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New diarylamides and diarylureas possessing 8-amino(acetamido) quinoline scaffold: Synthesis, antiproliferative activities against melanoma cell lines, kinase inhibition, and *in silico* studies

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ABSTRACT

Synthesis of a new series of diarylureas and diarylamides possessing 4-aryl-8-amino(acetamido)quinoline scaffold is described. Their *in vitro* antiproliferative activities against ten melanoma cell lines were tested. Compounds **1l**, **2l**, **3c**, and **4c** showed the highest potency against A375P cell line with IC_{50} values in sub-micromolar scale. Compound **4c** was equipotent to Vemurafenib against A375P. In addition, compounds **1l**, **2a**, and **2l** showed high potency over the NCI-9 tested melanoma cell line panel. The IC_{50} values of compounds **1l** and **2l** were in 2-digit nanomolar scale over four and five cell lines, respectively. Compound **2l** showed high, dose-dependent inhibition of ERK kinase. ADME profiling showed that compounds **1l**, **2l**, **3c**, **4c**, and **5b** are estimated to be orally bioavailable.

1. Introduction

Melanoma is the most aggressive type of skin cancer. It is considered as a malignant tumor of melanocytes. The major risk factors for melanoma development include exposure to solar ultraviolet irradiation, fair skin, dysplastic nevi syndrome, and a family history of melanoma. Melanomas can metastasize either by the lymphatic or by the hematogenous route [1]. Early stage melanoma (stage I/II) can be cured surgically with more than 95% success rate. But melanoma metastasizing to major organs (stage IV) is virtually incurable [2]. Patients with advanced melanoma have a median survival time of less than one year, and the estimated 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 RAS-RAF-MEK-ERK signaling pathway (ERK pathway) plays an important role in tumorigenesis and cancer progression [8]. Sorafenib (Nexavar®, Fig. 1), a diarylurea derivative, targets ERK pathway. It inhibits basal phosphorylation of ERK (p-ERK) in numerous cancer cell lines *in vitro*, including melanoma cell lines, independent of their K-RAS and B-RAF mutational status [9]. In addition, Sorafenib is a well known inhibitor of B-RAF. Dysregulated signaling through RAF kinase isoforms has been detected in ~30% of human cancers [10]. Constitutive B-RAF activity can be caused by activating oncogenic mutations, such as B-RAF V600E mutation, which is prevalent in melanomas (63%) [11]. Vemurafenib (PLX4032, Zelboraf®, Fig. 1) is another drug which targets ERK pathway through inhibition of V600E-B-RAF kinase. In 2011, it was approved by the U.S. Food and Drug Administration (FDA) for treatment of late-stage melanoma [12]. So inhibition of ERK signaling pathway is a very potential avenue for treatment of melanoma.

A number of reports have recently reported diarylamides and diarylureas with potential antiproliferative activities against melanoma cell lines [13–25]. Encouraged by the interesting antipr

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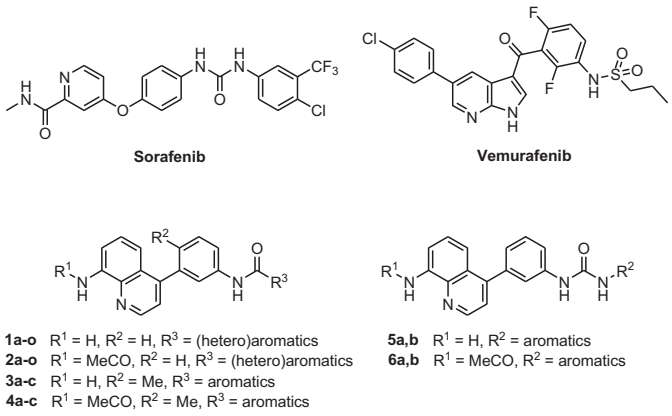

Fig. 1. Structures of Sorafenib, Vemurafenib, and the target compounds.

oliferative activities of diarylamide and diarylurea derivatives, a new series of diarylamides and diarylureas containing 8-amin o(acetamido)quinoline scaffold was synthesized (Fig. 1). Their *in vitro* antiproliferative activities against ten human melanoma cell lines are reported. MEK and ERK kinases inhibitory activity of compounds **2l** and **4c**, in addition to *in silico* calculations of steric factors and Lipinski's rule of five for the most potent target compounds are also reported.

2. Results and discussion

2.1. Chemistry

Synthesis of the target compounds was carried out as illustrated in Schemes 1 and 2. Nitration of 4-bromoquinoline (**7**) using nitric acid/sulfuric acid mixture produced 4-bromo-8-nitroquinoline (**8**). Compounds **9** and **10** were prepared by Suzuki coupling of the bromo compound **8** with the appropriate arylboronic acid derivatives in the presence of [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$) and potassium carbonate. Condensation of the amino compounds **9** and **10** with the appropriate aryl carboxylic acid derivatives in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA, Hünig's base) in dry DMF gave the corresponding amide derivatives **11a**–**o** and **12a**–**c**. The nitro groups of the latter products were reduced to the corresponding amino derivatives **1a**–**o** and **3a**–**c** using palladium over carbon in hydrogen atmosphere. Those produced amino groups of **1a**–**o** and **3a**–**c** were acetylated using acetic anhydride to yield the target bisamides **2a**–**o** and **4a**–**c** (Scheme 1).

For synthesis of the target urea derivatives **5a**,**b** and **6a**,**b**, the 3-step pathway illustrated in Scheme 2 was utilized. The amino compound **9** was treated with 4-chloro-3-(trifluoromethyl)phenylisocyanate or 3,5-bis(trifluoromethyl)phenylisocyanate to give the corresponding urea derivatives **13a**,**b**, respectively, possessing nitro group. The nitro group of **13a**,**b** was subsequently reduced with Pd/C in hydrogen atmosphere to give the corresponding target amino analogues **5a**,**b**. The amino group of compounds **5a**,**b** was acetylated in the same way of synthesis of compounds **2a**–**o** and **4a**–**c** using acetic anhydride to produce the target acetamido products **6a**,**b**.

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 4-aryl-8-amino(acetamido)quinoline diarylamides and diarylureas to inhibit the growth of A375P cell line is summarized in Tables 1–3. Sorafenib was selected as a reference standard because it has been extensively used in clinical trials for treatment of melanoma [5,26]. Vemurafenib was also utilized as a second reference standard in this experiment because of its high potency against melanoma cell lines [27], and it has been recently approved by the FDA for treatment of advanced melanoma [12].

Compounds **2a**, **2g**, **2i**–**l**, **2o**, and **6a** with acetamido moiety at position 8 of the quinoline ring were more potent than the corresponding amino compounds **1a**, **1g**, **1i**–**l**, **1o**, and **5a**. On the contrary, free amino analogues **1c**, **1e**, **1f**, **1n**, **3a**, **3b**, and **5b** showed higher potencies than the corresponding acetamido derivatives **2c**, **2e**, **2f**, **2n**, **4a**, **4b**, and **6b**. Compounds **1e**, **1k**, **2e**, and **2k** with *o*-unsubstituted phenyl ring attached to position 4 of the quinoline nucleus were more potent than the corresponding derivatives **3a**, **3b**, **4a**, and **4b** with *o*-tolyl ring. But on the other hand, compounds **3c** and **4c** with *o*-methylphenyl ring demonstrated higher potencies than compounds **1l** and **2l**.

Upon comparing the activities of derivatives with amide and urea linkers, it was found that compounds **1e** and **2k** with amide moiety were more potent than the corresponding urea derivatives **5a** and **6b**. But the urea analogue **5b** showed higher potency than compound **1k** with amide linker.

The effect of the terminal aryl ring on potency was also investigated. The terminal heteroaromatics; 2′,5′-dimethylfuran-3-yl, 2-thiazolyl, and benzo[*b*]thiazol-2-yl found in compounds **1a**–**m** and **2a**–**m** were unfavorable for activity. 3′,4′-Dimethoxyphenyl, 3′,4′-dimethylphenyl, and 4′-bromo-3′-methylphenyl terminal moieties were also unfavorable for activity. So we can conclude that the presence of heteroaromatics and/or electron-donating group(s) at the terminal aryl ring attenuates the potency of this series of compounds against A375P human melanoma cell line.

Compounds **1d** and **2d** possessing 3′-(trifluoromethyl)phenyl terminal ring showed moderate potency. Their potencies were enhanced by insertion of a chloro substituent at *para* position (compounds **1e** and **2e**) or another *meta*-(trifluoromethyl) group (compounds **1k** and **2k**). This may be attributed to the enhanced binding affinity produced by these substituents to the target protein. In addition, compounds **1l** and **2l**, possessing 2,3-dihydrobenzo[*b*][1,4]dioxine terminal ring, with sub-micromolar IC_{50} values were 11 times and 9.3 times, respectively, more potent than the corresponding 3′,4′-dimethoxyphenyl analogues **1a** and **2a**.

Among all the target compounds, the highest potencies were encountered with compounds **1l**, **2l**, **3c**, and **4c** with sub-micromolar IC_{50} values. All these four derivatives possess terminal 2,3-dihydrobenzo[*b*][1,4]dioxine ring. So this bicyclic ring was the most optimum terminal moiety for antiproliferative activity of this series against A375P melanoma cells. In addition, compounds **1e**, **2k**, and **5b** showed superior potency to Sorafenib but with IC_{50} values in micromolar range. Compound **4c** was equipotent to Vemurafenib. And the IC_{50} value of compound **3c** (0.28 μM) was very close to that of Vemurafenib (0.25 μM).

2.2.2. In vitro anticancer screening over nine melanoma cell lines at the NCI

After initial one-dose screening of the target compounds at the National Cancer Institute (NCI) [28], Bethesda, Maryland, USA, the eight compounds, **1e**, **1l**, **1n**, **2a**, **2e**, **2l**, **5b**, and **6b**, with interesting inhibitory activity in single-dose testing were further tested in a five-dose testing mode in order to determine their potencies over nine melanoma cell lines. For each of these compounds, the IC_{50} (the concentration producing 50% inhibition) values were recorded. The antiproliferative activity of these eight compounds over nine melanoma cell lines are summarized in Table 4.