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Discovery and Biological Evaluation of Potent Dual ErbB-2/EGFR Tyrosine Kinase Inhibitors: 6-Thiazolylquinazolines

Micheal D. Gaul,[†] Yu Guo, Karen Affleck, G. Stuart Cockerill,[‡] Tona M. Gilmer, Robert J. Griffin, Stephen Guntrip, Barry R. Keith, Wilson B. Knight, Robert J. Mullin, Doris M. Murray, David W. Rusnak, Kathryn Smith, Sarva Tadepalli, Edgar R. Wood and Karen Lackey*

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

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Abstract—We have identified a novel class of 6-thiazolylquinazolines as potent and selective inhibitors of both ErbB-2 and EGFR tyrosine kinase activity, with IC_{50} values in the nanomolar range. These compounds inhibited the growth of both EGFR (HN5) and ErbB-2 (BT474) over-expressing human tumor cell lines in vitro. Using xenograft models of the same cell lines, we found that the compounds given orally inhibited in vivo tumor growth significantly compared with control animals.

Epidermal growth factor receptor (EGFR) and ErbB-2 are members of the Type I receptor tyrosine kinase (TK) family (also known as the HER or ErbB family). Over-expression of these receptors is found in a number of cancers (e.g., breast, ovarian, colon, prostate) and has been associated with poor prognosis in patients.[1,2] Recent success in the clinical evaluation of TK inhibitors (Fig. 1) strongly suggests that these targets represent drug intervention opportunities.[3] Excellent descriptions of the involvement of erbB family proteins in cell physiology and disease applications have been published by Adams and Yarden.[4,5] There are also recent reviews that cover Type I Receptor TK inhibitors.[6,7]

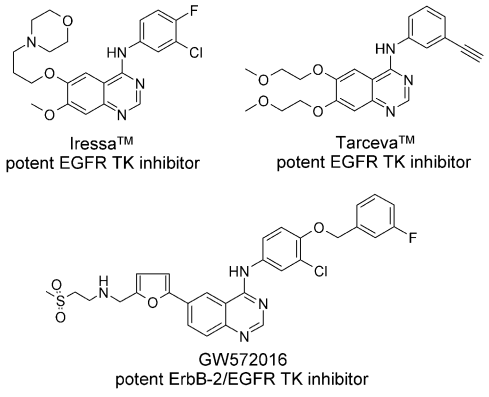

Figure 1. Examples of ErbB family TK inhibitors currently in clinical trials for anti-cancer therapy.

Type I receptors function as ligand receptors in a matrix of hetero- and homo-dimers. The greatest over amplification of signal is observed with erbB-2 heterodimers and this heterodimerization of the erbB-2 receptor may be the preferred initiating event for signaling with this receptor.[2,5] There appears to be a strong hierarchy of receptor dimerization, with those involving erbB-2 as the most stable and preferred.[2,5] For these reasons, we designed molecules to specifically target both erbB-2 and EGFR TK inhibition.

Because small substitution changes in kinase inhibitors greatly affect the kinase inhibition and drug properties, a series of compounds were generated to investigate the

*Corresponding author. Fax: + 1-919-315-1092; e-mail: karen.e.lackey@gsk.com
[†]Current address: 3-Dimensional Pharmaceuticals, 8 Clarke Drive, Cranbury, NJ 08512, USA.
[‡]Current address: Arrow Therapeutics, Britannia House, 7 Trinity Street, London SE1 1DS, UK.

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structure–activity relationships (SARs) of the 6-thiazolylquinazoline series. We report several dual erbB-2/EGFR TK inhibitors that possess efficacy in pre-clinical models for cancer and a facile synthetic route to obtain these compounds.

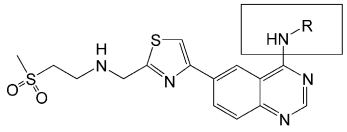
A straightforward five step synthetic route was used to generate 6-thiazolylquinazoline derivatives and is depicted in Figure 2. Representative 4-anilino groups used in step 1 are listed in Table 1 and the synthesis of the thioamide reagent in step 4 is shown in Figure 3.

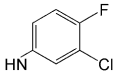
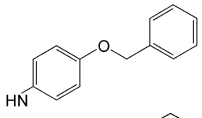
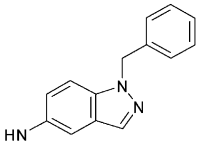
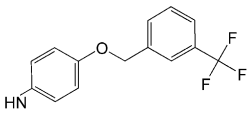
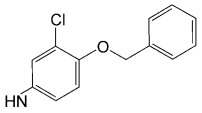
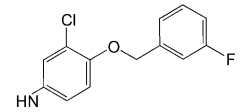
Treatment of 4-chloro-6-iodoquinazoline[8] with the appropriately substituted anilines in warm isopropanol afforded the 4-anilino-6-iodoquinazoline in high yields (>80%) using a simple filtration isolation. Stille coupling of the 4-anilino substituted iodoquinazoline with 1-ethoxy-1-vinyltributyltin gave the desired 6-(1-ethoxyvinyl)-quinazoline in 75% yield. Treatment of the 6-ethoxyvinylquinazoline with *N*-bromosuccinimide in dichloromethane followed by an immediate Hantzsch reaction[9] of the unstable α-bromoketone intermediate with the appropriate thioamide produced the substituted 6-(2,4-thiazole)-quinazoline in moderate (15–40%) yields. Hydrolysis of the trifluoroacetamide from the aminomethyl thiazole side chain with NaOH/MeOH and subsequent treatment with anhydrous HCl provided the desired final products as hydrochloride salts in good (70–80%) yields.

The tolerance for substitution in the 6-position of the quinazoline was generally large for potent enzyme inhibition, so we used cellular activity to develop the SAR to determine the preferred substitutions. Work in our laboratory had demonstrated that one of the best 6-position side chain substitutions for providing optimal cellular activity was achieved when the 2-(methylsulfonyl)ethyl amino group was the preferred side chain substitution linked via a methylene unit to a heterocyclic ring (Fig. 1, GW572016).[10] The synthetic route to the fully functionalized thioamide for use in the condensation reaction to generate the thiazole is shown in Figure 3. Beginning with the commercially available methylthioethylamine, an alkylation with chloroacetonitrile followed by acetylation with trifluoroacetic anhydride afforded the trifluoroacetamide in near quantitative yield. Oxidation with potassium peroxymonosulfate produced the sulfonyl compound, which was in turn converted to the thioamide by passing through hydrogen sulfide in the presence of triethylamine. This thioamide was then utilized in step 4 of the synthetic route illustrated in Figure 2.

The quinazoline scaffold provides the necessary binding properties for inhibition of the erbB family of tyrosine kinases.[7] The IC_{50} values for enzyme activity were generated by measuring the inhibition of the phosphorylation of a peptide substrate.[11] Smaller aniline

Table 1. Catalytic enzyme assay results of 6-thiazolylquinazoline aniline derivatives



Entry	Aniline group	ErbB-2 IC_{50}, μM[a]	EGFR IC_{50}, μM[a]
1		0.2 ±0.11	0.035 ±0.022
2		0.048 ±0.015	0.082 ±0.053
3		0.028 ±0.011	0.071 ±0.030
4		0.55 ±0.11	0.54 ±0.32
5		0.014 ±0.0016	0.008 ±0.0010
6		0.014 ±0.0002	0.01 ±0.0003

[a]Average values $n \geq 3$.

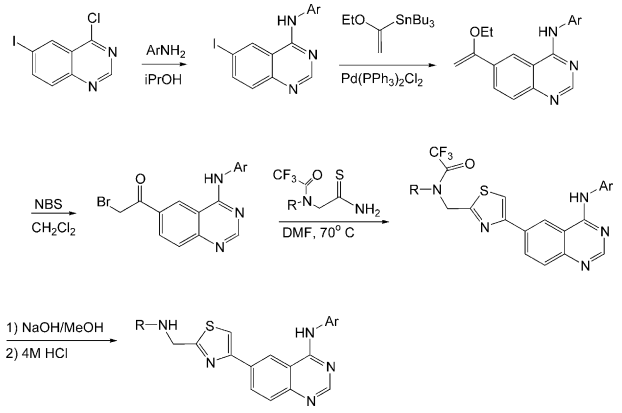


Figure 2. Synthetic route to 6-thiazolylquinazoline derivatives.

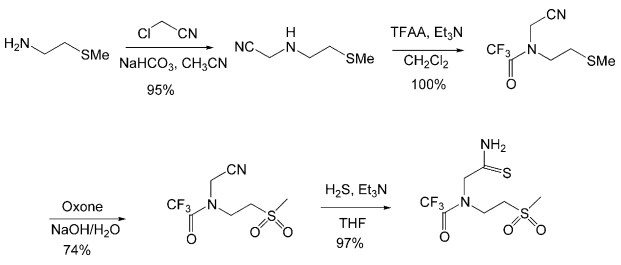


Figure 3. Synthetic route to the thioamide side chain.

substitutions generally provide compounds with potent EGFR TK inhibition, while larger aniline substitutions generally confer greater dual erbB-2 and EGFR tyrosine kinase inhibition. Using a mix and match strategy for lead optimization, the 'best' anilines for dual inhibition were combined with the optimized side chain. The results are listed in Table 1.

The choice of cell lines for evaluating these compounds as dual inhibitors was very important. The control cell line that was used was HFF, a normal human foreskin fibroblast and the desired compound profile should not inhibit the proliferation of these cells. A head and neck carcinoma cell line, HN5, which overexpresses EGFR, was used to determine the effectiveness of the EGFR TK inhibitory properties. Both N87, a gastric carcinoma cell line, and BT474, a breast carcinoma cell line, overexpress erbB-2 and should be potently inhibited by a dual erbB-2/EGFR TK inhibitor. Further, we used the same tumor cell lines in subcutaneous (sc) human xenograft murine models to evaluate the pre-clinical anti-tumor activity.

A good correlation is observed between the catalytic enzyme activity profile and the cellular efficacy of these derivatives (Table 2). For example, **1** is a potent EGFR TK inhibitor and it is relatively selective for the HN5 tumor line over the normal cell line and the lines over-expressing erbB-2 (N87 and BT474). Excellent growth inhibitory activity is observed in cell lines over-expressing EGFR (HN5) and erbB-2 (BT474 and N87) with dual inhibitors such as **3**, **5**, and **6**. The best overall cellular activity is observed with compounds **3** (*N*-1-benzylindazole) and **6** (4-[3-fluorobenzyloxy]-3-chloroaniline).

Compounds were progressed for further study provided that the IC_{50} values for cellular efficacy were below 300 nM and the selectivity ratio exceeded 20-fold tumor versus normal cells. The tabulated average dual enzyme inhibition values and averaged tumor cell line values are illustrated in Table 3. Since **1** would not be expected to function as a dual inhibitor, it does not meet the criteria for progression. However, the most promising 6-thiazolylquinazoline compounds (**3**, **5**, **6**) exceed 100-fold selectivity for tumor cells over normal cells.

Pharmacokinetic parameters were generated in female CD-1 mice ($n=2$ per time point), treated with a single IV or oral dose of compound at 10 mg/kg. The data are compiled in Table 4 and demonstrate a range of plasma exposures for these compounds with oral bioavailability ranging from 11 to 67%. The compounds were also administered orally at 100 mg/kg BID for 21 days in the sc xenograft studies of both an erbB-2 and EGFR over-expressing human tumor cell lines. The tumor inhibition, which was recorded for the final day of the study, is listed in Table 4.[12] The data illustrate that compound **6** was the most efficacious compound in this 6-thiazoylquinazoline series, displaying approximately 80% tumor inhibition in both the HN5 and BT474 xenograft models as further illustrated in Figures 4 and 5. It is noteworthy that the 6-thiazole substitution generally afforded better mouse oral exposures than the 6-furyl series that led to the discovery of GW572016.[13]

Table 2. Cellular activity results for a representative normal line (HFF) and tumor lines

Entry	HFF[a]	HN5[b]	N87[c]	BT474[d]
1	>10	0.57±0.14	4.4±0.26	2.4±3.3
2	>18	1.6±0.4	1.5±0.3	0.86±0.5
3	12	0.11±0.01	0.09±0.01	0.06±0.00
4[e]	>30	18	7.4	15
5	>30	0.23±0.02	0.36±0.12	0.28±0.11
6	>23	0.08±0.01	0.13±0.03	0.11±0.04

IC_{50} values reported as μM concentrations.
[a]Human foreskin fibroblasts.
[b]Head and neck tumor line over-expressing EGFR.
[c]Gastric tumor line over-expressing erbB-2.
[d]Breast tumor line over-expressing erbB-2.
[e]$n=1$.

Table 3. Averaged enzyme values, average tumor cell line values, and selectivity ratio

Entry	EGFR and ErbB-2 TK dual inhibition average IC_{50}, μM	Tumor cell lines average IC_{50}, μM	HFF average IC_{50}, μM	Selectivity[a] X-fold difference
1	0.054	2.45	>10	>4
2	0.028	1.32	>30	>14
3	0.012	0.089	12	135
4	0.32	7.4	>30	>4
5	0.011	0.29	>30	>105
6	0.012	0.11	>30	>210

[a]Ratio of the growth inhibitory IC_{50} values of normal cell line (HFF) to the average of the three tumor cell lines.

Table 4. Representative murine in vivo data

No.	IV AUC	PO AUC	F (%)	%TI[a] (HN5)[b]	%TI[a] (BT474)[c]
1	nd	nd	nd	55	nd
2	11,308	7534	66.6	43	33
3	2489	274	11	65	0
5	14,722	2439	16.6	84	59
6	19,040	7520	39.5	81	83

nd, no data.
[a]%TI = tumor inhibition.
[b]Head and neck tumor line.
[c]Breast tumor line.

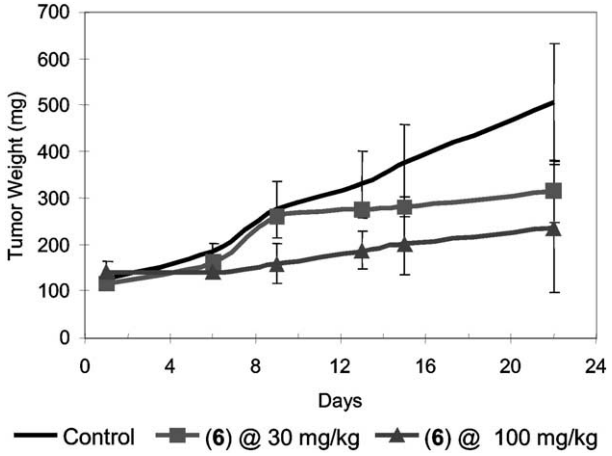


Figure 4. Inhibition of HN5 xenograft growth by **6**.

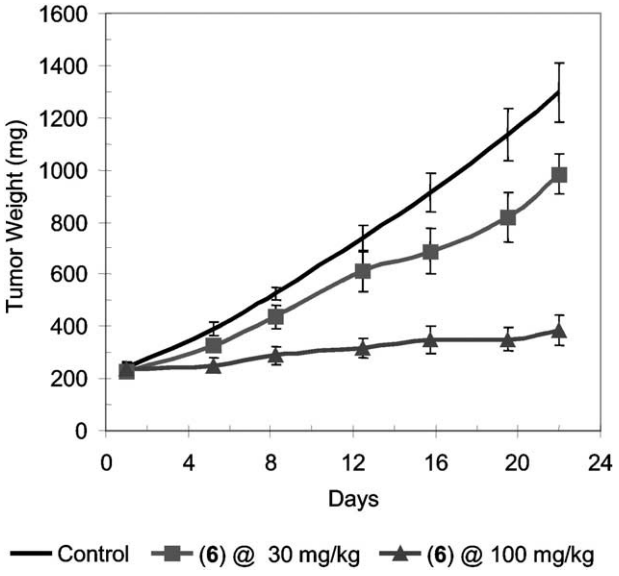


Figure 5. Inhibition of BT474 xenograft growth by **6**.

The data for tumor weights measured over the treatment period are shown graphically for **6,** one of the most efficacious compounds in this series, in both the erbB-2 and EGFR over-expressing lines. The control line represents a vehicle treated group of animals and approximately two tumor size doublings occur during the treatment period. A dose response can be seen in both models for a 0, 30, 100 mg/kg BID po of compound **6**. Lack of significant body weight loss (data not shown) during treatment with **6** in the subcutaneous in vivo xenograft mouse model suggests a good therapeutic index.

The quinazoline scaffold provides the necessary binding properties for inhibition of the ErbB family of tyrosine kinases. A series of 6-thiazolyl-quinazolines were discussed and the results of extensive SAR investigations were summarized using representative examples. The compounds possessed excellent in vitro tyrosine kinase inhibition as well as selective tumor cell-based activity. Furthermore, the compounds provided an acceptable pharmacokinetic profile to allow for oral dosing in a series of subcutaneous in vivo xenograft models. All of these studies, taken together, resulted in the discovery of compound **6**, which showed good anti-tumor activity with a lack of significant body weight loss during treatment, suggesting a good therapeutic index for this compound. Dual inhibition of EGFR and ErbB-2 may offer increased activity over agents which target only one of these receptor kinases. Our results suggest that these dual inhibitors of ErbB-2/EGFR tyrosine kinases have the potential for providing therapeutic benefit across a broad range of tumors.

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12. Cells were cultured in RPMI 1640 + 10% fetal bovine serum, sodium pyruvate and L-glutamine, at 37 °C in a 95/5 air/CO_2 atmosphere. HN5 tumors were initiated by injection of the cell suspension subcutaneously in the axillary region. BT474 tumors were initiated by injection of the tumor fragments subcutaneously in the axillary region. Solid tumors were measured by electronic caliper measurement. Tumor weights were calculated with the equation Tumor weight (mg) = (tumor length (mm) × tumor width $(mm)^2/2$. Compounds were administered po using a 10% sulfobutyl ether beta cyclodextrin formulation. These studies were performed under under a protocol approved by the institutional animal care committee.
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