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Alkynyl pyrimidines as dual EGFR/ErbB2 kinase inhibitors

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Abstract—Anilinoalkynylpyrimidines were prepared and evaluated as dual EGFR/ErbB2 kinase inhibitors. A preference was found for substituted phenyl and heteroaromatic rings attached to the alkyne. In addition, the presence of a potential hydrogen bond donor appended to this ring was favored. Selected molecules in the series demonstrated some activity against human tumor cell lines.

Protein tyrosine kinase inhibitors are a source of tremendous interest due to their promise as therapeutic agents for the treatment of a variety of disease states, particularly cancer.[1] One of the first kinases to be successfully targeted is the epidermal growth factor receptor (EGFR), with some inhibitors already demonstrating clinical benefit.[2] EGFR is one of a family of four related kinases, collectively called the ErbB family, that also includes ErbB2 (Her2/neu), kinase inactive ErbB3, and ErbB4.[3] Inhibition of multiple kinases within this family has emerged as a promising strategy for cancer treatment.[2b,4] A wide range of structural classes has been employed as kinase inhibitors.[1] The most commonly utilized template in inhibition of the ErbB family is the anilinoquinazoline.

Examples include the launched molecules gefitinib (**1**)[5] and erlotinib (**2**),[6] as well as the clinical candidate lapatinib (**3**) (GW572016)[7] (Fig. 1). While gefitinib and erlotinib are selective inhibitors of EGFR over ErbB2, lapatinib is a potent inhibitor of both.[7]

In this paper, dual EGFR/ErbB2 inhibitors based upon truncated quinazolines, specifically the pyrimidine substructure, are described. It appeared that attaching an alkyne to the 5 position of the pyrimidine nucleus would place functional groups into a similar orientation as the substituted furan of lapatinib (Fig. 2). In initial studies, the fluorobenzyloxy-chloroaniline present in lapatinib was conserved in order to further maximize the chances of identifying dual inhibitors.[8]

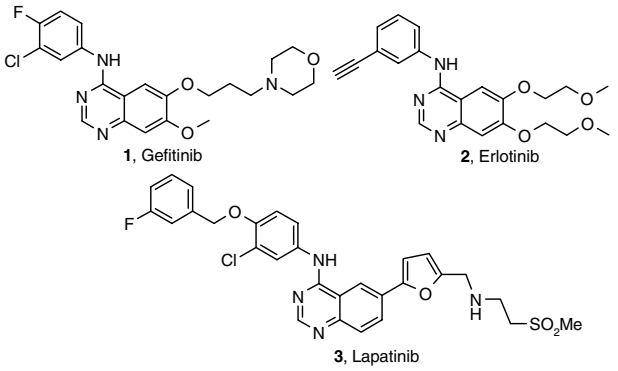

Figure 1. Examples of clinical ErbB family inhibitors.

Alkynyl pyrimidyl derivatives were synthesized according to the sequence outlined in Scheme 1. Treatment of pyrimidone **4** with sodium hydroxide and iodine was followed by chlorination with $POCl_3$ to give iodochloropyrimidine **5**. Displacement of the chloride with the appropriate aniline was performed in isopropanol in the presence of catalytic acid to give **6**. Analogs were constructed from this key intermediate in two ways.

Keywords: Receptor tyrosine kinase; Kinase inhibition; EGFR; ErbB2; Medicinal chemistry.
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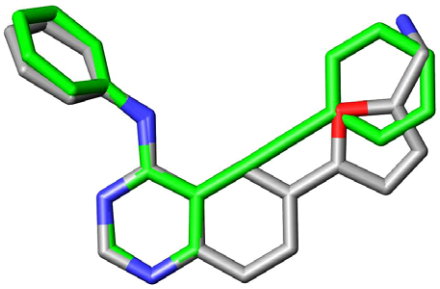
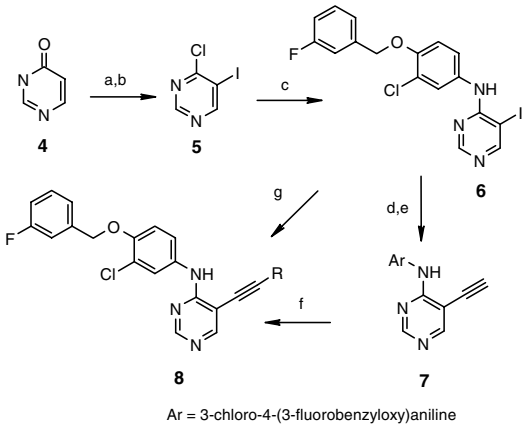


Figure 2. Overlay of anilinoquinazoline nucleus (gray) with anilinoalkynylpyrimidine (green).

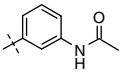
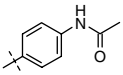
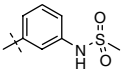
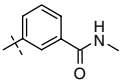
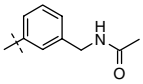
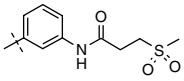
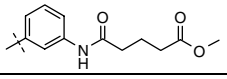


Scheme 1. General synthetic route. Reagents and conditions: (a) NaOH, I_2, 64%; (b) $POCl_3$, 88%; (c) $ArNH_2$, *i*-PrOH, cat HCl, 90 °C, 86%; (d) trimethylsilyl acetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, 65 °C, 83%; (e) TBAF, THF, 0 °C, 94%; (f) RBr, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, 65 °C, 20–75%; (g) R-alkyne, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, 65 °C, 19–78%.

Alkyne **7** was obtained by a Sonogashira coupling with trimethylsilyl acetylene, followed by removal of the silyl group with TBAF. A second Sonogashira coupling was then employed to introduce desired substituents. Alternatively, derivatives could be directly constructed from iodide **6** using an appropriately substituted alkyne. In some cases, additional functionality was introduced using known chemical transformations (vida infra).

The potency of several aryl ring derivatives is reported in Table 1. EGFR and ErbB2 enzyme inhibition values were obtained as previously described.[9] For comparison, lapatinib shows 10 nM potency against EGFR and 9 nM potency against ErbB2. Compounds in the alkynyl pyrimidine series that exhibit 20 nM or better potency on both kinases were desired. The parent anilinopyrimidine (des-iodo **6**) showed only modest inhibition of EGFR (0.32 μM) and was 21 μM against ErbB2. Unfunctionalized alkyne **7** showed a 4-fold improvement in EGFR potency, but was still only 3.8 μM against ErbB2. Placing a phenyl ring on the alkyne gave **8a**, with sub-micromolar potency on both enzymes. *ortho*-, *meta*-, and *para*-Amino phenyl analogs (**8b–d**) were prepared and were found to have a 9- to 20-fold increase in ErbB2 potency compared with the parent unsubstituted analog **8a**. Acetamide analogs were also prepared by acylation with acetic anhydride and demonstrated striking SAR. The *para*-acetamide **8f** was greater than 1 μM, while the *meta*-acetamide **8e** had a further 3-fold increase in dual EGFR/ErbB2 potency compared with **8c**, resulting in IC_{50}'s approaching 10 nM for both enzymes. Based on homology modeling, the *meta*-acetamide may occupy roughly the same physical space as the amine in lapatinib. It is possible that a heteroatom in this position is important for successful dual kinase inhibition in these compounds. It is speculated that the acetamide may be functioning as a hydrogen bond donor, interacting with Asp808 of ErbB2. In an attempt to optimize the character of this functionality, the simple acetamide was replaced with a number of related functional groups, including sulfonamide **8g** and reversed amide **8h**. Neither of these compounds improved potency. Analog **8i**, bearing an additional methylene unit between the aryl ring and the acetamide, was constructed by performing the Sonogashira coupling reaction with *N*-[(3-iodophenyl)methyl]acetamide, which was constructed from 3-iodobenzyl amine. This homolog likewise had decreased activity compared to **8e**. Amides **8j** and **k**, bearing extended alkyl chains, were generated by reacting **8c** with acyl chlorides. Both amides had EGFR/ErbB2 dual potencies lower than **8e**.

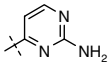
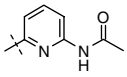
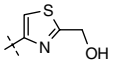
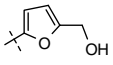
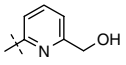
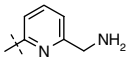
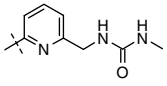
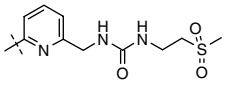
Table 1. Substituted anilinoalkynylpyrimidines and selected other compounds

Compound	R	EGFR IC_{50}[a]	ErbB2 IC_{50}[a]
3	—	0.010	0.009
Des-iodo **6**	—	0.320	21.0
7	H	0.085[b]	3.80
8a	–Ph	0.079[b]	0.78[b]
8b	2-Aminophenyl	0.063[b]	0.091[b]
8c	3-Aminophenyl	0.042[b]	0.044[b]
8d	4-Aminophenyl	0.030	0.036
8e		0.012	0.013
8f		25.1	1.82
8g		0.11[c]	0.045
8h		0.079	0.032
8i		0.060[b]	0.052[b]
8j		0.050	0.032
8k		0.031	0.029

[a] Mean values in micromolar, at least two determinations, standard deviation less than 0.025.
[b] One determination.
[c] Deviation <0.030.

In addition to phenyl-substituted alkynes, heterocyclic analogs were also evaluated (Table 2). The 3-pyridyl analog **8l** is similar to **8a** against EGFR and 5-fold better against ErbB2. The 2-pyridyl compound **8m** exhibited very good affinity for EGFR and also demonstrated a >10-fold improvement in ErbB2 potency compared with the phenyl derivative **8a**. Pyrimidine and pyrazole groups (**8n** and **8o**, respectively) were also synthesized and, despite similar ErbB2 potencies to **8m**, were less potent on EGFR than the pyridyl compound **8m**. In the phenyl series, the presence of a putative hydrogen bond donor in the *meta* position was important. Several heterocycles with small hydrogen bond donating substitutions were evaluated to determine if this was also important with the heterocyclic analogs. The aminopyrimidine **8p** demonstrated 4-fold improved activity against ErbB2 compared with **8m**. However, the pyridyl *meta*-acetamide **8q** was less effective in contrast to the phenyl series. The hydrogen bonding potential of the acetamide may be compromised due to the lower electron density of the pyridyl ring or by the proximity of the heterocyclic nitrogen's lone pair of electrons. Other heterocylic derivatives likewise met with mixed success. The hydroxymethyl thiazole **8r** showed reduced activity toward both kinases when compared to **8m**, while the hydroxymethyl furan **8s** demonstrated ErbB2 potency similar to that of **8m**. Placing the hydroxymethyl moiety onto a 2-pyridinyl ring with analog **8t** enhanced potency against ErbB2 with a 4-fold improvement over **8m**. A number of different functional groups were found to be tolerated in the pseudo-benzylic pyridyl position. The primary amine **8u**[10] was a 21 nM EGFR inhibitor with 38 nM potency against ErbB2. Methyl urea **8v** was also a good EGFR inhibitor, with potency similar to that of **8m**, but fell somewhat short against ErbB2. Adding tethered functionality resulted in sulfone analog **8w**, with an IC_{50} <15 nM in both the EGFR and ErbB2 enzyme assays.

Table 2. Heteroarylalkynylpyrimidines

Compound	R	EGFR IC_{50}[a]	ErbB2 IC_{50}[a]
8l	3-Pyridyl	0.074[c]	0.14
8m	2-Pyridyl	0.017	0.055[c]
8n	2-Pyrimidyl	0.077[c]	0.060
8o	3-Pyrazolyl	0.042[b]	0.066[b]
8p		0.032	0.012
8q		0.091	0.059
8r		0.076	0.16
8s		0.033	0.047
8t		0.040	0.013
8u		0.021	0.038[c]
8v		0.023	0.069
8w		0.015	0.009

[a] Mean values in micromolar, at least two determinations, standard deviation less than 0.025.
[b] One determination.
[c] Deviation <0.050.

It seems that having a potential hydrogen bond donor in the *meta* position of an aromatic ring can be important for ErbB2 inhibition. However, it appears that the effectiveness of the putative hydrogen bond can be affected by its electronic environment, whether via substitution around the donor or by changing the nature of the aromatic ring. Although no X-ray co-crystal structure yet exists for these compounds in ErbB2, a homology model (Fig. 3) suggests that the molecule binds in the same fashion as lapatinib, making a hinge contact at Met801. The aniline portion should extend past the Thr798 'gatekeeper' residue deep into a large hydrophobic pocket. This binding model places the potential hydrogen bond donor substituents in the proximity of Asp808 of ErbB2. We expect similar binding in EGFR.

Some of the more interesting compounds have been further profiled. Compounds **8e**, **k**, **s**, **t**, and **w** were greater than 100-fold selective against a panel of non-ErbB family kinases, including CDK2, GSK3, SRC, and VEGFR2.

These same compounds were also tested in cellular proliferation assays (Table 3).[11] Most of the inhibitors did not exhibit the level of cellular potency predicted by their enzyme activity. A number of potential explanations exist for this phenomenon. One possibility is a lack of cell penetration. Further investigation revealed that **8e** did not inhibit kinase activity in a cellular autophosphorylation assay (data not shown), possibly suggesting

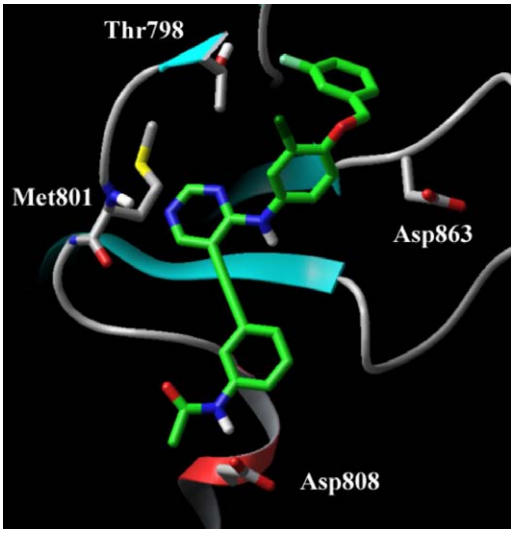


Figure 3. Acetamide **8e** docked into an ErbB2 homology model.

Table 3. Inhibition of cellular proliferation by dual inhibitors

Compound	HN5 IC_{50}[a]	BT474 IC_{50}[a]	HFF IC_{50}[a]
8e	13.7	21.6	>30.0
8k	3.47	4.24	22.4
8s	0.93	2.04	15.8
8t	4.89	5.79	>30.0
8w	0.77	0.84	>30.0

[a] Mean values in micromolar, at least two determinations.

a lack of cell penetration. Compound **8e** also exhibited a very poor permeation rate of <3 nm/s in an artificial membrane permeation assay.[12] Two compounds, **8s** and **w**, did exhibit sub-micromolar cell activity in both the cellular proliferation assays (Table 3) and in our autophosphorylation assay, with **8w** active against both EGFR- and ErbB2-driven cell lines. For reference, lapatinib (**3**) has an IC_{50} of approximately 25 nM on both HN5 and BT474 cell lines.

In summary, anilinoalkynylpyrimidines have been explored as dual EGFR/ErbB2 kinase inhibitors. The best examples showed very good inhibition in enzyme assays, with IC_{50}s approaching 10 nM. The most active dual inhibitors have polar functional groups attached to an aromatic ring that might act as hydrogen bond donors at physiological pH. This work suggests that this substitution can be important for successful dual inhibition within this series. In addition, while some compounds may have been limited by poor cell penetration, other compounds were found to be sub-micromolar inhibitors of kinase-driven cellular proliferation.

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10. Obtained from mesylation of **8t**, displacement with di-*tert*-butylimidocarbonate, and subsequent de-protection. Compounds **8v** and **w** were prepared by acylation of **8u**.
11. The HN5 cell line is a head and neck carcinoma line that overexpresses EGFR. The BT474 line is a breast carcinoma that overexpresses ErbB2. The HFF cell line is a human foreskin fibroblast line that represents normal cells. Methods of testing are previously described in Ref. 7b.
12. This assay is a parallel artificial membrane permeation assay similar to those widely reported in the literature. For leading references, see: (a) Kansey, M.; Senner, F.; Gubernator *J. Med. Chem.* **1998**, *44*, 923; (b) Cheng, Y. Y.; Song, J. C.; Liu, H.; Pidgeon, C. *Adv. Drug Delivery Rev.* **1996**, *23*, 229.