European Journal of Medicinal Chemistry 95 (2015) 453–463

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Design, synthesis, *in vitro* antiproliferative evaluation, and kinase inhibitory effects of a new series of imidazo[2,1-*b*]thiazole derivatives

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ARTICLE INFO

Article history:
Received 19 January 2015
Received in revised form
26 March 2015
Accepted 28 March 2015
Available online 30 March 2015

Keywords:
Antiproliferative activity
Arylsulfonamido
ERK pathway
Imidazo[2,1-*b*]thiazole
V600E-B-RAF
C-RAF

ABSTRACT

Design and synthesis of a new series of 5,6-diarylimidazo[2,1-*b*]thiazole derivatives possessing terminal aryl sulfonamide moiety are described. Their *in vitro* antiproliferative activities against a panel of 57 human cancer cell lines of nine different cancer types were tested at the NCI. Compounds **8a**, **8b**, **8n**, **8q**, **8t**, and **8u** showed the highest mean % inhibition values over the 57 cell line panel at 10 μM, and they were further tested in 5-dose testing mode to determine their IC_{50} values. Among the six compounds, compound **8u** possessing terminal *para*-hydroxybenzenesulfonamido moiety and ethylene linker showed the highest potency. It demonstrated superior potency than Sorafenib against eight different cell lines, and was equipotent to Sorafenib against COLO 205 colon cancer cell line. Its IC_{50} values over NCI-H460 non-small cell lung cancer cell line and MCF7 breast cancer cell line were 0.845 μM and 0.476 μM, respectively. Compounds **8a**, **8b**, **8q**, **8t**, and **8u** showed high selectivity indices towards cancer cells over L132 normal lung cell line. Compound **8u** showed potential inhibitory effects over the components of ERK pathway. Its IC_{50} value over V600E-B-RAF and C-RAF kinases were 39.9 nM and 19.0 nM, respectively.

1. Introduction

Imidazo[2,1-*b*]thiazole analogs exhibited potential antiproliferative activities against a variety of human cancer cell lines [1–11]. Some pyrimidinyl substituted imidazo[2,1-*b*]thiazole derivatives were reported as RAF kinases inhibitors (Fig. 1) [12].

The RAS-RAF-MEK-ERK signaling pathway (ERK pathway) plays an important role in tumorigenesis and cancer progression [13]. Sorafenib (Nexavar®, Fig. 1), a diarylurea derivative, is an example of kinase inhibitors targeting ERK pathway. Dysregulated signaling through RAF kinase isoforms has been detected in ~30% of human cancers [14]. Constitutive B-RAF activity can be caused by activating oncogenic mutations, such as B-RAF V600E mutation, which is associated with a variety of cancer types including melanoma, non-Hodgkin's lymphoma, colorectal adenocarcinoma, thyroid carcinoma, non-small cell lung carcinoma, renal cell carcinoma, hepatocellular carcinoma, ovarian cancer, gastrointestinal stromal tumors, and hairy cell leukemia [15]. Wild-type RAF1 (C-RAF) has been reported to prolong cell survival, independent of MAPK signaling, by direct interaction with anti-apoptotic and apoptotic regulatory proteins [14,16]. C-RAF kinase is over-expressed in cases

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http://dx.doi.org/10.1016/j.ejmech.2015.03.065
0223-5234/

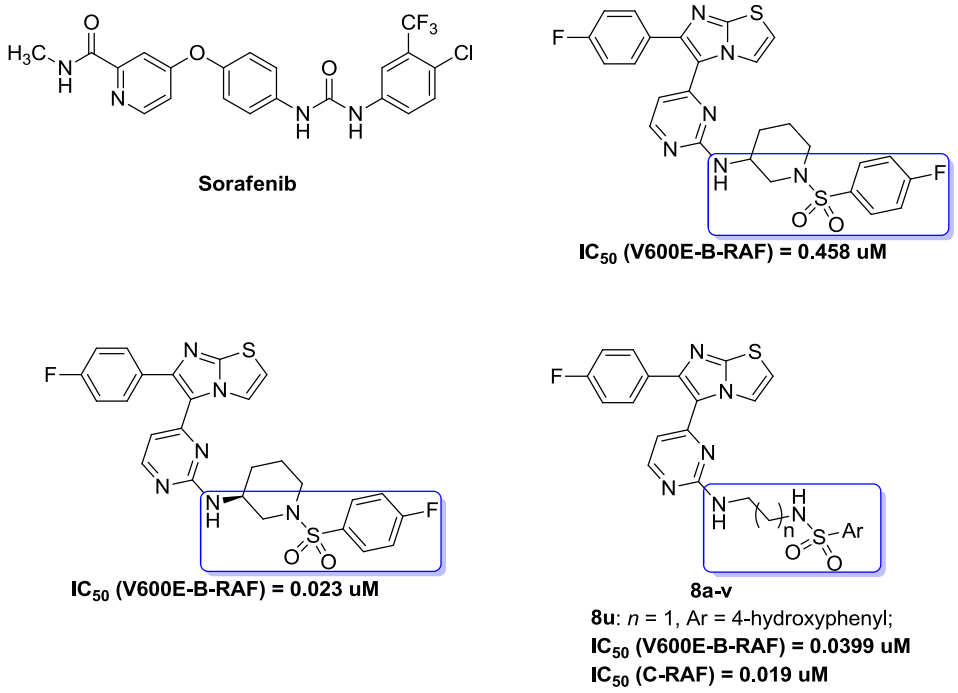

Fig. 1. Structures of Sorafenib, previously reported pyrimidinyl imidazo[2,1-*b*]thiazole derivatives [12], and the target compounds **8a**–**v**.

of renal cell carcinoma [17], hepatocellular carcinoma [18], and is associated with poor prognosis in ovarian [19] and androgen-insensitive prostate cancer [20]. There is also a relationship between C-RAF and disease progression and cell proliferation in melanomas [21].

Our target compounds in the present investigation were designed as open chain analogs to the previously reported RAF kinase inhibitors imidazo[2,1-*b*]thiazole derivatives (Fig. 1) [12]. Homologation of the spacer, ethylene or propylene, was carried out in order to study the effect of linker length on the activity. The target compounds were tested for *in vitro* antiproliferative activities against 57 human cancer cell lines of nine different cancer types, and B-RAF/V600E-B-RAF/C-RAF kinase inhibitory effect of the most potent compound was also examined in order to test the mechanism of action at molecular level.

2. Results and discussion

2.1. Chemistry

The target compounds **8a**–**v** were synthesized through the pathway illustrated in Scheme 1. It was important to synthesize the key mesyl intermediate compound **7** and the amino tail reagents **3a**–**t**. 1,2-Ethylenediamine (**1a**) or 1,3-propylenediamine (**1b**) were treated with the appropriate arylsulfonyl chloride derivatives **2a**–**j** in the presence of triethylamine as a base to afford the amino tail reagents **3a**–**t** [22,23]. Refluxing 2-aminothiazole (**4**) with α-bromo-4-fluoroacetophenone in ethanol led to cyclization to 6-(4-fluorophenyl)imidazo[2,1-*b*]thiazole (**5**) [24]. Coupling compound **5** with 4-iodo-2-(methylthio)pyrimidine in presence of palladium acetate, cesium carbonate, and triphenylphosphine produced compound **6**. Oxidation of the methylsulfide moiety of compound **6** using oxone produced the corresponding methylsulfonyl compound **7** [7]. This was confirmed through deshielding of the methyl signal in ^{1}H NMR from 2.64 ppm to 3.38 ppm. Heating compound **7** with the amino reagents **3a**–**t** in the presence of diisopropylethylamine led to displacement of the mesyl group of compound **7** by amino group and formation of the target compounds **8a**–**t**. This was confirmed by disappearance of the mesyl signal at 3.38 ppm in ^{1}H NMR analysis, and appearance of the aliphatic and aromatic signals related to the ethylene/propylene linker and terminal aromatic ring, respectively, in both ^{1}H NMR and ^{13}C NMR analyses. The methoxy compounds **8d,n** were demethylated using boron tribromide to obtain the corresponding hydroxyl compounds **8u,v**. The disappearance of methoxy signals at 3.76 and 3.79 ppm in ^{1}H NMR analysis confirms demethylation reaction. LC-MS analysis was another tool to confirm identity of the analyzed compounds. Table 1 illustrates the target compound structures and their yield percentages.

2.2. Biological evaluation

2.2.1. In vitro antiproliferative activity over NCI-57 cancer cell line panel

2.2.1.1. One-dose results. Structures of the target compounds were submitted to National Cancer Institute (NCI), Bethesda, Maryland, USA [25], and the fifteen derivatives shown in Fig. 2 were selected on the basis of degree of structural variation and computer modeling techniques for testing their antiproliferative activity. The selected compounds were subjected to *in vitro* anticancer assay against tumor cells in a full panel of 57 cell lines taken from nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast). The compounds were tested at a single-dose concentration of 10 μM, and the percentages of growth inhibition over the 57 tested cell lines were determined. The mean inhibition percentages for each of the tested analogs over the full panel of cell lines are illustrated in Fig. 3.

Upon investigating the effect of linker length on antiproliferative activity, it was found that compounds **8a**, **8b**, **8e**, and **8u** with ethylene linker were more active the corresponding propylene derivatives **8k**, **8l**, **8o**, and **8v**. On the other hand, some propylene analogs such as **8s** and **8t** showed higher activity than