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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6373–6377
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological evaluation of substituted 6-alkynyl-4-anilinoquinazoline derivatives as potent EGFR inhibitors

Lee Tai Liu,[a,*] Ta-Tung Yuan,[a] Hung-Huang Liu,[a] Shyh-Fong Chen[a] and Ying-Ta Wu[b]

[a]*Development Center for Biotechnology, 102, Lane 169, Kang Ning Street, Xi Zhi, Taipei, Taiwan, ROC*
[b]*Genomics Research Center, Academia Sinica, 128 Academia Road, Sect. II, Nan Kang, Taipei, Taiwan, ROC*

Received 4 May 2007; revised 15 August 2007; accepted 24 August 2007
Available online 28 August 2007

Abstract—A series of C-6 or C-3′ alkynyl-substituted 4-anilinoquinazoline derivatives was prepared straightforwardly by a Sonogashira reaction of the corresponding bromo-substituted 4-anilinoquinazolines. Bioactive assay of these compounds for in vitro EGFR kinase inhibition demonstrated that the novel 6-hydroxypropynyl-4-anilinoquinazoline **5e** was a very potent EGFR kinase inhibitor with an IC_{50} of 14 nM.

Overexpression of the epidermal growth factor receptor (EGFR) tyrosine kinases has been observed in many cancer patients and correlates with a poor prognosis for several human tumors including non-small-cell lung cancer (NSCLC), prostate, breast, stomach, colon, and ovarian cancers.[1–3] The binding of ligands to EGFR leads to the activation of the receptor tyrosine kinase via the dimerization and autophosphorylation of the receptor. The autophosphorylation, in turn, activates intracellular signaling pathways that regulate cellular functions including division, differentiation, and proliferation. These tyrosine kinases play a key role in signal transduction pathways, and therefore, have become important targets for drug design.

Small molecules that inhibit the kinase activity of EGFR are of considerable interest as new therapeutic antitumor agents for the treatment of EGFR-mediated cancers. Most of the effort in this area has been directed toward the development of tyrosine phosphorylation inhibitors. The selective inhibition of the ATP binding site to the receptor tyrosine kinase domain results in the inhibition of EGFR autophosphorylation and the blockade of the subsequent intracellular signal transduction cascades. And finally, the stop of the tumor cell growth.[1–4]

Three drugs, Gefitnib (Iressa), Erlotinib (Tarceva), and Lapatinib (Tykerb), have been approved and marketed for the treatment of non-small-cell lung cancer. And, several reversible and irreversible EGFR inhibitors are currently being investigated in various phases.[1–3] These small molecules have a scaffold that mimics the adenine moiety of ATP, and therefore are potent and competitive inhibitors of ATP.[4] One of the most extensively studied classes of these compounds is 4-anilino substituted quinazolines.[4] Variation of the C-4 substituted anilino moiety and at the side chain of the C-6 (such as Lapatinib) position or the side chains of the C-6/C-7 (such as Iressa and Tarceva) positions of the quinazoline scaffold induced the selective inhibition of the subfamily receptors.[5,6]

While the halide atoms (Cl, F) are placed at the C-3′ position at 4-anilinoquinazoline moiety of Iressa and Lapatinib, it is noticeable that there is an ethynyl group at the C-3′ anilino group in Tarceva. Though some of the alkynyl derivatives might be covered in the related patents, however, the influence of the substituted alkynyl derivatives on the inhibition of EGFR kinase has not been disclosed in the literature.

Keywords: EGFR kinase inhibitor; Alkynyl-substituted 4-anilinoquinazoline.
* Corresponding author. Tel.: +886 2 26956933; fax: +886 2 26957474; e-mail addresses: letliu@yahoo.com.tw; thomasyuan@mail.dcb.org.tw

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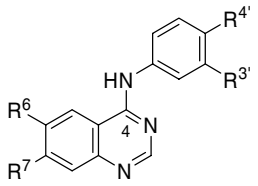
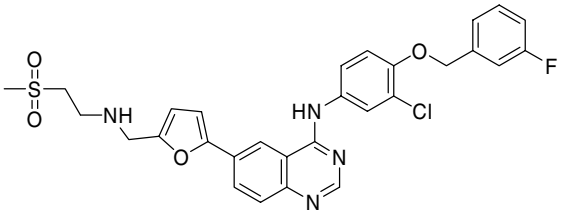
doi:10.1016/j.bmcl.2007.08.061

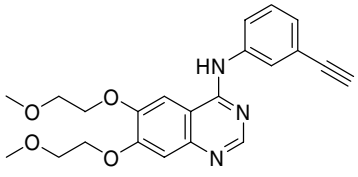
Because of the electrophilicity and the linear structure of the alkynes, attaching a substituted alkynyl group with a suitable chain length and hydrophilicity to replace the alkoxy groups at the C-6 of quinazoline moiety or to replace the chloride atom at the C-3′ of aniline moiety in Iressa may provide compounds with potential EGFR inhibitory activity. This report describes the investigational results on the synthesis of a series of C-6 or C-3′ alkynyl-substituted 4-anilinoquinazoline derivatives and their bioactivity as EGFR kinase inhibitors.



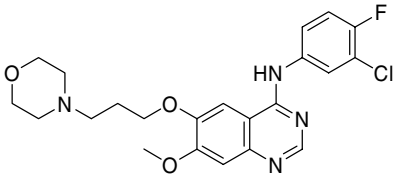
quinazoline



Lapatinib (Tykerb)



Erlotinib (Tarceva)



Gefitnib (Iressa)

The preparation procedure for alkynyl-substituted 4-anilinoquinazoline derivatives is much easier and straightforward than that of the marketed drugs such as Iressa and Lapatinib.[7b,c] As illustrated in Scheme 1, the bromo-substituted quinazoline derivatives **4a** and **b** were prepared from commercially available 2-amino benzoic acids **1a** and **b** and the corresponding anilines via three steps as described in the literature.[7] Palladium catalyzed Sonogashira reaction of aromatic bromide **2** with a series of terminal alkynes provided the corresponding substituted alkynyl-substituted 4-anilinoquinazolines **5a**–**i** and **6a**–**g**.[9] The hydroxy group in the propargyl alcohol **5a** was activated to the mesylate and was then converted to the alkynyl cyclic amino derivatives **7a** and **b**.[9]

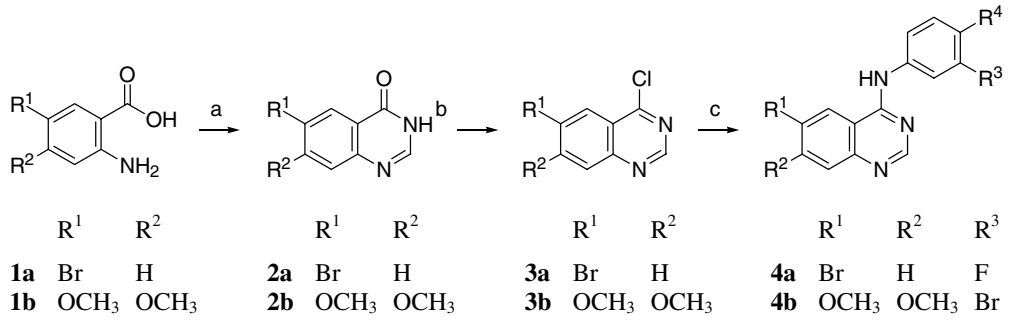
Table 1 shows the inhibition of EGFR kinase activity by 4-anilinoquinazoline derivatives **5**–**7**.[10] The IC_{50} values depend on the position, chain length, and hydrophilicity of the substituted alkynyl groups. Compound **5e** was more potent than the other alkynyl-substituted analogues in this investigation. In general, the C-6 alkynyl-substituted 4-anilinoquinazolines are far more potent than the C-3′ alkynyl-substituted 4-anilinoquinazoline counterparts (Table 1, entries 2–11 vs 12–17) except the acetylene derivative **6b**. Conversion of the hydroxy group in **5e** to the cyclic amino analogues **7a** and **b** greatly reduced their activity (entries 5 vs 10–11). It is noticeable that compound **5e** exhibited even more potent inhibition (entry 5, 14.1 nM) than the marketed drug Iressa (entry 1, 39.0 nM) for the in vitro EGFR kinase tests. This potent activity should be attributed to the hydrophilicity, linear structure, and optimal chain length of the 6-hydroxypropargyl group in the 4-anilinoquinazoline **5e**. To the best of our knowledge, no report about the bioactivity of the hydroxy alkynyl groups in 4-anilinoquinazoline derivatives for EGFR kinase inhibitors has been published to date.[8]

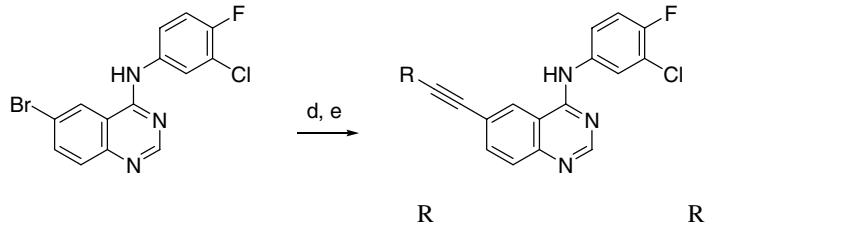
Computational modeling studies provided some explanation for these results.[11a] As shown in Figure 1, the EGFR binding pocket space is very limited to accommodate a C-3′ substituent. A drastic steric hindrance occurred when a C-3′ alkynyl group longer than acetylene (Tarceva and **6b**) directed toward the backbone Leu788 and Ilu789 residues.[11b] On the other hand, the C-6 substituents extended outward to solution, and therefore, the steric hindrance between the C-6 alkynyl group and EGFR was less serious than that of the C-3′ isomers. Besides, the

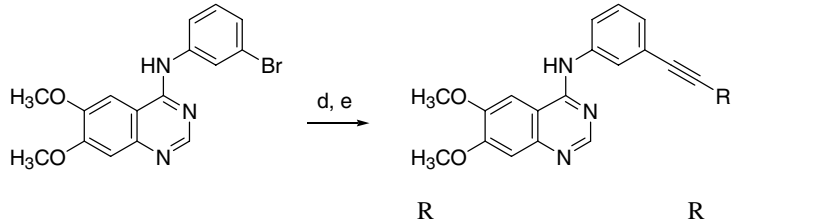
Table 1. In vitro inhibitory activity of quinazolines **5**–**7** to EGFR[10]

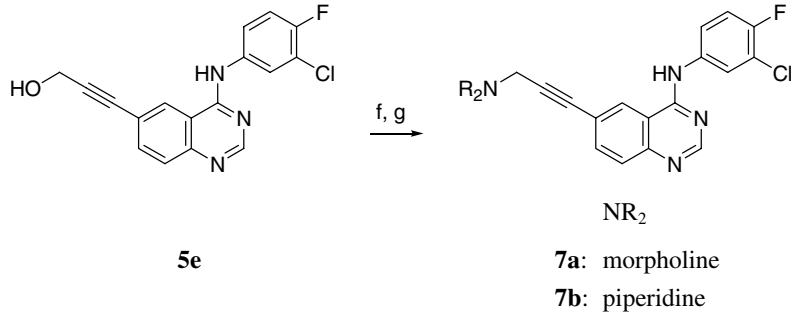
Entry	Compound	IC_{50}[a] (nM)
1	Gefitnib (Iressa)[b,c]	39.0 ± 0.02
2	**5b**	126.9 ± 2.7
3	**5c**	331.0 ± 41.6
4	**5d**	1640.6 ± 586.1
5	**5e**	14.1 ± 2.3
6	**5f**	185.8 ± 11.0
7	**5g**	114.2 ± 0.6
8	**5h**	94.5 ± 5.6
9	**5i**	151.5 ± 30.1
10	**7a**	50.3 ± 8.5
11	**7b**	88.7 ± 27.7
12	**6b**	28.8 ± 10.4
13	**6c**	921.0 ± 138.1
14	**6d**	1643.6 ± 318.9
15	**6e**	402.1 ± 89.3
16	**6f**	1362.6 ± 300.6
17	**6g**	205.8 ± 11.7

[a] Average values (at least two experiments) and standard deviations.[6,10]
[b] Iressa was prepared according to the literature.[7]
[c] Reference IC_{50} values: Gefitnib (Iressa), 23 nM; Erlotinib (Tarceva),









Scheme 1. Synthesis of quinazoline derivatives.[9] Reagents: (a) formamide, reflux (50–80%); (b) thionyl chloride, catalytic DMF (80%); (c) substituted aniline, 2-propanol (60%); (d) alkyne, copper(I) iodide, bis(triphenylphosphine)palladium (II) dichloride, tetrahydrofuran, reflux (60–80%); (e) tetrabutylammonium fluoride (95%); (f) methanesulfonyl chloride, triethylamine; (g) amine, sodium carbonate, acetonitrile, refluxed (80%).

propargyl group in **5e** provides a proper length of substituent for its terminal hydroxy group to form a hydrogen bonding with the backbone carbonyl oxygen atom of Leu718 to fix its position. This additional hydrogen bonding enhanced the binding affinity of **5e** to EGFR and explained why the IC_{50} value of **5e** was more potent than those of Iressa (entries 5 vs 1) as well as the cyclic amino derivatives **7a/b** (entries 5 vs 10/11).

In summary, a series of 4-anilinoquinazoline derivatives with various alkynyl groups at C-6 or C-3′ position were prepared via a Sonogashira reaction of the corresponding bromo-substituted 4-anilinoquinazolines. The procedure was much easier and straightforward than that of the current marketed drugs (Iressa and Lapatinib). Bioactive assay of these compounds for the in vitro EGFR kinase inhibition demonstrated that 6-hydroxypropynyl-4-anilinoquinazoline **5e** was a

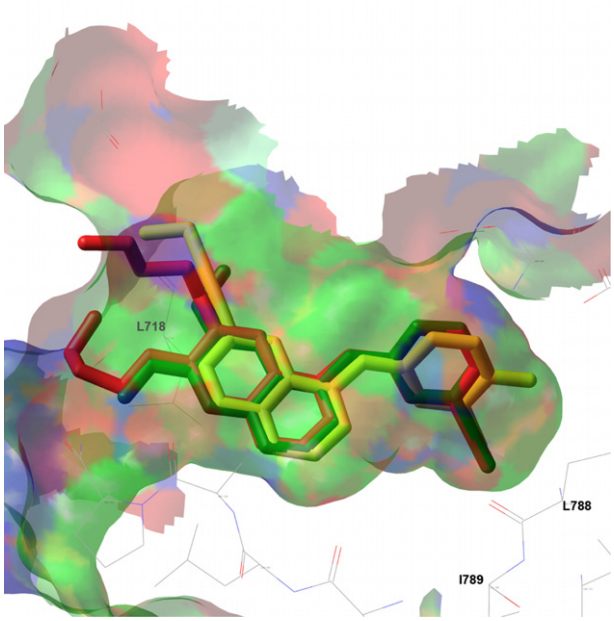


Figure 1. Interacting poses of Tarceva (colored in red), compounds **5e** (colored in yellow), and **6b** (colored in green) in EGFR (pdb code 2ito) from docking modeling.[11] The molecular surface colored by atom partial charges is plotted to show the binding pocket of EGFR. The distance of the C-3′ ethynyl end of Tarceva (also compound **6b**) is only 2.8 Å to the backbone carbonyl oxygen atom of Leu788. The C-6 hydroxy group in **5e** has an additional hydrogen bonding with the backbone carbonyl oxygen atom of Leu718.

very potent EGFR kinase inhibitor with an IC_{50} of 14 nM.

Acknowledgments

The authors thank the Ministry of Economy Affairs of ROC (Taiwan) for financially supporting this research. Ms. M.H. Lee is appreciated for assistance with the NMR analysis.

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9. All of the new compounds were characterized. Preparation of compound **5e** is described herein. A solution of compound **4**, propargyl alcohol, copper(I) iodide (0.05 equiv), bis(triphenylphosphine)palladium chloride (0.05 equiv), and triethylamine (2 equiv) in tetrahydrofuran was stirred and refluxed under nitrogen overnight. After the reaction was completed, the mixture was filtered through silica gel and eluted with ethyl acetate. The solvent was evaporated, and the mixture was purified by chromatography with hexane/ethyl acetate to give the desired product. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.99 (s, 1H), 8.72 (s, 1H), 8.65 (s, 1H), 8.23 (dd, J = 6.8, 2.3 Hz, 1H), 7.87–7.82 (m, 2H), 7.77 (m, 1H), 7.46 (t, J = 9.1 Hz), 5.44 (t, J = 5.6 Hz, 1H), 4.39 (d, J = 5.2 Hz, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.0, 154.9, 153.4, 149.1, 136.3, 135.3, 128.3, 126.2, 123.5, 122.3, 120.3, 118.8, 116.7, 114.9, 91.3, 83.1, 49.5. MS (EI) m/z 328 MH^+.
10. The kinase activity of EGFR was measured by the incorporated radiolabeled γATP into a tyrosine-containing peptide. In brief, 5 μl of testing compound was mixed well with 5 μl of 5× reaction buffer, 2.5 μl of EGFR (active, 25 ng), 1.25 μl of PolyGlu:Tyr (4/1, 5 μg), 1.25 μl of manganese chloride (10 mM), 5 μl of ammonium sulfate (0.8 M), and 5 μl of [γ-^{33}P] MgAc/ATP cocktail containing magnesium acetate (50 mM) and ATP (500 μM). The reaction mixture of a total volume of 25 μl was incubated at 30 °C for 30 min. Five microliters of phosphoric acid (3%) was added to stop the reaction. An aliquot of 20 μl from reaction was transferred onto the center of a 2 cm × 2 cm P81 paper and allow the radiolabeled substrate to bind to the filter paper for 30 s. Wash the assay squares five times for 5 min with water. Transfer the assay squares to vial and add 5 ml scintillation cocktail. Read in scintillation counter by Beckman LS-6500 counter.

11. (a) Docking modeling was applied to assist SAR study. Briefly, the 3D structure of EGFR (pdb coded 2ito) was obtained from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The structures of three compounds (Tarceva, **5e**, and **6b**) were generated and the energies were optimized on Meastro by using Ligpre (Schrödinger, USA). Molecular docking was conducted by using Glide with XP mode (detailed in user manual of Schrödinger), which allows flexible conformational search of the compounds against the target. For each inhibitor, five best docking modes were generated and its final interacting complex with EGFR was selected based on computed docking score of Glide.; (b) Glide docking scores of Iressa (−9.44), Tarceva (−10.83) as well as some active compounds such as **5e** (−10.87), **6b** (−9.88), and **6d** (−3.42) showed that compound **5e** is slightly favored over Iressa and Tarceva in EGFR kinase SAR.