**Design, Synthesis and Antitumor Activities of Novel Quinazolinone Derivatives as Potential EGFR Inhibitors**

Jing Wang, Liwei Huang, Xi Chen, Yangchen Yuan, Juan Sun, and Meng Yang

School of Pharmaceutical Engineering, Jiangsu Food & Pharmaceutical Science College; Huai’an 223005, China; School of Biological & Chemical Engineering, Zhejiang University of Science & Technology; Hangzhou 310023, P. R. China; and School of Health, Jiangsu Food & Pharmaceutical Science College; Huai’an 223005, China.

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Human epidermal growth factor (EGFR) is an important target for antitumor drug research. A series of novel quinazolinone derivatives were synthesized and developed as potent inhibitors of EGFR. The results showed that most of the aimed compounds had potential anti-tumor cell proliferation activities. Some compounds were tested for their EGFR inhibitory activity. Especially, compound 6d showed the most potent anti-tumor activity with IC50 values of 1.58 µM against human breast cancer (MCF-7) cell lines and exhibited the most potent EGFR inhibitory activity with IC50 of 0.77 µM. Docking simulation was performed to position compound 6d into the EGFR active site to determine the probable binding conformation.

**Key words** quinazolinone derivative; anti-tumor cell proliferation; epidermal growth factor receptor (EGFR) inhibitory; molecular docking

**Introduction**

Epidermal growth factor receptors (EGFRs), a 170KD transmembrane protein, is an important receptor that regulates downstream signaling pathways through autophosphorylation, thereby affecting cell proliferation, survival, adhesion, migration, and differentiation. Studies have found that EGFR dysregulation, transient expression or mutation can lead to various cancers. Based on the key role of EGFR in anti-tumor effects, many small molecule inhibitors have been developed. However, with the use of drugs, side effects and drug resistance problems have emerged. Currently, EGFR small molecule inhibitors have evolved to the fourth generation. The first generation features Gefitinib and Erlotinib as representative molecular inhibitors, the second generation of irreversible EGFR inhibitors features Afatinib as a representative molecular inhibitor, the third generation targets small molecule inhibitors carrying T790M mutant EGFR, such as Osimertinib and Rociletinib with exciting clinical efficacy, and the fourth generation features variant inhibitors of EGFR were developed, such as compound EA1001 with good inhibitory activity and significant selectivity for mutant EGFR, and EA1045 which significantly overcame the resistance problems caused by T790M and C797S (Fig. 1).

Nitrogen-containing heterocyclic compounds are the most abundant and complete skeletal materials and are widely found in various synthetic drugs, bioactive natural products, pharmaceutical and pesticide species. It has been found that quinazolinone derivatives can modulate the phosphorylation of EGFR and thus achieve anti-tumor cell proliferation effect. By analyzing the structure of existing EGFR kinase inhibitors, we found that the nitrogen atom in the quinazolinone ring is crucial for EGFR kinase inhibitory activity, so we retained this skeleton when designing the inhibitors. Besides, amino acids such as Met769, Phe699 and Cys773 at the active...
site of EGFR also play a role in enhancing the activity. Based on the structural characteristics of quinazolinones EGFR inhibitors, we designed and synthesized a series of novel EGFR small molecule inhibitors by introducing benzene rings on the quinazolinone backbone using amide bonds, through the substitution of structural fragments and the integration of favorable structures (Fig. 2).

Subsequently, in order to validate the ability of these designed compounds to target EGFR, molecular docking of these designed compounds and the positive compound (Gefitinib) to the ATP binding site of EGFR was performed (PDB code: 4WKQ). The obtained results were then plotted as line scattering maps and presented in Fig. 3, showing mainly the corresponding CDOCKER_INTERACTION_ENERGY in molecular docking studies. All compounds showed lower interaction energies compared to the positive drug Gefitinib. The results suggest that the target compounds have a strong inhibitory activity against EGFR tyrosine kinase. Thus, a new series of 2-((4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio)-N-phenylacetamide derivatives can be theoretically demonstrated as EGFR inhibitors by preliminary analysis.

Results and Discussion

Chemistry In this study, 15 quinazolinone derivatives were synthesized. The synthetic route of target compounds (6a–6o) were shown in Chart 1. Compound 3 was obtained from the reaction of Methyl anthranilate (1) and 1,3-dibromopropane (2) in CH3OH solvent. The reaction of compound 3 with bromoacetic acid in the presence of solvent N,N-dimethylformamide (DMF) gave compound 4. Then, compounds 6a–6o were accomplished by amidation of compound 4 with various amines using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and N,N-dimethyl-4-aminopyridine (DMAP) as the coupling reagents. Details of the whole synthesis process are shown in the Supplementary Materials.

Biological Activity All the synthesized quinazolinone derivatives 6a–6o were evaluated for their antitumor activities against two cancer cell lines: Human breast cancer cell line (MCF-7) and human liver cancer cell line (HepG2). The IC50 of those compounds against these cancer cell lines were presented in Table 1. Standard antitumor agent 5-Fluorouracil were also screened under identical conditions for comparison and the results revealed that most of the synthetic compounds exhibited certain antitumor activities, except for compounds with long fat chains (6j and 6l). In general, the inhibitory effect of the compound on MCF-7 was better than that of HepG2. Among them, compound 6d showed the best inhibitory activity (IC50 = 1.58 µM for MCF-7).

Structure–activity relationship analysis of these quinazolinone derivatives (targeting the MCF-7 cell line) showed that different substituent exhibited different degrees of biological activity. Among them, compounds with methoxyl and nitro substituents of the benzene ring showed higher inhibitory activities, probably because the oxygen atoms on the substituent group can form hydrogen bonds with the amino acids on the active site of EGFR, which significantly stable binding conformation. The activities of meta-substituted compounds were generally low, possibly because the conformation of meta-substituted compounds affected the binding energy of the backbone to the hydrophobic domain of EGFR.

In order to verify whether the antiproliferative effect is caused by the interaction between EGFR protein and target compound, the synthetic compounds with potent antitumor activities (6a, 6d, 6e, 6i, 6n, and 6o) were further evaluated against EGFR enzymes, and the IC50 values were summarized in Table 2. Most of the test compounds displayed potent inhibitory activity. In addition, the IC50 values of these compounds were similar to those of anti-proliferation tests. Therefore, we
further analyzed the relationship between the anti-tumor proliferation activity and the EGFR inhibitory activity of these compounds. As shown in Fig. 4, there is a correlation between the EGFR inhibitory activity and the inhibition of MCF-7 cell proliferation. The correlation coefficient \( r^2 \) is 0.9445 for MCF-7. Therefore, we can conclude that the synthesized inhibitors have the ability to inhibit EGFR, and part of the anti-proliferation effect is caused by the interaction between EGFR protein and target compound.

Not only antitumor activity, but also biological safety are the important indexes for effective drug. So the compounds selected above were also tested for their hemolytic activity and cytotoxic activity on a mouse embryonic fibroblast cell line (NIH-3T3) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In summary, as shown in Table 2, these compounds displayed low hemolytic activities. Moreover, the cytotoxicity data indicate that the compounds possessing inhibitory activity were low toxicity.

**Molecular Docking**

In order to determine the interaction binding mode between the target protein and small molecules, the protein crystal structure of EGFR (PDB entry code: 4WKQ) was employed for \textit{in silico} molecular docking as the receptor model. Docking procedure was followed using the standard protocol as well as some references. The three dimensional (3D) binding pose and the 2D interaction diagram between the key residues of EGFR active pocket and compound 6d and Gefitinib were presented in Figs. 5A–D, respectively.

The docking result showed compound 6d can be inserted into in the EGFR binding site with the docking energy of \(-32.5712\) kcal/mol. The oxygen atom on quinazolinone forms a hydrogen bond with the amino acid residue Gly719, which plays a vital role for the stabilization of its binding mode. In addition, the oxygen atom located on the acylhydrazine chain are the important indexes for effective drug. So the compounds selected above were also tested for their hemolytic activity and cytotoxic activity on a mouse embryonic fibroblast cell line (NIH-3T3) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In summary, as shown in Table 2, these compounds displayed low hemolytic activities. Moreover, the cytotoxicity data indicate that the compounds possessing inhibitory activity were low toxicity.

**Table 1. Antitumor Activities (IC\(_{50}\), \(\mu\)M) of Synthetic Compounds**

| Compound | IC\(_{50}\)(\(\mu\)M) ± S.D.\(^a\) |
|----------|---------------------------------|
|          | MCF-7                           |
| 6a       | 3.18 ± 0.12                     |
| 6b       | 5.22 ± 0.56                     |
| 6c       | 5.49 ± 1.12                     |
| 6d       | 1.58 ± 0.13                     |
| 6e       | 2.25 ± 0.29                     |
| 6f       | 3.01 ± 1.22                     |
| 6g       | 5.86 ± 1.56                     |
| 6h       | 3.78 ± 1.05                     |
| 6i       | 2.33 ± 0.88                     |
| 6j       | >50                             |
| 6k       | 3.77 ± 1.26                     |
| 6l       | >50                             |
| 6m       | 3.77 ± 1.09                     |
| 6n       | 2.56 ± 0.89                     |
| 6o       | 3.56 ± 0.88                     |
| 5-Fluorouracil | 15.22 ± 1.51 |

\(a\) Values are means ± standard deviation (S.D.) from triplicate determination.

**Table 2. EGFR Inhibitory Activity of Synthesized Compounds**

| Compound | EGFR IC\(_{50}\)(\(\mu\)M) ± S.D.\(^a\) | Hemolysis LC(mg/mL) | Cytotoxicity IC\(_{50}\)(\(\mu\)M) ± S.D.\(^a\) |
|----------|---------------------------------|----------------------|---------------------------------|
| 6a       | 1.29 ± 0.06                     | >10                  | 165.2 ± 10.22                   |
| 6d       | 0.77 ± 0.03                     | >10                  | 186.6 ± 12.12                   |
| 6e       | 1.05 ± 0.12                     | >10                  | 205.1 ± 15.21                   |
| 6f       | 0.88 ± 0.05                     | >10                  | 56.22 ± 8.65                    |
| 6i       | 1.08 ± 0.11                     | >10                  | 198.5 ± 14.05                   |
| 6n       | 1.22 ± 0.09                     | >10                  | 177.3 ± 12.31                   |
| 6o       | 2.35 ± 0.21                     | >10                  | 165.2 ± 10.18                   |
| Gefitinib | 0.82 ± 0.13                     | >10                  | 125.6 ± 10.11                   |

\(a\) Values are means ± standard deviation (S.D.) from triplicate determination.
forms a hydrogen bond with the amino acid residue Lys745, represented an opportunity to introduce electron-donating group into the active site of the binding pocket. Figures 5C and D showed the binding mode of crystal structure Gefitinib interacting with EGFR protein. The nitrogen atom on quinazolinone forms a hydrogen bond with the amino acid residue Met793. Besides, the oxygen atom on the methoxide of the quinazoline side chain forms a hydrogen bond with the amino acid residue Gly796.

Judged on the Co-crystal structures of 4WKQ, quinazolinone portion makes mostly lipophilic interactions, with the piperazine side chain too far from many amino acids to form strong beneficial interactions. The quinazoline skeleton entered the catalytic core region and formed hydrogen bonds with key amino acids (Fig. 5D), which is thought to facilitate the development of EGFR small molecule inhibitors. Therefore, compound 6d was designed and the quinazolinone skeleton was selected. The quinazolinone skeleton is inserted into the active cavity of EGFR and forms a stable force with key amino acid residues. In addition, we introduced benzene ring through the amide structure as a bridge, so that the side chain structure of the compound can better get close to the amino acid of the active site (Fig. 5B), form interaction force and improve the binding activity. As showed in Fig. 5E, the two compounds (6d and the crystal structure Gefitinib) can be overlapped well in the substrate binding pocket, which also verified the rationality of molecular docking.

**Conclusion**

In summary, a series of quinazolinone derivatives (6a–6o) were prepared and tested for their inhibitory activity against MCF-7 and HepG2 cell lines. Many synthesized compounds showed potent antitumor and EGFR inhibitory activities. Particularly, compound 6d showed the most potent EGFR inhibitory activity with an IC$_{50}$ value of 0.77 µM, which was compared with the positive control, 5-fluorouracil. Structure–activity relationships prediction and molecular modeling study provided further insight into interactions between the enzyme and its ligands. Based on the data obtained in this study, we conclude that compound 6d is the EGFR inhibitor most deserving of further research as a potential drug. In future studies, we will continue to study new quinazolinone derivatives and explore their potential functions.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials This article contains supplementary materials.

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