Synthesis of Quinazoline Analogues with Differential Activity for HER2-overexpressing Breast Cancer Cells

Sun-Young Jang,[†,‡,§] Seul Lee,[‡,§] Mira Kim,[†,‡] Young Jin Ham,[†] Mi Young Cha,[†] Kwee Hyun Suh,[†] Young Hoon Kim,[†] Ara Kwon,[‡] Hae Ju Kang,[‡] Kyung Hoon Min,[‡,*] and Kwang-Ok Lee[†,*]

[†]*Hanmi Research Center, Hanmi Pharm. Co. Ltd, Gyeonggi-do 445-813, Republic of Korea. *E-mail: kolee@hanmi.co.kr*
[‡]*College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea. *E-mail: khmin@cau.ac.kr*
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HER2 (also known as ErbB2) is a member of the epidermal growth factor receptor (EGFR) family, which also includes EGFR, HER2, HER3, and HER4. It is a receptor tyrosine kinase[1,2] and plays a pivotal role in oncogenic transformation and tumorigenesis via homodimerization or heterodimerization with EGFR family members to activate intracellular signaling.[3,4] Overexpression or amplification of HER2 receptor is seen in a variety of cancers, including breast, gastric, pancreatic, and bladder cancer.[5] Recent cancer genome sequencing has shown that somatic mutations in HER2 are driver events in breast[6] and non–small cell lung cancers.[7] Drugs targeting HER2 include monoclonal HER2 antibodies, such as trastuzumab[8] and pertuzumab,[9] and tyrosine kinase inhibitors, such as lapatinib.[10] These drugs are used clinically in combination with other anticancer drugs for the treatment of HER2-positive breast cancer. Lapatinib is an inhibitor of both EGFR and HER2 and exhibits potent antiproliferative activity against EGFR- and HER2-dependent cancer cells. However, potent inhibition of EGFR causes side effects, including diarrhea and skin rash, which diminish patient quality of life during long-term treatment. These adverse events are common with EGFR tyrosine kinase inhibitors such as gefitinib[11] and erlotinib[12] (Figure 1). Thus, when using lapatinib, it is important to minimize the side effects by reducing the inhibition of EGFR activity and maximizing the drug efficacy.[13] Herein, we describe potent inhibitors with differential activity in HER2-dependent breast cancer cells and EGFR-dependent cancer cells.

We commenced with the synthesis of a series of quinazoline derivatives (Table 1), in which an amide replaced the substituted furan moiety of lapatinib at the C_6 position, as well as the C_7 position (Figure 2). The aniline moiety at the C_4 position was fixed as 3-chloro-4-(pyridin-2-ylmethoxy)aniline, because it was a useful substituent to increase growth inhibitory activity against SkBr3 cells according to our previous report.[14]

The synthesis of C_6-modified quinazoline analogues is outlined in Scheme 1. 7-Nitro-4(3*H*)-quinazolinone **2** was obtained from the reaction of 2-amino-5-nitrobenzoic acid **1** with formamide at 170 °C. Chlorination of 7-nitroquinazoinone **2** using thionyl chloride in the presence of phosphoryl chloride and DMF (dimethylformamide) afforded 4-chloroquinazoline **3**, which was converted to aminoquinazoline **4** through the addition of 3-chloro-4-(pyridin-2-ylmethoxy) aniline and subsequent reduction using iron. Coupling of *N*-Boc-protected amino acids and aminoquinazoline **4** followed by deprotection of the *N*-Boc group using trifluoroacetic acid provided the desired analogues (**6–14**).

The synthetic route for C_7-modified quinazoline analogues is illustrated in Scheme 2. 6-Fluoro-7-nitroquinazolinone **15** was synthesized from commercially available 2-amino-4-fluoro-5-nitrobenzoic acid using the same synthetic method used for compound **2**. Nucleophilic substitution with alcohols or amine in the presence of potassium trimethylsilanolate in DMSO (dimethyl sulfoxide) gave the quinazolinone **16**, which was converted to chloroquinazoline **17** and reduced to 7-aminoquinazline **18**. Finally, *N*-Boc-$_L$-proline was coupled with 7-aminoquinazoline **18** in the presence of EDCI (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), and *N*-Boc group was deprotected to provide C_6-modified quinazoline analogues (**20–23**).

Antiproliferation assay[15] was performed to determine the selectivity between A-431, an EGFR-overexpressing cancer cell line, and SKBr-3, an HER2-overexpressing breast cancer cell line (Table 1). Gefitinib was used as an EGFR selective inhibitor and lapatinib was used as an EGFR/HER2 dual inhibitor. Acyclic amino acid derivative **6** showed higher selectivity for SKBr-3 cells than the corresponding cyclic derivative **8**. (*S*)-Isomer **7** exhibited ~10-fold greater potency than racemic compound **8**. Ring-expanded compounds (*R*)-**9** and (*S*)-**10** were also synthesized. (*S*)-Isomer **10**,[16] which had a GI_{50} of 8.8 nM, showed more than 200-fold greater potency than (*R*)-isomer **9**, which had a GI_{50} of 2148 nM in SKBr-3 cells. In terms of selectivity, the activity of (*S*)-isomer **10** in SKBr-3 cells was ~21-fold greater than that in A-431 cells. However, (*R*)-isomer **9** did not show differential activity in A-431 and SKBr-3 cells. *N*-methylation of the pyrrolidine group of compound **10** led to a complete loss of activity. Piperidine derivatives **12–14** had similar activity in both A431 and SKBr-3 cells, but were less active than compound **10**.

§ These authors contributed equally to this work.

Next, we synthesized the derivatives of compound **10** by introducing various substituents at the C_7 position. However, introduction of fluoro (**20**), dimethylamino (**22**), and 1-methyl-piperidinyl methyloxy (**23**) groups resulted in a loss of activity, except for the introduction of methoxy group. Only methoxy analogue **21** showed moderate activity and selectivity. Among the derivatives, (*S*)-proline derivative **10** was the most potent, with high selectivity for SKBr-3 over A431 cells. The kinase activity of compound **10** for EGFR and HER2 was explored *in* vitro.[17] Unlike the results from the cell-based assay, EGFR and HER2 were inhibited by 99% and 90%, respectively, by 1 μM of compound **10**. Data from cell-based assays and *in vitro* kinase assay were not consistent. Thus, further investigation, including biochemical experiments such as western blotting, is required to investigate the high differential activity of compound **10**.

In summary, we discovered novel quinazoline analogues that had high potency and selectivity for HER2-overexpressing cancer cells compared with EGFR-overexpressing cancer cells. The most potent compound, **10**, could be a useful chemical tool to find a therapeutic target for the treatment of HER2-dependent cancer patients.

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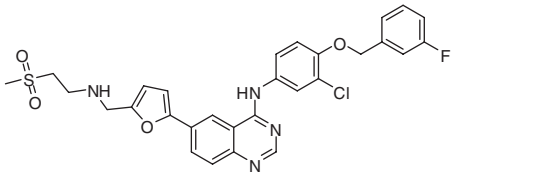


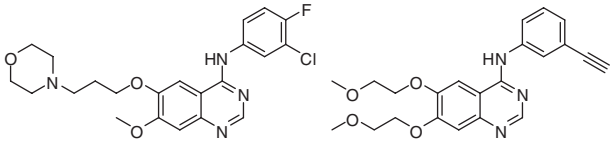
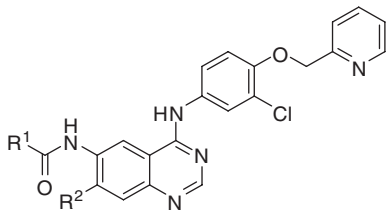


Figure 1. Chemical structures of EGFR tyrosine kinase inhibitors (gefitinib, erlotinib, and lapatinib).

Table 1. Activity of synthesized quinazoline derivatives in A-431 and SKBr-3 cells.[15]



Compound	(GI_{50}, nM) A-431	SKBr-3	Fold ratio[a]
6	4697	213	22
7	471	29	16
8	1298	276	4.7
9	1390	2148	0.65
10	184	8.8	21
11	>1000	>5000	–
12	208	58	3.6
13	323	73	4.4
14	142	51	2.8
20	>1000	>1000	–
21	483	56	8.5
22	>1000	>1000	–
23	2888	2487	1.2
Gefitinib	28	206	0.14
Lapatinib	98	29	3.4

[a] GI_{50} for A431/GI_{50} for SKBr3.

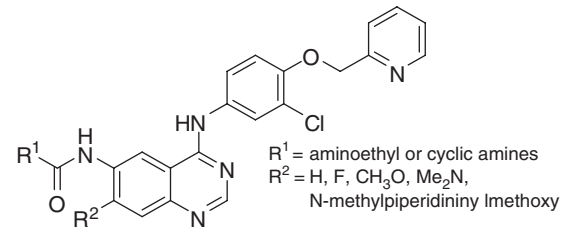
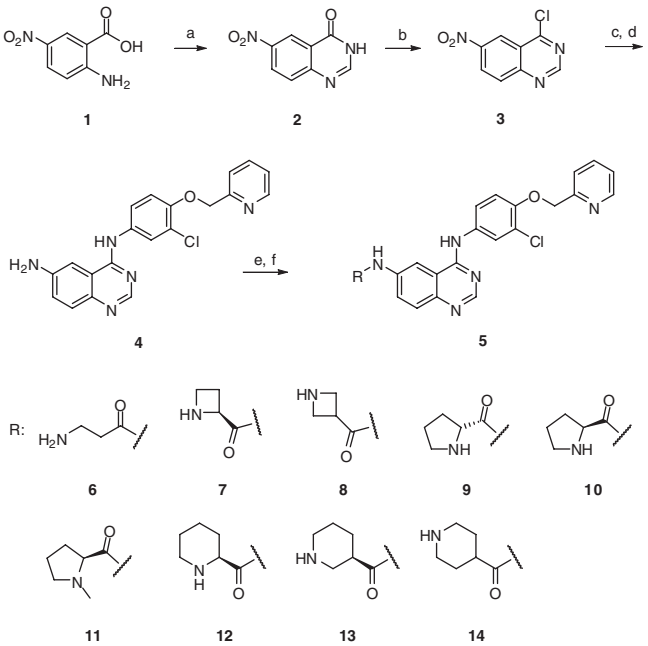
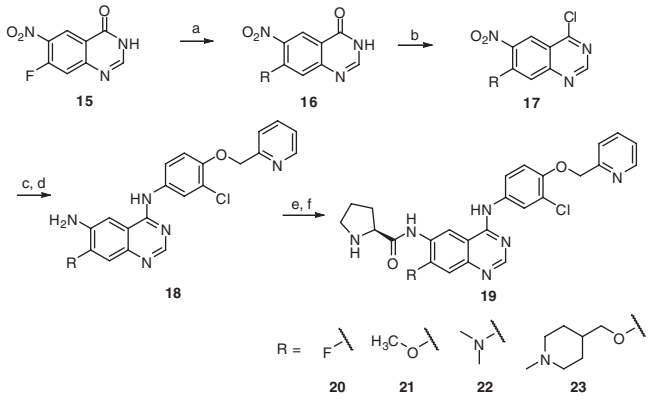


Figure 2. Design of quinazoline derivatives based on lapatinib.



Scheme 1. Reagents and conditions: (a) formamide, 170 °C; (b) $SOCl_2$, $POCl_3$, DMF (cat.); (c) 3-chloro-4-(pyridin-2-ylmethoxy) aniline, *i*PrOH, reflux; (d) Fe, *c*-HCl (cat.), 50% EtOH, reflux; (e) *N*-Boc-amino acid, EDCI, pyridine, rt; (f) TFA, DCM, rt.



Scheme 2. Reagents and conditions: (a) alcohols or amine, KOTMS, DMSO, rt; (b) $SOCl_2$, $POCl_3$, DMF (cat.); (c) 3-chloro-4-(pyridin-2-ylmethoxy)aniline, *i*PrOH, reflux; (d) Fe, *c*-HCl (cat.), EtOH/H_2O (1:1), reflux; (e) *N*-Boc-$_L$-proline, EDCI, pyridine, rt; (f) TFA, DCM, rt.

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15. The number of viable cells was determined using a Cell Counting Kit 8 (Dojindo Co. Japan) according to the manufacturer's instruction.
16. Spectral data of compound **13**: ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 10.12 (s, 1H), 8.87 (d, 1H, *J* = 2.4 Hz), 8.66 (1H, s), 8.09 (d, 1H, *J* = 4.2 Hz), 8.05 (d, 1H, *J* = 1.2 Hz), 7.82 (d, 1H, *J* = 9 Hz), 7.75(s, 1H), 7.53 (dd, 1H, *J* = 9 Hz, 2.4 Hz), 7.48 (dd, 1H, *J* = 9 Hz, 2.4 Hz), 7.53 (d, 1H, *J* = 9 Hz), 7.25–7.29 (m, 1H), 6.94–6.99 (m, 2H), 6.88 (dt 1H, *J* = 8.4 Hz, 1.8 Hz), 5.58 (s,2H), 3.92 (q, 1H, *J* = 9.6 Hz, 5.4 Hz), 3.12 (dt, 1H, *J* = 10.2 Hz, 6.6 Hz), 2.24–2.27 (m, 1H), 2.03–2.09 (m, 1H), 1.76–1.82 (m, 2H), ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 174.1, 163.8, 162.2, 157.8, 154.1, 146.9, 139.3, 139.2, 137.4, 135.8, 133.7, 131.5, 130.3, 130.3, 129.5, 125.5, 124.6, 123.9, 122.7, 122.7, 115.3, 114.9, 114.7, 114.2, 114.1, 109.5, 108.7, 61.01, 52.5, 52.5, 47.4, 30.8, 26.4, HRMS (ESI): exact mass calcd. for $C_{27}H_{24}FN_7O$ $[M+H]^+$, 482.2099, found 482.2086.
17. *In vitro* kinase assay was performed by Eurofins Pharma Discovery Service (Dundee, UK).

