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Novel morpholin-3-one fused quinazoline derivatives as EGFR tyrosine kinase inhibitors

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ABSTRACT

A series of novel morpholin-3-one-fused quinazoline derivatives were designed, synthesized and evaluated as EGFR tyrosine kinase inhibitors. Nineteen compounds showed significant inhibitory activities against EGFR^{wt} kinase ($IC_{50} < 1\ \mu M$). Compound **a8** demonstrated the most potent inhibitory activity toward EGFR^{wt} ($IC_{50} = 53.1$ nM). Compound **a7** and **a8** showed excellent inhibitory activities against mutant EGFR$^{T790M/L858R}$ and strong antiproliferative activity against H358 and A549 cell lines. Finally, molecular docking studies were performed to predict the possible binding mode of the target compounds. It is believed that this work would be very useful for designing a new series of tyrosine kinase inhibitors targeting EGFR.

The epidermal growth factor receptor (EGFR) tyrosine kinase is a key mediator in cellular signalling transduction pathways and is closely related to cell proliferation, survival, adhesion, migration, and differentiation.[1,2] Deregulation of EGFR signalling has been observed in many human cancers, including colorectal, head and neck, lung, breast, ovarian, and bladder cancers.[3] Thus, EGFR and its family members have emerged as attractive targets for anti-cancer therapy, especially for the treatment of non-small-cell lung cancer (NSCLC).[4,5] Several EGFR inhibitors, such as gefitinib and erlotinib (Fig. 1) belonging to a class of 4-anilinoquinazolines have made significant progress in the treatment of NSCLC patients harboring somatic EGFR mutations L858R and delE746_A750.[6–8] However, the undeniable success of EGFR tyrosine kinase inhibitors are limited by the up-regulation of bypass signalling pathways and acquired point mutations.[9,10] After treatment 10–16 months with these inhibitors in western countries, approximately 50% of the NSCLC patients gain an additional gate keeper mutation (T790M).[11,12] The secondary T790M mutation decreases sensitivity to gefitinib or erlotinib and increases the binding affinity for ATP.[13,14]

In order to overcome the T790M mutation related resistance, several irreversible EGFR second-generation covalent inhibitors (Fig. 1), which form a covalent bond with Cys797 within the EGFR active site, were developed.[15–17] Afatinib was the first approved irreversible EGFR inhibitor by the US FDA for the treatment of late-stage NSCLC patients with actively mutated EGFR.[18] However, these irreversible EGFR inhibitors lacked significant selectivity between EGFR T790M (EGFR^{T790M}) mutants and the wild-type kinase that leads to dose-limiting toxicities in these patients, including skin rash and diarrhea that can result in severe dehydration and kidney failure.[14,19] Recently, third-generation covalent inhibitors including AZD9291 and CO-1686 have been generated that demonstrate selectivity for EGFR^{T790M} mutants over wild-type EGFR (EGFR^{wt}), and early phase I data indicated promising efficacy.[20–22] Although, the number of potent EGFR inhibitors is constantly expanding, discovery of inhibitors with novel scaffolds remains a focus nowadays. Herein, we would like to report the design, synthesis and biological evaluation of morpholin-3-one fused quinazoline derivatives as new EGFR inhibitors.

The co-crystal structures of gefitinib or erlotinib[8,23] with EGFR have provided valuable structural information for helping the rational design of potent EGFR inhibitors. Binding mode analysis of gefitinib suggests several key interactions with the ATP binding pocket of EGFR, including the critical H-bond of quinazoline moiety with the hinge region, the hydrophobic pocket filled up by the 3-chloro-4-fluoro aniline substituent and the solvent region occupation of 6-morpholinopropoxy group. Solvent region generally accommodate a variety of polar functional groups with little affection on the efficacy.[24] The success of icotinib (Fig. 1), which mimics erlotinib with a crown ether fused quinazoline, is a good example of

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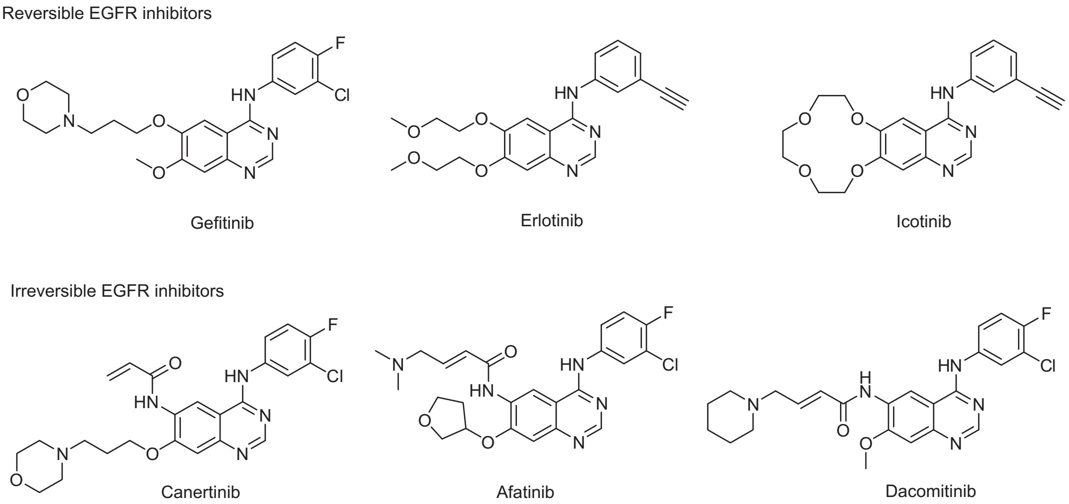

Figure 1. Chemical structures of EGFR-TKIs.

this strategy.[25,26] Accordingly, we have designed and synthesized a series of novel morpholin-3-one fused quinazoline derivatives through intramolecular cyclization as new EGFR inhibitors (Fig. 2).

The synthetic route of compounds **a1**–**a15** is illustrated in Scheme 1. Commercially available 4-hydroxy-3-nitrobenzoic acid **1** was esterified with methanol using concentrated sulfuric acid as a catalyst to give compound **2**.[27] Alkylation of methyl 4-hydroxy-3-nitrobenzoate **2** with ethyl bromoacetate and reduction of the nitro group with Fe/acetic acid followed by in situ cyclization yielded intermediate **4**. Subsequent nitration and alkylation provided **6**. Then reduction the nitro group of **6** using Fe/acetic acid gave **7**, which was subjected to cyclization to generate intermediate **8**. Following, **8** was chloridized by phosphorus oxychloride to generate intermediate **9**. Finally, condensation of **9** with substituted anilines afforded 4-anilinoquinazoline derivatives **a1**–**a15**.

Synthesis of compounds **b1**–**b6** is shown in Scheme 2. Intermediate **5**, which could be synthesized according to Scheme 1, was reacted with 1-bromo-3-chloropropane and piperidine in sequence to achieve **11**. The target compounds were synthesized in good yields by the next four step reactions according to Scheme 1.

To evaluate the EGFR inhibitory potency of the new compounds, Kinase-Glo luminescent assay is used to test their ability to block EGFR tyrosine kinase. Compounds were initially texted at final concentrations of 1000, 200, 40, 8, 1.6, 0.32, and 0.06 nM and performed in duplicate at each concentration with gefitinib as positive control. Compound inhibition curve was fitted using Graphpad Prism 5.0 software based on the inhibition rate of all compounds at different concentrations. The testing results are summarized in Table 1. Almost all the compounds displayed significant EGFR inhibitory activities ($IC_{50} < 1\ \mu M$). Among them, compound **a8** showed the most potent inhibitory activity against $EGFR^{wt}$ kinase ($IC_{50} = 53.1$ nM). When R was 3-morpholinopropyl (**a1**–**a15**), the inhibitory activities against $EGFR^{wt}$ ranged from 53.1 nM to 829.2 nM except **a5** and **a14** in which $IC_{50} > 1000$ nM. When R^1 was halogen (F, Cl or Br) and R^2 was hydrogen (**a1**, **a2**, **a3**), compounds substituted by Cl or Br (**a2**, $IC_{50} = 63.1$ nM; **a3**, $IC_{50} = 89.6$ nM) displayed 2-fold increase in potency than substituted by F (**a1**, $IC_{50} = 245.1$ nM). While R^1 was hydrogen and R^2 was halogen atom (**a9**, **a10**, **a11**), the results of inhibition against EGFR was unsatisfactory. This may be that meta-position compounds (**a1**, **a2**, **a3**) could form a stronger interaction with EGFR kinase than para-position compounds (**a9**, **a10**, **a11**). When R^1 was Cl and R^2 was F, compound **a8** displayed the most inhibitory activity toward $EGFR^{wt}$ ($IC_{50} = 53.1$ nM). When R^1 or R^2 was methoxy or trifluoromethyl group, compounds (**a5**, **a6**, **a14**,) displayed weaker potency against EGFR kinase. One explanation might be that a larger volume of R^1 group could not effectively fill up well the hydrophobic pocket of EGFR kinase. In summary, when R^1 was Cl or Br, and R^2 was H or F, compounds (**a2**, **a3**, **a8**) displayed excellent inhibitory activities against $EGFR^{wt}$ ($IC_{50} < 89.6$ nM). Among them, compound **a8** displayed the most inhibitory activity toward $EGFR^{wt}$ ($IC_{50} = 53.1$ nM).

To find a more potent EGFR inhibitor, R was substituted by 3-(piperidin-1-yl)propyl in the subsequent synthesis (**b1**–**b6**). Among them, **b3** was the most active compound against EGFR ($IC_{50} = 99$ nM). Compounds **b2**, **b3**, **b6** (R^1 = Cl or Br) showed much more potency than **b1** (R^1 = F). This was the same phenomena as the former discussion (**a1**–a3, R^1 = halogen). The reason may be that small fluorine atom could be too small to fill up well the hydrophobic pocket of EGFR kinase pocket. However, when R^1 was a relatively larger volume group methyl or ethynyl, compounds (**b4**, **b5**) did not showed good inhibitory activities towards $EGFR^{wt}$. As a whole, R^1 was Cl or Br would be beneficial for improving the inhibitory activities against $EGFR^{wt}$.

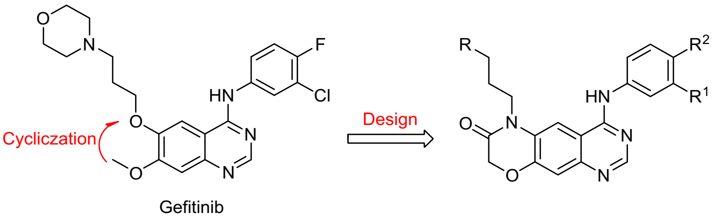

Figure 2. Design strategy and modification of novel EGFR inhibitors.