# DESIGN, SYNTHESIS AND BIOLOGICAL STUDY OF NOVEL EPIDERMAL GROWTH FACTOR RECEPTOR KINASE (EGFRK) INHIBITORS


Venkata Subbaiah D.R.C [1], Venkat R. Pallela [2], Stephen C. Cosenza [1], Gayathri Panda [1], Muralidhar M Mallireddigari [2], E Premkumar Reddy [1] and M V Ramana Reddy [1].

1. Mount Sinai School of Medicine, Oncological Sciences, New York, NY, 10029.
2. Onconova Therapeutics Inc., Medicinal Chemistry, Newtown, PA , 18940.

MOUNT SINAI SCHOOL OF MEDICINE

ONCONOVA
♦ TARGETING CANCER ♦ PROTECTING HEALTHY CELLS ♦


## Introduction

Epidermal growth factor receptor (EGFR/ErbB-1) and Her-2 (ErbB-2), members of the Type 1 receptor tyrosine kinase family, are frequently disregulated in human epithelial tumors, via autocrine stimulation, overexpression, or mutation and play a key role in cell proliferation and differentiation. Overexpression of these receptors is found in a variety of cancers such as breast, ovarian, colon, head and neck and prostate cancers. The ErbB receptors can be activated through homo or heterodimerization with other receptors resulting in phosphorylation events and downstream signaling that produces excessive growth by inducing cell proliferation and inhibiting apoptotic pathways.

In an attempt to identify most potent kinase inhibitors, we developed a library of small molecules containing a backbone of pyrimidines, quinolines, quinazolines and benzothiazinones. While screening these compounds in cytotoxicity assay, we found that 4-aryl/benzylthio quinazolines are found to be very effective in killing EGFR$^+$ cancer cells. The kinase profiling study of this chemotype showed that ON128030 and ON128060 are found to be selectively inhibiting EGFRK unlike Iressa® which inhibits both EGFR and ErbB2 (Her-2) receptor kinases.

In this presentation, we describe the synthesis, characterization, in vitro cytotoxicity and kinase profile of the lead molecule.

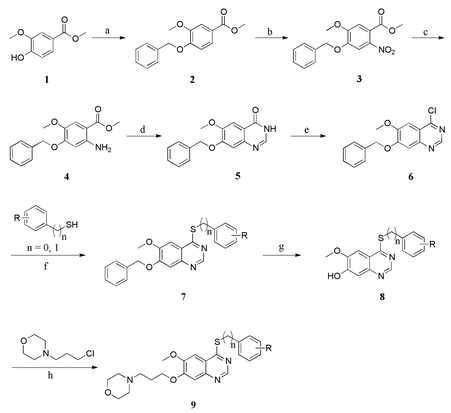
## Chemistry

The synthesis of 4,-7disubstitued 6-methoxyquinazolines (**9**) shown in **scheme** were prepared by using methyl vanillate (**1**) as the starting material. The hydroxy group of **1** was benzylated by using benzyl bromide in the presence of potassium carbonate in acetonitrile to get methyl 4-benzyoloxy-3-methoxybenzoate (**2**). The regiospecific nitration of **2** with fuming nitric acid afforded methyl 4-(benzyloxy)-5-methoxy-2-nitrobenzoate (**3**), which on subsequent reduction by using sodium borohydride and nickel(II)chloride hexahydrate resulted in the formation of methyl 2-amino-4-(benzyloxy)-5-methoxybenzoate (**4**). The cyclization of *o*-amino ester **4** with formamide and ammonium formate yielded 7-(benzyloxy)-6-methoxyquinazolin-4(3*H*)-one (**5**) which on treatment with phosphorus oxychloride resulted in the formation of 7-(benzyloxy)-4-chloro-6-methoxyquinazoline (**6**).

The substitution of chlorine of **6** with aryl/arylmethyl-thiols in the presence of sodium hydroxide in methanol resulted in the formation of 4-aryl/arylmethyl-thio-7-benzyloxy-6-methoxy quinazolines (**7**). Debenzylation was carried out by refluxing **7** with trifluoroacetic acid to get 6-methoxy-4-(aryl/arylmethyl-thio)quinazolin-7-ol (**8**). The final compounds 4-(3-((6-methoxy-4-(aryl/arylmethyl-thio)quinazolin-7-yl)oxy)propyl)morpholines (**9**) were achieved by the alkylation of **8** with *N*-(3-chloropropyl)morpholine in the presence of potassium carbonate in *N*,*N*-dimethylformamide in reasonably good yields (**Scheme**). The structures of all the compounds **2** to **9** were well established by NMR, LC–MS and HPLC.

## Scheme

### Scheme: Synthesis of 4,7-di-substitued 6-methoxyquinazolines




Reagents and conditions: (a) $PhCH_2Br$, $K_2CO_3$, MeCN, 60 °C, 2 h, 98%. (b) fuming $HNO_3$, AcOH, 15 °C– r.t., 3 h, 95%. (c) $NiCl_2.6H_2O$, $NaBH_4$, DCM:MeOH (2:1), 0–5 °C, 30 min., 96%. (d) $HCONH_2$, $HCO_2NH_4$, 140 °C, 4 h, 82%. (e) $POCl_3$, reflux, 2 h, 96%. (f) NaOH, MeOH, r.t., 3 h, 60–75%. (g) TFA, reflux, 1 h, 95–98%. (h) $K_2CO_3$, DMF, 100 °C, 10 h, 82–94%.

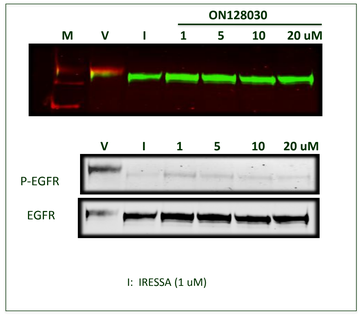
## Table 1. Invitro Cytotoxicity of 7, 8 and 9

| Comp. No. (ON No.) | R | n | Cell Lines ($IC_{50}$ nM) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | K562 | U87 | U87 EGFR VIII | DU145 | H1975 | A431 | BT474 |
| **7a** (ON128010) | 3-Cl,4-F | 0 | 10- 25 | - | - | 10- 25 | - | - | - |
| **7b** (ON128040) | 3-Cl,4-F | 1 | 10- 25 | - | - | >100 | - | - | - |
| **8a** (ON128020) | 3-Cl,4-F | 0 | 25- 50 | - | - | 50- 100 | - | - | - |
| **8b** (ON128050) | 3-Cl,4-F | 1 | 50- 100 | - | - | >100 | - | - | - |
| **9a** (ON128030) | 3-Cl,4-F | 0 | 10 | 4 | 12 | 5 | 2.5 | 3 | 9 |
| **9b** (ON128060) | 3-Cl,4-F | 1 | 5 | 2 | 4 | 5 | 1 | 2 | 8 |

Table-1: The compounds were tested for invitro cytotoxicity against various human cancer cell lines: Prostate (DU145), leukemic (K562), breast (BT474), non small cell lung (H1975), Glioblastoma (U87 & U87 EGFR VIII), Human epithelial carcinoma (A431).

## EGFR and HER-2 Assay of ON128030

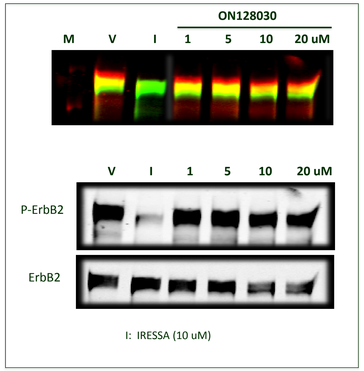
**Fig 1: EGFR ASSAY**




Inhibition of p-EGFR by ON128030 on human epithelial carcinoma cell line (A431). A431 cells were treated for 2 h followed by EGF stimulation for 10 min. Iressa was used as standard drug (1μM).

Result: ON128030 inhibits P-EGFR at low Nano molar concentration.

**Fig 2: HER-2 ASSAY**

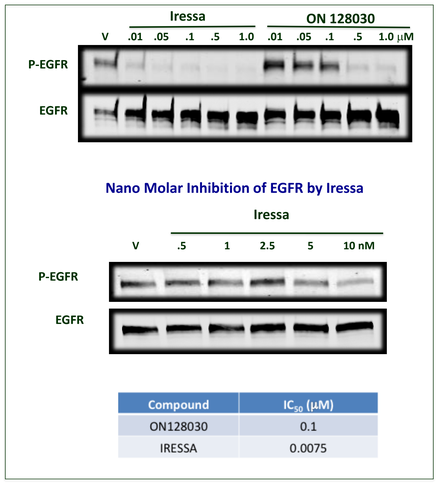



Inhibition of ErbB2 by ON128030 on A431 human epithelial carcinoma cell line. A431 cells were treated for 2 h followed by ErbB2 stimulation for 10 min. Iressa was used as standard drug (10μM).

Result: ON128030 has no inhibition effect on ErbB2 (HER-2) at micro molar concentration.

## EGFR Inhibition Between Iressa & ON128030

**Fig 3: Low Concentration Comparison of EGFR inhibition of Iressa & ON128030**



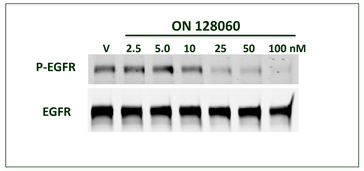

| Compound | $IC_{50}$ (μM) |
|---|---|
| ON128030 | 0.1 |
| IRESSA | 0.0075 |

A431 cells were treated for 2 h followed by EGF stimulation for 10 min. (50 ng/mL). The inhibition of ON128030 and Iressa was observed in different concentrations (10 nM to 1.0 μM).

Result: ON128030 inhibits EGFR at low Nano molar concentrations.

## EGFR Inhibition of ON128060

**Fig 4: Low Concentration Comparison of EGFR inhibition of Iressa & ON128060**




Treatment of A431 cells for 2 h followed by EGF stimulation for 10 min. (50 ng/mL) shows that ON128060 inhibits EGFR at low Nano molar concentrations.

## Table 2: $GI_{50}$ of ON128030

| EGFR | IRESSA (μM) | ON 128030 (μM) |
|---|---|---|
| Wildtype | 0.028 | 0.055 |
| L858R | 0.080 | 0.200 |
| L861Q | 0.074 | 0.600 |
| T790M | 2.5 | 2.5 |
| T790M-L858R | >5 | >5 |

ON128060 has $GI_{50}$ range >1<10 for K562 and DU145 cell lines.

## Table 3: $IC_{50}$ of Iressa & ON128030

| EGFR | IRESSA (μM) | ON 128030 (μM) |
|---|---|---|
| Wildtype | 0.028 | 0.055 |
| L858R | 0.080 | 0.200 |
| L861Q | 0.074 | 0.600 |
| T790M | 2.5 | 2.5 |
| T790M-L858R | >5 | >5 |

Inhibition of EGFR kinase activity invitro by ON128030 and Iressa tested for both wild type and mutant cell lines.

## Summary and Conclusions

- We have successfully designed and synthesized novel 4,6,7-trisubstituted quinazolines starting from methyl vanillate by following linear multi-step synthesis.
- The cytotoxicity of a series of compounds indicates that the morpholino-propyl at 7$^{th}$ position is found to be very important to enhance the activity of the lead molecules.
- The kinase profile study of ON128030 & ON128060 found to be selectively inhibiting EGFR unlike Iressa which inhibits both EGFR & ErbB2 (Her-2) receptor kinases.

## Acknowledgements


Authors are thankful to Mount Sinai School of Medicine, New York and Onconova Therapeutics Inc., Newtown, PA for the financial assistance and interest in this project.