Bioorganic & Medicinal Chemistry Letters 19 (2009) 21–26

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and stereochemical effects of pyrrolidinyl-acetylenic thieno[3,2-*d*]pyrimidines as EGFR and ErbB-2 inhibitors

Kirk L. Stevens [a,*], Krystal J. Alligood [b], Jennifer G. Badiang Alberti [a], Thomas R. Caferro [a,†], Stanley D. Chamberlain [a,‡], Scott H. Dickerson [c], Hamilton D. Dickson [a], Holly K. Emerson [a], Robert J. Griffin [d], Robert D. Hubbard [a,§], Barry R. Keith [b], Robert J. Mullin [b,¶], Kimberly G. Petrov [a], Roseanne M. Gerding [a], Michael J. Reno [a], Tara R. Rheault [a], David W. Rusnak [b], Douglas M. Sammond [a,‖], Stephon C. Smith [a], David E. Uehling [a], Alex G. Waterson [a], Edgar R. Wood [e]

[a] Department of Oncology Medicinal Chemistry, GlaxoSmithKline, Research Triangle Park, NC 27709, USA
[b] Department of Oncology Biology, GlaxoSmithKline, Research Triangle Park, NC 27709, USA
[c] Department of Computational and Structural Chemistry, GlaxoSmithKline, Research Triangle Park, NC 27709, USA
[d] Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, Research Triangle Park, NC 27709, USA
[e] Department of Biochemical and Cellular Targets, GlaxoSmithKline, Research Triangle Park, NC 27709, USA

ARTICLE INFO

Article history:
Received 9 October 2008
Accepted 10 November 2008
Available online 13 November 2008

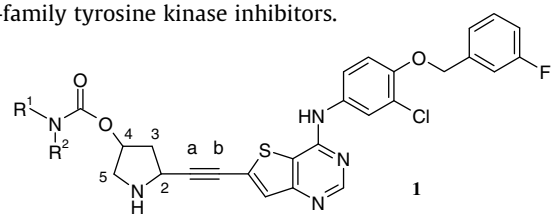
Keywords:
Epidermal growth factor receptor
EGFR
ErbB-2
Inhibitor
Thieno[3,2-*d*]pyrimidines
Proline

ABSTRACT

A novel class of pyrrolidinyl-acetyleneic thieno[3,2-*d*]pyrimidines has been identified which potently inhibit the EGFR and ErbB-2 receptor tyrosine kinases. Synthetic modifications of the pyrrolidine carbamate moiety result in a range of effects on enzyme and cellular potency. In addition, the impact of the absolute stereochemical configuration on cellular potency and oral mouse pharmacokinetics is described.

Overexpression of the epidermal growth factor receptor tyrosine kinases[1] EGFR (ErbB-1) and/or HER-2 (ErbB-2) has been implicated in poor prognosis for a variety of cancers.[2] Efforts to disrupt the ErbB-family signaling pathway with either monoclonal antibodies[3] or small molecule ATP-competitive kinase inhibitors have yielded novel anticancer agents.[4] The 4-anilinoquinazoline chemical class has provided reversible and irreversible ErbB-family inhibitors (Fig. 1). Iressa™ (gefinitib[5]), Tarceva™ (erlotinib[6]), and Tykerb™ (lapatinib[7]) are reversible ErbB-family kinase inhibitors approved in the U.S. by the FDA. Additionally, a number of irreversible quinazoline ErbB-family inhibitors shown in Figure 1 are in clinical trials (CI-1033,[8] HKI-272,[9] and BIBW-2992[10]). These inhibitors contain a Michael acceptor that forms a covalent bond with a cysteine residue near the front of the ATP-binding pocket (Cys797 in EGFR and Cys805 in ErbB-2). A potential advantage of an irreversible agent is prolonged inhibition of the enzyme, effectively competing with the high cellular concentrations of ATP to impair the ErbB-signaling pathway.[11]

Previously, we described our efforts to find effective dual inhibitors of EGFR and ErbB-2 in the pyrrolidinyl-acetylenic thienopyrimidine chemical class.[12,13] These compounds (**1**) are potent and selective EGFR/ErbB-2 enzyme inhibitors, with the ability to react covalently with Cys797 of EGFR at carbon 'a' of the acetylene. Prior SAR exploration indicated that the most desirable analogs contained a basic pyrrolidine nitrogen with a C-4 carbamate.[14] This paper describes further characterization of a variety of chiral carbamates around the 2,4-disubstituted pyrrolidine in this class of ErbB-family tyrosine kinase inhibitors.



1

* Corresponding author. Tel.: +1 919 483 7707; fax: +1 919 483 6053.
E-mail addresses: Kirk.L.Stevens@GSK.com, stevens@chemist.com (K.L. Stevens).
† Present address: Pfizer Ltd, Sandwich, UK.
‡ Present address: Inhibitex Inc., Alpharetta, GA, USA.
§ Present address: Abbott Labs, Abbott Park, IL, USA.
¶ Present address: Piedmont Research Center, Morrisville, NC, USA.
‖ Present address: Array BioPharma, Inc., Boulder, CO, USA.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.11.023

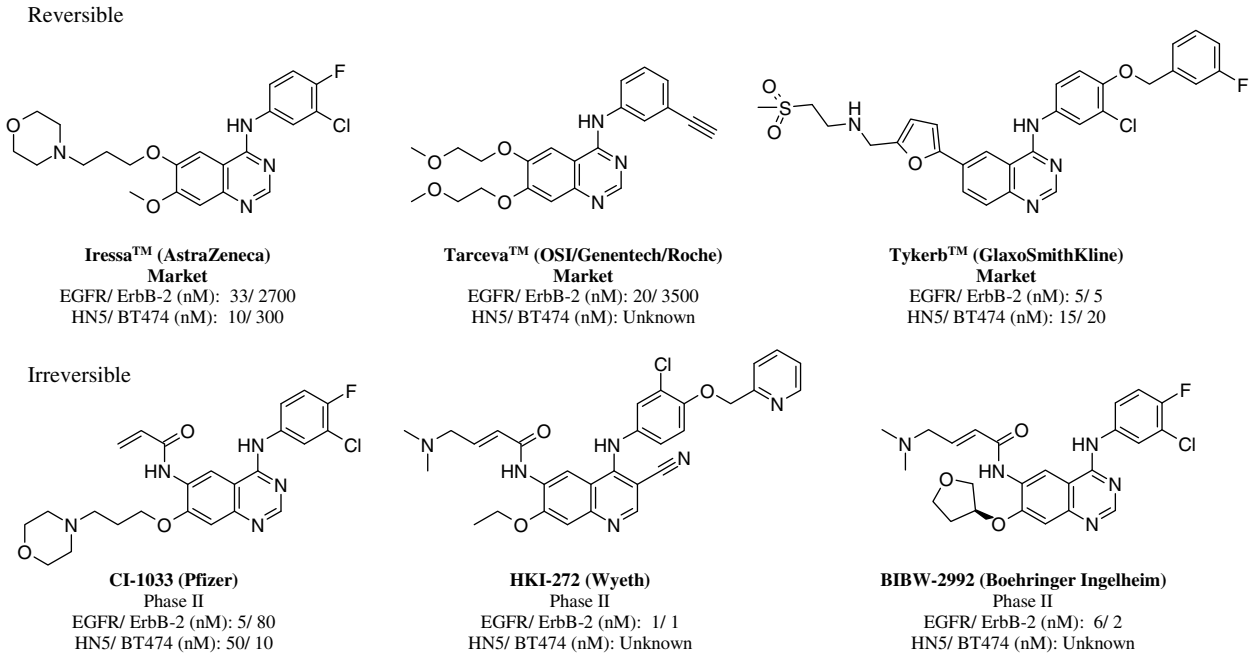
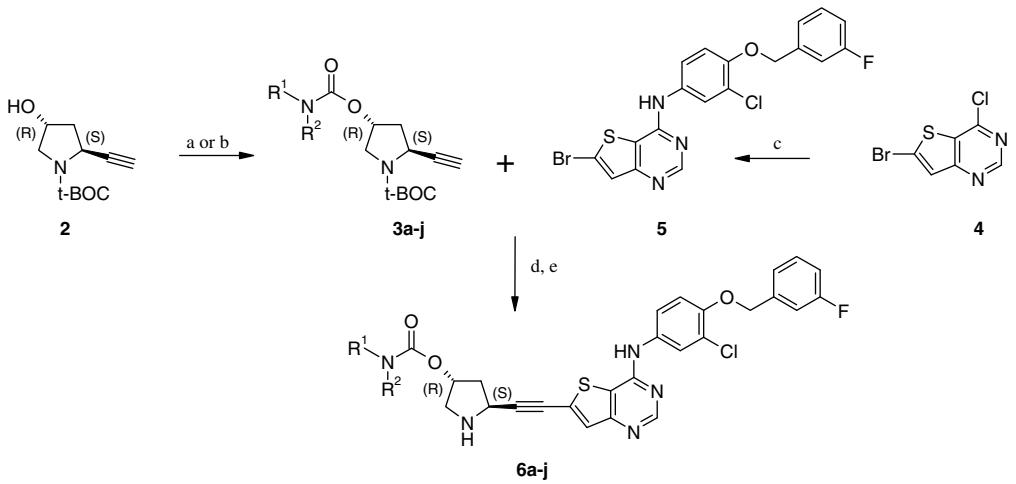


Figure 1. Chemical structures for reversible and irreversible EGFR/ErbB-2 kinase inhibitors. Enzyme inhibition data shown (EGFR/ ErbB-2) are IC_{50} values; cellular proliferation inhibition data shown (HN5/ BT474 cell lines) are EC_{50} values. Values are the mean of two or more experiments.[7]

Synthesis of the thienopyrimidine analogs was initiated from the known acetylenic pyrrolidine (**2**)[15] which is derived from the natural (*S*)-enantiomer of 4-hydroxy proline (Scheme 1). The C-4 alcohol in compound **2** was transformed to a variety of carbamates (**3a–j**) via reaction with commercially available carbamoyl chlorides or via reaction with diphosgene followed by amine addition (Scheme 1). These products were coupled with the known thienopyrimidinyl bromide **5**[16] via standard palladium-catalyzed Sonogashira conditions. Subsequent deprotection with TFA in CH_2Cl_2 yielded the desired acetylenic thieno[3,2-*d*]pyrimidines **6a–j**.

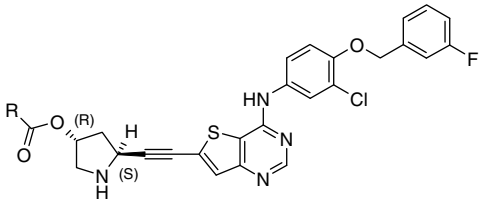
Analogs were initially assayed for their intrinsic potency against EGFR and ErbB-2 kinases. Since these carbamate analogs possess the ability to covalently modify either EGFR or ErbB-2, the IC_{50} values are time-dependent and may vary significantly as a function of incubation time.[17] The data presented are the apparent IC_{50} values obtained with a 40-min incubation. These carbamate analogs were also tested for inhibition of cellular proliferation against HN5 (head and neck) and BT474 (breast) transformed cell lines whose proliferation is driven by the overexpression of EGFR and ErbB-2, respectively.[18] EGFR covalent modification was determined at 3 h of incubation with methods described in Ref. 12. The compounds were also evaluated in mouse oral PK. The details are summarized in Table 1.

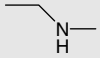
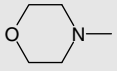
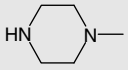
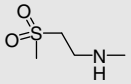
As expected, all compounds showed good enzyme potency, since the carbamate was identified as one of the most potent substituents on the pyrrolidine described in prior work from this series.[13] Smaller alkyl groups, both mono- and disubstituted, appeared to have the greatest EGFR and ErbB-2 enzyme potency, although the differences among the analogs were minor. For cellular potency in HN5 and BT474, the small alkyl substituents were again the best analogs (Table 1, **6b–d**). Interestingly, the morpholine analog (**6h**) showed comparable cell potency on the BT474 cell



Scheme 1. Reagents and conditions: (a) NaH, 0 °C to rt, DMF, then R^1R^2NCOCl (50–80%); (b) $ClC(O)OCCl_3$, CH_2Cl_2, TEA, then $R^1R^2NH_2$, 0 °C to rt, CH_2Cl_2 (50–80%); (c) 3-chloro-4-(3-fluorobenzyloxy)aniline, 60 °C, *i*-PrOH (80%); (d) $(Ph_3P)_2PdCl_2$, CuI, TEA, 60 °C, THF (50–80%); (e) TFA, 0 °C to rt, CH_2Cl_2 (90%).

Table 1
EGFR and ErbB-2 kinase inhibition, cell proliferation, covalent modification of EGFR, and mouse oral PK

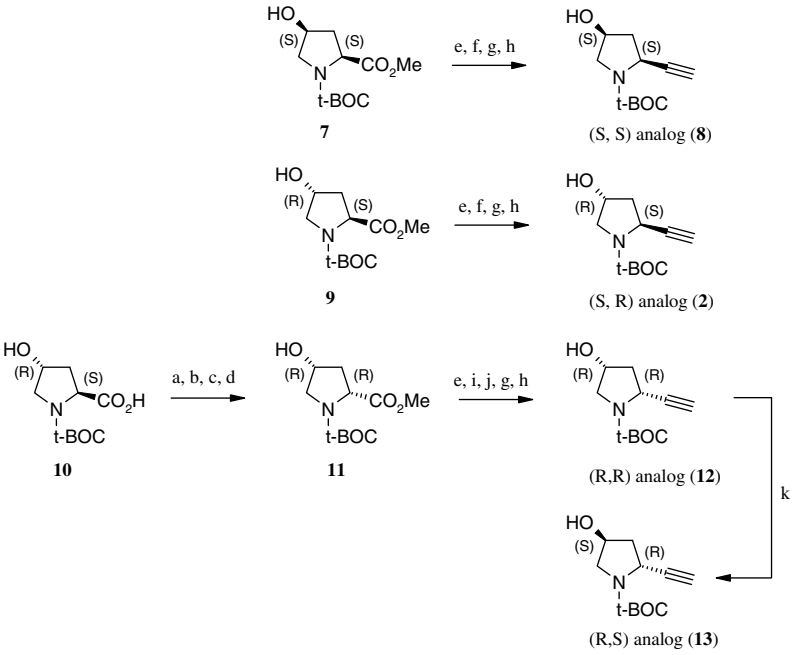


Compound		Enzyme IC_{50} (nM)[a,b]		Cellular EC_{50} (nM)[a]			EGFR Modification[c]	Mouse Oral DNAUC[e]
	R-	EGFR	ErbB-2	HN5	BT474	HFF	% @ 3 h	(ng h/mL/mg/kg)
6a		40	16	100	25	5500	–	12[f]
6b		50	20	60	30	5000	17[d]	18
6c		32	43	95	30	4200	24	61
6d		28	68	100	30	2800	11	93
6e		63*	50*	250	80	4300	8	25
6f		50	32	240	90	3000	–	–
6g		99*	32*	120	60	3500	8	63
6h		65	84	160	30	4900	5[d]	91
6i		63	20	400	100	5100	9[d]	–
6j		40	32	30	290	8200	20[d]	0

[a] Average values, $n \geqslant 2$.
[b] Homogeneous time resolved fluorescence assay. As these analogues may covalently modify either EGFR or ErbB-2, the apparent IC_{50} results are time-dependent and may vary significantly as a function of the 40 min incubation time (* denotes scintillation proximity assay). See Ref. 7.
[c] Value represents % covalent modification after 3 h at 0.002 mM compound concentration. For details see Ref. 12.
[d] Experiment run at 0.006 mM compound concentration.
[e] 10 mg/kg dosed as a solution in methanesulfonic acid:hydroxypropyl-β-cyclodextrin unless noted.
[f] Dosed as a solution in PolyEthylene Glycol:Tween 20.

line to the alkyl substituents, although the potency dropped off on the HN5 cell line. Most of the analogs displayed good selectivity over the HFF (Human Foreskin Fibroblast) control cell line (~30–150×). All carbamate analogs that were tested had measurable activity in the EGFR covalent modification assay. No obvious trend in the SAR could be determined, but all compounds modified EGFR at 3 h from 5% at the low end (**6h**) to 24% at the high end (**6c**).

Compounds that had promising enzyme and cell potency were advanced to mouse oral PK studies. In general, compounds in this series had poor to moderate oral exposure in mouse. Several com-



Scheme 2. Reagents and conditions: (a) Ac_2O, AcOH, 90 °C; (b) 2 N HCl, 100 °C; (c) $SOCl_2$, MeOH; (d) Boc_2O, TEA, DMAP (50%, four steps); (e) TBDPSCl, imidazole, DMAP (90%); (f) DIBAL-H, −78 °C then MeOH; (g) Bestmann–Ohira reagent, K_2CO_3, rt (75–88%, two steps); (h) TBAF, THF (90%); (i) $LiBH_4$, THF, 0 °C; (j) Dess–Martin Periodinane, CH_2Cl_2, rt (70%, two steps); (k) PPh_3, DIAD, pNO_2-PhCOOH (80%).

pounds had little or no exposure in these studies (**6a**, **6b**, **6e**, and **6j**). The ethyl, dimethyl, and morpholine derivatives (**6c**, **6d**, and **6h**) had the best combination of enzyme/cell potency, selectivity over HFF cells, and oral exposure in mouse PK studies. Compounds from this (2*S*, 4*R*)-enantiomer failed to demonstrate better mouse oral exposure than Tykerb (DNAUC = 150 ng h/mL/mg/kg). Therefore, we elected to explore the other stereoisomers of the 2,4-disubstituted pyrrolidine utilizing the three most promising carbamate substituents (ethyl, dimethyl, and morpholine). The synthesis of the enantiomers is shown in Scheme 2.

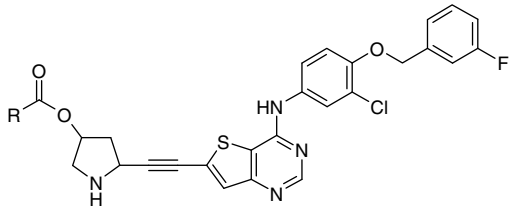
Amino acid derivatives of the natural 2-(*S*)-enantiomer of proline are readily available. The 4-(*R*)- and 4-(*S*)-diastereomers of commercially available N-Boc protected 4-hydroxy proline methyl ester (**7** and **9**, Scheme 2) were converted to acetylenes (**8** and **2**) in a convenient four step method. The alcohol is first protected as its TBDPS ether, followed by low temperature, selective DIBAL-H reduction of the ester to the corresponding aldehyde which is then converted in-situ to the acetylene using the Bestmann–Ohira modification of the Gilbert–Seyferth reagent.[19] The silyl group is removed with TBAF to provide the free hydroxyl acetylene derivatives **8** and **2**. For the compounds derived from the natural enantiomer of proline, a convenient one-pot transformation has since been developed which avoids the need for alcohol protection.[20]

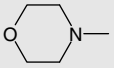
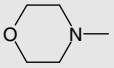
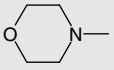
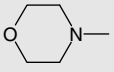
Amino acid derivatives of the unnatural 2-(*R*)-enantiomer of proline are not readily available or are extremely expensive.[21] For preparation of more than milligram quantities, we required a workable synthesis of unnatural 2-(*R*)-proline analogs. Commercially available 4-(*R*)-N-Boc-protected hydroxy 2-(*S*)-proline (**10**) was epimerized to a 2-(*R*)-proline analog by treatment in refluxing acetic anhydride followed by treatment in refluxing HCl.[22] The product crystallizes as the HCl salt which was free of the trans compound. Simple ester formation via $SOCl_2$ and MeOH followed by reaction with BOC anhydride gave the desired unnatural hydroxy proline derivative (**11**) in 50% yield over four steps. Acetylene analog (**12**) in the 2-(*R*)-proline series was prepared similarly to the previously described conditions, with one change being a two step conversion of the ester to aldehyde via $LiBH_4$ reduction and subsequent Dess–Martin periodinane oxidation. To our knowledge, the low temperature DIBAL-H reduction to the aldehyde should work as well as the one-pot protocol that was developed at a later date. The last of the four enantiomers (**13**) can be readily prepared by Mitsunobu reaction on alcohol (**12**). The enantiomeric set of nine compounds **14**–**22** was prepared from acetylenes **8**, **12**, and **13** utilizing conditions shown in Scheme 1 for carbamate analogs **6a–j**. Data for this set of compounds is shown in Table 2 with comparison to the original (2*S*,4*R*)- compounds (**6c**, **6d**, and **6h**).

All compounds in this set showed a similar level of enzyme inhibition (<100 nM in the EGFR and ErbB-2 assays). There were, however, interesting differences in the cell potencies that seem to correlate with the configuration around the pyrrolidine. In general, the trans isomers (2*S*,4*R*)- and (2*R*,4*S*)- were more potent on the BT474 cell line than their cis isomer counterparts (2*S*,4*S*)- and (2*R*,4*R*)-. The ethyl (**20**) and dimethyl (**21**) carbamates were the exceptions to this trend, which were much more potent than the morpholine carbamate in the (2*R*,4*R*) isomer. In order to quantify the inhibition of intracellular autophosphorylation, a DELFIA assay was developed for ErbB-2.[23] The results from the DELFIA assay clearly indicate the (2*S*,4*R*)-configuration is the most potent stereoisomer (Table 2). None of the tested enantiomeric compounds (**14–22)** had improved oral mouse PK compared with the original **6a–j** carbamates in the (2*S*,4*R*)-configuration. Overall, the original (2*S*,4*R*)-stereochemistry appeared to be the most preferred configuration across the three carbamates shown.

In conclusion, we have described a novel class of substituted pyrrolidinyl-acetylenic thieno[3,2-*d*]pyrimidines which are potent inhibitors of both the EGFR and ErbB-2 receptor tyrosine kinases and possess good anti-proliferative activity against EGFR and ErbB-2 overexpressing tumor lines. Synthetic modifications of the pyrrolidine carbamate moiety are found to result in a range of effects on enzyme and cellular potency. In addition, absolute stereochemical configuration around the pyrrolidine was found to impact the cellular potency and oral mouse pharmacokinetics. The natural,

Table 2
EGFR and ErbB-2 kinase inhibition, cell proliferation, cellular autophosphorylation, and mouse oral PK



Compound	Diastereomer	Enzyme IC_{50} (nM)[a,b]			Cellular IC_{50} (nM)[a]		Mouse oral DNAUC[d] (ng h/mL/mg/kg)
		R=	EGFR	ErbB-2	BT474	ErbB-2 Delfia[c]	
6c	(2*S*, 4*R*)		32	43	30	10	61
6d	(2*S*, 4*R*)		28	68	30	10	93
6h	(2*S*, 4*R*)		65	84	30	50	91
14	(2*R*, 4*S*)		50	32	60	50	1
15	(2*R*, 4*S*)		40	40	30	30	—
16	(2*R*, 4*S*)		40	32	50	50	37
17	(2*S*, 4*S*)		125	63	130	130	—
18	(2*S*, 4*S*)		79	63	230	220	3
19	(2*S*, 4*S*)		79	40	140	130	—
20	(2*R*, 4*R*)		32	20	20	10	1
21	(2*R*, 4*R*)		79	63	10	20	6
22	(2*R*, 4*R*)		50	63	120	120	—

[a] Average values, $n \geqslant 2$.
[b] HTRF assay. As these analogues may covalently modify either EGFR or ErbB-2, the apparent IC_{50} results are time-dependent and may vary significantly as a function of the 40 min incubation time. See Refs. 7 and 12.
[c] See Ref. 23.
[d] 10 mg/kg dosed in methanesulfonic acid:hydroxypropyl-β-cyclodextrin.

2-(*S*)-proline configuration was found to have the best overall properties in the series.

References and notes

1. Seymour, L. K. *Curr. Drug Targets* **2001**, *2*, 117.
2. Yarden, Y. *Eur. J. Cancer* **2001**, *37*, S3.
3. Rowinsky, E. K. *Annu. Rev. Med.* **2004**, *55*, 433.
4. Dumas, J. *Curr. Opin. Drug Discov. Dev.* **2001**, *4*, 378.
5. Baker, A. J.; Gibson, K. H.; Grudy, W.; Godfrey, A. A.; Barlow, J. J.; Healy, M. P.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Henthorn, L.; Richards, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1191.
6. Bonomi, P. *Expert Rev. Anticancer Ther.* **2003**, *3*, 367.
7. Rusnak, D. W.; Lackey, K. E.; Wood, E. R.; Alligood, K. J.; Rhodes, N.; Knight, W. B.; Gilmer, T. M. *Mol. Cancer Ther.* **2001**, *1*, 85.
8. Allen, L. F.; Eiseman, I. A.; Fry, D. W.; Lenehan, P. F. *Semin. Oncol.* **2003**, *30*, 65.
9. Rabindran, S. K.; Discafani, C. M.; Rosfjord, E. C.; Baxter, M.; Floyd, M. B.; Golas, J.; Hallett, W. A.; Johnson, B. D.; Nilakantan, R.; Overbeek, E.; Reich, M. F.; Shen, R.; Shi, X.; Tsou, H.-R.; Wang, Y.-F.; Wissner, A. *Cancer Res.* **2004**, *64*, 3958.
10. Reid, A.; Vidal, L.; Shaw, H.; de Bono, J. *Eur. J. Cancer* **2007**, *433*, 481.
11. Fry, D. W. *Anti-Cancer Drug Des.* **2000**, *15*, 3.
12. Wood, E. R.; Shewchuk, L. M.; Ellis, B.; Brignola, P.; Brashear, R. L.; Caferro, T. R.; Dickerson, S. D.; Dickson, H. D.; Donaldson, K. H.; Gaul, M.; Griffin, R. J.; Hassell, A. M.; Keith, B.; Mullin, R.; Petrov, K. G.; Reno, M. J.; Rusnak, D. W.; Tadepalli, S. M.; Ulrich, J. C.; Wagner, C. D.; Vanderwall, D. E.; Waterson, A. G.; Williams, J. D.; White, W. L.; Uehling, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2773.
13. Waterson, A. G.; Petrov, K. G.; Hornberger, K. R.; Hubbard, R. D.; Sammond, D. M.; Smith, S. C.; Hinkle, K. W.; Stevens, K. L.; Uehling, D. E.; Dickerson, S. H.; Rusnak, D. W.; Spehar, G. M.; Wood, E. R.; Griffin, R. J. *Bioorg. Med. Chem. Lett.*, submitted for publication.
14. Hubbard, R. D.; Dickerson, S. H.; Emerson, H. K.; Griffin, R. J.; Reno, M. J.; Hornberger, K. R.; Rusnak, D. W.; Wood, E. R.; Uehling, D. E.; Waterson, A. G *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5738.
15. Trybulski, E. J.; Kramss, R. H.; Mangano, R. M.; Rusinko, A. *J. Med. Chem.* **1990**, *33*, 3190.
16. Compound **4** (CAS#: 225385-03-5) has recently become commercially available through Medinoah Inc., Wallingford, CT. Synthetic details can be found in *PCT Int. Appl.* WO 99/24440. Compound **5** is prepared by displacement on the chloro of **4** with 3-chloro-4-(3-fluorobenzyloxy)aniline which is available through Affinitis Pharma LLC, New Haven, CT. Synthetic details can be found in *PCT Int. Appl.* WO 98/02437.
17. The modification data was only acquired for EGFR using methods described in Ref. 12. These analogs have not been assayed versus ErbB-2 for potential reactivity.
18. Thienopyrimidine analogs (**6a–j**) were found to be selective for the transformed cell lines (HN5 and BT474) versus a control human foreskin fibroblast cell line (HFF). Experimental protocols are described in Ref. 7.
19. Ohira, S. *Synth. Commun.* **1989**, *19*, 561.
20. Dickson, H. D.; Smith, S. C.; Hinkle, K. W. *Tetrahedron Lett.* **2004**, *45*, 5597.
21. (4*R*)-4-Hydroxy-(2*R*)-proline 5g, $153; (4*S*)-4-Hydroxy-(2*R*)-proline 5g, $291, both from AstaTech, Bristol, PA. Advanced derivatives of the 2-(*S*) enantiomer, such as **10**, are occasionally available, 5g, $169 from AstraTech.
22. Baker, G. L.; Fritschel, S. J.; Stille, J. R.; Stille, J. K. *J. Org. Chem.* **1981**, *46*, 2954.
23. Dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA), see: Braunwalder, A. F.; Yarwood, D. R.; Sills, M. A.; Lipson, K. E. *Anal. Biochem.* **1996**, *238*, 159. BT474 cells and their culture conditions are described in Ref. 7.