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Synthesis of aminoquinazoline derivatives and their antiproliferative activities against melanoma cell line

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ABSTRACT

The synthesis of a novel series of aminoquinazoline derivatives **1a–r** and their antiproliferative activities against A375 human melanoma cell line were described. Among them, six compounds showed superior antiproliferative activities to Sorafenib as a reference compound. In particular, the representative compound **1q** bearing chromen-4-one moiety exhibited excellent antiproliferative activity (IC_{50} = 0.006 μM) and good selectivity over HS27 fibroblast cell line.

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] The 5-year survival rate for patients with metastatic melanoma below 15% and median survival of about 6–8 months.[2–5]

The immunotherapy, interferon alfa-2b (Intron-A)[6] is approved by both the FDA and EMEA for adjuvant treatment of melanoma patients, and aldesleukin (Proleukin)[7,8] is also approved for the treatment of metastatic melanoma in US.

Decarbazine (DTIC)[9,10] is the only cytotoxic formally approved for the treatment of melanoma and Temozolomide (Temodar)[11] is an imidazotetrazine with a mechanism of action similar to DTIC. Both of them are used most frequency for stage IV melanoma patients as a chemotherapy. However, the intensive research and effort into new drugs and treatments[12–20] have not afforded the effective response rates yet.

In this paper, a novel scaffold by the introduction of aminoquinazoline and amide moieties as hinge and tail regions into benzene nucleus was designed as shown in Figure 1. The middle benzene nucleus with *m*-orientated configuration possesses the structural features containing an aminomethyl group as a spacer and a methyl group to afford the restricted conformation.

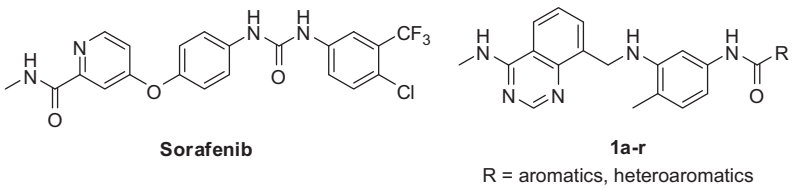

Figure 1. Structures of Sorafenib and aminoquinazoline derivatives **1a**–**r**.

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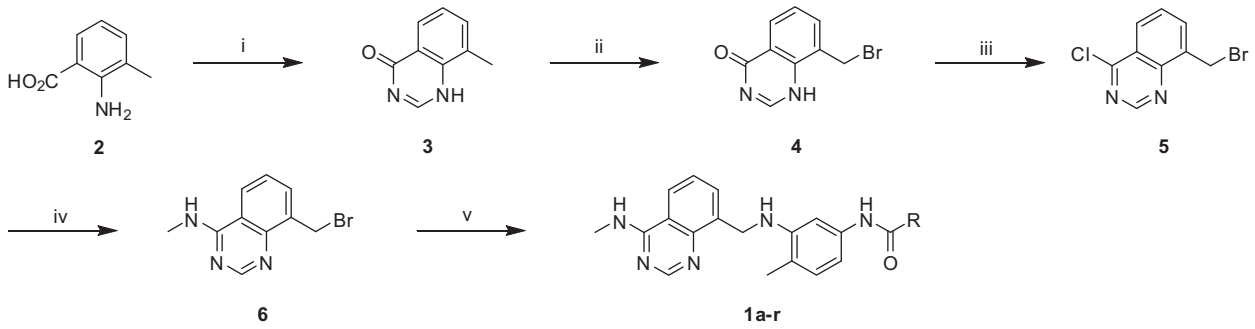
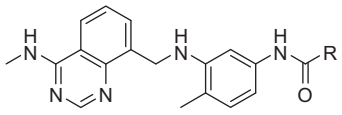


Scheme 1. Reagents and conditions: (i) formamidine acetate, $HCONH_2$, 160 °C, 12 h, 96%; (ii) *N*-bromosuccinimide, AIBN, CCl_4, rt, 24 h, 74%; (iii) $POCl_3$, *N*,*N*-dimethylaniline, toluene, reflux, 4 h, 85%; (iv) CH_3NH_2, THF, rt, 12 h, 68%; (v) **9**, K_2CO_3, DMF, 80 °C, 1.5 h.

Table 1
In vitro antiproliferative activity of aminoquinazoline derivatives **1a–r**



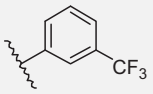
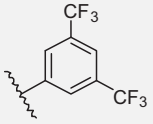
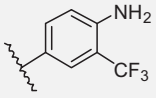
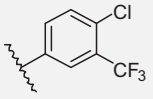
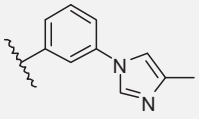
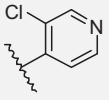
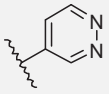
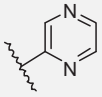
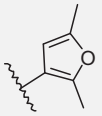
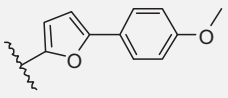
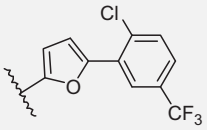
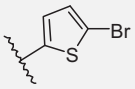
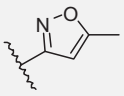
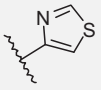
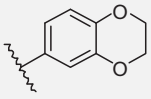
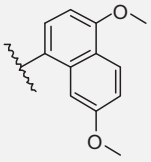
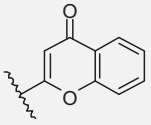
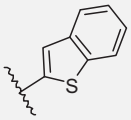
Compd	R	Yields[a] (%)	A375 (IC_{50}, μM)
1a		12	0.433
1b		9	0.769
1c		22	ND[b]
1d		11	9.70
1e		31	ND
1f		23	ND
1g		22	61.7
1h		21	ND
1i		7	ND
1j		22	ND

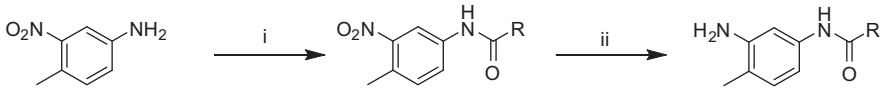
Table 1 (*continued*)

Compd	R	Yields[a] (%)	A375 (IC_{50}, μM)
1k		33	14.9
1l		32	10.5
1m		26	1.20
1n		74	1.90
1o[26]		24	0.137
1p		26	ND
1q[26]		31	0.006
1r		29	ND
Sorafenib	—	—	5.58

[a] Isolated yields from compound **6**.
[b] Not determined. Drug concentration of 100 μM did not inhibit growth of cell by 50%.

We report here the synthesis of a novel series of aminoquinazoline derivatives **1a–r** and their antiproliferative activities against A375 human melanoma cell line compared with Sorafenib (Nexavar).[21]

Aminoquinazoline derivatives **1a–r** with varying R group were prepared by the sequence of reactions shown in Scheme 1. Ring closure of 2-amino-3-methylbenzoic acid (**2**) with formamidine



7 8 9

Scheme 2. Reagents and conditions: (i) R-CO_2H, HOBt, EDCI, Et_3N, DMF, 90 °C, 20 h, 28–86%; (ii) Pd/C, H_2, MeOH, rt, 1 h, 24–89% (except **9l**); $SnCl_2$, EtOH, reflux, 2 h, 46% (for **9l**).

Table 2
Cell line selectivity and enzymatic activity of the selected compounds **1o** and **1q**

Compd	IC_{50} (μM)			
	A375	HS27	V600E-b-Raf	c-Raf
1o	0.137	1.20	—	—
1q	0.006	0.230	19.5	0.765
Sorafenib	5.58	—	0.038[a]	0.006[a]

[a] Enzymatic assay data were cited from the literature (Wilhelm, S. M. et al. *Cancer Res.* **2004**, *64*, 7099).

acetate in formamide[22] followed by bromination with *N*-bromosuccinimide in the presence of AIBN gave the bromomethylquinazolinone **4** in good yield. Compound **6** as a key intermediate was obtained by treatment of **4** with phosphoryl chloride in the presence of *N,N*-dimethylaniline, and subsequent amination of the resulting chloroquinoline **5** using methylamine. Coupling of **6** with the appropriate aminobenzenes **9** having amide moiety under basic condition led to the corresponding title compounds **1a–r**, respectively.[23]

The synthesis of **9**, which were served for the synthesis of **1a–r**, was outlined in Scheme 2. Amide coupling of 4-methyl-3-nitroaniline (**7**) with the appropriate benzoic acids using HOBt and EDCI in the presence of Et_3N[24] followed by reduction of the resulting nitrobenzamides **8** with Pd/C in MeOH or $SnCl_2$ in EtOH afforded the aminobenzenes **9**, respectively, in 24–89 yields.

In general, five-membered heterocycles **1i–n** possessed potent activities as compared to six-membered heterocyles **1f–h**. In five-membered heterocycles, compounds **1m,n** with two heteroatoms showed superior antiproliferative activities to compounds **1i–l** with one heteroatom. However, comparison of aromatic and heteroaromatic compounds did not show a meaningful trend.

As shown in Table 2, compounds **1o,q** having high potency against melanoma cell line displayed good selectivity over HS27 fibroblast cell line as a control. To identify whether mode of action is or not due to Ras/Raf/MAPK pathway, the representative compound **1q** was screened against V600E-b-Raf and c-Raf enzymes using HotSpot kinase assay by Reaction Biology Corp. Compound **1q** showed the marginal inhibitory activity against the Raf family. The precise identification of mode of action is under way.

Table 1 shows the in vitro antiproliferative activities[25] (IC_{50} values) of aminoquinazoline derivatives **1a–r** against A375 human melanoma cell line together with that of Sorafenib as a reference compound.

All the synthesized compounds were evaluated by MTT assays using CellTiter 96® (Promega). Six compounds showed better antiproliferative activity against A375 human melanoma cell line than Sorafenib. Among these compounds, **1a**, **1b**, **1o**, and **1q** possessed the nanomolar antiproliferative activities. Especially, compound **1q** bearing chromen-4-one moiety exhibited excellent antiproliferative activity with IC_{50} value of 0.006 μM.

In conclusion, a novel series of aminoquinazoline derivatives was designed and synthesized. In our series, the representative compound **1q** exhibited not only excellent antiproliferative activity against A375 human melanoma cell line but also good selectivity over HS27 fibroblast cell line. These results suggest that aminoquinazolines could be served as a scaffold for treatment of melanoma.

Acknowledgment

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25. 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.

26. Selected data. Compound **1o**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.14 (s, 3H), 3.01 (d, J = 3.88 Hz, 3H), 4.26 (d, J = 3.72 Hz, 4H), 4.79 (d, J = 5.08 Hz, 2H), 6.84 (s, 1H), 6.89–6.93 (m, 2H), 7.04 (d, J = 7.71 Hz, 1H), 7.38–7.43 (m, 3H), 7.60 (d, J = 6.72 Hz, 1H), 8.03 (d, J = 8.07 Hz, 1H), 8.28 (d, J = 4.09 Hz, 1H), 8.57 (s, 1H), 9.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.67, 28.21, 43.61, 64.44, 64.81, 102.50, 108.65, 115.20, 117.00, 117.79, 121.53, 121.53, 123.08, 125.49, 128.48, 130.10, 136.67, 138.62, 143.26, 146.53, 146.88, 147.47, 154.97, 160.57, 164.58; MS m/z 456 (M+H)$^+$. Compound **1q**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.18 (s, 3H), 3.02 (d, J = 3.60 Hz, 3H), 4.82 (s, 2H), 6.86 (s, 1H), 7.02 (d, J = 6.61 Hz, 2H), 7.41–7.54 (m, 3H), 7.62 (d, J = 6.93 Hz, 1H), 7.77–7.95 (m, 2H), 8.04 (t, J = 7.56 Hz, 2H), 8.36 (s, 1H), 8.60 (s, 1H), 10.38 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.81, 28.24, 102.86, 109.20, 111.26, 115.18, 119.36, 119.54, 121.26, 124.07, 125.31, 125.58, 126.51, 130.26, 135.40, 136.94, 147.11, 154.92, 155.58, 156.47, 157.72, 160.58, 177.79; MS m/z 466 (M+H)$^+$.