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Synthesis and biological evaluation of crown ether fused quinazoline analogues as potent EGFR inhibitors

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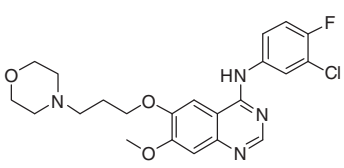
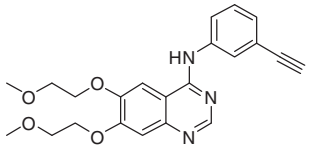
ABSTRACT

Crown ether fused anilinoquinazoline analogues were synthesized as novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. Representative compounds showed potent and selective EGFR inhibitory activities in an in vitro EGFR kinase assay and an EGFR-mediated intracellular tyrosine phosphorylation assay. The synthesis and preliminary biological, physical, and pharmacokinetic evaluation of these fused quinazoline compounds is reported.

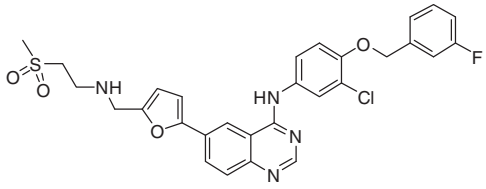
The epidermal growth factor receptor (EGFR), as a transmembrane glycoprotein, belongs to the erbB family of closely related cell membrane receptors that includes EGFR (erbB-1 or HER1), erbB-2 (HER2), erbB-3 (HER3), and erbB-4 (HER4).[1] Expression, overexpression, or dysregulated function of EGFR is observed in many human solid tumors, including breast, ovarian, non–small-cell lung (NSCLC), colorectal, and head and neck cancers.[2–5] In support of its important role in tumor biology, EGFR activation may assist tumor growth by increasing cell proliferation, motility,[6] adhesion, and invasive capacity[7] and by blocking apoptosis.[8] EGFR-dependent aberrant signaling, such as overexpression and dysregulation, is associated with indices of poorer prognosis in patients and is associated with metastasis, late-stage disease, and resistance to chemotherapy, hormonal therapy, and radiotherapy.[2,4,9–12] Small molecule EGFR inhibitors have been shown to be effective antitumor agents.[1] For example, two closely related anilinoquinazoline-containing EGFR inhibitors, gefitinib (Iressa™, **1**)[2] and erlotinib (Tarceva™, **2**)[3] have efficacy against several types of cancers in human clinical trials and were approved for the treatment of NSCLC and colon cancers. The dual EGFR/HER2 inhibitors lapatinib (Tykerb™, also known as GW-572016, **3**) was recently approved for the treatment of HER2-positive metastatic breast cancer.[4] Many more EGFR inhibitors including antibodies are either still under evaluation in clinical trials or been approved for the treatment of cancer.[1]



Iressa, **1**



Tarceva, **2**



Lapatinib, **3**

There are numerous crystal structures of EGFR available, including the co-crystal structures of EGFR with erlotinib or gefitinib, which have provided a rich set of structural information for our drug discovery effort. The structure–activity relationship (SAR) of these EGFR inhibitors are mostly well understood.[13] As shown in Figure 1, binding of erlotinib depends on several key interactions with the ATP binding pocket of EGFR, including the critical H-bond with the hinge region using its quinazoline core, the filling up of the so-called 'back pocket' by the ethynylphenyl substitution, and the interactions in the tail region. There is also a hydrophobic

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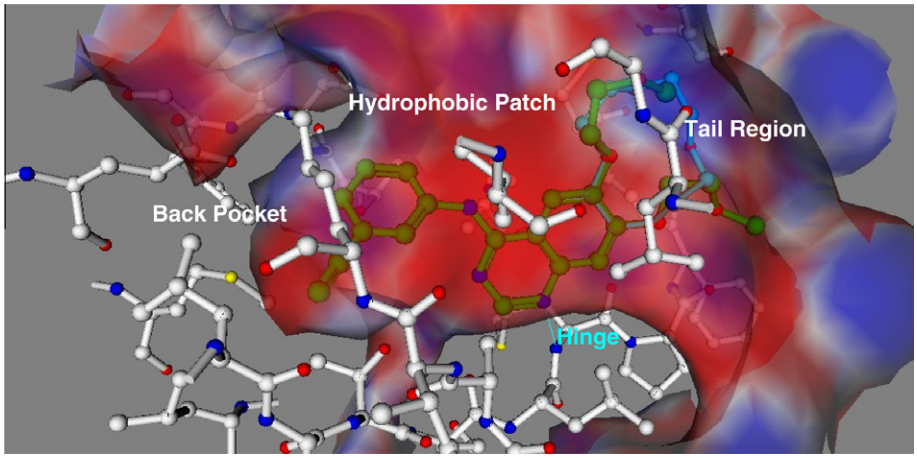
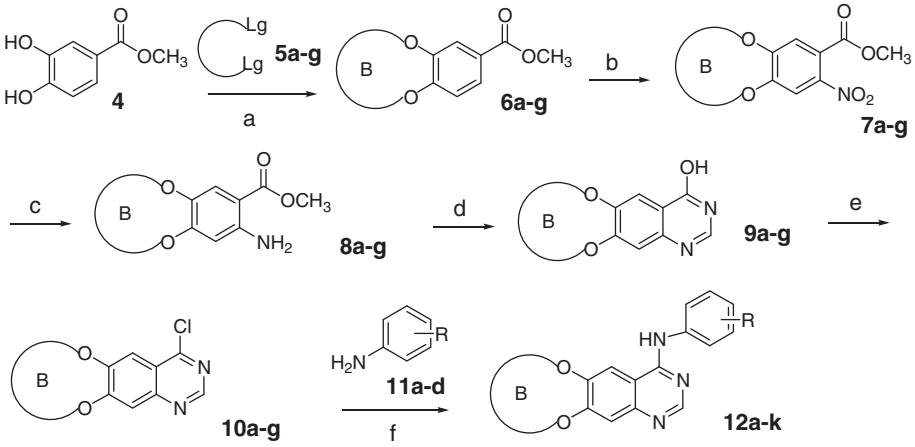


Figure 1. Crystal structure of erlotinib (gold) in EGFR (1M17 pdb). Compound **12k** (cyan) was modeled in for comparison. The protein surface of the ATP binding pocket of EGFR was shown, with red representing the hydrophobic areas and blue the hydrophilic areas.

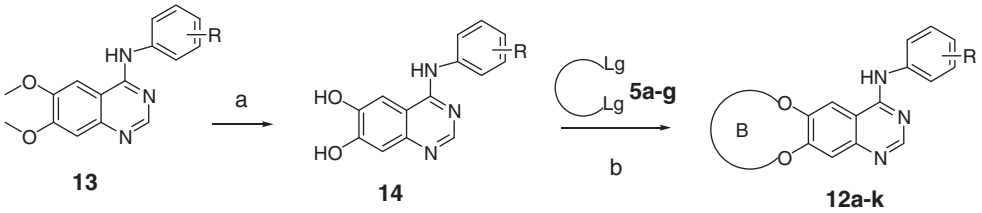


Scheme 1. General synthetic approach to fused quinazolines. Reagents and conditions: (a) K_2CO_3/DMF, 90 °C, 3 h, 24–45%; (b) HNO_3/H_2SO_4, HOAc, 72%; (c) H_2, Pd/C, 96%; (d) $HCONH_2/HCO_2NH_4$, 165 °C, 80%; (e) $POCl_3$, 77%; (f) *i*-PrOH/DMF,72%.

patch a little further away from the hinge, which may also provide additional opportunities for ligand optimization. In particular, the tail region is able to accommodate a variety of functional groups without affecting the activities of the compounds too much. The various substituents at the 6 and 7 positions of the quinazolines ring which occupies the tail region are able to significantly modulate the properties of the compounds, and such differences between gefitinib, erlotinib and lapatinib contribute in part to the distinct profiles of these three EGFR inhibitors. Therefore this is a major area to further optimize good lead molecules.

Our computer model study provides us much needed information to design new EGFR inhibitors. We have focused our development on the modification of tail region by attaching cyclic systems to the quinazoline ring. Therefore we synthesized a series of structural modified quinazolines compounds by fused with numerous ring systems, aimed at identifying potent, selective, and bioavailable EGFR inhibitors as anti-cancer agents. Herein we report their synthesis and preliminary biological evaluation.

A general approach to synthesize the designed crown ether fused quinazolines compounds **12a**–**k** is shown in Scheme 1, starting from commercially available methyl 2,3-dihydroxybenzoate **4**. The key intermediates, methyl benzoates **6a**–**g** with different ring system were synthesized from the reaction of methyl 2,3-dihydroxybenzoate **4** with various α,ω-dihalogens or α,ω-tosylates **5a**–**g** in the presence of abase such as potassium carbonate at elevated temperature. The standard nitration procedure is employed for the nitration of methyl benzoates **6a**–**g**, which was reduced by hydrogenation in methanol to give anilines **8a**–**g**. Compounds



Scheme 2. Alternative synthetic approach to fused quinazolines. Reagents: (a) BBr_3, THF, 63%; (b) K_2CO_3/DMF, 20–36%.