European Journal of Medicinal Chemistry 46 (2011) 3218–3226

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis, and antiproliferative activity of new 1*H*-pyrrolo[3,2-*c*]pyridine derivatives against melanoma cell lines

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ARTICLE INFO

Article history:
Received 1 February 2011
Received in revised form
29 March 2011
Accepted 10 April 2011
Available online 16 April 2011

Keywords:
Pyrrolo[3,2-*c*]pyridine
A375P
Antiproliferative activity
Melanoma
Diarylurea
Diarylamide

ABSTRACT

Synthesis of a new series of diarylureas and diarylamides having 1*H*-pyrrolo[3,2-*c*]pyridine scaffold is described. Their *in vitro* antiproliferative activity against A375P human melanoma cell line was tested and the effect of substituents on pyrrolo[3,2-*c*]pyridine nucleus was investigated. The newly synthesized compounds, except three *N*-tolyl derivatives (**8f**, **9f**, and **9h**), generally showed superior activity against A375P to Sorafenib. Among all of these derivatives, compounds **8b**, **8g**, and **9a**–**e** showed the highest potency against A375P with IC_{50} in nanomolar range. In addition, compounds **8d**, **8e**, **8h**, **9g**, **9i**, and **9j** were more potent than Sorafenib but with IC_{50} in micromolar range. Compounds **8b**, **8g**, **9b**–**d**, and **9i** demonstrated higher selectivity towards A375P compared with NIH3T3 fibroblasts. The most potent diarylurea **8g** and diarylamide **9d** were further tested and showed high potency over nine melanoma cell lines at the NCI.

1. Introduction

Melanoma is the most serious type of skin cancer as a malignant tumor of melanocytes. Incidence of melanoma has tripled in the last 4 decades, and more than 80% of skin cancer deaths are due to melanoma. Generally, two major risk factors for melanoma development are the individual's family history and environmental factors. The most important exogenous etiological factor is exposure to solar ultraviolet irradiation [1].

Early stage melanoma can be cured surgically. However, melanoma metastasizing to major organs (stage IV) is virtually incurable [2]. Patients with advanced melanoma have a median survival time of less than one year, and the estimated 5-year survival rate is less than 15% [3,4]. With the incidence of melanoma rapidly rising in the United States and other developed countries, there is an urgent need to develop more effective drugs [5–7].

The current treatments involve surgical removal of the tumor, immunotherapy, radiotherapy, chemotherapy, various combinations, or the use of new treatments in clinical trials. As for immunotherapy, interferon alfa-2b (Intron-A) [8] has been approved by both the FDA and EMEA for adjuvant treatment of melanoma patients, and aldesleukin (Proleukin) [9,10] has been also approved for the treatment of metastatic melanoma in the USA.

A number of reports have recently highlighted diarylureas and diarylamides as potential antiproliferative agents against melanoma cell line [11–18]. Sorafenib (Nexavar) is a diarylurea derivative that has been extensively used in clinical trials [19]. Encouraged by the interesting antiproliferative activity of diarylurea and diarylamide derivatives, a new series of diarylureas and diarylamides possessing 1*H*-pyrrolo[3,2-*c*]pyridine scaffold was synthesized (Fig. 1). Herein, we report the synthesis and antiproliferative activities of these compounds against human melanoma cell lines and NIH3T3 fibroblasts.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of the target compounds 8a–g and 9a–i (Scheme 1)

7-Hydroxy-1*H*-pyrrolo[2,3-*b*]pyridinium 3-chlorobenzoate (**2**) was prepared by reacting 7-azaindole (**1**) with 3-chloroperbenzoic acid [20,21]. Compound **2** was heated with phosphorus oxychloride to produce 4-chloro-7-azaindole (**3**) [21]. Compounds **4a,b** were prepared according to the literature procedure [22,23]. Fusion of **3**

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doi:10.1016/j.ejmech.2011.04.031

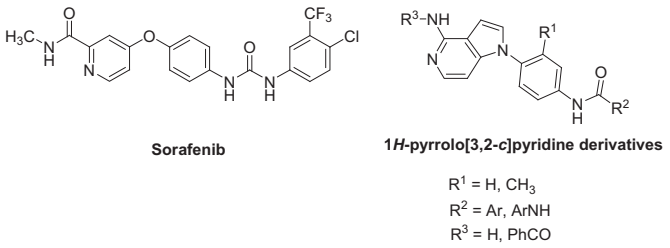


Fig. 1. Structures of Sorafenib and 1*H*-pyrrolo[3,2-*c*]pyridine derivatives.

with *p*-nitroaniline or 2-methyl-4-nitrobenzenamine neat led to nucleophilic displacement of the 4-chloro group by the aromatic amino group, followed by rearrangement of the resulting secondary amine to give the amine hydrochloride salts **4a,b**, albeit in low yields. The benzamido derivatives **5a,b** were obtained by reaction of the amino group of **4a,b** with benzoyl chloride in the presence of diisopropylamine as a base. Reduction of the nitro group of **5a,b** using Pd–C/H_2 gave the corresponding amino compounds **6a,b**. Treatment of **6a,b** with the appropriate aryl isocyanates produced the corresponding diarylurea derivatives **8a**–**f**. The bisamide derivatives **9a**–**i** were obtained by condensation of **6a,b** with the corresponding aromatic carboxylic acids in the presence of HOBt/EDCI/triethylamine.

The *N*-ethylpiperazinyl diarylurea derivative **8g** was prepared by a modified method through heating the aniline derivative **7** with *p*-nitrophenyl chloroformate in the presence of triethylamine as a base to form the corresponding carbamate intermediate, and subsequent heating with compound **6a**.

2.1.2. Synthesis of the target amino compounds **8h,i** and **9j,k** (Scheme 2)

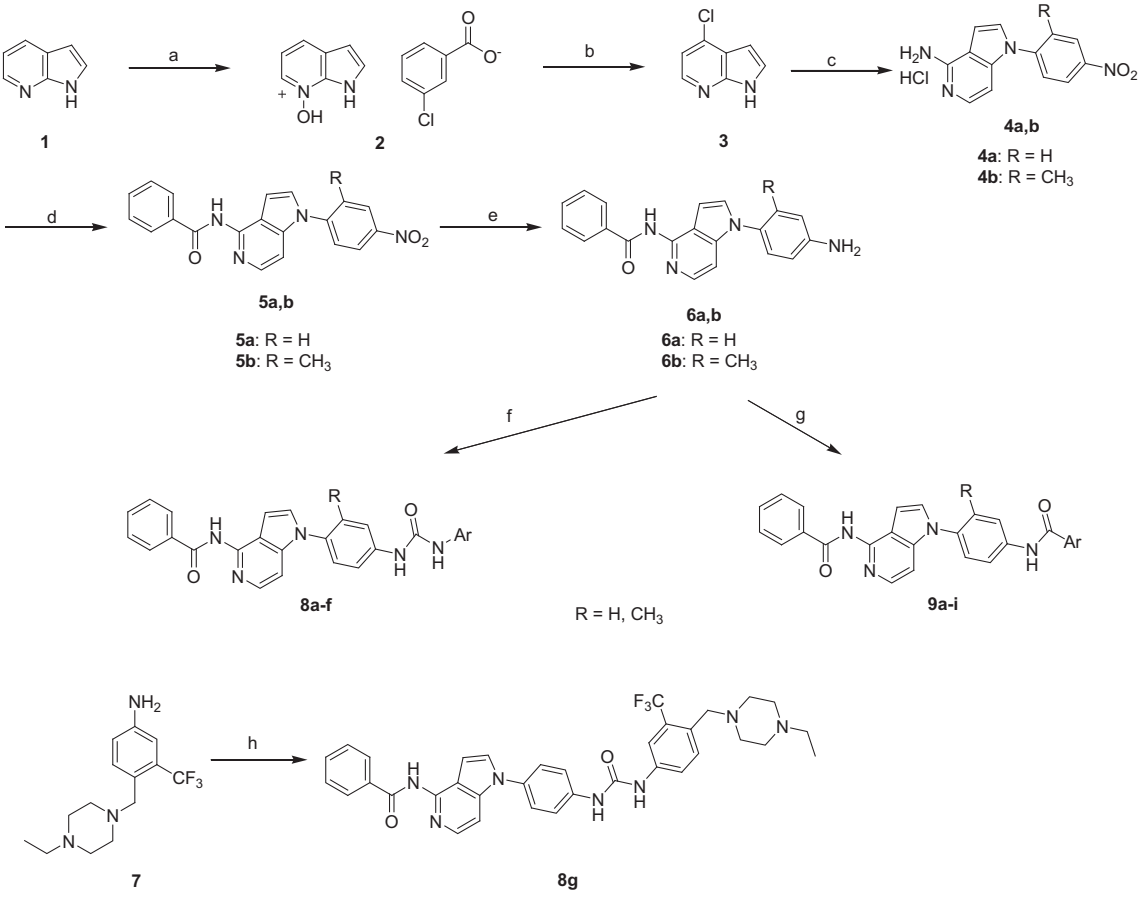
Refluxing the nitro compound **5a** with stannous chloride in ethanol led to simultaneous reduction of the nitro group and hydrolysis of the benzamido moiety to produce the diamino compound **10**. Interaction of the aniline amino group of **10** with 1-(trifluoromethyl)-3-isocyanatobenzene or 1-chloro-2-(trifluoromethyl)-4-isocyanato-benzene gave the target diarylurea derivatives **8h,i**, respectively, and the amino group at position 4 on the pyrrolo[3,2-*c*]pyridine nucleus remained unaffected. Preparation of compounds **9j,k** was carried out in a similar way as described for preparation of compounds **9a**–**i** and the amino group at position 4 on the pyrrolo[3,2-*c*]pyridine nucleus remained unaffected also.

2.2. Biological activity

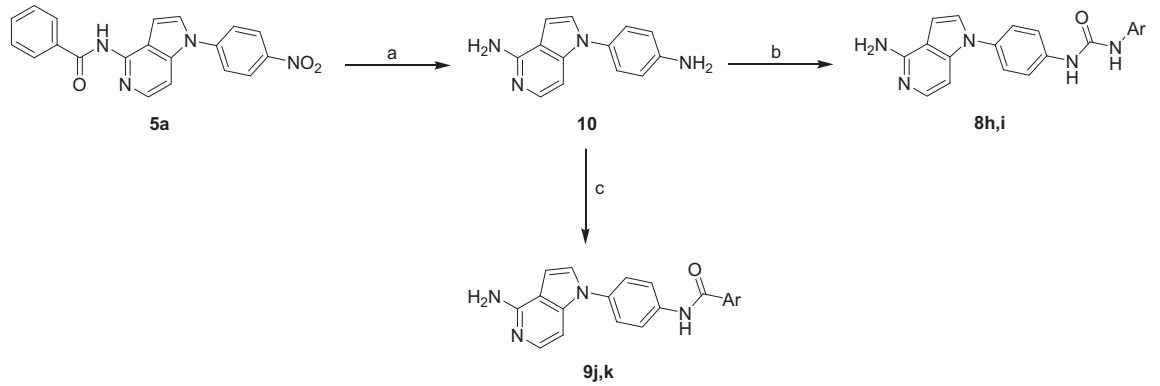
2.2.1. Antiproliferative activity against A375P human melanoma cell line

The antiproliferative activity of the newly synthesized compounds against A375P human melanoma cell line was tested. The ability of pyrrolo[3,2-*c*]pyridine diarylureas and diarylamides to inhibit the growth of A375P cell line is summarized in Tables 1 and 2. Sorafenib was selected as the reference standard.

The newly synthesized compounds, except three *N*-tolyl derivatives (**8f**, **9f**, and **9h**), generally showed superior activity against A375P to Sorafenib. Among all of these derivatives, compounds **8b**, **8g**, and **9a**–**e** showed the most potent antiproliferative activity against A375P with IC_{50} in nanomolar range. In addition,



Scheme 1. Reagents and conditions: (a) 3-chloroperoxybenzoic acid, DME:heptane (1:2), rt; (b) $POCl_3$, 55 °C then rt then 85–90 °C; (c) appropriate nitroaniline, 180 °C; (d) benzoyl chloride, diisopropylamine, CH_3CN, rt; (e) Pd/C, H_2, THF, rt; (f) aryl isocyanate, THF, rt; (g) carboxylic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C; (h) (i) 4-nitrophenyl chloroformate, TEA, 1,4-dioxane, 60 °C, (ii) **6a** in 1,4-dioxane, 90 °C.



Scheme 2. Reagents and conditions: (a) $SnCl_2 \cdot H_2O$, EtOH, reflux; (b) aryl isocyanate, THF, rt; (c) benzoic acid derivative, HOBt, EDCI, TEA, DMF, 80 °C.

compounds **8d**, **8e**, **8h**, **9g**, **9i**, and **9j** were more potent than Sorafenib against A375P but with IC_{50} in micromolar range.

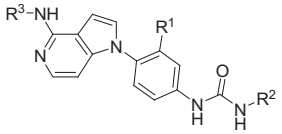
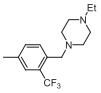
Compounds **9a**, **9b**, and **9d** with 2′-unsubstituted phenyl ring at position 1 on the pyrrolo[3,2-*c*]pyridine nucleus were more potent than compounds **9f**, **9g**, and **9h** having a tolyl ring against A375P. This suggests that introduction of 2′-methyl group on this phenyl ring is unfavorable.

The effect of substituents on the phenyl ring of the tail was also investigated. The introduction of *para*-chloro, *meta*-trifluoromethyl groups, or *para*-(4-ethylpiperazinyl)methyl moiety on the 3′-trifluoromethylphenyl ring of tail (compounds **8d**, **8e**, and **8g**, respectively) significantly enhanced the antiproliferative activity compared with compound **8c** with 4′,5′-unsubstituted trifluoromethylphenyl ring. This may be attributed to the enhanced binding affinity produced by these substituents to the target protein.

The enhanced antiproliferative activity produced by *para*-chloro substituent on the phenyl ring of the tail can be confirmed by comparing the IC_{50} values of compounds **9b** and **9g** with those of compounds **9a** and **9f**. In addition, compound **8b** with 3′,4′-dichlorophenyl moiety demonstrated more potent antiproliferative activity than compound **8a** with 2′,3′-dichlorophenyl moiety against A375P cell line. This also assures that *para*-chloro substituent on the phenyl ring of the tail is favorable.

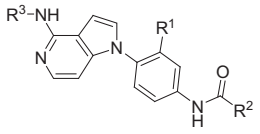
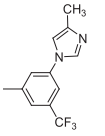
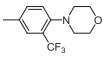
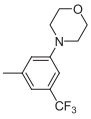
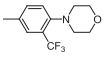
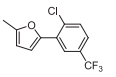
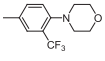
Upon comparing the activities of compounds **9c**–**e** with that of compound **9a**, substitution of the 3′-trifluoromethylphenyl ring of the tail with a morpholine moiety at position 4′ (compound **9d**) improved

Table 1
Antiproliferative activity of 1*H*-pyrrolo[3,2-*c*]pyridine ureas (**8a**–**i**).

Structure	Comp. no.	R^1	R^2	R^3	IC_{50} (μM)[a]	
					A375P	NIH3T3
	**8a**	H		PhCO	>10	–
	8b	H		PhCO	0.2	11.48
	8c	H		PhCO	7.0	–
	8d	H		PhCO	2.4	–
	8e	H		PhCO	1.8	–
	8f	CH_3		PhCO	>10	–
	8g	H		PhCO	95×10^{-3}	0.98
	8h	H		H	5.4	–
	8i	H		H	8.0	–
	Sorafenib				5.6	24.75

[a] Values are expressed as mean IC_{50} of the triplicate experiment.

Table 2
Antiproliferative activity of 1*H*-pyrrolo[3,2-*c*]pyridine amides (**9a**–**k**).

Structure	Comp. no.	R^1	R^2	R^3	IC_{50} (μM)[a]	
					A375P	NIH3T3
	**9a**	H		PhCO	0.1	0.05
	9b	H		PhCO	16.4×10^{-3}	5.65
	9c	H		PhCO	0.8	5.57
	9d	H		PhCO	7.0×10^{-3}	1.74
	9e	H		PhCO	0.3	0.27
	9f	CH_3		PhCO	>10	–
	9g	CH_3		PhCO	2.7	–
	9h	CH_3		PhCO	>10	–
	9i	H		PhCO	1.0	15.91
	9j	H		H	3.8	–
	9k	H		H	6.2	–
	Sorafenib				5.6	24.75

[a] Values are expressed as mean IC_{50} of the triplicate experiment.

the potency. On the other hand, introduction of methylimidazole or morpholine moieties at *meta* position on the same tail 3′-trifluoromethylphenyl ring (compounds **9c** and **9e**, respectively) slightly decreased the potency. This can be attributed to the influence of the substituents orientation at the receptor site on the activity.

Regarding the substituents on the 4-position of pyrrolo[3,2-*c*]pyridine, compounds **8d**, **9b**, and **9d** having benzoylamino moiety were more potent than the corresponding compounds **8i**, **9j**, and **9k** with unsubstituted amino group against A375P. This suggests that the aromatic amide substituent at this position is more favorable.

By comparing the activities of derivatives with amide and urea moieties at pyrrolo[3,2-*c*]pyridine side chain as a linker, it was found that the derivatives with amide moieties (**9a**, **9b**, and **9j**) were more potent than those with urea moieties (**8c**, **8d**, and **8i**). Moreover, most of the newly synthesized amide derivatives (**9a**–**e**, **9g**, **9i**, and **9j**) demonstrated more potent antiproliferative activity against A375P than that of Sorafenib with urea moiety. These results were seemed to indicate the effect of the linker on the activity.

2.2.2. Antiproliferative activity against NIH3T3 fibroblasts

Compounds **8b**, **8g**, **9a**–**e**, and **9i**, which showed the highest potency against A375P melanoma cell line, were tested against NIH3T3 fibroblasts in order to determine their selectivity for melanoma cells compared with normal cells. Sorafenib was considered as a reference drug in this experiment also.

Compounds **8b**, **8g**, **9b**–**d**, and **9i** showed higher selectivity for A375P cells than for NIH3T3 fibroblasts. Their selectivity indices were higher than that of Sorafenib. These compounds are promising leads for design of highly potent and highly selective antiproliferative agents for treatment of melanoma.

On the other hand, compounds **9a** and **9e** demonstrated low selectivity towards melanoma cells despite of their high potency. So it can be concluded that *para*-chloro, *meta*-methylimidazole, and *para*-morpholino substituents on the terminal 3′-trifluoromethylphenyl ring increased the selectivity of compounds **9b**, **9c** and **9d**, respectively, compared with **9a**. In addition, *para*-morpholino compound **9d** showed higher selectivity than *meta*-morpholino derivative **9e**.

Table 3
IC_{50} values in μM of compounds **8g** and **9d** over 9 melanoma cell lines.

Melanoma cell line	Compound no.	
	8g	**9d**
LOX IMVI	0.481	45.6×10^{-3}
MALME-3M	1.54	11.5
M14	1.22	0.763
MDA-MB-435	0.894	0.119
SK-MEL-2	3.62	0.637
SK-MEL-28	1.29	0.292
SK-MEL-5	0.764	0.138
UACC-257	1.59	1.98
UACC-62	1.27	0.485

*2.2.3. Antiproliferative activities of compounds **8g** and **9d** against nine human melanoma cell lines at the NCI*

The most potent diarylurea and diarylamide derivatives, **8g** and **9d**, respectively, against A375P cell line were selected by the National Cancer Institute (NCI) [24], Bethesda, Maryland, USA, for evaluation of their antineoplastic activity. After initial single dose screening of these two compounds, they were further tested in a five-dose testing mode in order to determine their potency. For each of these compounds, the IC_{50} value was recorded. The antiproliferative activities of **8g** and **9d** over 9 melanoma cell lines are summarized in Table 3.

As shown in Table 3, both the tested compounds showed good potency over the 9 tested cell lines. The IC_{50} values of the tested compounds were less than 4 μM over all tested cell lines, except for the IC_{50} of compound **9d** against MALME-3M cell line.

The IC_{50} of the diarylurea compound **8g** was in nanomolar range against 3 cell lines. In addition, compound **9d** with amide linker and 4-morpholino-3-(trifluoromethyl)phenyl terminal moiety generally showed higher potency against the 9-melanoma cell line panel, compared with **8g**. The IC_{50} value of compound **9d** was in nanomolar range over 7 cell lines. Its highest potency was recorded against LOX IMVI cell line ($IC_{50} = 45.6$ nM). This high potency together with its result against A375P cell line makes compound **9d** a promising lead for further design of antiproliferative agents for melanoma.

3. Conclusions

A new series of diarylureas and diarylamides containing 1*H*-pyrrolo[3,2-*c*]pyridine scaffold was synthesized based on our previous literature studies. Among all of these derivatives, compounds **8g**, **9b**, and **9d** demonstrated the highest potency against A375P human melanoma cell line with IC_{50} less than 0.1 μM. Another four compounds, **8b**, **9a**, **9c**, and **9e**, were more potent than Sorafenib against A375P with IC_{50} ranging from 0.1 to 0.8 μM. In addition, compounds **8d**, **8e**, **9g**, and **9i** were more potent than Sorafenib against A375P with IC_{50} in the range of 1.0–2.7 μM. Compounds **8b**, **8g**, **9b**–**d**, and **9i** showed superior selectivity towards melanoma cells than NIH3T3 fibroblasts, compared with Sorafenib.

The most potent diarylurea **8g** and diarylamide **9d** were further tested over nine melanoma cell lines at the NCI. Both compounds demonstrated high potency over the 9-cell line panel. The IC_{50} values of compounds **8g** and **9d** were in nanomolar range over 3 and 7 cell lines, respectively. Further modifications of these compounds in order to improve their potency are currently in progress.

4. Experimental

4.1. General

All melting points were obtained on a Walden Precision Apparatus Electrothermal 9300 apparatus and are uncorrected. Mass spectra (MS) were taken in ESI mode on a Waters 3100 Mass Detector (Waters, Milford, MA, USA). Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker ARX-300, 300 MHz spectrometer (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Purities of the target compounds (>95%) were determined by LC–MS analysis using the following system: Waters 2998 photodiode array detector, Waters 3100 mass detector, Waters SFO system fluidics organizer, Waters 2545 binary gradient module, Waters reagent manager, Waters 2767 sample manager, Sunfire™ C18 column (4.6 × 50 mm, 5 μm particle size); Solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) in water; solvent B: 0.035% TFA in CH_3OH; flow rate = 3.0 mL/min; the AUC was calculated using Waters MassLynx 4.1 software. All reagents and solvents were purchased from Aldrich chemical Co. and Tokyo Chemical Industry (TCI) Co., and used without further purification.

4.2. 7-Hydroxy-1H-pyrrolo[2,3-b]pyridinium 3-chlorobenzoate (**2**)

It was prepared following the literature procedure [20,21]. Mp: 141–143 °C (Lit. mp: 144.1–146 °C [21]).

4.3. 4-Chloro-7-azaindole (**3**)

It was prepared following the literature procedure [21]. Mp: 176–177 °C (Lit. mp: 175.3–177 °C [21]).

4.4. General procedure for preparation of compounds **4a,b**

A mixture of compound **3** (230 mg, 2.0 mmol) and the appropriate nitroaniline derivative (10.0 mmol) was fused at 180 °C for 2–5 h with stirring. The reaction mixture was cooled to room temperature and dissolved in ethanol (150 mL). The resulting suspension was filtered to remove the insoluble material and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, ethyl acetate–methanol 10:1 v/v then switching to ethyl acetate–methanol 5:1 v/v) to obtain the desired purified products.

*4.4.1. 1-(4-Nitrophenyl)-1H-pyrrolo[3,2-c]pyridin-4-amine hydrochloride (**4a**)*

Yield 18%; ^{1}H NMR (DMSO-d_6): δ 13.40 (brs, 1H), 8.42 (d, 2H, $J = 8.9$ Hz), 7.90 (d, 2H, $J = 9.0$ Hz), 7.80 (d, 1H, $J = 5.9$ Hz), 7.68 (d, 1H, $J = 6.8$ Hz), 7.34 (d, 1H, $J = 6.3$ Hz), 7.01 (d, 1H, $J = 7.2$ Hz); MS *m*/*z*: 255.95 ($M^+ + 2$, 90.57%), 254.65 (M^+, 100%).

*4.4.2. 1-(2-Methyl-4-nitrophenyl)-1H-pyrrolo[3,2-c]pyridin-4-amine hydrochloride (**4b**)*

Yield 9.5%; ^{1}H NMR (DMSO-d_6): δ 8.28–8.27 (m, 1H), 8.08 (d, 1H, $J = 5.2$ Hz), 7.53–7.50 (m, 2H), 7.43–7.36 (m, 2H), 7.30–7.25 (m, 3H), 6.76 (d, 1H, $J = 5.2$ Hz), 6.03–6.01 (m, 1H), 2.37 (s, 3H); MS *m*/*z*: 270.00 ($M^+ + 2$, 90%), 268.69 (M^+, 100%).

4.5. General procedure for preparation of compounds **5a,b**

To a stirred solution of compound **4a,b** (1.6 mmol) in acetonitrile (25 mL) at room temperature, diisopropylamine (0.7 mL, 4.0 mmol) was slowly added under nitrogen atmosphere. Benzoyl chloride (0.2 mL, 1.9 mmol) was slowly added and the reaction mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated under reduced pressure, and water (20 mL) and CH_2Cl_2 (20 mL) were added to the residue. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layer extracts were washed with brine, 1 N HCl, and then aqueous $NaHCO_3$, dried over

anhydrous $MgSO_4$, and filtered. The organic solvent was evaporated under reduced pressure and the residue was purified by column chromatography to give the desired products.

*4.5.1. N-(1-(4-nitrophenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl) benzamide (**5a**)*

It was purified by column chromatography (silica gel, hexane–ethyl acetate 3:1 v/v); yield 85%; ^{1}H NMR (DMSO-d_6): δ 8.49 (d, 2H, J = 5.0 Hz), 8.10 (d, 1H, J = 6.0 Hz), 7.88–7.82 (m, 6H), 7.63 (d, 1H, J = 6.0 Hz), 7.55–7.50 (m, 2H), 7.43–7.38 (m, 2H); MS m/z: 359.11 ($M^+ + 1$, 100%), 358.24 (M^+, 27.71%).

*4.5.2. N-(1-(2-methyl-4-nitrophenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl)benzamide (**5b**)*

It was purified by column chromatography (silica gel, hexane–ethyl acetate 1:1 v/v); yield 15%; ^{1}H NMR (DMSO-d_6): δ 11.86 (brs, 1H), 8.28 (d, 1H, J = 2.6 Hz), 8.09 (d, 1H, J = 5.2 Hz), 8.04–8.01 (m, 2H), 7.54–7.51 (m, 2H), 7.43–7.37 (m, 2H), 7.31–7.26 (m, 3H), 6.77 (d, 1H, J = 5.1 Hz), 6.02 (d, 1H, J = 2.8 Hz), 2.37 (s, 3H); MS m/z: 373.42 ($M^+ + 1$, 100%), 372.40 (M^+, 42%).

*4.6. General procedure for preparation of compounds **6a,b***

A mixture of compound **5a,b** (1.3 mmol) and Pd/C (10%) in anhydrous THF (20 mL) was stirred in hydrogen atmosphere at room temperature for 2 h. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography to give the purified desired products.

*4.6.1. N-(1-(4-aminophenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl) benzamide (**6a**)*

It was purified by column chromatography (silica gel, hexane–ethyl acetate 1:2 v/v); yield 48%; ^{1}H NMR (DMSO-d_6): δ 10.78 (brs, 1H), 8.12–7.93 (m, 3H), 7.63–7.46 (m, 5H), 7.20 (d, 2H, J = 8.6 Hz), 6.75–6.62 (m, 3H), 5.39 (brs, 2H); ^{13}C NMR (DMSO-d_6): δ 164.4, 152.9, 145.0, 141.0, 133.4, 132.8, 132.5, 132.3, 129.0, 127.3, 124.7, 120.7, 117.5, 117.3, 104.7; MS m/z: 330.24 ($M^+ + 2$, 23.65%), 329.21 ($M^+ + 1$, 100%).

*4.6.2. N-(1-(4-amino-2-methylphenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl)benzamide (**6b**)*

It was purified by column chromatography (silica gel, hexane–ethyl acetate 1:1 v/v); yield 32%; ^{1}H NMR (DMSO-d_6): δ 11.68 (brs, 1H), 7.94 (d, 2H, J = 6.9 Hz), 7.58–7.47 (m, 7H), 6.89 (d, 1H, J = 8.2 Hz), 6.45–6.39 (m, 2H), 4.94 (brs, 2H), 2.06 (s, 3H); MS m/z: 344.50 ($M^+ + 2$, 27%), 343.45 ($M^+ + 1$, 100%).

*4.7. N-(1-(4-[3-(2,3-dichlorophenyl)urea]phenyl)-1H-pyrrolo[3,2-c]pyridin-4-yl) benzamide (**8a**)*

To a solution of compound **6a** (22.0 mg, 0.06 mmol) in anhydrous THF (10 mL), 2,3-dichlorophenyl isocyanate (13.7 mg, 0.06 mmol) was added. The reaction mixture was stirred under nitrogen atmosphere at room temperature for 8 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (silica gel, hexane–ethyl acetate 3:1 v/v then switching to hexane–ethyl acetate 1:1 v/v) to obtain the purified product **8a** (9.0 mg, 26%). Mp: >280 °C; ^{1}H NMR (DMSO-d_6): δ 11.71 (brs, 1H), 10.84 (brs, 1H), 9.74 (brs, 1H), 8.50 (s, 1H), 8.20 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 8.12–8.09 (m, 2H), 7.69–7.63 (m, 3H), 7.58–7.52 (m, 5H), 7.45–7.30 (m, 3H), 6.66 (d, 1H, J = 3.2 Hz); MS m/z: 518.38 ($M^+ + 2$, 60%), 517.42 ($M^+ + 1$, 80%), 516.45 (M^+, 100%).

Synthesis of compounds **8b–g** was achieved by the same procedure as described for compound **8a**.

*4.7.1. 1-(4-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-3-(3,4-dichloro-phenyl)urea (**8b**)*

Yield 14%; mp: 172–175 °C;^{1}H NMR (DMSO-d_6): δ 10.87 (brs, 1H), 9.26–9.16 (m, 2H), 8.12–8.09 (m, 3H), 7.92 (d, 1H, J = 2.4 Hz), 7.74–7.61 (m, 3H), 7.57–7.48 (m, 5H), 7.41–7.31 (m, 2H), 6.64 (d, 1H, J = 3.2 Hz); ^{13}C NMR (DMSO-d_6): δ 170.8, 152.8, 140.4, 139.2, 131.5, 131.1, 129.8, 129.2, 128.9, 128.6, 127.4, 127.0, 125.2, 124.8, 123.7, 122.9, 121.8, 120.0, 119.9, 118.9, 104.7; MS m/z: 518.38 ($M^+ + 2$, 34%), 517.42 ($M^+ + 1$, 89%), 516.45 (M^+, 100%).

*4.7.2. 1-(4-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-3-(3-trifluoromethyl-phenyl)urea (**8c**)*

Yield 35%; mp: 158–160 °C; ^{1}H NMR (DMSO-d_6): δ 11.71 (brs, 1H), 10.83 (brs, 1H), 8.59–8.57 (m, 2H), 8.11–8.09 (m, 4H), 7.74 (d, 2H, J = 7.8 Hz), 7.66 (d, 2H, J = 3.2 Hz), 7.62–7.59 (m, 2H), 7.58–7.52 (m, 4H), 7.39 (d, 1H, J = 5.4 Hz), 6.66 (brs, 1H); MS m/z: 517.33 ($M^+ + 2$, 46.6%), 516.36 ($M^+ + 1$, 100%), 515.38 (M^+, 21%).

*4.7.3. 1-(4-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-3-(4-chloro-3-trifluoromethylphenyl)urea (**8d**)*

Yield 25%; mp: 204–206 °C; ^{1}H NMR (DMSO-d_6): δ 10.83 (brs, 1H), 9.37 (brs, 1H), 9.24 (brs, 1H), 8.15 (d, 1H, J = 2.0 Hz), 8.11–8.08 (m, 3H), 7.73–7.65 (m, 5H), 7.57–7.54 (m, 5H), 7.39 (d, 1H, J = 5.8 Hz), 6.65 (d, 1H, J = 3.2 Hz); ^{13}C NMR (DMSO-d_6): δ 164.8, 152.9, 141.0, 139.8, 139.0, 132.8, 132.5, 132.3, 129.7, 129.1, 128.8, 128.7, 127.3, 127.0, 125.7, 125.2, 124.7, 123.6, 122.8, 121.9, 120.1, 119.5, 117.3, 104.6; MS m/z: 552.22 ($M^+ + 2$, 38%), 551.18 ($M^+ + 1$, 95%), 550.14 (M^+, 100%).

*4.7.4. 1-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-(4-benzamido-1H-pyrrolo[3,2-c] pyridine-1-yl)phenyl)urea (**8e**)*

Yield 18%; mp: >280 °C; ^{1}H NMR (DMSO-d_6): δ 10.84 (brs, 1H), 9.50 (brs, 1H), 9.29 (brs, 1H), 8.17–8.03 (m, 4H), 7.73–7.67 (m, 4H), 7.62–7.52 (m, 5H), 7.39 (d, 1H, J = 5.3 Hz), 6.86 (s, 1H), 6.65 (brs, 1H); ^{13}C NMR (DMSO-d_6): δ 168.1, 153.0, 141.1, 139.1, 132.2, 131.9, 129.0, 128.6, 127.8, 126.8, 125.3, 124.9, 124.3, 123.1, 122.3, 121.6, 120.1, 117.4, 104.9; MS m/z: 584.52 ($M^+ + 1$, 100%), 583.56 (M^+, 31%).

*4.7.5. 1-(4-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-3-methylphenyl)-3-(2,3-dichlorophenyl)urea (**8f**)*

Yield 53%; mp: 181–184 °C; ^{1}H NMR (DMSO-d_6): δ 11.75 (brs, 1H), 9.68 (brs, 1H), 8.64 (brs, 1H), 8.12–8.04 (m, 2H), 7.50–7.45 (m, 2H), 7.40–7.23 (m, 7H), 7.04–6.96 (m, 1H), 6.74–6.65 (m, 2H), 6.00 (d, 1H, J = 2.4 Hz), 2.17 (s, 3H); MS m/z: 532.15 ($M^+ + 2$, 59.63%), 531.17 ($M^+ + 1$, 30.42%), 530.19 (M^+, 100%).

*4.8. 1-(4-(4-Benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-3-(4-((4-ethyl-piperazin-1-yl)methyl)-3-trifluoromethylphenyl)urea (**8g**)*

A mixture of 4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)aniline (**7**, 17.5 mg, 0.06 mmol), triethylamine (0.014 mL, 0.1 mmol), and *p*-nitrophenyl chloroformate (12.4 mg, 0.06 mmol) in 1,4-dioxane (5 mL) was heated at 60 °C for 2 h. A solution of **6a** (20 mg, 0.06 mmol) in 1,4-dioxane (5 mL) was slowly added thereto. The reaction mixture was heated at 90 °C overnight. The reaction mixture was concentrated under reduced pressure and then partitioned between water (5 mL) and ethyl acetate (5 mL). The organic layer was separated and the aqueous layer was then extracted with ethyl acetate (3 × 3 mL). 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 (silica gel, hexane–ethyl acetate 3:1 v/v then switching to hexane–ethyl acetate 1:1 v/v) to yield compound **8g** (6.0 mg, 15%). Mp: 265–267 °C; ^{1}H NMR (DMSO-d_6): δ 10.85 (brs, 1H), 9.19–9.14 (m, 2H), 8.11–8.08 (m, 3H), 8.02–7.97 (m, 2H), 7.72–7.66 (m, 3H), 7.63–7.53 (m, 3H), 7.42 (dd, 1H,

$J = 6.5$ Hz, $J = 5.6$ Hz), 2.73 (s, 4H), 2.38–2.27 (m, 6H), 1.23 (s, 4H), 0.98 (q, 2H, $J = 7.2$ Hz), 0.83 (t, 3H, $J = 7.3$ Hz); MS *m/z*: 642.75 ($M^+ + 1$, 38%), 641.72 (M^+, 100%).

4.9. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-3-trifluoromethyl-benzamide (**9a**)

A mixture of compound **6a** (20.0 mg, 0.06 mmol), 3-trifluoromethyl-benzoic acid (23.11 mg, 0.12 mmol), HOBt (18.1 mg, 0.13 mmol), and EDCI (29.1 mg, 0.15 mmol) in dry DMF (1.0 mL) was cooled to 0 °C under nitrogen atmosphere. To the reaction mixture, triethylamine (0.002 mL, 0.015 mmol) was added at 0 °C. The mixture was then stirred at 80 °C for 12 h. The reaction mixture was cooled and then partitioned between water (5 mL) and ethyl acetate (5 mL) and the organic layer was separated. The aqueous layer was then extracted with ethyl acetate (3 × 3 mL) 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 (silica gel, hexane–ethyl acetate 3:1 v/v then switching to hexane–ethyl acetate 1:1 v/v) to yield compound **9a** (5.0 mg, 16.4%). Mp: 166–169 °C (dec.); ^{1}H NMR (DMSO-d_6): δ 10.95–10.85 (m, 2H), 8.51 (s, 1H), 8.43–8.37 (m, 2H), 8.17–7.97 (m, 4H), 7.82–7.53 (m, 5H), 7.33–7.26 (m, 3H), 6.80 (d, 2H, $J = 8.5$ Hz); MS *m/z*: 501.50 ($M^+ + 1$, 80%), 500.46 (M^+, 100%).

Synthesis of compounds **9b**–**i** was achieved by the same procedure as described for compound **9a**.

4.9.1. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-4-chloro-3-trifluoromethyl-benzamide (**9b**)

Yield 21%; mp: 133–136 °C; ^{1}H NMR (DMSO-d_6): δ 10.86 (brs, 1H), 10.51 (brs, 1H), 8.43–8.30 (m, 2H), 8.13–7.96 (m, 7H), 7.68–7.45 (m, 6H), 6.74–6.67 (m, 1H); MS *m/z*: 537.02 ($M^+ + 2$, 44%), 536.00 ($M^+ + 1$, 90%), 535.00 (M^+, 100%).

4.9.2. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-3-(4-methyl-1H-imidazol-1-yl)-5-trifluoromethyl-benzamide (**9c**)

Yield 11%; mp: 206–207 °C; ^{1}H NMR (DMSO-d_6): δ 8.35 (brs, 1H), 8.31 (brs, 1H), 8.30–8.28 (m, 2H), 8.23 (d, 1H, $J = 1.4$ Hz), 8.16–8.02 (m, 5H), 7.93 (brs, 1H), 7.74–7.71 (m, 2H), 7.65–7.54 (m, 5H), 7.47 (d, 1H, $J = 5.5$ Hz), 6.89 (d, 1H, $J = 3.2$ Hz), 2.02 (s, 3H); MS *m/z*: 582.47 ($M^+ + 2$, 44.7%), 581.45 ($M^+ + 1$, 100%), 580.43 (M^+, 91%).

4.9.3. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-4-morpholino-3-trifluoromethyl-benzamide (**9d**)

Yield 11%; mp: 220–223 °C (dec.); ^{1}H NMR (DMSO-d_6): δ 10.92 (brs, 1H), 10.62 (brs, 1H), 8.43–8.28 (m, 2H), 8.11–8.05 (m, 2H), 8.01 (d, 2H, $J = 8.8$ Hz), 7.71 (d, 2H, $J = 2.6$ Hz), 7.68–7.52 (m, 4H), 7.50–7.44 (m, 2H), 7.29 (brs, 1H), 6.74–6.69 (m, 1H), 3.76 (t, 4H, $J = 3.1$ Hz), 2.96 (t, 4H, $J = 3.3$ Hz); ^{13}C NMR (DMSO-d_6): δ 164.5, 155.0, 141.0, 140.2, 134.3, 133.8, 133.5, 132.4, 130.9, 129.0, 128.8, 128.6, 127.5, 125.8, 124.8, 124.3, 123.1, 122.0, 119.8, 114.7, 104.8, 66.9, 53.6; MS (ESI) *m/z*: 587.63 ($M^+ + 2$, 38.7%), 586.60 ($M^+ + 1$, 100%), 585.56 (M^+, 87%).

4.9.4. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-phenyl]-3-morpholino-5-trifluoromethyl-benzamide (**9e**)

Yield 8%; mp: 233–234 °C; ^{1}H NMR (DMSO-d_6): δ 10.86 (brs, 1H), 10.59 (brs, 1H), 8.11–8.06 (m, 2H), 8.05–7.99 (m, 2H), 7.72–7.61 (m, 6H), 7.57–7.54 (m, 2H), 7.47–7.42 (m, 2H), 7.21 (d, 1H, $J = 8.3$ Hz), 6.73 (d, 1H, $J = 8.8$ Hz), 3.78 (t, 4H, $J = 4.3$ Hz), 2.74 (t, 4H, $J = 4.1$ Hz); ^{13}C NMR (DMSO-d_6): δ 164.5, 155.0, 141.1, 140.2, 134.3, 133.8, 133.5, 132.3, 130.9, 129.0, 128.8, 128.6, 127.5, 125.8, 124.8, 124.3, 123.0, 122.0, 119.7, 114.7, 104.7, 66.9, 53.6; MS *m/z*: 586.60 ($M^+ + 1$, 100%), 585.61 (M^+, 47%).

4.9.5. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-3-methylphenyl]-3-trifluoromethyl-benzamide (**9f**)

Yield 6.7%; mp: 48–51 °C; ^{1}H NMR (DMSO-d_6): δ 11.77 (brs, 1H), 10.50 (brs, 1H), 8.25 (dd, 1H, $J = 5.0$ Hz, $J = 8.3$ Hz), 8.08 (d, 1H, $J = 5.2$ Hz), 7.98 (d, 1H, $J = 7.8$ Hz) 7.81–7.72 (m, 2H), 7.58–7.49 (m, 3H), 7.40–7.34 (m, 2H), 7.26–7.10 (m, 2H), 7.00 (d, 1H, $J = 9.0$ Hz), 6.69 (d, 2H, $J = 5.2$ Hz), 6.01 (brs, 1H), 2.21 (s, 3H); MS *m/z*: 515.20 ($M^+ + 1$, 100%), 514.27 (M^+, 28.7%).

4.9.6. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-3-methylphenyl]-4-chloro-3-trifluoromethyl-benzamide (**9g**)

Yield 28%; mp: 105–106 °C; ^{1}H NMR (DMSO-d_6): δ 11.78 (brs, 1H), 10.53 (brs, 1H), 8.42–8.22 (m, 2H), 8.08 (d, 1H, $J = 5.2$ Hz), 7.93 (d, 1H $J = 5.6$ Hz), 7.73–7.62 (m, 2H), 7.54–7.49 (m, 3H), 7.42–7.26 (m, 2H), 7.13–7.08 (m, 2H), 6.69 (d, 1H, $J = 5.2$ Hz), 6.01 (brs, 1H), 2.21 (s, 3H); MS *m/z*: 551.00 ($M^+ + 2$, 88%), 550.00 ($M^+ + 1$, 100%), 549.00 (M^+, 33%).

4.9.7. N-[4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)-3-methylphenyl]-4-morpholino-3-trifluoromethyl-benzamide (**9h**)

Yield 26%; mp: 237–240 °C; ^{1}H NMR (DMSO-d_6): δ 11.77 (brs, 1H), 11.68 (brs, 1H), 8.21 (d, 1H, $J = 7.0$ Hz), 8.09–8.05 (m, 1H), 7.70–7.64 (m, 1H), 7.58–7.44 (m, 3H), 7.39–7.23 (m, 5H), 6.75–6.63 (m, 2H), 6.27 (d, 1H, $J = 2.6$ Hz), 6.00 (d, 1H, $J = 3.3$ Hz), 3.72 (t, 4H, $J = 3.8$ Hz), 2.96 (t, 4H, $J = 3.2$ Hz), 1.99 (s, 3H); MS *m/z*: 600.63 ($M^+ + 1$, 100%), 599.68 (M^+, 39.5%).

4.9.8. N-(4-(4-benzamido-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-5-(2-chloro-5-trifluoromethylphenyl)furan-2-carboxamide (**9i**)

Yield 29%; mp: 103–105 °C (dec.); ^{1}H NMR (DMSO-d_6): δ 10.84 (brs, 1H), 10.61 (brs, 1H), 8.27–8.14 (m, 4H), 8.02 (d, 2H, $J = 8.9$ Hz), 7.97–7.87 (m, 2H), 7.72–7.46 (m, 8H), 7.45 (d, 1H, $J = 5.9$ Hz), 6.75–6.67 (m, 1H); MS *m/z*: 603.07 ($M^+ + 2$, 90%), 602.05 ($M^+ + 1$, 100%), 601.03 (M^+, 28%).

4.10. 1-(4-Aminophenyl)-1H-pyrrolo[3,2-c]pyridin-4-amine (**10**)

A mixture of **6a** (0.47 g, 1.31 mmol) and $SnCl_2 \cdot H_2O$ (1.48 g, 6.55 mmol) in ethanol (20 mL) was heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the residue was partitioned between aqueous $NaHCO_3$ and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. 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 (silica gel, ethyl acetate) to yield compound **8** (0.12 g, 40.8%). MS *m/z*: 225.27 ($M^+ + 1$, 100%), 224.25 (M^+, 90%).

4.11. Synthesis of compounds **8h,i**

Synthesis of compounds **8h,i** was achieved by the same procedure as described for compound **8a**.

4.11.1. 1-(4-(4-Amino-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-3-(3-trifluoromethyl-phenyl)urea (**8h**)

It was purified by column chromatography (silica gel, hexane–ethyl acetate 2:1 v/v then switching to ethyl acetate); yield 8%; mp > 280 °C; ^{1}H NMR (DMSO-d_6): δ 8.56 (d, 1H, $J = 6.6$ Hz), 7.93–7.72 (m, 3H), 7.54–7.44 (m, 3H), 7.43–7.30 (m, 5H), 7.15 (d, 1H, $J = 6.4$ Hz); MS *m/z*: 412.40 ($M^+ + 1$, 100%), 411.43 (M^+, 30.5%).

4.11.2. 1-(4-(4-Amino-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-3-(4-chloro-3-trifluoro-methylphenyl)urea (**8i**)

It was purified by column chromatography (silica gel, hexane–ethyl acetate 2:1 v/v then switching to hexane–ethyl acetate 1:3 v/v); yield 27%; mp > 280 °C; ^{1}H NMR (CD_3OD): δ 8.06

(d, 1H, $J = 2.4$ Hz), 7.95 (d, 1H, $J = 2.2$ Hz), 7.72–7.64 (m, 4H), 7.59–7.51 (m, 3H), 7.44 (d, 2H, $J = 3.6$ Hz), 7.40–7.32 (m, 2H), 6.98 (d, 1H, $J = 3.1$ Hz), 6.87 (brd, 1H, $J = 6.8$ Hz); MS *m/z*: 447.90 ($M^+ + 2$, 60%), 446.86 ($M^+ + 1$, 43.7%), 445.82 (M^+, 100%).

4.12. Synthesis of compounds **9j,k**

Synthesis of compounds **9j,k** was achieved by the same procedure as described for compound **9a**.

4.12.1. N-(4-(4-Amino-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-4-chloro-3-trifluoromethylbenzamide (**9j**)

It was purified by column chromatography (silica gel, hexane–ethyl acetate 3:1 v/v then switching to hexane–ethyl acetate 1:1 v/v); Yield 12%; mp > 280 °C; ^{1}H NMR (DMSO-d_6): δ (ppm) 8.07 (d, 1H, $J = 2.3$ Hz), 7.94 (d, 1H, $J = 2.2$ Hz), 7.70–7.63 (m, 4H), 7.59–7.50 (m, 3H), 7.44 (d, 2H, $J = 3.7$ Hz), 7.40–7.33 (m, 2H), 7.00 (d, 1H, $J = 3.0$ Hz), 6.88 (brd, 1H, $J = 6.9$ Hz); MS *m/z*: 432.87 ($M^+ + 2$, 40%), 431.89 ($M^+ + 1$, 100%), 430.91 (M^+, 58%).

4.12.2. N-(4-(4-Amino-1H-pyrrolo[3,2-c]pyridin-1-yl)phenyl)-3-trifluoromethyl-4-morpholinobenzamide (**9k**)

It was purified by column chromatography (silica gel, hexane–ethyl acetate 3:1 v/v then switching to ethyl acetate); Yield 37%; mp: 253–256 °C (dec.); ^{1}H NMR (DMSO-d_6): δ (ppm) 8.38 (d, 1H, $J = 2.6$ Hz), 8.29–8.26 (m, 2H), 8.02–7.99 (m, 2H), 7.75–7.51 (m, 5H), 7.20 (brd, 1H, $J = 8.0$ Hz), 6.74 (brd, 1H, $J = 8.2$ Hz), 3.74–3.65 (m, 4H), 3.01–2.97 (m, 4H); MS *m/z*: 482.50 ($M^+ + 1$, 100%), 481.46 (M^+, 46.3%).

4.13. Evaluation of the antiproliferative activity against A375P human melanoma cell line and NIH3T3 fibroblasts

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.

The same procedure applied in case of A375P cell line was conducted for NIH3T3 fibroblasts, except for using bovine calf serum (BCS) instead of FBS.

4.14. Nine melanoma cell line screening at the NCI

Screening against a panel of nine melanoma cell lines was applied at the National Cancer Institute (NCI), Bethesda, Maryland, USA [24], applying the following procedure. The human cell lines are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96-well microtiter plates in 100 μL at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. Additional four, 10-fold or 1/2 log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 μL of these different drug dilutions are added to the appropriate microtiter wells already containing 100 μL of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are kept for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 μL of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

- $[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$.
- $[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Growth inhibition of 50% (IC_{50}) is calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation.

Acknowledgements

We would like to thank the National Cancer Institute (NCI), Bethesda, Maryland, USA, for performing the anticancer testing over nine melanoma cell lines. We are also grateful to Korea Institute of Science and Technology (KIST) for financial support.

References

[1] C. Garbe, A. Hauschild, M. Volkenandt, D. Schadendorf, W. Stolz, U. Reinhold, R.D. Kortmann, C. Kettelhack, B. Frerich, U. Keilholz, R. Dummer, G. Sebastian, W. Tilgen, G. Schuler, A. Mackensen, R. Kaufmann, Melanoma Res. 17 (2007) 393–399.
[2] E. Atallah, L. Flaherty, Curr. Treat. Options Oncol. 6 (2005) 185–193.
[3] A. Barth, L.A. Wanek, D.L. Morton, J. Am. Coll. Surg. 181 (1995) 193–201.
[4] C.M. Anderson, A.C. Buzaid, S.S. Legha, Oncol. (Williston Park) 9 (1995) 1149–1158.
[5] V. Gray-Schopfer, C. Wellbrock, R. Marais, Nature 445 (2007) 851–857.
[6] C. Garbe, T.K. Eigentler, Melanoma Res. 17 (2007) 117–127.
[7] H.B. Koon, M.B. Atkins, Expert Rev. Anticancer Ther. 7 (2007) 79–88.
[8] D.H. Lawson, Cancer Control 12 (2005) 236–241.
[9] S.A. Rosenburg, M.T. Lotze, J.C. Yang, P.M. Aebersold, W.M. Linehan, C.A. Seipp, D.E. White, Ann. Surg. 210 (1989) 474–484.
[10] M.B. Atkins, M.T. Lotze, J.P. Dutcher, R.I. Fisher, G. Weiss, K. Margolin, J. Abrams, M. Sznol, D. Parkinson, M. Hawkins, C. Paradise, L. Kunkel, S.A. Rosenberg, J. Clin. Oncol. 17 (1999) 2105–2116.
[11] M.-H. Jung, C.-H. Oh, Bull. Korean Chem. Soc. 29 (2008) 2231–2236.

[12] B.S. Nam, H. Kim, C.-H. Oh, S.H. Lee, S.J. Cho, T.B. Sim, J.-M. Hah, D.J. Kim, J.H. Choi, K.H. Yoo, Bioorg. Med. Chem. Lett. 19 (2009) 3517–3520.
[13] M.-H. Jung, H. Kim, W.-K. Choi, M.I. El-Gamal, J.-H. Park, K.H. Yoo, T.B. Sim, S.H. Lee, D. Baek, J.-M. Hah, J.-H. Cho, C.-H. Oh, Bioorg. Med. Chem. Lett. 19 (2009) 6538–6543.
[14] W.-K. Choi, C.-H. Oh, Bull. Korean Chem. Soc. 30 (2009) 2027–2031.
[15] H.J. Kim, M.-H. Jung, H. Kim, M.I. El-Gamal, T.B. Sim, S.H. Lee, J.H. Hong, J.-M. Hah, J.-H. Cho, J.H. Choi, K.H. Yoo, C.-H. Oh, Bioorg. Med. Chem. Lett. 20 (2010) 413–417.
[16] J. Lee, H. Kim, H. Yu, J.Y. Chung, C.-H. Oh, K.H. Yoo, T. Sim, J.-M. Hah, Bioorg. Med. Chem. Lett. 20 (2010) 1573–1577.
[17] H. Yu, Y. Jung, H. Kim, J. Lee, C.-H. Oh, K.H. Yoo, T. Sim, J.-M. Hah, Bioorg. Med. Chem. Lett. 20 (2010) 3805–3808.
[18] J. Lee, B.S. Nam, H. Kim, C.-H. Oh, S.H. Lee, S.J. Cho, T.B. Sim, J.-M. Hah, D.J. Kim, J. Tae, K.H. Yoo, Bioorg. Med. Chem. Lett. 20 (2010) 5722–5725.
[19] S.M. Wilhelm, L. Adnane, P. Newell, A. Villanueva, J.M. Llovet, M. Lynch, Mol. Cancer Ther. 7 (2008) 3129–3140.
[20] C.-C. Cheng, C.-P. Chang, W.-S. Yu, F.-T. Hung, Y.-I. Liu, G.-R. Wu, P.-T. Chou, J. Phys. Chem. A 107 (2003) 1459–1471.
[21] X. Wang, B. Zhi, J. Baum, Y. Chen, R. Crockett, L. Huang, S. Eisenberg, J. Ng, R. Larsen, M. Martinelli, P. Reider, J. Org. Chem. 71 (2006) 4021–4023.
[22] N.S. Girgis, S.B. Larson, R.K. Robins, H.B. Cottam, J. Heterocycl. Chem. 26 (1989) 317–325.
[23] M.I. El-Gamal, M.-H. Jung, C.-H. Oh, Bioorg. Med. Chem. Lett. 20 (2010) 3216–3218.
[24] NCI web site: www.dtp.nci.nih.gov.