Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 111–114
Bioorganic & Medicinal Chemistry Letters

Synthesis and SAR of potent EGFR/erbB2 dual inhibitors

Yue-Mei Zhang,[†] Stuart Cockerill,[‡] Stephen B. Guntrip, David Rusnak, Kathryn Smith, Dana Vanderwall, Edgar Wood and Karen Lackey*

GlaxoSmithKline, 5 Moore Drive, Research Triangle Park, NC 27709, USA

Received 28 August 2003; revised 29 September 2003; accepted 4 October 2003

Abstract—A series of 6-alkoxy-4-anilinoquinazoline compounds was prepared and evaluated for in vitro inhibition of the erbB2 and EGFR kinase activity. The IC_{50} values of the best compounds were below 0.10 uM. Further, several of these compounds inhibit the growth of erbB2 and EGFR over-expressing tumor cell lines at concentrations below 1 uM.

Overexpression of the epidermal growth factor receptor family members EGFR (also erbB1) and erbB2 kinases is reported in a variety of human tumors[1] and is associated with poor prognosis.[2] Within the signaling network of the type I tyrosine kinase receptor family, erbB2 plays a central role because it is the preferred hetero-dimerization partner for the other members.[3] Therefore, inhibition of EGFR and erbB2 kinase activity has emerged as a promising new approach to cancer therapy. This was validated by antibody-based therapy[4] (C225, Herceptin™) which is shown to increase mean survival time in metastatic breast cancer patients over-expressing EGFR and erbB2, respectively. Small molecule tyrosine kinase (TK) inhibitors are another class of promising new anticancer drugs[5] and several chemical series such as 4-anilinoquinazolines,[6] 4-anilinopyrido[*d*]pyrimidines,[7] 4-anilinopyrazolo[3,4-*d*]pyrimidines[8] and dianilinophthalimides[9] have been reported as EGFR TK inhibitors. Figure 1 includes some examples in the quinazoline series (Iressa™, Tarceva™, GW572016) that are currently approved drugs or in clinical trials.[10]

Here we wish to report the synthesis and biological activity of a series of dual EGFR and erbB2 TK inhibitors with an ether linker at the 6-position of quinazoline core coupled with a selection of optimal 4-anilino groups. We will describe the in vitro enzyme SAR and highlight a potential binding mode with useful interactions in the ATP binding site. Finally, the cellular activity in relevant tumor lines will be discussed.

Our chemical plan was to prepare a number of quinazoline derivatives with a variety of C-4 anilines and various C-6 ether linkers. Scheme 1 illustrates a six-step synthesis which allowed us to diversify position 6 via the key intermediate **4**. The synthesis and SAR of the 6-position side-chain modifications will initially be described where Ar = 4-benzyloxyaniline (labeled a). The remaining anilines studied are listed in Table 2 and are labeled b–f.

Chlorination of 6-hydroxy-4-quinazolinone **1** was initially attempted using classic conditions such as $POCl_3$

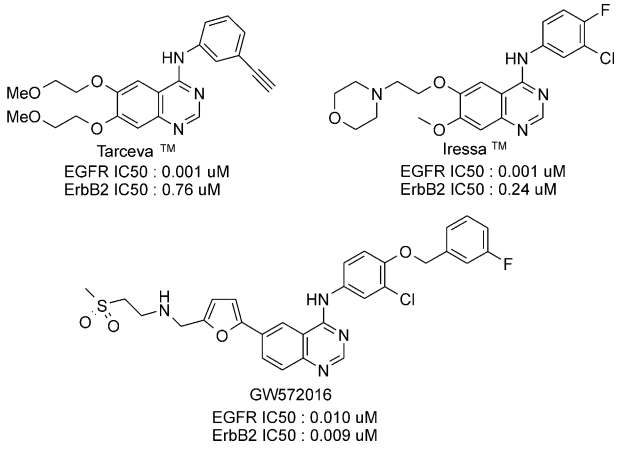
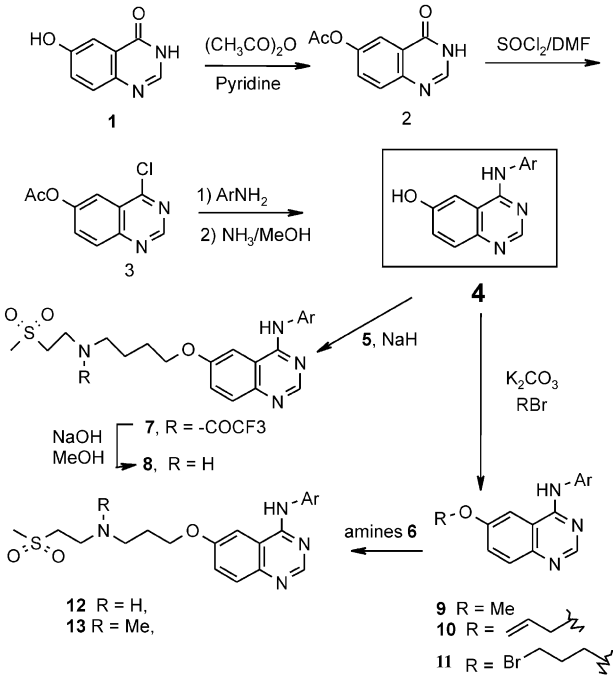


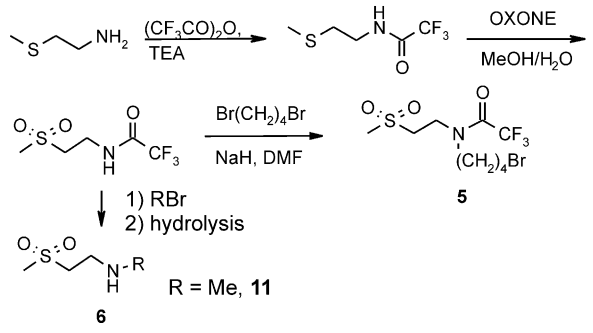
Figure 1. Leading erbB family TK inhibitors.

* Corresponding author. Tel.: +1-919-483-6240; fax: +1-919-315-1092; e-mail: karen.e.lackey@gsk.com
† Current address: J&J Pharm, 1000 Route 202, Raritan, NJ 08869, USA.
‡ Current address: Arrow Therapeutics, 7 Trinity Street, London SE1 1DA, UK.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.10.010



Scheme 1.



Scheme 2.

and $SOCl_2$, but only mixtures containing an unidentified by-product along with the desired product resulted. However, protection of the hydroxy group of quinazolinone **1**[11] and subsequent chlorination with thionyl chloride and DMF gave 4-chloroquinazoline **3** in high yield. Displacement with appropriate anilines and hydrolysis of the acetic ester afforded quinazolines **4**. These intermediates were *O*-alkylated with bromide **5** and then hydrolyzed to give quinazolines **7**. Scheme 2 depicts the synthesis of methylsulfonyl ethylamide **5** (a novel side chain previously developed in our group[12]) and amines **6** via *N*-acylation of methylthioethylamine with TFA anhydride and subsequent oxidation of sulfur with OXONE™, followed by alkylation with 1,4-dibromobutane gave 2-methyl sulfonylethyl trifluoroacetamide **5**.

Alkylation of secondary methylsulfonylethyl-trifluoracetamide with commercially available alkylhalides and basic hydrolysis provided amines **6**. The alternative synthesis for three carbon linker analogues **12** and **13** involved alkylation of key intermediate **4** with alkylhalides or amines **6** with **11**.

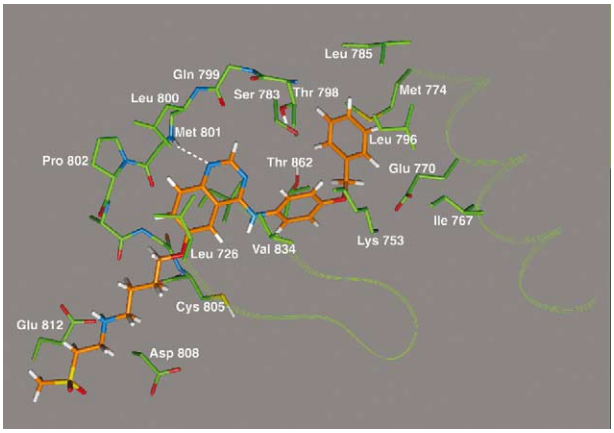


Figure 2. **8a** docked into homology model of erbB2.

1. Biological activity

Exploration of the SAR focused on C6 substitutions and C4 anilino variations to improve both EGFR and erbB2 activities based on results from previous work in the series and molecular modeling studies.[13] The in vitro enzyme assay methods were used as reported in the literature.[13]

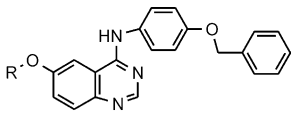
Means values of greater than three experiments are used for enzyme assays of EGFR/erbB2.

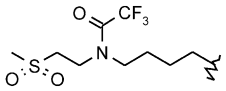
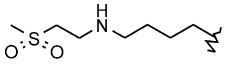
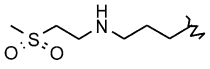
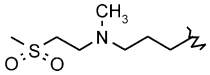
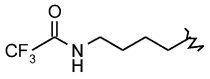
1.1. Ether linker at the 6-position of the quinazolines

The binding mode for the compounds described was proposed based on docking ligands into a homology model for erbB2. In this model, shown in Figure 2, the anilino portion is oriented deep in a hydrophobic region in the back of the active site not occupied by ATP. The 6-ether linker extends out of the ATP pocket into the solvent exposed region at the junction of the C- and N-terminal lobes of the kinase region. This proposal suggests that there is some room for modifications at the C6 position aimed at improving the PK properties and solubility. Therefore, several quinazolines with different ether linkers at C6 were prepared and evaluated for their inhibition of phosphorylation of the isolated EGFR and erbB2 enzymes, respectively (Table 1).

For the compounds not containing a water-solubilizing sulfonyl amine group (**4a**, **9a**, **10a**), the inhibition of erbB2 enzyme activity IC_{50} values fall in a range of 0.25–0.35 uM and for EGFR in a range of 0.068–0.098 uM. However, *N,N,N*-trisubstitution (both electron withdrawing TFA group and electron donating methyl group) on the side chain (**7a** and **13a**) decreased both EGFR and erbB2 enzyme potency 3.5- to 10-fold compared to *N,N*-disubstituted compounds (**8a**, **12a**, **14a**). In addition, erbB2 activity may be influenced by the linker length. A modest improvement (~2.4-fold) can be observed in the 4 carbon linker over the three-carbon linker. Several possibilities for specific hydrogen bonds with the inhibitor are suggested, but are speculative due to uncertainty in the protein conformation in this region. The best compound **8a** in Table 1 provided an excellent dual inhibitor enzyme profile (IC_{50} EGFR: 0.074 uM, erbB2: 0.095 uM).

Table 1. Enzyme inhibitory activity of quinazolines with various C6 substituents



Compd	R	Enzyme assay IC_{50}, nM[a]	
		EGFR	ErbB2
4a	H	98	263
7a		263	977
8a		74	95
9a	CH_3	68	309
10a		79	347
12a		104	234
13a		830	1778
14a		74	117

Mean values of greater than three experiments are used for enzyme assays of EGFR/erbB2.

[a] IC_{50} values are generated by measuring inhibition of phosphorylation of a peptide substrate added to enzyme reaction.[14]

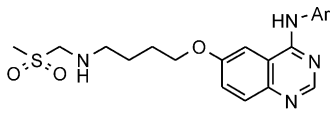
1.2. Anilino ring at the 4-position of quinazolines

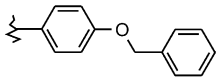
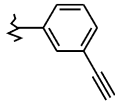
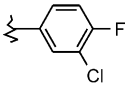
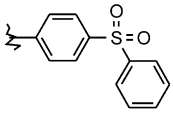
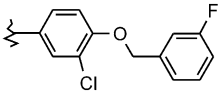
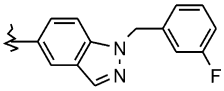
Given the proposed binding mode, it is readily apparent that the target affinity could be modulated to a greater extent via the 4-anilino substitutions. Therefore, the most potent side chain with a four-carbon linker was retained at the C-6 position combined variety of anilines. For comparison, 3-ethynylaniline and 3-chloro-4-fluoroaniline were introduced, which were reported to afford potent EGFR inhibitory activity.[10] The results are shown in Table 2.

All compounds in Table 2 showed relatively potent EGFR inhibition, of which 3-ethynylaniline appears to be the best EGFR inhibitor. Bicyclic anilines containing a benzyl group greatly improved erbB2 inhibition. Replacement of 4′-fluorine in **8c** (210 nM) with benzyloxy group in **8e** (10 nM) increased erbB2 potency 21 fold. The utility of small lipophilic groups in the 3′-position was reported for EGFR inhibition.[13] In our case, 3′-substitution also seems important for erbB2 inhibition (**8a**, 95 nM vs **8e**, 10 nM). The fusion of 3′- and 4′-substitutuents in the N1-benzylindazole **8f** retained excellent dual potency.

Although multiple binding modes in the ATP site are possible as substitutes change on kinase inhibitors, the

Table 2. Enzyme inhibitory activity of quinazolines with various C4 anilines



Compd	Ar	Enzyme assay IC_{50}, nM	
		EGFR	ErbB2
8a		74	95
8b		11	234
8c		20	210
8d		93	147
8e		27	10
8f		24	19

erbB2 inhibition data suggests that the anilino portion may play a strong role in determining the potency and selectivity of the quinazoline series. This observation supports our proposed binding mode in which the large substituted benzyloxyanilino group occupies the back pocket.

1.3. Cellular efficacy of EGFR/erbB2 dual inhibitors

Representative examples of cellular activity are shown in Table 3. The cellular assay methods were used as reported in the literature.[14] Consistent with the erbB family inhibition profile, compound **8b** and **8c** inhibit the proliferation of the HN5 cell line overexpressing EGFR, but are less active in a BT474 cell line overexpressing

Table 3. Cellular efficacy of EGFR/erbB2 dual inhibitors

Compd	Tumor cell IC_{50}, μM[a]		Cellular selectivity[b] HFF/avge tumor
	HN5	BT474	
8b	0.045	1.68	>35×
8c	0.071	1.38	>42×
8f	0.63	0.39	>59×

[a] Cell line BT474 (breast carcinoma) overexpresses erbB2; cell line NH5 (head/neck carcinoma) overexpresses EGFR.

[b] The value for cellular selectivity is calculated by dividing the IC_{50} value for the normal fibroblasts (not shown) by the average tumor cell IC_{50} value.

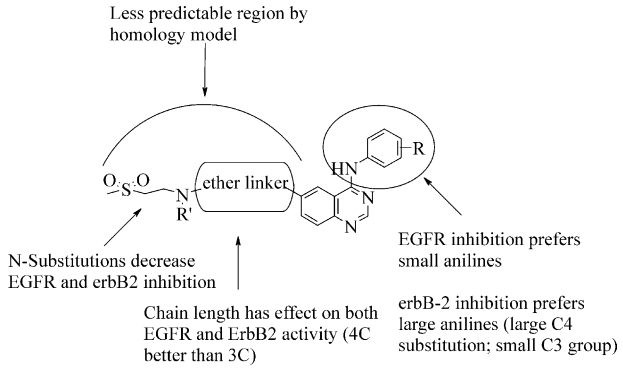


Figure 3. SAR summary.

erbB2. Compound **8f** showed potent EGFR/erbB2 inhibitory activity as well as excellent in-vitro efficacy (avge $IC_{50} = 0.54$ uM) against tumor cell lines. The cellular selectivity (normal cell vs tumor cell lines) also exceeded 50-fold. Very little difference was observed in the cellular assay systems between the trifluoracetylated side chains versus. the secondary amine comparators. Other compounds in the series, for example the three carbon linked ethers, appeared significantly less attractive in terms of cellular efficacy.

2. Conclusions

A series of 6-alkoxy-4-anilino as erbB2/EGFR TK inhibitors quinazolines were prepared via two six-step synthetic routes with a common intermediate **4** and assessed for their biological activity. The SAR of the 4-anilino portion and the 6-position was discussed and is summarized in Figure 3. A binding mode of these compounds docked into an erbB2 horology model is helpful for explaining the SAR of 4-aniline but less predictable at the 6-ether linker region.

References and notes

1. Aaronson, S. A. *Science* **1991**, *254*, 1146. Hung, M.; Lau, Y. *Semin. Oncol.* **1999**, *26*, 51.
2. Kersemaekers, A. M. F.; Fleuren, G. J.; Kenter, E. G.; Van den Broek, L. J. C. M.; Uljee, S. M.; Hermans, J.; Van de Vijver, M. *J. Clin. Cancer Res.* **1999**, *5*, 577.
3. Earp, H. S.; Dawson, T. L.; Li, X.; Yu, H. *Breast Cancer Res. Treat.* **1995**, *35*, 115. GRAU-Porta, D.; Beerli, R. R.; Daly, J. M.; Hynes, N. E. *EMBO J.* **1997**, *16*, 1647.
4. For Herceptin: Baselga, J.; Tripathy, D.; Mendelsohn, J.; Baughman, S.; Benz, C. C.; Dantis, L.; Sklarin, N. T.; Seidman, A. D.; Hudis, C. A.; Moore, J.; Rosen, P. P.; Twaddell, T.; Henderson, I. C.; Norton, L. *J. Clin. Oncol.* **1996**, *14*, 737. For C225: Waksal, H. W. *Cancer Metastasis Rev.* **2000**, *18*, 427.
5. Traxler, P. M. *Exp. Opin. Ther. Pat.* **1997**, *7*, 571. Traxler, P. M. *Exp. Opin. Ther. Pat.* **1998**, *8*, 1599.
6. Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E.; Showalter, H. D. H.; Vincent, P. W.; Elliott, W. L.; Denny, W. A. *J. Med. Chem.* **2000**, *43*, 1380, and references cited therein.
7. Smaill, J. B.; Palmer, B. D.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Bridges, A. J.; Zhou, H.; Showalter, H. D. H.; Winters, R. T.; Leopold, W. R.; Fry, D. W.; Nelson, J. M.; Slintak, V.; Elliott, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. *J. Med. Chem.* **1999**, *42*, 1803, and references cited therein.
8. Traxeler, P.; Bold, G.; Frei, J.; Lang, M.; Lydon, N.; Mett, H.; Buchdunger, E.; Meyer, T.; Mueller, M.; Furet, P. *J. Med. Chem.* **1997**, *40*, 3601, and references cited therein.
9. Trinks, U.; Buchdunger, E.; Furet, P.; Kump, W.; Mett, H.; Meyer, Th.; Muller, M.; Regenass, U.; Rihs, G.; Lydon, N. B.; Tralexer, P. *J. Med. Chem.* **1994**, *37*, 1015.
10. Iwata, K.; Miller, P. E.; Barbacci, E. G.; Arnold, L.; Doty, J.; Diorio, C. I.; Pustilnik, L. R.; Reynolds, M.; Thelemann, A.; Sloan, D.; Moyer, J. D. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, 633 (abstract 4248). Woodburn, J. R.; Barker, A. J.; Gibson, K. H.; Ashton, S. E.; Wakeling, A. E.; Curry, B. J.; Scarlett, L.; Henthorn, L. R. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, 633. Spector, N.; Raefsky, E.; Hurwitz, H.; Hensing, T.; Dowlati, A.; Dees, C.; O'Neil, B.; Koch, K.; Smith, D. A.; Mangum, S.; Burris, H. A. Safety, Clinical Efficacy, and Biologic Assessments from EGF10004: A Randomized Phase Ib Study of GW572016 for Patients with Metastatic Carcinomas Overexpressing EGFR or ErbB2. ASCO 2003. *Proc. Am. Soc. Clin. Oncol.* 22: page 193, 2003 (abstract 772).
11. Maillard, J.; Benard, M.; Vincent, M.; Vo-Van-Tri; Jolly, R.; Morin, R.; Benharkate, M.; Menillet, C. *Chim. Ther.* **1967**, *2*, 231.
12. Carter, M. C.; Cockerill, G. S.; Guntrip, S. B.; Lackey, K. E.; Smith, K. J. WO 9935146 A1.
13. Brignola, P. S.; Lackey, K.; Kadwell, S. H.; Hoffman, C.; Horne, E.; Carter, H. L.; Stuart, J. D.; Blackburn, K.; Moyer, M. B.; Alligood, K. J.; Knight, W. B.; Wood, E. R. *J. Biol. Chem.* **2002**, *277*, 1576.
14. Rusnak, D. W.; Lackey, K.; Affleck, K.; Wood, E. R.; Alligood, K. J.; Rhodes, N. R.; Keith, B.; Murray, D. M.; Mullin, R. J.; Knight, W. B.; Gilmer, T. M. *Mol. Cancer Ther.* **2001**, *1*, 85.