Thesis for the Degree Master of Science

# A Novel Quinazoline Derivative Acting on EGFR Mediated Signal Pathway in A431 Cells.


by

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Department of Microbiology

The Graduate School

Pukyong National University

February 2009

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## (A431 세포에서 EGFR 연관 신호전달 경로에 작용하는 새로운 퀴나졸린 유도체)

Advisor: Prof. Gun Do Kim

by

Chul Woo Jeong


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February 25, 2009

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# A Novel Quinazoline Derivative Acting on EGFR Mediated Signal Pathway in A431 Cells.

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## Abstract

Epidermal Growth Factor Receptor (EGFR) is involved in signaling pathways controlling cell growth and differentiation. The over-expression and loss of self-regulation of EGFR give rise to various solid tumors. Therefore, inhibitors of EGFR kinase activity may prove useful for therapeutic intervention in cancer as well as other proliferative diseases. Quinazoline is a compound made up of two fused six-membered simple aromatic rings, a benzene ring and a pyrimidine ring.

Medicinally it has been used in various areas especially as an anti-malarial agent and in cancer treatment. Both 63013 and 63033 compounds possess a [1,4]-dioxino quinazoline structure linking the alkoxy side chains together and their structural characteristic is considered to have better solubility than dialkoxyquinazoline derivatives. The purpose of this study was to investigate the inhibitory effects of EGFR activities by quinazoline derivatives (63013 and 63033). During incubation with 63013, 63033, and Iressa (ZD-1839) of various concentrations, EGFR activities of A431 cells (Human lung carcinoma) show concentration-dependent inhibition. Both 63013 and 63033 compounds inhibited EGFR phosphorylation on each tyrosine residue (Tyr-845, Tyr-992, Tyr-1045, Tyr-1068, and Tyr-1173) and inhibited the activities of MEK1/2, MAPK p44/42, STAT3, and EGF-related downstream molecules.

# Introduction

Epidemal Growth Factor Receptor (EGFR) is a kind of type 1 receptor tyrosine kinase or ErbB receptor (Casalini *et al.* 2004), and divided into four kinds, namely, EGFR (ErbB1/EGFR/HER1), ErbB2 (HER2/*neu*), ErbB3 (HER3) and ErbB4 (HER4) (Sedlacek 2000; Well 1999). ErbB receptor is divided into extracellular ligand-binding domain, transmembrane domain, intracellular tyrosine kinase (TK) domain. When ligand bound to ErbB receptor, forms homo-dimetric or heterodimetric complexes, intracellular tyrosine kinase domain is phosphorylated, and thus the intracellular signal transductions of Ras-Raf-MAP-kinase, phosphatidylinositol 3-kinase (PI3K), stress-activated protein kinase (protein kinase C & Jak/Stat) are activated (Olayioye *et al.* 2000).

Activation of the EGFR leads to receptor-associated tyrosine kinase activity that stimulates a cascade of events leading to cell cycle progression, as well as a number of other processes that are crucial to cancer progression (Downward *et al.* 1984; Herbst 2004).

The EGFR plays an important role in regulating cellular processes such as proliferation, differentiation, survival and is central to the maintenance of normal epidermal tissues where its expression is highly regulated (Klapper *et al.* 2000). The

over-expression and loss of self-regulation of EGFR give rise to various solid tumors, including breast, colorectal, ovarian, non-small-cell lung cancer (NSCLC) (Salomon *et al.* 1995; Porebska *et al.* 2000; Woodburn 1999). Furthermore, it is reported that when EGFR is over-expressed, it activates the signaling transduction system, and therefore cancer cells grow more aggressively, and, with the invasiveness increasing, the transitions occur more easily, affecting negative effects to the survival rate (Arteaga 2002; Hamid 2004). Therefore, the inhibitors preventing EGFR kinase activity can be the effective medicine for cancer and other proliferative diseases, and in this respect, a variety of researches on inhibitors have recently been conducted (De Luca *et al.* 2000; Mendelsohn *et al.* 2003; Ranson 2004). EGFR-targeted therapies in development include those that interact with the extracellular ligand-binding domain, and those that act intracellularly, such as the small-molecule EGFR tyrosine kinase inhibitors (EGFR-TKIs) that compete with ATP to bind to the receptor's ATP site.

Quinazoline is a compound made up of two fused six-membered simple aromatic rings, a benzene ring and a pyrimidine ring. Medicinally it has been used in various areas especially as an anti-malarial agent and in cancer treatment. However, quinazoline has poor bioavailability *in vivo*, because

of their low water solubility. So, to heighten bioavailability, numerous researches on quinazoline derivatives are conducted by researchers. 63013 and 63033 both compounds possess a [1,4]-dioxino quinazoline structure linking the alkoxy side chains together, because their structural characteristic is considered to have better solubility than dialkoxyquinazoline derivatives (Ha *et al.* 2005).

The purpose of this study was to investigate the inhibitive effects of EGFR and EGF-related downstream molecules activities by quinazoline derivatives (63013 and 63033).

# Materials and Methods

## Materials and antibodies

Media and cell culture reagents and materials were purchased from GIBCO BRL (baithersburg, MD). Anti-EGFR (pY845, pY992, pY1045, pY1068, total-EGFR), anti-STAT3 (pY705, pS727, total-STAT3), anti-MEK 1/2 (pS217/221, total-MEK 1/2), anti-MAPK p44/42 (pT202/pY204, total-MAPK p44/42), anti-AKT (pT308, pS473, total-AKT), and anti-PDK1 (pS241, total-PDK1) antibodies were obtained from Cell Signaling (Beverly, MA). Antibody against Phospho-tyrosine residue 1173 of EGFR was purchased from Calbiochem (San Diego, CA).

## *In vitro* assay for EGF Receptor Tyrosine Kinase

EGF Receptor Kinase assay was used AlphaScreen P-Tyr-100 assay kit system. EGFR (Affinity purified from human carcinoma A431 cells) was purchased from Sigma, substrate (poly[Glu:Tyr](4:1)) and AlphaScreen P-Tyr-100 assay kit were purchased from Packard BioScience company. The kinase reactions were performed in the mixture of EGFR

enzyme, ATP and biotinylated poly[Glu:Tyr] (4:1) in 50 mM Tris (pH7.5), 5mM $MgCl_2$, 5 mM $MnCl_2$, 2 mM DTT, 0.01% Tween-20; incubated for 1hr at room temperature (RT). Quenched by adding the detection buffer containing EDTA, Donor-Streptavidin and Acceptor-P-tyr-100 beads; incubated for 1hr at RT. Detected AlphaScreen signal using a Fusion alpha-microplate analyzer (Packard BioScience company, USA).

## Cell culture

A431 cells were cultured in DMEM (Life Technologies, Inc. , Rock ville, MD) supplemented with 10 % fetal bovine serum (FBS), 100 U/ml penicillin, and 100 ug/ml streptomycin (Life Technologies, Inc., Rock ville, MD) at 37 ℃ with 5 % $CO_2$. Hela and HEK293 cells were grown in DMEM containing heat-inactivated FBS, 100 U/ml penicillin, and 100 ug/ml streptomycin at 37 ℃ with 5 % $CO_2$. A549 and HT-29 cells were cultured in RPMI-1649 containing 10 % FBS, 100 U/ml penicillin, and 100 ug/ml streptomycin at same conditions as above. HUVEC cells were grown in liquid-endothelial cell basal medium-2 (EBM-2) purchased in Cambrex Bio Science Walkersville, lnc. (Walkersville, MD,) containing various growth factors (20 ml (20 %) FBS, Hydrocortisone (0.2 ml), hFGF (2 ml), vEGF (0.5 ml), R3-IGF (1.05 ml), Ascrobic acid (0.5 ml),

hEGF (0.5 ml), GA-1000 (0.5 ml), Heparin (0.5 ml) ). SK-Br-3 cells were cultured in McCoy's 5A medium supplemented with 10 % FBS. The cells were maintained by subculture in a T75 flask. For the experimental dishes, the confluent cells were subcultured in 6 cm dishes before performing experiments.

## Cell-based ELISA

For detection of phosphorylated (pY992) EGF receptors, I used cell-based ELISA kits from Active Motif (Carlsbad, California) following by manufacturer's recommended procedure. Briefly, A431 cells were seeded in 96-well plates at 1 x $10^4$ cells/well. After incubation for overnight, each compound was pre-treated for 45 min and then cultured in the presence or absence of 5 nM EGF for 10 min. The cells were fixed with 4% formaldehyde in PBS for 20 min at room temperature and washed three times with washing buffer (1 x PBS containing 0.1 % Triton X-100). Endogenous peroxidase was quenched with washing buffer containing 1 % $H_2O_2$ and 0.1 % Sodium azide for 20 min, washed three times in washing buffer, blocked with 10 % fetal calf serum in washing buffer for 1 hr and incubated overnight with phospho-specific EGFR antibody 1/200 diluted with dilution buffer at 4 ℃. Next day, cells were washed three times with washing buffer for 5 min and

incubated with secondary antibody (peroxidase-conjugated anti-rabbit antibody, 1/100 dilution) for 1 hr at room temperature and washed three times with washing buffer for 5 min and twice with PBS. Subsequently the cells were incubated with 50 ul chemiluminescent working solution to each well at room temperature. Luminescence activity was measured by luminometer. Luminescence activity on phospho-EGFR was calculated using the following formula: luminescence activity (%) = [(all sample treated each compound and EGF -sample average nontreated EGF)/ sample average treated only EGF] 100. All experiments were performed in duplicate.

## Western Blot Analysis

Cells were pre-cultured with indicated compound for 45 min and then treated with 5 nM EGF (Epidermal Growth Factor) for 10 min, To prepare whole cell lysates, cells were washed twice with cold-PBS and lysised by using 100 ul SDS-sample buffer (6.25 mM Tris-HCl (pH 6.8), 2 % w/v SDS, 10 % glycerol, 50 mM DTT, 0.1 % w/v bromophenol blue). Each lysate was electrophoresed on a 8 % or 10 % sodium dodecyl sulfate (SDS)-polyacrylamide gel after boiling 10 min in SDS sample buffer. Proteins were blotted onto cellulose nitrate membrane (Schleicher & Schuell, USA). After

electroblotting, the membranes were blocked with Tris-buffered saline and Tween 20 (10 mM Tris-HCl, pH 7.4; 150 mM NaCl; and 0.1% Tween 20) containing 5 % milk and incubated with the primary antibody diluted in blocking buffer for overnight in 4 ℃. Membranes were then washed, incubated with the appropriate second antibody in blocking buffer for 1 hr, and rewashed. Blotted proteins were detected by using the enhanced chemiluminescence detection system (Cell signaling, Beverly, MA) with Victor2 (PerkinElmer Life Sciences, Boston, MA) reading to luminescence activities. The methods used for molecular cloning were based on those of sambrook *et al.* (2000).

## MTT assay

Cells were seeded in 96 well plates at 5 x $10^3$ cells (for testing cell proliferation) and 1 x $10^4$ cells (for testing cell toxicity) confluence and cultured in appropriate medium for 48 hr. The proliferative effects of each compound against various cell lines were determined by a 3-(4,5-dimethylthiazol-2-yl)-2 ,5-diphen-yltetrazolium bromide (MTT) assay that measures cell proliferation based on the ability of live cells to use MTT and converts it to a dark blue formazan (Mosmann 1983). After incubation for 2 days the number of metabolically active cells

was determined by MTT assay as measured by a 96-well microtiter plate reader at absorbance 570 nm.

## Synthesis of compounds

After dihydroxybenzoate ethylester, 1 and epichlorohydrin were dissolved in ethanol, and heated and refluxed for 10 hr in the presence of $Na_2CO_3$, 3-hydroxymethyl dihydrobenzodioxane, 2 was obtained at 95 % yield rate. With compound 2 treated with sodium hydride (NaH), and methyl iodide (MeI) and benzyl bromide (BnBr) added to it, O-alkylation compound 3a and 3b were obtained at about 90 % yield rate (Fig. 1) (Ha *et al.* 2005).

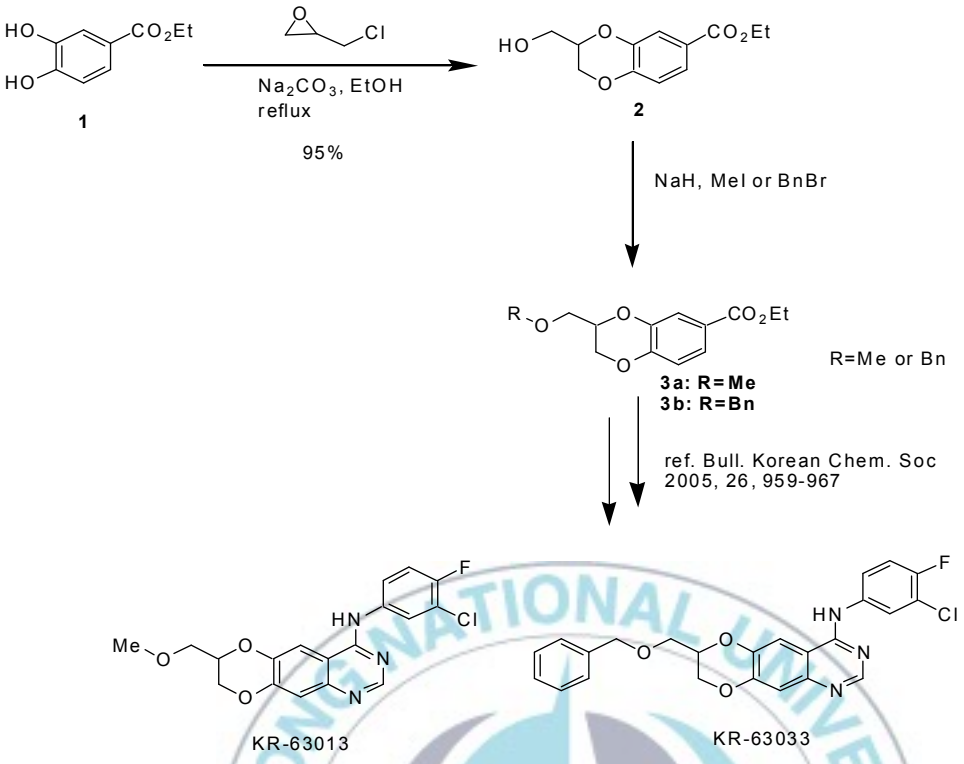


**Figure 1. Synthesis method of KR-63013 and KR-63033.** After dihydroxybenzoate ethylester, 1 and epichlorohydrin were dissolved in ethanol, and heated and refluxed for 10 hr in the presence of $Na_2CO_3$, 3-hydroxymethyl dihydrobenzodioxane, 2 was obtained at 95 % yield rate. With compound 2 treated with sodium hydride (NaH), and methyl iodide (MeI) and benzyl bromide (BnBr) added to it, O-alkylation compound 3a and 3b were obtained at about 90 % yield rate. The desired compounds, KR-63013 and KR-63033 can be manufactured with the method as indicated in Ha *et al.* 2005.

# Results and Discussion

## Inhibition effects on EGFR activities.

The quinazoline derivatives are the effective EGFR-activity inhibitors (Ranson *et al.* 2002). The EGFR-activity inhibiting effects of compound 66313 and compound 63033 are examined with A431 cell *in vitro* (Fig. 2). To compare the EGFR-activity inhibiting effects of compound 63013 and compound 63033, Iressa, well-known as a strong EGFR-activity inhibitor, is used as the control (Ranson *et al.* 2002). Iressa is a kind of Quinazoline derivatives, containing morpholinyl propoxy group at C-6. Using AlphaScreen P-Tyr-100 assay system, EGF Receptor Tyrosine Kinase is assayed *in vitro*. Each compound represented 50 % inhibition rate at 7 uM concentration level, showing the modality of concentration-depe-ndent inhibition. Like Iressa (positive control Iressa as a control group), both compounds, 63033 and 63013 does not have EGFR inhibiting effects *in vivo* and *in vitro*. Therefore, as these two compounds have the excellent ability to penetrate cell membrane, they can be used as drugs (Fig 2A, 2B).

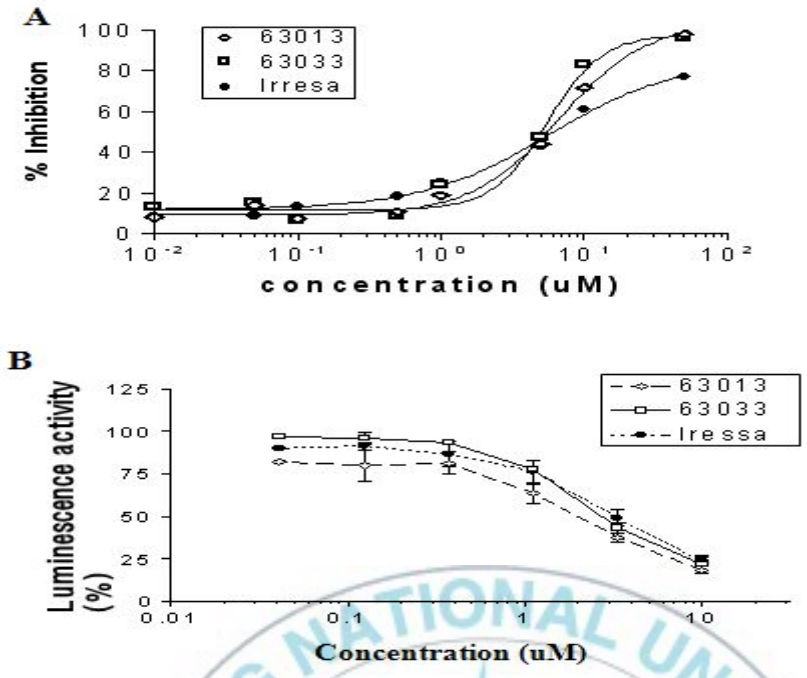


Figure 2. *In vitro* Inhibition effects of EGFR activities by 63013, 63033, and Iressa on A431 cell lines. (A) *In vivo*, (B) *In vitro*, measuring of the levels of EGFR activities was performed as described in materials and methods parts with 63013 (open diamond), 63033 (open quadrangle), and Iressa (as a positive control, closed circle). Reduction of phosphorylation of EGFR tyrosine residue 992 was measured by using cell based-ELISA as described in materials and methods parts in A431 cells. A431 cells were treated with 63013, 63033, and Iressa of various concentration as a indicated figure. Its results were established as a luminescence activities.

## The effect on each tyrosine residues of EGFR.

EGFR consists of ligand-binding region and kinase domain, and, when bound to EGF, is activated and, in turn, forms dimmer, and thus tyrosine residues in kinase domain become phosphorylated (Olayioye *et al.* 2000). EGFR inhibitors, including Quinazoline derivatives, inhibit phosphorylation of tyrosine residues in kinase domain of EGFR. Accordingly, with EGFR treated with Compound 63013 and Compound 63033, the effects of the compounds on each tyrosine residue in kinase domain of EGFR were tested (Fig. 3 and 4). Firstly, in the condition of A431 cell being treated for 10 min with an EGF at 5 nM concentration level and otherwise, the search was conducted with phosphorylated EGFR antibody against tyrosine residue 845. The result showed that in the condition of EFG at 5 nM concentration level, tyrosine residue 845 was phosphorylated (Fig. 3). A431 cell was cultivated for 10 min, in the condition of the cell being pre-treated with the two compounds at various concentration levels, and in the presence of 5 nM EGF. Next, EGFR phosphorylation in Tyr-845, Tyr-992, Tyr-1045, Tyr-1068 and Tyr-1173 was assessed through antibody recognizing various EGFR phosphopeptides. DMSO was used as positive control. It was found that tyrosine residue which resided in the EGFR was phosphorylated if there

was EGF (Fig. 3). The two compounds inhibited phosphorylation of the tyrosine residues, dependent on concentration levels (Fig. 4A). And, the results with HeLa cell reported to express EGFR, when treated with the two compounds in the same condition, also showed that phosphorylation of tyrosine residues was inhibited, dependent on concentration levels (Fig. 4B).

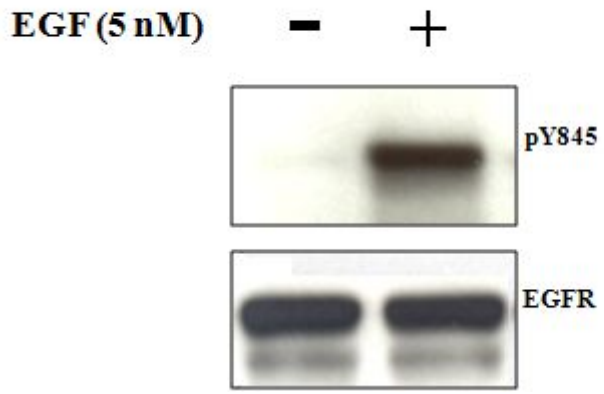


Figure 3. The effect of EGF on tyrosine residues in EGFR. A431 cells were treated with or without 5 nM EGF for 10 min and detected by phosphorylated EGFR antibody against tyrosine residue 845.

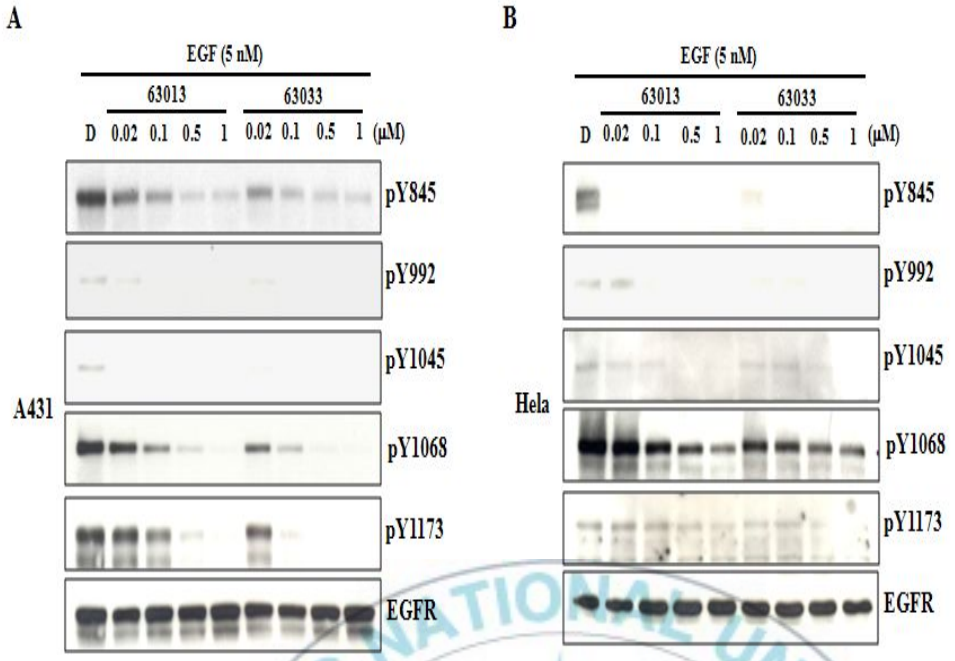


**Figure 4. The effect on each tyrosine residues of EGFR by both compounds.** (A) A431 cells were pre-treated with both compounds or DMSO (positive control) in indicated concentrations and then incubated in 5 nM EGF for 10 min. EGFR phosphorylation on Tyr-845, Tyr-992, Tyr-1045, Tyr-1068, and Tyr-1173 were subject to immunoblotting with antibodies that recognize various EGFR phosphopeptides. (B) In the same conditions as (A), EGFR phosphorylation on various phosphopeptides were tested in Hela cell lines.

## The effect on EGF-related downstream molecules.

When EGFR, bound with EGF, is activated, EGF-related downstream molecules (Olayioye *et al.* 2000), such as MEK 1/2, MAPK p44/42, PDK1, and STAT3, are phosphorylated. In this respect, it was tested whether the two compounds suppressed activity of each molecule using antibodies which recognize phosphorylation of each residue in MEK 1/2, MAPK p44/42, PDK1, and STAT3 (Fig. 5, 6, and 7). A431 cells, pre-treated with the two compounds with the concentrations indicated in figure 5, 6 and 7 were cultivated for 10 min in the presence of 5 nM EGF. And using antibodies recognizing phosphorylation of MEK 1/2, MAPK p44/42, PDK1, and STAT3, the experimentation was conducted to find whether the two compounds inhibited the activity of each molecule. In the case of MAPK p44/42, while the 63013 compound suppressed phosphorylation of pT202/pY204 residue in the 1 uM concentration, the 63033 compound did not show any suppressing effects (Fig. 5).

Therefore, it was suggested that there were little differences in their operating principles to inhibit EGFR although the two compounds, 63033 and 63013, had similar structure. In the case of PDK1, both compounds, 63033 and 63013 did not inhibit phosphorylation of a residue (Fig. 6). The results

showed that the two compounds inhibited the activities of MEK 1/2, MAPK p44/42, and STAT3.

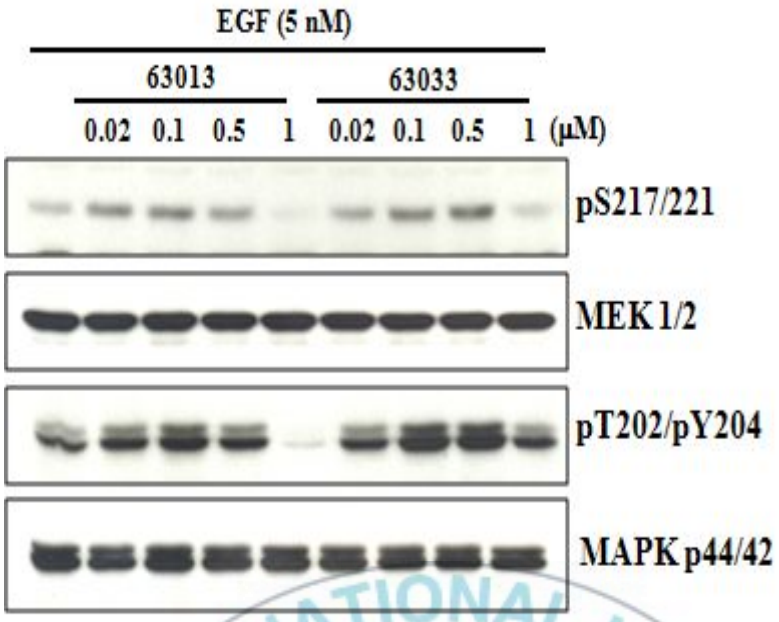


**Figure 5. 63013, and 63033 compound inhibited the activities of MEK1/2 and MAPK p44/42.** A431 cells seeded in 6 cm dishes were exchanged with serum free DMEM for 24 hour. Serum-starved A431 cells were pre-treated with both compounds or DMSO (positive control) and incubated with 5 nM EGF for 10 min. Phosphorylation of MEK 1/2 and MAPK p44/42 were detected by immunoblotting with antibodies that recognize phosphorylation site of each protein. After stripping each membrane used to detect phosphorylation of MEK 1/2 and MAPK p44/42 each membrane was rebloted with antibodies that recognize total proteins of MEK 1/2 and MAPK p44/42.

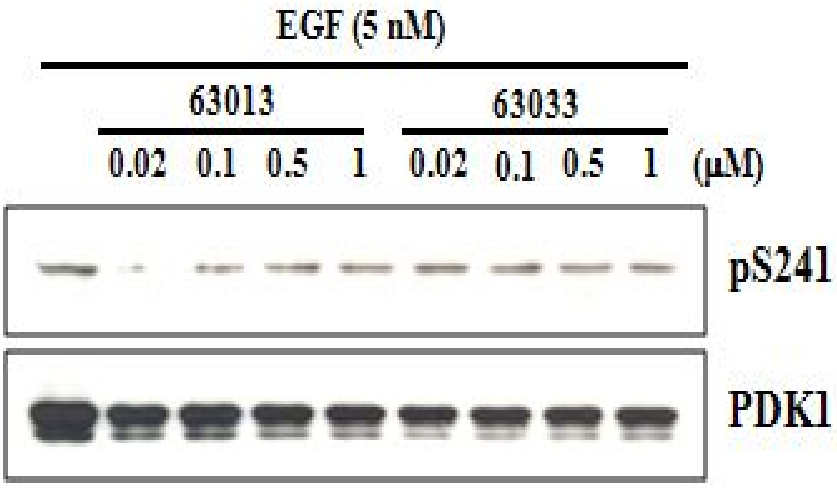


Figure 6. 63013, and 63033 compound inhibited the activities of PDK1.

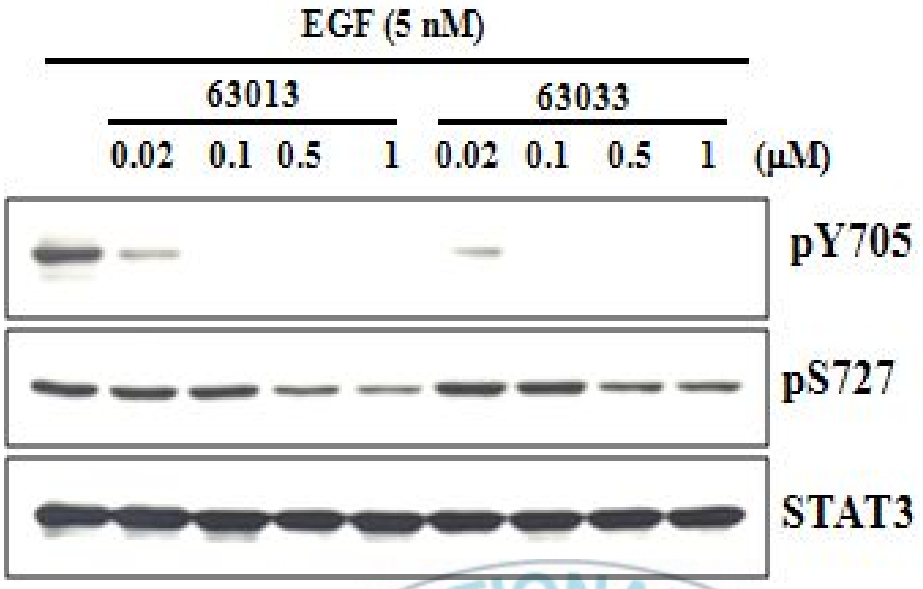


Figure 7. 63013, and 63033 compound inhibited the activities of STAT3.

## The comparison of phosphorylation and activation on EGFR and EGF-related downstream molecules.

In EGFR and EGF-related downstream molecules, the activity-inhibition of compound 63013 and compound 63033 was compared with that of Iressa, as a control group, which was reported to be a strong EGFR inhibitor (Ranson *et al.* 2002). A431 cells, pre-treated with 1 uM concentration of each compound, were cultivated for 10 minutes in the presence of 5 nM EGF. DMSO was used as positive control. The two compounds represented almost the same phosphrylation-inhibiti -on effects in tyrosine residues of EGFR as Iressa. Also, the two compounds, 63033 and 63013, had sometimes shown stronger inhibiting effects than those of Iressa (Fig. 8). In addition, the two compounds also represented the almost the same activity-inhibition effects in EGF-related downstream molecules, such as STAT3 (Fig. 9), and AKT (Fig. 11), as Iressa.

In the case of MEK1/2 and MAPK p44/42, while the 63013 compound had shown very similar effects with those of the Iressa, 63033 compound had relatively weaker inhibiting effects (Fig. 10).

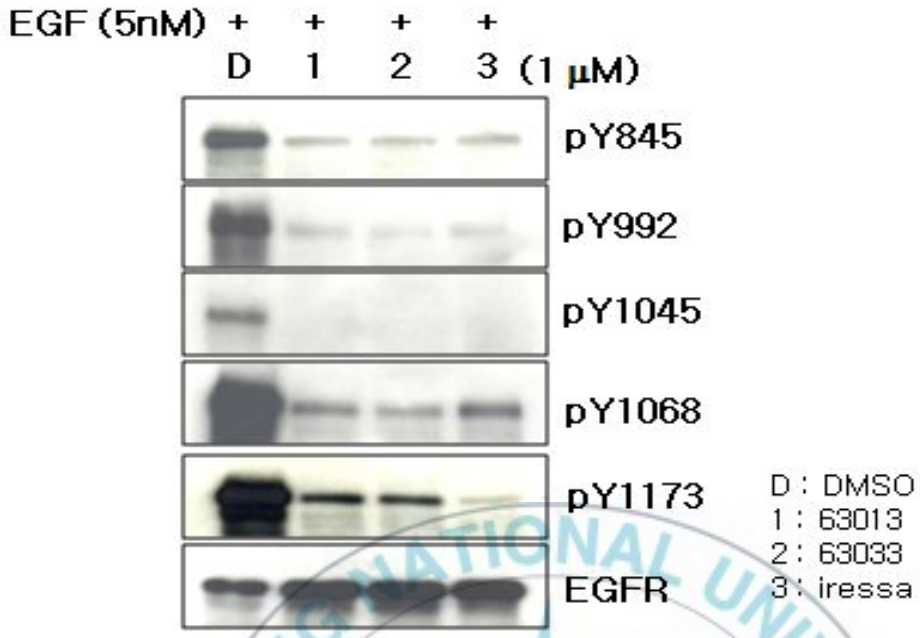


Fig. 8 The comparison of phosphorylation and activation on each tyrosine residue of EGFR. A431 cells were pre-treated with each compound described in figure at 1 uM concentration and stimulated with 5 nM EGF for 10 min. The results shown in figure were detected by western blotting using a various tyrosine phospho-specific antibodies against EGFR.

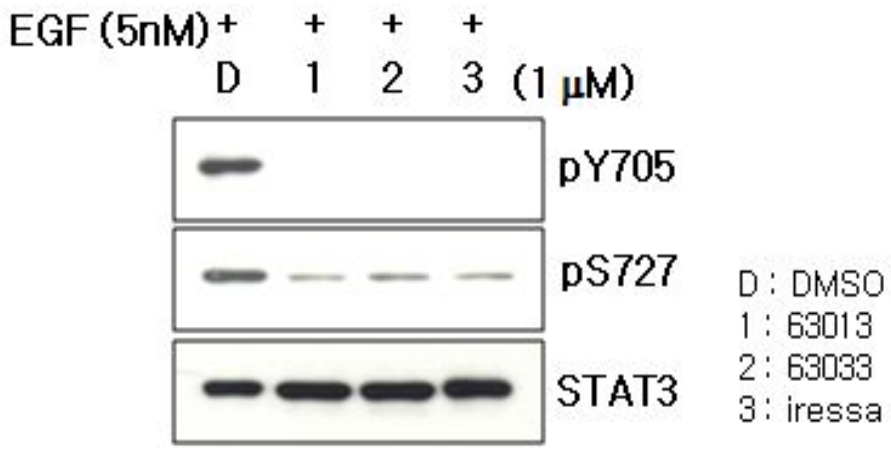


Fig. 9 The comparison of phosphorylation and activation on STAT3.

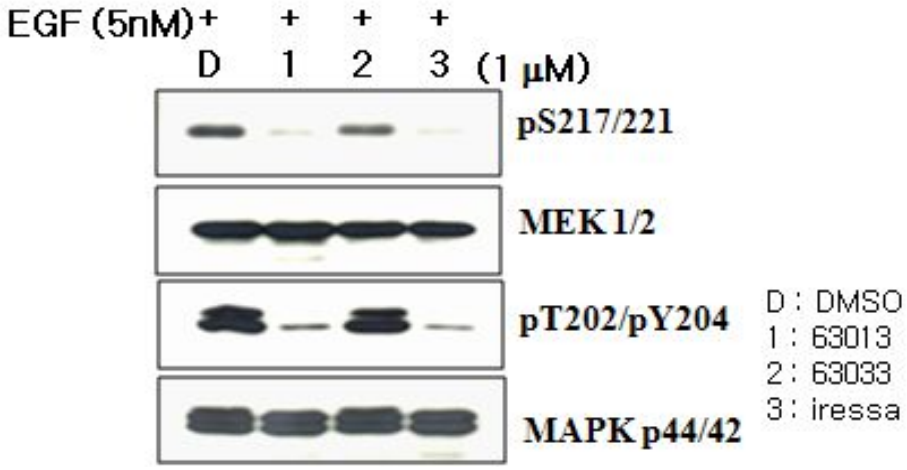


Fig. 10 The comparison of phosphorylation and activation on MEK1/2 and MAPK p44/42.

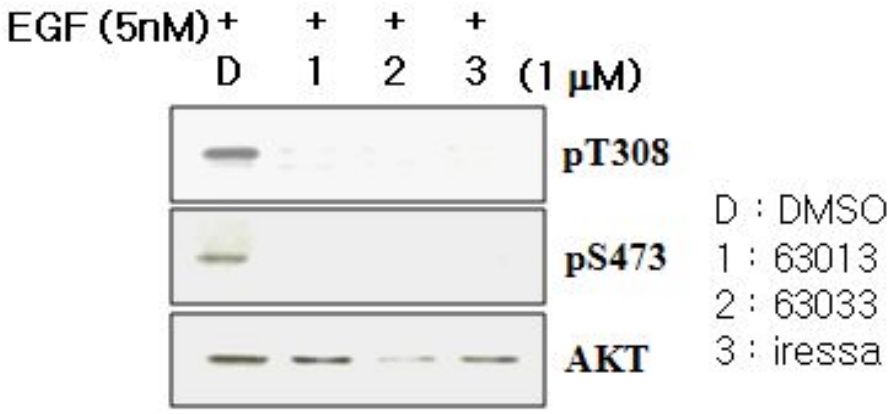


Fig. 11 The comparison of phosphorylation and activation on AKT.

## Inhibition effects of each compound on EGF-induced cell growth.

EGFR is activated by EGF, and involved in the growth of cells (Klapper *et al.* 2000). To examine whether EGF induces the growth of a cell or not, human cell lines reported to express EGFR, such as A431, Hela, A549, and SK-BR-3 were used, and the growth of a cell (%) was assessed through MTT assay (Fig. 12 A and B). Each cell line ($5 \times 10^3$) was cultured in media containing 5 nM EGF in presence or absence of 10% fetal bovine serum for 48 hour at 37 ℃, 5 % $CO_2$ and used cells cultured without EGF as a negative control. In each investigation using a media containing serum and serum free media, most of cell lines leaded growth of cells in the condition where EGF existed. Especially, A431 cell showed the highest growth rate of cell (Fig. 12A and 12B). The concentration of EGF which can induce the growth of a cell most effectively was examined. A431 cell, which showed the highest growth rate of a cell, was selected as the cell line. A431 cells were incubated in DMEM containing various concentrations of EGF for 48 hr at 37 ℃, 5 % $CO_2$. Cell growth (%) was detected by MTT assay. The results showed that at the 5 nM concentration level, the highest cell-growth rate was represented (Fig. 12C). So, all experiments were

performed in the 5 nM concentration of EGF. It was examined the effects of each compound on the growth of cell leaded by EGF (Fig. 12D). Under 1.9 uM concentration, the two compounds, 63033 and 63013 showed very similar growth of cells with Iressa. However, more than 1.9 uM concentration, two compounds had high cytotoxicity while Iressa had no cytotoxicity (Fig. 12D). As a result, the concentration of both 63013 and 63033 compound used in this study was maintained under 1.9 uM, which did not show cytotoxicity.

In sum up, although the two compounds, 63013 and 63033, have very similar EGFR inhibiting effects with Iressa, further research is needed to reduce cytotoxicity through changes in side chains, maintaining kinase inhibiting effects because the two compounds have higher cytotoxicity than Iressa.

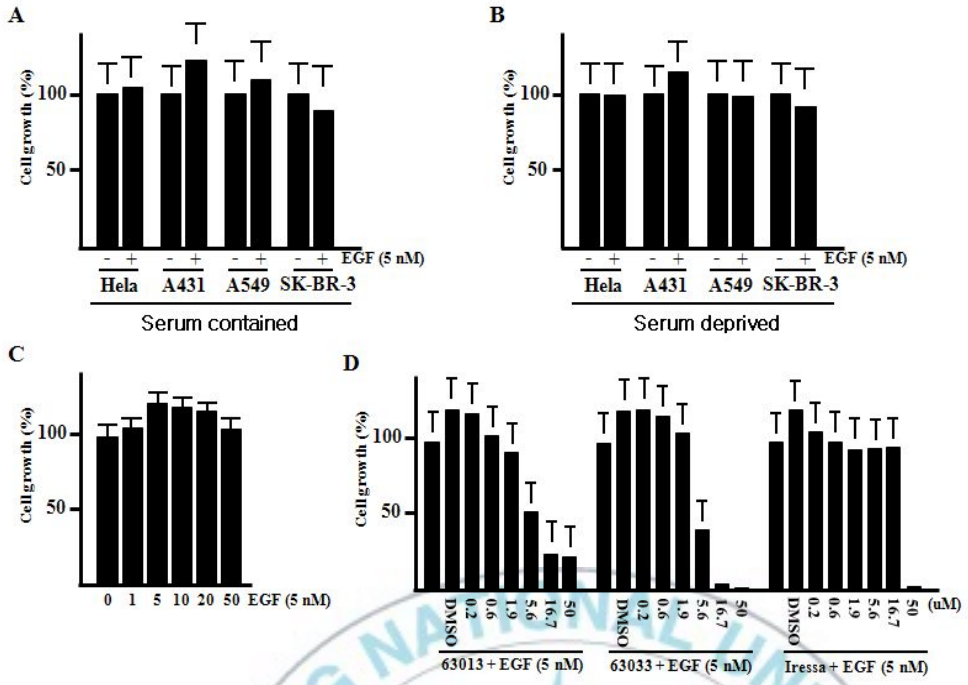


**Figure 12. Inhibition effects of each compound on EGF-induced A431 cell growth.** (A and B) To investigate whether EGF can induce a cell growth, I used a various human cell lines such as A431, Hela, A549, and SK-BR-3, reported that express EGFR. Each cell line ($5 \times 10^3$) were cultured in media containing 5 nM EGF in presence or absence of 10 % fetal bovine serum for 48 hour at 37 ℃, 5 % $CO_2$ and used cells cultured without EGF as a negative controls. (C) A431 cells were incubated in DMEM containing various concentrations of EGF for 48 hour at 37 ℃, 5 % $CO_2$. Cell growth (%) was detected by MTT assay. (D) A431 cells were cultured in media supplemeted with 5 nM EGF and various

concentrations of each compounds as a indicated in panel and cell growth (%) was determined by MTT activity. All experiment was performed as triplicate. These are the representative results from 3 separate experiments.

## Growth inhibition by 63013,63033, and Iressa.

A549 (expressed EGFR), HEK293 and HUVEC (non-expressed EGFR) were incubated with various concentration of each compound indicated in figure 13 for 48 hr at 37 ℃, 5 % $CO_2$ at a cell density of 5 x $10^3$. The results were determined as % growth inhibition on cells indicated in figure 13. Each compound had almost no cytotoxicity, but compound 63033 was shown to be a little cytotoxic at high concentration level.

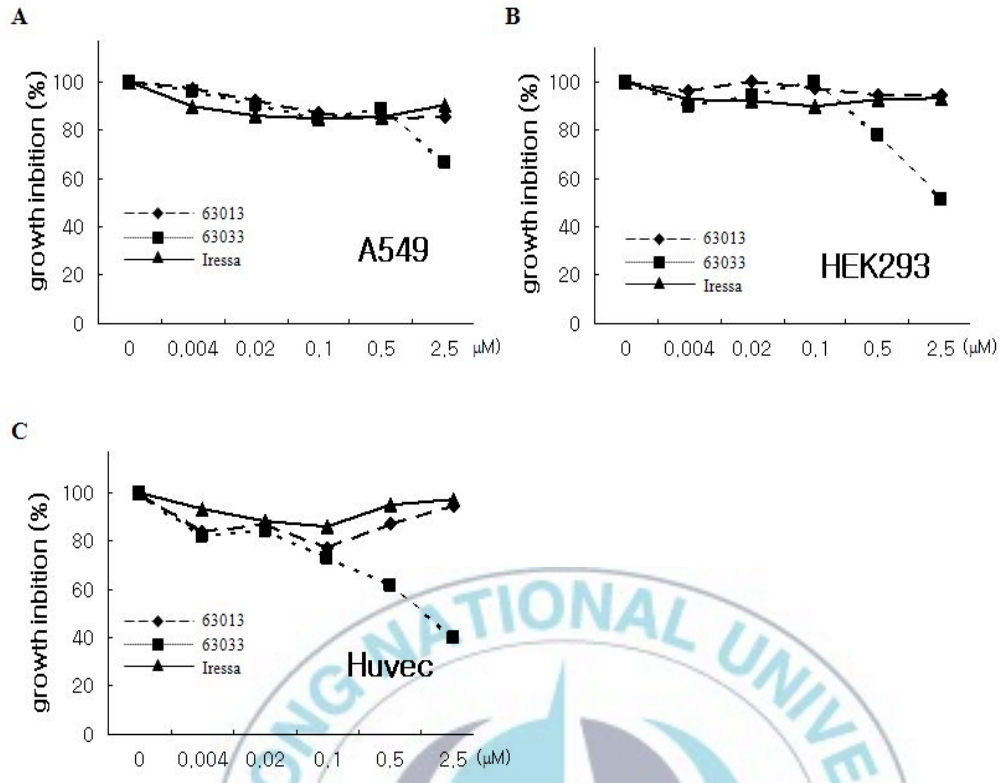


Figure 13. Growth inhibition by 630133, 63033, and Iressa. A549 (expressed EGFR), HEK293 and HUVEC (non-expressed EGFR) were incubated with various concentration of each compound indicated in figure for 48 hr at 37 ℃, 5 % $CO_2$ at a cell density of 5 x $10^3$. The results were determined as % growth inhibition on cells indicated in figure. The shape of each compound was marked closed diamond (63013), closed quadrangle (63033), and closed triangle (Iressa). These are the representative results from 3 separate experiments.

# 국문초록

상피 성장인자 수용체(EGFR)는 세포 성장과 분화를 조절하는 신호전달 경로에 관여한다. 이러한 EGFR의 과잉 발현과 자가 조절의 상실은 다양한 고형암을 유발한다. 그러므로 EGFR kinase 활성을 억제할 수 있는 억제제는 암뿐만 아니라 성장성 질환에도 효과적인 치료제가 될 수 있을 것이다. 퀴나졸린은 두 개의 단순한 방향성 고리(벤젠 고리와 피리미딘 고리)가 결합되어 만들어진 화합물이다. 퀴나졸린은 의학적으로 매우 다양한 부분에서 사용되고 있고, 그중에서도 항-말라리아 약제와 암치료제로 널리 사용되어 지고 있다. 63013, 63033 두 화합물의 구조적 특성은 디알콕시퀴나졸린 유도체보다 더 나은 용해성을 가지기 위해서 [1,4]-다이옥시노 퀴나졸린 구조를 가지고 있고, 알콕시 곁사슬로 서로 연결되어 있다. 이 연구의 목적은 63013, 63033 두 화합물의 EGFR 활성 억제효과를 조사하는 것이다. EGFR을 발현하는 인간 폐암 세포주인 A431 세포주에 두 화합물을 다양한 농도로 처리한 결과 농도 의존적인 억제를 나타냈다. EGF가 있는 조건에서 63013, 63033 두 화합물은 EGFR에 존재하는 각각의 티로신 잔기의 인산화를 억제하였고, 또한 EGF 연관 하위 분자들의 활성을 억제하였다. 63013, 63033 두 화합물의 EGFR 억제효과는 강력한 EGFR 억제제로 보고된 이레사(ZD-1839)와 비교했을 때 거의 유사하거나 더 높은 효과를 나타냈다.

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