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Synthesis and evaluation of aniline headgroups for alkynyl thienopyrimidine dual EGFR/ErbB-2 kinase inhibitors

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ABSTRACT

Aniline 'headgroups' were synthesized and incorporated into an alkynyl thienopyrimidine series of EGFR and ErbB-2 inhibitors. Potent inhibition of enzyme activity and cellular proliferation was observed. In certain instances, protein binding was reduced and oral exposure was found to be somewhat improved relative to compounds containing the reference aniline.

The inhibition of ErbB family receptor tyrosine kinases is an effective strategy for cancer therapy.[1] The ErbB family is comprised of four members, EGFR (ErbB-1), ErbB-2 (Her2/neu), catalytically inactive ErbB-3, and ErbB-4.[2] In addition to antibody therapy,[3] several small molecules have been approved or are under clinical investigation to treat diseases that are a result of aberrant signaling from one or more of these kinases. There are two clinically approved anilinoquinazoline inhibitors selective for EGFR inhibition (erlotinib[4] and gefitinib[5]) and one anilinoquinazoline (lapatinib[6]) which is an equipotent inhibitor of both EGFR and ErbB-2. Simultaneous inhibition of multiple ErbB family receptors may have significant therapeutic advantage, due to differing receptor expression patterns in human cancers and the cooperation of the ErbB receptors in inducing transformation.[7]

All of the small molecules approved to date for ErbB family inhibition are reversible kinase inhibitors. Irreversible inhibitors of the ErbB family have also been disclosed.[8] These inhibitors typically include a Michael acceptor in the molecule. We have recently reported potent alkynyl thienopyrimidine based ErbB family inhibitors that do not possess a typical Michael acceptor (Fig. 1) but nonetheless form a covalent bond with a conserved cysteine residue (C733 in EGFR, C805 in ErbB-2).[9]

It is known that the binding mode of quinazoline and related ErbB family inhibitors places the aniline portion (referred to hereafter as the 'headgroup') deep in the ATP binding site of the kinase, and that this can affect kinase selectivity.[10] In this communication, we report the effect of aniline 'headgroup' substitution on the activity of alkynyl thienopyrimidine ErbB family inhibitors.[11,12]

Headgroup design centered on three variations of the 4-(3-fluorobenzyloxy)-3-chloroaniline present in lapatinib: (1) additional benzyloxy substituted anilines, varying halo substitutions and incorporating heterocycles into the ring systems, (2) biaryl ether anilines and related compounds, removing the benzyl methylene, (3) fused bicyclic anilines. These changes were intended to produce dual EGFR/ErbB-2 inhibitors with reduced lipophilicity and protein

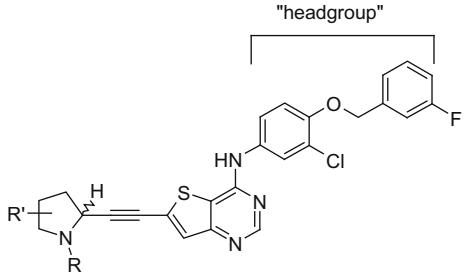

Figure 1. Previously reported covalent alkynyl thienopyrimidine EGFR/ErbB-2 inhibitors.

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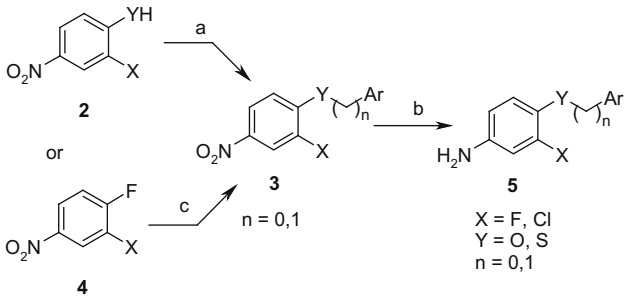
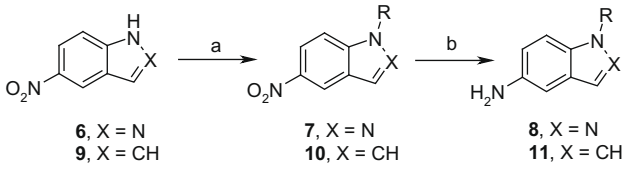


Scheme 1. Reagents and conditions: (a) 1—NaH, THF; 2—for $n = 1$: $BrCH_2Ar$, for $n = 0$: XAr (opt. Cu salt). (b) H_2, Pt/C, EtOH. (c) $HYCH_2Ar$ or HYAr, DMF, base.



Scheme 2. Reagents: (a) $ArCH_2X$, K_2CO_3 or $ArSO_2Cl$, Et_3N. (b) Pt/C, H_2 or Na_2S, EtOH.

binding relative to molecules containing the lapatinib headgroup. It was also felt that the benzyloxy group may be a potential metabolic liability.

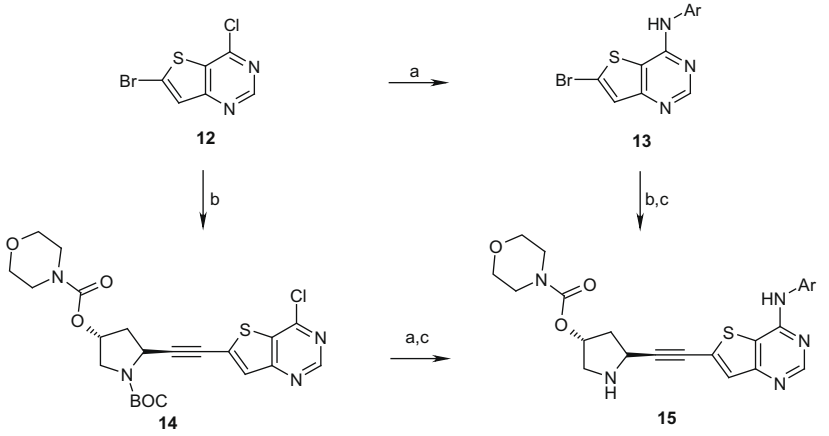
The synthetic route to benzyloxy substituted aniline headgroups and biaryl ether headgroups began with a sodium hydride mediated benzylation or arylation of nitro substituted phenols or thiophenols (**2**) (Scheme 1). Alternately, the nitro aromatic precursors were produced via a S_NAr displacement of the fluoride (**4**) with a benzyl alcohol or phenol. Selective reduction of the nitro group versus the aromatic halogen was accomplished by utilizing a catalytic hydrogenation with platinum on carbon to produce the desired aniline (**5**).

Fused bicyclic anilines were prepared by the route shown in Scheme 2. The appropriate nitroindazole (**6**) or nitroindole (**9**) was alkylated, acylated, or sulfonylated to afford substituted heterocycles (**7**, **10**). The nitro group was reduced using standard platinum catalyzed hydrogenation or treatment with sodium sulfide to give the desired bicyclic anilines (**8**, **11**).

The target molecules were constructed using one of two routes illustrated in Scheme 3. In the more commonly used method the aniline headgroups were combined with the known 6-bromo-4-chlorothieno[3,2-*d*]pyrimidine (**12**)[13] in the presence of catalytic acid. The bromides (**13**) were then subjected to a Sonogashira reaction with alkynes that have been described previously.[14] Final BOC deprotection provided the 6-ethynylthieno-[3,2-*d*]pyrimidin-4-anilines (**15**). Alternatively, the order of the reactions was switched, with the thienopyrimidine core (**12**) subjected to an initial Sonogashira coupling and then elaborated using a nucleophilic displacement as described above. Once again, a final deprotection of the pyrrolidine nitrogen generated the target molecules (**15**).

The target 6-ethynylthieno[3,2-*d*]-pyrimidin-4-anilines (**15**) were evaluated for EGFR and ErbB-2 potency using assays that have been described previously.[6,9,10] All molecules were also tested in cell proliferation assays, using cell lines that overexpress EGFR or ErbB-2.[15] These analogues have the potential to be irreversible inhibitors[9] by alkylating the conserved cysteine residue in the enzyme active site.[16] As a result of the time-dependent nature of these inhibitors, the IC_{50} values can change as a function of time. Therefore, the IC_{50} values presented are the apparent IC_{50}s based on the reaction time of the enzyme assay (40 min) and should not be used to draw conclusions about absolute potency. No consensus exists in the literature concerning the use of covalent inhibitors. Although toxicity risks may exist, it has been suggested that selective covalent binding may be a positive contributor to compound potency, due to prolonged inhibition of the enzyme and the ability to effectively compete with high concentrations of a natural ligand, in this case ATP.[17]

A morpholinocarbamate at the pyrrolidine C4 was chosen as a standard group for this study due to its favorable contribution to enzyme and cellular potency, coupled with a reduced degree of covalent reactivity and moderate pharmacokinetics.[14b] Compound **15a**, containing the reference fluorobenzyloxychloro-aniline, demonstrated potent inhibition of EGFR and ErbB-2 and displayed 3-fold better anti-proliferative activity on the ErbB-2 overexpressing BT474 cell line versus the EGFR driven HN5 line (Table 1). In general, headgroup substitution had only a small effect on the level of covalent reactivity toward the kinase. Most compounds showed rather modest covalent modification of EGFR enzyme, less than 20% after 3 h. However, notable exceptions were observed. For example, the napthyl derived compound **15e** displayed nearly complete modification of EGFR after only 3 h, and **15d**, **15g**, and **15k** were also significant covalent modifiers in this time frame. Interestingly, the compounds with the highest levels of covalent modification tended to be among the less potent compounds in the enzyme assays. The time-dependent nature of the inhibitor has the potential to complicate the interpretation of enzyme and cellular inhibition results. As the cellular assays are performed over 3 days, it is possible that all compounds may, in fact, be significant covalent modifiers of the kinases in this longer time frame, leading



Scheme 3. Reagents and condition: (a) $ArNH_2$, HCl, *i*-PrOH, 80 °C. (b) RCCH, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, 60 °C. (c) TFA, CH_2Cl_2.

Table 1
In vitro evaluation of inhibitors

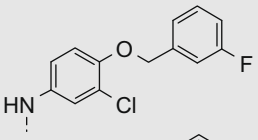
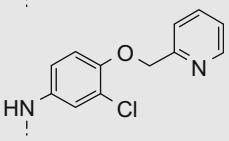
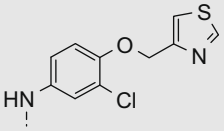
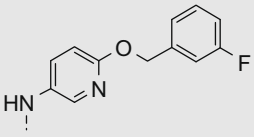
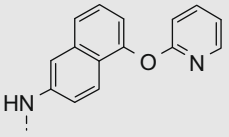
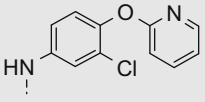
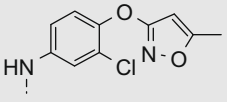
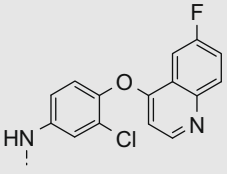
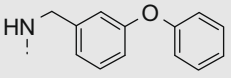
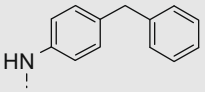
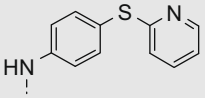
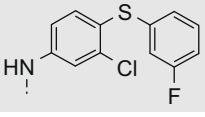
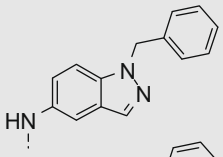
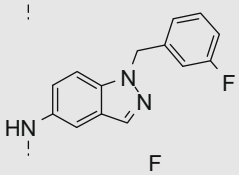
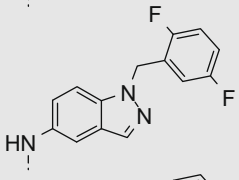
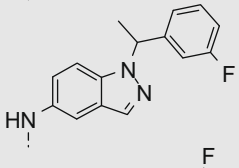
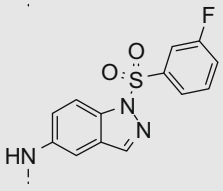
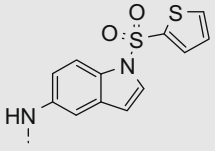
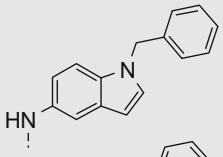
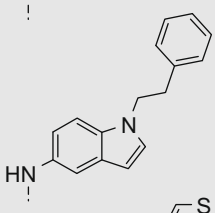
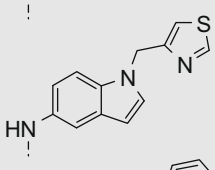
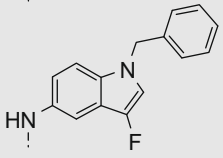
Compound	Ar	EGFR[a,b]	ErbB-2[a]	HN5[c]	BT474[c]
15a		80 (3)	80	260	90
15b		50[e] (7)	30[e]	240	60
15c		120 (2)	60	440	70
15d		2600 (43)	450	490	250
15e		1050 (89)	260	120	100
15f		190[e] (14)	60[e]	280	80
15g		400 (74)	370	1450	130
15h		280 (0)	180	390	140
15i		180 (0)	87	3470	2600
15j		240 (14)	60	270	70
15k		1650 (48)	220	380	100
15l		630 (0)	800	310	150

Table 1 (*continued*)

Compound	Ar	EGFR[a,b]	ErbB-2[a]	HN5[c]	BT474[c]
15m		80 (4)	50	70	20
15n		30 (6)	30	70	30
15o		33 (ND[d])	35	250	10
15p		120 (0)	130	70	130
15q		230 (0)	85	540	110
15r		150[e] (16)	120[e]	630	120
15s		90 (10)	60	190	110
15t		3160[e] (23)	2660[e]	2020	4850
15u		680 (0)	180	550	120
15v		240 (10)	210	320	120

[a] Apparent IC_{50} in nM, HTRF (Homogeneous Time-Resolved Fluorescence) assay format; mean values, $n \geqslant 2$.
[b] Number in parentheses is % covalent modification of EGFR after 3 h. See Ref. [14].
[c] IC_{50} in nM; mean values, $n \geqslant 2$.
[d] ND = not determined.
[e] SPA (Scintillation Proximity Assay) assay format.

to smaller potency differences than might have otherwise been anticipated.

The distal heterocycles **15b** and **15c** demonstrated a relatively similar enzymatic and cellular profile to **15a**, with **15b** being slightly more potent against the enzymes. However, the use of a pyridyl moiety as a replacement for the aniline phenyl group (**15d**) produced a compound that was significantly less potent against the kinases. A napthyl group in this position (**15e**) was also a less potent inhibitor of the kinases versus **15a**. Interestingly, for both **15d** and **15e** the cellular proliferation results, particularly for the EGFR driven HN5 line, showed far better potency than the enzyme assays would have predicted. This is likely a result of the increased covalent modification of the enzyme by these compounds. Eliminating the benzylic methylene of **15b** by use of a biaryl ether (**15f**) produced a compound that was somewhat selective for enzymatic ErbB-2 inhibition. Isoxazole (**15g**) and the larger quinoline (**15h**) based replacements of the pyridyl of **15f** were less successful. Interestingly, adding a methylene spacer between the thienopyrimidine and the phenyl ring of the headgroup via use of a benzylamine[18] produced a compound, **15i**, that was a relatively potent enzyme inhibitor, but performed poorly in the cellular proliferation assays. Replacing the biaryl ether oxygen with a methylene spacer (**15j**)[18] is tolerated in the active site, but the thioethers **15k** and **15l** showed significantly reduced inhibition of the kinases relative to **15a**.

Bicyclic derived aniline headgroups, **15m–v**, also enabled robust inhibition of EGFR and ErbB-2 in the enzyme and cellular proliferation assays (Table 1). Indeed, the indazole compounds **15m** and **15n** proved superior to the reference compound **15a**. Interestingly, adding a second halogen to the distal ring (**15o**), simultaneously enhanced ErbB-2 driven cellular potency and reduced EGFR driven potency, giving a 20-fold preference for ErbB-2 inhibition in the cellular assays. Changes in the benzylic linker between the heterocycle and the distal phenyl ring met with only limited success. A methyl group (**15p**) or sulfonyl group (**15q** and **15r**) reduced potency relative to **15n**. Indole based heterocycles were generally inferior to the indazoles. For example, indole **15s** proved inferior to **15m** in the cellular assays, although the potency in the kinase inhibition assays was similar to **15a** and **15m**. Extending the distal ring away from the indole, in **15t**, was very detrimental to kinase activity. Although the use of a distal heterocycle was quite successful in the phenyl based series (e.g., **15b** and **15c**), the use of a thiazole in **15u** displayed reduced potency in the indole series. Finally, the inclusion of a halogen on the indole ring (**15v**) also failed to improve kinase activity.

Selected compounds were evaluated for in vitro and in vivo ADMET properties,[19] including protein binding and oral exposure (Table 2). Of note is the distinctly reduced lipophilicity of the heterocyclic headgroups relative to the substituted phenyl compounds. The most dramatic improvement was realized with the inclusion of a 2-pyridyl moiety in **15b** or a thiazole in **15c**, both of which lower the *c*Log*P* of the inhibitors by over 1.5 units relative to the fluorophenyl group in reference compound **15a**. Other substitutions produced similar improvements. The bicyclic indazole containing compounds **15m** and **15n** exhibited 1 log unit improvements in lipophilicity. However, the presence of the quinoline moiety of **15h** or the sulfur linker of **15f** resulted in compounds that were more lipophilic than the reference compound **15a**. The retention time on an HPLC column containing immobilized HSA (human serum albumin) was used as a surrogate to measure the propensity of the compounds to bind to plasma proteins.[20] Several of the aniline headgroups displayed somewhat reduced protein binding. Notably, the heterocyclic derivatives **15b** and **15c**, in addition to the indazole **15n**, were improved over **15a**. Indeed, the 3% improvement in protein binding for **15c** and **15n** represents a potential doubling of the free fraction for these inhibitors relative to **15a**. Although many of the less lipophilic compounds did exhibit improved protein binding, there was no direct relationship observed between *c*Log*P* and protein binding in this series.[19]

Mouse oral exposure was also determined for several compounds (measured for 6 h following a 10 mg/kg oral dose). Although all compounds in this series tend toward poor to moderate murine exposure, aniline substitutions were found to affect the oral exposure. The reference compound **15a** had an oral DNAUC[21] of 91 ng h/mL/mg/kg. Pyridyl compound **15b** displayed a modest improvement. However, eliminating the methylene group actually led to a lower value for **15f**. The fused bicyclic headgroup compounds were also found to have suitable oral exposure. The indazole compound **15m** displayed exposure comparable to **15a**, while **15n** showed somewhat reduced oral exposure relative to **15a** or **15m**.

In summary, we have synthesized and evaluated a number of thieno[3,2-*d*]pyrimidines with changes in the aniline headgroup. These compounds are effective dual inhibitors of EGFR and ErbB-2 kinases and also display potent, selective inhibition of cellular proliferation. While most compounds, including **15b** and **15m**, displayed a slight preference (2- to 10-fold) for inhibition of ErbB-2 driven cellular proliferation, selected compounds, for example, **15e** and **15p**, were found to be essentially equipotent for EGFR and ErbB-2 driven cellular proliferation. In some cases, modest improvements in the ADMET profiles of the compounds relative to the reference aniline were noted. These improvements were realized by replacing phenyl rings with heterocyclic moieties (i.e., **15b**) to give compounds equal or superior to **15a** in potency, lipophilicity, and oral exposure. Bicyclic headgroups exemplified by **15m** were also found to reduce lipophilicity and protein binding.

Table 2
ADMET data for selected inhibitors

Compound	*c*Log*P*[a]	% Protein bound[b]	DNAUC[b,c]
15a	6.11	98.0	91
15b	4.46	96.9	120
15c	4.31	94.9	ND
15f	4.83	96.7	52
15h	6.42	97.0	ND
15j	5.79	96.4	ND
15l	6.98	97.6	ND
15m	5.02	97.3	98
15n	5.16	95.0	51
15s	5.96	ND	ND

[a] Calculated using ACD software, version 8.
[b] ND = not determined.
[c] DNAUC = dose normalized area under the curve; represented in ng h/mL/mg/kg; derived from murine oral PK experiment, measured for 6 h following a 10 mg/kg oral dose.

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