Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5863–5867
Bioorganic & Medicinal Chemistry Letters

Synthesis and inhibitory activity of 4-alkynyl and 4-alkenylquinazolines: Identification of new scaffolds for potent EGFR tyrosine kinase inhibitors

Yasunori Kitano,* Tsuyoshi Suzuki, Eiji Kawahara and Takahisa Yamazaki

Mitsubishi Pharma Corporation, Pharmaceuticals Research Division, 1000, Kamoshida-cho, Aoba-ku, Yokohama 227-0033, Japan

Received 18 June 2007; revised 7 August 2007; accepted 10 August 2007
Available online 15 August 2007

Abstract—The present study identified several 4-alkynyl and 4-alkenylquinazolines that serve as novel and potent EGFR tyrosine kinase inhibitors. The IC_{50} values of these compounds are in the nanomolar range. In addition, the 4-(4-phenylbut-1-yn/en-yl)quinazolines provided scaffolds for potent enzyme inhibition. Chiral discrimination was observed to occur in one of the 4-alkynylquinazoline derivatives with the (*R*)-isomer being more than 150 times as potent as the (*S*)-isomer.

Epidermal growth factor receptor (EGFR) is known to be over-expressed in a large percentage of clinical cancers of various types,[1–3] and to be closely related to a poor prognosis in patients.[4,5] Accordingly, the EGFR has become an important target for drug design.[6] Recent success in the clinical evaluation of EGFR tyrosine kinase (TK) inhibitors such as *gefitinib*[7] and *erlotinib*[8] (Fig. 1) strongly suggests small molecular EGFR TK inhibitors to be promising new anti-cancer drugs.

These quinazoline-based inhibitors have a substituted aniline group at the 4-position of the quinazoline nucleus. The 4-substitution is assumed to be optimal as an aniline or benzylamine, with other linkers being less effective,[9] and other variations have not been well investigated.[10] During the course of our exploration of the non-anilininoquinazoline scaffold, 4-alkynyl- and 4-alkenylquinazolines were found to be novel and potent EGFR TK inhibitors. Herein, we report the synthesis, inhibitory activity, and characteristic features in SAR of this series of compounds.

In initial studies, the 6,7-dimethoxyquinazoline nucleus present in early quinazoline-based inhibitors[11] was conserved for inter-comparisons of the effects of substitutions at the 4-position. The preparation of 4-alkynyl and 4-alkenylquinazolines is outlined in Scheme 1. The 4-alkynylquinazolines were synthesized by a Sonogashira coupling reaction[12] of 4-chloroquinazoline **1**[13] with terminal alkynes **3**. The corresponding alkynes were made available in several ways. Some alkynes, other than the commercially available **3a**–**c** and the one already known as **3e**,[14] were obtained using conventional transformation of an aldehyde into an acetylene sequence.[15] Other alkynes bearing amino-functionality, except for **3i**,[16] were constructed from ketones and the ethynyl Grignard reagent. Subsequent trapping in situ of the resulting adducts with acetic anhydride yielded acetates, which in turn upon treatment with appropriate amines by copper-catalyzed displacement[17] furnished aminoalkynes. The 4-alkenylquinazolines were obtained via hydro-zirconation of alkynes **3** followed by a Pd-catalyzed coupling reaction[18] with **1**.

Table 1 summarizes the structures and inhibitory activities of these compounds against the human EGFR TK.

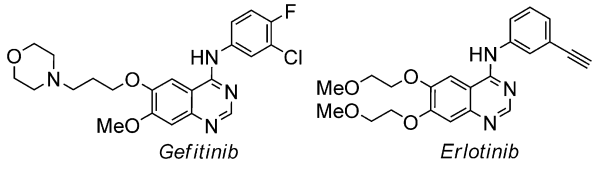
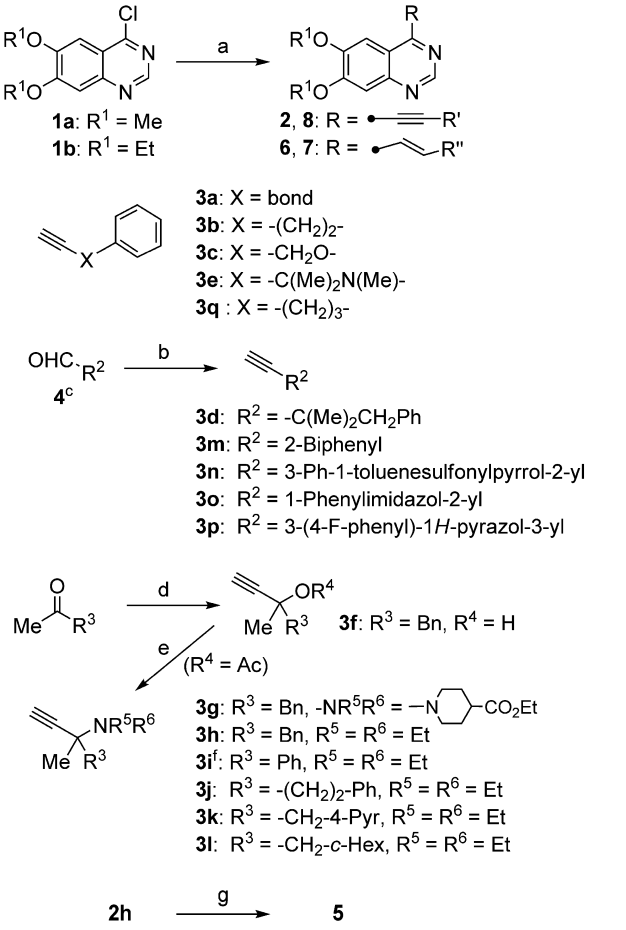


Figure 1.

Keywords: EGFR; Tyrosine kinase inhibitor; 4-Alkynylquinazoline; 4-Alkenylquinazoline.
* Corresponding author at present address: Mitsubishi Tanabe Pharma Corporation, Research Division, Medicinal Chemistry Laboratory, 16-89, Kashima 3-chome, Yodogawa-ku, Osaka 532-8505, Japan. Tel.: +81 6 6300 2562; fax: +81 6 6300 2564; e-mail: Kitano.Yasunori@mc.mt-pharma.co.jp

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.08.020



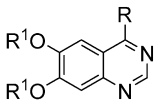
Scheme 1. Reagents: (a) for **2**, **8**: acetylene **3**, Et_3N, cat. $Pd(PPh_3)_4$ or $PdCl_2(PPh_3)_2$, CuI, DMF; for **2g** and **8n**: followed by hydrolysis (NaOH aq, MeOH); for **6b**, **7**: $Cp_2Zr(H)Cl$, acetylene, THF then **1**, cat. $Pd(PPh_3)_4$; (b) i—CBr_4, PPh_3, CH_2Cl_2; ii—2 equiv of *n*-BuLi, THF; (c) for preparation of **4**, see Ref. 20; (d) ethynyl magnesium chloride, THF then Ac_2O (for **3f**: then H^+); (e) R^5R^6NH, cat. CuCl, THF; (f) see Ref. 16; (g) H_2, Pd/C, EtOH.

For comparison, *gefitinib* and *erlotinib* have an IC_{50} of 1.4 and 1.0 nM, respectively, in this assay system.[19] The simple 4-phenylethynylquinazoline **2a** showed a modest inhibitory activity of 5.6 μM. In contrast, the two carbon homologue **2b** had a marked 400-fold increment in activity (14 nM). Its oxa-analogue **2c** also inhibited with comparable potency, implying the 4-phenylbut-1-ynyl substructure to be the preferred topology.

Compounds **2d** and **2e**, possessing a sterically demanding quaternary carbon or tertiary amine, retained their potencies, which were substantially comparable to that of **2b**. This result indicates a degree of tolerance for steric bulk at the propargyl position.

Incorporation of functionalities by taking advantage of the tolerance at the propargyl position was carried out to modulate physico-chemical properties. When employing a hydroxyl (**2f**), di-alkylamino (**2h**), or amino-acid (**2g**) functionality at the propargyl position, excellent inhibitory activity was observed, especially for **2h**, which had an IC_{50} value in the nanomolar range. To obtain

Table 1. Inhibitory activity of 4-alkynyl and 4-alkenylquinazolines



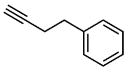
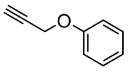
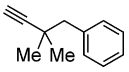
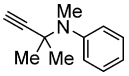
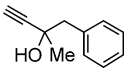
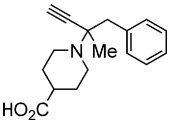
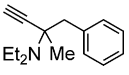
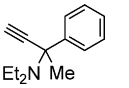
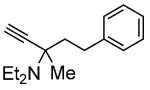
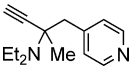
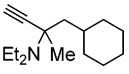
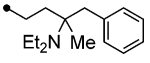
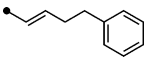
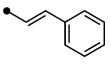
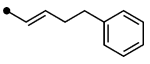
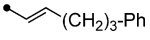
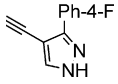
Compound			EGFR TK IC_{50} (nM)
No.	R^1	R	
2a	Me		5600
2b	Me		14
2c	Me		15
2d	Me		13
2e	Me		80
2f	Me		9
2g	Me		33
2h	Me		3
2i	Me		7300
2j	Me		1200
2k	Me		200
2l	Me		1100
5	Me		130
6b	Me		4
2m	Me		26
7a	Et		15
7b	Et		5
7q	Et		140

Table 1 (*continued*)

Compound			EGFR TK IC_{50} (nM)
No.	R^1	R	
8n	Et		10
8o	Et		12
8p	Et		1.8

further structural requirements for the most potent compound **2h**, related compounds **2i–l** were examined. With respect to the length of the side chain, the shorter (**2i**) and the longer methylene linker (**2j**) resulted in a three digits in magnitude drop in activity. These results reveal the optimal number of atoms between the acetylene β-carbon and the phenyl portion to be two [i.e., 4-(4-phenylbut-1-ynyl)quinazoline scaffold], as is consistent with the simple case of **2a–c**. The reduced activity observed with replacement of the phenyl portion in **2h** with pyridyl (**2k**) and cyclohexyl (**2l**) serves to highlight the importance of the phenyl aromatic nature.

To gain further insight into the structural requirements of EGFR TK inhibition, the saturation state of acetylene with respect to inhibitory activity was examined. It should be noted that compound **5** (full saturation at the triple bond of **2h**) showed significantly reduced activity, while the potency of compound **6b**, which possesses an (*E*)-double bond, was greater than that of the parent compound **2b**. It is of interest that the 4-(4-phenyl-but-1-enyl)quinazoline scaffold exhibited potent activity, although the 4-alkenyl group was anticipated to be placed in a different orientation with either the 4-alkynyl group or the aniline group of the 4-anilinoquinazolines. In order to understand the potent activities and interactions of these compounds with EGFR TK, a docking model of compounds **2b** and **6b** based on the crystal structure of EGFR TK complexed with *erlotinib*[21] (PDB code 1M17) was developed (Fig. 2).[22] This model shows that the quinazoline cores of **2b** and **6b** slightly deviate from that of *erlotinib*, but they bind in substantially the same fashion as *erlotinib*. It can thus be expected that each quinazoline N-1 atom of **2b** and **6b** forms a hydrogen bond to the backbone NH of Met769 in the hinge region, and each N-3 atom binds to a structural water molecule, which in turn is associated with the OH of Thr766 by another hydrogen bond (for clarity, the water molecule is not shown in Fig. 2), since the putative distance of each hydrogen bond is estimated to be ca. 3 Å. This model also shows there is a relatively large hydrophobic pocket containing Leu764-Thr766 and Lys721, which may lead to tolerance of the steric bulk at the propargyl position of the 4-alkynylquinazolines. A key feature of this model is that the phenyl portions of **2b** and **6b** point in similar directions and are buried deep in the hydrophobic pocket, where the acetylene moiety appended to the aniline of

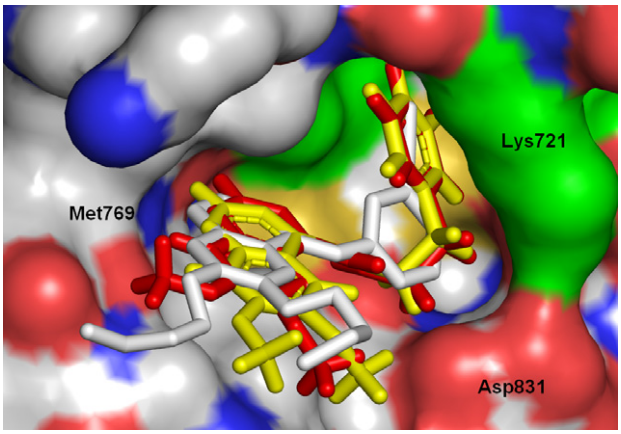


Figure 2. Docking of **2b** (yellow), **6b** (red) and *erlotinib* (white) with the EGFR TK catalytic domain. The hydrophobic pocket (Leu764-Thr766 and Lys721) is shown in green. The residues Val693-Ser696, Val702, Tyr703, and Ile720, and a structural water molecule have been removed for greater clarity of viewing.

erlotinib is located. Thus, this overlay model is different from our initial expectation, but explains the potent inhibition of **2b** and **6b** as well as their contact with the protein.

The results with **2b** prompted us to postulate that shifting of the ethylene in **2b** into part of the ring system could lead to potent inhibition through rigidity (Fig. 3). Compound **2m**, showing the incorporation of a benzene ring, was found to exhibit excellent potency against EGFR TK, proving this modification to be fruitful.

With the results of **6b** and **2m** in hand, their derivatives (**7**, **8**) were briefly examined. Since it has been reported that the potency of the 6,7-diethoxyquinazoline nucleus is greater than that of the 6,7-dimethoxy substitution,[13b] the 6,7-diethoxyquinazoline system was employed henceforth. Compound **7b** exhibited excellent activity at 5 nM, while direct connection of the phenyl ring with the ethenyl portion (**7a**) showed comparable to slightly detrimental results and the longer methylene linker (**7q**) led to decreased activity. A similar tendency was observed for the 4-alkynyl-6,7-dimethoxyquinazoline series, but even **7a** and **7q** were still potent at less than micromolar concentrations.

Replacement of the central phenyl portion in compound **2m** with a 5-membered heteroaryl such as pyrrole (**8n**), imidazole (**8o**), or pyrazole (**8p**) was generally effective and all compounds showed relatively potent inhibition. Among them, the pyrazole derivative **8p**, possessing a

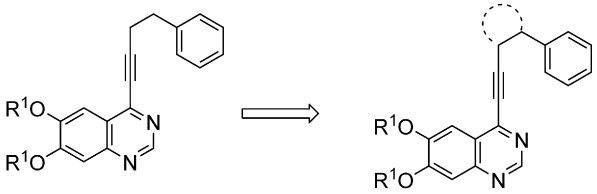
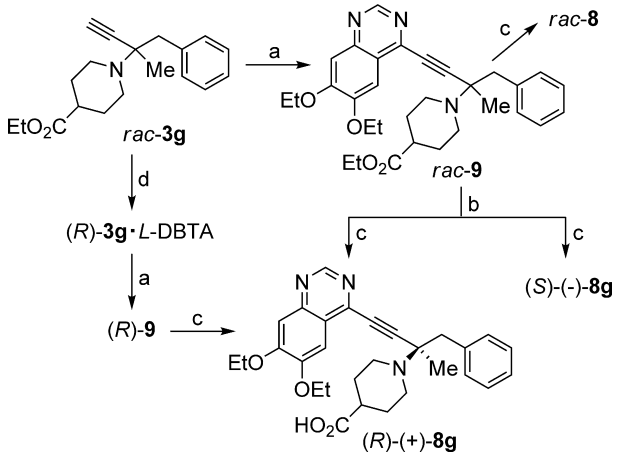


Figure 3.

halogenated phenyl ring, was the most potent, with an IC_{50} of 1.8 nM. This observation suggests an opportunity for further optimization by decorating the phenyl ring and/or refining the heteroaromatic ring. These results, combined with that obtained for compound **2m**, demonstrate that a 1-ethynyl-2-phenyl-aryl/heteroaryl substructure constitutes a new subclass of 4-alkynylquinazolines.

Finally, we turned our attention to assessing whether inhibition was enantio-selective, since the potent compounds **2f**–**h** had an asymmetric center. The compound **8g** was chosen and both enantiomers were successfully prepared, as outlined in Scheme 2. The racemic precursor *rac*-**9** was resolved by HPLC separation on a chiral stationary column[23] followed by hydrolysis to produce the enantiomers (+)- and (−)-**8g**, each with >99% ee.[23] To determine the absolute configuration of **8g** thus obtained, a stereorational synthesis of **8g** was carried out. It was found that the acetylene **3g** formed a crystalline salt with (2*R*,3*R*)-(−)-di-*O*-benzoyltartaric acid [*L*-(−)-DBTA] in acetone.[24] These crystals were determined by X-ray crystallographic analysis to be (*R*)-**3g** · *L*-(−)-DBTA. The optical rotation and mobility profile of **8g** prepared from (*R*)-**3g** on chiral column chromatography correlated well with those of (+)-**8g**, thus proving that (*R*) corresponds to (+) in **8g**. Table 2 summarizes the optical purities, specific rotations, and inhibitory activities of racemic and optically active **8g**.

It can be seen from the table that (*R*)-**8g** had a potent activity of 4.2 nM while the activity of the (*S*)-isomer was markedly reduced, revealing that EGFR TK discriminates the enantiopairs by more than 150-fold. With this result, future efforts will be directed toward understanding the interactions of the enantiomers from a structural point of view.



Scheme 2. Preparation of optically active **8g**. Reagents: (a) **1b**, cat. $Pd(PPh_3)_4$, CuI, Et_3N, DMF; (b) HPLC separation; (c) NaOH aq MeOH; (d) *L*-(−)-DBTA, acetone, re-crystallization.

Table 2. Inhibitory activities and physicochemical properties of **8g**

Compound	EGFR TK IC_{50} (nM)	$[\alpha]_D^{20}$ (*c* in $CHCl_3$)	% ee[a]
rac-**8g**	11.7	—	
(*R*)-**8g**	4.2	+34.4° (0.99)	>99
(*S*)-**8g**	662	−35.7° (1.02)	>99

[a] Determined by HPLC analysis using a chiral stationary column.[23]

In summary, non-anilinoquinazolines were explored in the search for EGFR TK inhibitors and 4-(4-phenyl-but-1-yn/en-yl)quinazolines were identified as new and potential scaffolds. In addition, 1-ethynyl-2-phenyl-aryl/heteroaryl was found to be a novel substructure at the 4-position of quinazolines. To our knowledge, these chemotypes represent the first illustration of carbon-substituted quinazolines at the 4-position with potent inhibitory activity and serve as new starting points for a structurally diverse class of EGFR TK inhibitors.

Acknowledgment

We thank Dr. Hideo Kubodera for helpful suggestions on the docking study.

References and notes

1. El-Zayat, A. A. E.; Pingree, T. F.; Mock, P. M.; Clark, G. M.; Otto, R. A.; Von Hoff, D. D. *Cancer J.* **1991**, *4*, 375.
2. Morishige, K.; Kurachi, H.; Amemiya, K.; Fujita, Y.; Yamamoto, T.; Miyake, A.; Tanizawa, O. *Cancer Res.* **1991**, *51*, 5322.
3. Jardines, L.; Weiss, M.; Fowble, B.; Greene, M. *Pathobiology* **1993**, *61*, 268.
4. Hickey, K.; Grehan, D.; Reid, I. M.; O'Brian, S.; Walsh, T. N.; Hennessy, T. P. *Cancer* **1994**, *74*, 1693.
5. Delarue, J. C.; Terrier, P.; Terrier-Lacombe, M. J.; Mouriesse, H.; Gotteland, M.; May-Levin, F. *Bull. Cancer* **1994**, *81*, 1067.
6. Dobrusin, E. M.; Fry, D. W. *Annu. Rep. Med. Chem.* **1992**, *27*, 169.
7. (a) Vansteenkiste, J. F. *Expert Rev. Anticancer Ther.* **2004**, *4*, 139; (b) Tiseo, M.; Loprevite, M.; Ardizzoni, A. *Curr. Med. Chem.: Anti-Cancer Agents* **2004**, *4*, 139.
8. Bonomi, P. *Exper. Opin. Invest. Drugs* **2003**, *12*, 139.
9. Bridges, A. J. *Curr. Med. Chem.* **1999**, *6*, 825.
10. For non-4-anilinoquinazoline EGFR-TK inhibitors, see (a) Bridges, A. J.; Cody, D. R.; Zhou, H.; McMichael, A.; Fry, D. W. *Bioorg. Med. Chem.* **1995**, *3*, 1651; (b) Gibson, K. H.; Grundy, W.; Godfrey, A. A.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Barker, A. J.; Brown, D. S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2723; (c) Bouey-Bencteux, E.; Loison, C.; Pommery, N.; Houssin, R.; Hénichart, J.-P. *Anti-Cancer Drug Des.* **1998**, *13*, 893.
11. Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Conners, R. W.; Bridges, A. J. *Science* **1994**, *265*, 1093.
12. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron lett.* **1975**, *50*, 4467.
13. (a) Barker, A. J. EP 566,226, 1995; (b) Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D. H.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 267.
14. Hennion, G. F.; Hanzel, R. S. *J. Am. Chem. Soc.* **1960**, *82*, 4908.
15. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769; For a review, see Eymery, F.; Iorga, B.; Savignac, P. *Synthesis* **2000**, 185.
16. Frey, H.; Kaupp, G. *Synthesis* **1990**, 931.

17. Imada, Y.; Yuasa, M.; Nakamura, I.; Murahashi, S.-I. *J. Org. Chem.* **1994**, *59*, 2282.
18. Negishi, E.; Takahashi, T.; Van Horn, D. E.; Okukado, N. *J. Am. Chem. Soc.* **1987**, *109*, 2393.
19. TK assay was performed using a partially purified EGFR-TK isolated from human epidermoid carcinoma A431 cells according to the procedure of Pike et al. with minor modification Pike, L. J.; Gallis, B.; Casnellie, J. E.; Bornstein, P.; Krebs, E. G. *Proc. Nat. Acad. Sci. USA* **1982**, *79*, 1433. IC_{50} values are representative of at least two independent experiments.
20. Compound **4d**: Purohit, V. G.; Subramanian, R. *Chem. Ind.* **1978**, 731; Compound **4n**: Ghosez, L.; Cécile, F.; Frédéric, D.; Cuisinier, C.; Touillaux, R. *Can. J. Chem.* **2001**, *79*, 1827; Compound **4n**: Dority, J.A. Jr.; Earley, W.G.; Kumar, V.; Mallamo, J.P.; Miller, M.S.; Subramanyanm, C.U.S. Patent 5,569,655, 1996; Compound **4p**: Kira, M. A.; Aboul-Enein, M. N.; KorKor, M. I. *J. Heterocycl. Chem.* **1970**, *7*, 25; Hamatake, R.K.; Chen, H.; Raney, A.; Allan, M.J.; Lang, S. WO 2006033995.
21. Stamos, J.; Sliwkowski, M. X.; Eigenbrot, C. *J. Biol. Chem.* **2002**, *277*, 46265.
22. The conformational ensemble and the local energy minimization of the poses (~1 kcal/mol) for **2b** and **6b** were performed using the Insight II software (Accelrys Software Inc.).
23. Separation and determination of optical purity were performed using CHIRALPAK AD (Daicel Chemical Industries, Ltd).
24. Re-crystallization from acetone three times and liberation yielded optically active **3g** with 96% ee with a 17% theoretical yield. (*R*)-**3g**: $[\alpha]_D^{18} = -12.7^\circ$ (*c* 1.36, $CHCl_3$).