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Research paper

Design, synthesis and biological activities of novel oxazolo[4,5-*g*]quinazolin-2(1H)-one derivatives as EGFR inhibitors

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ABSTRACT

A series of oxazolo[4,5-*g*]quinazolin-2(1H)-one derivatives employing Erlotinib as lead compound were synthesized and evaluated for their EGFR inhibition activity. These compounds having variation at the 1 and 8-position, included ether and esters hydrophilic side-chain and aromatic head fragment, respectively. All these compounds were evaluated by EGFR inhibition and two anti-proliferation assays *in vitro*. Four compounds were found more potent than Erlotinib in EGFR-TK assay. Furthermore, compounds **18**, **42** and **50** also had good to excellent anti-proliferation activity against human epidermoid cancer cell line (KB) and renal cell carcinoma cell line (A498). Finally, compound **50** presented remarkably higher inhibition efficacy towards tumor growth than Erlotinib in a mouse lewis lung cancer (LLC) xenograft model. Furthermore, compound **50** displayed the most distinguished effect on extending the survival period of the tumor-bearing mice.

1. Introduction

Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor which plays an essential role in normal cell growth and differentiation, and is involved in tumor proliferation and survival [1,2]. EGFR over-expression is a common feature in many human solid malignancies including non-small-cell lung cancer, ovarian cancer, breast cancer, etc., which is associated with poor clinical prognosis [3–6].

Quinazoline-containing derivatives form an important class of synthetic products and represent an attractive scaffold for EGFR inhibitors. Huge interest has been attracted over the past years because of their varied biological activities, notably as kinase inhibitors [7–10]. The 4-anilinoquinazoline scaffold (Fig. 1) has led to the development and the marketing of a series of anti-tumor agents such as Gefitinib [11], Erlotinib [12], and Lapatinib [13].

Recently, we developed oxazolo[4,5-*g*]quinazolin-2(1H)-one derivatives, using Gefitinib as a lead compound. In that work, we found the potential of building oxazolo[4,5-*g*]quinazolin-2(1H)-one scaffold and adopting the hydrophilic side-chain at its 1-position for EGFR small molecule inhibitors [14]. So based on this hypothesis that oxazolo[4,5-g]quinazolin-2(1H)-one scaffold is useful as a template for creation of new EGFR small molecule inhibitors, we synthesized a novel series of oxazolo[4,5-*g*]quinazolin-2(1H)-one derivatives adopting 2-methoxyethoxyl and 4-ethyloxy-4-oxobutyl group as side-chain at 1-position respectively and evaluated their EGFR inhibition activity comparing with Erlotinib (Fig. 2). Eventually, compounds **18**, **42** and **50** were discovered as promising inhibitors against EGFR Therefore, we reported compounds **18**, **42** and **50** as promising candidates for clinical development as novel EGFR kinase inhibitors.

2. Results and discussion

2.1. Chemistry

The synthetic method for the target compounds is showed in Scheme 1 and Scheme 2. Nitration of the starting material ethyl 4-hydroxybenzoate (**4**) led to ethyl 4-hydroxy-3-nitrobenzoate (**5**), followed by reduction with tin (II) chloride dihydrate to give ethyl 3-amino-4-hydroxybenzoate (**6**), which was cyclocondensed with di-(1H-imidazol-1-yl)-methanone (CDI) in anhydrous THF to afford ethyl 2-oxo-2,3-dihydrobenzo-oxazole-5-carboxylate (**7**). Nitration

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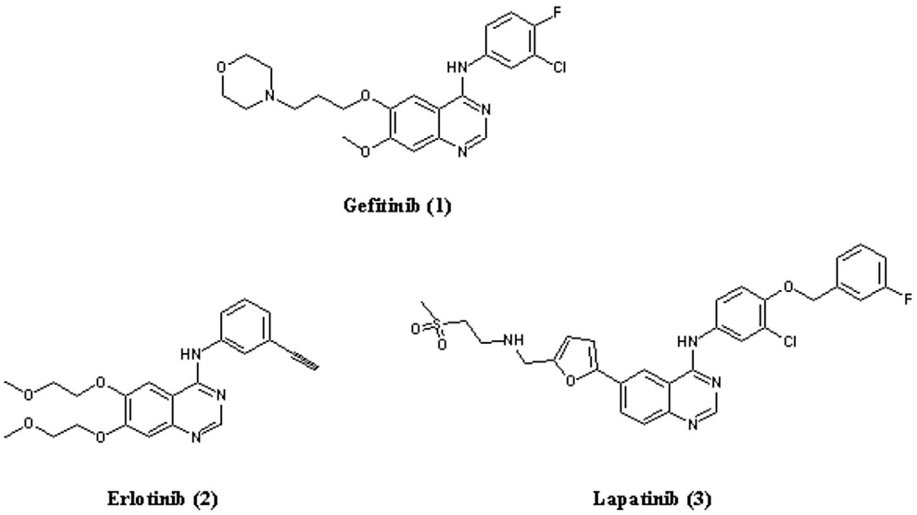

Fig. 1. The structures of Gefitinib, Erlotinib and Lapatinib.

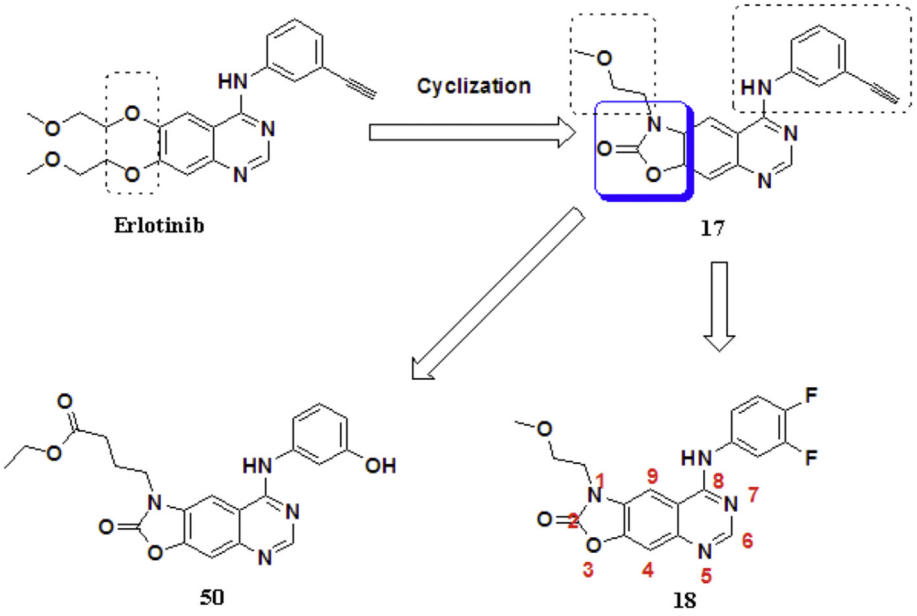

Fig. 2. The design inhibitors of EGFR.

of compound **7** with concentrated HNO_3, and subsequent purification via column chromatography, yielded compound **8** which was then reduced with iron power to produce compound **9**.

The series I compounds were obtained by alkylation of compound **9** with 1-chloro-2-methoxyethane, cyclization with formamidine acetate in EtOH, chlorination with thionyl chloride using DMF as catalyst, and nucleophilic displacement with aniline (Scheme 1). Likewise, the series II compounds were synthesized as described in Scheme 2.

2.2. Biological activities

2.2.1. Kinase inhibitory activity

Kinase assay was carried out to test the inhibitory activity of the designed compounds at the concentration of 0.04 mg/mL. As shown in Table 1, most of compounds inhibited EGFR dramatically, whereas compounds **21**, **22**, **36**, and **44** had no obvious inhibitory activity. The IC_{50} values of EGFR inhibition were further tested using Erlotinib as the positive control.

Most compounds exhibited moderate to high inhibition activities with EGFR kinase. These results revealed that introducing the moderate hydrophilic groups to the 1-position contributed to the activity. As indicated by the results of compounds **49**, **50**, **52** on compared to compounds **32**, **38**, **39**, aniline moieties directly conjuncted to the 8-position increased EGFR inhibitory activities as compared to benzyl amino groups. Compounds containing some functional groups at 8-position, such as halogens, hydroxyl groups, alkoxyl groups etc., caused an increase in EGFR inhibition with the exception of compounds **36**, **44**, **48** and **22**. This is due in part to their relatively large structures, which sterically hindered their interaction with the active site of the kinase. Additionally, the compounds which contained functional groups such as t-butyl, sulfanilamido group, etc., also demonstrated decreased inhibition activities like compounds **37**, **52** and **20**.

The IC_{50} values of compounds **18**, **42** and **50** were 0.026 μM, 0.0073 μM and 0.0087 μM respectively. This is a significant