 (m, 1H), 7.55 (d, $J = 4.74$ Hz, 1H), 7.17–7.12 (m, 2H), 6.32 (d, $J = 5.64$ Hz, 1H).

*3-Chloro-5-trifluoromethylphenyl-N4-(8-nitroquinolin-4-yl) pyrimidine-2,4-diamine (**6h**)*

Brown solid, yield 18%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.85 (d, $J = 4.77$ Hz, 1H), 8.50 (d, $J = 9.42$ Hz, 1H), 8.40 (d, $J = 9.41$ Hz, 1H), 8.27 (d, $J = 5.62$ Hz, 1H), 7.94 (s, 1H), 7.86 (s, 1H), 7.61 (s, 1H), 7.56 (d, $J = 4.76$ Hz, 1H), 7.29 (s, 1H), 6.37 (d, $J = 5.66$ Hz, 1H).

*2,4-Bis(trifluoromethyl)phenyl-N4-(8-nitroquinolin-4-yl) pyrimidine-2,4-diamine (**6i**)*

White solid, yield 17%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.85 (d, $J = 4.76$ Hz, 1H), 8.56 (d, $J = 8.79$ Hz, 1H), 8.40 (d, $J = 9.42$ Hz, 1H), 8.30 (s, 1H), 8.29 (d, $J = 2.63$ Hz, 1H), 8.03–7.99 (m, 2H), 7.87 (s, 1H), 7.70 (d, $J = 9.17$ Hz, 1H), 7.57 (d, $J = 4.76$ Hz, 1H), 7.46 (s, 1H), 6.46 (d, $J = 5.68$ Hz, 1H).

*3,5-Bis(trifluoromethyl)phenyl-N4-(8-nitroquinolin-4-yl) pyrimidine-2,4-diamine (**6j**)*

White solid, yield 19%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.86 (d, $J = 4.76$ Hz, 1H), 8.45 (d, $J = 9.43$ Hz, 1H), 8.34 (d, $J = 9.42$ Hz, 1H), 8.30 (d, $J = 5.64$ Hz, 1H), 8.06 (s, 2H), 7.84 (s, 1H), 7.57 (d, $J = 4.76$ Hz, 1H), 7.49 (s, 1H), 7.29 (s, 1H), 6.38 (d, $J = 5.64$ Hz, 1H).

*8-Nitroquinolin-4-yl-N2-(3-phenoxyphenyl)pyrimidine-2, 4-diamine (**6k**)*

Orange solid, yield 17%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.82 (d, $J = 4.75$ Hz, 1H), 8.62 (d, $J = 9.44$ Hz, 1H), 8.32 (d, $J = 9.44$ Hz, 1H), 8.20 (d, $J = 5.60$ Hz, 1H), 7.83 (s, 1H), 7.51 (d, $J = 4.76$ Hz, 1H), 7.48 (t, $J = 4.24$ Hz, 1H), 7.31–7.26 (m, 2H), 7.22 (d, $J = 8.06$ Hz, 1H), 7.16 (d, $J = 8.31$ Hz, 1H), 7.06 (t, $J = 7.35$ Hz, 1H), 7.01 (d, $J = 7.90$ Hz, 1H), 6.65 (d, $J = 7.80$ Hz, 1H), 6.26 (d, $J = 5.60$ Hz, 1H).

General procedure for preparation of aminoquinolinylaminopyrimidines 1a-k

A mixture of the appropriate nitroquinolinylaminopyrimidines **6a**–**k** and 5% Pd/C in MeOH was stirred in hydrogen atmosphere at room temperature for 1 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 to afford the corresponding compounds **1a**–**k**.

*(2-(3,4-Dimethylphenylamino)pyrimidin-4-yl)quinoline-4, 8-diamine (**1a**)*

White solid, yield 80%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.82 (s, 1H), 8.66 (s, 1H), 8.26–8.22 (m, 2H), 7.85 (s, 1H), 7.52 (s, 1H), 7.29–7.22 (m, 5H), 6.22 (s, 1H), 2.22 (s, 6H); MS *m/z*: 357 [M+1]$^+$.

*5-(4-(8-Aminoquinolin-4-ylamino)pyrimidin-2-ylamino)-2-methylbenzonitrile (**1b**)*

Brown solid, yield 56%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.59 (d, $J = 4.64$ Hz, 1H), 8.04 (s, 1H), 8.02 (s, 1H), 7.90 (d, $J = 5.88$ Hz, 1H), 7.52 (d, $J = 8.93$ Hz, 1H), 7.45–7.38 (m, 4H), 7.08 (d, $J = 8.48$ Hz, 1H), 6.76 (s, 1H), 5.88 (d, $J = 5.84$ Hz, 1H), 4.26 (s, 1H), 2.33 (s, 3H); MS *m/z*: 368 [M+1]$^+$.

*(2-(4-Trifluoromethylphenylamino)pyrimidin-4-yl)quinoline-4, 8-diamine (**1c**)*

White solid, yield 75%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.66 (d, $J = 4.65$ Hz, 1H), 8.03 (d, $J = 8.03$ Hz, 1H), 7.68 (d, $J = 8.45$ Hz, 3H), 7.57 (d, $J = 8.87$ Hz, 1H), 7.51–7.43 (m, 6H), 6.73 (s, 1H), 6.0 (d, $J = 3.24$ Hz, 1H), 5.23 (s, 2H); MS *m/z*: 397 [M+1]$^+$.

*(2-(3-Trifluoromethylphenylamino)pyrimidin-4-yl)quinoline-4, 8-diamine (**1d**)*

White solid, yield 72%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.66 (d, $J = 4.42$ Hz, 1H), 8.03 (d, $J = 4.42$ Hz, 1H), 7.94 (s, 1H), 7.70 (d, $J = 6.71$ Hz, 1H), 7.57 (d, $J = 8.82$ Hz, 1H), 7.49 (t, $J = 8.84$ Hz, 2H), 7.43–7.38 (m, 3H), 7.21 (d, $J = 6.72$ Hz, 1H), 6.74 (s, 1H), 5.97 (d, $J = 5.38$ Hz, 1H), 5.22 (s, 2H); MS *m/z*: 397 [M+1]$^+$.

*(2-(2-Chloro-4-trifluoromethylphenylamino)pyrimidin-4-yl) quinoline-4,8-diamine (**1e**)*

Pale brown solid, yield 59%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.81 (d, $J = 2.76$ Hz, 1H), 8.76–8.73 (m, 1H), 8.15–8.10 (m, 2H), 7.64–7.61 (m, 2H), 7.52 (d, $J = 4.65$ Hz, 1H), 7.44 (q, $J = 4.19$ Hz, 1H), 7.38 (d, $J = 8.58$ Hz, 1H), 7.20 (d, $J = 8.64$ Hz, 1H), 6.54 (s, 1H), 6.05 (d, $J = 2.60$ Hz, 1H), 5.22 (s, 2H); MS *m/z*: 431 [M+1]$^+$; HRMS (ESI, positive) calcd for $C_{20}H_{15}ClF_3N_6$ [(M+H)$^+$] 431.0921, found 431.0989.

*(2-(4-Chloro-3-trifluoromethylphenylamino)pyrimidin-4-yl) quinoline-4,8-diamine (**1f**)*

White solid, yield 60%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.66 (d, $J = 4.67$ Hz, 1H), 8.05 (d, $J = 5.79$ Hz, 1H), 8.01 (d, $J = 2.58$ Hz, 1H), 7.72 (d, $J = 2.51$ Hz, 1H), 7.57 (d, $J = 8.88$ Hz, 1H), 7.53–7.48 (m, 2H), 7.34 (d, $J = 8.86$ Hz, 1H), 7.08 (s, 1H), 6.42 (s, 1H), 5.99 (d, $J = 5.73$ Hz, 1H), 5.19 (s, 2H); MS *m/z*: 431 [M+1]$^+$; HRMS (ESI, positive) calcd for $C_{20}H_{15}ClF_3N_6$ [(M+H)$^+$] 431.0921, found 431.0997.

*(2-(4-Fluoro-3-trifluoromethylphenylamino)pyrimidin-4-yl) quinoline-4,8-diamine (**1g**)*

Pale brown solid, yield 70%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.66 (d, $J = 4.76$ Hz, 1H), 8.02 (d, $J = 7.88$ Hz, 1H), 7.90 (d, $J = 8.08$ Hz, 1H), 7.75–7.72 (m, 1H), 7.58 (d, $J = 11.91$ Hz, 1H), 7.51 (d, $J = 6.48$ Hz, 2H), 7.14 (s, 1H), 7.09 (t, $J = 12.2$ Hz, 1H), 6.58 (s, 1H), 5.98 (d, $J = 7.68$ Hz, 1H), 5.21 (s, 2H); MS *m/z*: 415 [M+1]$^+$; HRMS (ESI, positive) calcd for $C_{20}H_{15}F_4N_6$ [(M+H)$^+$] 415.1216, found 415.1226.

*(2-(3-Chloro-5-trifluoromethylphenylamino)pyrimidin-4-yl) quinoline-4,8-diamine (**1h**)*

Pale brown solid, yield 50%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 9.64 (s, 1H), 9.01 (s, 1H), 8.68 (d, $J = 4.65$ Hz, 1H), 8.14 (s, 1H), 8.07 (d, $J = 5.77$ Hz, 1H), 7.85 (s, 1H), 7.71 (s, 1H), 7.69 (t, $J = 2.47$ Hz, 1H), 7.41 (d, $J = 8.94$ Hz, 1H), 7.13 (s, 1H), 6.30 (d, $J = 5.81$ Hz, 1H), 5.83 (s, 2H); MS *m/z*: 421 [M+1]$^+$.

*(2-(2,4-Bis(trifluoromethyl)phenylamino)pyrimidin-4-yl) quinoline-4,8-diamine (**1i**)*

Pale brown solid, yield 48%; ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm): 8.81 (d, $J = 1.64$ Hz, 1H), 8.80 (d, $J = 8.78$ Hz, 1H), 8.12 (d, $J = 8.28$ Hz, 1H), 8.06 (d, $J = 5.84$ Hz, 1H), 7.82 (s, 1H), 7.61 (d, $J = 7.09$ Hz, 1H), 7.47–7,42 (m, 2H), 7.36 (d, $J = 8.62$ Hz, 1H), 7.20 (d, $J = 8.57$ Hz, 1H), 6.56 (s, 1H), 6.03 (d, $J = 5.72$ Hz,

1H), 5.26 (s, 2H); MS *m/z*: 465 $[M+1]^+$; HRMS (ESI, positive) calcd for $C_{21}H_{15}F_6N_6$ $[(M+H)^+]$ 465.1184, found 415.0611.

*(2-(3,5-Bis(trifluoromethyl)phenylamino)pyrimidin-4-yl) quinoline-4,8-diamine (**1j**)*

White solid, yield 70%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.76 (d, $J = 1.64$ Hz, 1H), 8.06 (d, $J = 1.61$ Hz, 1H), 7.37 (t, $J = 4.27$ Hz, 1H), 7.32 (d, $J = 7.63$ Hz, 1H), 7.15 (d, $J = 8.10$ Hz, 1H), 6.93 (d, $J = 1.02$ Hz, 1H), 5.0 (s, 2H); MS *m/z*: 465 $[M+1]^+$; HRMS (ESI, positive) calcd for $C_{21}H_{15}F_6N_6$ $[(M+H)^+]$ 465.1184, found 415.0611.

*(2-(3-Phenoxyphenylamino)pyrimidin-4-yl)quinoline-4, 8-diamine (**1k**)*

Brown solid, yield 60%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.62 (d, $J = 4.58$ Hz, 1H), 7.99 (d, $J = 5.66$ Hz, 1H), 7.55 (d, $J = 9.11$ Hz, 2H), 7.46 (t, $J = 4.66$ Hz, 2H), 7.34 (t, $J = 7.56$ Hz, 4H), 7.21–7.16 (m, 2H), 7.10 (t, $J = 7.21$ Hz, 2H), 7.05 (d, $J = 8.06$ Hz, 2H), 6.61 (d, $J = 7.38$ Hz, 2H), 5.88 (d, $J = 5.60$ Hz, 1H), 5.20 (s, 2H); MS *m/z*: 421 $[M+1]^+$.

*8-Methyl-4-quinazolinone (**8**)*

A mixture of 2-amino-3-methylbenzoic acid (**7**) (25.0 g, 0.165 mol), formamidine acetate (50.0 g, 0.496 mol), and formamide (6.6 mL, 0.165 mol) was heated at 160 °C for 2 h. Upon completion, the reaction mixture was cooled to room temperature and treated with 10% NaOH. After stirring at 60 °C for 20 min, the reaction mixture was neutralized with 1 N HCl at ice bath temperature. The resulting precipitate was collected by filtration, washed with water, and dried to give the title compound **8** (25.4 g, 96%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.16 (s, 1H), 8.50 (s, 1H), 7.94 (d, $J = 7.94$ Hz, 1H), 7.66 (d, $J = 7.38$ Hz, 1H), 7.38 (t, $J = 7.63$ Hz, 1H), 2.51 (s, 3H).

*8-Bromomethyl-4-quinazolinone (**9**)*

To a solution of 8-methyl-4-quinazolinone (**8**) (5.0 g, 31.2 mmol) in CCl_4 (150 mL), *N*-bromosuccinimide (6 g, 34.3 mmol) and AIBN (1.0 g, 6.3 mmol) were added. The mixture was stirred at room temperature for 24 h. Upon completion, the reaction mixture was filtered through a pad of celite. The filtrate was evaporated under reduced pressure, and the resulting residue was triturated with dichloromethane to afford the title compound **9** (5.5 g, 74%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 12.42 (s, 1H), 8.23 (s, 1H), 8.10 (d, $J = 7.86$ Hz, 1H), 7.96 (d, $J = 6.45$ Hz, 1H), 7.50 (t, $J = 7.65$ Hz, 1H), 5.06 (s, 2H).

*8-Bromomethyl-4-chloroquinazoline (**10**)*

To a solution of 8-bromomethyl-4-quinazolinone (**9**) (3.0 g, 12.5 mmol) in toluene (100 mL), $POCl_3$ (2.92 mL, 31.3 mmol) and *N,N*-dimethylaniline (2.38 mL, 18.8 mmol) were added. The mixture was refluxed for 4 h and then cooled to room temperature. The excess of $POCl_3$ was removed by distillation under reduced pressure. H_2O was carefully added to the residue, and the suspension was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography to afford the title compound **10** (2.7 g, 84%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.12(s, 1H), 8.29(d, $J = 8.4$ Hz, 1H), 8.13(d, $J = 7.1$ Hz, 1H), 7.76(t, $J = 8.0$ Hz, 1H), 5.26(s, 2H).

*8-Bromomethyl-N-methylquinazolin-4-amine (**11**)*

To a solution of 8-bromomethyl-4-chloroquinazoline (**10**) (2.0 g, 7.8 mmol) in THF (50 mL), methylamine (40% solution in water, 1.8 mL, 23.4 mmol) was added. The mixture was stirred at room temperature for 12 h. The solvent was removed by distillation under reduced pressure, the residue was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography to afford the title compound **11** (1.3 g, 68%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.56 (s, 1H), 8.40 (d, $J = 4.18$ Hz, 1H), 8.18 (d, $J = 1.12$ Hz, 1H), 7.90 (d, $J = 6.28$ Hz, 1H), 7.50 (t, $J = 7.42$ Hz, 1H), 5.18 (s, 2H), 3.01 (d, $J = 4.54$ Hz, 3H).

General procedure for preparation of quinazolinyl-methylaminopyrimidines 2a–i

To a solution of 8-bromomethyl-*N*-methylquinazolin-4-amine (**11**) (0.50 mmol) in DMF, the appropriate pyrimidin-2,4-diamines **5** (0.55 mmol) and NaO^{t}Bu (0.75 mmol) were added. After stirring at room temperature for 4 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography to afford the corresponding compounds **2a–i**.

*2-Methyl-5-(4-(4-methylaminoquinazolin-8-yl)methylaminopyrimidin-2-ylamino)benzonitrile (**2a**)*

Brown solid, yield 15%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.64 (s, 1H), 8.01(s, 1H), 7.93 (d, $J = 5.68$ Hz, 1H), 7.63 (d, $J = 2.27$ Hz, 1H), 7.59 (d, $J = 7.24$ Hz, 1H), 7.54 (d, $J = 8.17$ Hz, 1H), 7.49 (d, $J = 8.37$ Hz, 1H), 7.30 (t, $J = 7.52$ Hz, 1H), 7.18 (d, $J = 8.41$ Hz, 1H), 6.25 (s, 1H), 5.89 (d, $J = 5.66$ Hz, 1H), 5.73 (s, 1H), 4.78 (s, 2H), 3.15 (s, 3H); MS *m/z*: 397 [M+1]+; HRMS (ESI, positive) calcd for $C_{22}H_{21}N_8$ $[(M+H)^+]$ 397.1811, found 397.1877.

*2-Chloro-5-trifluoromethylphenyl-N4-(4-methylaminoquinazolin-8-yl)methylpyrimidine-2,4-diamine (**2b**)*

Brown solid, yield 14%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.68(s, 1H), 8.12 (s, 1H), 7.87 (s, 1H), 7.59 (s, 1H), 7.49 (d, $J = 6.0$ Hz, 1H), 7.34 (m, 2H), 7.18 (d, $J = 8.40$ Hz, 1H), 6.01 (s, 1H), 5.61 (s, 2H), 5.30 (d, $J = 8.0$ Hz, 1H), 3.16 (s, 3H); MS *m/z*: 460 [M+1]+; HRMS (ESI, positive) calcd for $C_{21}H_{18}ClF_3N_7$ $[(M+H)^+]$ 460.1186, found 460.1264.

*3-Chloro-4-trifluoromethylphenyl-N4-(4-methylaminoquinazolin-8-yl)methylpyrimidine-2,4-diamine (**2c**)*

White solid, yield 14%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.69 (s, 1H), 7.96 (d, $J = 5.6$ Hz, 1H), 7.78 (d, $J = 2.5$ Hz, 1H), 7.62 (d, $J = 6.9$ Hz, 1H), 7.53 (d, $J = 8.1$ Hz, 1H), 7.46 (dd, $J = 2.5$ Hz, 1H), 7.36 (d, $J = 2.5$ Hz, 1H), 7.34 (d, $J = 3.5$ Hz, 1H), 5.92 (d, $J = 5.7$ Hz, 1H), 5.79 (s, 2H), 4.63 (s, 2H), 3.20 (d, $J = 5.5$ Hz, 3H); MS *m/z*: 460 [M+1]+.

*4-Fluoro-3-trifluoromethylphenyl-N4-(4-methylaminoquinazolin-8-yl)methylpyrimidine-2,4-diamine (**2d**)*

Brown solid, yield 13%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.63 (s, 1H), 7.92 (d, $J = 5.68$ Hz, 1H), 7.63 (m, 2H), 7.50 (m, 1H), 7.46 (d, $J = 8.21$ Hz, 1H), 7.28 (t, $J = 7.42$ Hz, 1H), 7.06 (d, $J = 9.44$ Hz, 1H), 6.15 (s, 1H), 5.88 (d, $J = 5.71$ Hz, 1H), 5.72 (s, 1H), 4.75 (s, 2H), 3.15 (s, 3H); MS *m/z*: 444 [M+1]+; HRMS (ESI, positive) Calcd for $C_{21}H_{18}F_4N_7$ $[(M+H)^+]$ 444.1482, found 444.1551.

*4-(4-(4-Methylaminoquinazolin-8-yl)methylaminopyrimidin-2-ylamino)-3-trifluoromethylbenzonitrile (**2e**)*

Brown solid, yield 14%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.71 (d, $J = 5.84$ Hz, 1H), 8.19 (d, $J = 9.68$ Hz, 1H), 8.10 (d, $J = 8.68$ Hz, 1H), 7.89 (t, $J = 9.16$ Hz, 1H), 7.84 (s, 1H), 7.62 (m, 1H), 7.51 (d, $J = 2.64$ Hz, 1H), 7.46 (s, 1H), 7.42 (d, $J = 3.04$ Hz, 1H), 7.38 (d, $J = 10.6$ Hz, 1H), 5.62 (s, 2H), 7.51 (d, $J = 9.72$ Hz, 1H), 3.21 (s, 1H); MS *m/z*: 451 [M+1]+; HRMS (ESI, positive) calcd for $C_{22}H_{18}F_3N_8$ [$(M+H)^+$] 451.1528, found 451.1608.

*(4-Methylaminoquinazolin-8-yl)methyl-N2-(3-nitro-4-trifluoromethylphenyl)pyrimidine-2,4-diamine (**2f**)*

Brown solid, yield 13%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.73 (s, 1H), 8.07 (s, 1H), 8.03 (d, $J = 5.2$ Hz, 1H), 7.83 (d, $J = 8.9$ Hz, 1H), 7.56 (d, $J = 8.7$ Hz, 2H), 7.53 (d, $J = 7.3$ Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 6.05 (d, $J = 5.6$ Hz, 1H), 5.94 (s, 1H), 5.84 (s, 1H), 4.77 (s, 2H), 3.22 (d, $J = 4.68$ Hz, 1H); MS *m/z*: 471 [M+1]+; HRMS (ESI, positive) calcd for $C_{21}H_{18}F_3N_8O_2$ [$(M+H)^+$] 471.1427, found 471.1503.

*3-Chloro-5-trifluoromethylphenyl-N4-(4-methylaminoquinazolin-8-yl)methylpyrimidine-2,4-diamine (**2g**)*

Brown solid, yield 12%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.62 (s, 1H), 7.91 (d, $J = 5.7$ Hz, 1H), 7.58 (d, $J = 7.70$ Hz, 2H), 7.54 (d, $J = 7.12$ Hz, 1H), 7.43 (d, $J = 8.02$ Hz, 1H), 7.27 (s, 1H), 7.16 (t $J = 8.85$, 1H), 6.51 (d, $J = 4.6$ Hz, 1H), 5.88 (d, $J = 5.71$ Hz, 1H), 5.75 (s, 2H), 4.92 (s, 2H), 3.05 (s, 3H); MS *m/z*: 460 [M+1]+; HRMS (ESI, positive) Calcd for $C_{21}H_{18}ClF_3N_7$ [$(M+H)^+$] 460.1186, found 460.1255.

*2,4-Bis(trifluoromethyl)phenyl-N4-(4-methylaminoquinazolin-8-yl)methylpyrimidine-2,4-diamine (**2h**)*

Brown solid, yield 16%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 8.73 (s, 1H), 8.07 (s, 1H), 8.03 (d, $J = 5.2$ Hz, 1H), 7.83 (d, $J = 8.9$ Hz, 1H), 7.56 (d, $J = 8.7$ Hz, 2H), 7.53 (d, $J = 7.3$ Hz, 1H), 7.32 (t, $J = 7.8$ Hz, 1H), 6.05 (d, $J = 5.6$ Hz, 1H), 5.94 (s, 1H), 5.84 (s, 1H), 4.77 (s, 2H), 3.22 (d, $J = 4.68$ Hz, 1H); MS *m/z*: 494 [M+1]+.

*2,4-Bis(trifluoromethyl)phenyl-N4-(4-methylaminoquinazolin-8-yl)methylpyrimidine-2,4-diamine (**2i**)*

White solid, yield 15%; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.98 (d, $J = 5.66$ Hz, 1H), 7.87 (s, 2H), 7.63 (d, $J = 7.2$ Hz, 1H), 7.54 (m, 2H), 7.36 (t, $J = 7.6$ Hz, 1H), 5.96 (t, $J = 5.6$ Hz, 1H), 5.86 (s, 1H), 5.75 (s, 2H), 3.2 (d, $J =$ Hz, 3H); MS *m/z*: 494 [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) and maintained in Dulbecco's modified eagle medium (DMEM, Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS, Welgene, Daegu, Korea) and 1% penicillin/streptomycin (Welgene, Daegu, 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 test 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). The IC_{50} was calculated using GraphPad Prism 4.0 software.

Kinase Screening

Immunoblot analysis

For immunoblotting, A375P melanoma cells grown to 70–80% confluence were harvested in RIPA lysis buffer and disrupted by sonication and centrifuged at 12 000 rpm for 15 min. The quantity of protein was determined with DC protein assay kit (Bio-Rad Lab., Hercules, CA). Protein samples were subjected to SDS-PAGE and immunoblotted with the appropriate primary antibody overnight at 4 °C. The protein bands were visualized using chemiluminescence detection kit (Amersham HRP Chemiluminescent Substrates, Amersham Biosciences, Piscataway, NJ) after hybridization with the HRP-conjugated secondary antibody from rabbits or mice. The LAS4000-mini (Fujifilm, Tokyo, Japan) was used for chemiluminescence detection.

Compound treatment in A375P cells

To assess the effect of the target compounds on the RAF-1/MEK/ERK signaling pathway, A375P cells were treated with the tested compounds and Sorafenib in a dose dependent way (1, 3, and 10 μM) for 24 h and immunoblotted with antibodies against phospho-MEK1/2, ERK1/2 and β-actin, respectively.

Results and discussion

Chemistry

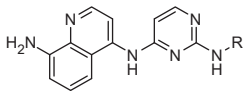
Quinolinylaminopyrimidines **1a–k** were synthesized by the pathways illustrated in Scheme 1. Nitration of 4-chloroquinoline (**2**) using a mixture of nitric acid and sulfuric acid yielded 4-chloro-8-nitroquinoline (**3**). Nucleophilic substitution of the chloro group of compound **3** with the appropriate pyrimidinyl amines **5** was carried out using sodium *t*-butoxide to produce compounds **6a–k**. Pyrimidinyl amines **5** were obtained by treatment of 2-chloropyrimidin-4-amine (**4**) with various aromatic amines in the presence of (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl [(±)-BINAP], palladium (II) acetate, and cesium carbonate according to the procedure of Buchwald-Hartwig amination[23]. Reduction of the nitro group of **6a–k** using Pd/C in hydrogen atmosphere gave the title compounds **1a–k**.

Synthesis of quinazolinylmethylaminopyrimidines **2a–i** was carried out by the sequence of reactions shown in Scheme 2. Ring closure of 2-amino-3-methylbenzoic acid (**7**) with formamidine acetate in formamide[24] followed by bromination with *N*-bromosuccinimide in the presence of AIBN gave the bromomethylquinazolinone **9** in good yield. Compound **11** as a key intermediate was obtained by treatment of **9** with phosphoryl chloride in the presence of *N,N*-dimethylaniline, and subsequent amination of the resulting chloroquinazoline **10** using methylamine. Coupling of **11** with the appropriate pyrimidinyl amines **5** by the similar procedure as described for the preparation of **6a–k** led to the title compounds **2a–i**.

Biological activity

The antiproliferative activity of the newly synthesized compounds against A375P human melanoma cell line was tested. The ability of quinolinylaminopyrimidines **1a–k** and quinazolinylmethylaminopyrimidines **2a–i** to inhibit the growth of A375P cell line is

Table 1. Antiproliferative activity of quinolinylaminopyrimidines **1a–k** against A375P human melanoma cell line.



1a-k

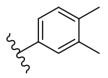
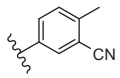
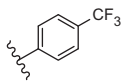
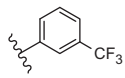
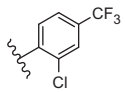
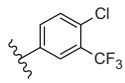
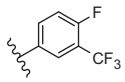
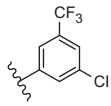
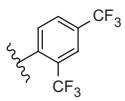
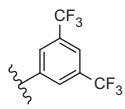
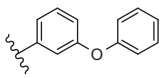
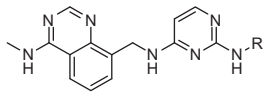
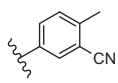
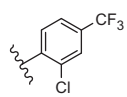
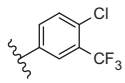
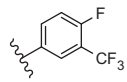
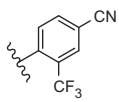
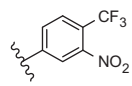
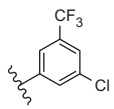
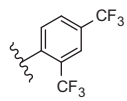
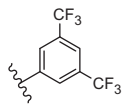
Compd. No.	R	IC_{50} (μM)	Compd. No.	R	IC_{50} (μM)
1a		6.5	**1g**		>20
1b		15.3	**1h**		0.57
1c		14.0	**1i**		3.3
1d		9.3	**1j**		10.6
1e		>20	**1k**		0.94
1f		9.2			
Sorafenib		3.4	Vemurafenib		0.25

Table 2. Antiproliferative activity of quinazolinylmethylamino pyrimidines **2a–i** against A375P human melanoma cell line.



2a-i

Compd. No.	R	IC_{50} (μM)	Compd. No.	R	IC_{50} (μM)
2a		19.3	**2f**		8.5
2b		4.8	**2g**		5.8
2c		10.2	**2h**		10.6
2d		9.4	**2i**		12.0
2e		8.8			
Sorafenib		3.4	Vemurafenib		0.25

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[5,25]. Vemurafenib was also utilized as a second reference standard in this experiment because of its high potency against melanoma cell lines[26], and it has been recently approved by the FDA for treatment of advanced melanoma[10].

In general, compounds **1a–k** with quinolinylamino moiety were more potent than compounds **2a-i** having quinazolinylmethylamino moiety. This may be attributed to that the spacer length may geometrically permit appropriate fitting of the molecule at the receptor site. As shown in Table 1, compounds **1h** and **1k** with sub-micromolar IC_{50} values displayed superior antiproliferative activity against A375P human melanoma cell line to Sorafenib, even though their potencies were less than that of Vemurafenib. In addition, compound **1i** showed higher potency than Sorafenib. Among all the target compounds, compound **1h** possessing 3-chloro-5-trifluoromethylphenyl as a terminal group exhibited the highest potency against A375P cell line with IC_{50} value of 0.57 μM. Compound **1h** with 3,5-disubstituted phenyl group was more potent than compounds **1e** and **1f** having the different position of substituents. This may be rationalized that the orientation of substituents at receptor site may affect the affinity and potency. As a whole, the structure–activity relationships did not show a well-defined trend. In quinazolinylmethylaminopyrimidine series, compounds **2b** and **2g** showed similar antiproliferative activities to Sorafenib (Table 2). In order to study the mechanism of action, compound **1h** with high potency against

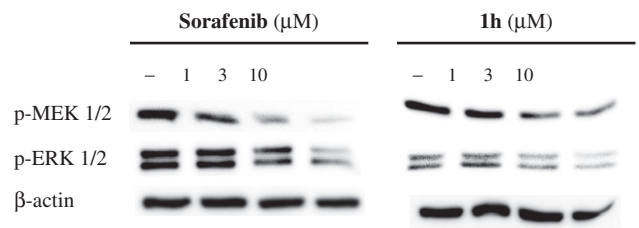
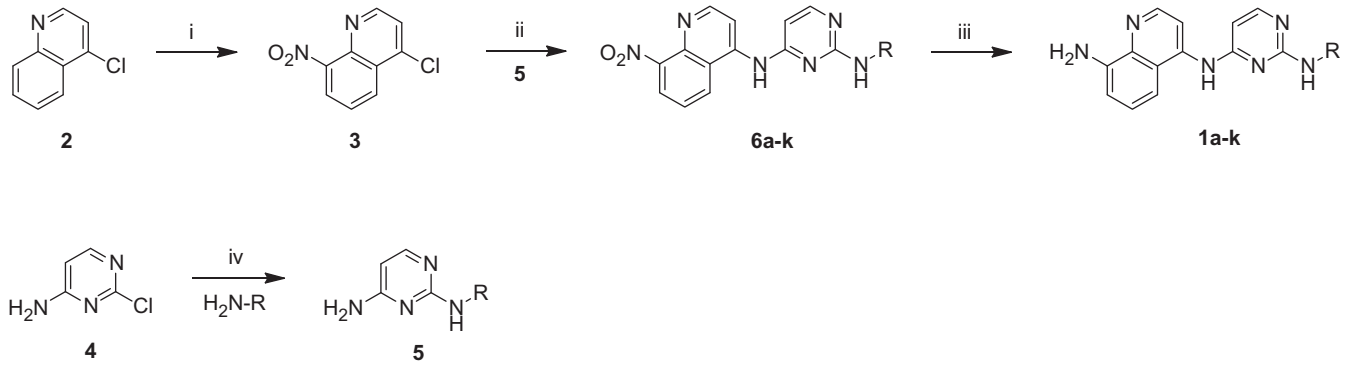


Figure 2. Western blot analyses of p-MEK 1/2 and p-ERK 1/2 expression in presence of compound **1h** and Sorafenib.

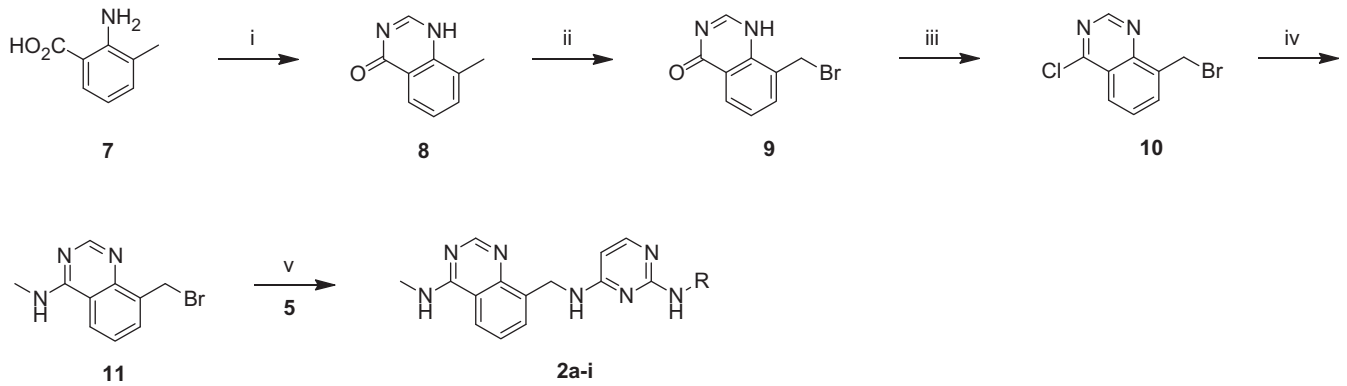
A375P human melanoma cell line was selected as a representative example of this series to be screened for its inhibitory effects on MEK and ERK kinases (Figure 2). A375P cell lysate was treated with three different concentrations of the test compound (1, 3, and 10 μM), and its inhibitory activity was compared with that of Sorafenib. The results showed that compound **1h** and Sorafenib markedly suppressed phosphorylation of MEK1/2 and ERK1/2 in a dose-dependent manner. Compound **1h** inhibited ERK kinase more than MEK kinase at the same concentrations. Therefore, it can be concluded that this compound may inhibit melanoma cell proliferation through ERK kinase inhibition.

Conclusion

A new series of quinolinylaminopyrimidines **1a–k** and quinazolinylmethylaminopyrimidines **2a–i** was designed and synthesized as a continuation of our ongoing anticancer development research project. The target compounds were evaluated for antiproliferative



Scheme 1. Reagents and reaction conditions: (i) HNO_3, H_2SO_4, rt, 4 h; (ii) NaO^tBu, DMF, rt, 4 h; (iii) 5% Pd/C, H_2, MeOH, rt, 1 h; (iv) $Pd(OAc)_2$, BINAP, $CsCO_3$, toluene, reflux, 6 h.



Scheme 2. Reagents and reaction conditions: (i) formamidine acetate, $HCONH_2$, 160 °C, 12 h; (ii) *N*-bromosuccinimide, AIBN, CCl_4, rt, 24 h; (iii) $POCl_3$, *N,N*-dimethylaniline, toluene, reflux, 4 h; (iv) methylamine, THF, rt, 12 h; (v) NaO^tBu, DMF, rt, 4 h.

activities against A375P human melanoma cell line. Compounds **1h** and **1k** with sub-micromolar IC_{50} values exhibited superior antiproliferative activity to Sorafenib. The representative compound **1h** possessing 3-chloro-5-trifluoromethylphenyl as a terminal group showed significant and dose-dependent ERK kinase inhibitory activity. It can be considered as a promising lead for future development of antiproliferative agents targeting ERK pathway for treatment of melanoma.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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