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The discovery of highly selective erbB2 (Her2) inhibitors for the treatment of cancer

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Abstract—The synthesis and biological evaluation of potent and selective inhibitors of the erbB2 kinase is presented. Based on the 4-anilinoquinazoline chemotype, the syntheses of several new series of erbB2 inhibitors are described with quinazoline and pyrido[4,3-*d*]pyrimidine cores. The vast majority of these compounds are found to be >100× selective over the closely related EGFR kinase. Two lead compounds are further shown to have low clearance and moderate bioavailability in rat.

Breast cancer is the second leading cause of cancer related death in American women. It is estimated that 214,640 new cases of breast cancer were diagnosed and 41,430 deaths occured in the U.S. during 2006.[1] Current statistics suggest that 12.7% of American women born today (~1/8) will be diagnosed with breast cancer at some point in their life. While advances continue to be made in the treatment of this disease, current chemotherapy options are often limited by a lack of efficacy and toxicity; thus new therapies are urgently needed to treat this disease.[2]

The erb family of receptors are transmembrane receptor tyrosine kinases involved in a wide range of signal transduction and cellular functions, and have become a very fruitful area for the successful development of drugs to treat cancer.[3] erbB2 (Her2) is found to be significantly overexpressed in 20–30% of human breast cancers and is associated with a poor prognosis.[4] The humanized antibody herceptin (trastuzumab) from Genentech targets erbB2 and has been approved for the treatment of breast cancer.[5] EGFR (erbB1) has also been targeted for the treatment of cancer, and several agents have been approved with this mode of action.[6]

At Pfizer, an effort to selectively target erbB2 was initiated. Despite >80% homology between EGFR and erbB2 in their kinase domains,[7,8] a kinase selective approach was chosen for two reasons: (1) to avoid unwanted toxicity from inhibiting EGFR, such as acneiform skin rashes, and (2) to facilitate the ability to combine a selective erbB2 agent with other selective drugs, including an EGFR agent, in a tunable fashion so that different relative levels of selective inhibition could be achieved. We believed that this approach is advantageous from a scientific viewpoint in the long term when compared to being limited to a fixed selectivity profile inhibiting multiple kinases from an unselective single agent.

Previously, Pfizer reported on a series of anilinoquinazolines exemplified by compounds **1** and **2** (Fig. 1).[9–11]

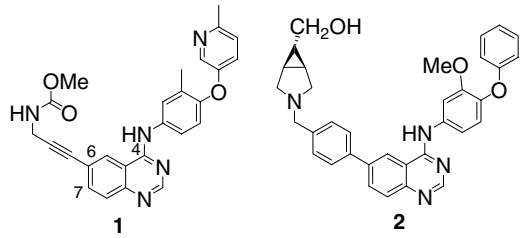

Figure 1. erbB2 selective anilinoquinazolines.[9–11]

Keywords: erbB2; Her2; Anilinoquinazoline; Kinase; Cancer.
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These inhibitors were shown to be 11–42 nM erbB2 antagonists with 60–100× selectivity over EGFR. Based on a homology model and comparisons with the crystal structure of an inhibitor bound to EGFR,[12] it is believed that the N1 of the quinazoline makes a key hydrogen bond to the hinge region of the protein, and that the aniline portion of the inhibitors is largely responsible for kinase selectivity because it is buried deep in a hydrophobic pocket. The quinazoline 6 position substituents are believed to be pointed toward a solvent accessible portion of the protein, and thus this position may be an ideal place to introduce modifications designed to improve ADME properties.

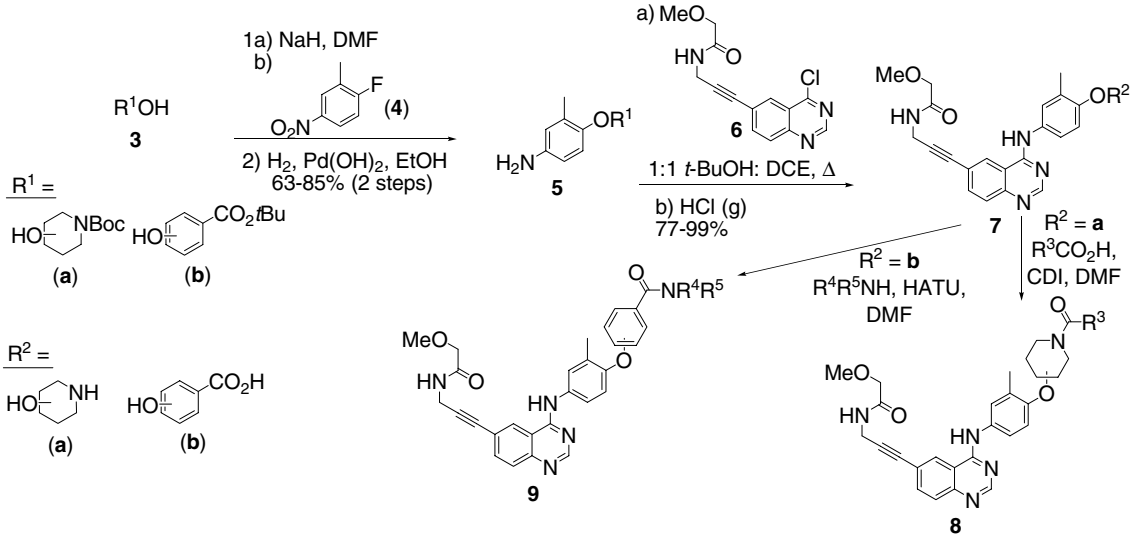
The goal of the effort described herein was to build on this level of potency, increase selectivity, and further elaborate SAR within this exciting chemotype, which is already present in three approved cancer drugs.[6] In particular, a focus was on elaborating the anilino portion of the molecule with more polarity by utilizing motifs that have been shown to induce a DFG out conformation in other kinases.[13] Thus, amide substituents were envisioned on the terminal phenyl group. In addition, replacement of this terminal phenyl group with substituted piperidines was also sought.

The initial analogs in this series were made with the C6 alkyne substituent of the quinazoline. The synthesis begins with a nucleophilic displacement of the fluoro group of **4** with either 3- or 4-substituted piperidine alcohols or phenols (**3a** and **3b**, Scheme 1). Reduction of the nitro group affords **5** in good yield. The resulting amine is then added to the C6-alkyne substituted 4-chloroquinazoline (**6**),[14] followed by acid catalyzed deprotection of either the *t*Boc group of piperidine containing intermediates, or the *t*-Bu ester of the phenol containing intermediates. The resulting amine and acid substrates (**7**) are then subjected to standard high speed analoging protocols resulting in final analogs **8** and **9**.

Table 1 shows the potency of piperidine compounds in inhibiting the isolated erbB2 kinase,[15] and erbB2 in a cellular system, as well as their selectivity in inhibiting EGFR in the same cellular system.[16,17] Initial unsubstituted piperidine and piperidine alkyl analogs were found to be inactive (**8a** and **b**). Simple amide derivatives were also inactive (**8c,g**), however, bulky amides (**8d–f**) and urea (**8i**) showed surprising potency and selectivity. Notably, these 4-piperidines were significantly more potent than 3-piperidines (data not shown). Table 2 contains compounds in the phenyl amide class. These compounds showed moderate to excellent potency and remarkable selectivity over EGFR when substituted at the 3′ position with a bulky amide (**9c–f**). Simple amides caused little inhibition (**9a**,**b**). At the 4′ position, the SAR was considerably more narrow, with only the neopentyl amide displaying significant potency (**9k**). For all of these series, the distance between the amide and the N1 of the quinazoline as well as the need for large groups on the amide for potency suggests the possibility that these compounds require a protein conformational change to a DFG out form to bind, although no definitive kinetic or crystallographic data has been generated to support this hypothesis.[13]

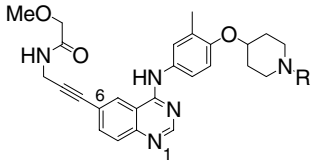
To further explore the SAR of these novel quinazoline C4 substituents, we sought to combine them with other quinazoline C6 and C7 substituents, as well as C6 substituted pyridopyrimidines. As described previously, this portion of the inhibitors is believed to point toward a solvent accessible portion of the binding pocket, and thus polar substituents were envisioned. In particular, ethers and amines were chosen due to their intrinsic polarity and based on the precedents of previous erb inhibitors.[6,18,19]

The readily available 4-chloro-6,7-dimethoxy and 6,7-dimethoxyethoxy ether starting materials were first utilized to synthesize di-ether analogs in an analogous



Scheme 1. The synthesis of amide substituted anilinoquinazoline analogs. DMF = *N*,*N*-dimethylformamide; DCE = 1,2-dichloroethane; CDI = 1,1′-carbonyldiimidazole; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.

Table 1. erbB2 kinase and cell inhibition, and EGFR cell selectivity for the piperidine sub-class of anilinoquinazolines **8a–i**



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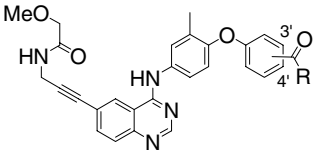
Compound	R	erbB2 kinase inhibition IC_{50}[a] (nM)	erbB2 cell inhibition IC_{50}[a] (nM)	Cell selectivity versus EGFR[a]
8a	H	>10,000	NT	NT
8b	Me	>10,000	NT	NT
8c	Ac	>10,000	NT	NT
8d	CO(*c*-Bu)	150	51	>200×
8e	CO(*m*-ClPh)	210	75	92×
8f	$COCH_2$*t*-Bu	94	16	400×
8g	$COCH_2OMe$	>10,000	NT	NT
8h	CO(2-THF)	550	2900	NT
8i	CONH(2,6-difluoroPh)	76	190	>53×

NT, not tested.
[a] Values are means of at least two experiments, assay error is <2×.

Table 2. erbB2 kinase and cell inhibition, and EGFR cell selectivity for phenyl amide analogs **9a–k**



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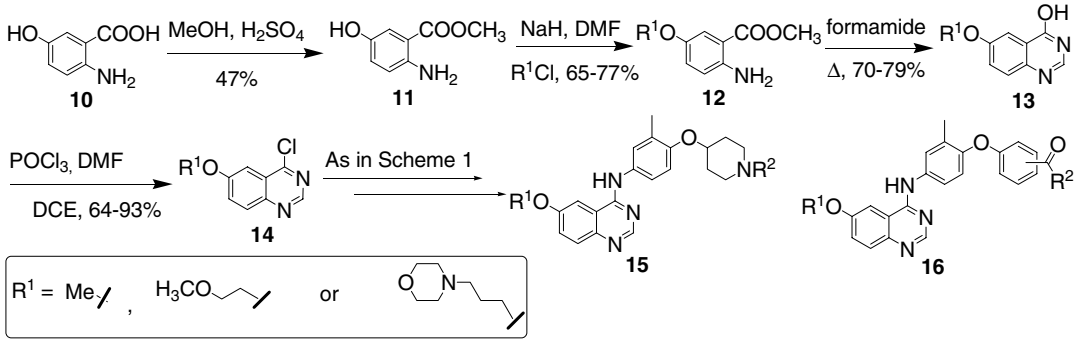
Compound	Amide position	R	erbB2 kinase inhibition IC_{50}[a] (nM)	erbB2 cell inhibition IC_{50}[a] (nM)	Cell selectivity versus EGFR
9a	3′	NHMe	>10,000	NT	NT
9b	3′	$N(Me)_2$	>10,000	NT	NT
9c	3′	NHPh	140	NT	NT
9d	3′	NH*c*-Bu	230	160	>64×
9e	3′	NH*t*-Bu	18	30	250×
9f	3′	$NHCH_2$*t*-Bu	130	110	91×
9g	4′	NHMe	>10,000	NT	NT
9h	4′	$N(Me)_2$	>10,000	NT	NT
9i	4′	NHPh	>10,000	NT	NT
9j	4′	NH*t*-Bu	>10,000	NT	NT
9k	4′	$NHCH_2$*t*-Bu	140	23	200×

NT, not tested.
[a] Values are means of at least two experiments, assay error is <2×.

fashion as that described in Scheme 1. Subsequently, C6-monoethers were synthesized (Scheme 2) beginning with the esterification of anthranilic acid **10** followed by alkylation of the phenol under standard conditions to provide **12** where R1 = Me, MeOEt, and morpholine propyl. Cyclization of the anthranilic acid with



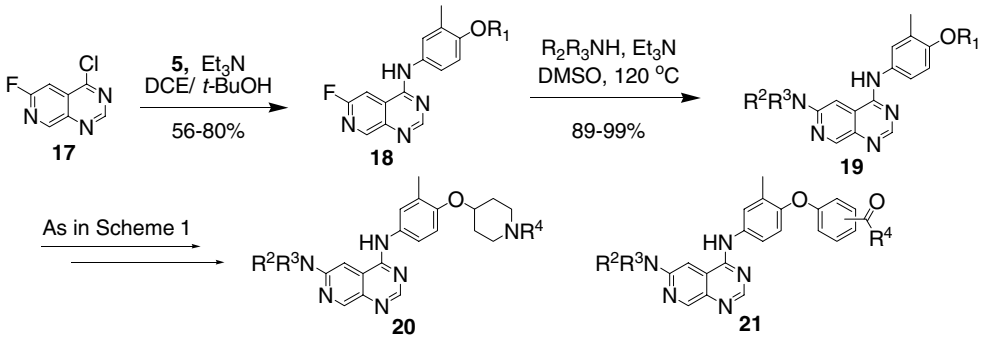
Scheme 2. The synthesis of C6 monoether analogs. DMF = *N*,*N*-dimethylformamide; DCE = 1,2-dichloroethane.

formamide to the quinazoline followed by chlorination afforded the key 4-chloro-6-ether **14**. Elaboration of this intermediate into final analogs took place as described in Scheme 1.

The synthesis of C6-aminopyridopyrimidine inhibitors is shown in Scheme 3. Beginning with 4-chloro-6-fluoropyrido[3,4-*d*]pyrimidine (**17**)[19] the 4-chloro group is first displaced with the aniline intermediates (**5**) of Scheme 1 to provide **18**. Amine nucleophiles are then utilized to displace the 6-fluoro group with high heat in DMSO to provide intermediate **19**. Analogs are then completed as described previously.

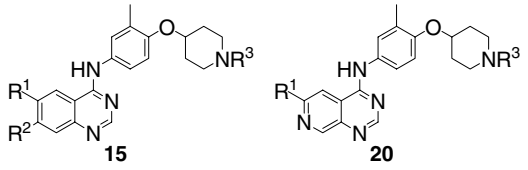
Tables 3 and 4 show the potency and selectivity of C6 and C7 analogs. In general, all analogs in these series show moderate to excellent kinase and cell potency, as well as EGFR selectivity. Particularly with regard to erbB2 cell potency, the pyridopyrimidine analogs are superior. For example, compound **20b** was shown to have a potency of <10 nM in the erbB2 cell assay, whereas its EGFR cell potency was >10 μM resulting in >1000× selectivity.

Two of the most promising pyridopyrimidine compounds were assayed to determine their pharmacokinetic properties in rat. Compound **20f** as the HCl salt showed low clearance (Cl) of 2.5 mL/min/kg and a low volume of distribution (V_d) of 0.2 L/kg resulting in a half-life of 1 h after a single 2 mg/kg IV dose. When dosed orally (5 mg/kg) in 0.5% methylcellulose, this compound was 54% bioavailable (F). When dosed IV,



Scheme 3. The synthesis of pyrido[4,3-*d*]pyrimidine analogs. DCE = 1,2-dichloroethane; DMSO = dimethylsulfoxide.

Table 3. erbB2 kinase and cell inhibition, and EGFR cell selectivity for the piperidine sub-class of quinazoline ether and C6-amino substituted pyrido[4,3-*d*]pyrimidines **15a**–**l** and **20a**–**f**

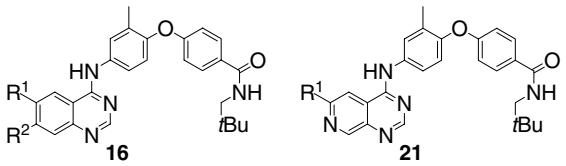


Compound	R^1	R^2	R^3	erbB2 kinase inhibition IC_{50}[a] (nM)	erbB2 cell inhibition IC_{50}[a] (nM)	Cell selectivity versus EGFR[a]
15a	OMe	OMe	$COCH_2t$-Bu	100	76	>130×
15b	$O(CH_2)_2OMe$	$O(CH_2)_2OMe$	$COCH_2t$-Bu	260	530	11×
15c	OMe	H	$COCH_2t$-Bu	96	NT	NT
15d	$O(CH_2)_2OMe$	H	$COCH_2t$-Bu	120	19	>500×
15e	$O(CH_2)_3$ morpholine	H	$COCH_2t$-Bu	75	34	>290×
20a	NMe_2	—	$COCH_2t$-Bu	51	<10	>230×
20b	Morpholine	—	$COCH_2t$-Bu	23	<10	>1000×
15f	OMe	OMe	CONH(2,6-difluoroPh)	19	67	23×
15g	$O(CH_2)_2OMe$	$O(CH_2)_2OMe$	CONH(2,6-difluoroPh)	230	840	6.5×
15h	OMe	H	CONH(2,6-difluoroPh)	46	250	11×
15i	$O(CH_2)_2OMe$	H	CONH(2,6-difluoroPh)	27	33	>300×
15j	$O(CH_2)_3$ morpholine	H	CONH(2,6-difluoroPh)	55	55	32×
20c	NHMe	—	CONH(2,6-difluoroPh)	25	180	25×
20d	NMe_2	—	CONH(2,6-difluoroPh)	21	20	68×
20e	Morpholine	—	CONH(2,6-difluoroPh)	7	16	210×
15k	$O(CH_2)_2OMe$	$O(CH_2)_2OMe$	CO(*c*-pent)	77	530	>19×
15l	$O(CH_2)_2OMe$	H	CO(*c*-pent)	44	29	>350×
20f	Morpholine	—	CO(*c*-pent)	18	33	>300×

NT, not tested.

[a] Values are means of at least two experiments, assay error is <2×.

Table 4. erbB2 kinase and cell inhibition, and EGFR cell selectivity for the phenyl amide sub-class of quinazoline ethers and C6-amino substituted pyrido[4,3-*d*]pyrimidines **16a** and **21a–f**



16 **21**

Compound	R^1	R^2	erbB2 kinase inhibition IC_{50}[a] (nM)	erbB2 cell inhibition IC_{50}[a] (nM)	Cell selectivity versus EGFR[a]
16a	OMe	OMe	77	310	1×
21a	NHMe	—	30	13	230×
21b	$NH(CH_2)_2$ morpholine	—	280	15	230×
21c	NMe_2	—	48	<10	>770×
21d	Pyrrolidine	—	36	<10	>100×
21e	Morpholine	—	21	24	>410×
21f	NMe Piperazine	—	130	15	>660×

NT, not tested.
[a] Values are means of at least two experiments, assay error is <2×.

compound **21e** was also found to have low Cl of 2.5 mL/min/kg, and a slightly higher V_d of 0.4 L/kg, resulting in a half-life of 1.9 h. An initial oral formulation of this compound in 0.5% methyl cellulose provided only 5% F, presumably due to poor absorption. An in vitro measurement of permeability in the Caco2 system predicted good permeability ($A \rightarrow B$: $15.4 \pm 1.8 \times 10^{-6}$ cm/s), however the solubility of this compound was found to be poor, likely resulting in the low bioavailability. To address this issue, **21e** was dosed in a self-emulsifying drug delivery system (SEDDS) formulation consisting of a mixture of Miglyol 812, Polysorbate 80, and Capmul MCM. This formulation caused bioavailability to increase to 37%, presumably due to enhanced solubility in this formulation.[20] Compound **21e** was further screened against 14 diverse kinases and found to have >10 μM potency against all kinases tested (EGFR, IRK, IGF-1R, CDK2, CDK5, cSRC, FES, JAK3, PKCα, PKCθ, LCK, PDGFRB, PKA, and Wee-1) thus demonstrating broad kinase selectivity.

In conclusion, several new classes of anilinoquinazoline inhibitors were synthesized. These inhibitor classes consist of novel piperidine and benzamide functionality on the northern portion of the molecule combined with a quinazoline C6-alkyne amide, C6,C7-bisethers, C6 monoethers, and amine substituted pyridopyrimidines. These compounds were shown to be potent inhibitors of erbB2 in kinase and cellular assays, and are extremely selective over EGFR, despite the high homology that exists between these kinases. Two representative compounds are further shown to have moderate oral bioavailability with low clearance. It is expected that agents of this type could be effective in the treatment of cancer either as single agents, or in combination with other chemotherapeutic drugs. The exquisite kinase selectivity of these compounds should offer the scientific community excellent tools for further understanding the erbB2 signaling cascade, and the implications for inhibiting this pathway. Other future studies should include combining these agents with EGFR inhibitors to determine an optimal ratio of erbB2/EGFR inhibition for anticancer activity.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.03.046.

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15. erbB2 kinase assays were conducted as described in Refs. 9,10. Briefly, recombinant erbB intracellular domain (residues 675–1255) was expressed in Baculovirus-infected Sf9 cells as GST fusion proteins and purified. The phosphorylation of poly(Glu, Tyr) was then measured in the presence of ~15 ng of this recombinant protein,1 mM ATP, and inhibitors with a 6 min. incubation time. The degree of phosphorylation was then measured with HRP conjugated-PY-54 antiphosphotyrosine antibody. The colorimetric signal was then developed with TMB microwell peroxidase substrate and the absorbance was measured at 450 nm.
16. erbB2 and EGFR cell assays were conducted as described in Refs. 9,10. Briefly, NIH3T3 cells transfected with either human EGFR or a chimeric receptor with EGFR extracellular domain and erbB2 intracellular domain were seeded in a 96-well tissue culture plate. Inhibitors were added and incubated for 2 h. Cells were stimulated with human recombinant EGF for 15 min. The degree of phosphorylation was then measured with HRP conjugated-PY-54 antiphosphotyrosine antibody. The colorimetric signal was then developed with TMB microwell peroxidase substrate and the absorbance was measured at 450 nm.
17. EGFR kinase selectivity was not measured routinely because it is thought that the cellular system is more biologically relevant with both cell assays performed under identical conditions, and because EGFR kinase and cell selectivity have correlated well in previous studies.[9,10] Examples in this publication include: **15f**: kinase sel. = 6×, cell sel. = 23×; **15i**: kinase sel. = 178×, cell sel. = >300×; **21e**: kinase sel. = >480×, cell sel. = >410×.
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