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Synthesis and antiproliferative activity of pyrrolo[3,2-*b*]pyridine derivatives against melanoma

Hee Jin Kim [a], Myung-Ho Jung [a], Hwan Kim [a], Mohammed I. El-Gamal [a], Tae Bo Sim [a], So Ha Lee [a], Jun Hee Hong [c], Jung-Mi Hah [a], Jung-Hyuck Cho [a], Jung Hoon Choi [b], Kyung Ho Yoo [a], Chang-Hyun Oh [a,*]

[a] *Biomaterials Research Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea*
[b] *Department of Chemistry, Hanyang University, Seoul 133-791, Republic of Korea*
[c] *College of Pharmacy, Chosun University, Gwangju 501-759, Republic of Korea*

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ABSTRACT

Synthesis of a new series of diarylureas and amides having pyrrolo[3,2-*b*]pyridine scaffold is described. Their in vitro antiproliferative activity against human melanoma cell line A375 and HS 27 human fibroblast cell line was tested and the effect of substituents on the pyrrolo[3,2-*b*]pyridine was investigated. The newly synthesized compounds, except *meta*-substituted derivatives (**Ij–k** and **Iv–w**), generally showed superior or similar activity against A375 to Sorafenib. Among all of these derivatives, compounds **Ir** and **It** having 5-benzylamide substituted 4′-amide moieties showed the most potent antiproliferative activity against A375.

Melanoma is the most aggressive form of skin cancer and is the fastest growing type of cancer in the United States.[1,2] 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-years survival rate is less than 15%.[2,3] 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]

The current treatments involve surgical removal of the tumor, immunotherapy, radiotherapy, chemotherapy, various combinations, or use of new treatments in clinical trials. As for immunotherapy, interferon alfa-2b (Intron-A)[7] is approved by both FDA and EMEA for adjuvant treatment of melanoma patients, and aldesleukin (Proleukin)[8,9] is also approved for the treatment of metastatic melanoma in the USA.

It is recently reported that diarylurea derivatives such as Sorafenib are promising, potent, and selective antiproliferative agents for treatment of melanoma.[10–15] There is a considerable number and variety of diarylureas identified as anticancer agents.[16–21]

Encouraged by the interesting antiproliferative activity of diarylurea derivatives, a new series of diarylureas and amides containing pyrrolo[3,2-*b*]pyridine moiety was synthesized. We now report the synthesis and antiproliferative activity against human melanoma cell line A375 and HS 27 fibroblast of these compounds (Fig. 1).

The general method for synthesis of 5-substituted pyrrolo[3,2-*b*]pyridine ureas and amides is shown in Schemes 1 and 4. The methyl ester **2** can be prepared by treating **1**[22] with thionyl chloride in methonal. Synthesis of compound **3** can be achieved by heating **2** with iodine and sodium periodate in a solvent such as DMF. Compound **4** can be synthesized by reacting **3** with trimethylsilyl acetylene in the presence of $Pd(PPh_3)_2$, CuI, and an amine base and subsequent treatment with acetyl chloride and pyridine in dichloromethane. Cyclization to **5** can be performed by refluxing a solution of **4** in THF with tetrabutylammonium fluoride.

Preparation of the 5-ester substituted *p*-nitrophenyl compound **6** was achieved by treatment of **5** with 1-iodo-4-nitrobenzene in the presence of potassium carbonate, copper iodide, and L-proline. Reduction of nitro compound **6** using $Pd–C/H_2$ gave amino compound **7**, which was treated with the corresponding isocyanates to form the 5-ester substituted urea derivatives (**Ia–c**). The amide derivatives (**Il–o**) were obtained by condensation with the corresponding carboxylic acid derivative using EDCI/HOBt (Scheme 1).

The 5-ester substituted pyrrolo[3,2-*b*]pyridine intermediate **7** was converted into 5-amide substituted compound **8** by ammonolysis using aqueous ammonia and subsequently treated with the corresponding isocyanates or carboxylic acids and coupling reagents to provide urea derivatives (**Id–f**) or amide derivatives (**Ip–q**), respectively (Scheme 2).

* Corresponding author. Tel.: +82 2 958 5160; fax: +82 2 958 5189.
E-mail address: choh@kist.re.kr (C.-H. Oh).

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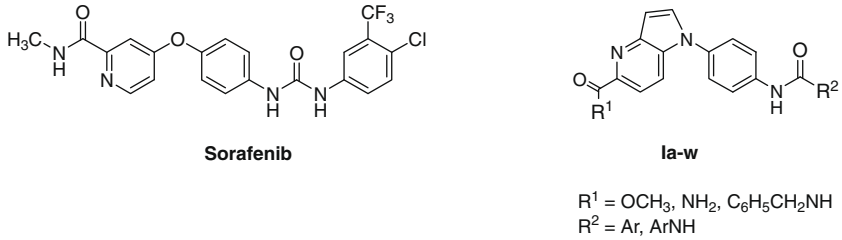
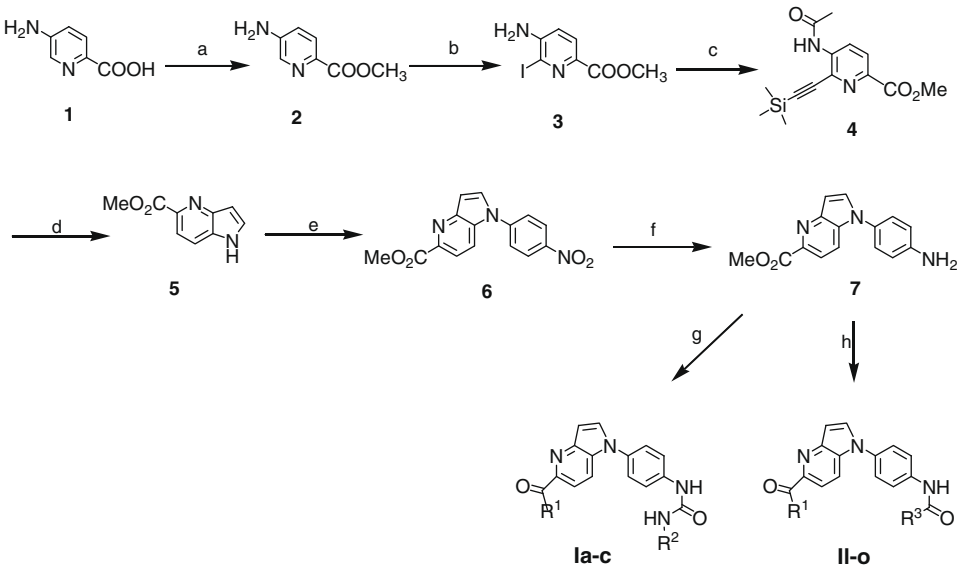


Figure 1. Structures of Sorafenib and pyrrolo[3,2-*b*]pyridine derivatives.



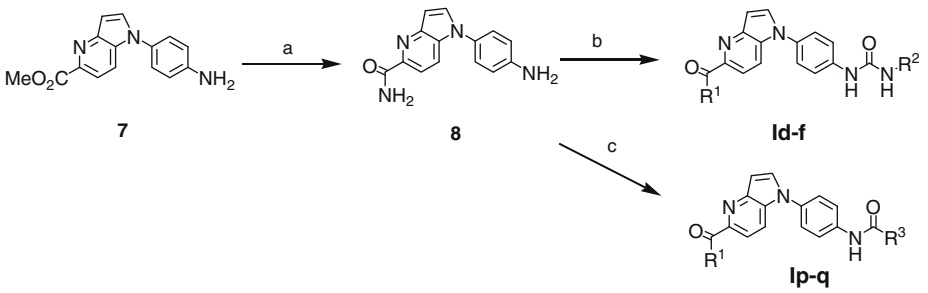
Scheme 1. Reagents and conditions: (a) $SOCl_2$, methanol, reflux, 98.7%; (b) I_2, $NaIO_4$, DMF, 60 °C, 72%; (c) (i) TMS acetylene, $Pd(PPh_3)_2$, CuI, amine base, (ii) acetyl chloride, pyridine, CH_2Cl_2, 78%; (d) TBAF, THF, 66 °C, 93%; (e) 1-iodo-4-nitrobenzene, K_2CO_3, CuI, L-proline, DMSO, 90 °C, 66%; (f) Pd/C, H_2, THF, 92%; (g) phenyl isocyanate, THF (**Ia**: 41%, **Ib**: 35%, **Ic**: 30%); (h) benzoic acid derivative, HOBt, EDCI, TEA, DMF (**II**: 50%, **Im**: 43%, **In**: 38%, **Io**: 42%).

The 5-benzyl amide substituted pyrrolo[3,2-*b*]pyridine intermediate **9** was obtained by reacting the 5-ester substituted compound **7** with benzylamine. Synthesis of compounds **Ig–i** and **Ir–u** was carried out by the same procedure as described for the preparation of **Ia–f** and **II–q** using the corresponding isocyanates and acids, respectively (Scheme 3).

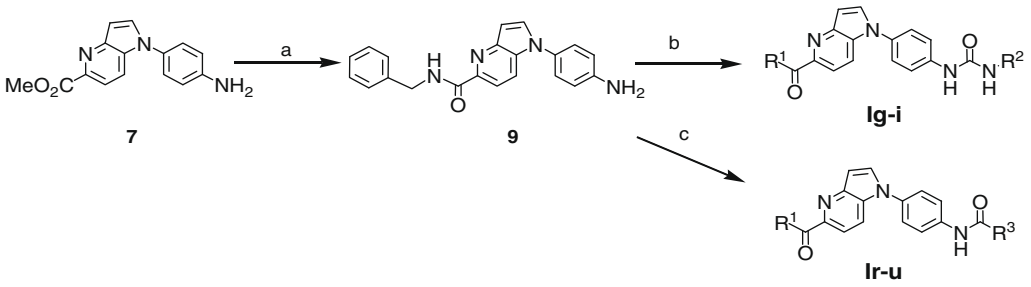
Preparation of the 5-ester substituted *m*-nitrophenyl compound **10** was accomplished by the same procedure as described for the preparation of **6** using 1-iodo-3-nitrobenzene. Reduction of the nitro group into amino group can be achieved as described for the preparation of compound **7**. Compounds **Ij–k** and **Iv–w** were also prepared as described for the preparation of compounds **Ia–i** and **II–u**, respectively (Scheme 4).

The antiproliferative activity of these newly synthesized compounds against human melanoma cell line A375 and HS 27 fibroblasts was tested.[23] The ability of pyrrolo[3,2-*b*]pyridine derivatives to inhibit the growth of A375 and HS 27 cell lines is summarized in Tables 1 and 2. Sorafenib was selected as the reference standard, because it has been extensively used in clinical trials for melanoma.[4,24]

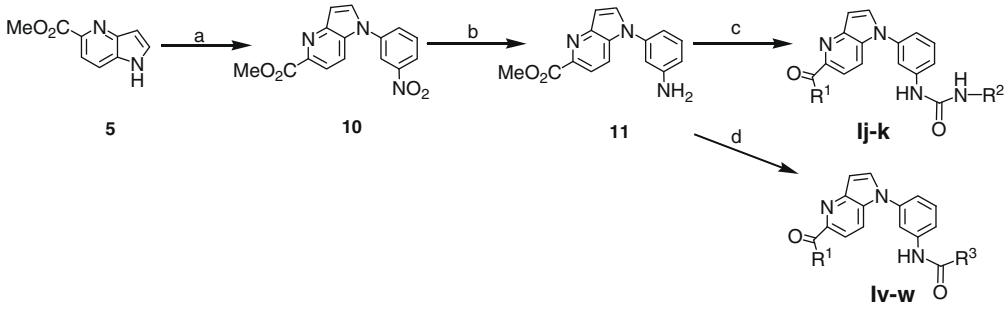
The newly synthesized compounds, except *meta*-substituted derivatives (**Ij–k** and **Iv–w**), generally showed superior or similar



Scheme 2. Reagents and conditions: (a) ammonia (aq), 60 °C, 65%; (b) phenyl isocyanate, THF (**Id**: 29%, **Ie**: 39%, **If**: 33%); (c) benzoic acid derivative, HOBt, EDCI, TEA, DMF (**Ip**: 35%, **Iq**: 31%).



Scheme 3. Reagents and conditions: (a) benzylamine, 50 °C, 98%; (b) phenyl isocyanate, THF (**Ig**: 43%, **Ih**: 39%, **Ii**: 38%); (c) benzoic acid derivative, HOBt, EDCI, TEA, DMF (**Ir**: 33%, **Is**: 47%, **It**: 31%, **Iu**: 45%).



Scheme 4. Reagents and conditions: (a) 1-iodo-3-nitrobenzene, K_2CO_3, CuI, L-proline, DMSO, 90 °C, 66%; (b) Pd/C, H_2, THF, 59%; (c) phenyl isocyanate, THF (**Ij**: 31%, **Ik**: 27%); (d) benzoic acid derivative, HOBt, EDCI, TEA, DMF (**Iv**: 33%, **Iw**: 30%).

Table 1
Antiproliferative activity of pyrrolo[3,2-*b*]pyridine ureas (**Ia–k**)

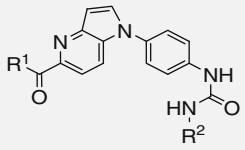
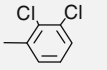
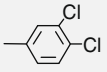
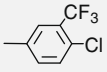
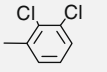
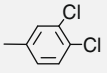
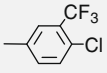
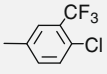
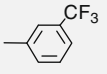
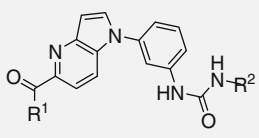
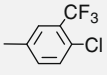
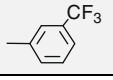
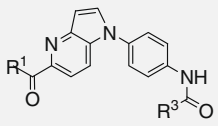
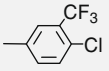
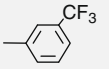
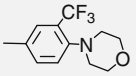
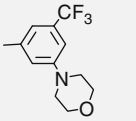
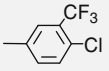
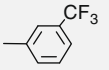
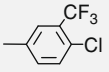
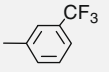
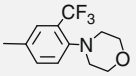
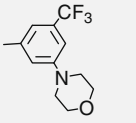
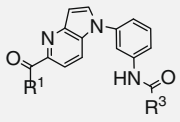
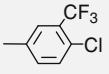
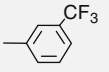
Structure	Compd no.	R^1	R^2	IC_{50} (μm)	
				A375P	HS 27
	**Ia**	OCH_3		6.4	>10
	Ib	OCH_3		3.8	2.8
	Ic	OCH_3		3.6	8.2
	Id	NH_2		4.6	9.8
	Ie	NH_2		3.2	6.6
	If	NH_2		9.9	>10
	Ig			>10	>10
	Ih			1.1	3.0
	Ii			7.9	>10
	**Ij**	OCH_3		9.5	>10
	Ik	OCH_3		>10	>10
Sorafenib				5.6	7.8

Table 2
Antiproliferative activity of pyrrolo[3,2-*b*]pyridine amides (**Il**–**w**)

Structure	Compd no.	R^1	R^3	IC_{50} (μm)	
				A375P	HS 27
	**Il**	OCH_3		3.0	2.9
	Im	OCH_3		9.5	9.8
	In	OCH_3		9.8	>10
	Io	OCH_3		>10	>10
	Ip	NH_2		8.9	>10
	Iq	NH_2		>10	>10
	Ir			0.7	1.5
	Is			3.0	>10
	It			0.9	2.6
	Iu			>10	>10
	**Iv**	OCH_3		>10	>10
	Iw	OCH_3		>10	>10
Sorafenib				5.6	7.8

activity against A375 to Sorafenib. Among these compounds, **Ib**–**e**, **Ih**, **Il**, and **Ir**–**t** showed excellent activity against A375 compared to Sorafenib.

Regarding the substituents on the 5-position of pyrrolo[3,2-*b*]pyridine, compounds **Ih**, **Ir**, **Is**, and **It** having benzyl amide moiety were generally more potent than compounds **Ic**, **Il**, **Im**, and **In** having methyl ester moiety and amides (**If**, **Ip**, and **Iq**) against A375. This suggests that the hydrophobic substituents at this position are favorable.

The effect of substituents on the phenyl ring of the tail was also investigated. The introduction of chloro group at *para* position in 3′-trifluoromethylphenyl ring of tail (**Ih**, **Ij**, **Il**, **Ip**, and **Ir**) significantly enhanced the antiproliferative activity compared to compounds (**Ii**, **Ik**, **Im**, **Iq**, and **Is**). This can be attributed to the different electronic characters at the phenyl ring of the tail. Compounds **In** and **It** having the *para*-substituted morpholino-3′-trifluoromethyl-phenyl were more potent than *meta*-substituted derivatives (**Io** and **Iu**). As to the substituent position on the phenyl chain, the *para* oriented compounds (**Ic** and **Il**) were generally more potent than the *meta* oriented compounds (**Ij** and **Iv**).

By comparing the activity of derivatives substituted with amide and urea moieties at pyrrolo[3,2-*b*]pyridine side chain as a linker, it was found that the derivatives with amide moieties (**Il**, **Ip**, **Ir**, and **Is**) were generally more potent than that of urea moieties (**Ic**, **If**, **Ih**, and **Ii**). These results seemed to indicate the effect of the linker on the activity.

In conclusion, a new series of pyrrolo[3,2-*b*]pyridine derivatives was synthesized based on our previous literature studies, by focusing on the structure–activity relationship studies of the pyrrolo[2,3-*d*]pyrimidine derivatives. Among all of these derivatives, compounds **Ir**[25] and **It**[25] having 5-benzylamide substituted 4′-amide moieties showed the most potent antiproliferative activity against A375 human melanoma cell line. Further modification of these compounds in order to improve their potency is currently in progress. Our ultimate goal is to identify several compounds that are highly potent and highly selective against melanoma cells.

Acknowledgments

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23. A375P cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, US) and maintained in DMEM medium (Welgene, Daegu, Korea) supplemented with 10% FBS (Welgene) and 1% penicillin/streptomycin (Welgene) in a humidified atmosphere with 5% CO_2 at 37 °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 of various concentration (threefold serial dilution, 12 points) of test compounds. The A357P cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. MTT assays were carried out with CellTiter 96® (Promega) according to the manufacturer's instructions. The absorbance at 590 nm was recorded using EnVision 2103 (Perkin Elmer; Boston, MA, US). The IC_{50} was calculated using GraphPad Prism 4.0 software.
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25. **Ir**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.74 (br s, 1H), 9.24 (br s, 1H), 8.42 (s, 1H), 8.30 (d, J = 8.1 Hz, 1H), 8.15 (d, J = 3.2 Hz, 1H), 8.10 (d, J = 8.6 Hz, 1H), 8.01–7.90 (m, 3H), 7.67 (d, J = 8.9 Hz, 1H), 7.34–7.21 (m, 6H), 6.93–6.71 (m,1H), 5.39 (br s, 1H), 4.53 (d, J = 5.9 Hz, 2H). MS m/z 549.13 $(M+H)^+$. HRMS (FAB) Calcd for $C_{29}H_{20}ClF_3N_4O_2$ 548.1227, found 548.1224.**It**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.59 (br s, 1H), 9.26 (br s, 1H), 8.27 (d, J = 6.2 Hz, 2H), 8.14–8.09 (m, 2H), 8.01–7.90 (m, 3H), 7.69–7.56 (m, 3H), 7.37–7.29 (m, 4H), 7.25–7.21 (m, 1H), 6.92 (d, J = 3.5 Hz, 1H), 4.53 (d, J = 6.3 Hz, 2H), 3.73 (br s, 4H), 2.96 (br s, 4H). MS m/z 600.22 $(M+H)^+$. HRMS (FAB) Calcd for $C_{33}H_{28}F_3N_5O_3$ 599.2144, found 599.2143.