Synthesis of 4-Anilinoquinazoline-Derivative Dual Kinase Inhibitors Targeting EGFR and VEGFR-2

Keuk Chan Bang,[†,‖] Tae Hun Song,[†,‡,‖] Young Jin Park,[†,‡] Jong Soo Lee,[†,‡] Seungah Jun,[‡] Seung Hyun Jung,[‡] Young-Jin Chun,[†,*] and Ha Hyung Kim[†,*]

[†]*College of Pharmacy, Chung-Ang University, Seoul 06974, Korea.*
**E-mail: yjchun@cau.ac.kr; hahyung@cau.ac.kr*
[‡]*Department of Drug Discovery, Hanmi Research Center, Gyeonggi-do 18469, Korea*
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The epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor-2 (VEGFR-2) signaling pathway have been clinically validated in solid tumors.[1,2] EGFR is highly overexpressed in a variety of cancers and plays a role in enhanced cell proliferation, in escape from apoptosis to ensure cancer cell survival, and finally, in the aggressive growth of invasive tumors.[3]

VEGFR-2 is the principal receptor in angiogenesis. Inhibition of VEGFR-2 signaling pathway influences tumor growth and metastasis by inhibiting tumor angiogenesis.[4,5]

Although single-pathway inhibitors have shown potential in cancer therapy, these pathways have shown limited clinical efficacy because cancer has complex pathology.[6,7] However, it is highly recommended to treat cancer with dual-pathway inhibitors because of the heterogeneous characteristics of cancer. In addition, dual kinase inhibitors could synergistically inhibit tumor growth.[8]

In our study, a series of novel dual-acting compounds were designed and synthesized to inhibit EGFR and VEGFR-2. *In vitro* kinase profiling and cell-based screening together with Structure–Activity Relationship (SAR) studies finally led to the discovery of compound **1**, which was a hybrid structure containing both acryl amide of CI-1033 and 4-bromo-2-fluoroaniline moiety of ZD-6474 (Figure 1).[9,10]

The general synthetic procedure for the preparation of compound **1** derivatives is summarized in Scheme 1.[11] A substituted quinazoline **2** was reacted with a substituted aniline, and subsequent nucleophilic aromatic substitution by *N*-Boc amino alcohol afforded the alkoxy quinazoline **3**. After deprotection of *N*-Boc, the carboxylic acid group was coupled with an amine to provide the amide **4**. Subsequent reduction of the nitro group on compound **4** and acrylation using acryloyl chloride yielded the target compounds **5**–**13**.

The enzyme inhibitory activities of EGFR and VEGFR-2 are summarized in Table 1. In order to investigate the SAR, the chain length (*n*) at site C-7 of the quinazoline was modified to vary the number of carbons (**5**–**7**). All of the modified compounds showed good IC_{50} values for EGFR in the range of 2 to 10 nM; in particular, **7** (n = 1) displayed better VEGFR-2 activity than **5** (n = 3) and **6** (n = 2). Thus, the short chain was superior to the long chains. Next, we evaluated the influence of R_1-substituted analogues, which were derived with various alkyl (**7**–**10**) or hetero alkyl (**11**–**13**) substituents on EGFR and VEGFR-2, for which the carbon chain was fixed at n = 1. The hetero alkyl analogues (**11** and **13**) exhibited better inhibitory activities than the alkyl analogues (**7**–**10**). Among the analogues, 11[12] showed stronger inhibition targeting EGFR and VEGFR-2 than the other derivatives (Table 1). In addition, **11** demonstrated potent inhibitory activities against mutated EGFRs (Table 2) as well as A431, VEGF-induced HUVEC, and H1975 cell lines. However, **11** did not inhibit Hs27, the human normal cell line (Table 3). Thus, **11** showed strong inhibitory activities toward EGFR and VEGFR-2 overexpressed cells as well as 1st generation EGFR inhibitor-resistant cell, which was expressed as T790 M mutation of EGFR in non-small cell lung cancer.[13] On the other hand, Iressa and ZD-6474 did not show any inhibitory activity against EGFR T790 M mutated cell and

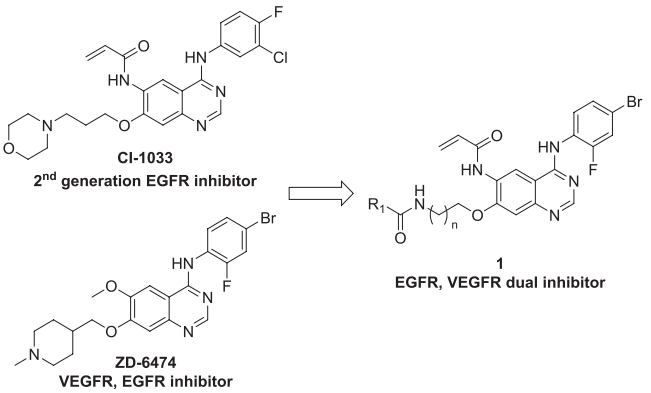

Figure 1. Schematic showing the design of compound **1** for the merged EGFR–VEGFR pharmacophore.

‖These authors contributed equally to this work.

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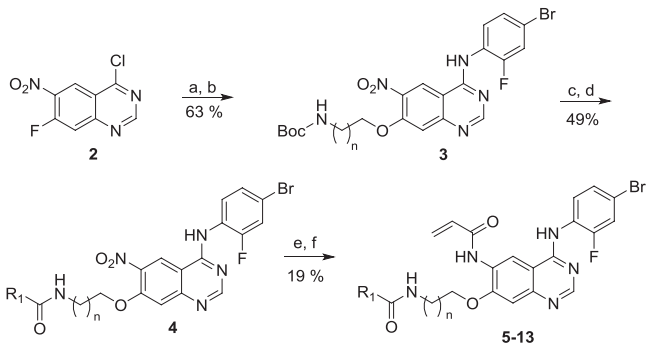
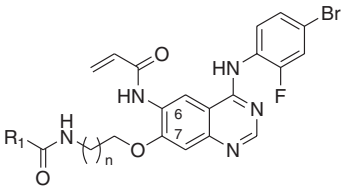


Scheme 1. (a) 4-bromo-2-fluoroaniline, iPrOH, reflux, 4 h; (b) *N*-Boc amino alcohol, KOTMS, DMSO, rt., 4 h; (c) TFA, CH_2Cl_2, rt., 0.5 h; (d) R_1CO_2H, EDCI, pyridine, rt., 4 h; (e) Fe, AcOH, reflux, 2 h; (f) acryloyl chloride, DMF, rt., 1 h.

enzymes. In addition, **11** significantly suppressed angiogenesis dose-dependently in mice because of VEGFR-2 inhibition (Figure 2).

In summary, we developed a series of compounds having 4-anilinoquinazoline as the key structure. The compounds were synthesized and evaluated for dual inhibitory activities against EGFR and VEGFR-2. Compound **11** showed excellent inhibitory activities against kinases and cells in EGFR and VEGFR-2 as well as T790 M mutant EGFR pathway. In addition, **11** demonstrated anti-angiogenic effect. Thus, compound **11** could serve as a guide for the development of an EGFR and VEGFR-2 dual inhibitor.

Table 1. Inhibitory kinase activities of derivatives for EGFR and VEGFR-2



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Compound	*n*	R_1	IC_{50} (nM) EGFR	IC_{50} (nM) VEGFR-2
5	2	CH_3	2	157
6	3	CH_3	10	545
7	1	CH_3	2	139
8	1	CH_2CH_3	3	250
9	1	$CH_2CH_2CH_3$	32	954
10	1	cyclopropyl	8	513
11	1	$CH_2N(CH_3)_2$	2	103
12	1	CH_2piperidine	7	857
13	1	CH_2OCH_3	14	161
ZD-6474			800	35
CI-1033			9	>1,000

Table 2. Inhibitory activities against mutated EGFRs.

Compound	IC_{50} (nM) EGFR	$EGFR^{T790M}$	$EGFR^{T790M/L858R}$
11	2	11	3
Iressa	530	> 1,000	> 1,000
ZD6474	800	> 1,000	> 1,000

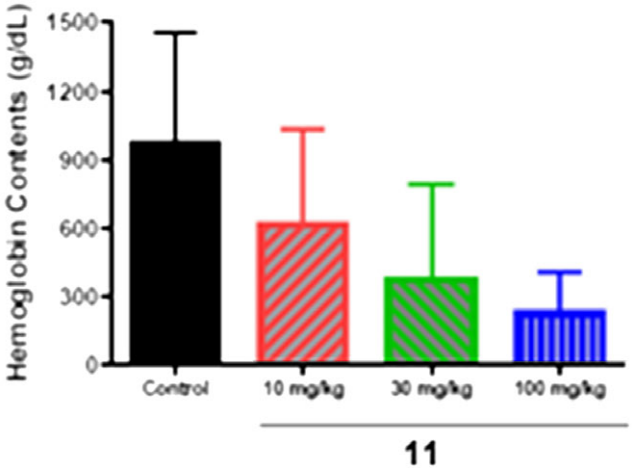

Figure 2. Compound **11** inhibit angiogenesis in the mice Matrigel Plug assay.[14]

Table 3. Inhibitory activities in cell-based assay for A431, VEGF-induced HUVEC, H1975, and Hs27.

Compound	IC_{50} (nM) A431	HUVEC	H1975	Hs27
11	14	93	130	> 1,000
Iressa	45	> 1,000	> 1,000	> 1,000
ZD6474	142	43	> 1,000	> 1,000

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