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Dual EGFR/ErbB-2 inhibitors from novel pyrrolidinyl-acetylenic thieno[3,2-*d*]pyrimidines

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ABSTRACT

A novel class of substituted pyrrolidinyl-acetylenic thieno[3,2-*d*]pyrimidines has been identified that are potent and selective inhibitors of both EGFR/ErbB-2 receptor tyrosine kinases. The inhibitors are found to display a range of enzyme and cellular potency and also to display a varying level of covalent modification of the kinase targets. Selected molecules, including compound **15h**, were found to be potent in enzymatic and cellular assays while also demonstrating exposure in the mouse from an oral dose.

Overexpression of the epidermal growth factor receptor tyrosine kinases[1] EGFR (ErbB-1) and/or HER-2 (ErbB-2) has been implicated in a variety of cancers.[2] The ability to disrupt the ErbB-family signaling pathway either by monoclonal antibody therapy[3] or small molecule ATP-competitive kinase inhibitors has yielded novel anticancer agents.[4] A common chemical class of the initial TK inhibitors is the 4-anilinoquinazolines. This class has provided the reversible ErbB-family inhibitors gefinitib,[5] erlotinib,[6] and lapatinib.[7] The latter possesses a novel anilino-aryl head-group that yields dual EGFR/ErbB-2 potency (Fig. 1).[8] The quinazoline ErbB-family inhibitor canertinib[9] contains an acrylamide Michael acceptor that forms a covalent bond with a Cys residue (Cys^{733} in EGFR and Cys^{805} in ErbB-2).[10] A potential advantage of a covalent agent is the prolonged inhibition of the enzyme, thereby competing with the high intracellular concentrations of ATP to effectively impair the ErbB-signaling pathway.[11]

In an effort to find effective dual inhibitors of EGFR and ErbB-2 from a distinct structural class relative to lapatinib, we discovered that pyrrolidinyl-acetylenic thienopyrimidines (**1**) are potent and selective EGFR/ErbB-2 enzyme inhibitors, with the ability to react irreversibly with the Cys^{733} of EGFR.[12] Previous SAR exploration had indicated that analogs with a basic propargylic nitrogen tend to show greater covalent reactivity.[12] This paper describes our further synthetic explorations and the in vitro characterization of selected members of this class of receptor tyrosine kinase inhibitors.

As shown in Scheme 1, the synthesis of the analogs begins with the known acetylenic pyrrolidines (**2**, **3**)[13] which were separately coupled to the known thienopyrimidinyl bromide **4**[14] via Sonogashira reaction to afford the desired alkynes **5, 6**. Subsequent deprotection with TFA in CH_2Cl_2 yielded the desired analogs **7** and **8**. In order to assess the role of the basic pyrrolidine nitrogen

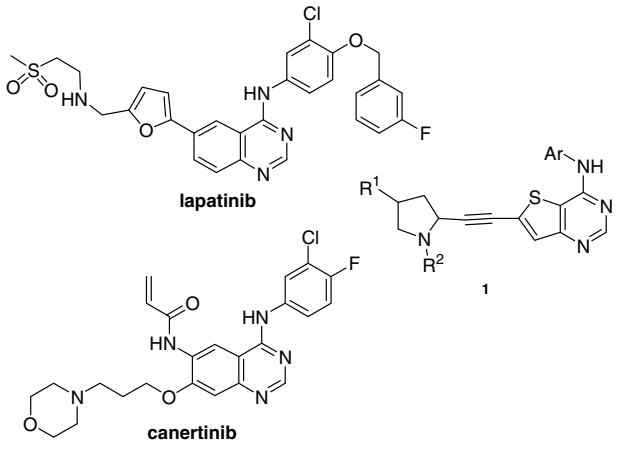

Figure 1. Quinazoline and thienopyrimidine-based ErbB-family inhibitors.

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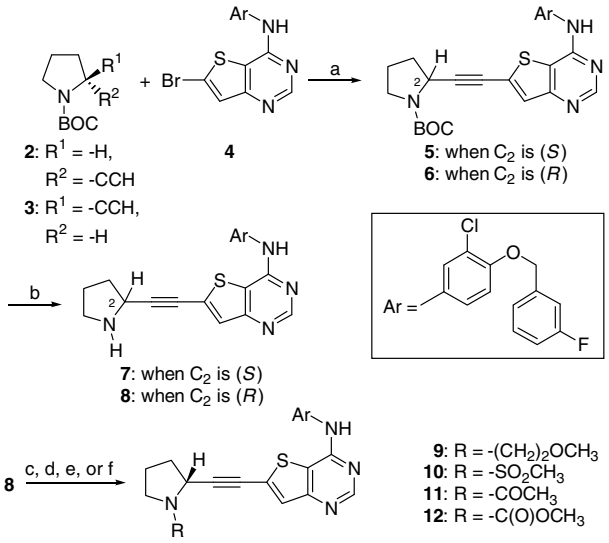


Scheme 1. Reagents and conditions: (a) $(Ph_3P)_2PdCl_2$, CuI, Et_3N, 60 °C, THF ; (b) TFA, 0 °C to rt, CH_2Cl_2; (c) 2-chloroethyl methyl ether, K_2CO_3, sealed tube, 100 °C, CH_3CN; (d) for the sulfonamide: MsCl, 0 °C, CH_2Cl_2; (e) for the acetamide, Ac_2O, 0 °C, CH_2Cl_2; (f) for the carbamate: methyl chloroformate, 5 N NaOH, 0 °C, Et_2O.

present in **8**, we performed a series of chemoselective reactions, in which the pyrrolidine nitrogen was alkylated (**9**), sulfonylated (**10**), acylated (**11**), or carbamoylated (**12**).

Analogs were initially assayed for their intrinsic potency against EGFR and ErbB-2. Since these analogs possess the ability to covalently modify either EGFR or ErbB-2, it should be noted that the IC_{50} values are time-dependent and may vary significantly as a function of incubation time.[15] The data presented is the apparent IC_{50} values obtained with a 40-min reaction. The analogs were further progressed to assess their inhibition of cellular proliferation against two transformed cell lines, whose proliferation was driven by the targets of interest: EGFR (HN5) and ErbB-2 (BT474).[16]

Comparison of the unsubstituted pyrrolidines **7** and **8** revealed that **8** was clearly more potent on the enzyme and also somewhat more effective at inhibiting cellular proliferation (Table 1). Interestingly, pyrrolidine **8** was also significantly more reactive, modifying the EGFR kinase 47% after 3 h, versus 15% for pyrrolidine **7**.

Functionalization of the pyrrolidine nitrogen with electron withdrawing substituents yielded analogs with generally reduced cellular efficacy (**7** vs **10, 12**), despite roughly similar enzyme potencies. Interestingly, amide **11** was a more potent inhibitor of EGFR than **7**. Reducing the basicity of the pyrrolidine nitrogen in analogs **10, 11**, and **12** also abrogated their ability to covalently

Table 1
EGFR and ErbB-2 kinase and cell proliferation inhibition

Analog	Enzyme IC_{50}[a,b] (nM)		Cellular IC_{50}[a] (nM)		EGFR mod.[c] (%)
	EGFR	ErbB-2	HN5	BT474	
7	109	54	536	205	15
8	7	13	238	94	47
9	130	110	190	110	19
10	120	88	550	650	0
11	58	38	37	670	0
12	140	120	590	1700	0

[a] Average values, $n > 2$.
[b] Since these analogs possess the ability to covalently modify either EGFR, ErbB-2 or ErbB-4 it should be noted that the IC_{50} results are time-dependent and may vary significantly as a function of incubation time. The data presented is the apparent IC_{50} obtained with the 40-min reaction.
[c] Value represents % covalent modification after 3 h. For experimental details, see Ref. 12.

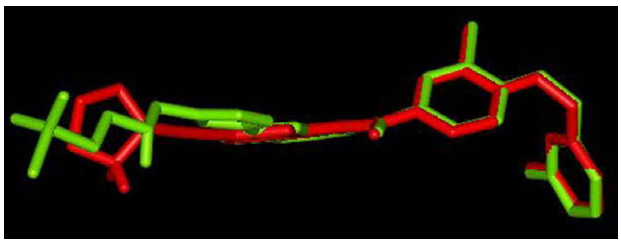
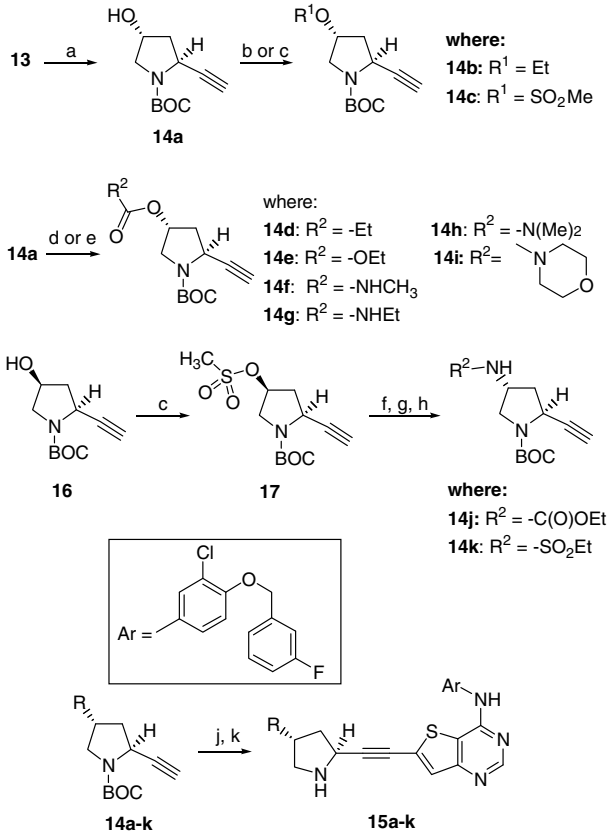


Figure 2. Superimposition of a possible binding conformation of lapatinib (green) and **8** (red) docked into ErbB-2.

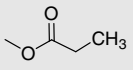
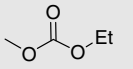
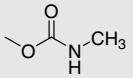
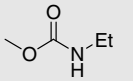
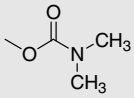
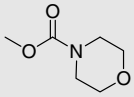
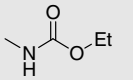
modify EGFR. Therefore, while derivatization of the pyrrolidine nitrogen yielded potent enzyme inhibitors, these analogs were generally less effective at inhibiting cell proliferation than the parent analogs **7** and **8**, particularly against the BT474 line.

An overlay of lapatinib and **8**, each docked into a model of ErbB-2, revealed that functionalization of C_4 of the pyrrolidine ring might access similar chemical space as the sulfone tail present in lapatinib (Fig. 2). We found that the 4-hydroxy group of the commercially available *N*-BOC-*trans*-4-hydroxy-L-proline methyl ester (**13**) afforded access to a variety of such analogs. The ester **13** was treated sequentially with DIBAL-H, then with the modified Seyferth–Gilbert reagent[17] under previously disclosed one-pot conditions,[18] to afford the key alkyne intermediate **14a** (Scheme 2). Generation of the sodium alkoxide derived from **14a** followed by addition of the appro-



Scheme 2. Reagents and conditions: (a) 1—DIBAL-H, −78 °C, toluene; then, MeOH; 2—MeC(=O)C(=N_2)P(=O)(OCH_3)$_2$, K_2CO_3, 0 °C to rt; (b) NaH, 0 °C, DMF; then EtI, rt; (c) MsCl, Et_3N, 0 °C to rt, CH_2Cl_2; (d) for **14d** and **14e**: $CH_3CH_2C(O)Cl$ or EtOC(O)Cl, Et_3N, 0 °C to rt, CH_2Cl_2; (e) for **14f–i**: isocyanate, DMAP, 60 °C, DMF; (f) NaN_3, DMF, 70 °C; (g) Ph_3P, H_2O, 25 °C, THF; (h) for **14j**: EtOC(O)Cl, Et_3N, 0 °C to rt; (i) for **14k**: MsCl, Et_3N, 0 °C to rt, CH_2Cl_2; (j) **4**, $(Ph_3P)_2PdCl_2$, CuI, Et_3N, 60 °C, THF; (k) TFA, 0 °C to rt, CH_2Cl_2.

Table 2
Comparison of enzyme and cellular potency

Analog	R=	Enzyme IC_{50}[a,b,c] (nM)		Cellular IC_{50}[a] (nM)	
		EGFR[c]	ErbB-2	HN5	BT474
15a	–OH	14 (ND)[d]	11	160	39
15b	–OEt	115 (21%)	98	99	45
15c	$-OSO_2CH_3$	81 (ND)	40	380	86
15d		63 (0%)	90	52	18
15e		141 (0%)	135	200	34
15f		50 (17%)	20	58	28
15g		32 (24%)	43	95	30
15h		28 (11%)	68	100	30
15i		65 (5%)	84	160	30
15j		60 (41%)	78	113	40
15k	$-NHSO_2Et$	50 (26%)	87	127	42

[a] Average values $\geqslant 2$.
[b] Number in parentheses is % covalent modification of EGFR after 3 h.
[c] See footnote b in Table 1.
[d] ND, not determined.

priate alkyl halide provided **14b**. Mesylation of **14a** with MsCl and Et_3N afforded sulfonate **14c**. The hydroxyl moiety was also functionalized with either acid chlorides or chloroformates affording alkynes **14d** and **14e**, respectively. Heating **14a** with an isocyanate in presence of DMAP yielded carbamates **14f–i**. In addition to oxygen-based functional groups, we also explored other substituents at C_4 of the pyrrolidine. Mesylation of the 4*R* antipode of **14a**[19] with MsCl and Et_3N afforded mesylate **17**, and subsequent reaction with NaN_3 followed by a Staudinger reduction provided a crude aminopyrrolidine. This aminopyrrolidine was treated with an isocyanate to give carbamate **14j**, or with methanesulfonyl chloride to yield the sulfonamide **14k**. The individual functionalized pyrrolidine alkynes **14a–k** were then coupled under Sonogashira reaction conditions with bromide **4**. Deprotection of the BOC-group with TFA afforded the desired analogs (**15a–15k**).

The initial lead for this SAR endeavor, **15a** (R = (*R*)-OH), was quite potent relative to **7** on both the enzyme and on transformed cell lines, particularly the ErbB-2 overexpressing BT474 line (Table 2); unfortunately, the murine oral exposure was quite low (DNAUC = 9 ng h/mL/mg/kg). A simple alkyl ether **15b** managed to retain cellular activity, but also suffered from poor oral exposure. A survey of other functionalities such as sulfonate (**15c**), ester (**15d**), or carbonate (**15e**), also provided analogs with generally comparable cellular activity to **15a**. Carbamate **15f** retained enzyme and cell activity but possessed only marginally better oral exposure (DNAUC = 18 ng h/mL/mg/kg). In contrast, the ethyl carbamate **15g** provided significantly increased mouse oral exposure (DNAUC = 61 ng h/mL/mg/kg) to complement its potency. Secondary carbamates were also explored. The dimethyl amino carbamate **15h** demonstrated potent inhibition in the enzyme and cell assays, displayed reduced covalent modification as compared with **7** or **15g**, and also showed good oral exposure in the mouse (DNAUC = 93 ng h/mL/kg). However, the morpholine analog **15i** showed somewhat reduced potency compared with **15g**, particularly on the EGFR enzyme and the HN5 (EGFR overexpressing) cell line. The amine attached sulfonamide and carbamate, **15j** and **15k**, respectively, were also found to be potent in the primary assays and were more reactive in the covalent modification assay than many of the other C_4-substituted analogs.

In conclusion, we describe novel acetylenic pyrrolidine thieno[3,2-*d*]pyrimidines that are potent, selective dual EGFR/ErbB-2 enzyme inhibitors with good anti-proliferative activity against EGFR and ErbB-2 overexpressing tumor lines. In addition, we have presented our SAR findings around both the pyrrolidine nitrogen and the C_4-substituent. The C_4-carbamoyl analog **15g** was found to have desirable enzyme and cell potency while displaying good mouse oral PK. In addition, the secondary carbamate **15h** was found to be among the most potent molecules in the series and also demonstrated exposure from an oral dose in the mouse. On the basis of these encouraging results, the SAR of C_4-carbamates has been further developed and the pre-clinical developability of these compounds has been probed. The results will be disclosed in a future communication.

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