Journal of Medicinal Chemistry

Article

Tyrosine Kinase Inhibitors. 20. Optimization of substituted quinazoline and pyrido[3,4-d]pyrimidine derivatives as orally active, irreversible inhibitors of the epidermal growth factor receptor family.

Jeff Bruce Smaill, Andrea J Gonzales, Julie A Spicer, Helen Lee, Jessica E. Reed, Karen Sexton, Irene W Althaus, Tong Zhu, Shannon L Black, Adrian Blaser, William A. Denny, Paul A Ellis, Stephen Fakhoury, Patricia J Harvey, Ken Hook, Florence O.J. McCarthy, Brian D. Palmer, Freddy Rivault, Kevin Schlosser, Teresa Ellis, Andrew M. Thompson, Erin Trachet, R. Thomas Winters, Haile Tecle, and Alexander Bridges

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b00883 • Publication Date (Web): 04 Aug 2016

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

	Bridges, Alexander; Pfizer Global Research and Development Ann Arbor

Tyrosine Kinase Inhibitors. 20. Optimization of substituted quinazoline and pyrido[3,4-*d*]pyrimidine derivatives as orally active, irreversible inhibitors of the epidermal growth factor receptor family.

Jeff B. Smaill,[a,b*] Andrea J. Gonzales,[c] Julie A. Spicer,[a,b] Helen Lee,[c] Jessica E. Reed,[c] Karen Sexton,[c] Irene W. Althaus,[c] Tong Zhu,[c] Shannon L. Black,[a] Adrian Blaser,[a] William A. Denny,[a,b] Paul A. Ellis,[c] Stephen Fakhoury,[c] Patricia J. Harvey,[c] Ken Hook,[c] Florence O. J. McCarthy,[a] Brian D. Palmer,[a,b] Freddy Rivault,[a] Kevin Schlosser,[c] Teresa Ellis,[c] Andrew M. Thompson,[a] Erin Trachet,[c] R. Thomas Winters,[c] Haile Tecle[c] and Alexander Bridges.[c]

[a]*Auckland Cancer Society Research Centre, School of Medical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand.* [b]*Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand.* [c]*Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Rd, Ann Arbor, Michigan, 48105-1047, USA.*

Abstract

Structure-activity relationships for inhibition of erbB1, erbB2 and erbB4 were determined for a series of quinazoline- and pyrido[3,4-*d*]pyrimidine-based analogues of the irreversible pan-erbB inhibitor, canertinib. Cyclic amine bearing crotonamides were determined to provide rapid inhibition of cellular erbB1 autophosphorylation and good metabolic stability in liver microsome and hepatocyte assays. The influence of 4-anilino substitution on pan-erbB inhibitory potency was investigated. Several anilines were

identified as providing potent, reversible pan-erbB inhibition. Optimum 4- and 6-substituents with known 7-substituents provided preferred irreversible inhibitors for pharmacodynamic testing *in vivo*. Quinazoline **54** and pyrido[3,4-*d*]pyrimidine **71** were identified as clearly superior to canertinib. Both compounds possess a piperidinyl crotonamide Michael acceptor and a 3-chloro-4-fluoroaniline, indicating these as optimized 6- and 4-substituents respectively. Pharmacokinetic comparison of compounds **54** and **71** across three species selected compound **54** as the preferred candidate. Compound **54** (PF-00299804) has been assigned the nomenclature of dacomitinib and is currently under clinical evaluation.

Introduction

There is considerable interest in small molecule inhibitors of the human epidermal growth factor receptor family of tyrosine kinases for the treatment of cancer. The family consists of four members, erbB1 (EGFR, HER-1), erbB2 (HER-2, neu), erbB3 (HER-3) and erbB4 (HER-4). Hyperactivation of this family due to overexpression of the receptors, mutations leading to constitutive activation or overexpression of the growth factors themselves results in inappropriate signal transduction cascades driving cellular functions such as proliferation, differentiation, migration and angiogenesis, in a process known to correlate with poor prognosis in patients.[1-4]

The erbB1 selective, reversible ATP-competitive inhibitors gefitinib (**1**)[5, 6] and erlotinib (**2**)[7] are approved as front line therapy for non-small cell lung cancer (NSCLC) that has been determined through genetic analysis to harbor somatic mutations in erbB1. In this setting they demonstrate impressive response rates (60-80%) and significant

improvements in progression free survival over chemotherapy.[8] While some patients may experience a durable response, resistance to these agents eventually emerges, with approximately half of patients having acquired a second T790M mutation in the kinase active site.[9, 10] Early attempts to improve the efficacy of these inhibitors was centered around improving their potency for inhibition of erbB2 which is known to be overexpressed in 25-30% of breast cancers.[11] This was achieved by introducing large lipophilic substituents to the 4-position of the aniline ring, as typified by lapatinib (**3**),[12] which is approved for use in combination with Xeloda® as the last line treatment for erbB2 positive breast cancer.[13, 14]

The recognition that erbB1, 2, and 4 possess a unique unpaired cysteine residue (Cys 773, 784 and 778 respectively)[15, 16] at the mouth of the ATP-binding domain, in a position normally occupied by a glutamine or serine in other kinases, has led ourselves[15, 17-20] and others[21-29] to develop small molecule irreversible inhibitors of the erbB family of tyrosine kinases. The design concept is typified by canertinib (CI-1033; **4**),[18] the first irreversible pan-erbB inhibitor to enter clinical trials.[30, 31] This compound bears an electrophilic 6-acrylamide substituent ideally positioned to alkylate the key cysteine residues of erbB1, 2, and 4, providing for low nanomolar potency against these kinases. Potent inhibition of erbB3 signalling is also achieved through blockade of its transphosphorylation partners.

While a number of alternate Michael acceptors have been explored in the development of irreversible erbB inhibitors, the most widely employed is the dimethylamino crotonamide originally developed by Tsou and colleagues.[22] This is exemplified in the quinoline-3-carbonitrile pelitinib (EKB-569; **5**),[23] which was investigated in human clinical trials.[32] Here the dimethylamine group plays a critical role as an intramolecular base between

reaction of a sulfhydryl group and the Michael acceptor through a 5-membered transition state, increasing the reactivity of the Michael acceptor towards sulfur nucleophiles when compared to the unsubstituted crotonamide.[22, 23] Optimisation of pelitinib focused on improving erbB2 potency,[23] resulting in the irreversible erbB1/2 inhibitor neratinib (HKI-272; **6**),[24] currently in Phase III clinical trial for erbB2-positive breast cancer.[33] Application of the dimethylamino crotonamide Michael acceptor to a quinazoline scaffold provided the potent irreversible erbB1/2 inhibitor afatinib (BIBW-2992; **7**),[34] which is approved for mutant erbB1-positive NSCLC.[35]

Irreversible inhibitors of the erbB family have been proposed for the treatment of T790M-mediated relapse of first generation erbB1 inhibitors based on preclinical efficacy against T790M-positive cell lines,[36, 37] and human tumor xenograft models, although this efficacy may be at supraphysiological concentrations.[38-40] Third generation inhibitors that are selective for mutant forms of erbB1 (including T790M), while sparing wild-type erbB1, have now been reported. Examples include WZ4002,[38, 41] rociletinib (CO-1686)[42] and osimertinib (AZD9291).[43] The latter derivative demonstrates impressive efficacy in clinical trials against T790M-positive NSCLC,[44] and is now approved by the FDA in this setting.

Returning to canertinib, Phase I trials of this agent administered orally for 7 or 14 days of a 21-day cycle established 650 mg/day[45] or 450 mg/day[46] as the respective maximum tolerated doses. Diarrhea, acneiform rash, emesis and anorexia were described as the dose-limiting toxicities and are typical of the class. Other toxicities such as transient thrombocytopaenia and hypersensitivity reactions were also reported.[45, 46] It has been speculated that these toxicities may be due to off-mechanism kinase activity.[45] Indeed,

canertinib is reported to be a potent inhibitor of BMX and BTK, TEC-kinases that possess a target cysteine in the same relative position of the ATP-binding domain as erbB1, 2 and 4.[47] Kinase screening against 317 protein kinases reveled off-target micromolar binding affinity to approximately 12.6% of kinases tested and submicromolar affinity to 5.7%, with particularly strong affinity to BLK, GAK, LCK, MKNK1 and RIPK2.[48] We herein also identify canertinib as a potent inhibitor of JAK3 (IC50 = 128 nM), another kinase to share an appropriately positioned cysteine in its ATP-binding domain. Phase II studies of canertinib in previously treated advanced ovarian cancer were disappointing with a lack of responses precluding further clinical development.[49] The modest pharmacokinetic profile of canertinib in humans, in particular a short plasma elimination half-life (4 h) has been proposed as limiting efficacy,[45] while the observed lack of canertinib excretion in urine indicates elimination is primarily metabolic.[46] Consistent with this, the major metabolite in cellular studies is the glutathione adduct of the Michael acceptor.[18]

In the present study we describe our work to develop an improved analogue of canertinib. We sought to identify a window of opportunity whereby the metabolic stability of the Michael acceptor could be improved without disrupting the desired irreversible inhibition of erbB1, 2, and 4. We argued this would minimize first-pass metabolism and provide improved pharmacokinetic parameters. Analogues were screened *in vitro* against recombinant JAK3 kinase seeking to eliminate off-target potency against this enzyme, thought to contribute to the myleotoxicity observed for canertinib. Dose-potency and duration of suppression of erbB1 autophosphorylation was assessed in nude mice bearing tumor xenografts. Herein we discuss the strategy we adopted to develop an optimized irreversible quinazoline or pyrido[*3,4-d*]pyrimidine pan-erbB inhibitor with improved

pharmacokinetics, tumor pharmacodynamics, tumor efficacy in animal models and potential toxicity profile in humans, relative to canertinib itself.

Chemistry

The synthesis of 6-amide substituted quinazolines, pyrido[3,4-*d*]pyrimidines and quinoline-3-carbonitriles is well precedented,[17-24] and the present work proceeded along similar lines. The substituted acrylamides (**8**-**11**) of Table 1 were prepared by reaction of the 6-aminoquinazoline[18] (**85**) with the respective acid chlorides (Scheme 1).

The 4-anilinoquinazolines (**12**-**43**) of Table 2 were prepared by aniline displacement of the 4-chloro substituent of quinazoline **89**. This array substrate was prepared from 7-fluoro-6-nitroquinazolone[50] (**86**) by methoxide displacement of the activated fluorine to give **87**, followed by iron dust reduction of the nitro group and acylation with propionic anhydride to give propanamide **88**. Chlorination in refluxing $POCl_3$ then gave **89** (Scheme 2). The majority of the anilines of Table 2 were commercially available. The remainder were previously reported (A7-10, A12) or prepared (A6, A11, A13, A14, A31, A32) by reduction of their nitro precursors, which were obtained by standard techniques (see Supporting Information).

The quinazolines (**44**-**52**) of Table 3 were prepared by ethoxide displacement on **86** followed by $POCl_3$ mediated chlorination and displacement with 1-benzyl-1*H*-indazol-5-amine to give **91**. Nitro group reduction *via* hydrogenation over Raney nickel then gave amine **92** which was acylated with the freshly prepared acid chloride of *E*-4-bromobut-2-

enoic acid to give bromide **93**. Low temperature displacement of the bromide with a range of secondary amines then gave compounds **44**-**52** of Table 3 (Scheme 3).

The quinazolines (**53**-**69**) of Table 4 were prepared in an analogous manner to those of Table 3 described above. The pyrido[3,4-*d*]pyrimidines (**70**-**82**) of Table 4 were prepared similarly from 6-fluoropyrido[3,4-*d*]pyrimidin-4(3*H*)-one[51] (**95**) by chlorination with $SOCl_2$ and displacement with the respective anilines to give compounds **96**. Fluorine displacement with 4-methoxybenzylamine then gave the PMB-protected amines (**97**), which were deprotected using TFA to give the 6-amino derivatives (**98**). Acylation of these with the freshly prepared acid chloride of *E*-4-bromobut-2-enoic acid gave the bromides (**99**). Low temperature displacement of the bromides with a range of secondary amines then gave compounds **70**-**82** of Table 4 (Scheme 4).

Results and Discussion

Kinase and Reactivity Assays. The relative reactivity towards reduced glutathione for several Michael acceptors (A-E, Figure 1), on a common quinazoline scaffold, has been reported.[22] The dimethylamino crotonamide (A) was found to be more reactive than the acrylamide (B) which was in turn more reactive than the alkylacrylamides (C and D) and the morpholino crotonamide (E). The greater chemical reactivity of the dimethylamino crotonamide (A) towards reduced gluthathione relative to the acrylamide (B) intrigued us because pelitinib bears this Michael acceptor yet demonstrates a significantly longer human plasma half-life than canertinib (21.7 hours[52] *cf* 4 hours[45]). This led us to prioritise microsome and hepatocyte stability testing to predict compounds with improved first pass metabolism, oral bioavailability and plasma half-life, rather than chemical glutathione

reactivity assays. With respect to alkylacrylamides (C and D) we reasoned that these Michael acceptors were understudied and further work was merited using the exact canertinib scaffold. With respect to the decreased reactivity of the morpholino crotonamide relative to the dimethylamino crotonamide (E *cf* A) it was unclear whether this was due to changes in steric bulk, amine pKa or both. To investigate we therefore targeted quinazolines and pyrido[*3,4-d*]pyrimidines substituted at the 6-position with crotonamides bearing cyclic amines of varying steric bulk and pKa.

Table 1 describes the structures and kinase inhibitory properties against erbB1, 2, and 4 of a subset of the compounds synthesized to explore alkylacrylamide (C and D, Figure 1) Michael acceptors on the canertinib scaffold. In previous publications we[17-24] and others[21-24] have noted that care needs to be taken when interpreting IC_{50} values for putative irreversible inhibitors. These values are essentially a composite of both the covalent interaction with the enzyme over the time frame of any particular assay and the reversible binding provided by the scaffold. As reference we prepared the reversible propanamide **11** for comparative purposes. Table 1 also describes the results of a time-course washout assay where A431 cells were treated with potential irreversible inhibitors for 1, 5, 10, 30, 60 and 120 minutes before being washed free of drug, stimulated with EGF, lysed and western blotted for inhibition of erbB1 autophosphorylation. This determines the time required for the inhibitor to completely ablate autophosphorylation of erbB1 in A431 cells, providing an indication of the kinetics of alkylation of Cys 773 for each Michael acceptor.

Relative to canertinib (**4**) compounds **8**-**10** showed a modest loss of potency (3 to 11-fold) for inhibition of erbB1 in the isolated enzyme assay and a greater loss of potency in the cellular erbB1 assay (13 to 38-fold). Compounds **8-10** further showed a considerable loss

of potency against erbB2 (> 31-fold) and erbB4 (>37-fold) in cellular and biochemical assays, respectively. Compounds **8** and **9** also gave incomplete inhibition of erbB1 autophosphorylation after 2 h drug exposure in the washout assay, although compound **10** was a little more encouraging in this regard. In general, the data for **8**-**10** was comparable to that for **11**, with the erbB1 selective nature of the compounds indicating inefficient alkylation of the target cysteines of erbB1, 2 and 4. It became apparent from this study that in order to reduce the reactivity of the Michael acceptor while retaining potent pan-erbB activity it may be first be preferable to increase the reversible binding affinity of the kinase inhibitor scaffold to erbB2 and 4. A study to investigate this was undertaken prior to an examination of the type E Michael acceptors (Figure 1).

The SAR for erbB2 potency of 4-aniline substituents of quinazoline and quinoline-3-carbonitrile inhibitors has been reasonably well explored during the development of both lapatinib[53-56] and neratinib.[24] In this latter study however, we felt it was difficult to separate the relative contribution made to the inhibitory potency of the test compounds from the aniline substituent versus that from the Michael acceptor. Further, to our knowledge, no erbB4 data has been reported for an SAR study around 4-aniline substituents. For these reasons we undertook such a study in a reversible 7-methoxy-6-propanamide series. The aniline pharmacophore outlined in Figure 2 was explored to determine its influence on pan-erbB potency. This approach had the added advantage that each aniline studied required only one synthetic step to provide final compounds for testing (aniline displacement of the 4-chloroquinazoline **89**). Approximately 95 anilines were explored in this series. Table 2 shows the erbB1, 2 and 4 isolated enzyme activities for the 32 that provided the most potent pan-erbB activity (compounds **12**-**43**). We reasoned that any compound with erbB1, 2 and 4 activity of < 500 nM would be

sufficiently interesting to synthesise comparative irreversible analogues (see Tables 3 and 4). Of the 32 compounds, 6 fitted this profile (A1, A4, A5, A10, A17, A24 of Table 2). A further 8 of the 32 had erbB1, 2 and 4 activity of < 1 μM (A6, A11, A12, A13, A25, A26, A30, A32 of Table 2).

The erbB1 selective nature of the 1*H*-indol-5-amine (A2) and the 1*H*-indazol-5-amine (A3) are readily apparent, while the 3-chloro-4-fluoroaniline (A1) is 203-fold erbB1 selective (over erbB2) but possesses acceptable erbB2 and 4 potency (< 500 nM). As previously described[53] introduction of a benzyl substituent to the *N*-1 position of the indole (A4) and indazoleamines (A5) provided a significant improvement in the erbB2 and 4 potency giving a potent pan-erbB scaffold. *N*-1-Phenylsulfonylindole (A10) was also potent in this regard, while only the 2″-pyridomethyl substituted indole (A6) was tolerated by erbB1 and 4 (and to a lesser extent erbB2) with the 3″- and 4″-pyridine isomers (A7, A8) showing a considerable loss of erbB1 potency. The erbB1 preference for a 2″-pyridyl isomer is noted for the other pyridines in this study where a direct comparison could be made (A21, A22 and A26, A27), consistent with the preferred 2″-pyridomethyloxy substituent of neratinib (**6**).[24] The 1-(2-furylmethyl)-1*H*-indol-5-amine (A11) and indoline (A12, A13) analogues were detrimental to overall potency when compared to the analogous benzylindole (A4) and the need for an aromatic substituent at the indole *N*-1 position was confirmed by the lack of potency for the *N*-1-*iso*-butyl derivative (A14). The benzylamine (A15) and α-methylbenzylamine (A16) substituents were demonstrated to be erbB1 selective. The 4-phenoxyaniline (A17) showed pan-erbB potency as did the 4-(benzyloxy)aniline derivatives (A24, A25), typical of that present in lapatinib.[56] The 4-phenylsulfonamide (A30) was also considered to be of interest. Several representative anilines with pan-erbB activity (erbB1, 2 and 4 mostly < 500 nM) from

Table 2 (A1, A5, A10, A17, A21, A25, A26, A30) were selected for studies incorporating a range of Michael acceptors and 7-substituents (see Table 4).

Variations in the terminal dialkylamino group of the crotonamide Michael acceptor (e.g. A and E; Figure 1) have been widely reported in the quinazoline[22] and quinoline-3-carbonitrile[23, 24] scaffolds. However, to our knowledge only morpholine has been directly studied and determined to be less reactive towards reduced gluthathione.[22] For this example, it is not clear if it is the reduced amine pKa or increased steric demand of the cyclic amine that is responsible for this attenuated reactivity. To investigate the influence of amine structure on the reactivity of the crotonamide Michael acceptor both metabolically and towards covalent reaction with the target Cys-773 of erbB1, we utilized the most potent pan-erbB *N*-1-benzylindazole (A5) scaffold of Table 2. Table 3 shows the erbB1, 2 and 4 isolated enzyme data, inhibition of erbB1 cellular autophosphorylation, time-course washout data and rat and human liver microsome and hepatocyte stability for 9 amine variations of the solubilized crotonamide Michael acceptor. Isolated enzyme inhibition of JAK3 kinase is included as an important off-target counterscreen. The most striking observation was that the dimethylamine (**44**), diisopropylamine (**45**), pyrrolidine (**46**), piperidine (**47**), morpholine (**49**), 4-methylpiperazine (**50**) and 3,5-dimethylpiperazine (**51**) crotonamides were all potent low nanomolar inhibitors of erbB1, 2 and 4 that irreversibly inhibited 100% of erbB1 cellular autophosphorylation after only one minute of compound exposure.The 2,6-dimethyl substituted piperidine (**48**) and piperazine (**52**), possessing additional steric bulk around the amine nitrogen, showed an approximate 10-fold reduction in erbB1 and 2 isolated enzyme activity, consistent with reported observations of poorer performance.[24] These compounds were not progressed to further studies. Of the compounds advanced to rat and human liver microsome and

hepatocyte stability assays only the diisopropylamine (**45**) was considered unstable. All of the compounds of Table 3 showed a favourable selectivity profile for the erbB family relative to JAK3 (approximately 100 to 1000-fold). The cyclic amine crotonamides tested displayed excellent metabolic stability and this in conjunction with rapid inhibition of cellular erbB1and a favourable profile against JAK3 kinase indicated this class of Michael acceptor warranted further investigation in combination with preferred anilines of Table 2.

To prepare an optimized 4-anilinoquinazoline or 4-anilinopyrido[3,4-*d*]pyrimidine, preferred 4-, 6-, and 7-substituents were selected. At the 4-position, the most potent pan-erbB anilines of Table 2 (A1, A5, A10, A17, A21, A25, A26, A30). At the key 6-position, optimized cyclic amine-bearing crotonamide Michael acceptors of Table 3, with a number of dimethylamino crotonamides prepared for comparison, while at the 7-position (where present), alkoxy substituents selected from those of gefitinib (OMe), neratinib (OEt) and erlotinib (OCH_2CH_2OMe) were included. Table 4 shows the erbB1, 2 and 4 isolated enzyme activity, erbB1 cellular autophosphorylation inhibitory activity and JAK3 isolated enzyme inhibitory activity as an off-target counter screen, for these compounds (**44**, **46**, **47**, **50**, **55**-**82**). A number of trends were apparent. The anilines that generally provided the best pan-erbB potency on either the quinazoline or pyrido[3,4-*d*]pyrimidine scaffolds, when prepared with a crotonamide Michael acceptor at the 6-position, were the 3-chloro-4-fluoroaniline (A1), the 1-benzyl-1*H*-indazol-5-amine (A5) and the lapatinib aniline (A25). Within this set of anilines ranging the dialkylamine substituted crotonamide had very little influence on the potencies against the isolated erbB1, 2, and 4 enzymes, or the cellular erbB1 potency. The anilines that generally provided less potent pan-erbB activity, when prepared with a crotonamide Michael acceptor at the 6-position, were the 1-(phenylsulfonyl)-1*H*-indol-5-amine (A10), the 4-phenoxyaniline (A17), the 4-(2-

pyridinylmethyl)aniline (A21), the 4-(2-pyridinylmethoxy)aniline (A26) and the *N*-(4-aminophenyl)benzenesulfonamide (A30). Within this set of anilines ranging the dialkylamine substituted crotonamide had more influence on the erbB1, 2, and 4 isolated enzyme potency, and the cellular erbB1 potency, with the most basic amines (dimethylamine, piperidine and pyrrolidine) providing the superior potency when compared to the less basic cyclic amines (morpholine and 4-methylpiperazine) (compare **58**, **59** with **60**; **65** with **66**; **67**, **68** with **69; 78**, **79** with **80**). Where a comparison of 7-substituent could be made between 7-OEt and 7-OCH_2CH_2OMe, the overall potencies were comparable (compare **44**, **46** and **50** with **55**, **56** and **57**). With the exception of compound **71** (JAK3 IC_{50} = 0.793 μM) all of the compounds of Table 4 were micromolar inhibitors of isolated JAK3.

A selection of compounds from Table 4 were investigated in the erbB1 cellular autophosphorylation wash-out assay and the rat and human liver microsome and hepatocyte stability assays. The results are shown in Table 5. With the exception of compound **69**, all of the compounds gave rapid and complete shutdown of cellular erbB1autophosphorylation after 1 to 5 minutes of drug exposure, indicating rapid alkylation of Cys-773. With the exception of compound **53**, all of the compounds tested were stable in rat and human microsomes for the duration of the assay (40 min) and gave comparable or improved rat and human hepatocyte stability relative to canertinib (**4**).

Pharmacodynamic testing *in vivo*. The majority of the compounds of Table 4 demonstrated potent pan-erbB activity in enzyme and cellular assays, an acceptable counter-screening profile against JAK3 and good metabolic stability in microsome and hepatocyte assays. It appeared likely that physicochemical and pharmacokinetic

differences between analogues would be the main determinant of *in vivo* efficacy. We therefore decided to advance most of the compounds of Table 4 to *in vivo* pharmacodynamic testing, where it was anticipated analogues optimal in this regard would show superior target modulation. Mice (3 per cohort) bearing NIH3T3 tumors were dosed orally with each test compound of Table 4 at 30, 65 and 130 mg/kg twice daily (QDx2). Tumors were harvested 6 and 24 h after the final dose, digested and Western blotted for the ability of each compound to inhibit the autophosphorylation of erbB1 (Table 6). Gefitinib (**1**) and canertinib (**4**) were included in this assay as reference compounds. As expected, the irreversible inhibitor canertinib showed superior inhibition of erbB1 autophosphorylation when compared to gefitinib, which only gave complete inhibition at the earliest time point and highest dose (6h after 130 mg/kg dose). In contrast canertinib showed some return of phosphorylated erbB1 24 h after the lowest dose (30 mg/kg), while all other doses and time points gave complete inhibition.

We sought to identify compounds of Table 6 with pharmacodynamic profiles comparable to or better than canertinib for more advanced investigation. Very few compounds qualified, despite being potent inhibitors of both isolated enzyme and cellular erbB1 (for example **44**, **46**, **47**, **50**, **58**, **59**, **60**, **62**, **67**, **74**, **75**, **79**, **80**). We attribute this poor target modulation *in vivo* to a combination of factors including poor solubility, permeability, bioavailability, pharmacokinetics and tissue penetration. We note that when dialkylamino crotonamide Michael acceptors are compared across scaffolds, the higher molecular weight compounds, bearing the more structurally complex anilines, are generally less effective at providing dose-potent and prolonged inhibition of erbB1 autophosphorylation. Quinazoline **54** and pyrido[3,4-*d*]pyrimidines **71** and **73** stood out as having superior activity to canertinib in the erbB1 pharmacodynamic assay, providing complete inhibition

of phosphorylated erbB1 24 h after the lowest dose tested (30 mg/kg). These compounds possess the same 3-chloro-4-fluoroaniline (A1) of canertinib (gefitinib, peltinib and afatinib) and a piperidine (**54**, **71**) or methylpiperazine (**73**) solubilized crotonamide Michael acceptor. We propose for simplicity that the common features of **54** and **71** represent the optimal 4- and 6-substituents for irreversible inhibition of erbB1 *in vivo*. These optimized substituents appear to be equally effective on a quinazoline or pyrido[3,4-*d*]pyrimidine scaffold.

An improvement in the duration of *in vivo* activity for a cyclic amine bearing crotonamide relative to a dimethylamino crotonamide is evident when comparing dimethylamine (**74)** and pyrrolidine (**75)**. These compounds are identical in terms of reversible scaffold, isolated enzyme erbB1, 2 and 4 activity and erbB1 cellular activity. Both compounds were effective inhibitors of erbB1 *in vivo* at the highest dose tested (130 mg/kg) while only the cyclic amine derivative **75** was effective 24 h after the middle and lower doses (65 and 30 mg/kg respectively). It was however slow to inhibit erbB1 at the 30 mg/kg dose with 2 of 3 mice showing considerable erbB1 autophosphorylation at the 6 h time point which had been completely inhibited after 24 h.

Pharmacokinetic comparison of compounds 54 and 71 in rat, dog and monkey. A pharmacokinetic comparison of compounds **54** and **71** was undertaken in three preclinical species (rat, dog and monkey) following a single oral and intravenous dose (Table 7). As previously described[57] compound **54** displayed an excellent pharmacokinetic profile across all three species demonstrating high oral bioavailability (>50%), a long plasma half-life ($t_{1/2}$ > 12 hours) and a large volume of distribution (>17 L/kg). Indeed compound 54 was determined to have significantly improved pharmacokinetics relative to

canertinib.[57] Compound **71** however was considerably more variable across species, showing a volume of distribution (24.8 L/kg) and oral bioavailability that was acceptable in rat (60%), but with considerably poorer parameters in dog and particularly monkey, where plasma half-life following intravenous administration (0.7 hours) and oral bioavailability (4%) were disappointing. This pharmacokinetic variability was deemed to be of concern, sufficiently so to recommend compound **54** to advance to human trial over compound **71**.

Conclusions

We have described an approach to develop an irreversible inhibitor of erbB1, 2 and 4 with an improved pharmacokinetic and toxicity profile relative to canertinib. We have investigated the reactivity of alkyl substituted acrylamides and dialkylamino crotonamides in microsome and hepatocyte assays, while ascertaining their relative rate of reaction with Cys-773 of erbB1. This has allowed identification of drug candidates with the potential for rapid irreversible inhibition of the target erbB family, minimized glutathione conjugation and therefore improved first-pass metabolism, oral bioavailability, plasma half-life and tumor pharmacodynamics. We have counter-screened analogues against JAK3 kinase to identify compounds with minimized potency against this enzyme, an off-target event thought to contribute to the undesirable thrombocytopenia observed during human trials of canertinib.

Cyclic amine solubilized crotonamides have been discovered to be optimal in terms of their rapid alkylation of erbB1 and desirable metabolic stability. We have characterized the pan-erbB potency imparted by a range of 4-aniline substituents to a reversible

quinazoline scaffold and established one of the broadest SAR datasets for the inhibition of erbB4 reported to date. We have determined that 3-chloro-4-fluoroaniline, 5-amino-*N*-1-benzylindole, 5-amino-*N*-1-benzylindazole, 5-amino-*N*-1-phenylsulfonylindole, 4-phenoxyaniline and 4-benzyloxyaniline all provide sufficiently potent pan-erbB isolated enzyme inhibition (IC_{50} < 500 nM). Compounds with cyclic amine solubilized crotonamides and pan-erbB anilines on a quinazoline or pyrido[3,4-*d*]pyrimidine scaffold have been prepared. These compounds have been tested for their ability to inhibit erbB1, 2 and 4 isolated enzyme activity and erbB1 cellular activity. They have further been analyzed in functional assays to determine their erbB1 alkylation kinetics and pharmacodynamic inhibition of erbB1 *in vivo*. Compounds **54** and **71** were identified as clear lead candidates for advanced studies, having demonstrated selective, potent, irreversible inhibition of erbB1 *in vitro* and prolonged inhibition of erbB1 autophosphorylation *in vivo*. The sustained target modulation for **54** *in vivo* is consistent with dramatically improved pharmacokinetics relative to canertinib in rat, dog and monkey,[57] a consequence of the metabolic stability of the piperidinyl crotonamide Michael acceptor. We propose that this Michael acceptor at the 6-position, particularly with a 3-chloro-4-fluoroanilino group at the 4-position, provides an optimum pharmacophore for irreversible inhibition of erbB1, 2 and 4 on both quinazoline and pyrido[3,4-*d*]pyrimidine scaffolds. Pharmacokinetic comparison of compounds **54** and **71** in rat, dog and monkey identified compound **54** over (the more variable) compound **71** as the preferred clinical candidate.

Compound **54** (PF-00299804), has now been widely studied in our laboratory and by several other groups. The irreversible nature of erbB1 inhibition was confirmed at the isolated enzyme level employing an ELISA-based enzyme assay and at the cellular level

by assaying inhibition of erbB1autophosphorylation following drug exposure time course and extensive wash-out in A431 cells.[57] This was further supported by solution state protein mass spectrometry experiments and an erbB1 T790M X-ray co-crystal structure at 1.8 Å resolution, clearly showing the presence of PF-00299804 covalently linked to the target cysteine.[58] PF-00299804 has been screened against a panel of 38 protein kinases to assess off-target interactions and was found to be particularly selective for the erbB family with only Lck, Src and JAK3 returning isolated enzyme inhibitory IC50's < 10 μM (IC50 = 0.094, 0.110 and 3.57 μM, respectively).[59]

Growth inhibitory activity has been observed for PF-00299804 in several cancer cell lines including models of squamous cell carcinoma of the head and neck that exhibit low response to cetuximab,[60] biliary tract cancer cell lines either as a single agent or in combination with gemcitabine,[61] erbB2-amplified breast cancer cell lines resistant to trastuzumab and lapatinib[62] and erbB2-amplified gastric cancer cell lines.[63]

PF-00299804 has shown excellent distribution to human tumor xenografts in nude mice and admirable pharmacokinetic properties across species.[57] Features resulting in efficacy superior to canertinib, demonstrating marked tumor regression in several xenograft models including the wild-type erbB1 over-expressing A431 epidermoid carcinoma xenograft, the erbB2/3 expressing H125 non-small-cell lung cancer xenograft and the erbB2 over-expressing SKOV3 ovarian carcinoma xenograft.[57] The striking erbB1 pharmacodynamics reported herein is matched by greater than 99% inhibition of phospho-erbB2 observed in SKOV3 tumor xenografts following a well tolerated dose.[57] In addition, PF-00299804 has been shown pre-clinically to be a potent inhibitor of erbB1-activating mutations (L858R, exon 19 deletions) as well as the erbB1 T790M resistance mutation, both *in vitro* and *in*

vivo. Robust anti-tumor activity is observed in the NCI-H1975 and engineered HCC827-del/T790M NSCLC xenograft models that are resistant to erlotinib/gefitinib.[57, 59]

Phase I trials of PF-00299804 have now been conducted in the USA,[64] South Korea[65] and Japan[66] providing broadly similar results. Dose-limiting toxicities include rash, diarrhea, paronychia, dehydration and stomatitis. The maxium tolerated daily oral dose has been established to be 45 mg and the mean human plasma half-life ($t_{1/2}$) was 59 – 85 h, with dosing ranging from 30 to 60 mg. The improved human half-life relative to canertinib (~ 4 h) and the elimination of thrombocytopenia as a problematic toxicity validates the drug optimisation approach reported herein.

The efficacy of PF-00299804 has to date most widely been assessed in advanced or metastatic NSCLC.[67, 68] Phase II studies evaluating PF-00299804 as salvage therapy in advanced NSCLC patients who progressed following chemotherapy and erlotinib demonstrated preliminary evidence of activity,[65, 69] however a double-blind, randomised Phase III trial (BR-26) in this setting determined PF-00299804 did not improve overall survival compared with placebo (NCT01000025).[70] Phase II evaluation of PF-00299804 as a second-line therapy for NSCLC in comparison to erlotinib, successfully met the primary endpoint of a statistically significant improvement in progression-free survival over erlotinib.[71] Unfortunately, the double-blind, randomised phase III trial (ARCHER 1009) comparing the efficacy of PF-00299804 to erlotinib as second- or third-line NSCLC therapy failed to establish superiority of PF-00299804 over erlotinib in this unselected patient population (NCT01360554).[72] A subanalysis of this trial has since demonstrated superiority of PF-00299804 over erlotinib among NSCLC patients harbouring erbB1 mutations,[73] while Phase II evaluation of PF-00299804 in a first-line setting in this patient

population has demonstrated significant activity with an objective response rate of 74%.[74] A randomised phase III study of PF-00299804 versus gefitinib (ARCHER 1050) in this setting is on-going (NCT01774721). Outside of lung cancer, PF-00299804 has also demonstrated promising efficacy in Phase II trial as a first-line treatment in recurrent and/or metastatic squamous-cell carcinoma of the head and neck where 12.7% of patients achieved a partial response and 57.1% of patients experienced stable disease.[75]

In summary, PF-00299804, now assigned the nomenclature of dacomitinib, has demonstrated encouraging anti-tumor activity in human trials.[73,76] A large multi-center randomised Phase III trial in the appropriately selected NSCLC EGFR mutant positive patient population is currently in progress. Only when this is complete will we further understand the role of this second generation irreversible pan-erbB inhibitor for the treatment of NSCLC.

Experimental Section

Combustion analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ, or by the Analytical Department, Pfizer Global Research and Development, Michigan Laboratories. Purity of the compounds was confirmed to be ≥95% by combustion analysis with found values for carbon, hydrogen and nitrogen being within 0.4% of the calculated values for the proposed formula. The compounds were recrystallized from ethyl acetate unless otherwise stated. Melting points were determined using either an Electrothermal Model 9200 or a Gallenkamp digital melting point apparatus, and are as read. ^{1}H NMR spectra were measured either on a Bruker Avance-400, or a Varian INOVA 400 MHz spectrometer, and are referenced to Me_4Si.

High resolution mass spectra were recorded on a Finnigan MAT 900Q or a Varian VG-70SE spectrometer at nominal 5000 resolution. Liquid chromatography-mass spectrometry (LCMS) was performed either on an Agilent 1100 LC system interfaced with an Agilent MSD mass detector or on a Micromass Platform LC mass spectrometer. In both cases, mass detection was performed with an APCI source, using simultaneous positive and negative ion acquisition. Unless otherwise indicated, compounds were purified by flash column chromatography on Silica gel 60 support (Scharlau, 230-400 mesh ASTM), using the indicated eluants.

General Procedure 1. Synthesis of Quinazolines of Scheme 1

The appropriate acid chloride (1.25 eq) was added to a solution of 6-aminoquinazoline[18] (**85**) (1.0 eq) and triethylamine (1.25 eq) in THF. The reaction was stirred at room temperature until tlc indicated the reaction is complete. The reaction was then diluted with water and extracted into EtOAc. The organic extract was dried over $MgSO_4$, filtered, concentrated and purified by column chromatography eluting with a gradient of $MeOH/CH_2Cl_2$.

***N*-{4-(3-Chloro-4-fluoroanilino)-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl}-2-methylacrylamide (8).** Reaction of amine **85** with methacryloyl chloride according to General Procedure 1, gave the title compound **8** as a cream solid (6%), m.p. 137-138ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.78 (s, 1 H), 9.19 (s, 1 H), 8.71 (s, 1 H), 8.51 (s, 1 H), 8.12 (dd, *J* = 2.7, 6.8 Hz, 1 H), 7.78 (m, 1 H), 7.39 (t, *J* = 9.3 Hz, 1 H), 7.25 (s, 1 H), 5.91 (s, 1 H), 5.56 (s, 1 H), 4.20 (t, *J* = 6.1 Hz, 2 H), 3.54 (t, *J* = 4.6 Hz, 4 H), 2.44 (m, 4 H), 2.33 (br s, 2 H), 1.99 (s, 3 H), 1.93 (m, 2 H). Anal. ($C_{25}H_{27}N_5O_3FCl.1^1/_5H_2O$) C, H, N.

(2*E*)-*N*-{4-(3-Chloro-4-fluoroanilino)-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl}-2-butenamide (9). Reaction of amine **85** with crotonyl chloride according to General Procedure 1, gave the title compound **9** as a cream solid (1%), m.p. 117-118ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.76 (s, 1 H), 9.33 (s, 1 H), 8.83 (s, 1 H), 8.49 (s, 1 H), 8.10 (dd, *J* = 2.6, 6.8 Hz, 1 H), 7.39 (t, *J* = 9.3 Hz, 1 H), 7.24 (s, 1 H), 6.70 (m, 1H), 6.39 (m, 1H), 4.20 (t, *J* = 6.3 Hz, 1 H), 3.54 (t, *J* = 4.6 Hz, 4 H), 2.44 (m, 4 H), 2.33 (br s, 2 H), 1.99 (s, 2 H), 1.85 (m, 3 H) (^{1}H NMR contains 1 equivalent of crotonic acid). Anal. ($C_{25}H_{27}N_5O_3FCl.C_4H_6O_2.1^1/_5H_2O$) C, H, N.

***N*-{4-(3-Chloro-4-fluoroanilino)-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl}-1-cyclopentene-1-carboxamide (10).** Reaction of amine **85** with 1-cyclopentenecarboxylic acid chloride according to General Procedure 1, gave the title compound **10** as a cream solid (42%), m.p. 165-166ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.76 (s, 1 H), 8.97 (s, 1 H), 8.74 (s, 1 H), 8.48 (s, 1 H), 8.07 (m, 1 H), 7.75 (m, 1 H), 7.38 (t, *J* = 9.0 Hz, 1 H), 7.20 (s, 1 H), 6.72 (s, 1 H), 4.19 (m, 2 H), 3.52 (m, 4 H), 2.57 (m, 2 H), 2.42 (m, 6 H), 1.93 (m, 4 H). Anal. ($C_{27}H_{29}N_5O_3FCl.1^1/_{10}H_2O$) C, H, N.

***N*-{4-(3-Chloro-4-fluoroanilino)-7-[3-(4-morpholinyl)propoxy]-6-quinazolinyl}propanamide (11).** Reaction of amine **85** with propionyl chloride according to General Procedure 1, gave the title compound **11** as a cream solid, m.p. 201-202ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.73 (s, 1 H), 9.24 (s, 1 H), 8.70 (s, 1 H), 8.47 (s, 1 H), 8.10 (m, 1 H), 7.75 (m, 1 H), 7.36 (t, *J* = 9.0 Hz, 1 H), 7.20 (s, 1 H), 4.19 (t, *J* = 6.3 Hz, 2 H), 3.54 (m, 4 H), 2.44 (m, 8 H), 1.90 (m, 2 H), 1.09 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{24}H_{27}N_5O_3FCl$) C, H, N.

General Procedure 2. Synthesis of Quinazolines of Scheme 2

7-Methoxy-6-nitro-4(3*H*)-quinazolinone (87). Sodium (2.3 g, 0.1 mol) was dissolved in methanol (150 mL). This solution was then added to 7-fluoro-6-nitro-4(3*H*)-quinazolinone[77] **86** (4.01 g, 19.1 mmol) in a sealed pressure vessel and heated to 100ºC over night. The solution was neutralised with AcOH and diluted with water. The solid was filtered off to give the title compound **87** as light brown solid (3.63 g, 86%), m.p. (EtOH) 287°C (dec). ^{1}H NMR [$(CD_3)_2SO$] δ 12.4 (br s, 1 H), 8.53 (s, 1 H), 8.23 (s, 1 H), 7.43 (s, 1 H), 4.05 (s, 3 H). Anal. ($C_9H_7N_3O_4$) C, H, N.

***N*-(7-Methoxy-4-oxo-3,4-dihydro-6-quinazolinyl)propanamide (88)**. Compound **87** (5.1 g, 23.2 mmol) was suspended in EtOH (200 mL) and water (100 mL) and heated to 100ºC. After addition of glacial acetic acid (3 mL), Fe dust (activated by 1N HCl wash, 6.8 g, 121 mmol) was added and the mixture was heated at 100ºC for 1 h. The hot solution was basified with 25% ammonia solution and filtered through a pad of Celite, then cooled to r.t. The pH of the solution was adjusted to pH 6 (HCl) and basified again to pH 8 ($NaHCO_3$). Ethanol was removed under reduced pressure, then water was added and the solid was collected by filtration to give the amine[78] as a brown solid (3.98 g, 90%). The amine was mixed with propionic anhydride (200 mL) and heated at 120ºC. Once the solution turned dark brown it was cooled down and water (250 mL) was added. The mixture was then heated to quench the excess anhydride. The cooled solution was neutralised with 25% ammonia solution followed by saturated $NaHCO_3$ solution. Filtration of the solid gave the title compound **88** as light brown solid (4.63 g, 90%), m.p. (EtOH) 298-301°C. ^{1}H NMR [$(CD_3)_2SO$] δ 12.0 (br s, 1 H), 9.23 (s, 1 H), 8.76 (s, 1 H), 7.99 (s, 1

H), 7.17 (s, 1 H), 4.16 (s, 3 H), 2.45 (q, *J* = 7.5 Hz, 2 H), 1.09 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{12}H_{13}N_3O_3$) C, H, N.

***N*-(4-Chloro-7-methoxy-6-quinazolinyl)propanamide (89)**. Compound **88** (6.14 g, 25 mmol) was suspended in $POCl_3$ (80 mL) and heated at reflux for 90 min. The $POCl_3$ was distilled off under reduced pressure and the resulting yellow-cream solid dissolved in EtOAc (700 mL), then treated with a mixture of ice and saturated $NaHCO_3$ solution. The aqueous layer was further extracted with EtOAc (100 mL), then the combined organic fractions were washed with brine (2x250 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford the title compound **89** as a pale yellow solid (5.64 g, 86%), m.p. 165-168°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.59 (s, 1 H), 9.08 (s, 1 H), 8.90 (s, 1 H), 7.52 (s, 1 H), 4.10 (s, 3 H), 2.55 (q, *J* = 7.5 Hz, 2 H), 1.11 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{12}H_{12}ClN_3O_2$) C, H, N.

***N*-[4-(3-Chloro-4-fluoroanilino)-7-methoxy-6-quinazolinyl]propanamide (12)**. Compound **89** (50 mg, 0.19 mmol) and 3-chloro-4-fluoroaniline (64 mg, 0.44 mmol) in isopropanol (10 mL) with 1 drop of conc. HCl was heated to 100ºC. After 45 min TEA was added until the solution was pH 7-8, then the solvent was removed under reduced pressure. The resulting solid was purified by chromatography on silica gel (2-10% MeOH/EtOAc as eluant) to afford the title compound **12** as a white solid (64 mg, 90%), m.p. 253-256°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.78 (s, 1 H), 9.43 (s, 1 H), 8.84 (s, 1 H), 8.52 (s, 1 H), 8.12 (dd, *J* = 6.9, 2.5 Hz, 1 H), 7.79, (ddd, *J* = 9.0, 6.9, 2.5 Hz, 1 H), 7.42 (t, *J* = 9.1 Hz, 1 H), 7.39 (s, 1 H), 4.01 (s, 3 H), 2.48 (q, *J* = 7.5 Hz, 2 H), 1.13 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{18}H_{16}ClFN_4O_2$) C, H, N.

***N*-[4-(1*H*-Indol-5-ylamino)-7-methoxy-6-quinazolinyl]propanamide (13)**. Reaction of chloride **89** with 1*H*-indol-5-amine according to General Procedure 2, gave the title compound **13** as a cream solid (62%), m.p. 256-258ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 10.89 (s, 1 H), 9.42 (s, 1 H), 9.22 (s, 1 H), 8.67 (s, 1 H), 8.24 (s, 1 H), 7.71 (d, *J* = 1.6 Hz, 1 H), 7.26-7.19 (m, 3 H), 7.08 (s, 1 H), 6.29 (m, 1 H), 3.86 (s, 3 H), 2.36 (q, *J* = 7.5 Hz, 2 H), 1.00 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{20}H_{19}N_5O_2.{}^1/_8$MeOH) C, H, N.

***N*-[4-(1*H*-Indazol-5-ylamino)-7-methoxy-6-quinazolinyl]propanamide (14).** Reaction of chloride **89** with 5-aminoindazole according to General Procedure 2, gave the title compound **14** as a cream solid (39%), m.p. 280-282ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 12.98 (s, 1 H), 9.70 (s, 1 H), 9.37 (s, 1 H), 8.81 (s, 1 H), 8.40 (s, 1 H), 8.09 (d, *J* = 1.8 Hz, 1 H), 8.04 (d, *J* = 1.4 Hz, 1 H), 7.60 (m, 1 H), 7.50 (d, *J* = 9.0 Hz, 1 H), 7.21 (s, 1 H), 3.98 (s, 3 H), 2.44 (q, *J* = 7.4 Hz, 2 H), 1.11 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{19}H_{18}N_6O_2.1^4/_5\ H_2O$) C, H, N.

***N*-{4-[(1-Benzyl-1*H*-indol-5-yl)amino]-7-methoxy-6-quinazol0inyl}propanamide (15).** Reaction of chloride **89** with 1-benzyl-1*H*-indol-5-amine according to General Procedure 2, gave the title compound **15** as a cream solid (62%), m.p. 208-212ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (s, 1 H), 9.34 (s, 1 H), 8.80 (s, 1 H), 8.36 (s, 1 H), 7.87 (d, *J* = 1.8 Hz, 1 H), 7.50 (d, *J* = 3.1 Hz, 1 H), 7.21-7.42 (m, 8 H), 6.48 (d, *J* = 3.1 Hz, 1 H), 5.42 (s, 2 H), 3.98 (s, 3 H), 2.45 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{27}H_{25}N_5O_2$) C, H, N.

***N*-{4-[(1-Benzyl-1*H*-indazol-5-yl)amino]-7-methoxy-6-quinazolinyl}propanamide (16).** Reaction of N^4-(1-benzyl-1*H*-indazol-5-yl)-7-methoxy-4,6-quinazolinediamine[79] with propionyl chloride according to General Procedure 1, gave the title compound **16** as a

cream solid (26%), m.p. 211-212ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.69 (s, 1 H), 9.38 (s, 1 H), 8.77 (s, 1 H), 8.36 (s, 1 H), 8.05 (s, 2 H), 7.60 (m, 2 H), 7.29-7.18 (m, 6 H), 5.61 (s, 2 H), 3.94 (s, 3 H), 1.19 (d, *J* = 13.4 Hz, 2 H), 1.07 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{25}H_{29}N_5O_2FCl.1^1/_5H_2O$) C, H, N.

***N*-(7-Methoxy-4-{[1-(2-pyridinylmethyl)-1*H*-indol-5-yl]amino}-6-quinazolinyl)propanamide (17)**. Reaction of chloride **89** with 1-(2-pyridinylmethyl)-1*H*-indol-5-amine (Supporting Information) according to General Procedure 2, gave the title compound **17** as a cream solid (91%), m.p. 219-221ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (s, 1 H), 9.36 (s, 1 H), 8.79 (s, 1 H), 8.55 (m, 1 H), 8.36 (s, 1 H), 7.87 (d, *J* = 1.8 Hz, 1 H), 7.72 (dt, *J* = 7.7, 1.8 Hz, 1 H), 7.50 (d, *J* = 3.1 Hz, 1 H), 7.26-7.39 (m, 3 H), 7.19 (s, 1 H), 6.99 (d, *J* = 7.7 Hz, 1 H), 6.50 (d, *J* = 3.1 Hz, 1 H), 5.51 (s, 2 H), 3.98 (s, 3 H), 2.44 (q, *J* = 7.4 Hz, 2 H), 1.12 (t, *J* = 7.4 Hz, 3 H). Anal. ($C_{26}H_{24}N_6O_2.^1/_2H_2O$) C, H, N.

***N*-(7-Methoxy-4-{[1-(3-pyridinylmethyl)-1*H*-indol-5-yl]amino}-6-quinazolinyl)propanamide (18)**. Reaction of chloride **89** with 1-(3-pyridinylmethyl)-1*H*-indol-5-amine[80] according to General Procedure 2, gave the title compound **18** as a cream solid (88%), m.p. 213-217ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (s, 1 H), 9.35 (s, 1 H), 8.79 (s, 1 H), 8.54 (d, *J* = 1.7 Hz, 1 H), 8.46 (dd, *J* = 4.8, 1.6 Hz, 1 H), 8.37 (s, 1 H), 7.88 (d, *J* = 1.8 Hz, 1 H), 7.58 (m, 1 H), 7.54 (d, *J* = 3 .1 Hz, 1 H), 7.47 (d, *J* = 8.8 Hz, 1 H), 7.32-7.38 (m, 2 H), 7.21 (s, 1 H), 6.50 (dd, *J* = 3.1, 0.7 Hz, 1 H), 5.47 (s, 2 H), 3.98 (s, 3 H), 2.45 (q, *J* = 7.6 Hz, 2 H), 1.13 (t, *J* = 7.6 Hz, 3 H). HRMS (FAB^+) calcd for $C_{26}H_{25}N_6O_2$: 453.2039 (MH^+). Found 453.2034.

***N*-(7-Methoxy-4-{[1-(4-pyridinylmethyl)-1*H*-indol-5-yl]amino}-6-quinazolinyl)propanamide (19)**. Reaction of chloride **89** with 1-(4-pyridinylmethyl)-1*H*-indol-5-amine[80] according to General Procedure 2, gave the title compound **19** as a cream solid (92%), m.p. 168-171ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.58 (s, 1 H), 9.35 (s, 1 H), 8.79 (s, 1 H), 8.49 (m, 2 H), 8.37 (s, 1 H), 7.91 (m, 1 H), 7.51 (d, *J* = 3.1 Hz, 1 H), 7.36 (d, *J* = 1.3 Hz, 2 H), 7.21 (s, 1 H), 7.10 (m, 2 H), 6.53 (d, *J* = 3.1 Hz, 1 H), 5.50 (s, 2 H), 3.97 (s, 3 H), 2.45 (q, *J* = 7.6 Hz, 2 H), 1.12 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{26}H_{24}N_6O_2.1^1/_2H_2O$) C, H, N.

***N*-(7-Methoxy-4-{[1-(2-pyridinylmethyl)-1*H*-indazol-5-yl]amino}-6-quinazolinyl)propanamide (20).** Reaction of 7-methoxy-*N*^{4}-(1-(pyridin-2-ylmethyl)-1*H*-indazol-5-yl)quinazoline-4,6-diamine[81] with propionyl chloride according to General Procedure 1, gave the title compound **20** as a cream solid (58%). ^{1}H NMR [$(CD_3)_2SO$] δ 11.47 (s, 1 H), 9.65 (s, 1 H), 9.11 (s, 1 H), 8.55 (d, *J* = 4.1 Hz, 1 H), 8.17 (s, 1 H), 7.93 (d, *J* = 1.5 Hz, 1 H), 7.85 (t, *J* = 7.5 Hz, 1 H), 7.75 (d, *J* = 9.0 Hz, 1 H), 7.55 (dd, *J* = 1.7, 8.8 Hz, 2 H), 7.40 (t, *J* = 5.6 Hz, 2 H), 7.12 (d, *J* = 7.8 Hz, 1 H), 5.83 (s, 2 H), 4.01 (s, 3 H), 2.44 (q, *J* = 1.7 Hz, 2 H), 1.07 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{25}H_{23}N_7O_2.3HCl$) C, N. H: calcd, 4.7; found, 5.2.

***N*-(7-Methoxy-4-{[1-(phenylsulfonyl)-1*H*-indol-5-yl]amino}-6-quinazolinyl)propanamide (21).** Reaction of chloride **89** with 1-(phenylsulfonyl)-1*H*-indol-5-amine[24] according to General Procedure 2, gave the title compound **21** as a cream solid (85%), m.p. 270-275ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.69 (s, 1 H), 9.37 (s, 1 H), 8.81 (s, 1 H), 8.42 (s, 1 H), 7.99 (m, 3 H), 7.91 (d, *J* = 8.9 Hz, 1 H), 7.79 (d, *J* = 3.7 Hz, 1 H), 7.70-

7.59 (m, 4 H), 7.23 (s, 1 H), 6.86 (dd, *J* = 3.7, 0.6 Hz, 1 H), 3.99 (s, 3 H), 2.45 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{26}H_{23}N_5O_4S.^1/_2MeOH$) C, H, N.

***N*-(4-{[1-(2-Furylmethyl)-1*H*-indol-5-yl]amino}-7-methoxy-6-quinazolinyl)propanamide (22)**. Reaction of chloride **89** with 1-(2-furylmethyl)-1*H*-indol-5-amine (Supporting Information) according to General Procedure 2, gave the title compound **22** as a cream solid (82%), m.p. 221-224ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.58 (s, 1 H), 9.36 (s, 1 H), 8.80 (s, 1 H), 8.37 (s, 1 H), 7.85 (d, *J* = 1.8 Hz, 1 H), 7.58 (m, 1 H), 7.53 (d, *J* = 8.8 Hz, 1 H), 7.39 (m, 2 H), 7.21 (s, 1 H), 6.40-6.47 (m, 3 H), 5.41 (s, 2 H), 3.99 (s, 3 H), 2.45 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{25}H_{23}N_5O_3.^1/_3THF$) C, H, N.

***N*-{4-[(1-Benzyl-2,3-dihydro-1*H*-indol-5-yl)amino]-7-methoxy-6-quinazolinyl}propanamide (23)**. Reaction of chloride **89** with 1-benzyl-2,3-dihydro-1*H*-indol-5-ylamine[24] according to General Procedure 2, gave the title compound **23** as a yellow solid (80%), m.p. 169-171ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.39 (s, 1 H), 9.33 (s, 1 H), 8.73 (s, 1 H), 8.35 (s, 1 H), 7.22-7.41 (m, 7 H), 7.19 (s, 1 H), 6.57 (d, *J* = 8.4 Hz, 1 H), 4.27 (s, 2 H), 3.97 (s, 3 H), 3.26 (t, *J* = 8.2 Hz, 2 H), 2.92 (t, *J* = 8.2 Hz, 2 H), 2.44 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{27}H_{27}N_5O_2.^1/_4EtOAc$) C, H, N.

***N*-(7-Methoxy-4-{[1-(2-pyridinylmethyl)-2,3-dihydro-1*H*-indol-5-yl]amino}-6-quinazolinyl)propanamide (24).** Reaction of chloride **89** with 1-(2-pyridinylmethyl)-2,3-dihydro-1*H*-indol-5-ylamine (Supporting Information) according to General Procedure 2, gave the title compound **24** as a brown solid (79%), m.p. 90-94ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.40 (s, 1 H), 9.34 (s, 1 H), 8.72 (s, 1 H), 8.54 (d, *J* = 4.0 Hz, 1 H), 8.35 (s, 1 H), 7.78 (dt,

J = 7.7, 1.8 Hz, 1 H), 7.41 (m, 2 H), 7.30-7.15 (m, 3 H), 6.51 (d, *J* = 8.4 Hz, 1 H), 4.37 (s, 2 H), 3.97 (s, 3 H), 3.44 (m, 2 H), 2.96 (t, *J* = 8.2 Hz, 2 H), 2.45 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). HRMS (FAB^+) calcd for $C_{26}H_{26}N_6O_2$: 454.2117 (M^+). Found 454.2110.

***N*-{4-[(1-Isobutyl-1*H*-indol-5-yl)amino]-7-methoxy-6-quinazolinyl}propanamide (25).** Reaction of chloride **89** with 1-isobutyl-2,3-dihydro-1*H*-indol-5-ylamine (Supporting Information) according to General Procedure 2, gave the title compound **25** as a cream solid (91%), m.p. 202-204ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (s, 1 H), 9.35 (s, 1 H), 8.81 (s, 1 H), 8.37 (s, 1 H), 7.86 (d, *J* = 1.8 Hz, 1 H), 7.46-7.31 (m, 3 H), 7.21 (s, 1 H), 6.41 (d, *J* = 3.0 Hz, 1 H), 3.99 (s, 3 H), 3.96 (d, *J* = 5.7 Hz, 2 H), 2.45 (q, *J* = 7.5 Hz, 2 H), 2.13 (m, 1 H), 1.13 (t, *J* = 7.5 Hz, 3 H), 0.87 (d, *J* = 7.8 Hz, 6 H). Anal. ($C_{24}H_{27}N_5O_2.{}^2/_9H_2O$) C, H, N.

***N*-[4-(Benzylamino)-7-methoxy-6-quinazolinyl]propanamide (26).** Reaction of chloride **89** with benzylamine according to General Procedure 2, gave the title compound **26** as a cream solid (45%), m.p. 166-168ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.29 (s, 1 H), 8.61 (s, 1 H), 8.57 (t, *J* = 6.0 Hz, 1 H), 8.31 (s, 1 H), 7.34-7.25 (m, 4 H), 7.22-7.17 (m, 1 H), 7.14 (s, 1 H), 4.70 (d, *J* = 5.7 Hz, 1 H), 3.94 (s, 3 H), 2.41 (q, *J* = 7.4 Hz, 2 H), 1.08 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{19}H_{20}N_4O_2.H_2O$) C, H, N.

***N*-(7-Methoxy-4-{[(1*R*)-1-phenylethyl]amino}-6-quinazolinyl)propanamide (27).** Reaction of chloride **89** with α-methylbenzylamine according to General Procedure 2, gave the title compound **27** as a cream solid (44%), m.p. 157-159ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.31 (s, 1 H), 8.62 (s, 1 H), 8.26 (m, 2 H), 7.42 (m, 2 H), 7.27 (t, *J* = 7.2 Hz, 2 H), 7.16

(m, 1 H), 7.11 (s, 1 H), 5.57 (m, 1 H), 3.92 (s, 3 H), 2.41 (q, *J* = 7.2 Hz, 2 H), 1.55 (d, *J* = 7.0 Hz, 3 H), 1.09 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{20}H_{22}N_4O_2 .H_2O$) C, H, N.

***N*-[7-Methoxy-4-(4-phenoxyanilino)-6-quinazolinyl]propanamide (28)**. Reaction of chloride **89** with 4-phenoxyaniline according to General Procedure 2, gave the title compound **28** as a cream solid (91%), m.p. 137-140ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.67 (s, 1 H), 9.38 (s, 1 H), 8.82 (s, 1 H), 8.46 (s, 1 H), 7.79 (m, 2 H), 7.39 (m, 2 H), 7.25 (s, 1 H), 7.12 (m, 1 H), 7.07-7.00 (m, 4 H), 4.00 (s, 3 H), 2.47 (q, *J* = 7.5 Hz, 2 H), 1.13 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{24}H_{22}N_4O_3.{}^1/_2$MeOH) C, H, N.

***N*-{4-[4-(3-Fluorophenoxy)anilino]-7-methoxy-6-quinazolinyl}propanamide (29).** Reaction of chloride **89** with 4-(3-fluorophenoxy)aniline according to General Procedure 2, gave the title compound **29** as a cream solid (29%), m.p. 144-146ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.78 (s, 1 H), 9.42 (s, 1 H), 8.81 (s, 1 H), 8.46 (s, 1 H), 7.79 (d, *J* = 8.8 Hz, 2 H), 7.39 (q, *J* = 8.1 Hz, 1 H), 7.22 (s, 1 H), 7.08 (d, *J* = 8.8 Hz, 1 H), 6.92 (t, *J* = 8.1 Hz, 1 H), 6.81 (m, 2 H), 3.95 (s, 3 H), 2.42 (q, *J* = 8.0 Hz, 2 H, under DMSO peak), 1.09 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{24}H_{21}N_4O_3F.H_2O$) C, H. N: calcd, 12.4; found, 13.2.

***N*-{4-[4-(1,3-Benzodioxol-5-yloxy)anilino]-7-methoxy-6-quinazolinyl}propanamide (30).** Reaction of chloride **89** with 4-(benzo[d][1,3]dioxol-5-yloxy)benzenamine according to General Procedure 2, gave the title compound **30** as a cream solid (24%). ^{1}H NMR [$(CD_3)_2SO$] δ 9.60 (s, 1 H), 9.35 (s, 1 H), 8.75 (s, 1 H), 8.38 (s, 1 H), 7.67 (d, *J* = 9.0 Hz, 2 H), 7.18 (s, 1 H), 6.93-6.84 (m, 3 H), 6.67 (d, *J* = 2.4 Hz, 1 H), 6.42 (m, 1 H), 5.98 (s, 2 H), 3.94 (s, 3 H), 2.41 (q, *J* = 7.3 Hz, 2 H under DMSO peak), 1.06 (t, *J* = 7.6 Hz, 3 H).

***N*-{7-Methoxy-4-[4-(3-pyridinyloxy)anilino]-6-quinazolinyl}propanamide (31).** Reaction of chloride **89** with 3-(4-aminophenoxy)pyridine according to General Procedure 2, gave the title compound **31** as a cream solid (77%), m.p. 163-167ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.66 (s, 1 H), 9.36 (s, 1 H), 8.78 (s, 1 H), 8.42 (s, 1 H), 8.32 (d, *J* = 17.8 Hz, 2 H), 7.77 (d, *J* = 9.0 Hz, 2 H), 7.37 (d, *J* = 1.7 Hz, 1 H), 7.20 (s, 1 H), 7.05 (d, *J* = 9.0 Hz, 2 H), 3.94 (s, 3 H), 2.42 (q, *J* = 7.6 Hz, 2 H under DMSO peak), 1.07 (t, *J* = 7.6 Hz, 3 H). Anal. ($C_{23}H_{21}N_5O_3.H_2O$) C, H, N.

***N*-{7-Methoxy-4-[4-(2-pyridinylmethyl)anilino]-6-quinazolinyl}propanamide (32).** Reaction of chloride **89** with 4-(2-pyridinylmethyl)aniline according to General Procedure 2, gave the title compound **32** as a cream solid (94%), m.p. 195-198ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.62 (s, 1 H), 9.41 (s, 1 H), 8.80 (s, 1 H), 8.50 (m, 1 H), 8.43 (s, 1 H), 7.75-7.65 (m, 3 H), 7.32-7.18 (m, 5 H), 4.07 (s, 2 H), 3.99 (s, 3 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{24}H_{23}N_5O_2.{}^1/_4$MeOH) C, H, N.

***N*-{7-Methoxy-4-[4-(4-pyridinylmethyl)anilino]-6-quinazolinyl}propanamide (33).** Reaction of chloride **89** with 4-(4-pyridinylmethyl)aniline according to General Procedure 2, gave the title compound **33** as a yellow solid (85%), m.p. 227-230ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.61 (s, 1 H), 9.37 (s, 1 H), 8.80 (s, 1 H), 8.48-8.42 (m, 3 H), 7.71 (br d, *J* = 8.5 Hz, 2 H), 7.29-7.23 (m, 5 H), 3.99 (s, 3 H), 3.96 (s, 2 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{24}H_{23}N_5O_2$) C, H, N.

***N*-(4-{4-[Hydroxy(phenyl)methyl]anilino}-7-methoxy-6-quinazolinyl)propanamide (34)**. Reaction of chloride **89** with (4-aminophenyl)(phenyl)methanone according to General Procedure 2, followed by reduction of the ketone with $NaBH_4$ in MeOH, gave the

title compound **34** as a white solid (37%), m.p. 239-242ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.60 (s, 1 H), 9.36 (s, 1 H), 8.79 (s, 1 H), 8.42 (s, 1 H), 7.68 (br d, *J* = 8.6 Hz, 2 H), 7.40 (br d, *J* = 7.2 Hz, 2 H), 7.36-7.27 (m, 4 H), 7.25-7.17 (m, 2 H), 5.81 (d, *J* = 4.0 Hz, 1 H), 5.69 (d, *J* = 3.8 Hz, 1 H), 3.99 (s, 3 H), 2.45 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{25}H_{24}N_4O_3$) C, H, N.

***N*-{4-[4-(Benzyloxy)anilino]-7-methoxy-6-quinazolinyl}propanamide (35)**. Reaction of chloride **89** with 4-(benzyloxy)aniline according to General Procedure 2, gave the title compound **35** as a cream solid (96%), m.p. 189-192ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.54 (s, 1 H), 9.36 (s, 1 H), 8.77 (s, 1 H), 8.40 (s, 1 H), 7.63 (br d, *J* = 9.1 Hz, 2 H), 7.47 (m, 2 H), 7.44-7.37 (m, 2 H), 7.35-7.29 (m, 1 H), 7.22 (s, 1 H), 7.02 (br d, *J* = 9.1 Hz, 2 H), 5.12 (s, 2 H), 3.99 (s, 3 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{25}H_{24}N_4O_3$) C, H, N.

***N*-(4-{3-Chloro-4-[(3-fluorobenzyl)oxy]anilino}-7-methoxy-6-quinazolinyl)propanamide (36)**. Reaction of chloride **89** with 3-chloro-4-[(3-fluorobenzyl)oxy]aniline according to General Procedure 2, gave the title compound **36** as a cream solid (95%), m.p. 240-243ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.65 (s, 1 H), 9.42 (s, 1 H), 8.80 (s, 1 H), 8.47 (s, 1 H), 7.96 (d, *J* = 2.6 Hz, 1 H), 7.69 (dd, *J* = 8.9, 2.6 Hz, 1 H), 7.47 (m, 1 H), 7.36-7.28 (m, 2 H), 7.26-7.22 (m, 2 H), 7.21-7.15 (m, 1 H), 5.25 (s, 2 H), 4.00 (s, 3 H), 2.47 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{25}H_{22}ClFN_4O_3$) C, H, N.

***N*-{7-Methoxy-4-[4-(2-pyridinylmethoxy)anilino]-6-quinazolinyl}propanamide (37)**. Reaction of chloride **89** with 4-(2-pyridinylmethoxy)aniline according to General

Procedure 2, gave the title compound **37** as a white solid (64%), m.p. 216-218ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.55 (s, 1 H), 9.35 (s, 1 H), 8.78 (s, 1 H), 8.59 (ddd, *J* = 4.8, 1.7, 0.9 Hz, 1 H), 8.40 (s, 1 H), 7.85 (dt, *J* = 7.7, 1.8 Hz, 1 H), 7.64 (br d, *J* = 9.1 Hz, 2 H), 7.54 (d, *J* = 7.8 Hz, 1 H), 7.35 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1 H), 7.22 (s, 1 H), 7.04 (br d, *J* = 9.1 Hz, 2 H), 5.19 (s, 2 H), 3.99 (s, 3 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.13 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{24}H_{23}N_5O_3.{}^1/_4$MeOH) C, H, N.

***N*-{7-Methoxy-4-[4-(4-pyridinylmethoxy)anilino]-6-quinazolinyl}propanamide (38).** Reaction of chloride **89** with 4-(4-pyridinylmethoxy)aniline according to General Procedure 2, gave the title compound **38** as a white solid (20%), m.p. 212-214ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.55 (s, 1 H), 9.35 (s, 1 H), 8.78 (s, 1 H), 8.59 (dd, *J* = 4.4, 1.6 Hz, 2 H), 8.40 (s, 1 H), 7.65 (br d, *J* = 9.1 Hz, 2 H), 7.46 (br d, *J* = 6.0 Hz, 2 H), 7.22 (s, 1 H), 7.03 (br d, *J* = 9.1 Hz, 2 H), 5.20 (s, 2 H), 3.99 (s, 3 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{24}H_{23}N_5O_3.{}^1/_3$MeOH) C, H, N.

***N*-{7-Methoxy-4-[4-(2-phenylethyl)anilino]-6-quinazolinyl}propanamide (39)**. Reaction of chloride **89** with 4-(2-phenylethyl)aniline according to General Procedure 2, gave the title compound **39** as a cream solid (65%), m.p. 170-172ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.56 (s, 1 H), 9.37 (s, 1 H), 8.80 (s, 1 H), 8.44 (s, 1 H), 7.67 (br d, *J* = 8.5 Hz, 2 H), 7.31-7.15 (m, 8 H), 3.99 (s, 3 H), 2.89 (m, 4 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.13 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{26}H_{26}N_4O_2$) C, H, N.

***N*-{4-[4-(Benzylamino)anilino]-7-methoxy-6-quinazolinyl}propanamide (40)**. Reaction of chloride **89** with N^1-benzyl-1,4-benzenediamine according to General Procedure 2, gave the title compound **40** as a yellow solid (76%), m.p. 146-149ºC. ^{1}H NMR [$(CD_3)_2SO$] δ

9.35 (s, 1 H), 9.32 (s, 1 H), 8.70 (s, 1 H), 8.32 (s, 1 H), 7.39 (br d, *J* = 7.0 Hz, 2 H), 7.35-7.28 (m, 4 H), 7.23 (m, 1 H), 7.17 (s, 1 H), 6.58 (br d, *J* = 8.9 Hz, 2 H), 6.13 (t, *J* = 6.2 Hz, 1 H), 4.28 (d, *J* = 6.0 Hz, 2 H), 3.97 (s, 3 H), 2.44 (q, *J* = 7.5 Hz, 2 H), 1.11 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{25}H_{25}N_5O_2$) C, H, N.

***N*-(7-Methoxy-4-{4-[(phenylsulfonyl)amino]anilino}-6-quinazolinyl)propanamide (41)**. Reaction of chloride **89** with *N*-(4-aminophenyl)benzenesulfonamide according to General Procedure 2, gave the title compound **41** as a yellow solid (87%), m.p. 155-165ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 10.17 (s, 1 H), 9.83 (br s, 1 H), 9.40 (s, 1 H), 8.81 (s, 1 H), 8.47 (s, 1 H), 7.77 (br d, *J* = 7.0 Hz, 2 H), 7.65-7.52 (m, 5 H), 7.23 (s, 1 H), 7.08 (br d, *J* = 8.9 Hz, 2 H), 3.99 (s, 3 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.11 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{24}H_{23}N_5O_4S.{}^1/_4EtOAc.{}^1/_2MeOH$) C, H, N.

***N*-(4-{3-Fluoro-4-[(phenylsulfonyl)amino]anilino}-7-methoxy-6-quinazolinyl)propanamide (42).** Reaction of chloride **89** with *N*-(4-amino-2-fluorophenyl)benzenesulfonamide (Supporting Information) according to General Procedure 2, gave the title compound **42** as a cream solid (78%), m.p. 157-162ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.98 (s, 1 H), 9.74 (s, 1 H), 9.41 (s, 1 H), 8.80 (s, 1 H), 8.51 (s, 1 H), 7.79 (dd, *J* = 13.0, 2.3 Hz, 1 H), 7.72 (br d, *J* = 7.1 Hz, 2 H), 7.64 (m, 1 H), 7.60-7.50 (m, 3 H), 7.26 (s, 1 H), 7.16 (t, *J* = 8.9 Hz, 1 H), 3.99 (s, 3 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). Anal. ($C_{24}H_{22}FN_5O_4S.{}^1/_2H_2O.{}^1/_4MeOH$): C, H, N.

***N*-[7-Methoxy-4-({6-[(phenylsulfonyl)amino]-3-pyridinyl}amino)-6-quinazolinyl]propanamide hydrochloride (43)**. *N*-(5-nitro-2-pyridinyl)benzenesulfonamide (280 mg, 1.0 mmol) was dissolved in EtOH/EtOAc (80

mL, 1:1) to which a catalytic amount of Raney nickel was added. The reaction was stirred vigourously under an atmosphere of hydrogen (40 psi) for 2.5 hours. The catalyst was removed by filtration (caution) and the residue concentrated under reduced pressure to give a quantative yield of *N*-(5-amino-2-pyridinyl)benzenesulfonamide (**A32**) which was used directly. Reaction of **A32** with chloride **89** according to General Procedure 2, gave the title compound **43** as a brown solid (99%), m.p. 244-247ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 11.0 (br s, 1 H), 9.73 (s, 1 H), 9.39 (s, 1 H), 8.79 (s, 1 H), 8.49 (s, 1 H), 8.43 (s, 1 H), 8.07 (dd, *J* = 9.0, 2.6 Hz, 1 H), 7.91 (br d, *J* = 6.9 Hz, 2 H), 7.54-7.65 (m, 4 H), 7.24 (s, 1 H), 7.17 (d, *J* = 8.9 Hz, 1 H), 3.99 (s, 3 H), 2.46 (q, *J* = 7.5 Hz, 2 H), 1.12 (t, *J* = 7.5 Hz, 3 H). HRMS (FAB$^+$) calcd for $C_{23}H_{23}N_6O_4S$: 479.1502 (MH^+). Found 479.1499. Anal. ($C_{23}H_{22}N_6O_4S.2.75HCl$): C, H, N.

General Procedure 3. Synthesis of Quinazolines of Schemes 3 and 4

***N*-(1-Benzyl-1*H*-indazol-5-yl)-7-ethoxy-6-nitroquinazolin-4-amine (91).** 7-Ethoxy-6-nitroquinazolin-4(3*H*)-one (**90**) (2.50 g, 10.6 mmol) prepared using the same method as described for **87** (using ethanol at 50 ^{o}C for 4 hours), was suspended in $POCl_3$ (50 mL) and heated at reflux for 3 h. The $POCl_3$ was distilled off under reduced pressure and the resulting yellow-cream solid was dissolved in CH_2Cl_2 (100 mL) and then treated with a mixture of ice and saturated $NaHCO_3$ solution until the pH was slightly basic. The aqueous layer was further extracted with CH_2Cl_2 (100 mL), then the combined organic fractions were washed with brine (200 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford crude 4-chloro-7-ethoxy-6-nitroquinazoline. This solid was dissolved directly in a mixture of CH_2Cl_2 (40 mL) and iPrOH (10 mL), then added to a suspension of 1-benzyl-1*H*-indazol-5-amine (2.61 g, 11.7 mmol) in iPrOH (50 mL)

which contained 1 M HCl (5 drops). This mixture was heated at 50°C for 15 hours, resulting in precipitation of the desired product as the hydrochloride salt. The reaction was allowed to cool, concentrated *in vacuo*, then poured into saturated $NaHCO_3$ solution (300 mL) and stirred for 5 minutes. The resulting precipitate was collected by filtration and washed well with water and Et_2O. This material was dried to give the title compound **91** as a yellow solid (4.59 g, 98%), m.p. 222-224°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.15 (br s, 1 H), 9.23 (s, 1 H), 8.56 (s, 1 H), 8.19-8.11 (m, 2 H), 7.74-7.68 (m, 1 H), 7.65 (dd, *J* = 8.8, 1.8 Hz, 1 H), 7.42 (s, 1 H), 7.35-7.21 (m, 5 H), 5.67 (s, 2 H), 4.36 (q, *J* = 6.9 Hz, 2 H), 1.41 (t, *J* = 6.9 Hz, 3 H). Anal. ($C_{24}H_{20}N_6O_3$) C, H, N.

***N*^{4}-(1-Benzyl-1*H*-indazol-5-yl)-7-ethoxyquinazoline-4,6-diamine (92).** Compound **91** (1.00 g, 2.27 mmol) was dissolved in a mixture of EtOAc (60 mL) and MeOH (60 mL), then Raney nickel (1.12 g wet from a 50% slurry in water) was added and the mixture stirred under a 40 psi atmosphere of hydrogen in a pressure vessel for 1.75 h. The Raney nickel was then carefully removed by filtration over celite (*caution:* Raney nickel highly flammable when dry) and the solvent removed from the filtrate under reduced pressure. The resulting solid was purified by chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) to afford the title compound **92** as a crystalline pink-brown solid (556 mg, 60%), m.p. 210-212°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.25 (s, 1 H), 8.27 (s, 1 H), 8.21-8.19 (m, 1 H), 8.08 (d, *J* = 0.6 Hz, 1 H), 7.67 (dd, *J* = 9.0, 1.8 Hz, 1 H), 7.63 (d, *J* = 9.0 Hz, 1 H), 7.44 (s, 1 H), 7.34-7.21 (m, 5 H), 7.05 (s, 1 H), 5.65 (s, 2 H), 5.21 (br s, 2 H), 4.21 (q, *J* = 7.0 Hz, 2 H), 1.45 (t, *J* = 7.0 Hz, 3 H). LCMS (APCI$^+$) 311. Anal. ($C_{24}H_{22}N_6O.^1/_4H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-bromobut-2-enamide (93). (*E*)-4-Bromobut-2-enoic acid (2.65 g, 15.9 mmol) was dissolved in dry

CH_2Cl_2 (25 mL), to which was added oxalyl chloride (2.22 g, 17.5 mmol) and DMF (1 drop). This mixture was stirred at room temperature for 3 hours, then all solvent was removed under reduced pressure to give a pale yellow oil which was dissolved in THF (25 mL). Compound **92** (5.00 g, 12.1 mmol) was also dissolved in THF (350 mL), TEA (3.06 g, 30.3 mmol) added, and the flask sealed under nitrogen and cooled to -20°C (ice/salt/water). The above THF solution of acid chloride was added dropwise over 0.25 h., then the reaction mixture was stirred for a further 0.5 h. at this temperature. The mixture was then diluted with saturated $NaHCO_3$ solution (200 mL) and extracted with EtOAc (2x200 mL). The combined EtOAc fractions were washed with brine (200 mL), dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure to give a crude solid which was purified by chromatography on silica gel (2% MeOH/CH_2Cl_2 as eluant). The title compound **93** was isolated as an unstable orange foam (4.28 g, 63%). ^{1}H NMR [$(CD_3)_2SO$] δ 9.75 (s, 1 H), 9.52 (s, 1 H), 8.80 (s, 1 H), 8.42 (s, 1 H), 8.10 (s, 2 H), 7.68 (d, *J* = 9.0 Hz, 1 H), 7.63 (dd, *J* = 9.0, 1.6 Hz, 1 H), 7.20-7.34 (m, 6 H), 6.62-6.67 (m, 1 H), 6.48-6.57 (m, 1 H), 5.66 (s, 2 H), 4.26 (q, *J* = 7.0 Hz, 2 H), 3.49-3.41 (m, 2 H; by D_2O exchange), 1.45 (t, *J* = 6.9 Hz, 3 H). LCMS ($APCI^+$) 557, 559.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-(dimethylamino)but-2-enamide (44). Compound **93** (500 mg, 0.90 mmol) was dissolved in DMA (10 mL) and cooled to -20°C (ice/salt/water). Aqueous dimethylamine solution (40%, 1.01 mL) was added and the reaction monitored by TLC until complete reaction was achieved after 2 h. The reaction mixture was diluted with water (50 mL) and poured into saturated $NaHCO_3$ solution (100 mL). The resulting aqueous solution was extracted with EtOAc (2x100 mL) and the combined EtOAc fractions washed with brine (100 mL) and dried (Na_2SO_4). Filtration, followed by removal of the solvent under reduced pressure

afforded a crude residue which was purified by column chromatography on silica gel (2% MeOH/CH_2Cl_2) to give the title compound **44** as a pale yellow solid (333 mg, 71%), m.p. (Et_2O) 150-154°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H), 9.47 (s, 1 H), 8.92 (s, 1 H), 8.41 (s, 1 H), 8.12 (br s, 1 H), 8.10 (s, 1 H), 7.69-7.63 (m, 2 H), 7.35-7.20 (m, 6 H), 6.80 (dt, *J* = 15.4, 6.0 Hz, 1 H), 6.57 (br d, *J* = 15.1 Hz, 1 H), 5.66 (s, 2 H), 4.28 (q, *J* = 7.0 Hz, 2 H), 3.09 (br d, *J* = 5.0 Hz, 2 H), 2.19 (s, 6 H), 1.46 (t, *J* = 7.0 Hz, 3 H). LCMS (APCI$^+$) 522. Anal. ($C_{30}H_{31}N_7O_2.1^1/_2H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-(diisopropylamino)but-2-enamide (45). Reaction of **93** with diisopropylamine, using the same method as described for **44**, afforded the title compound **45** as a pale yellow solid (32%), m.p. 133-137°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.70 (s, 1 H), 9.42 (s, 1 H), 8.87 (br s, 1 H), 8.41 (s, 1 H), 8.13 (br s, 1 H), 8.10 (s, 1 H), 7.69-7.62 (m, 2 H), 7.35-7.19 (m, 6 H), 6.83 (dt, *J* = 15.2, 5.5 Hz, 1 H), 6.54 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.28 (q, *J* = 7.0 Hz, 2 H), 3.30-3.25 (m, 2 H; by D_2O exchange), 3.01 (septet, *J* = 6.5 Hz, 2 H), 1.45 (t, *J* = 6.9 Hz, 3 H), 1.00 (d, *J* = 6.5 Hz, 12 H). LCMS (APCI$^+$) 578. Anal. ($C_{34}H_{39}N_7O_2.1^1/_2H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-(pyrrolidin-1-yl)but-2-enamide (46). Reaction of **93** with pyrrolidine, using the same method as described for **44**, afforded the title compound **46** as a pale yellow solid (61%), m.p. 131-136°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H), 9.47 (s, 1 H), 8.91 (s, 1 H), 8.41 (s, 1 H), 8.12 (s, 1 H), 8.10 (s, 1 H), 7.69-7.63 (m, 2 H), 7.35-7.21 (m, 6 H), 6.84 (dt, *J* = 15.5, 5.8 Hz, 1 H), 6.57 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.30-3.25 (m, 2

H; by D_2O exchange), 2.51-2.49 (m, 4 H), 1.76-1.68 (br m, 4 H), 1.46 (t, *J* = 7.0 Hz, 3 H). LCMS ($APCI^+$) 548. Anal. ($C_{32}H_{33}N_7O_2.1^1/_2H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-(piperidin-1-yl)but-2-enamide (47). Reaction of **93** with piperidine, using the same method as described for **44**, afforded the title compound **47** as a pale yellow solid (69%), m.p. 140-144°C. ^1H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H), 9.46 (s, 1 H), 8.89 (br s, 1 H), 8.41 (s, 1 H), 8.15-8.08 (m, 2 H), 7.69-7.63 (m, 2 H), 7.35-7.20 (m, 6 H), 6.79 (dt, *J* = 15.4, 6.1 Hz, 1 H), 6.54 (br d, *J* = 15.6 Hz, 1 H), 5.66 (s, 2 H), 4.28 (q, *J* = 7.0 Hz, 2 H), 3.11 (dd, *J* = 6.1, 1.2 Hz, 2 H), 2.40-2.30 (br m, 4 H), 1.56-1.48 (br m, 4 H), 1.45 (t, *J* = 7.0 Hz, 3 H), 1.43-1.35 (br m, 2 H). LCMS ($APCI^+$) 562. Anal. ($C_{33}H_{35}N_7O_2.H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-((2*R*,6*S*)-2,6-dimethylpiperidin-1-yl)but-2-enamide (48). Reaction of **93** with *cis*-2,6-dimethylpiperidine, using the same method as described for **44**, afforded the title compound **48** as a cream solid (62%), m.p. 143-146°C. ^1H NMR [$(CD_3)_2SO$] δ 9.70 (s, 1 H), 9.46 (s, 1 H), 8.89 (s, 1 H), 8.42 (s, 1 H), 8.13 (s, 1 H), 8.10 (s, 1 H), 7.69-7.63 (m, 2 H), 7.35-7.21 (m, 6 H), 6.98 (dt, *J* = 15.3, 6.1 Hz, 1 H), 6.59 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.28 (q, *J* = 7.0 Hz, 2 H), 3.48 (br d, *J* = 5.6 Hz, 2 H), 2.47-2.37 (br m, 2 H), 1.63-1.52 (br m, 4 H), 1.45 (t, *J* = 7.0 Hz, 3 H), 1.34-1.12 (br m, 2 H), 1.06 (d, *J* = 6.2 Hz, 6 H). LCMS ($APCI^+$) 590. Anal. ($C_{35}H_{39}N_7O_3.H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-morpholinobut-2-enamide (49). Reaction of **93** with morpholine, using the same method as described for **44**, afforded the title compound **49** as a cream solid (62%), m.p. 177-

181°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H), 9.48 (s, 1 H), 8.90 (s, 1 H), 8.42 (s, 1 H), 8.13 (s, 1 H), 8.10 (s, 1 H), 7.69-7.62 (m, 2 H), 7.35-7.21 (m, 6 H), 6.79 (dt, *J* = 15.4, 6.1 Hz, 1 H), 6.57 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.28 (q, *J* = 7.0 Hz, 2 H), 3.61 (t, *J* = 4.6 Hz, 4 H), 3.15 (dd, *J* = 6.1, 1.1 Hz, 2 H), 2.40 (t, *J* = 4.4 Hz, 4 H) 1.45 (t, *J* = 7.0 Hz, 3 H). LCMS (APCI$^+$) 564. Anal. ($C_{32}H_{33}N_7O_3.^1/_2H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-(4-methylpiperazin-1-yl)but-2-enamide (50). Reaction of **93** with *N*-methylpiperazine, using the same method as described for **44**, afforded the title compound **50** as a pale yellow solid (80%), m.p. 138-141°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H), 9.47 (s, 1 H), 8.89 (s, 1 H), 8.42 (s, 1 H), 8.12 (s, 1 H), 8.10 (s, 1 H), 7.69-7.62 (m, 2 H), 7.35-7.21 (m, 6 H), 6.78 (dt, *J* = 15.4, 6.1 Hz, 1 H), 6.55 (br d, *J* = 15.5 Hz, 1 H), 5.66 (s, 2 H), 4.28 (q, *J* = 7.0 Hz, 2 H), 3.14 (br d, *J* = 5.0 Hz, 2 H), 2.46-2.26 (br m, 8 H), 2.16 (s, 3 H), 1.45 (t, *J* = 7.0 Hz, 3 H). LCMS (APCI$^+$) 577. Anal. ($C_{33}H_{36}N_8O_2.1^1/_2H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-((3*S*,5*R*)-3,5-dimethylpiperazin-1-yl)but-2-enamide (51). Reaction of **93** with *cis*-2,6-dimethylpiperazine, using the same method as described for **44**, afforded the title compound **51** as a pale yellow solid (45%), m.p. 140-144°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H), 9.47 (s, 1 H), 8.89 (s, 1 H), 8.42 (s, 1 H), 8.12 (s, 1 H), 8.10 (s, 1 H), 7.69-7.62 (m, 2 H), 7.34-7.21 (m, 6 H), 6.79 (dt, *J* = 15.3, 6.2 Hz, 1 H), 6.55 (br d, *J* = 15.3 Hz, 1 H), 5.66 (s, 2 H), 4.28 (q, *J* = 7.0 Hz, 2 H), 3.13 (br d, *J* = 5.8 Hz, 2 H), 2.90-2.78 (br m, 2 H), 2.72 (br d, *J* = 9.9 Hz, 2 H), 1.59 (br t, *J* = 9.6 Hz, 2 H), 1.45 (t, *J* = 7.0 Hz, 3 H), 0.96 (br d, *J* = 6.2 Hz, 6 H). LCMS (APCI$^+$) 591. Anal. ($C_{34}H_{38}N_8O_2.1^1/_2H_2O$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)-7-ethoxyquinazolin-6-yl)-4-((2*R*,6*S*)-2,6-dimethylpiperazin-1-yl)but-2-enamide (52). Reaction of **93** with BOC-*cis*-2,6-dimethylpiperazine,[82] using the same method as described for **44**, gave the BOC-protected precursor of the title compound as a viscous oil which, without further purification, was deprotected with 1:1 CH_2Cl_2/TFA. The title compound **52** was isolated as a pale yellow solid (25%), m.p. 240-244°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.70 (s, 1 H), 9.46 (s, 1 H), 8.88 (s, 1 H), 8.42 (s, 1 H), 8.14 (s, 1 H), 8.10 (s, 1 H), 7.69-7.62 (m, 2 H), 7.35-7.20 (m, 6 H), 6.98 (dt, *J* = 15.3, 6.2 Hz, 1 H), 6.58 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.29 (q, *J* = 7.0 Hz, 2 H), 3.46 (br d, *J* = 5.5 Hz, 2 H), 2.73 (dd, *J* = 12.0, 1.9 Hz, 2 H), 2.47-2.37 (m, 2 H), 2.27 (br t, *J* = 11.9 Hz, 2 H), 1.45 (t, *J* = 7.0 Hz, 3 H), 0.96 (d, *J* = 6.1 Hz, 6 H). HRMS (EI^+) calcd. for $C_{34}H_{38}N_8O_2$: 590.3118. Found 590.3114. Anal. ($C_{34}H_{38}N_8O_2.{}^1/_2H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Chloro-4-fluoroanilino)-7-methoxy-6-quinazolinyl]-4-(diisopropylamino)-2-butenamide (53). Application of General Procedure 3 to compound **87** using 3-chloro-4-fluoroaniline as the aniline and diisopropylamine as the amine, gave the title compound **53**, m.p. 170-171°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.76 (s, 1 H), 9.60 (s, 1 H), 8.87 (s, 1 H), 8.51 (s, 1 H), 8.10 (m, 1 H), 7.77 (m, 1 H), 7.40 (t, *J* = 9.2 Hz, 1 H), 7.26 (s, 1 H), 6.80 (m, 1 H), 6.56 (m, 1 H), 3.99 (s, 3 H), 3.25 (d, *J* = 4.1 Hz, 2 H), 2.99 (m, 2 H), 0.97 (d, *J* = 6.6 Hz, 12 H). Anal. ($C_{25}H_{29}N_5O_2FCl.1{}^1/_{10}H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Chloro-4-fluoroanilino)-7-methoxy-6-quinazolinyl]-4-(1-piperidinyl)-2-butenamide (54). Application of General Procedure 3 to compound **87** using 3-chloro-4-fluoroaniline as the aniline and piperidine as the amine, gave the title compound **54**, m.p. 149-151°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.76 (s, 1 H), 9.64 (s, 1 H), 8.87 (s, 1 H), 8.48 (s, 1 H),

8.07 (dd, *J* = 2.7, 6.8 Hz, 1 H), 7.74 (m, 1 H), 7.37 (t, *J* = 9.3 Hz, 1 H), 7.24 (s, 1 H), 6.74 (m, 1 H), 6.50 (m, 1 H), 3.96 (s, 3 H), 3.04 (d, *J* = 5.1 Hz, 2 H), 2.30 (m, 4 H), 1.46 (m, 4 H), 1.34 (m, 2 H). LCMS (APCI +ve) 470.1 (M+H). Anal. ($C_{24}H_{25}N_5O_2FCl.H_2O$) C, H, N.

(2*E*)-*N*-[4-[(1-Benzyl-1*H*-indazol-5-yl)amino]-7-(2-methoxyethoxy)-6-quinazolinyl]-4-(dimethylamino)-2-butenamide (55). 7-(2-Methoxyethoxy)-6-nitro-4(3*H*)-quinazolinone **94** (2.50 g, 10.6 mmol) was prepared using the same method as described for **87** (using THF as the solvent). General Procedure 3 was then followed to give the title compound **55** as a pale yellow solid, m.p. 123-127°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.73 (s, 1 H), 9.48 (s, 1 H), 8.90 (s, 1 H), 8.42 (s, 1 H), 8.13 (s, 1 H), 8.11 (s, 1 H), 7.67 (m, 2 H), 7.35-7.20 (m, 6 H), 6.80 (dt, *J* = 15.4, 6.0 Hz, 1 H), 6.55 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.36 (m, 2 H), 3.80 (m, 2 H), 3.35 (s, 3 H), 3.09 (br d, *J* = 5.2 Hz, 2 H), 2.20 (s, 6 H). Anal. ($C_{31}H_{33}N_7O_3.1^1/_3H_2O$) C, H, N.

(2*E*)-*N*-[4-[(1-Benzyl-1*H*-indazol-5-yl)amino]-7-(2-methoxyethoxy)-6-quinazolinyl]-4-(1-pyrrolidinyl)-2-butenamide (56). Application of General Procedure 3 to compound **94**, using pyrrolidine as the amine, gave the title compound **56** as a pale yellow solid, m.p. 117-121°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.73 (s, 1 H), 9.48 (s, 1 H), 8.90 (s, 1 H), 8.42 (s, 1 H), 8.13 (s, 1 H), 8.10 (s, 1 H), 7.66 (m, 2 H), 7.35-7.20 (m, 6 H), 6.84 (dt, *J* = 15.4, 5.8 Hz, 1 H), 6.55 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.36 (m, 2 H), 3.80 (m, 2 H), 3.36 (s, 3 H), 3.17 (d, *J* = 5.3 Hz, 2 H), 2.52-2.48 (m, 4 H), 1.76-1.68 (br m, 4 H). Anal. ($C_{33}H_{35}N_7O_3.2H_2O$) C, H, N.

(2*E*)-*N*-[4-[(1-Benzyl-1*H*-indazol-5-yl)amino]-7-(2-methoxyethoxy)-6-quinazolinyl]-4-(4-methyl-1-piperazinyl)-2-butenamide (57). Application of General Procedure 3 to

compound **94**, using *N*-methylpiperazine as the amine, gave the title compound **57** as a pale yellow solid, m.p. 123-127°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.73 (s, 1 H), 9.48 (s, 1 H), 8.88 (s, 1 H), 8.42 (s, 1 H), 8.13 (s, 1 H), 8.11 (s, 1 H), 7.66 (m, 2 H), 7.36-7.21 (m, 6 H), 6.78 (dt, *J* = 15.4, 6.1 Hz, 1 H), 6.53 (br d, *J* = 15.4 Hz, 1 H), 5.66 (s, 2 H), 4.36 (m, 2 H), 3.80 (m, 2 H), 3.35 (s, 3 H), 3.14 (br d, *J* = 5.3 Hz, 2 H), 2.50-2.30 (m, 8 H), 2.18 (s, 3 H). Anal. ($C_{34}H_{38}N_8O_3.1^1/_2H_2O$) C, H, N.

(2*E*)-4-(Dimethylamino)-*N*-(7-ethoxy-4-{[1-(phenylsulfonyl)-1*H*-indol-5-yl]amino}-6-quinazolinyl)-2-butenamide (58). The title compound was prepared according to General Procedure 3 using 1-(phenylsulfonyl)-1*H*-indol-5-amine[24] as the aniline, m.p. 154-158ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H), 9.46 (s, 1 H), 8.91 (s, 1 H), 8.42 (s, 1 H), 8.03 (m, 3 H), 7.93 (d, *J* = 8.9 Hz, 1 H), 7.79 (d, *J* = 3.7 Hz, 1 H), 7.70-7.59 (m, 4 H), 7.22 (s, 1 H), 6.87 (dd, *J* = 3.7, 0.4 Hz, 1 H), 6.80 (m, 1 H), 6.56 (d, *J* = 15.5 Hz, 1 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.09 (dd, *J* = 6.0, 1.1 Hz, 2 H), 2.19 (s, 6 H), 1.45 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{30}H_{30}N_6O_4S.1^3/_4H_2O$) C, H, N.

(2*E*)-*N*-(7-Ethoxy-4-{[1-(phenylsulfonyl)-1*H*-indol-5-yl]amino}-6-quinazolinyl)-4-(1-pyrrolidinyl)-2-butenamide (59). The title compound was prepared according to General Procedure 3 using 1-(phenylsulfonyl)-1*H*-indol-5-amine[24] as the aniline and pyrrolidine as the amine, m.p. 158-162ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H), 9.46 (s, 1 H), 8.90 (s, 1 H), 8.42 (s, 1 H), 8.02 (m, 3 H), 7.91 (d, *J* = 8.9 Hz, 1 H), 7.78 (d, *J* = 3.7 Hz, 1 H), 7.70-7.59 (m, 4 H), 7.22 (s, 1 H), 6.87 (dd, *J* = 3.7, 0.4 Hz, 1 H), 6.85 (m, 1 H), 6.56 (d, *J* = 15.4 Hz, 1 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.25 (dd, *J* = 6.0, 1.1 Hz, 2 H), 2.47 (m, 4 H), 1.72 (m, 4 H), 1.45 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{32}H_{32}N_6O_4S.1^1/_2H_2O$) C, H, N.

(2*E*)-*N*-(7-Ethoxy-4-{[1-(phenylsulfonyl)-1*H*-indol-5-yl]amino}-6-quinazolinyl)-4-(4-morpholinyl)-2-butenamide (60). The title compound was prepared according to General Procedure 3 using 1-(phenylsulfonyl)-1*H*-indol-5-amine[24] as the aniline and morpholine as the amine, m.p. 227-230ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.70 (s, 1 H), 9.48 (s, 1 H), 8.89 (s, 1 H), 8.42 (s, 1 H), 8.03 (m, 3 H), 7.91 (d, *J* = 8.9 Hz, 1 H), 7.78 (d, *J* = 3.7 Hz, 1 H), 7.70-7.59 (m, 4 H), 7.22 (s, 1 H), 6.87 (dd, *J* = 3.7, 0.4 Hz, 1 H), 6.76 (m, 1 H), 6.59 (d, *J* = 15.4 Hz, 1 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.61 (t, *J* = 4.5 Hz, 4 H), 3.15 (dd, *J* = 6.0, 1.1 Hz, 2 H), 2.40 (t, *J* = 4.4 Hz, 4 H), 1.45 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{32}H_{32}N_6O_5S.^1/_2H_2O$) C, H, N.

(2*E*)-*N*-[7-Methoxy-4-(4-phenoxyanilino)-6-quinazolinyl]-4-(1-piperidinyl)-2-butenamide (61). Application of General Procedure 3 to compound **87** using 4-phenoxyaniline as the aniline and piperidine as the amine, gave the title compound **61**, m.p. 115-116ºC. ^{1}H NMR [$(CD_3)_2SO$] δ 9.62 (d, *J* = 19.0 Hz, 2 H), 8.85 (s, 1 H), 8.41 (s, 1 H), 7.75 (m, 2 H), 7.32 (m, 2 H), 7.21 (s, 1 H), 7.05 (t, *J* = 7.3 Hz, 1 H), 7.00 (m, 4 H), 6.75 (m, 1 H), 6.48 (d, *J* = 15.4 Hz, 1 H), 3.95 (s, 3 H), 3.05 (d, *J* = 4.9 Hz, 2 H), 2.30 (m, 4 H), 1.47 (m, 4 H), 1.34 (m, 2 H). Anal. ($C_{30}H_{31}N_5O_3.1^1/_5H_2O$) C, H, N.

(2*E*)-*N*-{7-Methoxy-4-[4-(2-pyridinylmethyl)anilino]-6-quinazolinyl}-4-(1-pyrrolidinyl)-2-butenamide trihydrochloride (62). Application of General Procedure 3 to compound **87** using 4-(2-pyridinylmethyl)aniline as the aniline and pyrrolidine as the amine, gave the title compound **62** as a yellow solid, m.p. 188-193^{o}C (dec.). ^{1}H NMR [$(CD_3)_2SO$] δ 15.2 (very br s, 1 H), 11.47 (br s, 1 H), 11.34 (s, 1 H), 10.16 (s, 1 H), 9.18 (s, 1 H), 8.81 (s, 1 H), 8.72 (br s, 1 H), 8.23 (br s, 1 H), 7.78-7.65 (m, 2 H), 7.61 (d, *J* = 8.4 Hz, 2 H), 7.50-7.43 (m, 3 H), 6.93 (dt, *J* = 15.4, 6.7 Hz, 1 H), 6.79 (br d, *J* = 15.5 Hz,

1 H), 4.37 (br s, 2 H), 4.07 (s, 3 H), 4.02 (m, 2 H), 3.54 (m, 2 H, by D_2O exchange), 3.03 (m, 2 H), 2.04 (m, 2 H), 1.90 (m, 2 H). Anal. ($C_{29}H_{33}Cl_3N_6O_2.H_2O.EtOAc$) C, H, N.

(2*E*)-*N*-(4-{3-Chloro-4-[(3-fluorobenzyl)oxy]anilino}-7-ethoxy-6-quinazolinyl)-4-(1-pyrrolidinyl)-2-butenamide dihydrochloride (63). The title compound was prepared according to General Procedure 3 using 3-chloro-4-[(3-fluorobenzyl)oxy]aniline as the aniline and pyrrolidine as the amine, m.p. 177-181 ^oC. ^1H NMR [$(CD_3)_2SO$] δ 11.41 (br s, 1 H), 11.26 (br s, 1 H), 10.00 (s, 1 H), 9.14 (s, 1 H), 8.85 (s, 1 H), 7.83 (d, *J* = 2.5 Hz, 1 H), 7.57 (dd, *J* = 8.9, 2.5 Hz, 1 H), 7.51-7.46 (m, 1 H), 7.45 (s, 1 H), 7.36-7.29 (m, 3 H), 7.19 (ddd, *J* = 9.0, 8.9, 1.8 Hz, 1 H), 6.97-6.90 (m, 1 H), 6.79 (d, *J* = 15.4 Hz, 1 H), 5.30 (s, 2 H), 4.35 (q, *J* = 6.9 Hz, 2 H), 4.03 (t, *J* = 5.8 Hz, 2 H), 3.55-3.43 (m, 4 H), 3.08-2.97 (m, 2 H), 2.08-1.98 (m, 2 H), 1.96-1.85 (m, 2 H), 1.50 (t, *J* = 6.9 Hz, 3 H). LCMS ($APCI^+$) 577 (100%). Anal. ($C_{31}H_{31}ClFN_5O_3.2HCl.1^1/_2H_2O$) C, H, N.

(2*E*)-*N*-(4-{3-Chloro-4-[(3-fluorobenzyl)oxy]anilino}-7-ethoxy-6-quinazolinyl)-4-(4-methyl-1-piperazinyl)-2-butenamide dihydrochloride (64). The title compound was prepared according to General Procedure 3 using 3-chloro-4-[(3-fluorobenzyl)oxy]aniline as the aniline and 4-methylpiperazine as the amine, m.p. 178-180 ^oC. ^1H NMR [$(CD_3)_2SO$] δ 11.30 (br s, 1 H), 9.92 (s, 1 H), 9.15 (s, 1 H), 8.84 (s, 1 H), 7.82 (d, *J* = 2.5 Hz, 1 H), 7.57 (dd, *J* = 8.9, 2.5 Hz, 1 H), 7.51-7.46 (m, 1 H), 7.45 (s, 1 H), 7.36-7.29 (m, 3 H), 7.19 (ddd, *J* = 9.0, 8.9, 1.8 Hz, 1 H), 6.95-6.86 (m, 1 H), 6.75 (d, *J* = 15.4 Hz, 1 H), 5.30 (s, 2 H), 4.35 (q, *J* = 6.9 Hz, 2 H), 3.73 (m, obscured by water), 3.17 (s, 3 H), 2.80 (br s, 2 H), 1.50 (t, *J* = 6.9 Hz, 3 H). LCMS ($APCI^+$) 606 (100%). Anal. ($C_{32}H_{34}ClFN_6O_3.2HCl.4H_2O$) C, H, N.

(2*E*)-*N*-{7-Ethoxy-4-[4-(2-pyridinylmethoxy)anilino]-6-quinazolinyl}-4-(1-pyrrolidinyl)-2-butenamide (65). The title compound was prepared according to General Procedure 3 using 4-(2-pyridinylmethoxy)aniline as the aniline and pyrrolidine as the amine, m.p. 169-172 ^{o}C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.57 (br s, 1 H), 9.47 (br s, 1 H), 8.86 (br s, 1 H), 8.59 (d, *J* = 4.1 Hz, 1 H), 8.40 (s, 1 H), 7.85 (ddd, *J* = 7.7, 7.7, 1.7 Hz, 1 H), 7.64 (d, *J* = 9.0 Hz, 2 H), 7.54 (d, *J* = 7.8 Hz, 1 H), 7.36 (dd, *J* = 5.1, 6.8 Hz, 1 H), 7.21 (s, 1 H), 7.04 (d, *J* = 9.0 Hz, 2 H), 6.84 (ddd, *J* = 5.8, 5.8, 15.4 Hz, 1 H), 6.57 (br d, *J* = 15.4 Hz, 1 H), 5.19 (s, 2 H), 4.27 (q, *J* = 6.9 Hz, 2 H), 3.3 (obscured d, *J* = 5.9 Hz, 2 H), 2.50 (br s, 4 H), 1.73 (br s, 4 H),1.45 (t, *J* = 6.9 Hz, 3 H). Anal. ($C_{30}H_{32}N_6O_3.2^1/_5H_2O$) C, N. H: calcd, 6.50; found, 5.96.

(2*E*)-*N*-{7-Ethoxy-4-[4-(2-pyridinylmethoxy)anilino]-6-quinazolinyl}-4-(4-methyl-1-piperazinyl)-2-butenamide trihydrochloride (66). The title compound was prepared according to General Procedure 3 using 4-(2-pyridinylmethoxy)aniline as the aniline and 4-methylpiperazine as the amine, m.p. 194-197^{o}C. ^{1}H NMR [$(CD_3)_2SO$] δ (free base): 9.56 (s, 1 H), 9.48 (s, 1 H), 8.85 (s, 1 H), 8.59 (d, *J* = 4.1 Hz, 1 H), 8.40 (s, 1 H), 7.85 (dt, *J* = 7.7, 1.7 Hz, 1 H), 7.64 (d, *J* = 9.0 Hz, 2 H), 7.54 (d, *J* = 7.8 Hz, 1 H), 7.35 (dd, *J* = 6.7, 4.9 Hz, 1 H), 7.21 (s, 1 H), 7.04 (d, *J* = 9.0 Hz, 2 H), 6.78 (dt, *J* = 15.4, 6.2 Hz, 1 H), 6.54 (br d, *J* = 15.3 Hz, 1 H), 5.19 (s, 2 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.14 (br d, *J* = 5.5 Hz, 2 H), 2.46-2.26 (br m, 8 H), 2.16 (s, 3 H), 1.45 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{31}H_{38}Cl_3N_7O_3.2H_2O.EtOAc$) C, H, N.

(2*E*)-*N*-(7-Ethoxy-4-{4-[(phenylsulfonyl)amino]anilino}-6-quinazolinyl)-4-(1-pyrrolidinyl)-2-butenamide (67). The title compound was prepared according to General Procedure 3 using *N*-(4-aminophenyl)benzenesulfonamide as the aniline and pyrrolidine

as the amine, m.p. 115-119°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.12 (s, 1 H), 9.57 (s, 1 H), 9.44 (s, 1 H), 8.85 (s, 1 H), 8.41 (s, 1 H), 7.76 (br d, *J* = 7.0 Hz, 2 H), 7.65-7.50 (m, 5 H), 7.21 (s, 1 H), 7.06 (br d, *J* = 8.9 Hz, 2 H), 6.83 (dt, *J* = 15.4, 5.8 Hz, 1 H), 6.56 (br d, *J* = 15.6 Hz, 1 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.30 (d, *J* = 5.6 Hz, 2 H; by D_2O exchange), 2.51-2.49 (m, 4 H), 1.76-1.68 (br m, 4 H), 1.45 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{30}H_{32}N_6O_4S.^1/_2H_2O.1^1/_4MeOH$) C, H, N.

(2*E*)-*N*-(7-Ethoxy-4-{4-[(phenylsulfonyl)amino]anilino}-6-quinazolinyl)-4-(4-morpholinyl)-2-butenamide (68). The title compound was prepared according to General Procedure 3 using *N*-(4-aminophenyl)benzenesulfonamide as the aniline and morpholine as the amine, m.p. 157-160°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.12 (br s, 1 H), 9.57 (s, 1 H), 9.46 (s, 1 H), 8.84 (s, 1 H), 8.41 (s, 1 H), 7.76 (m, 2 H), 7.66-7.50 (m, 5 H), 7.21 (s, 1 H), 7.06 (m, 2 H), 6.78 (dt, *J* = 15.4, 6.1 Hz, 1 H), 6.56 (br d, *J* = 15.3 Hz, 1 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.61 (t, *J* = 4.6 Hz, 4 H), 3.15 (dd, *J* = 6.1, 1.2 Hz, 2 H), 2.40 (t, *J* = 4.4 Hz, 4 H) 1.44 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{30}H_{32}N_6O_5S.^1/_2MeOH$) C, H, N.

(2*E*)-*N*-(7-Ethoxy-4-{4-[(phenylsulfonyl)amino]anilino}-6-quinazolinyl)-4-(4-methyl-1-piperazinyl)-2-butenamide (69). The title compound was prepared according to General Procedure 3 using *N*-(4-aminophenyl)benzenesulfonamide as the aniline and 4-methylpiperazine as the amine, m.p. 151-155°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.12 (br s, 1 H), 9.56 (s, 1 H), 9.45 (s, 1 H), 8.84 (s, 1 H), 8.41 (s, 1 H), 7.76 (m, 2 H), 7.66-7.52 (m, 5 H), 7.21 (s, 1 H), 7.06 (br d, *J* = 8.8 Hz, 2 H), 6.77 (dt, *J* = 15.4, 6.1 Hz, 1 H), 6.54 (br d, *J* = 15.4 Hz, 1 H), 4.27 (q, *J* = 7.0 Hz, 2 H), 3.13 (br d, *J* = 5.1 Hz, 2 H), 2.45-2.25 (br m, 8 H), 2.16 (s, 3 H), 1.44 (t, *J* = 7.0 Hz, 3 H). Anal. ($C_{31}H_{35}N_7O_4S.1^3/_4H_2O.^3/_4MeOH$) C, H, N.

General Procedure 4. Synthesis of Pyrido[3,4-*d*]pyrimidines of Scheme 4

***N*-(1-Benzyl-1H-indazol-5-yl)-6-fluoropyrido[3,4-*d*]pyrimidin-4-amine (96A5).** 6-Fluoropyrido[3,4-*d*]pyrimidin-4(3*H*)-one (**95**)[51] (1.00 g, 6.06 mmol) was suspended in $SOCl_2$ (80 mL), to which was added DMF (1 drop). The mixture was heated at reflux for 2 h., then the solvent was distilled off under reduced pressure. The resulting crude 4-chloro-6-fluoropyrido[3,4-*d*]pyrimidine was then dissolved in a mixture of iPrOH (80 mL) and CH_2Cl_2 (50 mL), 1-benzyl-1*H*-indazol-5-amine (1.62 g, 7.27 mmol) was added, and the mixture heated at reflux for 0.75 h. After cooling, the reaction mixture was concentrated under reduced pressure, then the suspension was poured into saturated $NaHCO_3$ solution (400 mL). The resulting solid was extracted with 10% MeOH/CH_2Cl_2 (4x100 mL) then the combined organic extracts were washed with brine (200 mL) and dried (Na_2SO_4). Filtration, followed by removal of the solvent under reduced pressure, afforded a crude solid which was purified by chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant). The title compound **96A5** was isolated as yellow-orange plates (1.94 g, 87%), m.p. 202-205^{o}C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.15 (br s, 1 H), 8.92 (s, 1 H), 8.63 (s, 1 H), 8.27 (s, 2 H), 8.16 (s, 1 H), 7.75 (d, *J* = 9.0 Hz, 1 H), 7.69 (dd, *J* = 9.0, 1.8 Hz, 1 H), 7.35-7.22 (m, 5 H), 5.68 (s, 2 H). LCMS (APCI^{+}) 371. Anal. ($C_{21}H_{15}FN_6$) C, H, N.

***N*^{4}-(1-Benzyl-1*H*-indazol-5-yl)-*N*^{6}-(4-methoxybenzyl)pyrido[3,4-*d*]pyrimidine-4,6-diamine (97A5).** Compound **96A5** (3.85 g, 10.4 mmol) was dissolved in DMSO (50 mL), to which was added 4-methoxybenzylamine (7.14 g, 52.0 mmol). The mixture was heated to 120^{o}C with stirring for 24 h. The reaction was allowed to cool, then partitioned between water (200 mL) and EtOAc (200 mL). A bright yellow solid crystallised out of this

mixture and was collected by filtration and dried, affording the title compound **97A5** (3.21 g, 63%), m.p. 204-206°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.68 (s, 1 H), 8.71 (s, 1 H), 8.28 (s, 1 H), 8.20 (d, *J* = 1.2 Hz, 1 H), 8.12 (d, *J* = 0.8 Hz, 1 H), 7.71 (d, *J* = 9.0 Hz, 1 H), 7.66 (dd, *J* = 9.0, 1.9 Hz, 1 H), 7.37-7.16 (m, 9 H), 6.91-6.85 (m, 2 H), 5.67 (s, 2 H), 4.49 (d, *J* = 6.4 Hz, 2 H), 3.71 (s, 3 H). LCMS (APCI$^+$) 488. Anal. ($C_{29}H_{25}N_7O$) C, H, N.

N^4-(1-Benzyl-1*H*-indazol-5-yl)pyrido[3,4-*d*]pyrimidine-4,6-diamine (98A5). Compound **97A5** (1.46 g, 3.00 mmol) was dissolved in TFA (15 mL) and stirred at room temperature for 6 h. The TFA was then evaporated off under a stream of nitrogen to give an oil which was dissolved in EtOAc (200 mL) and washed with saturated $NaHCO_3$ solution (2x200 mL), brine (200 mL) and dried (Na_2SO_4). Filtration, followed by removal of the solvent under reduced pressure, afforded a residue which was purified by chromatography on silica gel (10% MeOH/CH_2Cl_2 as eluant) to give the title compound **98A5** as a yellow crystalline solid (763 mg, 69%), m.p. 231-234°C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.72 (s, 1 H), 8.67 (s, 1 H), 8.29 (s, 1 H), 8.25 (t, *J* = 1.2 Hz, 1 H), 8.12 (s, 1 H), 7.68 (d, *J* = 1.1 Hz, 2 H), 7.34-7.22 (m, 5 H), 7.17 (d, *J* = 0.6 Hz, 1 H), 6.20 (br s, 2 H), 5.66 (s, 2 H). Anal. ($C_{21}H_{17}N_7$) C, H, N.

(*E*)-*N*-(4-(1-Benzyl-1*H*-indazol-5-ylamino)pyrido[3,4-*d*]pyrimidin-6-yl)-4-bromobut-2-enamide (99A5). (*E*)-4-Bromobut-2-enoic acid (420 mg, 2.51 mmol) was converted to the corresponding acid chloride using the same method as described for **93**. The resulting oil was dissolved in dry THF (10 mL) and added dropwise to a solution of **98A5** (710 mg, 1.93 mmol) and TEA (390 mg, 3.86 mmol) in THF (50 mL) which had been previously cooled to -20°C (ice/salt/water) under nitrogen. The resulting reaction mixture was stirred at this temperature for 4 h., then partitioned between EtOAc (100 mL) and saturated

$NaHCO_3$ solution (100 mL). One further extraction was performed with EtOAc, then the organic extracts combined, washed with brine (100 mL), and dried (Na_2SO_4). Filtration, followed by removal of the solvent under reduced pressure, afforded a residue which was purified by filtration through a plug of silica gel (5% MeOH/CH_2Cl_2 as eluant). The title compound **99A5** was isolated as an unstable orange foam (707 mg, 71%) which was used immediately in the next step. LCMS ($APCI^+$) 514, 516.

(2*E*)-*N*-{4-[(1-Benzyl-1*H*-indazol-5-yl)amino]pyrido[3,4-*d*]pyrimidin-6-yl}-4-(dimethylamino)-2-butenamide (74). Compound **99A5** (324 mg, 0.63 mmol) was dissolved in DMA (10 mL), cooled to -20°C (ice/salt/water), then 40% aqueous dimethylamine added (709 mg, 6.30 mmol) and stirring continued at this temperature for 1 h. The reaction mixture was diluted with water (50 mL) and poured into saturated $NaHCO_3$ solution (100 mL). The resulting yellow precipitate was extracted into EtOAc (3x80 mL) and the combined EtOAc fractions washed with brine (100 mL) and dried (Na_2SO_4). Filtration, followed by removal of the solvent under reduced pressure, afforded a residue which was purified by chromatography on silica gel (5% MeOH/CH_2Cl_2 as eluant) to give the title compound **74** as a pale yellow solid (218 mg, 72%), m.p. 237-240°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.88 (s, 1 H), 10.29 (s, 1 H), 9.00 (d, 1 H), 8.98 (s, 1 H), 8.52 (s, 1 H), 8.18 (d, 1 H), 8.13 (s, 1 H), 7.73-7.66 (m, 2 H), 7.36-7.23 (m, 5 H), 6.87 (dt, *J* = 15.4, 6.0 Hz, 1 H), 6.52 (dt, *J* = 15.4, 1.5 Hz, 1 H), 5.69 (s, 2 H), 3.09 (dd, *J* = 6.0, 1.3 Hz, 2 H), 2.19 (s, 6 H). Anal. ($C_{27}H_{26}N_8O.^1/_2H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Chloro-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(dimethylamino)-2-butenamide (70). The title compound was prepared according to General Procedure 4 using 3-chloro-4-fluoroaniline as the aniline, m.p. 182-186°C. ^{1}H NMR [$(CD_3)_2SO$] δ

10.90 (s, 1 H), 10.30 (s, 1 H), 9.01 (s, 1 H), 8.97 (s, 1 H), 8.62 (s, 1 H), 8.12 (s, 1 H), 7.81 (s, 1 H), 7.45 (t, *J* = 9.1 Hz, 1 H), 6.88 (dt, *J* = 15.4, 6.1 Hz, 1 H), 6.51 (d, *J* = 15.4 Hz, 1 H), 3.09 (d, *J* = 6.4 Hz, 2 H), 2.18 (s, 6 H). LCMS ($APCI^+$) 401 (100%). HRMS (FAB^+) calcd for $C_{19}H_{19}ClFN_6O$: 401.1293 (M^+). Found 401.1294.

(2*E*)-*N*-[4-(3-Chloro-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(1-piperidinyl)-2-butenamide (71). The title compound was prepared according to General Procedure 4 using 3-chloro-4-fluoroaniline as the aniline and piperidine as the amine, m.p. 187-190°C. ^1H NMR [$(CD_3)_2SO$] δ 10.93 (s, 1 H), 10.31 (s, 1 H), 8.99 (s, 1 H), 8.95 (s, 1 H), 8.60 (s, 1 H), 8.10 (d, *J* = 5.4 Hz, 1 H), 7.79 (m, 1 H), 7.43 (t, *J* = 9.0 Hz, 1 H), 6.83 (m, 1 H), 6.48 (d, *J* = 15.4 Hz, 1 H), 3.0 (d, *J* = 5.4 Hz, 2 H), 2.32 (br s, 4 H), 1.49 (t, *J* = 5.1 Hz, 4 H), 1.36 (m, 2 H). MS (APCI+) 441.1 (M+H). Anal. ($C_{22}H_{22}N_6OFCl.H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Chloro-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(4-morpholinyl)-2-butenamide (72). The title compound was prepared according to General Procedure 4 using 3-chloro-4-fluoroaniline as the aniline and morpholine as the amine, m.p. 218-220°C. ^1H NMR [$(CD_3)_2SO$] δ 10.93 (s, 1 H), 10.32 (s, 1 H), 9.01 (s, 1 H), 8.97 (s, 1 H), 8.62 (s, 1 H), 8.13 (br s, 1 H), 7.82 (br s, 1 H), 7.46 (t, *J* = 9.1 Hz, 1 H), 6.86 (dt, *J* = 15.4, 5.8 Hz, 1 H), 6.55 (d, *J* = 15.4 Hz, 1 H), 3.61 (t, *J* = 4.5 Hz, 4 H), 3.16 (d, *J* = 5.8 Hz, 2 H), 2.41 (t, *J* = 4.3 Hz, 4 H). LCMS ($APCI^+$) 443 (100%). Anal. ($C_{21}H_{20}ClFN_6O_2.H_2O$) C, H, N.

(2*E*)-*N*-[4-(3-Chloro-4-fluoroanilino)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(4-methyl-1-piperazinyl)-2-butenamide (73). The title compound was prepared according to General Procedure 4 using 3-chloro-4-fluoroaniline as the aniline and 4-methylpiperazine as the

amine, m.p. 138-141°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.93 (s, 1 H), 10.31 (s, 1 H), 9.02 (s, 1 H), 8.97 (s, 1 H), 8.63 (s, 1 H), 8.17-8.11 (m, 1 H), 7.87-7.80 (m, 1 H), 7.45 (t, *J* = 9.1 Hz, 1 H), 6.86 (td, *J* = 15.4, 5.9 Hz, 1 H), 5.53 (d, *J* = 15.4 Hz, 1 H), 3.15 (d, *J* = 5.9 Hz, 2 H), 2.41-2.36 (m, 8 H), 1.99 (s, 3 H). LCMS ($APCI^+$) 456 (100%). Anal. ($C_{22}H_{23}ClFN_7O.^1/_2MeOH.^1/_4CH_2Cl_2$) C, H, N.

(2*E*)-*N*-{4-[(1-Benzyl-1*H*-indazol-5-yl)amino]pyrido[3,4-*d*]pyrimidin-6-yl}-4-(1-pyrrolidinyl)-2-butenamide (75). Reaction of bromide **99A5** with pyrrolidine, using the same method as described for **74**, afforded the title compound **75** as a pale yellow solid (30%), m.p. 224-226°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.86 (s, 1 H), 10.29 (s, 1 H), 9.00 (s, 1 H) 8.98 (s, 1 H), 8.52 (s, 1 H), 8.16 (s, 1 H), 8.14 (d, 1 H), 7.73-7.65 (m, 2 H), 7.35-7.23 (m, 5 H), 6.92 (dt, *J* = 15.4, 5.8 Hz, 1 H), 6.53 (d, *J* = 15.4 Hz, 1 H), 5.67 (s, 2 H), 3.24 (d, *J* = 5.1 Hz, 2 H by D_2O exchange), 2.46 (br s, 4 H by D_2O exchange), 1.69 (br s, 4 H). Anal. ($C_{29}H_{28}N_8O.^1/_2H_2O$) C, H, N.

(2*E*)-4-(Dimethylamino)-*N*-[4-(4-phenoxybenzyl)pyrido[3,4-*d*]pyrimidin-6-yl]-2-butenamide dihydrochloride (76). The title compound was prepared according to General Procedure 4 using 4-phenoxyaniline as the aniline, m.p. 229-235°C. ^{1}H NMR [$(CD_3)_2SO$] δ 11.57 (br, 1H), 11.41 (s, 1 H), 11.12 (br, 1H), 9.16 (s, 1 H), 9.15 (s, 1 H), 8.82 (s, 1H), 7.73 (d, *J* = 6.7 Hz, 2H), 7.45-7.41 (m, 2H), 7.20-6.95 (m, 6H), 6.70 (d, *J* = 15.4 Hz, 1H), 3.97 (dd, *J* = 7.0, 5.5 Hz, 2H), 2.77, 2.76 (2s, 6H). LCMS ($APCI^+$) 440 (100%). Anal. ($C_{26}H_{25}N_5O_2.2HCl.H_2O.MeOH$) C, H, N.

(2*E*)-*N*-[4-(4-Phenoxybenzyl)pyrido[3,4-*d*]pyrimidin-6-yl]-4-(1-pyrrolidinyl)-2-butenamide dihydrochloride (77). The title compound was prepared according to

General Procedure 4 using 4-phenoxyaniline as the aniline and pyrrolidine as the amine, m.p. 200-205°C. ^{1}H NMR [$(CD_3)_2SO$] δ 11.64 (br, 2H), 11.43 (s, 1 H), 9.21 (s, 1 H), 9.16 (s, 1 H), 8.85 (s, 1H), 7.73 (d, *J* = 6.8 Hz, 2H), 7.45-7.40 (m, 2H), 7.20-6.97 (m, 6H), 6.71 (d, *J* = 15.5 Hz, 1H), 4.04 (dd, *J* = 6.6, 5.8 Hz, 2H), 3.48 (m, 2H), 3.02 (m, 2H), 2.02 (m, 2H), 1.91 (m, 2H). LCMS (APCI$^+$) 466 (100%). Anal. ($C_{28}H_{27}N_5O_2.2HCl.2H_2O.^1/_2Et_2O$) C, H, N.

(2*E*)-4-(Dimethylamino)-*N*-{4-[4-(2-pyridinylmethyl)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-2-butenamide (78). The title compound was prepared according to General Procedure 4 using 4-(2-pyridinylmethyl)aniline as the aniline, m.p. 196-199°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.92 (s, 1 H), 10.20 (s, 1 H), 8.98 (s, 1 H), 8.97 (s, 1 H), 8.53 (s, 1 H), 8.49 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1 H), 7.75-7.65 (m, 3 H), 7.35-7.25 (m, 3 H), 7.22 (ddd, *J* = 7.5, 4.9, 1.0 Hz, 1 H), 6.86 (dt, *J* = 15.4, 6.0 Hz, 1 H), 6.50 (d, *J* = 15.4 Hz, 1 H), 4.09 (s, 2 H), 3.08 (br d, *J* = 5.6 Hz, 2 H), 2.17 (s, 6 H). LCMS (APCI$^+$) 440 (100%). Anal. ($C_{25}H_{25}N_7O.^1/_3H_2O$) C, H, N.

(2*E*)-*N*-{4-[4-(2-Pyridinylmethyl)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-4-(1-pyrrolidinyl)-2-butenamide (79). The title compound was prepared according to General Procedure 4 using 4-(2-pyridinylmethyl)aniline as the aniline and pyrrolidine as the amine, m.p. 159-163°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.90 (s, 1 H), 10.20 (s, 1 H), 8.98 (s, 1 H), 8.97 (s, 1 H), 8.53 (s, 1 H), 8.49 (ddd, *J* = 4.9, 1.8, 0.8 Hz, 1 H), 7.75-7.65 (m, 3 H), 7.35-7.25 (m, 3 H), 7.22 (ddd, *J* = 7.5, 4.9, 1.1 Hz, 1 H), 6.90 (dt, *J* = 15.4, 5.8 Hz, 1 H), 6.52 (d, *J* = 15.4 Hz, 1 H), 4.09 (s, 2 H), 3.25 (dd, *J* = 5.8, 1.4 Hz, 2 H), 2.48 (br s, 4 H), 1.71 (br s, 4 H). LCMS (APCI$^+$) 466 (100%). Anal. ($C_{27}H_{27}N_7O.H_2O$) C, H, N.

(2*E*)-4-(4-Methyl-1-piperazinyl)-*N*-{4-[4-(2-pyridinylmethyl)anilino]pyrido[3,4-*d*]pyrimidin-6-yl}-2-butenamide (80). The title compound was prepared according to General Procedure 4 using 4-(2-pyridinylmethyl)aniline as the aniline and 4-methylpiperazine as the amine, m.p. 176-180°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.88 (s, 1 H), 10.17 (s, 1 H), 8.97 (s, 1 H), 8.96 (s, 1 H), 8.53 (s, 1 H), 8.50 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1 H), 7.75-7.65 (m, 3 H), 7.35-7.25 (m, 3 H), 7.22 (ddd, *J* = 7.5, 4.9, 1.0 Hz, 1 H), 6.86 (dt, *J* = 15.4, 6.0 Hz, 1 H), 6.50 (d, *J* = 15.4 Hz, 1 H), 4.09 (s, 2 H), 3.13 (br d, *J* = 5.6 Hz, 2 H), 2.41 (br s, 4 H), 2.35 (br s, 4 H), 2.16 (s, 3 H). LCMS (APCI$^+$) 495 (100%). Anal. ($C_{28}H_{30}N_8O.^3/_5CH_2Cl_2$) C, H, N.

(2*E*)-*N*-(4-{3-Chloro-4-[(3-fluorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)-4-(dimethylamino)-2-butenamide (81). The title compound was prepared according to General Procedure 4 using 3-chloro-4-[(3-fluorobenzyl)oxy]aniline as the aniline, m.p. 210-214°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.88 (s, 1 H), 10.19 (s, 1 H), 8.99 (s, 1 H), 8.96 (s, 1 H), 8.58 (s, 1 H), 7.98 (s, 1 H), 7.72 (d, *J* = 7.3 Hz, 1 H), 7.50-7.43 (m, 1 H), 7.35-7.25 (m, 3 H), 7.21-7.14 (m, 1 H), 6.87 (dt, *J* = 15.4, 6.0 Hz, 1 H), 6.51 (d, *J* = 15.4 Hz, 1 H), 5.26 (s, 2 H), 3.09 (dd, *J* = 6.0, 1.3 Hz, 2 H), 2.19 (s, 6 H). LCMS (APCI$^+$) 507 (100%). Anal. ($C_{26}H_{24}ClFN_6O_2$) C, H, N.

(2*E*)-*N*-(4-{3-Chloro-4-[(3-fluorobenzyl)oxy]anilino}pyrido[3,4-*d*]pyrimidin-6-yl)-4-(1-pyrrolidinyl)-2-butenamide (82). The title compound was prepared according to General Procedure 4 using 3-chloro-4-[(3-fluorobenzyl)oxy]aniline as the aniline and pyrrolidine as the amine, m.p. 188-192°C. ^{1}H NMR [$(CD_3)_2SO$] δ 10.87 (s, 1 H), 10.20 (s, 1 H), 8.99 (s, 1 H), 8.96 (s, 1 H), 8.58 (s, 1 H), 7.99 (d, *J* = 2.6 Hz, 1 H), 7.73 (dd, *J* = 8.9, 2.6 Hz, 1 H), 7.51-7.44 (m, 1 H), 7.35-7.25 (m, 3 H), 7.21-7.15 (m, 1 H), 6.91 (dt, *J* =

15.4, 5.8 Hz, 1 H), 6.53 (d, *J* = 15.4 Hz, 1 H), 5.27 (s, 2 H), 3.23 (d, *J* = 5.3 Hz, 2 H), 1.72 (p, *J* = 3.1 Hz, 4 H) (other 4 H peak under DMSO-d_6). LCMS ($APCI^+$) 533 (100%). Anal. ($C_{28}H_{26}ClFN_6O_2.H_2O$) C, H, N.

ELISA-based erbB and JAK3 kinase assays. Inhibitory activity against the catalytic domains of erbB1, erbB2, erbB4 and JAK3 was assessed using ELISA-based receptor tyrosine kinase assays, as described previously.[20, 83]

Inhibition of erbB1 and erbB2 autophosphorylation in cells. Inhibition of erbB1 tyrosine kinase activity was evaluated in NIH3T3 cells transfected with the full-length human erbB1 receptor, and inhibition of erbB2 tyrosine kinase activity was evaluated in T24 NIH 3T3 cells transfected with a chimeric receptor with the extracellular binding domain of erbB1 and the intracellular kinase domain of erbB2. These lines were a gift from Dr. Bruce Cohen (National Cancer Institute, Bethesda, MD) and the derivation of these lines has been previously described.[84] Inhibition of erbB1 and erbB2 autophosphorylation was detected using BioVeris technology, as previously described.[20]

Inhibition of EGF-stimulated erbB1 autophosphorylation in A431 cells. To determine the rate at which test compounds could irreversibly inhibit EGF-induced phosphorylation of erbB1 in cells, A431 cells were incubated with 2 μmol/L of test compound for varying lengths of time (1, 5, 30, 60, or 120 min). Medium was removed, and cells were repeatedly washed thrice with serum-free and inhibitor-free medium every hour for 2h. After the last wash, cells were stimulated with 100 ng/mL of EGF (Sigma) for 5 min at 37°C. Medium was removed, and cells were immediately lysed in buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 1% Triton, 10 mM β-

glycerophosphate, 0.1 mM NaVa, 1 mM NaF, 0.25% deoxycholic acid, 10 μg/mL aprotinin, 10 μg/mL leupeptin) for 15 min at room temperature, mixing gently. Inhibition of erbB1 phosphorylation was assessed using a sandwich ELISA approach employing the BioVeris technology as previously described.[20]

Human and rat liver microsome and hepatocyte stability assessment. Cryopreserved rat and human hepatocytes were obtained from XenoTech, LLC (Lenexa, Kansas). Liver microsomes were obtained from BD Gentest™ (Woburn**,** Massachusetts). Test compoundss were incubated at 37ºC with hepatocytes in Leibovitz's L-15 Medium (Invitrogen, Carlsbad, California) and with liver microsomes in a shaking water bath. Aliquots (100 μL) from incubations were harvested at various timepoints thereafter, where 100 μL of sample was dried down under a steady stream of nitrogen before being reconstituted with 100 μL of mobile phase and analysed with mass spectrometric detection.

***In vivo* assessment of erbB1 pharmacodynamic effects**. Test compounds were given orally to mice implanted subcutaneously with NIH 3T3 cells transfected with the full-length human erbB1 receptor (described above). Modulation of erbB1 autophosphorylation was assessed in tumors after dosing using western blotting techniques. Briefly, mice (3 per cohort) were dosed once a day for 2 days with test compounds at a dose of 30, 65 and 130 mg/kg. Tumor samples were harvested at 6 or 24 h after the last dose. Excised tumors were immediately frozen in liquid nitrogen and ground into a powder over liquid nitrogen using a mortar and pestle. Powders (100–500 mg) were suspended in 0.1 to 1.5 mL of ice-cold cell lysis buffer [50 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 5 mmol/L EDTA, 50 mmol/L β-glycerophosphate, 0.1% SDS, 1% Triton,

0.2% deoxycholate, 1 mmol/L sodium vanadate, and 1 EDTA-free Complete protease inhibitor tablet from Roche] and homogenized with a Polytron hand-held tissue homogenizer. Protein concentrations were determined on clarified lysates using a Bradford Protein Assay (Bio-Rad) or BCA reagents (Pierce Biotechnology). For each sample, 50 μg of protein were denatured and separated by SDS PAGE. Proteins were transferred to 0.2-μm nitrocellulose membranes (Invitrogen). Anti-phosphorylated erbB1 (Tyr^{1068}) from Sigma-Aldrich, Co., was used to detect phosphorylated erbB1.

Pharmacokinetics. Male Sprague-Dawley or Wistar rats (Charles River Laboratories), male purebred beagle dogs (Covance), and male cynomolgus monkeys (Biomedical Resources Foundation/Charles River Laboratories) were used for pharmacokinetic assessments. Animals were housed and maintained in accordance with Pfizer Institutional Animal Care and Use Committee, State, and Federal guidelines for the humane treatment and care of laboratory animals. All protocols involving animals were approved by the Pfizer Research and Development Institutional Animal Care and Use Committee. All animals were fasted overnight prior to dosing the next morning, and food was withheld until 4 h following dosing. Water was allowed ad libitum throughout the studies. For each study, blood samples were collected from a jugular cannula into EDTA/ascorbic acid tubes. Sampling occurred prior to dosing and at time points up to 144 h after the i.v. and the p.o. dose. To assess the concentration of **54** or **71** in plasma, protein was precipitated with acetonitrile (50 μL of), and 100 μL of sample was dried down under a steady stream of nitrogen. Samples were reconstituted with 100 μL of mobile phase [40:60 acetonitrile/10 mmol/L ammonium formate (pH 3.5) with formic acid]. Liquid chromatography-tandem mass spectrometry was conducted using a Sciex API 4000 equipped with a Leap Technologies CTCPAL autosampler and a Shimadzu LC-10Avp

Pump. Drug concentration below the limit of quantitation (< 5 ng/mL) was treated as zero for pharmacokinetic and statistical calculations.

Corresponding author. Jeff B. Smaill, Telephone: 64 9 373 7599 x86789; Fax: 64 9 373 7502; E-Mail: j.smaill@auckland.ac.nz

Author Contributions. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Acknowledgment. This work was partially supported by the Auckland Division of the Cancer Society of New Zealand and the Maurice Wilkins Centre for Molecular Biodiscovery.

Supporting Information Available: Synthetic procedures for the synthesis of anilines (A6, A11, A13, A14, A31, A32) of Table 2 and combustion analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

Abbreviations Used: APCI, Atmospheric pressure chemical ionization; BLK, B lymphocyte kinase; BMX, Bone marrow kinase in chromosome X; BTK, Bruton's tyrosine kinase; CL, Clearance; C_{max}, maximum serum concentration; GAK, Cyclin G associated kinase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; JAK3, Janus kinase 3; LCK, Lymphocyte-specific protein tyrosine kinase; MKNK1, MAP kinase-interacting serine/threonine-protein kinase 1; RIPK2, Receptor-interacting serine/threonine-protein kinase 2; T790M, mutation of threonine 790 to methionine; TEA, Triethylamine; V_{obs}, Apparent volume of distribution

References

(1) Reid, A.; Vidal, L.; Shaw, H.; de Bono, J. Dual inhibition of ErbB1 (EGFR/HER1) and ErbB2 (HER2/neu). *Eur. J. Cancer* **2007**, *43*, 481-489.

(2) Nyati, M. K.; Morgan, M. A.; Feng, F. Y.; Lawrence, T. S. Integration of EGFR inhibitors with radiochemotherapy. *Nat. Rev. Cancer* **2006**, *6*, 876-885.

(3) Mendelsohn, J.; Baselga, J. Epidermal growth factor receptor targeting in cancer. *Semin. Oncol.* **2006**, *33*, 369-385.

(4) Hynes, N. E.; Lane, H. A. ErbB receptors and cancer: the complexity of targeted inhibitors. *Nat. Rev. Cancer* **2005**, *5*, 341-354.

(5) Landi, L.; Cappuzzo, F. Front-line therapy in lung cancer with mutations in EGFR. *Nat. Rev. Clin. Oncol.* **2011**, *8*, 571-573.

(6) Blackhall, F.; Ranson, M.; Thatcher, N. Where next for gefitinib in patients with lung cancer? *Lancet Oncol.* **2006**, *7*, 499-507.

(7) Goodin, S. Erlotinib: Optimizing therapy with predictors of response? *Clin. Cancer Res.* **2006**, *12*, 2961-2963.

(8) Gorden, K. J.; Mesbah, P.; Kolesar, J. M. EGFR inhibitors as first-line therapy in EGFR mutation-positive patients with NSCLC. *J. Onc. Pharm. Practice* **2012**, *18*, 245-249.

(9) Pao, W.; Miller, V. A.; Politi, K. A.; Riely, G. J.; Somwar, R.; Zakowski, M. F.; Kris, M.; Varmus, H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* **2005**, *2*, 1-11.

(10) Jänne, P. A.; Gray, N.; Settleman, J. Factors underlying sensitivity of cancers to small-molecule kinase inhibitors. *Nat. Rev. Drug Discovery* **2009**, *8*, 709-723.

(11) Nass, S. J.; Hahm, H.A.; Davidson, N. E. Breast cancer biology blossoms in the clinic. *Nat. Med. (N. Y., NY, U. S.)* **1998**, *7*, 761-762.

(12) Lackey, K. E. Lessons from the drug discovery of Lapatinib, a dual ErbB1/2 tyrosine kinase inhibitor. *Curr. Top. Med. Chem.* **2006**, *6*, 435-460.

(13) Tsang, R. Y.; Sadeghi, S.; Finn, R. S. Lapatinib, a dual-targeted small molecule inhibitor of EGFR and HER2, in HER2-amplified breast cancer: from bench to bedside. *Clin. Med. Insights: Ther.* **2011**, *3*, 1-13.

(14) Hurvitz, S. A.; Hu, Y.; O'Brien, N.; Finn, R. S. Current approaches and future directions in the treatment of HER2-positive breast cancer. *Can. Treat. Rev.* **2013**, *39*, 219-229.

(15) Fry, D. W.; Bridges, A. J.; Denny, W. A.; Doherty, A.; Greis, K. D.; Hicks, J. L.; Hook, K. E.; Keller, P. R.; Leopold, W. R.; Loo, J. A.; McNamara, D. J.; Nelson, J. M.; Sherwood, V.; Smaill, J. B.; Trumpp-Kallmeyer, S. Dobrusin, E. M. Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 12022-12027.

(16) Fry, D. W.; Mechanism of action of erbB tyrosine kinase inhibitors. *Exp. Cell Res.* **2003**, *284*, 131-139.

(17) Smaill, J. B.; Palmer, B. D.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Bridges, A. J.; Zhou, H.; Showalter, H. D. H.; Winters, R. T.; Leopold, W. R.; Fry, D. W.; Nelson, J. M.; Slintak, V.; Elliot, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. Tyrosine kinase inhibitors. 15. 4-(Phenylamino)quinazoline and 4-(phenylamino)pyrido[*d*]pyrimidine acrylamides as irreversible inhibitors of the ATP binding site of the epidermal growth factor receptor. *J. Med. Chem*. **1999**, *42*, 1803-1815.

(18) Smaill, J. B.; Rewcastle, G. W.; Bridges, A. J.; Zhou, H.; Showalter, H. D. H.; Fry, D. W.; Nelson, J. M.; Sherwood, V.; Elliott, W. L.; Vincent, P. W.; DeJohn, D.; Loo, J. A.; Gries, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E., Denny, W. A.

Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-*d*]pyrimidine-6-acrylamides bearing additional solubilizing functions. *J. Med. Chem.* **2000***, 43*, 1380-1397.

(19) Smaill, J. B.; Showalter, H. D. H.; Zhou, H.; Bridges, A. J.; McNamara, D. J.; Fry, D. W.; Nelson, J. M.; Sherwood, V.; Vincent, P. W.; Roberts, B. J.; Elliott, W. L.; Denny, W. A. Tyrosine kinase inhibitors. 18. 6-Substituted 4-anilinoquinazolines and 4-anilinopyrido[3,4-*d*]pyrimidines as soluble, irreversible inhibitors of the epidermal growth factor receptor. *J. Med. Chem*. **2001***, 44*, 429-440.

(20) Klutchko, S. R.; Zhou, H.; Winters, R. T.; Tran, T. P.; Bridges, A. J.; Althaus, I. W.; Amato, D. M.; Elliott, W. L.; Ellis, P. A.; Meade, M. A.; Roberts, B. J.; Fry, D. W.; Gonzales, A. J.; Harvey, P. J.; Nelson, J. M.; Sherwood, V.; Han, H. K.; Pace, G.; Smaill, J. B.; Denny, W. A.; Showalter, H. D. H. Tyrosine kinase inhibitors. 19. 6-Alkynamides of 4-anilinoquinazolines and 4-anilinopyrido[3,4-*d*]pyrimidines as irreversible inhibitors of the erbB family of tyrosine kinase receptors. *J. Med. Chem*. **2006**, *49*, 1475-1485.

(21) Discafani, C. M.; Carroll, M. L.; Floyd, M. B.; Hollander, I. J.; Husain, Z.; Johnson, B. D.; Kitchen, D.; May, M. K.; Malo, M. S.; Minnick, A. A.; Nilakantan, R.; Shen, R.; Wang, Y.-F.; Wissner, A.; Greenburger, L. M. Irreversible inhibition of epidermal growth factor receptor tyrosine kinase with in vivo activity by N-[4-(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide (CL-387,785). *Biochem. Pharmacol. (Amsterdam, Neth.)* **1999**, *57*, 917-925.

(22) Tsou, H.-R.; Mamuya, N.; Johnson, B. D.; Reich, M. F.; Gruber, B. C.; Ye, F.; Nilakantan, R.; Shen, R.; Discafani, C.; DeBlanc, R.; Davis, R.; Koehn, F. E.; Greenberger, L. M.; Wang, Y. F.; Wissner, A. 6-Substituted-4-(3-bromophenylamino)quinazolines as putative irreversible inhibitors of the epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER-2) tyrosine kinases with enhanced antitumor activity. *J. Med. Chem.* **2001***, 44*, 2719-2734.

(23) Wissner, A.; Overbeek, E.; Reich, M. F.; Brawner Floyd, M.; Johnson, B. D.; Mamuya, N.; Rosfjord, E. C.; Discafani, C.; Davis, R.; Shi, X.; Rabindran, S. K.; Gruber, B. C.; Ye, F.; Hallett, W. A.; Nilakantan, R.; Shen, R.; Wang, Y.-F.; Greenberger, L. M.; Tsou, H.-R. Synthesis and structure-activity relationships of 6, 7-disubstituted 4-anilinoquinoline-3-carbonitriles. The design of an orally active, irreversible inhibitor of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER-2). *J. Med. Chem.* **2003***, 46*, 49-63.

(24) Tsou, H.-R.; Overbeek-Klumpers, E. G.; Hallett, W. A.; Reich, M. F.; Brawner Floyd, M.; Johnson, B. D.; Michalak, R. S.; Nilakantan, R.; Discafani, C.; Golas, J.; Rabindran, S. K.; Shen, R.; Shi, X.; Wang, Y.-F.; Upeslacis, J.; Wissner, A. Optimization of 6, 7-disubstituted-4-(arylamino)quinoline-3-carbonitriles as orally active, irreversible inhibitors of human epidermal growth factor receptor-2 kinase activity. *J. Med. Chem*. **2005**, *48,* 1107-1131.

(25) Wood, E. R.; Shewchuk, L. M.; Byron Ellis, B.; Brignola, P.; Brasheard, R. L.; Caferro, T. R.; Dickerson, S. H.; 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. 6-Ethynylthieno[3,2-d]- and 6-ethynylthieno[2,3-d]pyrimidin-4-anilines as tunable covalent modifiers of ErbB kinases. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 2772−2778.

(26) Carmi, C.; Cavazzoni, A.; Vezzosi, S.; Bordi, F.; Vacondio, F.; Silva, C.; Rivara, S.; Lodola, A.; Alfieri, R. R.; La Monica, S.; Galetti, M.; Ardizzoni, A.; Petronin, P. G.; Mor, M. Novel irreversible epidermal growth factor receptor inhibitors by chemical modulation of the cysteine-trap portion. *J. Med. Chem.* **2010**, *53*, 2038−2050.

(27) Cha, M. Y.; Lee, K.-O.; Kim, J. W.; Lee, C. G.; Song, J. Y.; Kim, Y. H.; Lee, G. S.; Park, S. B.; Kim, M. S. Discovery of a novel Her-1/Her-2 dual tyrosine kinase inhibitor for the treatment of Her-1 selective inhibitor-resistant non-small cell lung cancer. *J. Med. Chem.* **2010**, *52*, 6880−6888.

(28) Coumar, M. S.; Chu, C.-Y.; Lin, C.-W.; Shiao, H.-Y.; Ho, Y.-L.; Reddy, R.; Lin, W.-H.; Chen, C.-H.; Peng, J.-S.; Lien, T.-W.; Huang, C.-T.; Fang, M.-Y.; Wu, S.-H.; Wu, J.-S.; Chittimalla, S. K.; Song, J.-S.; Hsu, J. T.-A.; Wu, S.-Y.; Liao, C.-C.; Chao, Y.-S.; Hsieh, H.-P. Fastforwarding hit to lead: Aurora and epidermal growth

factor receptor kinase inhibitor lead identification. *J. Med. Chem*. **2010**, *53*, 4980−4988.

(29) Wu, C.-H.; Coumar, M. S.; Chu, C.-Y.; Lin, W.-H.; Chen, Y.-R.; Chen, C.-T.; Shiao, H.-Y.; Rafi, S.; Wang, S.-Y.; Hsu, H.; Chen, C.-H.; Chang, C.-Y.; Chang, T.-Y.; Lien, T.-W.; Fang, M.-Y.; Yeh, K.-C.; Chen, C.-P.; Yeh, T.-K.; Hsieh, S.-H.; Hsu, J. T.-A.; Liao, C.-C.; Chao, Y.-S.; Hsieh, H.-P. Design and synthesis of tetrahydropyridothieno-[2,3-d]pyrimidine scaffold based epidermal growth factor receptor (EGFR) kinase inhibitors: the role of side chain chirality and Michael acceptor group for maximal potency. *J. Med. Chem*. **2010**, *53*, 7316−7326.

(30) Allen, L. F.; Eiseman, I. A.; Fry, D. W.; Lenehan, P. F. CI-1033, an irreversible pan-erbB receptor inhibitor and its potential application for the treatment of breast cancer. *Semin. Oncol*. **2003**, *30 (Suppl. 16)*, 65-78.

(31) Calvo, E.; Tolcher, A. W.; Hammond, L. A.; Patnaik, A.; de Bono, J. S.; Eiseman, I. A.; Olson, S. C.; Lenehan, P. F.; McCreery, H.; LoRusso, P.; Rowinsky, E. K. Administration of CI-1033, an irreversible pan-erbB tyrosine kinase inhibitor, is feasible on a 7-day on, 7-day off schedule: a phase I pharmacokinetic and food effect study. *Clin. Cancer Res*. **2004**, *10*, 7112-7120.

(32) Nunes, M.; Shi, C.; Greenberger, L, M. Phosphorylation of extracellular signal-regulated kinase 1 and 2, protein kinase B, and signal transducer and activator of transcription 3 are differently inhibited by an epidermal growth factor receptor

inhibitor, EKB-569, in tumor cells and normal human keratinocytes. *Mol. Cancer Ther*. **2004**, *3,* 21-27.

(33) Chan, A.; , Delaloge, S.; Holmes, F. A.; Moy, B.; Iwata, H.; Harvey, V. J.; Robert, N. J.; Silovski, T.; Gokmen, E.; Von Minckwitz, G.; Ejlertsen, B.; Chia, S. K. L.; Mansi, J.; Barrios, C. H.; Gnant, M.; Wong, A.; Bryce, R.; Yao, B.; Martin, M. Neratinib after adjuvant chemotherapy and trastuzumab in HER2-positive early breast cancer: Primary analysis at 2 years of a phase 3, randomized, placebo-controlled trial (ExteNET). *J. Clin. Oncol.* **2015**, *33* (suppl; abstr 508).

(34) Solca, F.; Dahl, G.; Zoephel, A.; Bader, G.; Sanderson, M.; Klein, C.; Kraemer, O.; Himmelsbach, F.; Haaksma, E.; Adolf, G. R. Target binding properties and cellular activity of afatinib (BIBW 2992), an irreversible ErbB family blocker. *J. Pharmacol. Exp. Ther*. **2012**, *343*, 342-350.

(35) Sanderson, K. Irreversible kinase inhibitors gain traction. *Nat. Rev. Drug Discovery*, **2013**, *12*, 649-651.

(36) Kwak, E. L.; Sordella, R.; Bell, D. W.; Godin-Heymann, N.; Okimoto, R. A.; Brannigan, B. W.; Harris, P. L.; Driscoll, D. R.; Fidias, P.; Lynch, T. J.; Rabindran, S. K.; McGinnis, J. P.; Wissner, A.; Sharma, S. V.; Isselbacher, K. J.; Settleman, J.; Haber, D. A. Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 7665-7670.

(37) Carter, T. A.; Wodicka, L. M.; Shah, N. P.; Velasco, A. M.; Fabian, M. A.; Treiber, D. K.; Milanov, Z. V.; Atteridge, C. E.; Biggs, W. H., III; Edeen, P. T.; Floyd, M.; Ford, J. M.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Mehta, S. A.; Patel, H. K.; Pao, W.; Sawyers, C. L.; Varmus, H.; Zarrinkar, P. P.; Lockhart, D. J. Inhibition of drug- resistant mutants of ABL, KIT, and EGF receptor kinases. *Proc. Natl. Acad. Sci. U. S. A*. **2005**, *102*, 11011-11016.

(38) Zhou, W.; Ercan, D.; Chen, L.; Yun, C.-H.; Li, D.; Capelletti, M.; Cortot, A. B.; Chirieac, L.; Lacob, R. E.; Padera, R.; Engen, J. R.; Wong, K.-K.; Eck, M. J.; Gray, N. S.; Jänne, P. A. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature* **2009**, 462, 1070-1074.

(39) Sos, M.L.; Rode, H. B.; Heynck, S.; Peifer, M.; Fischer, F.; Klüter, S.; Pawar, V.G.; Reuter, C.; Heuckmann, J. M.; Weiss, J.; Ruddigkeit, L.; Rabiller, M.; Koker, M.; Simard, J. R.; Getlik, M.; Yuza, Y.; Chen, T.-H.; Greulich, H.; Thomas, R. K.; Rauh, D. Chemogenomic profiling provides insights into the limited activity of irreversible EGFR inhibitors in tumor cells expressing the T790M EGFR resistance mutation. *Cancer Res*. **2010**, *70*, 868-874.

(40) Kim, Y.; Ko, J.; Cui, Z.-Y.; Abolhoda, A.; Ahn, J. S.; Ou, S.-H.; Ahn, M.-Y.; Park, K. The EGFR T790M mutation in acquired resistance to an irreversible second-generation EGFR inhibitor. *Mol. Cancer Ther*. **2012**, *11*, 784-791.

(41) Zhou, W.; Ercan, D.; Jänne, P. A.; Gray, N. S. Discovery of selective irreversible inhibitors for EGFR-T790M. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 638-643.

(42) Walter, A. O.; Tjin Tham Sjin, R.; Haringsma, H. J.; Ohashi, K.; Sun, J.; Lee, K.; Dubrovskiy, A.; Labenski, A.; Zhu, Z.; Wang, Z.; Sheets, M.; St Martin, T.; Karp, R.; van Kalken, D.; Chaturvedi, P.; Niu, D.; Nacht, M.; Petter, R. C.; Westlin, W.; Lin, K.; Jaw-Tsai, S.; Raponi, M.; Van Dyke, T.; Etter, J.; Weaver, Z.; Pao, W.; Singh, J.; Simmons, A. D.; Harding, T. C.; Allen, A. Discovery of a mutant-selective covalent inhibitor of EGFR that overcomes T790M mediated resistance in NSCLC. *Cancer Discovery* **2013**, *3*, 1404-1415.

(43) Cross, D. A. E; Ashton, S. E.; Ghiorghiu, S.; Eberlein, C.; Nebhan, C. A.; Spitzler, P. J.; Orme, J. P.; Finlay, M. R. V.; Ward, R. A.; Mellor, M. J.; Hughes, G.; Rahi, A.; Jacobs, V. N.; Brewer, M. R.; Ichihara, E.; Sun, J.; Jin, H.; Ballard, P.; Al-Kadhimi, K.; Rowlinson, R.; Klinowska, T.; Richmond, G. H. P.; Cantarini, M.; Kim, D-W.; Ranson, M. R.; Pao, W. AZD9291, an Irreversible EGFR TKI, Overcomes T790M-Mediated Resistance to EGFR Inhibitors in Lung Cancer. *Cancer Discovery* **2014**, *4*, 1046-1061.

(44) Mitsudomi, T., Tsai, C., Shepherd, F., Bazhenova, L., Lee, J., Chang, G., Crino, L., Satouchi, M., Chu, Q., Lawrance, R., Cantarini, M., Ghiorghiu, S., Goss, G. MINI16.08: AZD9291 in pre-treated T790M positive advanced NSCLC: AURA2 Phase II study. *J. Thorac. Oncol*. Sept. **2015**, *10(9)*, Suppl. 2, Abstract 1406.

(45) Zinner, R. G.; Nemunaitis, J.; Eiseman, I.; Shin, H. J. C.; Olson, S. C.; Christensen, J.; Huang, X.; Lenehan, P. F.; Donato, N. J.; Shin, D. M. Phase I

clinical and pharmacodynamic evaluation of oral CI-1033 in Patients with refractory cancer. *Clin. Cancer Res*. **2007**, *13*, 3006-3014.

(46) Nemunaitis, J.; Eiseman, I.; Cunningham, C.; Senzer, N.; Williams, A.; Lenehan, P. F.; Olson, S. C.; Bycott, P.; Schlicht, M.; Zentgraff, R.; Shin, D. M.; Zinner, R. G. Phase 1 clinical and pharmacokinetics evaluation of oral CI-1033 in patients with refractory cancer. *Clin. Cancer Res*. **2005**, *11*, 3846-3853.

(47) Hur, W.; Velentza, A.; Kim, S.; Flatauer, L.; Jiang, X.; Valente, D.; Mason, D. E.; Suzuki, M.; Larson, B.; Zhang, J.; Zagorska, A.; DiDonato, M.; Nagle, A.; Warmuth, M.; Balk, S. P.; Peters, E. C.; Gray, N. S. Clinical stage EGFR inhibitors irreversibly alkylate Bmx kinase. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5916-5919.

(48) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol*. **2008**, *26*, 127-132.

(49) Campos, S.; Hamid, O.; Seiden, M. V.; Oza, A.; Plante, M.; Potkul, R. K.; Lenehan, P. F.; Kaldjian, E. P.; Varterasian, M. L.; Jordan, C.; Charbonneau, C.; Hirte, H. Multicenter, randomized phase II trial of oral CI-1033 for previously treated advanced ovarian cancer. *J. Clin. Oncol*. **2005**, *23*, 5597-5604.

(50) Rewcastle, G. W.; Palmer, B. D.; Bridges, A. J.; Showalter, H. D. H.; Sun, L.; Nelson, J.; McMichael, A.; Kraker, A. J.; Fry, D. W.; Denny, W. A. Tyrosine kinase inhibitors. 9. Synthesis and evaluation of fused tricyclic quinazoline analogs as ATP site inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor. *J. Med. Chem*. **1996**, *39*, 918-928.

(51) Rewcastle, G. W.; Palmer, B. D.; Thompson, A. M.; Bridges, A. J.; Cody, D. R.; Zhou, H.; Fry, D. W.; McMichael, A.; Kraker, A. J.; Denny, W. A. Tyrosine Kinase Inhibitors. 10. Isomeric 4-[(3-Bromophenyl)amino]pyrido[*d*]pyrimidines Are Potent ATP Binding Site Inhibitors of the Tyrosine Kinase Function of the Epidermal Growth Factor Receptor. *J. Med. Chem.* **1996**, *39*, 1823-1835.

(52) Erlichman, C.; Hidalgo, M.; Boni, J. P.; Martins, P.; Quinn, S. E.; Zacharchuk, C.; Amorusi, P.; Adjei, A. A.; Rowinsky, E. K. Phase I study of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor, in patients with advanced solid tumors. *J. Clin. Oncol*. **2006**, *24*, 2252-2260.

(53) Cockerill, S.; Stubberfield, C.; Stables, J.; Carter, M.; Guntrip, S.; Smith, K.; McKeown, S.; Shaw, R.; Topley, P.; Thomsen, L.; Affleck, K.; Jowett, A.; Hayes, D.; Willson, M.; Woollard, P.; Spalding, D. Indazolylamino quinazolines and pyridopyrimidines as inhibitors of the EGFr and c-erbB-2. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1401-1405.

(54) Gaul, M. D.; Guo, Y.; Affleck, K.; Cockerill, G. S.; Gilmer, T. M.; Griffin, R. J.; Guntrip, S.; Keith, B. R.; Knight, W. B.; Mullin, R. J.; Murray, D. M.; Rusnak, D.

W.; Smith, K.; Tadepalli, S.; Wood, E. R.; Lackey, K. Discovery and Biological Evaluation of Potent Dual ErbB-2/EGFR Tyrosine Kinase Inhibitors: 6-Thiazolylquinazolines. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 637-640.

(55) Zhang, Y.-M.; Cockerill, S.; Guntrip, S. B.; Rusnak, D.; Smith, K.; Vanderwall, D.; Wood, E.; Lackey, K. Synthesis and SAR of potent EGFR/erbB2 dual inhibitors. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 111-114.

(56) Petrov, K. G.; Zhang, Y.-M.; Carter, M.; Cockerill, G. S.; Dickerson, S.; Gauthier, C. A.; Guo, Y.; Mook, R. A.; Rusnak, D. W.; Walker, A. L.; Wood, E. R.; Lackey, K. E. Optimization and SAR for dual ErbB-1/ErbB-2 tyrosine kinase inhibition in the 6-furanylquinazoline series. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 4686-4691.

(57) Gonzales, A. J.; Hook, K. E.; Althaus, I. W.; Ellis, P. A.; Trachet, E.; Delaney, A. M.; Harvey, P. J.; Ellis, T. A.; Amato, D. M.; Nelson, J. M.; Fry, D. W.; Zhu, T.; Loi, C.-M.; Fakhoury, S. A.; Schlosser, K. M.; Sexton, K. E.; Winters, R. T.; Reed, J. E.; Bridges, A. J.; Lettiere, D. J.; Baker, D. A.; Yang, J.; Lee, H. T.; Tecle, H.; Vincent, P. W. Antitumor activity and pharmacokinetic properties of PF-00299804, a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor. *Mol. Cancer Ther.* **2008**, *7*, 1880-1889.

(58) Gajiwala, K. S.; Feng, J.; Ferre, R.; Ryan, K.; Brodsky, O.; Weinrich, S.; Kath, J. C.; Stewart, A. Insights into the aberrant activity of mutant EGFR kinase domain and drug recognition. *Structure* **2013**, *21*, 209-219.

(59) Engelman, J. A.; Zejnullahu, K.; Gale, C.-M.; Lifshits, E.; Gonzales, A. J.; Shimamura, T.; Zhao, F.; Vincent, P. W.; Naumov, G. N.; Bradner, J. E.; Althaus, I. W.; Gandhi, L.; Shapiro, G. I.; Nelson, J. M.; Heymach, J. V.; Meyerson, M.; Wong, K.-K.; Jänne, P. A. PF00299804, an irreversible pan-erbB inhibitor, is effective in lung cancer models with EGFR and erbB2 mutations that are resistant to gefitinib. *Cancer Res.* **2007**, *67*, 11924-11932.

(60) Ather, F.; Hamidi, H.; Fejzo, M. S.; Letrent, S.; Finn, R. S.; Kabbinavar, F.; Head, C.; Wong, S. G. Dacomitinib, an irreversible pan-ErbB inhibitor significantly abrogates growth in head and neck cancer models that exhibit low response to cetuximab. *PLoS One* **2013**, *8*, e56112.

(61) Nam, H.-J.; Kim, H.-P.; Yoon, Y.-K.; Song, S.-H.; Min, A.-R.; Han, S.-W.; Im, S.-A.; Kim, T.-Y.; Oh, D.-Y.; Bang, Y.-J. The irreversible pan-HER inhibitor PF00299804 alone or combined with gemcitabine has an antitumor effect in biliary tract cancer cell lines. *Invest. New Drugs* **2012**, *30*, 2148-2160.

(62) Kalous, O.; Conklin, D.; Desai, A. J.; O'Brien, N. A.; Ginther, C.; Anderson, L.; Cohen, D. J.; Britten, C. D.; Taylor, I.; Christensen, J. G.; Slamon, D. J.; Finn, R. S. Dacomitinib (PF-00299804), an irreversible pan-HER inhibitor, inhibits proliferation of HER2-amplified breast cancer cell lines resistant to trastuzumab and lapatinib. *Mol. Cancer Ther.* **2012**, *11*, 1978-1987.

(63) Nam, H.-J.; Ching, K. A.; Kan, J.; Kim, H.-P.; Han, S.-W.; Im, S.-A.; Kim, T.-Y.; Christensen, J. G.; Oh, D.-Y.; Bang, Y.-J. Evaluation of the antitumor effects and

mechanisms of PF00299804, a pan-HER inhibitor, alone or in combination with chemotherapy or targeted agents in gastric cancer. *Mol. Cancer Ther*. **2012**, *11*, 439-451.

(64) Jänne, P. A.; Schellens, J. H.; Engelman, J. A.; Eckhardt, S. G.; Millham, R.; Denis, L. J.; Britten, C. D.; Wong, S. G.; Boss D. S.; Camidge, D. R. Preliminary activity and safety results from a phase I clinical trial of PF-00299804, an irreversible pan-HER inhibitor, in patients (pts) with NSCLC. *J. Clin. Oncol.* **2008**, *26*, 15S, Abstract 8027.

(65) Park, K.; Heo, D. S.; Cho, B.; Kim, D.; Ahn, M.; Lee, S.; Millham, R. D.; Campbell, A.; Zhang, H.; Kim, J. PF-00299804 (PF299) in asian patients (pts) with non-small cell lung cancer (NSCLC) refractory to chemotherapy (CT) and erlotinib (E) or gefitinib (G): A phase (P) I/II study. *J. Clin. Oncol.* **2010**, *28*, 15S, Abstract 7599.

(66) Takahashi, T.; Boku, N.; Murakami, H.; Naito, T.; Tsuya, A.; Nakamura, Y.; Ono, A.; Machida, N.; Yamazaki, K.; Watanabe, J.; Ruiz-Garcia, A.; Imai, K.; Ohki, E.; Yamamoto, N. Phase I and pharmacokinetic study of dacomitinib (PF-00299804), an oral irreversible, small molecule inhibitor of human epidermal growth factor receptor-1, -2, and -4 tyrosine kinases, in Japanese patients with advanced solid tumors. *Invest. New Drugs* **2012**, *30*, 2352-2363.

(67) Brzezniak, C.; Carter, C. A.; Giaccone, G. Dacomitinib, a new therapy for the treatment of non-small cell lung cancer. *Expert Opin. Pharmacother.* **2013**, *14*, 247-253.

(68) Mok, T.; Lee, K.; Tang, M.; Leung, L. Dacomitinib for the treatment of advanced or metastatic non-small-cell lung cancer. *Future Oncol.* **2014**, *10*, 813-822.

(69) Campbell, A.; Reckamp, K. L.; Camidge, D. R.; Giaccone, G.; Gadgeel, S. M.; Khuri, F. R.; Engelman, J. A.; Denis, L. J.; O'Connell, J. P.; Jänne, P. A. PF-00299804 (PF299) patient (pt)-reported outcomes (PROs) and efficacy in adenocarcinoma (adeno) and nonadeno non-small cell lung cancer (NSCLC): A phase (P) II trial in advanced NSCLC after failure of chemotherapy (CT) and erlotinib (E). *J. Clin. Oncol.* **2010**, *28*, 15S, Abstract 7596.

(70) Ellis, P. M.; Shepherd, F. A.; Millward, M.; Perrone, F.; Seymour, L.; Liu, G.; Sun, S.; Cho, B. C.; Morabito, A.; Leighl, N. B.; Stockler, M. R.; Lee, C. W.; Wierzbicki, R.; Cohen, V.; Blais, N.; Sangha, R. S.; Favaretto, A. G.; Kang, J. H.; Tsao, M-S.; Wilson, C. F.; Goldberg, Z.; Ding, K.; Goss, G. D.; Bradbury, P. A. Dacomitinib compared with placebo in pretreated patients with advanced or metastatic non-small-cell lung cancer (NCIC CTG BR.26): a double-blind, randomized, phase 3 trial. *Lancet Oncol.* **2014**, *15*, 1379-1388.

(71) Ramalingam, S. S.; Blackhall, F.; Krzakowski, M.; Barrios, C. H.; Park, K.; Bover, I.; Seog Heo, D.; Rosell, R.; Talbot, D. C.; Frank, R.; Letrent, S. P.; Ruiz-Garcia, A.; Taylor, I.; Liang, J. L.; Campbell, A. K.; O'Connell, J.; Boyer, M. Randomized

phase II study of dacomitinib (PF-00299804), an irreversible pan–human epidermal growth factor receptor inhibitor, versus erlotinib in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* **2012**, *30*, 3337-3344.

(72) Ramalingam, S. S.; Jänne, P. A.; Mok, T.; O'Byrne, K.; Boyer, M. J.; Von Pawel, J.; Pluzanski, A.; Shtivelband, M.; Docampo, L. I.; Bennouna, J.; Zhang, H.; Liang, J. Q.; Doherty, J. P.; Taylor, I.; Mather, C. B.; Goldberg, Z.; O'Connell, J.; Paz-Ares, L. Dacomitinib versus erlotinib in patients with advanced-stage, previously treated non-small-cell lung cancer (ARCHER 1009): a randomized, double-blind, phase 3 trial. *Lancet Oncol.* **2014**, *15*, 1369-1378.

(73) Ramalingam, S. S; O'Byrne, K.; Boyer, M.; Mok, T.; Jänne, P.A.; Zhang, H.; Liang, J.; Taylor, I.; Sbar, E.I.; Paz-Ares, L.; Dacomitinib versus erlotinib in patients with EGFR mutated advanced non small-cell lung cancer (NSCLC): pooled subset analyses from two randomized trials. *Ann. Oncol.* **2016**, *3*, 423-9.

(74) Kris, M. G.; Mok, T.; Ignatius Ou, S.-H.; Martins, R.; Kim, D.-W.; Goldberg, Z.; Zhang, H.; Taylor, I.; Letrent, S. P.; Jänne, P. A. First-line dacomitinib (PF-00299804), an irreversible pan-HER tyrosine kinase inhibitor, for patients with EGFR-mutant lung cancers. *J. Clin. Oncol.* **2012**, *30*, 487S, Abstract 7530.

(75) Abdul Razak, A. R.; Soulieres, D.; Laurie, S. A.; Hotte, S. J.; Singh, S.; Winquist, E.; Chia, S.; Le Tourneau, C.; Nguyen-Tan, P. F.; Chen, E. X.; Chan, K. K.; Wang, T.; Giri, N.; Mormont, C.; Quinn, S.; Siu, L. L. A phase II trial of dacomitinib, an oral pan-human EGF receptor (HER) inhibitor, as first-line treatment in recurrent

and/or metastatic squamous-cell carcinoma of the head and neck. *Ann. Oncol*. **2013**, *24*, 761-769.

(76) Jänne, P. A.; Ou, S. H.; Kim, D. W.; Oxnard, G. R.; Martins, R.; Kris, M. G.; Dunphy, F.; Nishio, M.; O'Connell, J. Paweletz, C.; Taylor, I.; Zhang, H.; Goldberg, Z.; Mok, T. Dacomitinib as first-line treatment in patients with clinically or molecularly selected advanced non-small-cell lung cancer: a multicentre, open-label, phase 2 trial. *Lancet Oncol.* **2014**, *13*, 1433-41.

(77) Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D. H.; Fry, D. W.; Kraker, A. J.; Denny, W. A. Tyrosine kinase inhibitors: 8. An unusually steep structure-activity relationship for analogs of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor. *J. Med. Chem*. **1996**, *39*, 267-276.

(78) Nomoto, Y.; Obase, H.; Takai, H.; Hirata, T.; Teranishi, M.; Nakamura, J.; Kubo, K. Studies on cardiotonic agents. I. Synthesis of some quinazoline derivatives. *Chem. Pharm. Bull*. **1990**, *38*, 1591-1595.

(79) Ham, Y. J.; Gong, J. H.; Cha, M. Y.; Kim, J. W.; Kim, M. S.; Kim, E. Y.; Song, J. Y.; Kim, C. I.; Kim, S. Y.; Lee, G. S. Preparation of quinazoline derivatives as inhibitors of epidermal growth factor receptor and growth of cancer cells. *PCT Int. Appl*. **2006**, WO 2006071017 A1.

(80) Gaster, L. M.; Wyman, P. A.; Mulholland, K. R.; Davies, D. T.; Duckworth, D. M.; Forbes, I. T.; Jones, G. E. Preparation of indolines as 5-HT2B/2C receptor antagonists. *PCT Int. Appl*. **1996**, WO 9623783 A1.

(81) Cockerill, G. S.; Carter, M. C.; Guntrip, S. B.; Smith, K. J. Preparation of azolylquinazolines and related compounds as protein tyrosine kinase inhibitors. *PCT Int. Appl*. **1998**, WO 9802434 A1.

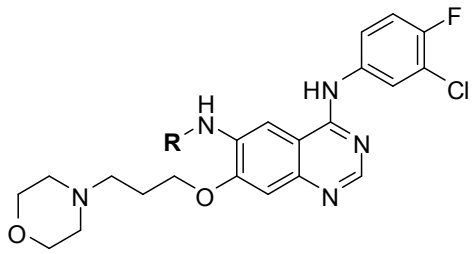
(82) Jacobsen, E. J.; Stelzer, L. S.; TenBrink, R. E.; Belonga, K. L.; Carter, D. B.; Im, H. K.; Im, W. B.; Sethy, V. H.; Tang, A. H.; VonVoigtlander, P. F.; Petke, J. D.; Zhong, W. Z.; Mickelson, J. W. Piperazine imidazo[1,5-*a*]quinoxaline ureas as high-affinity GABAA ligands of dual functionality. *J. Med. Chem*. **1999**, *42*, 1123-1144.

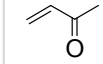
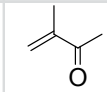
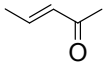
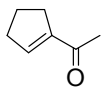
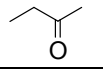
(83) Flanagan, M. E.; Blumenkopf, T. A.; Brissette, W. H.; Brown, M. F.; Casavant, J. M.; Shang-Poa, C.; Doty, J. L.; Elliott, E. A.; Fisher, M. B.; Hines, M.; Kent, C.; Kudlacz, E. M.; Lillie, B. M.; Magnuson, K. S.; McCurdy, S. P.; Munchhof, M. J.; Perry, B. D.; Sawyer, P. S.; Strelevitz, T. J.; Subramanyam, C.; Sun, J.; Whipple, D. A.; Changelian, P. S. Discovery of CP-690,550: A potent and selective Janus kinase (JAK) inhibitor for the treatment of autoimmune diseases and organ transplant rejection. *J. Med. Chem*. **2010**, *53*, 8468–8484.

(84) Cohen, B. D.; Goldstein, D. J.; Rutledge, L.; Vass, W. C.; Lowy, D. R.; Schlegel, R.; Schiller, J. T. Transformation-specific interaction of the bovine papillomavirus E5 oncoprotein with the platelet-derived growth factor receptor transmembrane

domain and the epidermal growth factor receptor cytoplasmic domain. *J. Virol.* **1993**, *67*, 5303-5511.

Table 1. Exploration of substituted acrylamides on the canertinib scaffold



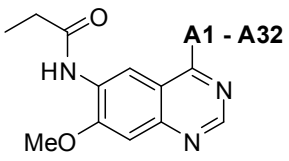
		kinase assays IC_{50} (μM)					reactivity assay
		erbB1		erbB2		erbB4	100% inhib (min)[f]
compd	R	enzyme[a]	cellular[b]	enzyme[a]	cellular[c]	enzyme[a]	
4		0.002	0.002	0.011	0.016	0.027	1
8		0.021	0.067	NA	40%[d]	12%[e]	64% at 2h
9		0.022	0.075	NA	38%[d]	26%[e]	75% at 2h
10		0.007	0.026	NA	36%[d]	43%[e]	97% at 2h
11		0.009	0.025	0.509	39%[d]	38%[e]	54% at 2h

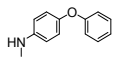
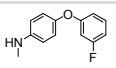
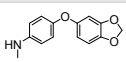
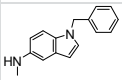
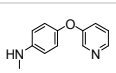
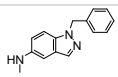
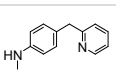
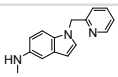
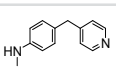
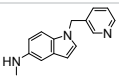
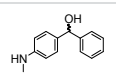
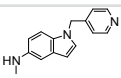
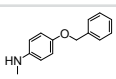
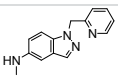
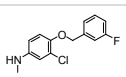
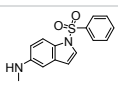
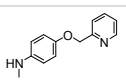
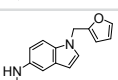
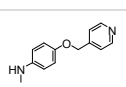
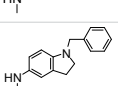
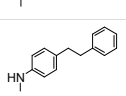
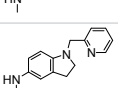
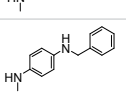
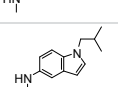
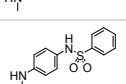
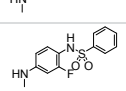
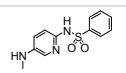
Footnote for Table 1

[a]Concentration for 50% inhibition of phosphorylation of a Glu/Tyr copolymer (see Experimental Section). Values are the average of at least two separate determinations, with a CV of ±22 %. [b]Inhibition of erbB1 autophosphorylation in NIH3T3 cells transfected with the full-length human erbB1 (see Experimental Section). [c]Inhibition of ligand-regulated erbB2 autophosphorylation in T24 NIH fibroblasts expressing a chimeric receptor with the extracellular binding domain of erbB1 and the intracellular kinase domain of erbB2 (see Experimental Section). [d]Percentage inhibition at a drug concentration of 0.5 μM. [e]Percentage inhibition at a drug concentration of 1.0 μM. [f]Length

of drug exposure (minutes) required to provide 100% inhibition of erbB1 autophosphorylation in A431 cells, or conversely the percentage of inhibition after 2 hours drug exposure (see Experimental Section). NA = data not available.

Table 2. Exploration of pan-erbB potency for anilines on a reversible scaffold.

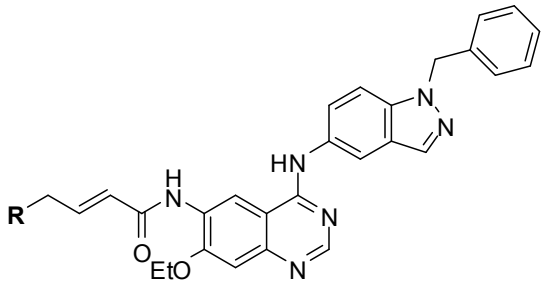


			isolated enzyme IC_{50} (μM)[a]						isolated enzyme IC_{50} (μM)[a]		
compd	aniline	structure	erbB1	erbB2	erbB4	compd	aniline	structure	erbB1	erbB2	erbB4
12	A1		0.002	0.406	0.376	**28**	A17		0.065	0.419	0.048
13	A2		0.006	0.800	33%[b]	**29**	A18		0.881	5%[b]	0.097
14	A3		0.079	18%[b]	16%[b]	**30**	A19		0.509	14%[b]	0.040
15	A4		0.021	0.124	0.038	**31**	A20		0.531	22%	0.285
16	A5		0.017	0.153	0.018	**32**	A21		0.205	NA	0.676
17	A6		0.061	0.642	0.095	**33**	A22		0.388	NA	45%[b]
18	A7		0.714	NA	0.328	**34**	A23		0.343	44%[b]	0.078
19	A8		43%[b]	NA	0.213	**35**	A24		0.036	0.404	0.460
20	A9		0.094	47%[b]	0.015	**36**	A25		0.067	0.458	0.636
21	A10		0.127	0.286	0.063	**37**	A26		0.116	0.815	0.108
22	A11		0.086	0.782	0.022	**38**	A27		34%[b]	0%[b]	0.428
23	A12		0.175	0.556	0.082	**39**	A28		0.747	NA	0.653
24	A13		0.519	0.897	0.514	**40**	A29		0.383	NA	0.064
25	A14		0.421	NA	34%[b]	**41**	A30		0.065	0.798	0.408
26	A15		0.136	15%[b]	4%[b]	**42**	A31		0.593	25%[b]	0.583
27	A16		0.086	17%[b]	3%[b]	**43**	A32		0.522	0.958	0.487

Footnotes for Table 2

[a]See footnotes to Table 1. [b]Percentage inhibition at a drug concentration of 1 μM. NA = data not available.

Table 3. Investigation of cyclic amine bearing crotonamides on a pan-erbB scaffold.



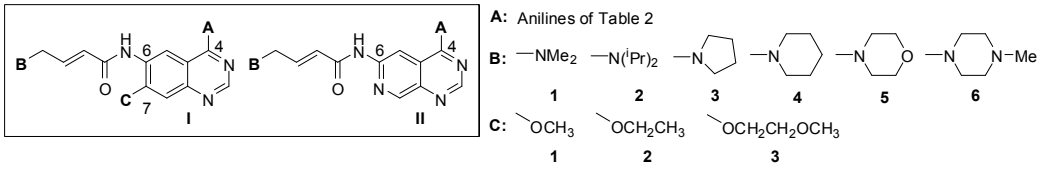
		kinase assays IC_{50} (μM)					reactivity assays				
		erbB1		erbB2	erbB4	JAK3					
compd	R	enzyme[a]	cellular[b]	enzyme[a]	enzyme[a]	enzyme[c]	100% inhib (min)[d]	RLM Stability (min)[e]	HLM Stability (min)[e]	RH Stability (min)[f]	HH Stability (min)[f]
44		0.006	0.002	0.012	0.003	6.88	1	>40	>40	83	121
45		0.015	0.007	0.03	0.006	8.15	1	28	14	35	NA
46		0.007	0.003	0.019	0.004	4.96	1	>40	>40	NA	135
47		0.008	0.002	0.019	0.004	7.15	1	>40	>40	NA	NA
48		0.079	0.026	0.128	0.005	7.67	NA	NA	NA	NA	NA
49		0.026	0.004	0.027	0.006	>10	1	>40	>40	95	97
50		0.023	0.008	0.023	0.004	8.75	1	>40	>40	NA	234
51		0.017	0.019	0.019	0.004	6.94	1	>40	>40	NA	154
52		0.072	NA	0.198	0.004	8.88	NA	NA	NA	NA	NA

Footnotes for Table 3

[a, b] See footnotes to Table 1. [c]Concentration for 50% inhibition of phosphorylation of polyglutamic acid-tyrosine [PGT (4:1), Sigma catalog no. P0275] by recombinant JAK3 (see Experimental Section). Values are the average of at least two separate determinations, with a CV of ±22 %. [d]Length of drug exposure (minutes) required to provide 100% inhibition of erbB1 autophosphorylation in A431 cells (see Experimental Section).

[e]Length of rat liver microsome (RLM) or human liver microsome exposure (minutes) required to give 50% loss of parent compound (starting concentration 5 μM) (see Experimental Section). [f]Length of rat hepatocyte (RH) or human hepatocyte exposure (minutes) required to give 50% loss of parent compound (starting concentration 1 μM) (see Experimental Section). NA = data not available.

Table 4. Pan-erbB anilines and cyclic amino crotonamides on quinazoline and pyrido[3,4-*d*]pyrimidine scaffolds.



					kinase assays IC_{50} (μM)[a]										kinase assays IC_{50} (μM)[a]				
					erbB1		erbB2	erbB4	JAK3						erbB1		erbB2	erbB4	JAK3
compd	series	A	B	C	enzyme	cellular	enzyme	enzyme	enzyme	compd	series	A	B	C	enzyme	cellular	enzyme	enzyme	enzyme
1					0.003	0.014	0.343	0.476	>1.0	**65**	I	26	3	2	0.098	0.012	0.278	0.041	>10
4					0.002	0.002	0.011	0.027	0.128	**66**	I	26	6	2	0.164	0.061	>1.0	0.094	>10
53	I	1	2	1	0.002	0.009	0.032	0.088	7.40	**67**	I	30	3	2	0.021	0.061	0.045	0.003	>10
54	I	1	4	1	0.006	0.006	0.046	0.074	3.57	**68**	I	30	5	2	0.069	0.014	0.174	0.018	>10
44[b]	I	5	1	2	0.006	0.002	0.012	0.003	6.88	**69**	I	30	6	2	0.082	0.390	0.290	0.029	>10
46[b]	I	5	3	2	0.007	0.003	0.019	0.004	4.96	**70**	II	1	1		0.002	0.001	0.002	0.028	>10
47[b]	I	5	4	2	0.008	0.002	0.019	0.004	7.15	**71**	II	1	4		0.014	0.002	0.014	0.007	0.793
50[b]	I	5	6	2	0.023	0.008	0.023	0.004	8.75	**72**	II	1	5		0.012	0.002	0.035	0.043	1.71
55	I	5	1	3	0.007	0.005	0.004	0.001	7.10	**73**	II	1	6		0.019	0.009	0.089	0.302	2.42
56	I	5	3	3	0.010	0.008	0.005	0.002	4.80	**74**	II	5	1		0.041	0.003	0.062	0.003	7.44
57	I	5	6	3	0.016	0.026	0.019	0.003	>10	**75**	II	5	3		0.057	0.003	0.062	0.004	7.62
58	I	10	1	2	0.038	0.016	0.038	0.010	>10	**76**	II	17	1		0.201	0.014	0.100	0.003	8.01
59	I	10	3	2	0.036	0.053	0.030	0.011	3.79	**77**	II	17	3		0.180	0.008	0.102	0.002	5.97
60	I	10	5	2	0.122	0.030	0.100	0.027	>10	**78**	II	21	1		0.304	0.010	0.011	0.004	>10
61	I	17	4	1	0.132	0.016	0.073	0.003	7.1	**79**	II	21	3		0.405	0.010	0.017	0.004	>10
62	I	21	3	1	0.148	0.029	0.413	0.013	>10	**80**	II	21	6		0.857	0.100	>1.0	0.094	>10
63	I	25	3	2	0.060	0.023	0.010	0.004	6.45	**81**	II	25	1		0.025	0.011	0.042	0.003	1.22
64	I	25	6	2	0.023	0.010	0.020	0.010	3.69	**82**	II	25	3		0.037	0.016	0.053	0.005	3.49

Footnotes for Table 4

[a]See footnotes to Table 1 and Table 3. [b]Data repeated from Table 3 for clarity.

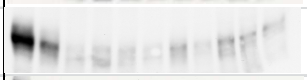
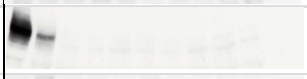
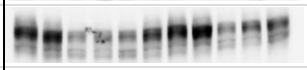
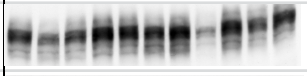
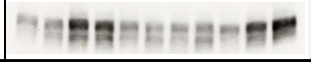
Table 5. Reactivity and stability of selected compounds from Table 4.

	reactivity assays[a]				
compd	100% inhib (min)	RLM Stability (min)	HLM Stability (min)	RH Stability (min)	HH Stability (min)
1	90% at 2h	>40	>40	101	225
4	1	>40	>40	28.2	159
44	1	>40	>40	83.1	121
46	1	>40	>40	NA	135
50	1	>40	>40	NA	234
53	3	26.3	27	52.9	52
54	1	>40	>40	143	249
59	1	>40	>40	NA	86.5
60	1	>40	>40	53.1	NA
67	1	>40	NA	NA	139
68	5	>40	>40	NA	NA
69	30	>40	>40	329	273
71	1	>40	>40	NA	NA

Footnote to Table 5

[a] See footnotes to Table 3.

Table 6. Inhibition of erbB1 phosphorylation *in vivo* for selected compounds from Table 4.

	erbB1 pharmacodynamic assay (3 mice per cohort)[a]									
	control		dose po			control		dose po		
			130mg/kg		65mg/kg			65mg/kg	30mg/kg	
compd	6h	24h	6h	24h	6h	6h	24h	24h	6h	24h
1										
4										
44										
46										
47										
50										
54										
58										
59										
60										
62										
67										
71										
73										
74										
75										
79										
80										

Footnote to Table 6

[a] Western blots demonstrating inhibition of erbB1 autophosphorylation in NIH3T3 tumors 6 and 24 hours following oral administration of the specified dose on a QDx2 schedule (see Experimental Section).

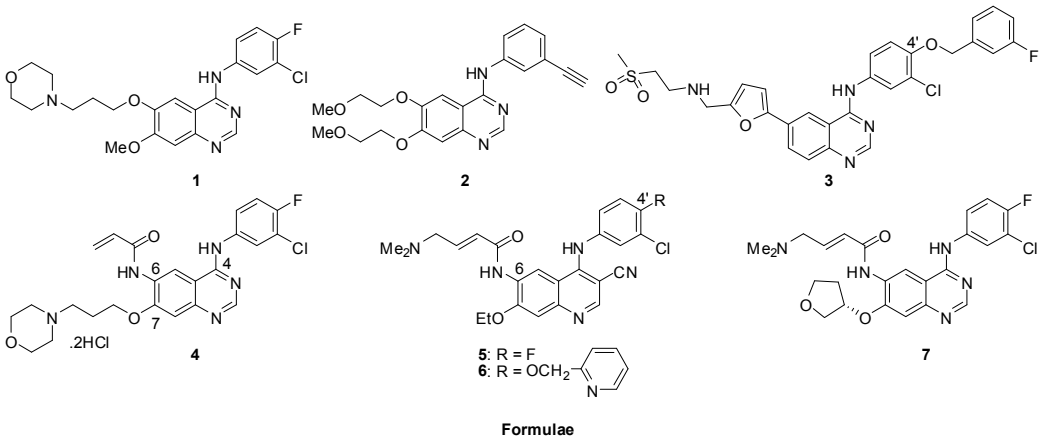
Table 7. Mean pharmacokinetic parameters of compounds **54** and **71** in rat, dog and monkey following a single oral and intraveneous dose.

Species	Dose (mg/kg/d)	Route	C_{max} (μg/mL)	AUC (μg.h/mL)	CL (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	F (%)
Compound **54**[a]								
Rat	50	p.o.	0.63	20.5			19.4	80
Dog	50[b]	p.o.	0.87	25.2			20.9	74
Monkey	25	p.o.	0.22	5.4			12.1	56
Rat	5	i.v.		2.1	49.1	34.2	9.8	
Dog	5	i.v.		3.4	24.4	28.0	15.9	
Monkey	5	i.v.		1.9	44.1	17.3	5.7	
Compound **71**								
Rat	50	p.o.	0.69	13.5			9.8	60
Dog	40[b]	p.o.	1.04	11.0			7.9	13
Monkey	25	p.o.	0.04	0.2			NR	4
Rat	5	i.v.		1.4	62.0	24.8	5.4	
Dog	5	i.v.		10.3	9.0	4.0	7.6	
Monkey	2.5	i.v.		0.6	69.8	4.3	0.7	

Footnotes for Table 7

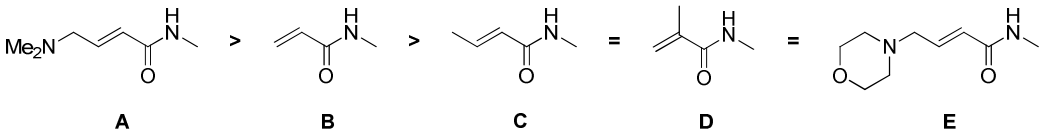
[a]As report in Gonzales *et al*, 2008 (reference 57). [b]Fed condition, milled to reduce particle sizes; for further methods see Experimental Section. NR: not reported due to insufficient data.

Formulae



Formulae

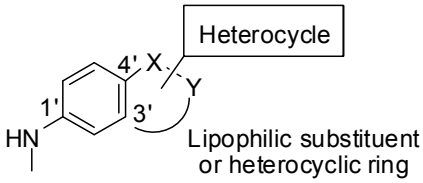
Figure 1



Legend for Figure 1

Reported relative reactivity of various Michael acceptors towards reduced glutathione.[22]

Figure 2

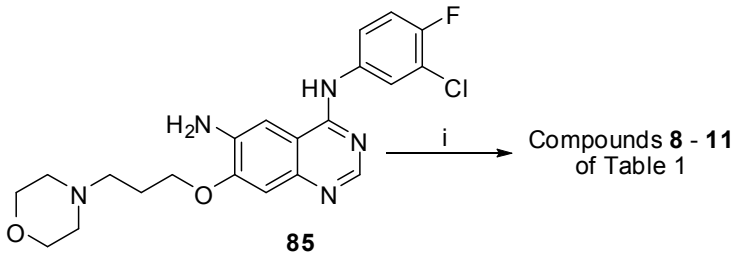


Legend for Figure 2

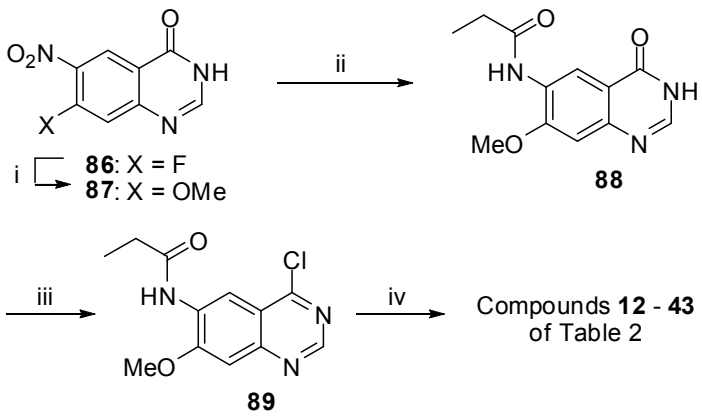
General pharmacophore explored for anilines that provide pan-erbB activity.

Schemes

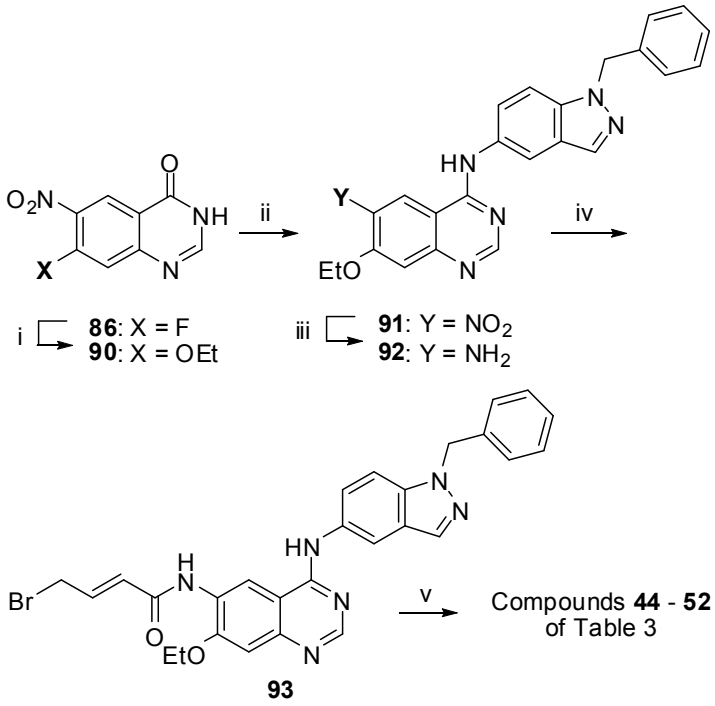
Scheme 1



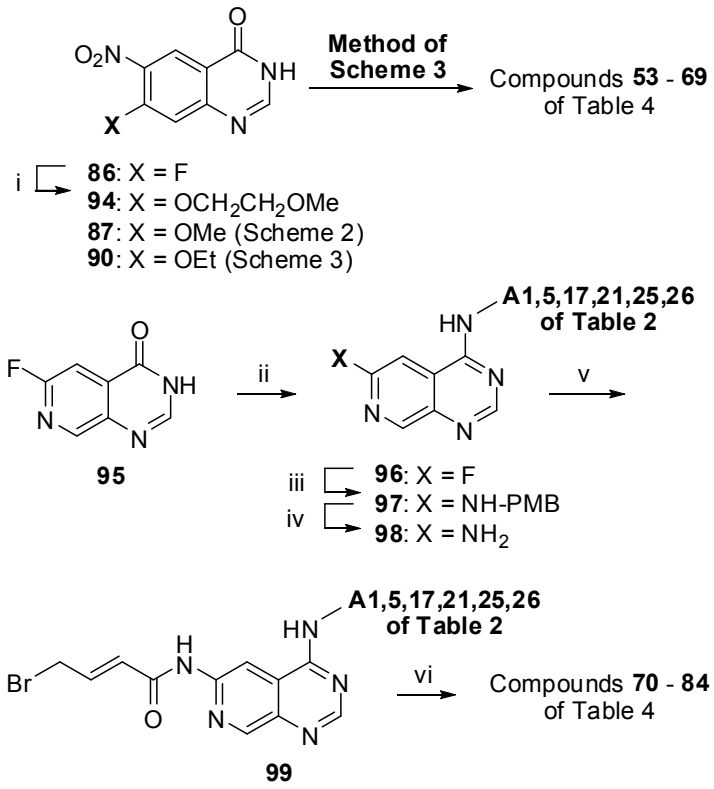
Scheme 2


O2N
X
NH
N
O
i
86: X = F
87: X = OMe
ii
HN
MeO
88
iii
Cl
89
iv
Compounds 12 - 43
of Table 2

Scheme 3


O2N
X
NH
O
N
ii
Y
EtO
HN
N
N
iv
i
86: X = F
90: X = OEt
iii
91: Y = NO2
92: Y = NH2
Br
H
N
O
EtO
v
Compounds 44 - 52
of Table 3
93

Scheme 4


Method of Scheme 3
Compounds 53 - 69 of Table 4
i
86: X = F
94: X = OCH2CH2OMe
87: X = OMe (Scheme 2)
90: X = OEt (Scheme 3)
95
ii
v
A1,5,17,21,25,26 of Table 2
iii
96: X = F
iv
97: X = NH-PMB
98: X = NH2
99
vi
Compounds 70 - 84 of Table 4

Legends for reaction schemes

Scheme 1

Reagents and conditions: (i) acid chloride, Et_3N, THF, R.T.

Scheme 2

Reagents and conditions: (i) NaOMe, MeOH, 100ºC, 18 h; (ii) (a) Fe, AcOH, EtOH, water, 100ºC, 1 h, (b) propionic anhydride, 120°C, 2 h; (iii) $POCl_3$, reflux, 90 min; (iv) subst. aniline, ^{i}PrOH, c. HCl (cat.), 100°C, 0.5-1h.

Scheme 3

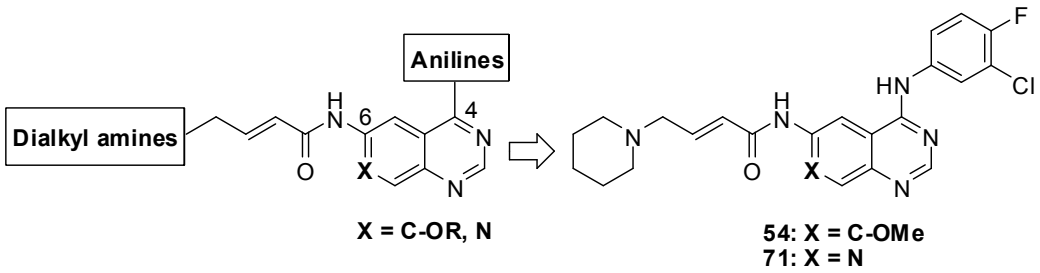
Reagents and conditions: (i) NaOEt, EtOH, 50ºC, 4 h; (ii) (a) $POCl_3$, reflux, 3h., (b) 1-benzyl-1*H*-indazol-5-amine, ^{i}PrOH/CH_2Cl_2, 50°C, 15 h.; (iii) Raney nickel, H_2, EtOAc/MeOH; (iv) (a) *E*-4-bromobut-2-enoic acid, oxalyl chloride, cat. DMF, CH_2Cl_2, R.T., (b) acid chloride from (a), THF, -20°C, 0.75 h.; (v) amine, DMA, -20°C, 2h.

Scheme 4

Reagents and conditions: (i) $NaO(CH_2)_2OCH_3$, THF, reflux; (ii) (a) $SOCl_2$, cat. DMF, reflux, 2 h., (b) subst. aniline, ^{i}PrOH/CH_2Cl_2, reflux, 0.75 h.; (iii) 4-methoxybenzylamine, DMSO, 120°C, 24 h.; (iv) TFA, 6 h., R.T.; (v) (a) *E*-4-bromobut-2-enoic acid, oxalyl

chloride, cat. DMF, CH_2Cl_2, R.T., (b) acid chloride from (a), THF, -20^{o}C, 0.75 h.; (vi) amine, DMA, -20^{o}C, 2h.

TOC graphic



Compound **54** (dacomitinib) is under clinical evaluation.