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Aminoquinoline derivatives with antiproliferative activity against melanoma cell line

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ABSTRACT

The synthesis of a novel series of aminoquinoline derivatives **1a–p** and their antiproliferative activities against A375 human melanoma cell line were described. Most compounds showed superior antiproliferative activities to Sorafenib as a reference compound. Among them, quinolinyloxymethylphenyl compounds **1k** and **1l** exhibited potent activities (IC_{50} = 0.77 and 0.79 μM, respectively) and excellent selectivity against melanoma and fibroblast cell lines.

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

In early-stage melanoma without metastasis, treatment for localized melanoma normally involves surgery to remove the lesion. If it is not detected early, melanomas can grow down into the skin and spread to other parts of the body either by the lymphatic or by the hematogenous route.[2] Metastatic melanoma can be fatal with the 5-year survival rate below 15% and median survival of about 6–8 months.[3–6]

The current treatment involves 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 the FDA and EMEA for adjuvant treatment of melanoma patients, and aldesleukin (Proleukin)[8,9] is also approved for the treatment of metastatic melanoma in US.

Temozolomide (Temodar)[10] or decarbazine (DTIC)[11,12] as a chemotherapy are used most frequently for stage IV melanoma patients. Decarbazine is the only cytotoxic formally approved for the treatment of melanoma and Temodar is currently in preregistration. However, due to the development of metastatic disease which is highly resistant to conventional chemotherapeutics and radiation,[13] the intensive research and effort into new drugs and treatments[14–19] afforded merely the response rates of approximately 20% or less.

Based on the structural features of Sorafenib (Nexavar)[20] that has been used extensively in clinical trials, an aminoquinoline scaffold for b-Raf kinase inhibitors was designed as shown in Figure 1. We report here the synthesis of a novel series of aminoquinoline derivatives **1a–p** and their antiproliferative activities and selectivities against human melanoma cell line and fibroblast cell line.

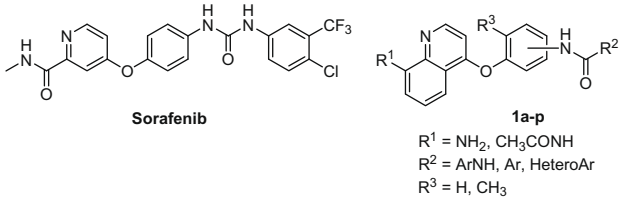

Figure 1. Structures of Sorafenib and aminoquinoline derivatives.

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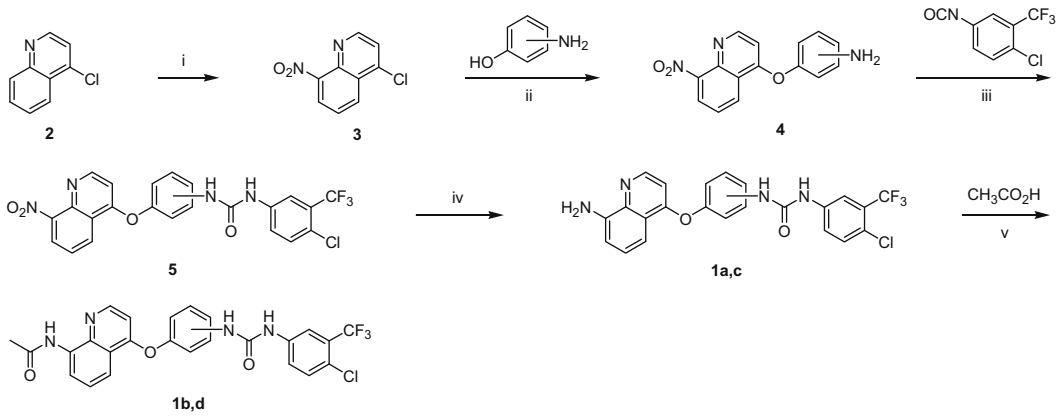


Scheme 1. Reagents and reaction conditions: (i) HNO_3, H_2SO_4, 0 °C to rt, 4 h, 56%; (ii) ^{t}BuOK, DMF, 110 °C, 7 h, 59% (*p*-Ph), 23% (*m*-Ph); iii) THF, rt, 12 h, 89% (*p*-Ph), 54% (*m*-Ph); (iv) $SnCl_2$, EtOH, reflux, 30 min, 75% (**1a**), 85% (**1c**); (v) HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 81% (**1b**), 31% (**1d**).

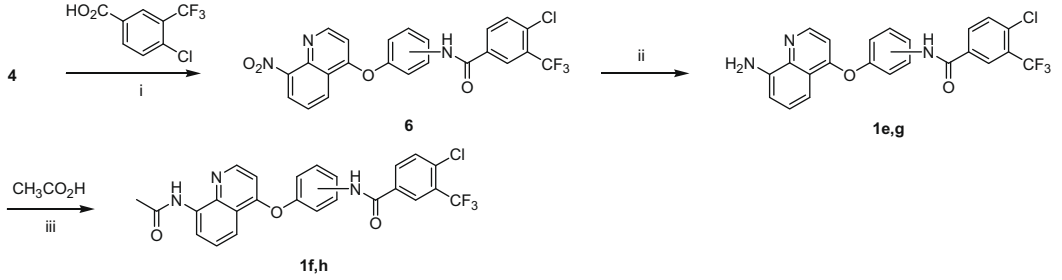
Quinolinyloxyphenyl derivatives **1a–d** with urea moiety at the terminal part were prepared by the sequence of reactions shown in Scheme 1. 4-Chloroquinoline (**2**) with nitric acid in the presence of sulfuric acid gave 4-chloro-8-nitroquinoline (**3**),[21] which was then coupled with aminophenols to give quinolinyloxy compounds **4** as key intermediates.[22] The title compounds **1a,c** were obtained by treatment of **4** with phenylisocyanate,[23] and subsequent reduction of nitro group of the resulting ureas **5** using stannous chloride, respectively.[24] Acetylation of **1a,c** using HOBt and EDCI provided the corresponding title compounds **1b,d**, respectively.[25]

The synthesis of **1f,h** with amide moiety at the terminal part was outlined in Scheme 2. Amide coupling of **4** with 4-chloro-3-trifluoromethylbenzoic acid using HOBt and EDCI followed by reduction of **6** led to the title compounds **1e,g**, respectively. Acetylation of **1e,g** was carried out by using procedures analogous to those described above to provide **1f,h**.

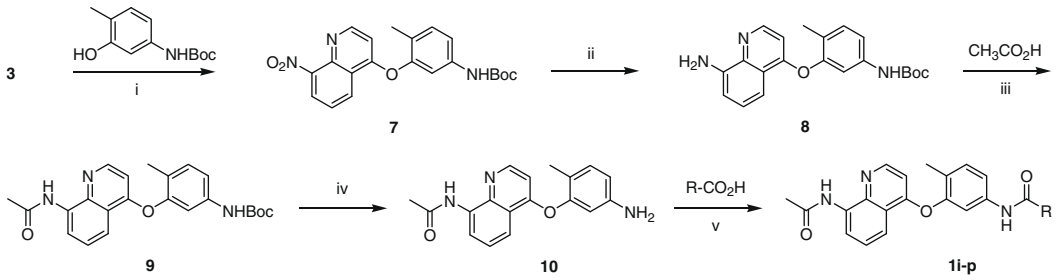
Quinolinyloxymethylphenyl compounds **1i–p** having an additional methyl group to afford the restricted conformation were prepared in several steps using nitroquinoline **3** as a starting material (Scheme 3). Coupling of **3** with Boc-protected methylaminophenol by potassium *t*-butoxide gave nitroquinolinyloxy compound **7**, and subsequent reduction of **7** with Pd/C afforded amino compound **8**. Acetylation of **8** followed by deprotection with trifluoroacetic acid gave the compound **10**, which was reacted with the appropriate carboxylic acids to give the corresponding title compounds **1i–p**, respectively.

Table 1 shows the antiproliferative activities[26] (IC_{50} values) of quinolinyloxyphenyl derivatives **1a–h** against A375 human melanoma cell line and HS27 fibroblast cell line together with that of Sorafenib as a reference compound.

Most compounds showed better antiproliferative activity against A375 human melanoma cell line than Sorafenib. In gen-

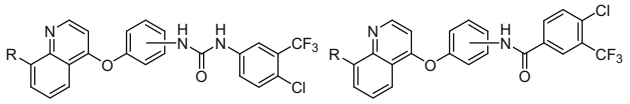


Scheme 2. Reagents and reaction conditions: (i) HOBT, EDCI, TEA, DMF, 80 °C, 12 h, 71% (*p*-Ph), 97% (*m*-Ph); (ii) $SnCl_2$, EtOH, reflux, 30 min, 26% (**1e**), 65% (**1g**); (iii) HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 41% (**1f**), 31% (**1h**).



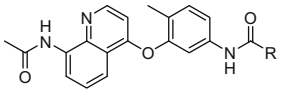
Scheme 3. Reagents and reaction conditions: (i) ^{t}BuOK, DMF, 110 °C, 7 h, 68%; (ii) 5% Pd/C, H_2, MeOH, rt, 3 h, 73%; (iii) HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 82%; (iv) CH_2Cl_2/TFA (1:1) solution, rt, 1 h, 89%; (v) acid, HOBt, EDCI, TEA, DMF, 80 °C, 12 h, 26–91% (**1i–p**).

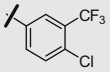
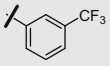
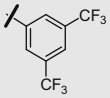
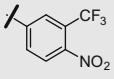
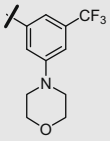
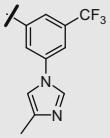
Table 1
Antiproliferative activity of quinolinyloxyphenyl derivatives **1a–h**



Compd	Orientation	R	IC_{50} (μM)	
			A375	HS27
1a	*p*-Phenyl	NH_2	3.74 ± 0.16	6.71 ± 1.62
1b	*p*-Phenyl	CH_3CONH	4.19 ± 2.13	6.02 ± 0.29
1c	*m*-Phenyl	NH_2	2.56 ± 0.09	10.39 ± 1.91
1d	*m*-Phenyl	CH_3CONH	2.32 ± 0.54	5.68 ± 0.18
1e	*p*-Phenyl	NH_2	5.69 ± 0.13	7.63 ± 1.01
1f	*p*-Phenyl	CH_3CONH	3.14 ± 0.36	5.47 ± 0.74
1g	*m*-Phenyl	NH_2	6.73 ± 0.24	>20
1h	*m*-Phenyl	CH_3CONH	3.49 ± 0.35	>20
Sorafenib			5.58	7.85

Table 2
Antiproliferative activity of quinolinyloxymethylphenyl derivatives **1i–p**



Compds	R	IC_{50} (μM)	
		A375	HS27
1i		1.30 ± 0.08	>20
1j		1.28 ± 0.19	>20
1k[27]		0.77 ± 0.07	>20
1l[27]		0.79 ± 0.02	>20
1m		>20	>20
1n		>20	>20
1o		2.12 ± 0.14	>20
1p		>20	>20
Sorafenib		5.58	7.85

eral, urea compounds **1a–d** possessed slightly potent activities as compared to amide compounds **1e–h**. Positional effect according to *p*- or *m*-orientation of middle benzene ring did not make any significant difference of activity. Acetylamino compound **1h** with *m*-orientation displayed very good selectivity against HS27 fibroblast cell line as a control in addition to high potency against melanoma cell line. However, conversion of the amino group to acetylamino group on the quinoline nucleus did not show a meaningful trend.

Shown in Table 2, some of quinolinyloxymethylphenyl derivatives **1i–p** with acetylamino and *m*-oriented structure showed superior antiproliferative activities to Sorafenib. Furthermore, they possessed excellent selectivity against melanoma compared to fibroblast cell lines. In particular, compounds **1k** and **1l** having electron-withdrawing groups on the terminal benzene nucleus exhibited excellent antiproliferative activities (0.77 and 0.79 μM, respectively) with IC_{50} values in the nanomolar range.

The representative compound **1k** was screened against V600E-b-Raf enzyme in vitro using cascade assay system. However, compound **1k** did not show a meaningful b-Raf inhibitory activity. Accordingly, the selectivity profile of **1k** was assessed against a panel of 30 protein kinases at a single concentration of 10 μM to find kinase target. This compound exhibited good selectivity profile for c-Raf and Abl inhibition with significant activity over other kinases tested. Verification of mode of action is under way.

In conclusion, a series of aminoquinoline derivatives based on the structural features of Sorafenib showed potent antiproliferative activities against A375 human melanoma cell line. In our series, quinolinyloxymethylphenyl compounds **1k** and **1l** exhibited both potent activity and excellent selectivity compared to Sorafenib. These results suggest that aminoquinoline derivatives have potentials as a therapeutic agent for treatment of melanoma.

Acknowledgments

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26. 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.
27. Selected data. Compound **1k:** ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.14 (s, 1H), 2.28 (s, 1H), 6.65 (d, J = 5.08 Hz, 1H), 7.47 (d, J = 8.81 Hz, 1H), 7.61–7.70 (m, 3H), 8.02 (d, J = 8.78 Hz, 1H), 8.37 (s, 1H), 8.58 (s, 2H), 8.68 (d, J = 7.76 Hz, 1H), 8.72 (d, J = 5.09 Hz, 1H), 10.12 (s, 1H), 10.76 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.47, 25.00, 105.04, 113.67, 115.45, 117.87, 118.58, 120.79, 121.74, 125.36, 126.01, 127.30, 128.98, 130.30, 130.74, 131.19, 131.63, 132.44, 135.27, 137.33, 138.59, 140.14, 150.28, 152.09, 161.10, 163.02, 169.44; MS m/z 548 $(M+H)^+$. Compound **1l:** ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.12 (s, 3H), 2.26 (s, 3H), 6.62 (d, J = 5.12 Hz, 1H), 7.44 (d, J = 8.34 Hz, 1H), 7.59–7.69 (m, 4H), 8.00 (d, J = 8.33 Hz, 1H), 8.30 (d, J = 8.20 Hz, 1H), 8.42 (d, J = 8.45 Hz, 1H), 8.47 (s, 1H), 8.67 (d, J = 7.66 Hz, 1H), 8.70 (d, J = 5.14 Hz, 1H), 10.10 (s, 1H), 10.76 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.43, 24.94, 104.98, 113.57, 115.52, 117.93, 118.53, 120.76, 121.64, 122.08, 124.12, 126.13, 126.18, 127.28, 127.68, 132.46, 134.25, 135.16, 138.483, 139.12, 140.111, 149.12, 150.30, 152.04; MS m/z 525 $(M+H)^+$.