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# Optimization and SAR for dual ErbB-1/ErbB-2 tyrosine kinase inhibition in the 6-furanylquinazoline series

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**Abstract**—Synthetic modifications on a 6-furanylquinazoline scaffold to optimize the dual ErbB-1/ErbB-2 tyrosine kinase inhibition afforded consistent SAR whereby a 4-(3-fluorobenzyloxy)-3-haloanilino provided the best enzyme potency and cellular selectivity. Changes made to the 6-furanyl group had little impact on the enzyme activity, but appeared to dramatically affect the cellular efficacy. The discovery of lapatinib emerged from this work.


The Type I receptors represent an ideal point for drug intervention in cancer therapy due to aberrant regulation in certain solid tumors and correlation with disease progression and outcome.[2] The quinazoline scaffold features in several well-known examples of potent ErbB-1 (EGFR) tyrosine kinase (TK) inhibitors that are currently in clinical trials or are used in anti-cancer therapy.[3] Several reviews have been published covering these and other small molecule Type 1 receptor inhibitors and provide excellent information regarding the current state of the art in this class of drug candidates.[4,5] Our medicinal chemistry lead optimization efforts in the quinazoline series to produce a dual ErbB-1/ErbB-2 inhibitor led to the discovery of lapatinib (GW572016), which is currently in clinical trials.[6] The structure is shown in Figure 1.

Many compounds were synthesized in the 6-furanyl quinazoline series using facile syntheses to investigate the structure–activity relationships (SAR) for dual inhibition of ErbB-1/ErbB-2 focused on the ATP binding region of the protein. Herein we will describe the synthesis, kinase enzyme inhibition, and cellular activity of this important series of compounds.

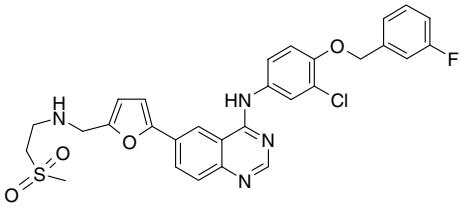

**Figure 1.** Lapatinib.

The general strategy for synthetic modifications of the core 6-furanyl quinazoline to establish the SAR is summarized in Scheme 1. The orientation of the substituents on the furan ring was found to dramatically affect the cellular activity (discussed below), so most of our work focused on the 5-substituted-2-furanylquinazoline series. The introduction of a side chain via a facile reductive amination provided an excellent scaffold to optimize the cellular activity of the series with modifications in chain length, heteroatom linker, and terminal R-group changes. This chemistry discussion will cover the general synthesis, and the generation of a focused set of anilines and selected amines used to optimize dual ErbB-1 and ErbB-2 TK inhibition.

Beginning with commercially available 4-chloro-6-iodoquinazoline, the desired compounds can be synthesized

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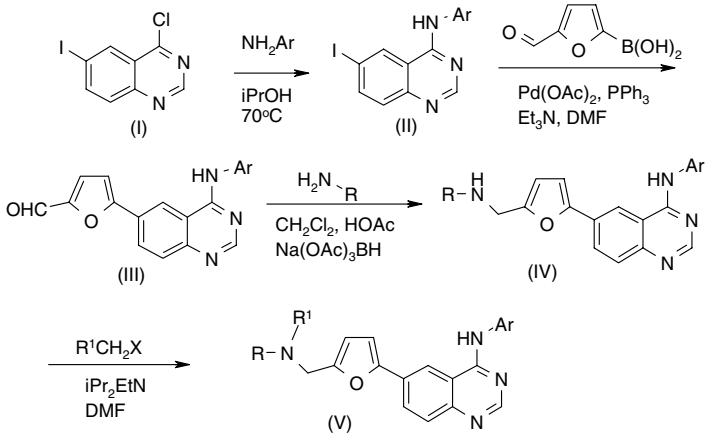
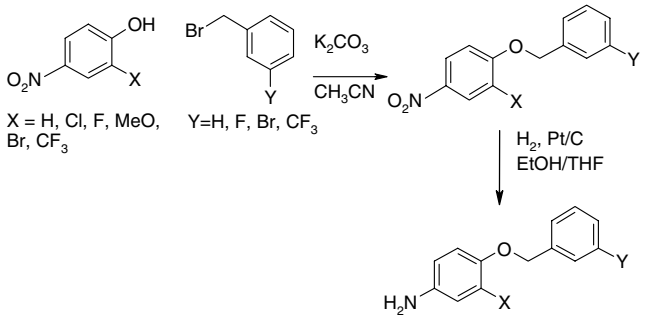



**Scheme 1.** General synthetic route for 4-anilino-6-furanylquinazoline derivatives.

in three convergent steps with the capability to build in highly functionalized groups at each step as shown in Scheme 1. Optimization work was necessary to improve the yield and reproducibility of the palladium coupling reaction to afford analogs of (III) in sufficient scale for in vivo evaluation studies.[7,8] The furylaldehyde moiety could easily be functionalized to include substitutions designed to optimize cellular activity.

Previous research at GlaxoSmithKline had demonstrated the need for large anilino substitutions to retain both ErbB-1 and ErbB-2 inhibition activity.[9] Since the most potent dual enzyme inhibition could be obtained when the aryl group was phenylsulfonylphenyl, *N*1-benzylindazolyl, and benzyloxyphenyl, we focused our optimization efforts on these anilines or further functionalized versions of these anilines.[10] The most interesting SAR trends were found in the benzyloxyaniline substituted series and are summarized in this report. In-house data had demonstrated that the optimal substitution pattern involved a *para*-benzyloxy group.[11] Compounds with substitutions in the 2- and 6-position of the aniline ring had diminished ErbB family enzyme inhibition, so our work focused only on combining 3- and 4-position substituents shown in Scheme 2. These anilines were synthesized by alkylating the appropriately substituted nitrophenol followed by a platinum or palladium catalyzed hydrogenation. Care must be taken to use less than 1% catalyst due to the exothermic nature of some of the reactions.

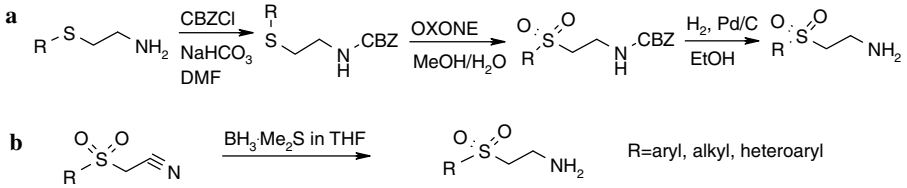



**Scheme 2.** Benzyloxyaniline building blocks used for dual ErbB-1/2 inhibition SAR.

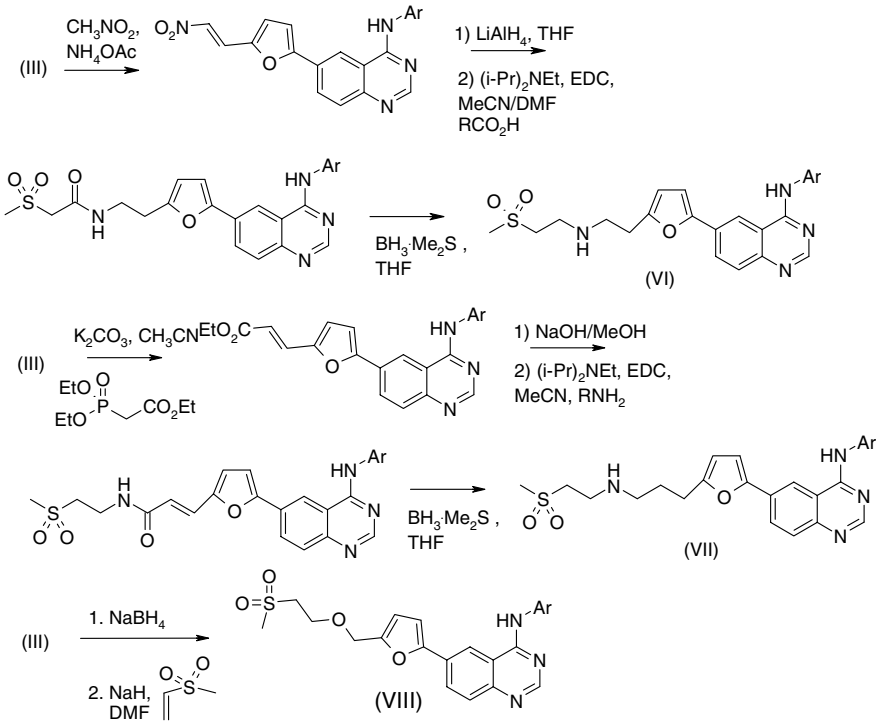
While the reductive amination step was amenable to a variety of amines, the data suggested that basic amines were much less active in the cellular assays (unpublished data). The preferred amines were substituted sulfonylethyl amines and thiomorpholine oxide to form (IV). Thiomorpholine was easily converted to thiomorpholine oxide using sodium periodate in water.[12] There were two methods for generating the substituted sulfonylethylamines, selected largely on the basis of available starting materials.[13] In Scheme 3a, the longer route is shown beginning with a substituted thioethylamino reagent that must first be *N*-protected followed by oxidation. Upon removal of the protecting group, the amine was then used in the reductive amination reactions with the furanylaldehyde quinazoline intermediate (III). The second sequence shown in Scheme 3b begins with commercially available sulfonylacetonitriles. In a single-step reduction, the substituted sulfonylethylamine is generated.

To generate compounds with modifications of the sulfonylethylamine side chain, homologations and ether linkages were examined. The representative syntheses are shown in Scheme 4 each beginning with the advanced furanylquinazoline intermediate (III). To form the homolygated compound (VI), a nitrovinyl group was installed, then subsequently reduced to the aminoethyl. Standard amide coupling conditions are used to form the amide intermediate prior to the reduction. To generate the 3-carbon linked side chain (VII), a four-step synthetic route starting with (III) was used. A vinyl ester was installed, hydrolyzed to the carboxylic acid, subjected to standard amide coupling, and then reduction conditions applied. The final reaction sequence described in Scheme 4 is the conversion of (III) to the ether linked series (VIII) by reducing the aldehyde to the hydroxymethyl group followed by treatment with vinyl sulfone.

Inhibition of ErbB-1 and ErbB-2 tyrosine kinase activity was evaluated in enzyme assays using the purified recombinant human intracellular domain of each receptor. The expression, purification, and assay methods were conducted as described by Brignola and co-workers using the scintillation proximity assay detection method.[14] The average standard deviation of the mean




**Scheme 3.** Substituted sulfonylethylamine or ether functional groups.



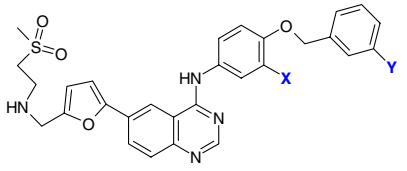

**Scheme 4.** Synthesis of furan side-chain substitutions on the 4-anilinoquinazoline scaffold.

for the enzyme results described in Tables 1–5 is equal to 0.2 units of the log of the $IC_{50}$ value (ErbB-1 and ErbB-2).

The cellular assay data shown in Tables 1–5 are included to demonstrate the efficacy of the substituted quinazolines in tumor lines driven by the ErbB family targets. A thorough explanation of the assay system and the methods used are included in published work by Rusnak and co-workers.[10] HN5, derived from a head and neck cancer line, over-expresses ErbB-1.[15] BT474, a breast line, over-expresses ErbB-2.[16] While N87, a gastric tumor line, over-expresses both receptors, ErbB-2 is present at significantly greater levels.[10] The $IC_{50}$ values listed in the tables are the average of 3 or more determinations where the cells were treated with compound for 72 h. Effective dual ErbB-1 and ErbB-2 tyrosine kinase inhibitors should be efficacious in all three cell lines. We observed distinct SAR in the correlation between the kinase enzyme potency and cellular activity.

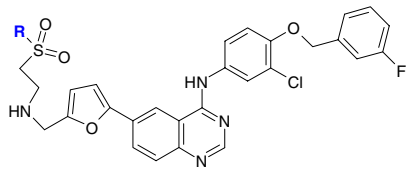
Well over 1000 compounds were generated with the 4-benzyloxyaniline group or a substituted analog thereof on a quinazoline core, it was determined that the 3-anilino (X) and 3-benzyloxy (Y) positions were optimal for dual ErbB-1 and ErbB-2 inhibition designated in the structure for Table 1. Illustrative examples have been selected for this report to demonstrate the SAR. Using

**Table 1.** Type I receptor inhibition activity and cellular efficacy results for representative examples of the modified benzyloxyaniline analogs



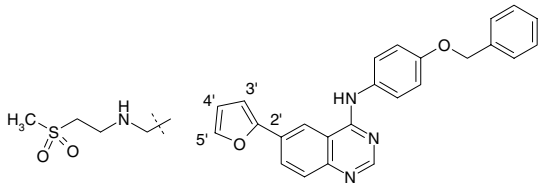

| Compound | X | Y | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|---|
| | | | ErbB-2 | ErbB-1 | HN5 | BT474 | N87 |
| **1** | Cl | F | 0.010 | 0.012 | 0.12 | 0.08 | 0.08 |
| **2** | H | H | 0.026 | 0.027 | 0.65 | 0.28 | 0.87 |
| **3** | H | F | 0.031 | 0.024 | 0.84 | 0.77 | 0.86 |
| **4** | H | Br | 0.10 | 0.043 | 2.56 | 2.13 | 3.46 |
| **5** | H | $CF_3$ | 0.36 | 0.091 | 6.9 | 4.2 | 5.2 |
| **6** | Cl | H | 0.022 | 0.018 | 0.25 | 0.25 | 0.28 |
| **7** | Br | H | 0.025 | 0.025 | 0.26 | 0.27 | 0.37 |
| **8** | MeO | F | 0.09 | 0.10 | 3.33 | 2.07 | 1.90 |
| **9** | $CF_3$ | H | 0.20 | 0.16 | 5.90 | 10.14 | 6.50 |
| **10** | $CF_3$ | F | 0.22 | 0.08 | 1.94 | 4.49 | 2.89 |

**Table 2.** Type I receptor inhibition activity and cellular efficacy results for representative examples of terminal side-chain modifications



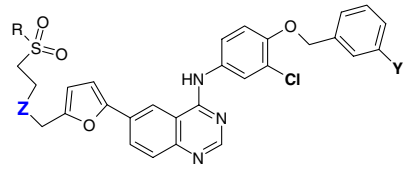
| Compound | R | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | ErbB-2 | ErbB-1 | HN5 | BT474 | N87 |
| **1** | $-CH_3$ | 0.010 | 0.022 | 0.12 | 0.08 | 0.08 |
| **11** | *n*-Pr | 0.026 | 0.021 | 1.6 | 3.14 | 8.66 |
| **12** | *i*-Pr | 0.024 | 0.018 | 0.28 | 0.20 | 0.13 |
| **13** | –Ph | 0.030 | 0.019 | 1.07 | 1.04 | 0.87 |
| **14** | 2-Pyridyl | 0.030 | 0.019 | 1.55 | 0.97 | 0.79 |
| **15** |  | 0.026 | 0.017 | 0.50 | 0.39 | 0.23 |

**Table 3.** Type I receptor inhibition activity and cellular efficacy results for regiochemical analogs of **2**



| Compound | Position | $IC_{50}$ (μM) | |
|---|---|---|---|
| | | Average ErbB-2/ErbB-1 | Average tumor HN5/BT474/N87 |
| **16** | None | 0.074 | 2.98 |
| **2** | 5′ | 0.027 | 0.60 |
| **17** | 4′ | 0.023 | 2.68 |
| **18** | 3′ | 0.068 | 3.81 |

**Table 4.** Type I receptor inhibition activity and cellular efficacy results for representative examples of side-chain modifications

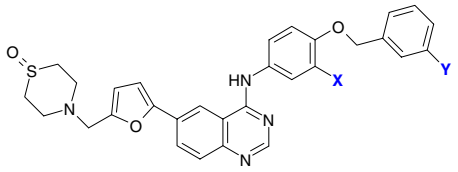


| Compound | R | Y | Z | $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|---|
| | | | | ErbB-2 | ErbB-1 | BT474 |
| **19** | Me | F | –O– | 0.017 | 0.026 | 0.17 |
| **20** | Ph | F | –O– | 0.028 | 0.025 | NT |
| **21** | Ph | H | $-NCH_2CH_2-$ | 0.038 | 0.050 | NT |
| **22** | Me | F | –N-(*n*-Pr) | 0.02 | 0.085 | 0.25 |
| **23** | Me | F | $-N-(CH_2CN)$ | 0.022 | 0.027 | 0.067 |
| **24** | Me | F | –N-(Bn) | 0.141 | 0.090 | 0.61 |

NT, not tested.

a fixed 6-position modification, 5-methylsulfonylethylamino-2-furanyl group, many combinations of substituents were explored and some representative examples

**Table 5.** Type I receptor inhibition activity and cellular efficacy results for representative examples of thiomorpholine side chain with benzyloxyaniline changes



| Compound | X, Y | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | ErbB-2 | ErbB-1 | HN5 | BT474 | N87 |
| **25** | $CF_3$, F | 0.141 | 0.089 | 1.11 | 3.00 | 2.09 |
| **26** | Cl, F | 0.018 | 0.028 | 0.06 | 0.10 | 0.08 |
| **27** | F, H | 0.035 | 0.016 | 1.0 | 1.20 | 1.13 |
| **28** | Cl, H | 0.023 | 0.012 | 0.32 | 0.42 | 0.46 |

are listed in Table 1. Chlorine at position X afforded insignificant increases in enzyme potency, but appeared to offer improvements in cellular efficacy. Larger groups in position X generally diminished activity. In combination with suitable X substituents, fluorine at position Y yielded compounds with greater potency in the cellular assay panel. Larger groups at Y also reduced activities. The binding pocket for this moiety in the ErbB-1 crystal structure appears as a well-defined lipophilic pocket (Fig. 2) which tends to support the observed size tolerance and position of the benzyloxyaniline substitutions.[17]

The exploration of the terminal substitution on the 6-position side chain was performed to determine if

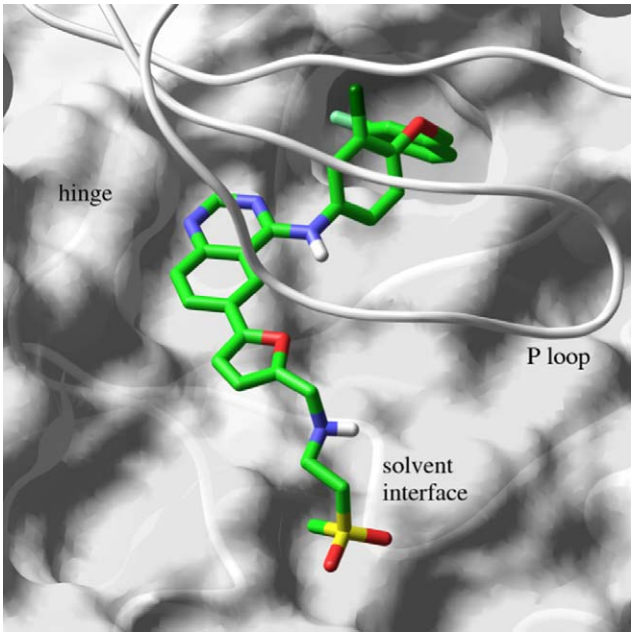



**Figure 2.** A view through the N-terminal lobe of ErbB-1 of the bound conformation of **1** as determined by X-ray crystallography.[17] The benzyloxyaniline moiety binds in a well-defined lipophilic pocket, the quinazoline makes a hydrogen bond to the hinge region, and the methylsulfonylethylamino group binds at the solvent interface. Image created with Maestro from Schrodinger, LLC.

changes would significantly affect the enzyme and tumor cell inhibition potency. The crystal structure of lapatinib places the side-chain pointing toward and extending into the protein/solvent interface.[17] Enzyme inhibition $IC_{50}$ values for all analogs stayed within the range of 10–35 nM for both ErbB-1 and ErbB-2, as shown for representative examples in Table 2. While little or no changes in potency were observed for the enzyme assay, the inhibition of tumor cell proliferation varied significantly. No substituent proved to be superior to the simple methyl sulfone.

Another question we sought to understand was the regiochemistry of the linking furan ring with the side-chain substituent. Table 3 lists a set of analogs synthesized in a similar fashion as described in Scheme 1 and tested to determine the optimal connection chemistry. While the substitution at the 4′ and 5′ position of the furan appeared to be equally potent at inhibiting ErbB-1/ErbB-2 enzymes, the 5′ substituted analog was 4–5 times more potent against the tumor cell lines than either the 3′ or 4′ regioisomer.

Linker side-chain SAR showed modifications were well tolerated in the kinase enzyme inhibition assays. It is interesting to note that there is virtually no difference in the enzyme potency of a small linkage group, oxygen, and larger groups of *n*-propylamino or benzylamine (data shown in Table 4). The ether **19** and N-$CH_2CN$ **23** analogs in particular were relatively potent in the cellular assays. Because of the mixing and matching of functional group combinations and the space required to depict the compounds, only BT474 cellular assay data are shown in the Table 4. However, it is noteworthy that the values in the HN5 and N87 cell lines were comparable (unpublished). No advantage was observed in relative potency in the in vitro assay systems of the tertiary amines (e.g., **21**–**24**) compared with the secondary amino linker (e.g., **1**, **12**), except possibly improved selectivity for tumor versus normal cells (see discussion below). The extra synthetic steps and increased molecular weight were deemed non-beneficial for optimizing the properties of our best compounds that contained the methylsulfonylethylamine with a one-carbon tether to the furan ring.

Since the disubstituted amine was tolerated in the examples above, a cyclic version was investigated for potential value. Some examples of those derivatives are shown in Table 5. Similar trends in activity were observed for the substitution of the benzyloxy aniline moiety in this series and the optimally substituted compound (**26**) compared very favorably to **1**.

A control cell line, derived from human foreskin fibroblasts, HFF, was used to assess the selectivity for tumor cells (T) versus normal cells (N).[13] Table 6 contains representative examples from each of the groups of modifications covered in the discussion thus far. The average of the tumor cell $IC_{50}$ values was used to assess the dual ErbB-1 and ErbB-2 inhibitory properties in assays that are run under similar conditions. Greater than 100-fold selectivity was observed for all of these analogs which bolstered our confidence in the therapeutic potential of this series. A more thorough analysis of the enzyme/cell activity was done for lapatinib, and the results suggest that the improved cellular activity may be due to the slow binding kinetics.[16]

**Table 6.** Cellular activity and selectivity for representative substituted 6-furanylquinazolines

| Compound | $IC_{50}$ (μM) | | Selectivity N/T |
|---|---|---|---|
| | Average tumor[a] | HFF | |
| **1** | 0.09 | 9.9 | 111 |
| **12** | 0.20 | >30 | >150 |
| **19** | 0.16 | >30 | >188 |
| **23** | 0.11 | >30 | >273 |
| **26** | 0.08 | 8.18 | 102 |

[a] Average $IC_{50}$ value for N87, BT474, and HN5 cellular data combined.

The 6-furanyl-4-(4-benzyloxyanilino)-quinazoline scaffold afforded the necessary drug-like properties and dual ErbB-2/ErbB-1 tyrosine kinase inhibition to discover a potential anti-cancer therapeutic agent. The halogen substitution on the benzyloxyanilino group was key to improving the enzyme/cell ratio of activity, with 4-(3-fluorobenzyloxy)-3-chloroanilino providing the most promising cellular efficacy. The substitutions on the furanyl ring were important to be in a 2,5-orientation for the desired cellular activity, while there was no apparent difference in the enzyme activity. Quite a diversity of amine substitutions were tolerated, presumably due to the binding mode of these inhibitors where the aniline is tucked into the back of the ATP binding pocket, and the side chain on the furanyl portion extends out toward solvent. Overall, GW572016 (lapatinib, **1**) possessed the desired enzyme potency, cellular activity in a panel of tumor cell lines, and selectivity.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.05.090.

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