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Research paper

Design, synthesis and biological evaluation of *N*-phenylquinazolin-4-amine hybrids as dual inhibitors of VEGFR-2 and HDAC

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ABSTRACT

A single agent that simultaneously inhibits multiple targets may offer greater therapeutic benefits in cancer than single-acting agents through interference with multiple pathways and potential synergistic action. In this work, a series of hybrids bearing *N*-phenylquinazolin-4-amine and hydroxamic acid moieties were designed and identified as dual VEGFR-2/HDAC inhibitors. Compound **6fd** exhibited the most potent inhibitory activity against HDAC with IC_{50} of 2.2 nM and strong inhibitory effect against VEGFR-2 with IC_{50} of 74 nM. It also showed the most potent inhibitory activity against a human breast cancer cell line MCF-7 with IC_{50} of 0.85 μM. Docking simulation supported the initial pharmacophoric hypothesis and suggested a common mode of interaction at the active binding sites of VEGFR-2 and HDLP ((Histone Deacetylase-Like Protein), which demonstrates that compound **6fd** is a potential agent for cancer therapy deserving further researching.

1. Introduction

Angiogenesis, the process of new blood vessels formation from existing vasculature, is a normal physiological event for organ development. It occurs during tissue growth from embryonic development through to maturity. It is also activated during wound repairment and certain stages of menstrual cycle [1,2]. However, abnormal regulation of angiogenesis has been involved in the development of various diseases such as rheumatoid arthritis, psoriasis, diabetic retinopathy, tumor growth, and tumor metastasis [2–7]. Among the angiogenic factors identified to date, vascular endothelial growth factor (VEGF) and its receptor tyrosine kinase VEGFR-2 or kinase insert domain receptor (KDR) are the most important regulator of tumor angiogenesis [8,9]. It is well demonstrated that upon binding its ligand, VEGFR-2 undergoes receptor dimerization and autophosphorylation and initiates downstream signaling, ultimately leading to tumor angiogenesis, vascular permeability enhancement, proliferation, and migration [10,11]. Consequently, inhibition of the VEGF/VEGFR-2 signaling pathway has become a valuable approach in the treatment of cancers. Indeed, a number of angiogenesis inhibitors of VEGFR-2 have been approved by FDA or effectively demonstrated in preclinical and clinical settings. Bevacizumab, a monoclonal antibody to VEGF, has been approved by the FDA for the treatment of non-small-cell lung cancer [12] and metastatic colorectal cancer [13]. Ramucirumab, a mAb antibody (human IgG1) directed against VEGFR-2, has been approved by FDA in 2014 for advanced gastric or gastro-esophageal junction adenocarcinoma and metastatic non-small-cell lung carcinoma [14]. In addition, small-molecule tyrosine kinase inhibitors of VEGFR-2, such as Sunitinib [15], Sorafenib [16], and Vandetanib [17] have been approved for treatment of various types of cancers including renal cell carcinoma, gastrointestinal stromal tumor (GIST), hepatocellular carcinoma, thyroid cancer, and soft tissue sarcom.

However, a significant number of patients do not respond to VEGFR-2 targeted therapy. Furthermore, the effectiveness of these angiogenesis inhibitors is also limited by the drug resistance that

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frequently emerges following treatment [18–20]. To overcome the low response rate and acquired drug resistance to tyrosine kinase inhibitors (TKIs), a number of strategies have been tested, including combination therapies and development of multi-targeted inhibitors [21,22].

Histone deacetylases (HDACs) are a class of enzymes that catalyze the removal of acetyl groups from the ε-amino groups of lysine residues present within the N-terminal extension of the core histones, leading to chromatin condensation and transcriptional repression [23,24]. In addition to regulating the acetylation state of histones, HDACs can bind to, deacetylate and regulate the activity of a number of non-histone proteins, including transcription factors such as p53, E2F1, and NF-κB and proteins with diverse biological functions such as α-tubulin, Ku70 and Hsp90 [24]. Eighteen HDACs have been identified in humans, and they are subdivided into four structurally and functionally different phylogenetic classes. Among them, Class I HDACs (1, 2, 3, and 8) and Class II HDACs (4, 5, 6, 7, 9, and 10) are zinc dependent proteases [25]. It has been widely recognized that zinc-containing HDACs are promising targets for therapeutic interventions intended to reverse aberrant epigenetic states associated with cancer [26–28]. Consequently, considerable effort has been devoted to develop HDAC inhibitors recently [29–34].

HDAC inhibitors have been demonstrated to synergize with other antitumor agents, including RTK inhibitors, to suppress proliferation and induce apoptosis in tumor cells, even to overcome TKI resistance [35–39]. Recently, multi-acting inhibitors against HDAC and RTK have been reported [40–44]. But the report of VEGFR-2/HDAC dual inhibitors is rare [45]. In this study, a series of *N*-phenylquinazolin-4-amine hybrids were rationally designed and synthesized as dual VEGFR-2/HDAC inhibitors by combination of pharmacophores of two reference drugs, Vandetanib and Vorinostat, which were used to achieve VEGFR-2 and HDAC inhibition, respectively (Fig. 1).

2. Results and discussion

2.1. Chemistry

The synthetic route to obtain the desired target compounds is outlined in Scheme 1 according to the literature with some modifications [40]. Compounds **3a**-**3f** were prepared through the coupling of substituted anilines with 4-chloro-7-methoxyquinazolin-6-yl acetate (**2**), which was prepared through chlorination of 7-methoxy-4-oxo-3,4-dihydroquinazolin-6-yl acetate (**1**). Hydrolysis of the acetyl group on compounds **3a**-**3f** using lithium hydroxide gave corresponding phenol intermediates **4a**-**4f**. Alkylation of the phenolic hydroxyl group on compounds **4a**-**4f** with various chain lengths of ethyl bromoalkanoate gave ethyl ester intermediates **5aa**-**5fd**. Conversion of ethyl esters **5aa**-**5fd** to hydroxamic acids using freshly prepared hydroxylamine furnished target *N*-phenylquinazolin-4-amine hybrids **6aa**-**6fd**.

2.2. Biological evaluation

The *in vitro* enzymatic inhibitory activities of the target compounds against VEGFR-2 and HDAC were evaluated. Additionally, the *in vitro* antiproliferative effects of the targeted compounds against MCF-7, a human breast adenocarcinoma cell line, were also tested. The results were summarized in Table 1.

2.2.1. VEGFR-2 inhibition

As shown in Table 1, the target compounds **6aa**-**6fd** exhibited mild to moderate VEGFR-2 inhibitory activities compared to the reference compound Vandetanib. Among them, compounds **6fa**-**6fd** with *para*-bromo substituent on the phenyl ring exhibited potent inhibitory activity against VEGFR-2 kinase with IC_{50} values ranging from 74 nM to 153 nM, which was comparable to the positive drug Vandetanib (IC_{50} = 54 nM). Among them, compound **6fb** exhibited the most potent VEGFR-2 inhibitory activity with IC50 of 59 nM. It seems that the change of the length of carbon chain (n = 2 to 5) does not influence VEGFR-2 inhibition significantly. On the contrary, the type and position of halogen substituent on the phenyl ring play important roles in the VEGFR-2 inhibition. Compared with compounds **6fa**-**6fd**, the inhibitory activity of compounds **6ea**-**6ed** with *ortho*-bromo substituent decreased dramatically with IC_{50} over 10,000 nM against VEGFR-2. It is likely that *ortho*-Br restricts the free rotation of the benzene ring. This is supported by the fact that the rank order of the VEGFR-2 inhibitory activity is *ortho*-Br < *ortho*-Cl<= *ortho*-F < *ortho*-H. Compounds **6ba**-**6bd** with *para*-fluoro substituent showed stronger inhibitory activity against VEGFR-2 (IC_{50} ranged from 270 nM to 524 nM) than compounds **6aa**-**6ad** with *ortho*-fluoro substituent (IC_{50} ranged from 754 nM to 918 nM). Similarly, compounds **6cd**-**6dd** with *para*-chloro substituent showed weaker inhibition against VEGFR-2 (IC_{50} ranged from 364 nM to 857 nM) than compounds **6da**-**6dd** with *ortho*-fluoro substituent (IC_{50} ranged from 182 nM to 313 nM). All the above results indicated that introduction of bromo substituent at *para* position on the phenyl ring is favorable for the VEGFR-2 inhibition. It is worth noting that 7-(4-(3-ethynylphenylamino)-7-methoxyquinazolin-6-yloxy)-N-hydroxyheptanamide (CUDC-101) reported by Cai et al. exhibited potent

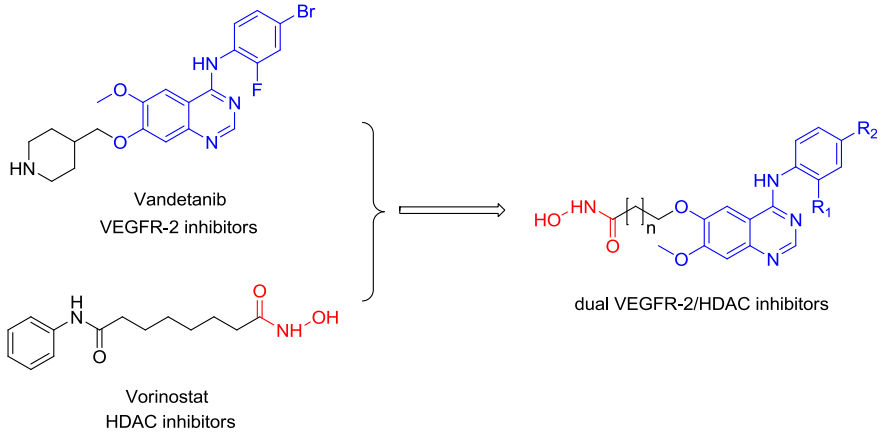

Fig. 1. Design of dual inhibitors against VEGFR-2 and HDAC.