Discovery of a New Series of Naphthamides as Potent VEGFR-2 Kinase Inhibitors

Yongcong Lv,†§ Mengyuan Li,‡§,⊥ Linjiang Tong,‡ Ting Peng,‡ Lixin Wei,§ Jian Ding,‡ Hua Xie,*‡ and Wenhu Duan*†

†Department of Medicinal Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China
‡Division of Anti-Tumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China
§Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 23 Xinning Road, Qinghai 81008, China
⊥Medical College of Nanchang University, 461 Bayi Road, Nanchang, Jiangxi 330066, China

Supporting Information

ABSTRACT: Inhibition of VEGFR-2 signaling pathway has already become one of the most promising approaches for the treatment of cancer. In this study, we describe the design, synthesis, and biological evaluation of a new series of naphthamides as potent inhibitors of VEGFR-2. Among these compounds, 14c exhibited high VEGFR-2 inhibitory potency in both enzymatic and HUVEC cellular proliferation assays, with IC50 values of 1.5 and 0.9 nM, respectively. Kinase selectivity profiling revealed that 14c was a multitargeted inhibitor, and it also exhibited good potency against VEGFR-1, PDGFR-β, and RET. Furthermore, 14c effectively blocked tube formation of HUVEC at nanomolar level. Overall, 14c might be a promising candidate for the treatment of cancer.

KEYWORDS: Angiogenesis, HUVEC, inhibitor, naphthamide, VEGFR-2

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is essential for both embryonic growth and normal physiological processes, such as reproduction and wound healing.1,2 Aberrant angiogenesis has been observed in a wide range of diseases including atherosclerosis, retinopathies, endometriosis, arthritis, and cancer.3 As angiogenesis plays an important role in the process of tumor growth and metastasis, it has been proved in humans that restricting the blood supply could limit the growth of a solid tumor.4 Many factors are involved in tumor angiogenesis, among which the vascular endothelial growth factors (VEGFs) play a major role.5 Overactivation of vascular endothelial growth factor receptors (VEGFRs), especially VEGFR-2, is closely related to poor prognosis and metastasis in the majority of solid tumor patients.6,7 The VEGF-mediated VEGFR-2 signaling promotes several endothelial responses required for the formation of new blood vessels, such as cell proliferation, migration, and survival.8 As VEGFR-2 signaling is important in tumor angiogenesis, blocking VEGFR-2 signaling pathway has become an attractive approach for the treatment of cancers. Receptor tyrosine kinase (RTK) inhibitors that target VEGFR-2 have been demonstrated to possess substantial antitumor activity, and generally they are ATP-competitive small molecule inhibitors.9–13 In fact, six such inhibitors (sorafenib,12 sunitinib,13 pazopanib,14 axitinib,15 vandetanib,16 and regorafenib17) targeting VEGFR-2 and/or other kinases have been approved for the treatment of cancers, such as advanced renal cell carcinoma (RCC) and hepatocellular carcinoma (HCC). In this communication, we report the design, synthesis, and biological evaluation of a new series of naphthamides as potent VEGFR-2 inhibitors (Figure 1).

Linifanib (1) (ABT869, Figure 1), a multitargeted RTK inhibitor bearing an N,N’-diaryl urea moiety, is a potent VEGFR-2 inhibitor (IC50 = 4 nM). It also inhibits other kinases such as VEGFR-1 (Flt-1), Flt-3, Kit, and PDGFR-β with IC50 values of 3, 4, 14, and 66 nM, respectively. It is currently in phase III clinical trials for the treatment of advanced HCC.18 In 2008, Amgen Inc. researchers reported a series of naphthamides19 and 2,3-dihydro-1,4-benzoxazines20 as potent VEGFR inhibitors. Of naphthamides, representative compound 2 (Figure 1) showed IC50 values of 0.5 and 8 nM for VEGFR-2 and VEGF-induced proliferation of human umbilical vein endothelial cells (HUVEC), respectively. In benzoxazine series,

Received: January 29, 2014
Accepted: February 24, 2014
Published: February 24, 2014
compound 3 (Figure 1) showed IC_{50} values of 0.5 and 4 nM for VEGFR-2 and HUVEC, respectively.

Linifanib binds to the ATP binding site of VEGFR-2 and forces the protein to adopt a DFG-out conformation. This conformation is incompatible with ATP binding, enabling an appropriately substituted inhibitor to penetrate into the extended hydrophobic pocket. Linifanib forms several hydrogen bonds with the amino acid residues in the catalytic site of VEGFR-2: in the hinge region, the 3-amino group on the indazole and the backbone carbonyl of Glu917, the ring N2 nitrogen, and the backbone amide NH of Cys919; the urea carbonyl and the backbone amide NH of Asp1046; and finally the urea external NH and Glu885 carboxylate. Compounds 2 and 3 have similar interaction patterns with VEGFR-2: both bind to the ATP binding site in the DFG-out conformation, enabling the p-chlorophenyl to penetrate into the extended hydrophobic pocket; the nitrogen of the quinoline forms a hydrogen bond with the backbone amide NH of Cys919; the carbonyl oxygen and the amide NH in compound 2 or the urea NH in compound 3 form two hydrogen bonds with the backbone amide NH of Asp1046 and the side chain of Glu885, respectively. As the N,N'-diaryl urea moiety of 1, the...
naphthamide moiety of 2, and the urea moiety of 3 occupy similar areas of kinase region, forming two hydrogen bonds with Asp1046 and Glu885. We envisioned that cyclization of 1 to the corresponding naphthamide or 2,3-dihydro-1,4-benzoxazine may form new VEGF-2 inhibitor scaffolds. To test the hypothesis, naphthamide 4a and 2,3-dihydro-1,4-benzoxazine 5 (Figure 1) were prepared, and the enzymatic assay showed that both 4a and 5 were potent VEGF-2 inhibitors with IC_{50} values of 1.6 and 40.8 nM, respectively. Encouraged by the high potency of 4a, we chose 4a as the lead molecule for further structure–activity relationship (SAR) exploration.

The general synthetic strategy for indazolynaphthamides 4a–v is outlined in Scheme 1. Triflate 8 was synthesized in two steps starting with the conversion of the 6-hydroxy-1-naphthoic acid 6 to the corresponding methyl ester 7 followed by triflation in the presence of DIPEA. Palladium-catalyzed coupling of the triflate 8 with bis(pinacolato)-diboron afforded the naphthylborate 9, which was then hydrolyzed to the carboxylic acid 10. Treatment of 10 with the corresponding amines in the presence of EDCI and DMAP gave naphthamides 11a–v. Preparation of 4a–v was achieved by Suzuki coupling between 4-iodo-1H-indazol-3-amine 12 and 11a–v. Derivatives 14a–c were similarly prepared by using a coupling reaction of 11a with corresponding heteroaryl bromides 13a–c (Scheme 2).

The synthesis of 5 is illustrated in Scheme 3. Reduction of 5-bromo-2-nitrophenol 15 followed by cyclization gave 17, which was then reduced to benzoxazine 18. The palladium-catalyzed coupling of 18 with bis(pinacolato)-diboron afforded the naphthylborate 19, which was reacted with phenyl isocyanate to provide 20. Finally, coupling 20 with iodide 12 afforded 5.

**Scheme 2. Synthesis of Naphthamides 14a–c**

**Scheme 3. Synthesis of 2,3-Dihydro-1,4-benzoxazine 5**

**Table 1. Biological Activity of Compounds 4a and 5**

The synthesis of 5 is illustrated in Scheme 3. Reduction of 5-bromo-2-nitrophenol 15 followed by cyclization gave 17, which was then reduced to benzoxazine 18. The palladium-catalyzed coupling of 18 with bis(pinacolato)-diboron afforded the naphthylborate 19, which was reacted with phenyl isocyanate to provide 20. Finally, coupling 20 with iodide 12 afforded 5.

**Table 1. Biological Activity of Compounds 4a and 5**

The synthesis of 5 is illustrated in Scheme 3. Reduction of 5-bromo-2-nitrophenol 15 followed by cyclization gave 17, which was then reduced to benzoxazine 18. The palladium-catalyzed coupling of 18 with bis(pinacolato)-diboron afforded the naphthylborate 19, which was reacted with phenyl isocyanate to provide 20. Finally, coupling 20 with iodide 12 afforded 5.

All compounds depicted in Tables 1–3 were evaluated for their inhibitory activity against VEGF-2 and VEGF-induced proliferation of HUVEC, using linifanib as reference. As shown in Table 1, the first two compounds 4a and 5, the biological data revealed that naphthamide 4a displayed more potent VEGF-2 inhibition than that of 2,3-dihydro-1,4-benzoxazine 5 (IC_{50}: 1.6 nM for 4a vs 40.8 nM for 5, Table 1). The result was similar in the further HUVEC cellular assay: 4a exhibited higher cellular potency than 5 (IC_{50}: 3.8 nM for 4a vs 12.9 nM for 5). Accordingly, 4a was chosen as the lead molecule for further SAR exploration.

We first examined the impact of the amide variations on their biological activity. Table 2 contains derivatives differing in the substitution patterns of the benzene rings, the isosteres of the benzene ring, and the types of alkyl groups. Incorporation of a methyl at ortho-position of the phenyl moiety led to a significant loss in both enzymatic and cellular potency by 16.7-fold and 22.7-fold, respectively (4b vs 4a). A methyl at the meta-position also reduced enzymatic potency but improved cellular potency by 1.8-fold (4c vs 4a). The para-methyl analogue 4d showed similar inhibition of the enzyme (IC_{50} = 1.9 nM) but improved cellular potency (IC_{50} = 1.3 nM) compared to 4a. meta-Ethyl substitution (4e) reduced enzymatic potency by 11.4-fold but improved cellular potency by 2.7-fold (4e vs 4a). Besides, meta-trifluoromethyl analogue 4f showed the same enzymatic potency (IC_{50} = 7.3 nM) but a drastically decreased cellular potency (IC_{50} > 100 nM) compared to 4c. Incorporation of an additional fluorine atom at the para-position to the methyl on the phenyl in 4c provided a 2-fold improvement in enzymatic potency accompanied by a 3-fold reduction in cellular potency (4g vs 4c). However, placing an additional fluorine atom on the phenyl of 4d made the molecule less active in both enzymatic and cellular assays (4h and 4i). In halogen-containing analogues 4j–r, no improvement was observed in the enzymatic assay compared to 4a, while compounds 4k, 4m, 4n, and 4o showed a slightly improved cellular potency (IC_{50} = 2.3, 2.6, 1.6, and 3.1 nM, respectively). Isostere replacement of the amide benzene ring...
exhibited matched enzymatic potency (IC\textsubscript{50} = 1.5 nM) but cyclopropyl groups were prepared (derivatives in which phenyls were replaced with ethyl or respectively), which was comparable to the phenyl derivatives.

To further explore the variations of amide moiety, two and a 5.4-fold reduction in cellular potency (IC\textsubscript{50} = 3.8 vs 20.5 nM) improved cellular potency (IC\textsubscript{50} = 0.9 nM).

addition to VEGFR-2, PDGFR-\(\beta\) is also an important anticancer target through antiangiogenic pathway. Therefore, inhibiting VEGFR-2 and PDGFR-\(\beta\) simultaneously may be beneficial for antiangiogenic tumor therapy. However, 14c displayed high selectivity over PDGFR-\(\alpha\) (365-fold, IC\textsubscript{50} = 548.6 nM) and FGFR-1 (429-fold, IC\textsubscript{50} = 643.7 nM) and excellent selectivity (>6666-fold) over Flt-3, EGFR, ErbB2, ErbB4, EGF (T790M/L858R), c-Src, ABI, EPHE-A2, and IGFR-1.

We finally investigated the in vitro antiangiogenic activity of compound 14c in a tube formation assay. HUVEC were placed on growth factor reduced matrigel and cultured with di

Inhibition of VEGF-induced proliferation of human umbilical vein endothelial cells.

**IC\textsubscript{50} values were the mean ± SD of two or more independent experiments.**

addition to VEGFR-2, PDGFR-\(\beta\) is also an important anticancer target through antiangiogenic pathway. Therefore, inhibiting VEGFR-2 and PDGFR-\(\beta\) simultaneously may be beneficial for antiangiogenic tumor therapy. However, 14c displayed high selectivity over PDGFR-\(\alpha\) (365-fold, IC\textsubscript{50} = 548.6 nM) and FGFR-1 (429-fold, IC\textsubscript{50} = 643.7 nM) and excellent selectivity (>6666-fold) over Flt-3, EGFR, ErbB2, ErbB4, EGF (T790M/L858R), c-Src, ABI, EPHE-A2, and IGFR-1.

We finally investigated the in vitro antiangiogenic activity of compound 14c in a tube formation assay. HUVEC were placed on growth factor reduced matrigel and cultured with different concentration of 14c in the presence of VEGF. As shown in Figure 2, compound 14c effectively inhibited endothelial cell tube formation induced by VEGF in a concentration-dependent manner, and tube formation was significantly inhibited by 10 nM of 14c.

In conclusion, we report the design, synthesis, and biological evaluation of novel naphthamides as a new class of VEGFR-2 inhibitors. The hybrid compound 4a served as a starting point for SAR exploration. SAR studies demonstrated that this new scaffold was tolerable to most of the modifications, such as isostere replacement of indazole and benzene and various

Table 2. Variations of the Naphthamide*"
substitution patterns of benzene ring. Privileged compound 14c exhibited high potency in both enzymatic and cellular assays. Kinase selectivity studies revealed that, in addition to VEGFR inhibition, 14c also inhibited PDGFR and RET kinases but showed high selectivity over other kinases. Moreover, 14c effectively inhibited angiogenesis in HUVEC tube formation assay. Therefore, our hybrid design of new VEGFR-2 inhibitor scaffolds and an understanding of their SARs offer promising opportunities to develop more effective anticancer agents.

**ASSOCIATED CONTENT**

* Supporting Information
General synthetic schemes and details of experimental procedures for the synthesis of all compounds, analytical data, and biological assay descriptions. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

*Corresponding Authors*


