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Synthesis and biological evaluation of novel tricyclic oxazine and oxazepine fused quinazolines. Part 1: Erlotinib analogs

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ABSTRACT

Two series of novel tricyclic oxazine and oxazepine fused quinazolines have been designed and synthesized. The in vitro antitumor effect of the title compounds was screened on N87, A431, H1975, BT474 and Calu-3 cell lines. Compared to erlotinib and gefitinib, compounds **1a**–**1h** were found to demonstrate more potent antitumor activities. Several derivatives could counteract EGF-induced phosphorylation of EGFR in cells, and their potency was comparable to the reference compounds. Compounds **1a**–**1h** were chosen for further evaluation of EGFR and HER2 in vitro kinase inhibitory activity. Compounds **1b**–**1f**, **1h** effectively inhibited the in vitro kinase activity of EGFR and HER2 with similar efficacy as erlotinib and gefitinib.

Lung cancer is the number one cause of cancer mortality in men globally, with an estimated 13% (1.6 million) of total cases and accounting for 18% (1.4 million) of total deaths worldwide in 2008.[1] Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited mainly due to undesirable side effects and a limited choice of available anticancer drugs. This clearly underlies the urgent need of developing novel chemotherapeutic agents with more potent antitumor activities.[2]

EGFR is a member of a family of closely related receptors, including EGFR (ErbB1), human epidermal growth factor receptor-2 (HER2)/neu (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). EGFR is overexpressed in the majority of NSCLCs and its expression is inversely related to survival outcome.[3] The two main signaling pathways activated by EGFR are the RAS/RAF/MEK/ERK pathway and the PI3K/AKT pathway, which lead to evasion of apoptosis (cell survival) and cell proliferation.[4,5]

Based on the critical role of the ErbB family of receptors in the growth and metastases of NSCLC and other human malignancies, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have been developed as targeted antitumor agents.[6] Currently gefitinib (Iressa™, AstraZeneca) and erlotinib (Tarceva™, Genentech) (Fig. 1), were approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with non–small cell lung cancer (NSCLC) in May 2003 and November 2004, respectively.[7,8] Both are reversible competitive inhibitors at the adenosine triphosphate (ATP) binding site of the EGFR TK domain. Despite the benefits of reversible EGFR TKIs, the efficacy of these agents has been limited by the development of resistance in most, if not all, initially responsive patients, which leads to tumor progression and relapse after a median time of 12 months.[9]

Recently, second generation inhibitors, designed to address resistance, are currently under investigation in clinical trials. The two most advanced compounds are dacomitinib (PF-00299804, Pfizer) and afatinib (BIBW-2992, Boehringer–Ingelheim) (Fig. 1) which are currently approved and in phase III clinical trials.[10] Both of these compounds are structurally very similar to gefitinib and erlotinib with the exception that they harbor Michael acceptors in the 6-position side chain of the quinazoline core. This leads to dacomitinib and afatinib to be irreversible inhibitors of EGFR.[11] The results of several phase III clinical trials for dacomitinib and afatinib are expected to be completed in 2013. Moreover, many studies have been targeted at finding new structures based on quinazolines that are potent EGFR inhibitors.[12–14]

Based on erlotinib as the leading compound, we have devised and synthesized two series of novel tricyclic oxazine and oxazepine fused quinazolines through intramolecular cyclization which possessed a functional Michael acceptor group, with the aim of obtaining agents displaying more potent antitumor activities. The antitumor effect of all the newly synthesized compounds on the in vitro growth of five cell lines, namely human gastric carcinoma cell line NCI-N87 (HER2 overexpression), human epidermoid carcinoma cell line A431 (EGFR overexpression), human adenocarcinoma cell line NCI-H1975 (EGFR L858R/T790M mutation), human breast cancer cell line BT-474 (HER2 overexpression) and human adenocarcinoma cell line Calu-3(HER2 overexpression), was evaluated. Apparent growth inhibition was observed for most of the

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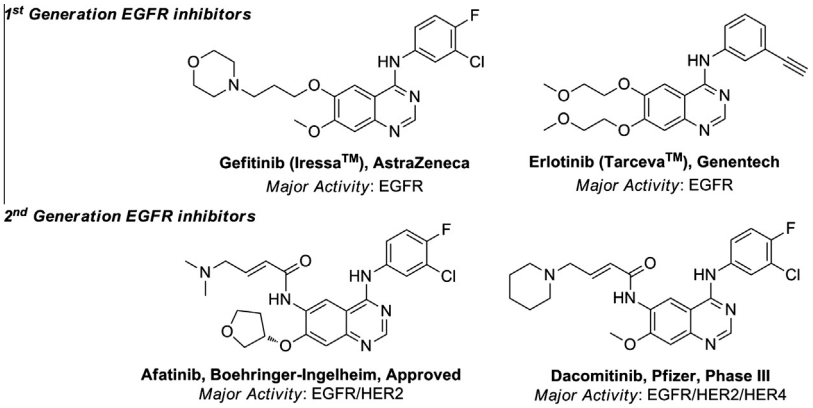


Figure 1. Selected 1st, and 2nd generation EGFR inhibitors for NSCLC.

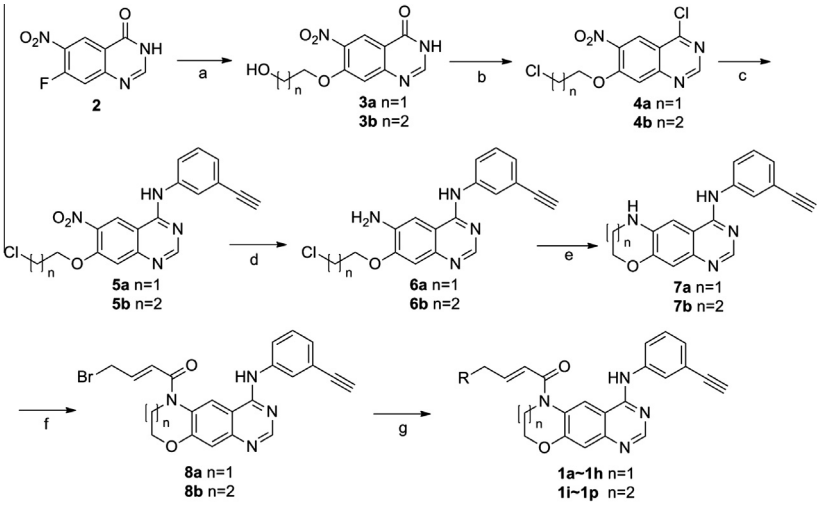
compounds, with **1a**–**1h** demonstrating more potent activities against all five cell lines as compared to gefitinib and erlotinib, respectively. Furthermore, all the synthesized compounds were assessed the in vitro inhibition of EGF-induced receptor autophosphorylation in the KB nasopharyngeal carcinoma cell line. Finally, compounds **1a**–**1h** were chosen for further evaluation of EGFR and HER2 in vitro kinase inhibitory activity.

The synthetic route to tricyclic oxazine and oxazepine fused quinazolines (**1a**–**1p**) was illustrated in Scheme 1. Conversion of the fluoro group of **2** to the corresponding hydroxylethoxy (hydroxylpropoxy) compound **3**. Treatment of **3** with phosphoryl chloride and thionyl chloride gave the dichloride **4**. Coupling of **4** with 3-aminophenylacetylene **9** gave nitro compound **5**. Reduction of **5** with iron/ammonium chloride gave the corresponding amine **6**. Intramolecular cyclization of **6** with potassium carbonate gave **7**. Acylation of **7** with the bromocrotonic acid chloride **10** generated from bromocrotonic acid and oxalyl chloride gave the bromocrotonamide **8**, which was finally submitted to bromide displacement with different aliphatic amines to yield the corresponding target compounds **1a**–**1p**.

All the 16 newly synthesized erlotinib derivatives (**1a**–**1p**) were tested for cytoxicity[15] toward cancer cell lines either with EGFR (A431) or HER2 (N87, BT474, Calu-3) overexpression. The data are summarized in Table 1. The tricyclic oxazine compounds (**1a**–**1h**) demonstrated more remarkable inhibition activity against the four cell lines (IC_{50}: 0.046–2.06 μM) compared with erlotinib (IC_{50}: 0.75–>10 μM) and gefitinib (IC_{50}: 0.36–1.00 μM). The activity of compound **1h**[16] (IC_{50}: 0.046–0.24 μM) was found to be 3 to >210 fold and 1.5 to 22 fold more potent than erlotinib (IC_{50}: 0.75–>10 μM) and gefitinib (IC_{50}: 0.36–1.00 μM) anaginst the four cell lines, respectively.

The in vitro sensitivity of NSCLC cell lines to gefitinib was most closely associated with the presence of activating mutations in EGFR. However, some EGFR mutations, such as T790M or exon 20 insertion mutations, were associated with gefitinib resistance in vitro and in vivo.[17] Hence the efficacy of target compounds (**1a**–**1p**) to gefitinib and erlotinib in H1975 cell line (EGFR L858R/T790M mutation) was compared (Table 1). The tricyclic oxazine compounds (**1a**–**1h**) demonstrated more remarkable inhibition activity against the H1975 cell line (IC_{50}: 0.30–1.46 μM) compared with gefitinib (IC_{50}: >10 μM) and erlotinib (IC_{50}: 5.51 μM). The activity of compound **1h** was found to be 33 fold and 18 fold more potent than gefitinib and erlotinib, respectively.

To determine the potency of target compounds against EGFR autophosphorylation in intact cells, we performed ELISA tests[18] with EGFR-specific antibodies and measured levels of receptor phosphorylation with increasing drug concentrations (Table 1). Compounds **1a**–**1h** demonstrated potent cellular effects on EGFR



Scheme 1. Synthetic route for the preparation of the target compounds **1a**∼**1p**. Reagents and conditions: (a) ethylene glycol, NaH, THF, 75 °C, 20 h, 99% of **3a**; (a') propylene glycol, NaH, THF, 75 °C, 20 h, 99% of **3b**; (b) $POCl_3$, $SOCl_2$, 90 °C, 3 h, 83% of **4a** and **4b**; (c) 3-aminophenylacetylene (9), isopropanol, 50 °C, 2 h, 99% of **5a** and **5b**; (d) Fe/NH_4Cl, DMF/H_2O, 80 °C, 1 h, 80% of **6a** and 76% of **6b**; (e) K_2CO_3, KI, DMF, 110 °C, 24 h, 27% of **7a** and 23% of **7b**; (f) bromocrotonic acid chloride (10), Et_3N, DCM, 35 °C, 24 h, 76% of **8a** and 70% of **8b**; (g) aliphatic amines, DMF, r.t, 1 h.