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Thienopyrimidine-based dual EGFR/ErbB-2 inhibitors

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ABSTRACT

Two new series of potent and selective dual EGFR/ErbB-2 kinase inhibitors derived from novel thienopyrimidine cores have been identified. Isomeric thienopyrimidine cores were evaluated as isosteres for a 4-anilinoquinazoline core and several analogs containing the thieno[3,2-*d*]pyrimidine core showed anti-proliferative activity with IC_{50} values less than 1 μM against human tumor cells in vitro.

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ErbB family type I receptor tyrosine kinases (EGFR, ErbB-2, ErbB-3, and ErbB-4) play a critical role in mediating growth factor signaling.[1] After ligand binding, these RTKs propagate signaling through formation of hetero- and homo-dimers with heterodimerization of ErbB-2 preferred for maximum signaling.[2] Overexpression EGFR and ErbB-2 has been associated with oncogenic activity such as unregulated cell growth, proliferation, differentiation and survival.[3] In fact, elevated levels of these receptors have been observed in a number of human cancers including breast, lung, colon, and prostate cancer.[4] Disruption of ErbB family signaling by treatment with monoclonal antibodies (either anti-EGFR or anti ErbB-2 MAbs) or small molecule inhibitors of EGFR has led to significant advances in cancer treatment and has largely validated this targeted therapy approach.[2,3,5]

Two small molecule EGFR-selective inhibitors, Iressa™ (gefitinib,[6] **1**) and Tarceva™ (erlotinib,[7] **2**) have recently been approved by the FDA for treatment of certain cancers over-expressing EGFR (Fig. 1).[8] Tykerb™ (lapatinib, **3**) is the first example of a dual EGFR/ErbB-2 inhibitor also recently approved by the FDA.[9] It was postulated during the development of Tykerb™ that targeting both

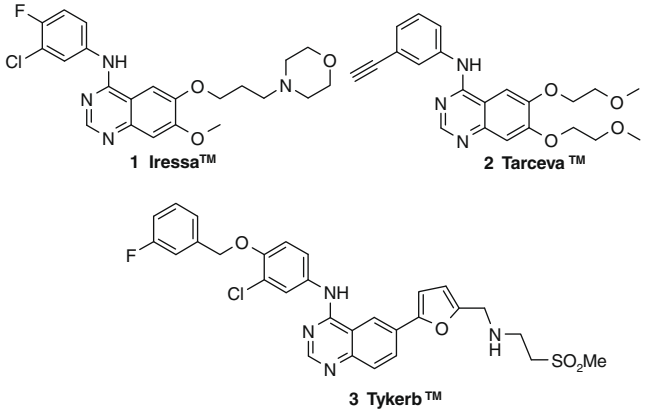

Figure 1. Examples of ErbB family inhibitors. Iressa™ and Tarceva™ are FDA-approved potent EGFR inhibitors. Tykerb™ is a potent inhibitor of both EGFR and ErbB-2, also recently approved by the FDA.

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ErbB-2 and EGFR with a dual inhibitor may offer advantages relative to selective inhibition of either enzyme.[8b,9]

Iressa™ (**1**), Tarceva™ (**2**), and Tykerb™ (**3**) are selective, ATP-competitive inhibitors that contain a 4-anilinoquinazoline scaffold as a common structural feature. Inhibition of ErbB-2 activity with Tykerb™ is approximately 100-fold greater than that of either Iressa™ or Tarceva™. It is thought that the 4-benzyloxy aniline moiety present in Tykerb™ is the structural feature responsible for the potent ErbB-2 inhibitory activity observed with that molecule.[10]

In an effort to develop novel non-quinazoline dual inhibitors of EGFR and ErbB-2, thieno[3,2-*d*]pyrimidine and thieno[2,3-*d*]pyrimidine cores were evaluated (Core A and Core B, Scheme 1). The 4-benzyloxyaniline portion of Tykerb™ was conserved in order to maintain sufficient ErbB-2 activity, and a survey of both thienopyrimidine cores combined with a variety of heterocycle attachments was performed. The structure–activity relationships and biological evaluation of these compounds are described herein.[11]

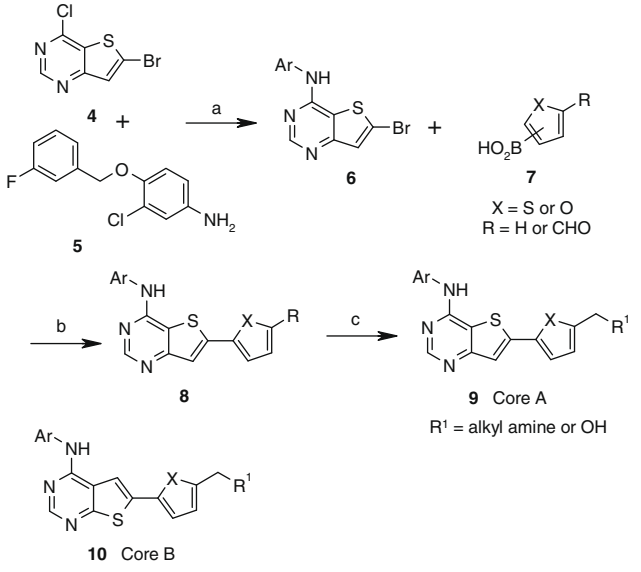
Thieno[3,2-*d*]pyrimidine (Core A) and thieno[2,3-*d*]pyrimidine (Core B) analogs with furan and thiophene linkers were synthesized according to the sequence shown in Scheme 1. Nucleophilic displacement of known 6-bromo-4-chlorothieno[3,2-*d*]pyrimidine (**4**) with aniline **5** provided bromothienopyrimidine intermediate **6**.[12] Suzuki coupling of bromide **6** with boronic acids **7** gave heteroaryl (R = H) or heteroaryl aldehyde (R = CHO) products **8**. Finally, reduction or reductive amination of the aldehyde functionality yielded the desired analogs **9** (Core A). The corresponding thieno[2,3-*d*]pyrimidine analogs **10** (Core B), could be made from the same reaction sequence starting with 6-bromo-4-chlorothieno[2,3-*d*]pyrimidine.

Pyrrole-linked thienopyrimidines were prepared according to Scheme 2. Suzuki coupling followed by thermal BOC-deprotection gave pyrrole **13**. Subsequent reaction with Vilsmeier reagent gave the desired pyrrole-2-aldehyde **14**, which was further transformed into secondary amines **15** by reductive amination.[13] Alternatively, tertiary amines were obtained in one step from pyrrole **13** by a Vilsmeier-type reaction followed by in situ reductive amination with a secondary amine. An analogous sequence was carried out to arrive at compounds **16** (Core B).

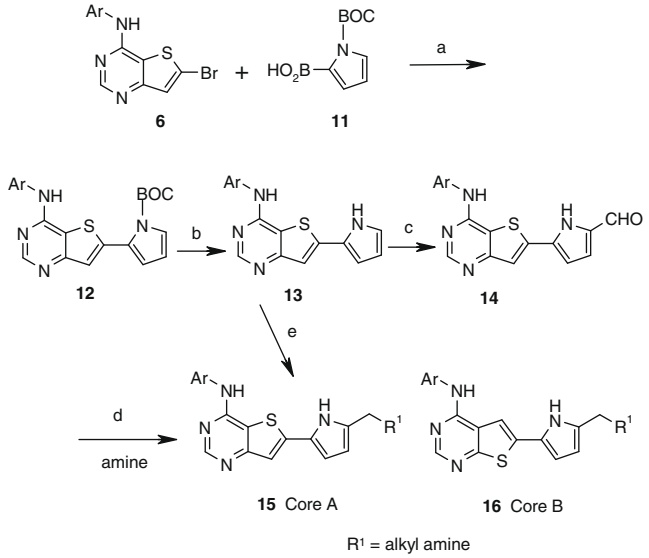
By choosing to maintain the Tykerb™ head group, optimized for inhibition of both ErbB-2 and EGFR, we were able to focus SAR efforts on the thienopyrimidine core isomer, heteroaryl linker and hydrophilic side chain. Table 1 shows enzyme inhibition data for thieno[3,2-*d*]pyrimidine (A) with furan, thiophene and pyrrole linkers and a variety of hydrophilic side chains.[14] Compounds containing Core A were potent inhibitors of EGFR, despite changes in the hydrophilic side chain, heteroaryl functionality (see compounds **17**–**23**, **25**, and **29**) and orientation (compare **22** and **23**). Compounds containing a furan linker with a hydrophilic side chain (**18**–**23**) were more potent inhibitors of ErbB-2 than unsubstituted furan **17**. Interestingly, changing the heteroaryl linker from a furan or thiophene, containing an H-bond acceptor atom (**17** and **24**), to a pyrrole, containing an H-bond donor (**26**), resulted in reduced potency at EGFR but not for ErbB-2. Also, addition of hydrophilic side chains to a pyrrole linker did not improve potency at ErbB-2 to the same degree that the addition enhanced potency for both furan and thiophene analogs (see **17**, **22**, **24**, **25**, **26**, and **29**).

Table 2 shows enzyme data for a similar series of analogs containing thieno[2,3-*d*]pyrimidine (Core B). Compounds in this series were generally less potent inhibitors of EGFR compared to analogs containing Core A (compare **22** and **32**, **25** and **35**, **29** and **39**). Benzyl amine substitution (**31**) was not tolerated in ErbB-2 and thiophene linker orientation had a major impact on both EGFR and ErbB-2 potency (compare **34** and **35**). Compounds **30** and **32** were significantly more effective dual inhibitors of EGFR and ErbB-2 compared to their Core A counterparts **17** and **22**. This level of enzyme inhibition is similar to that reported for Tykerb™ (**3**), 11 nM and 9 nM, respectively.[9d]

Figures 2 and 3 show molecular models of both thienopyrimidine isomers **22** and **32**, containing the furan linker and pendant amine side chain of **3**, overlayed with a crystal structure of **3** in EGFR. The benzyloxy aniline head group of molecules **3**, **22** and **32** neatly fills the back pocket of the ATP binding region of EGFR.[11] The quinazoline (**3**) or thienopyrimidine (**22** and **32**) functions as point-binder to the hinge region and the heteroaryl linker and hydrophilic side chain extends into the solvent exposed region. The trajectory of the amine-containing side chain appears to be a key difference when comparing both thienopyrimidine core isomers A and B. Core B (**32**, Fig. 3) has a very high degree of overlap with **3**. This is in support of the observation that **32** has similar EGFR and ErbB-2 enzyme potencies to that obtained with **3**.

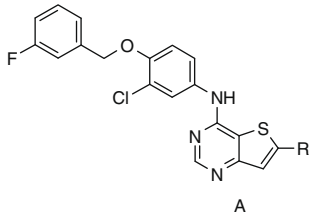


Scheme 1. General synthetic route. Reagents and conditions: (a) *i*-PrOH, 60 °C, 80–100%; (b) Pd(dppf)Cl_2, DMF:toluene 1:1 or DMA, 2 M Na_2CO_3, 27–64%; (c) AcOH, DCM:MeOH 2:1, $NaBH(OAc)_3$, 37–67%.

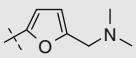
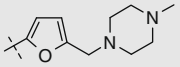
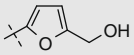
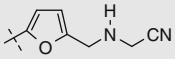
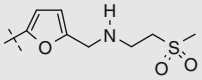
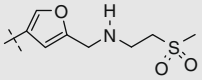
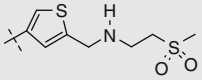
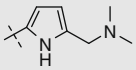
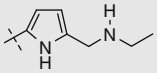
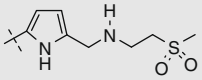


Scheme 2. General synthetic route. Reagents and conditions: (a) Pd(dppf)Cl_2, DMF:toluene 1:1 or DMA, 2 M Na_2CO_3, 64–96%; (b) DMA, 150 °C, 96%; (c) i—Vilsmeier reagent, DMF; ii—10% NaOH, 19–46%; (d) 1° amine, AcOH, DCM:MeOH 2:1, $NaBH(OAc)_3$, 46–79%; (e) formaldehyde, 2° amine, AcOH, 99%.

Table 1
Enzyme activities of compounds containing core A

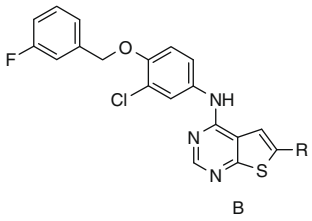


A

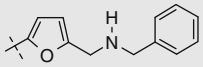
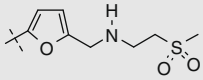
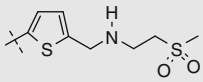
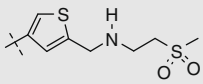
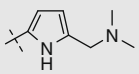
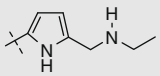
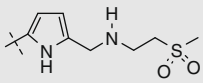
Compound	R	EGFR IC_{50}[a]	ErbB-2 IC_{50}[a]
17		10	709
18		2	146
19		5	250
20		3	122
21		3	68
22		1	71
23		4	86
24		8	113
25		3	31
26		68	121
27		4	76
28		6	68
29		7	113

[a] Mean values in nanomolar, >1 determination.

Table 2
Enzyme activities of compounds containing core B



B

Compound	R	EGFR IC_{50}[a]	ErbB-2 IC_{50}[a]
30		15	14
31		54	898
32		12	6
33		9	43
34		96	95
35		304	299
36		115	203
37		68[b]	246[b]
38		121	111
39		138	153

[a] Mean values in nanomolar, >1 determination.
[b] One determination.

Another key conformational difference between thienopyrimidine molecules and quinazolines such as **3** is the presence of dipole–dipole interactions within the molecule that favor the furan oxygen atom orienting in the opposite direction to the sulfur atom in the thienopyrimidine core. Figure 2 shows that not only does Core A (**22**) orient the hydrophilic tail in a different trajectory than **3**, but it also forces the furan oxygen atom in the opposite direction. Additionally, pyrroles (**26**–**29** and **36**–**39**) may prefer an intramolecular hydrogen bond to the sulfur of the thienopyrimidine and orient opposite their furan or thiophene counterparts

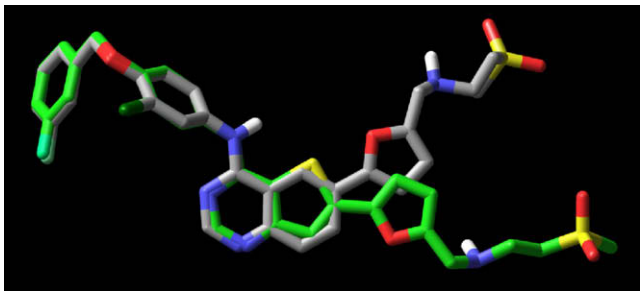


Figure 2. Overlay of **22** (green, Core A) model with crystal structure of **3** (gray) in EGFR.

Representative compounds from both the Core A and Core B series were also evaluated in cellular proliferation assays. (Table 3)[15] Unsubstituted furan and thiophene analogs **17**, **24**, **30**, and **33**, from both the Core A and B series showed minimal potency

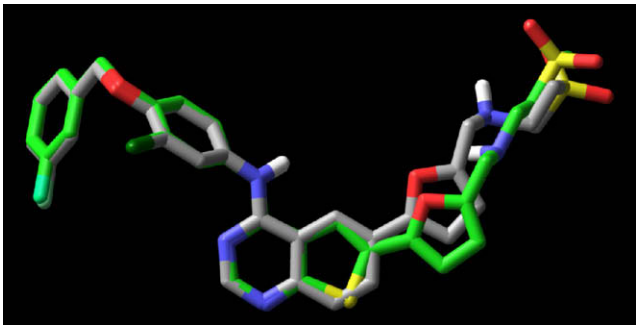


Figure 3. Overlay of **32** (green, Core B) model with crystal structure of **3** (gray) in EGFR.

Table 3
Inhibition of cellular proliferation

Compound (Core)	HN5 IC_{50}[a] (μM)	BT474 IC_{50}[a] (μM)	HFF IC_{50}[a] (μM)
17 (A)	8.09	21.25	>30.00
18 (A)	1.22	1.88	4.46
20 (A)	0.10[c]	0.72[c]	9.39[c]
22 (A)	0.41	0.42	>30.00
23 (A)	0.14[c]	0.73[c]	7.95[c]
24 (A)	2.19	5.77	10.74
25 (A)	0.10[c]	0.60[c]	>30.00[c]
26 (A)	0.57[c]	0.54[c]	2.37[c]
29 (A)	0.77	0.63	3.73
30 (B)	>30.00	>30.00	>30.00
32 (B)	0.42	0.42	19.54
33 (B)	10.08	3.22	9.72[b]
36 (B)	2.71[c]	1.98[c]	>3.00[c]

[a] Values are means of two experiments, CTG assay format.
[b] $n = 1$.
[c] MEB assay format.

in proliferation assays in cancer cell lines driven by over-expression of EGFR (HN5) and ErbB-2 (BT474) even though they all had reasonable levels of target potency at the enzyme level. For comparison, **3** has a reported IC_{50} of 120 nM and 100 nM on HN5 and BT474 cancer cell lines, respectively.[9d] Conversely, unsubstituted pyrrole **26** demonstrated sub-micromolar potency despite having similar or less potent enzyme inhibition compared to **17**, **24**, and **30**. Pyrroles **26** and **29** did show more potent inhibition of normal cell proliferation (HFF) compared to furan analogs **17** and **22**. However, inhibition of intracellular autophosphorylation with pyrroles **26** and **29** probed by an ErbB-2 DELFIA assay[16] did show IC_{50}s consistent with the BT474 anti-proliferative activity observed, 826 nM and 431 nM, respectively. The presence of an H-bond donor in the hydrophilic tail appeared to improve potency on cancer cell proliferation (compare **18** to **20** and **22**). Finally, compounds **22** (Core A) and **32** (Core B) were found to be equipotent inhibitors of HN5 and BT474 proliferation, despite the observation that **32** is approximately 12 times more potent on ErbB-2 at the enzyme level.

In conclusion, we have been able to develop dual inhibitors of EGFR and ErbB-2 containing a novel thienopyrimidine core. We have also been able to demonstrate sub-micromolar inhibition of cancer cell proliferation in the series containing thienopyrimidine Core A. Finally, compounds in both series, **22** and **32**, are 10- to 1000-fold selective for EGFR and ErbB-2 over a variety of other kinases including SRC, CDK2, P38, VEGFR2, and Aurora A and B.

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15. The HN5 cell line is a head and neck carcinoma line that over-expresses EGFR. The BT474 line is a breast carcinoma that over-expresses ErbB-2. The HFF cell line is a human foreskin fibroblast line that represents normal cells. The MEB proliferation assay was previously described in Ref. 9d. The CTG proliferation assay protocol was as follows: cells were treated with compounds in 0.1% DMSO and incubated for 72 h at 37 °C, 5% CO_2. Viable cells were quantified using CellTiter-Glo reagent (Promega, Madison, WI) and luminescence detection on a Victor 2V plate reader (Perkin-Elmer, Turku, Finland). Either proliferation assay format gives an IC_{50} within a 2-fold range for this chemical series.
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