

Chemical Inhibitors of Protein Kinases

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Contents

I. Introduction	2541
II. Drug Discovery Process	2542
A. Some Useful Definitions	2542
B. Quick Overview of the Standard Drug Discovery Process	2542
C. Problems Inherent in Exploiting a Novel Target Class	2543
III. Protein Kinases	2544
A. Cellular Signaling and Protein Kinases	2544
B. Could Kinases Make Good Drug Targets?	2544
C. Kinases and Cancer	2545
D. Cytotoxicity, Cytostasis, Combinations, and Conundrums	2546
E. Choice of Kinase Targets for Cancer Therapy	2547
IV. Kinase Inhibitors	2548
A. PKC Inhibitors	2548
B. BCR-ABL Inhibitors	2549
V. Epidermal Growth Factor Receptor TK Inhibitors	2551
A. Introduction	2551
B. Early Natural Product Leads	2551
C. Erbstatin-Based EGFr Inhibitors	2554
D. Initial Parke-Davis Approach to EGFr Inhibitors	2555
VI. Anilinopyrimidine Inhibitors	2555
A. Discovery of the Anilinopyrimidine Pharmacophore	2555
B. Exploration and Development of the Anilinopyrimidine SAR	2557
1. EGFr Enzyme SAR	2557
2. Molecular Modeling of the EGFr SAR	2558
3. ErbB Family Selectivity and Potency	2559
4. In Vivo Activity of the EGFr Inhibitors	2560
5. Other Inhibitors Based on the Anilinoquinazoline Pharmacophore	2561
VII. Making an Irreversible Change in Strategy	2563
A. Reasons for Returning to Covalence	2563
B. Development of Irreversible Quinazoline EGFR Inhibitors	2564
C. Discovery of CI-1033: A Soluble, Irreversible, Inhibitor of the ErbB Family with Excellent in Vivo Potency	2565
D. Summary	2567
E. Epilogue	2567
VIII. Acknowledgments	2568
IX. References	2568



Alexander J. Bridges obtained his B.A. degree in Chemistry from Oxford University in 1972 and his Ph.D. degree under the supervision of Dr. Gordon Whitham in 1974, working on *trans*-cyclooctenes. He was a NATO Fellow with Professor Barry Trost at Wisconsin for the following two years, making novel dienes for Diels–Alder reactions and spent a year with Professor Pierre Potier at the CNRS at Gif-sur-Yvette working on the synthesis of vinblastine. This was followed by a final postdoctoral position at the University of Toronto with Professor J. B. Jones, working on enzymes as chiral synthetic reagents. In 1978 he joined the chemistry faculty of Northern Illinois University in DeKalb, IL, taught organic chemistry for six years, and worked on sulfur-substituted allenes and dienes. In 1984 he joined Parke-Davis in Ann Arbor, MI, and worked on adenosine agonists and quinolone anti-infectives. He moved to the Eisai Research Institute of Boston in 1988 and worked on Lipid A analogues for sepsis and urokinase inhibitors for cancer. He moved back to Parke-Davis (now Pfizer Global Research and Development, Ann Arbor Laboratories) in 1992 and worked on kinase inhibitors for cancer, especially EGFr and MEK inhibitors. He then moved to metabolic diseases chemistry and is currently Director of Cancer Chemistry at Pfizer, Ann Arbor.

I. Introduction

Many excellent and up to date reviews on kinase inhibitors exist, and this area certainly does not meet the normal criteria for a *Chemical Reviews* article. However, most of these reviews are written by Medicinal Chemists for Medicinal Chemists and largely by the pharmaceutical industry for the pharmaceutical industry. Such reviews make many assumptions about the (relatively specialized) knowledge base of the average reader and tend to take for granted that the readers all know what drug companies are after in their research process. Therefore, I will spend a part of this review trying to elucidate a pharmaceutical industry perspective and then put kinase inhibitors into that background. An overview of the way new drugs are looked for will be discussed, along with some of the perils in looking for a new mechanistic class of drugs, over and above looking in areas which are well explored and understood.

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Then this review will try to tell something of the story of the emergence of kinases as potential drug targets and the way that the pharmaceutical industry has responded to the current biological revolution in signal transduction. This review will touch briefly on classes of inhibitors for the kinases PKC and BCR-ABL, as both contain very interesting lessons, and then concentrate on the EGFr family inhibitors, especially the Parke-Davis effort for the remainder of the review, trying to show how concerns discussed in the first part of the review affected many of the decisions taken in that program.

The initial references in this manuscript are (1997–2000) general reviews of the area.^{1–18} They are followed by sets of reviews which cover several important classes of kinases where inhibitors have been developed but are not covered in this review. These are to the cyclin-dependent kinases,^{19–26} the p38 MAP kinase pathway,^{27–31} angiogenic growth factors,^{32–34} the neurotrophic TRK receptors,³⁵ and the inflammation-related tyrosine kinases.³⁶

II. Drug Discovery Process

A. Some Useful Definitions

An IC_{50} , the concentration required to inhibit a biochemical process (enzyme activity or ligand binding) by 50%, is the most easily obtained measure of potency for a potential drug in biochemical systems, but it has the problem that it is actually very dependent on the assay conditions. The units used are concentrations, in micromolar (μM) or nanomolar (nM) usually, although occasionally picomolar (pM) is used with very potent drugs. IC_{50} s can be corrected to give an inhibition constant, K_i , which is a measure of the thermodynamic binding of the ligand/inhibitor to the target protein. For enzymes, this is measured by seeing how much the rate of an enzyme-catalyzed reaction is slowed, but for receptors, there is no reaction to look at but there are two things quite readily measured. One is displacement of another ligand (often radioactive) from the receptor. From these data alone, one cannot tell if the new ligand has activated the receptor (agonist) or blocked activation of the receptor (antagonist). A second type of assay is a functional assay whereby one measures a response in the cell to the receptor. For example, most G-protein-coupled receptors (GPCRs) are coupled to adenylate cyclases and their cellular product cyclic AMP is easy to measure. For GPCRs, coupled in a stimulatory fashion to cyclases, agonists would be expected to increase cellular cyclic AMP levels whereas antagonists will block the rise caused by a reference agonist. The IC_{50} s and K_i s can be used for the antagonistic effect again, whereas an EC_{50} (effective concentration), the concentration to produce a half-maximal response, is reported for agonist potency. The ED_{50} , effective dose for a 50% of maximal response, is a similar measure that is often used to report results in animal experiments, and the most usual way of reporting these is in mg/kg, which is the dose in milligrams per kilogram of animal body weight. Thus, a 1 mg dose in a 25 g mouse is the equivalent of a 2 g dose in a 50 kg (small) adult, being

40 mg/kg in both cases. This explains why most medicinal chemists tend to regard the mouse as a better stand-in for humans than a 1000 kg horse.

B. Quick Overview of the Standard Drug Discovery Process

In principle, the strategy to find a new drug is very simple. One needs to have a set of assays which will detect a change induced by drug candidates, which should be useful for a therapeutic intervention in a disease state. One then finds, or “borrows”, compounds which behave favorably in these assays, and these “chemical leads” are put through an iterative process of design, testing, and redesign, which improves this activity to the point where it is felt to be both useful and competitive. This generates a pattern of activity, which can be correlated with chemical structure, called a structure–activity relationship (SAR). Simultaneously, one may be using similar iterative processes to optimize other properties, such as solubility, actual amount of drug delivered to the target tissue, and selectivity for the chosen target over closely related proteins. When it is felt that a drug candidate satisfies enough of these properties, it is taken into safety and toxicology testing to determine whether it is safe enough to go into humans at a dose where efficacy may be expected. It is then tested in humans in clinical trials, which are conducted in three phases. In Phase I trials, the compound is dosed to healthy volunteers and only three questions are asked: Is it safe at the proposed doses? What are the limiting side effects likely to be? And how much of the drug is absorbed, and how long does it stay in the system? Once these questions are answered, Phase II clinical trials look for some signs of efficacy in a sample of patients with the disease. If there are signs that the compound is active enough, it can then go into Phase III clinical trials, which tend to be very big and expensive and are designed to answer questions such as the following: How well does the drug work? What are its side effects at the proposed efficacy doses? What kind of a dosing schedule is optimal? How does it interact, favorably or unfavorably, with other drugs for the same or related conditions? If the compound has a favorable therapeutic index, i.e., does more good than harm, and does better than currently marketed therapies, it will probably be approved for sale for the tested indications. This is, of course, not a guarantee that anyone will wish to buy it.

The beguiling simplicity of this strategy hides a multitude of problems, for pharmaceutical research is one of the prime examples of “the devil is in the detail”, with an endless detail list to follow. Rather than discuss any of these problems in depth, it can be noted that probably at least 25 000 compounds have to be made in order to get one drug on the market currently and that the amortized costs of finding a drug are now around \$500 million, with a lead time of 7–10 years.

Figures such as these, coupled with the fact that only around one approved drug in five actually breaks even in sales, explain the industrial fascination with patenting. For that one successful drug to lead to a

profitable company, it must rack up at least \$5 billion in total sales. Much of the cost of the drug is not in the manufacture and distribution of the chemical entity, but in the costs that it has accumulated during development. Clearly, if anyone was free to sell the product immediately, it could be sold much more cheaply by manufacturers who had no research to support and the company which did all of the work to develop the compound would go bankrupt. Patents grant a period of exclusive sales to the patent holder, giving an opportunity to charge enough to recoup costs and make a profit as a spur to further innovation. However, a drug can only be patented if it is novel in some way, and the system is set up so that discoveries are secret until the application for a patent is published. Under current rules in the United States, this means that an application disappears for 18 months after it is filed and, at any time in that 18 months, one may learn from somebody else's published application that they had the idea first and will get the patent, and the exclusivity to sell the compound.

Although the compounds have to be novel, the targets do not. Developing a drug for a well understood target brings many advantages because, when pushing an agent forward from such a program, one often has well-benchmarked published assays at every stage of the discovery process, so that one knows the kind of profile to aim for right from the beginning. Once a clinical candidate is developed, one has a good idea that it should work in the clinic, what kind of dosing to use to demonstrate efficacy, and the most likely side effects that one needs to avoid. Clearly, all of these facts reduce the risk (somewhat) in trying to develop the compound.

C. Problems Inherent in Exploiting a Novel Target Class

One of the complaints against the pharmaceutical industry is that most drugs are designed to interfere with a very small range of target proteins. It has been estimated that almost all drugs marketed to date have less than 200 discrete molecular targets between them, and at least one-half of these targets are GPCRs. Such targets as the histamine receptors (for allergy and ulcers), dopamine receptors (for antipsychotics and Parkinsonism), and serotonin receptors (antidepressants and weight loss) bear this out.

This complaint is clearly valid, but in reality before the large scale cloning of genes, there was a very limited selection of discrete molecular targets. Many of the targets interacted with relatively common small biochemicals which had already been isolated, especially hormones. This explains why biogenic amine GPCRs and steroid receptors were rapidly targeted. Others were proteins of known function, which were found in large amounts in the body, and could be isolated in pure form. As there were a limited number of these targets, much of the driving force in industry was to find novel classes of compound which interacted with them.

With the decoding of the human genome, 30 000 distinct proteins (excluding splice variants) will be

found. This will provide a plethora of targets for new mechanism drugs. In fact, the ability to clone genes using PCR-based techniques means that a modest percentage of the human genome has already been deciphered, and several thousand potential novel targets have appeared in the past decade or so. The mere knowledge of a protein's primary sequence is not very useful in itself, as there are many things that must be known before a protein can be useful as a drug target. Some sort of function has to be assigned to the protein. This is often done by assigning it to a protein class by sequence homology with known members of that class. Thus, all kinases, for example, have several highly conserved residues which are absolutely required in the catalytic mechanism or for the maintenance of certain structural features. If these residues are present in the sequence at the appropriate spots, the chances are overwhelming that the protein is a kinase. Both mechanistic function and biochemical pathways that the protein is involved in, must be elucidated, and some sort of guess as to its role in a disease state must be made and then used to validate the target in model systems. Even after one has identified a target and shown that it is behaving abnormally in a pathological state, there are still questions to answer before it can be considered a good drug candidate. The target may play an obvious role in a disease, which turns out to be not causative or functionally redundant, so if the activity is knocked out, some cellular homologue simply takes over the function, leaving the disease course unmodified. Protein function is another important decision factor in choosing a target. Small molecules are good at inhibiting enzymes, turning receptors on and off, and general mimicry of small biological molecules such as cofactors. They are not usually good at changing the location of proteins in the cell, adding function to proteins, or interfering with protein-protein interactions, although exceptions to all of these can be found. Therefore, the thrust is still by and large to target receptors and enzymes.

Once the novel protein is successfully targeted with potential drug candidates, these become very interesting biochemical tools and often allow a great deal of novel science to be done on the target protein, but there is still an enormous amount to be learned before a decision can be made as to whether the mechanistic approach is a viable one. The protein may well be involved in processes other than the targeted activity, which may induce mechanism-associated side effects serious enough to kill the approach. Behavior in a biochemical assay may not correlate with behavior in a cellular assay, and neither may correlate with effects in animal models, leaving several conflicting SARs to follow and a real suspicion that the whole system is not properly understood. Alternately, the human and rodent proteins can turn out to be too different to develop a compound which allows one to optimize the series against both species at the same time. The series may have very poor physical properties, such as low solubility, making it very difficult to formulate the compound or ensure that it is absorbed. Once in the

bloodstream, the compounds may be rapidly excreted or metabolized and may not distribute into the target organ. Once in the clinic, one quite often finds that the human does not use the target in quite the same way as rodents do, and even if both species have the target modulated in the same way, the effects can be different enough to render them unsuitable for human therapy. Once one is out of controlled clinical trials in a heterogeneous group of basically unsupervised patients, one may find all kinds of surprises, from unsuspected abuse potential to deadly drug–drug interactions. The general experience with new targets is that few if any of the surprises inherent in the process are pleasant and that the later in the process the surprises occur the more expensive they are.

III. Protein Kinases

A. Cellular Signaling and Protein Kinases

During the late 1970s it became clear that proteins are not simply translated at the ribosome and then optionally posttranslationally modified by glycosylation but may be reversibly modified in response to many stimuli, both extracellular and intracellular. The first such mechanism found was phosphorylation, whereby a phosphate group is added to an appropriate hydroxyl bearing side chains on serines and threonines. Later, it was found that about 1% of eukaryotic phosphorylation is on the phenolic hydroxyl of tyrosine. The ready availability and detectability of ^{32}P allowed this phenomenon to be explored relatively easily, and it was soon found that a large percentage of the protein in a cell might be phosphorylated and that the degree of phosphorylation of any particular protein might change very dramatically over time. Furthermore, quiescent cells tended to have very low levels of phosphorylation, and once stimulated in any way, there would be rapid and often transient bursts of protein phosphorylation. In particular, mitogen-induced progress through the cell cycle (cellular reproduction) is accompanied by intense, ever-changing patterns of cellular phosphorylation. This suggested that phosphorylation might be involved in intracellular signal transduction, and such has proved to be the case.

Reacting ATP, which contains a somewhat activated linkage between the β - and γ -phosphate groups, and an alcohol or phenol will produce very little phosphate ester, as the reaction is kinetically very slow and also rather improbable in an aqueous medium, where the solvent is a competing nucleophile. Therefore, for the reaction to be useful, it must be catalyzed; enzymes, called protein kinases, were found which transfer the γ -phosphate from ATP to the side-chain hydroxyls of substrate proteins. Similarly, spontaneous hydrolysis of phosphate monoesters is very slow under normal physiological conditions, and phosphatase enzymes are needed to make the hydrolysis reversible on a useful time scale. Thus, protein phosphorylation is reversible and controlled in both directions by enzymes. In mammalian signaling systems there appear to be three distinct classes of kinases, classified by their sub-

strate preferences. The most common are serine–threonine kinases (S/TKs), followed by tyrosine kinases (TKs), with the rarest being both S/TKs and TKs, the so-called dual-function kinases (DFKs). Similarly, phosphatases have been found which are specific for either phosphoserine/threonine or phosphotyrosine, with dual function phosphatases, which cleave both substrate types, being less common.

Although the kinases and phosphatases are divided into broad specificity classes, individual enzymes are usually very specific in which substrates they modify. As much of cellular signaling and the timing of the most important cellular function, cell division, relies upon the phosphorylation and dephosphorylation of very specific residues in single target proteins, the kinases and phosphatases have to be very precisely targeted and their activity precisely regulated. Interestingly, many pathogens have incorporated eukaryotic kinases or phosphatases into their genomes, and these stolen proteins have often been mutated to the point where they have lost their original specificity and are highly indiscriminate. Such enzymes are usually used by the pathogens as toxins, perhaps the most notorious being the deregulated, indiscriminate YopJ tyrosine phosphatase of yersinia, the bubonic plague pathogen, which is one of yersinia's main virulence factors. Such examples certainly lend credence to the notion that specificity in signal transduction agents will be required to avoid toxicities.

B. Could Kinases Make Good Drug Targets?

It is evident that these enzymes, which are an integral part of controlling cellular signaling, would be of intrinsic interest as potential targets for drugs designed to fight diseases where cellular signaling is aberrant. Such a sentiment by itself did not trigger a “kinase rush” by the pharmaceutical industry for several very good reasons. How many of these kinases are there? Although not many kinases were known in the early 1980s, it was evident that those known were only the tip of the iceberg, and current estimates run to around 2000 kinases in the human genome. How closely related to one another are they? Experience suggests that the more structurally similar they are, the harder it will be to find molecules which selectively affect only the kinases one wants to affect. Virtually all other drug projects show that only bad things (non-mechanism-related side effects) happen when one interferes strongly with pathways which are not the ones targeted. What kind of signaling are the kinases involved in? Do these pathways produce rapid changes, like secretion of stored hormones, or long-term changes in gene expression? If kinases are involved in the signaling pathway one is interested in, which kinases are involved? Are there functionally redundant kinases for this signaling pathway? How are the kinases themselves regulated, as it is clear that they cannot be catalytically active all of the time? Do discrete kinases control discrete signaling pathways? What does any particular pathway have to do with any particular disease? Will the same pathway be used for different purposes in tissues other than the target

tissue, leading to potential toxicities? If a kinase-containing signal pathway is blocked, how much will it need to be blocked, 30%, 70%, 95%? And how much of the time, occasionally or almost all of the time? What disease states might be beneficially impacted by inhibiting or activating kinases? Similar questions arise for all drug projects, but all of them are initially unanswered (along with all of the other normal questions of drug development) in the case of novel targets, such as the kinases.

Several things suggested a possible utility for inhibitors of protein kinases and identified some signaling pathways to work on. The initial indication was cancer, which from a pharmaceutical development point of view was both good news and bad news. The good news was that the drugs and treatments that were (and to a large extent still are) available were generally very poor. The treatments work on the theory that the very toxic insults that the patient receives will damage the tumor more than healthy tissues. This is usually a marginal assumption, with the result that most cancer treatments are very unpleasant, often dangerous, and only moderately successful. Thus, if one is to come in with a new treatment modality, the current treatment should be not too difficult to improve upon in cancer. In very well developed areas like hypertension, it would be very difficult to come in with an approach largely made up of unknowns and meet the competition for safety, efficacy, and cost. Part of the bad news was that the overall anticancer market was very small, probably below \$3 billion worldwide at the time, including all palliatives and co-therapies. Even in 1998, the top three antiulcer drugs outsold *all* anticancer treatments and palliatives combined, an incredible statistic when one considers the different mortality tolls of the two conditions. However, when treatments are toxic and of marginal efficacy, patients and physicians tend to use them sparingly. Another part of the bad news is that cancer is not a homogeneous disease like diphtheria but a very wide range of conditions which all lead to an uncontrollably growing mass of cells which will kill one eventually if not checked. Thus, there are good chances that no single overall pathway can be found which would affect all tumors. Last, cancers tend to be both very aggressive and difficult to kill and become more resistant if not treated appropriately at first, something which is difficult to do if one has not yet learned how to do it properly.

C. Kinases and Cancer

One of the most intriguing pointers to the importance of kinases in cancer came from experiments done on tumor promoters. These are compounds which do not themselves induce tumors but which, when given after a carcinogen, greatly increase the number of tumors which are induced. Phorbol esters from *Euphorbia* shrubs were identified as very powerful tumor promoters, and areas of the West Indies where they are consumed (along with croton oil, a known tumor inducer) have extraordinarily high cancer rates, demonstrating a real-life correlation. The mechanism of action of these compounds

was shown to be activation of PKC, an S/TK. Then a natural product, staurosporin, was identified, which very potently inhibited PKC, and in cells this prevented the activity associated with phorbol ester tumor promotion. Thus, there was not only a potential indication, but something of a proof of concept from this system. As we shall see later, there are several other proof of concept experiments required on the drug development pathway, and this was definitely a case of "Two swallows do not make a summer".

From the mid-1970s onward, genes which transform cells from normal cells to tumor cells have been identified, and such genes are called oncogenes and their protein products oncoproteins. The oncogene of the Rous Sarcoma Virus, the classical infectious cancer agent known since the pioneering work of Peyton Rous in the early 1900s, turned out to be v-SRC, a TK. A bigger surprise came when gene sequence comparisons showed that v-SRC is a mutated version of a cellular TK, c-SRC, which is mutated to be permanently active. It was concluded that the virus had picked up the mutated kinase during its evolution, presumably because v-SRCs ability to push the host cell to reproduce its DNA is vital for viral replication. A second viral oncogene, the erbB oncogene, was soon identified as a mutated, intrinsically activated form of another cellular TK, the epidermal growth factor receptor (EGFr). Many tumors were already known to overexpress EGFr, and there are cell lines which undergo the classical cell shape changes (morphology) of transformed cells upon treatment with epidermal growth factor (EGF) the EGFr ligand, which is also a potent mitogen. A third line of evidence came from a relatively rare leukemia, chronic myelogenous leukemia (CML). Around 90% of CML patients show a chromosomal abnormality, the "Philadelphia chromosome", in their leukemia cells. This is a translocation, whereby during replication parts of chromosome 8 and 22 are interchanged, leading to two hybrid chromosomes. Examining the Philadelphia chromosome at the translocation point shows that a fusion protein is now coded for, which has at its N-terminus either 426 or over 900 amino acids of an atypical S/TK, BCR, fused to all but the four N-terminal amino acids of a TK c-ABL to give fusion proteins of 190 and 220 kD, respectively. This in itself is very interesting as c-ABL had been identified as the eukaryotic precursor of v-ABL, the oncoprotein of the Abelson leukemia virus. The BCR-ABL fusion proteins, just like the v-ABL protein, are constitutively active, completely unregulated TKs, and the degree of transforming ability, v-ABL > 220 kD BCR-ABL > 190 kD BCR-ABL, exactly tracks their activity as TKs. The first unequivocally successful kinase inhibitor in the clinic is targeted against BCR-ABL and will be discussed later in considerable detail as a great deal can be learned from it, both about cancer and the requirements for drug candidates in this arena.

Other factors pointed toward kinase activity being very important in cancer. Transformed cells contain a lot more phosphoprotein than normal cells, and this imbalance is much more marked for phosphotyrosine

than for phosphoserine or phosphothreonine. To reproduce, cells have to go through the cell cycle. A nonreplicating cell (said to be in G_0) has no machinery to replicate DNA or separate its chromosomes, and once the decision is made to replicate, it first expresses genes which produce the proteins which allow DNA to be duplicated, which is done in the G_1 phase of the cell cycle. (G stands for gap because it was originally thought cells were doing nothing at the gap times.) Then the DNA is reproduced during the S (synthesis) phase. In the G_2 phase, the S-phase proteins are demolished and the machinery to separate the chromosomes is built. Then in the final phase, the M-phase (mitosis), the chromosomes are separated and packed into two different nuclei, the cells are physically separated, the M-phase machinery is demolished, and the cells either stop reproducing (G_0) or start another cell cycle (G_1). Each of these phases of the cell cycle is marked by very heavy and distinctive bursts of phosphorylation, with a very highly regulated family of kinases, the so-called cyclin-dependent kinases (CDKs), playing a primary role.

Not surprisingly, the above-mentioned facts created great interest in pharmaceutical and academic circles about the possible use of kinase inhibitors in cancer therapy. This explosion in functional knowledge came at about the same time as a new generation of carefully designed cytotoxic drugs went into the clinic and proved to be only a marginal advance. Thus, there was some disillusionment with the old approach, just as the new science suggested novel targets and approaches.

D. Cytotoxicity, Cytostasis, Combinations, and Conundrums

One obvious advantage of a successful cytotoxic approach to cancer is that the tumor cells are fatally poisoned and that the tumor is cured. It is difficult to see why targeting a growth-inducing protein in a tumor cell should kill it. If a kinase helps tumors to proliferate, blocking the kinase should prevent proliferation, but the tumor may just be sitting there in (cyto)stasis, not growing but not dying. This is a somewhat mixed blessing as the patient will probably not deteriorate while the tumor is not growing but the tumor may well regrow if treatment is discontinued or if it mutates to circumvent the block. Tamoxifen, an antagonist of the estrogen receptor, appears to be a cytostatic signal transduction inhibitor. In estrogen-dependent breast cancers, estrogen is a mitogen and Tamoxifen blocks that mitogenicity. Five years of Tamoxifen treatment leads to a 5-year increase in time before relapse, but 10 years of treatment only leads to about 6 years of time before relapse, suggesting that cytostatic blocks can be evaded even by apparently quiescent tumors. If this is generally true, it suggests that cytostatic agents could be a part of the anticancer picture but not the whole picture.

A very attractive scenario is to combine the cytotoxic and cytostatic approaches. Cytotoxic agents generally run out of efficacy because one needs to use them at high rates, which sooner or later become

unsustainable, shutting down a vital system in the patient through cumulative toxicity. If time could be bought between cytotoxic treatments, when the tumor was not growing, or if blocking certain signaling pathways made the tumor more vulnerable to cytotoxic insults, then a combination approach might work very successfully. At the very least, the cytostatic approach might add a fixed period of time to the life-prolonging effects of the cytotoxic, and one can easily envision mechanisms whereby the two treatments would have synergistic effects, perhaps even increasing the overall cure rate as well as slowing disease progression.

This scenario has attracted me and many others in the pharmaceutical industry and on the face of it appears to be a "no-brainer". However, it actually contains several traps for the unwary. Apoptosis does not just occur. For a cell's death-inducing machinery to be primed, it must enter a cell cycle. The machinery is then triggered if irreparable DNA damage is noted or if certain signaling pathways are working in a "discordant" fashion. This is exploited by most cytotoxics, which are DNA-damaging agents or inhibitors of certain cell cycle machinery. In rapidly dividing cells, this triggers apoptosis if the damage cannot be repaired before the next cell cycle (when the damage would be duplicated), whereas quiescent cells simply repair the lesions. This explains why rapidly replaced cells such as white blood cells and intestinal lining tend to show limiting toxicity and why certain very slowly dividing tumors such as prostate tumors are essentially completely resistant to most cytotoxics. Thus, if a cell cycle is required for apoptosis and a signaling inhibitor can suppress cell cycling, then the inhibitor could well protect tumor cells from the cytotoxic agent. Such functional antagonism is observed in cell culture, but sometimes the same combinations of agents given at different times can show additive or synergistic activity. This suggests several operational principles. The first is that not all combinations will be of equal utility; some may be always contraindicated, either because they are inherently antagonistic in mechanism or because they have synergistic toxicities. A second is that different tumors may respond in different ways to the same combination. The third and potentially most difficult is that the effects of any given combination on any particular tumor may be schedule dependent. One can imagine that a signaling inhibitor taken before a cytotoxic could protect the cells by closing down cell cycling, but taken after the cytotoxic, once the next cycle is under way, it may prevent the cells from calling upon certain anti-apoptotic mechanisms and thereby increase the damage done by the cytotoxic. Unlike most therapies (take one pill a day until better), cancer treatments already involve very sophisticated and often optimized dosing schedules. Where signal transduction inhibitors would best fit into such schedules and how the schedules should be otherwise modified is in itself a very difficult problem.

This brings one back to one of the fundamental problems with any new mode of therapy: how does one actually use novel preclinical agents in a clinical

setting? Trying to develop a novel agent by itself can be difficult enough. As discussed previously, the safety of the agent is tested in healthy volunteers in Phase I trials to establish exposure levels and a safety dose for later trials. Most anticancer agents are themselves very toxic and carcinogenic, so Phase I clinical trials cannot be done in healthy volunteers but are done in terminal cancer patients, who have failed all conventional therapy and have very little left to lose. These patients have very aggressive, highly resistant tumors which are extremely difficult to treat at all. Thus, one must start with an agent that one does not know how to use and learn on the patient population in which it is least likely to work. On top of that, these Phase I trials must give some hint of activity for the drug as a *single agent*, a mode we do not expect to use much. Needless to say, cancer drugs tend to go into more tractable Phase II patients on rather small hints of activity if the preclinical data looks solid, but often the Phase I trials prove more predictive than the preclinical science.

Given all of these difficulties, why is there such a strong push to get novel agents such as kinase inhibitors into clinical trials? In the opinion of the author, there are several reasons. The first is that most people involved have seen someone close to them die very prematurely from cancer, so the unmet medical need is very self-evident. Second, it is very hard not to be carried along by the explosion of knowledge and the belief that difficulties will be avoided by those smart enough to "ride the knowledge wave". Third, the small current size of the cancer market offers an incredible opportunity. "If you build it, they will come" and a series of efficacious, relatively nontoxic anticancer drugs could easily turn this into the largest single market for drugs. Last and maybe most dangerously, the scientific rationale appears to be very strong and we now have agents which preclinically behave exactly as predicted, and it is very difficult for those of us closely involved not to believe that some will work as designed (preferably ours).

E. Choice of Kinase Targets for Cancer Therapy

Having been carried away on a tide of novel science and optimism, there are the not entirely trivial matters of which actual kinases to inhibit and how to judge whether one has succeeded in a clinically meaningful fashion. With around 2000 potential targets, this choice is not trivial but ignorance is a great help in this matter at present. If the kinase or its function is completely unknown, or there is no way of proving that it has been inhibited, or it is not known to be of any significance in cancer, it can be discarded. This still gets rid of 95% of the possibilities today and a decade ago got rid of about 99%. With the ignorance filter so satisfactorily reducing target numbers, one can use a knowledge filter to decide between the remaining few.

There is no doubt that kinases carry out vital functions throughout the cell cycle and carry out many functions other than straight proliferative ones, but the only parts of proliferation where we know enough to identify potential kinase targets at present

are in the early parts of the cell cycle, G_1 , although the CDKs are obviously potential targets throughout the cell cycle. Therefore, almost all cancer kinase inhibitor programs are targeted at the G_0 to G_1 transition or the CDKs.

What pushes a cell into the cell cycle? Normal cells cannot spontaneously enter the cell cycle as they are only allowed to replicate if more of that cell lineage is required when cell surface receptors pick up proliferative signals from other cells. Many hormones can induce replication, but the most important are the growth factor hormones. These are polypeptides which bind to the extracellular domains of large transmembrane receptor molecules which contain a tyrosine kinase moiety in their intracellular portion, of which around 100 have been identified. These receptor tyrosine kinases (RTKs) are not active when the ligand is not bound, but when it is bound, they dimerize and become active. The appearance of tyrosine-phosphorylated proteins near the membrane leads to a flurry of biochemical activity in the cell with a large number of signaling pathways turned on. Adding RTK inhibitors prevents all of this activation and cellular proliferation as well. We have already come across one of these RTKs, EGFr, as being the source of the v-erbB oncoprotein, suggesting a link with cancer, and EGFr inhibitors will be discussed later. Several other RTKs and/or their cognate ligands have also been implicated in lesser but still significant percentages of tumors, such as the platelet-derived growth factor receptor (PDGFr), the fibroblast growth factor receptor (FGFr), the hepatic growth factor receptor (HGFr), and the insulin-like growth factor receptor (IGFr). The vascular endothelial growth factor receptors (VEGFr) are not found in tumors but appear to be vital for the tumors to develop their own blood vessels. As tumors cannot grow to a dangerous size without vascularization, these are also attractive targets.

Once the tyrosine phosphorylation signal has been established, it passes through several signal mediators which are not kinases, including the RAS switching proteins, and then reappears in the activation of the catalytic activity of at least two classes of kinases. The first is the PKCs, which were mentioned earlier. These kinases tend to be activated by various lipid derivatives, which in turn are produced by enzymes activated by tyrosine phosphorylation and will be discussed in detail below.

The second class of mitogenic kinase activated involves a cascade of kinases, downstream of the RAS protein. RAS binds to and leads to the activation of a very complex S/TK, RAF. RAF in turn when activated, either by autophosphorylation or another activated kinase, phosphorylates MEK, a dual-function kinase. This in turn phosphorylates an S/TK ERK on both threonine and tyrosine, which then phosphorylates a large variety of targets both in the nucleus and in the cytoplasm, and these phosphorylations seem to trigger gene expression leading to cell cycle progression. This cascade is known as the RAS-ERK MAP kinase cascade and is another obvious point to go after inhibitors for antiproliferative therapies. Two other MAP kinase cascades appear to

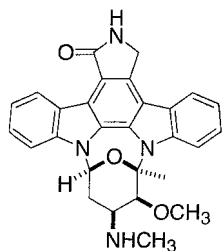


Figure 1. Staurosporin, 1.

be very important: the p38 MAP kinase cascade in immune cell signaling and the JNK MAP kinase cascade in some immune signaling and in initiating apoptosis. p38 inhibitors are being developed primarily as antiinflammatory compounds, and the JNK cascade inhibitors revealed are claimed to be neuroprotective.

IV. Kinase Inhibitors

A. PKC Inhibitors

As described earlier, PKC was among the earliest kinases identified³⁷ and a clear cancer indication came from its identification as a target for tumor promoters. Then in 1986 staurosporin **1** (Figure 1) was identified as a very potent low-nanomolar inhibitor of PKC.³⁸ At this point there was an enormous surge of interest in PKC and many labs, both academic and industrial, started to look for PKC inhibitors and staurosporin analogues. This fast start could not be rapidly followed up, and even today there are only a few PKC inhibitors in clinical trials³⁹ and few recent reviews.^{40–42} As some of the reasons for this are tied in with the problems of being on the leading edge in new drug development, it is worth examining them in some detail.

Four basic factors seem to have turned PKC inhibition from a fast breaking project into a very slow process. All four of them are inherent in working on systems which are only slightly characterized and hence liable to produce unwanted surprises. The first was the nature of PKC itself. As the protein was better characterized, it turned out to be not one kinase but at least 12 isoforms which fall into three distinct subfamilies, the conventional or c-PKCs, activated by diacylglycerol and calcium, the novel or n-PKCs, which do not require calcium, and the atypical or a-PKCs, which do not require calcium or diacylglycerol to activate them.^{43,44} There are also some kinases very closely related to the PKCs but not generally included in the family.⁴⁵ Most of the early data obtained on inhibitors was gained on at best poorly characterized mixtures of PKC isoforms and is thus of somewhat dubious meaning and reproducibility. Just to isolate and assay the isoforms is in itself a major undertaking. As of 1997, even when assays were being run on the different isoforms, no group had taken a look at the substrate or inhibitor selectivities for the whole family under a standard set of assay conditions.⁴⁶ Looking at inhibitor profiles against all of the PKCs shows that there is a tendency for some isoforms to produce very similar inhibition, meaning that separating the SARs

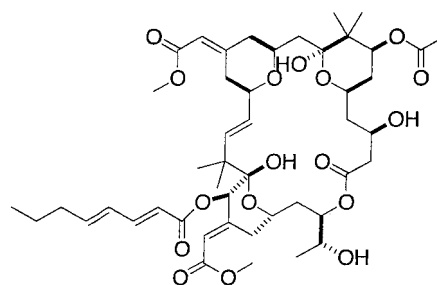


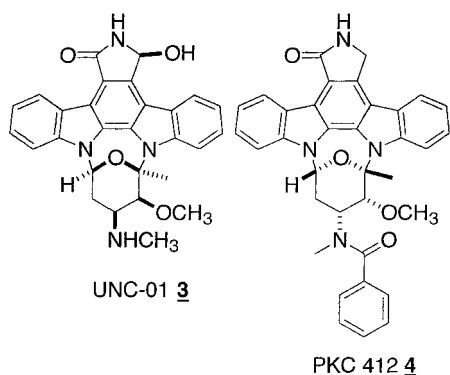
Figure 2. Bryostatin 1, 2.

for these isoforms will be very difficult, whereas others tend to have quite different potencies, meaning that results gained on mixtures can be very deceptive.

The second problem is working out what was actually done in any given experiment in cells. Most cell types seem to express a mixture of several of the isoforms, and this varies widely from tissue to tissue.⁴⁷ It is very rare to find tissues which express only one isoform or where only one isoform seems to be heavily activated in a disease state. PKCs tend to be activated in part by lipids,^{43,48} the c-PKCs by diacylglycerols, at least one of the a-PKCs by PIP₃ (phosphatidyl-3,4,5-triphosphate a very uncommon phospholipid).⁴⁹ All these lipids bind to PKC, making it more lipophilic and recruiting it to the membrane where it can be activated.⁵⁰ Bryostatin **1, 2** (Figure 2),⁵¹ is a very potent activator of the c-PKCs, recruiting them to the membrane. However, it appears that most PKC targets are not located at the cell membrane and that PKCs are only transiently at the membrane to be activated. Bryostatin binds the PKCs strongly to the plasma membrane where they are strongly inhibited from carrying out many of their normal functions and become more susceptible to ubiquitin-induced degradation.⁵² Thus, Bryostatin is functionally an inhibitor of PKC signaling in cells, although it appears to be an activator of the enzyme, and it is in clinical trials as an anticancer agent at present, working as a PKC inhibitor.

There are subtler effects that the reliance on unnatural activators can have on the interpretation of PKC signaling. The activators such as phorbol myristoyl acetate mimic lipids, which are generally short-lived, but exogenous activators tend to be very long-lived, and a long duration signal may be very differently interpreted in the cell from a transient one. The second is that long-term stimulation of PKCs often leads to drastic down regulation of the proteins, both by suppression of gene expression and by enhanced proteolysis.⁵³ Many of the early effects attributed to activation of PKCs may in fact have been largely due to inhibition via this down regulation. To complicate matters further, there is some evidence that even when equally stimulated, different isoforms may be down regulated very differently.

The third problem was in working out which isoform is responsible for a given effect. The isoforms seem to have largely but not completely overlapping substrates *in vivo* and to be vital modulators of pathways rather than generally direct transducers, meaning that it is difficult to assign roles to individual isoforms with current cell biology techniques.

**Figure 3.**

As there are not a series of isoform-selective inhibitors and substrate specificities are too hazily understood, the pharmacological approach has not clarified matters as much as in many other cases.⁵⁴

Despite these problems, some general principles of isoform function do seem to be emerging. PKC α seems to be involved with proliferation, and it is usually thought that it should be inhibited in anti-cancer strategies.⁵⁵ In contrast, PKC δ appears to have antiproliferative effects and is thought to be a target to avoid in anticancer strategies,⁵⁶ and given its role in the suppression of AKT⁵⁷ (perhaps the most important anti-apoptotic kinase discovered to date) and the mitogenic RAF kinase,⁵⁸ the same seems likely to be true of PKC ζ . As PKC ϵ appears to suppress apoptosis when activated and may in fact be an oncogene⁵⁹ and PKC ι protects leukemia cells from drug-induced apoptosis,⁶⁰ both isoforms may be good cancer targets. One disease where the involvement, if not the actual role, of a PKC isoform may be clear is diabetic vascular complications where PKC β 1 has been shown to be strongly activated in the kidney and retina⁶¹ with no evidence of any other isoform being involved, suggesting that other disease states may be found where a particular PKC isoform is implicated in a particular tissue. Given these trends and if, in addition, one of the current class of PKC inhibitors shows real clinical utility, we may well see an upsurge in interest in PKC inhibitors over the next few years.

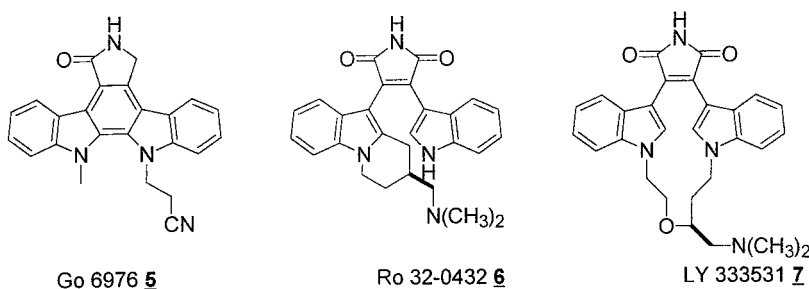
The fourth problem was in finding selective PKC inhibitors. The indolylcarbazole staurosporin **1** is an excellent start for an enzyme inhibitor, as it is extraordinarily potent for a screening lead with an IC₅₀ value for PKC α of 2.5 nM. However, it inhibits at least four of the other isoforms with IC₅₀s below 10 nM, and worse yet, the basic staurosporin scaffold is very promiscuous, inhibiting most of the kinases

examined to date with good potency. This means that a lot of work has to be done to get rid of unwanted kinase activity and to demonstrate that it has been done. However, its very close analogues UNC-01 **3**⁶² and CGP41251 (PKC 412) **4** (Figure 3)⁶³ are in the clinic for solid tumors and leukemia/lymphoma, respectively.

The extensive work done on the staurosporin nucleus has improved selectivities quite dramatically. For example, replacement of the bicyclic saccharide ring of staurosporin with much simpler side chains, typified by Go 6976, **5** (Chart 1),^{64,65} weakened many non-PKC inhibitory activities into the micromolar range while having little effect on PKC activity. Removal of the bond between the two indoles to give the bis-indolylmaleimides such as Ro 32-0432, **6**,⁶⁶ led to considerably increased selectivity for PKCs over many of the other kinases. Putting a mimic of the sugar back onto a bis-indolylmaleimide to give bis-seco-staurosporin analogues led to LY 333531, **7**, which is very selective for the β -isoforms over other PKCs and over other kinases.⁶⁷ This compound is in the clinic for diabetic complications, especially retinopathy and nephropathy. There are several other families of PKC inhibitors, but all seem to suffer from the problems of the indolylcarbazole type, and none appear likely to produce serious drug candidates at present.⁶⁸

B. BCR-ABL Inhibitors

As discussed previously, chronic myelogenous leukemia, CML, is a cancer, apparently completely caused by a single genetic defect, probably the only cancer known where this is true. The genetic defect, as discussed earlier, produces a fusion protein between the BCR S/TK and the ABL TK. There are several reasons why this appears to be enough to cause full transformation of the pre-B cells in which this leukemia arises. As mentioned earlier, several mutations are generally required to transform cells, because there are many levels of control of cellular behavior. Earlier, angiogenesis was mentioned as being a necessity for solid tumors, but as leukemias occur in the circulatory system, they have no need of angiogenesis. Another requirement for most cells is that they only grow when attached to appropriate substratum, and often if they do not have the appropriate adhesion receptors activated, they will undergo apoptosis. Clearly, freely circulating cells do not have such restraints to begin with. Thus, hematopoietic tumors already avoid two of the major requirements for transformation. What they still

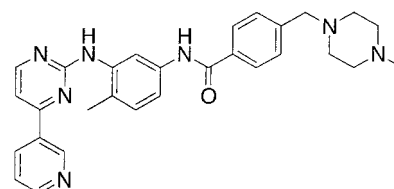
Chart 1

appear to need to do is to have their growth deregulated and their apoptotic mechanisms suppressed, and the BCR-ABL oncoprotein appears to do both. The ABL protein appears to normally be largely nuclear in localization, containing both a DNA-binding domain and multiple nuclear localization signals (highly basic sequences which bind to nuclear import chaperone proteins, leading to the protein being actively imported into the nucleus), as well as a nuclear export sequence and an actin-binding domain, which are associated with cytoplasmic activity.⁶⁹ In the nucleus, c-ABL is a negative regulator of cell growth⁷⁰ and is associated with several of the best known tumor-suppressor genes. It is activated in a cell-cycle-dependent manner by binding to the RB (retinoblastoma) protein, which sequesters it until the end of G₁,⁷¹ and it increases the activity of the p53 (Li-Fraumeni syndrome) growth inhibitory and pro-apoptotic protein^{72,73} as well as being required for the pro-apoptotic effects of the p53 homologue p73.^{74,75} It is activated by DNA-damaging events by activation of the ATM (ataxia-telangiectasia mutant) S/TK⁷⁶ and helps activate the pro-apoptotic JNK MAP kinase cascade.⁶⁹

In contrast, the BCR-ABL protein appears to be wholly a cytoplasmic enzyme,⁷⁷ so in CML cells, the inhibitory nuclear activity of ABL is largely lost. In addition, because of a "coiled-coil" oligomerization domain in BCR, the BCR-ABL oncoprotein is a tetrameric, intrinsically active TK⁷⁸ and has also lost much of its normal substrate specificity, becoming a rather indiscriminate kinase.⁷⁹ This new kinase appears to activate the RAS-ERK proliferative signaling pathway through several adapter proteins; GRB-2 via interaction with the Y¹⁷⁷ BCR site, now autophosphorylated by the ABL TK moiety,⁸⁰ and ABL-phosphorylated SHC, via interaction with the ABL SH2 domain,⁸¹ in effect inserting itself in place of mitogenic RTKs and some of their immediate effector proteins.⁸² It also leads to activation of the oncogenic transcription factor c-Myc via RAS and RAF but not ERK.⁸³ Several antiapoptotic pathways including PI3K-AKT⁸⁴ and BCL-X_L⁸⁵ are also activated by BCR-ABL.

Thus, this one genetic lesion may well overcome both of the barriers to transformation in these cells, and one can make a plausible case that this is enough to cause transformation. Therefore, inhibition of the kinase activity of BCR-ABL^{86,87} should at the very least slow proliferation of these cells and quite conceivably could be enough to reverse transformation, although as nuclear ABL signaling would not be restored, it could not completely repair the damage.

The one serious effort to make BCR-ABL inhibitors has been carried out by the Novartis group and culminated in the synthesis of CPG 57148B (now called STI 571), **8** (Figure 4). This compound was developed out of an SAR, which was aimed at platelet-derived growth factor receptor (PDGFr) TKs inhibitors, by counterscreening against the oncogenic v-ABL TK.⁸⁸ The compound has an IC₅₀ of 25 nM against both ABL and BCR-ABL and 50 nM against PDGFr but no activity against the rest of the panel

CPG 57148/ STI571 **8****Figure 4.**

of kinases tested,⁸⁹ although activity against the c-Kit RTK has been subsequently demonstrated.^{90,91} Recently a crystal structure of a very close analogue of STI-571 bound to the ABL tyrosine kinase has been published revealing the mode of inhibition of the compound.⁹² Interestingly, the inhibitor binds to an inactivated form of the kinase, stabilizing that form and preventing activation. As the activation is achieved by phosphorylation of the kinase and there are many phosphatases around in the cell, the overall activity probably comes in part from active BCR-ABL being dephosphorylated and then trapped in the inactive form, a mechanism which has previously been revealed for the MEK inhibitor PD 0098059.⁹³ In cells, ABL was inhibited with IC₅₀s in the 100–500 nM range^{94,95} and both v-ABL and a PDGF-transformed cell line were prevented from growing in vivo in nude mice.⁹⁶ Leukemic cells appear to be killed by this agent by suppression of a pathway known to involve activation of the anti-apoptotic oncogene Bcl_{XL} via tyrosine phosphorylation.⁹⁷

This compound went into CML patients in 1998^{98–100} and showed quite extraordinary efficacy, with all patients not in blast crisis responding at the 300 mg/day dose. No patients have been on the drug for 2 years yet and there are relapses,¹⁰¹ but for many patients it does appear that the next blast crisis has been postponed. Even for those patients where no leukemic cells can be detected, PCR can still detect the BCR-ABL transcript, so it appears that, as expected for a cytostatic approach, the malignancy has not been completely eradicated. The lack of toxicity, especially considering the PDGFr and c-Kit activity, was rather unexpected, and plans are now being made to look at glioblastomas, where PDGFr signaling is often implicated, and small cell lung cancer, where c-Kit is implicated.^{102,103}

Events are now moving very fast in this area, and several interesting discoveries have been made since this review was first submitted. In the examination of mechanisms of resistance, all mice with large KU812 tumors (BCR-ABL expressing human leukemia) initially responded to and then failed upon prolonged treatment with STI-571 whereas mice with small tumors did not show resistance. Upon examination of the tumors, the cells were as susceptible to STI-571 as before treatment but the resistant animals showed greatly elevated plasma levels of α 1 acid glycoprotein (AGP), a protein that binds strongly to STI-571, effectively sequestering it in the plasma. When another AGP binder, erythromycin, was dosed simultaneously to resistant animals, the efficacy of STI-571 was largely restored (10/12 animals survived 180 days as opposed to 1/13 without erythromycin).

If (and it is a big if) this mechanism proves to be the same as in humans, it suggests that similar cotherapy or working out how to block the ASP induction could greatly improve STI-571 therapy.

In what can only be described as a dazzling intellectual *tour de force*, Vigneri and Wang^{104,105} showed how a deep understanding of the biology of BCR-ABL can lead to the exploitation of the abilities of STI-571 in a manner never originally conceived of, but which greatly increases its potential utility. The authors examined what drives the cytoplasmic localization of BCR-ABL. Leptomycin B (LMB), an antiproliferative agent which was dropped from Phase I trials due to (reversible) toxicity,¹⁰⁶ inhibits CRM1, the transporter responsible for nuclear export of NLS-containing proteins.¹⁰⁷ Treatment of BCR-ABL transfected cells with LMB, or use of BCR-ABL with an inactivating mutation in the NLS still led to no nuclear localization, proving that the localization defect in the protein lay in the inability of BCR-ABL to be imported into the nucleus. Further studies implicate both the ABL kinase activity and some segments of BCR in this localization; a kinase defective BCR-ABL mutant was also cytoplasmic, but upon treatment with LMB, about 35% of it ended up in the nucleus, close to the fraction normally seen for c-ABL. The authors then examined the effect of STI-571 on the NLS-inactivated BCR-ABL mutant and discovered that after 24 h there was a dose-dependent accumulation of the mutated BCR-ABL in the nucleus, peaking again at ~35% of the total. Cotherapy of the cells with STI-571 and LMB led to exactly the same results. Thus, kinase inhibition had led to nuclear localization, and continuing the LMB treatment while washing the STI-571 out of the system allowed the authors to examine what happens when there is both active BCR-ABL in the nucleus and in the cytoplasm. Most gratifyingly in both the defective NES and the LMB case, the presence of constitutive ABL activity in the nucleus proved to be strongly apoptogenic, overriding the anti-apoptotic effects of the cytoplasmic BCR-ABL. In the classic K562 CML blast tumor line, this regimen led to complete loss of viability by the end of 9 days, and in leukemic mouse bone marrow, the combination killed about 75% of normal cells but 97% of the BCR-ABL positive cells. The authors suggest that such results could be used in autologous transplantation, whereby patient bone marrow is removed, stripped of BCR-ABL positive cells by STI-571/LMB treatment, and then transplanted back into the patient after total marrow ablation, offering a possible cure of the disease.

These results are very encouraging for the whole field of kinase inhibition and cancer. STI-571 does not have exceptional potency or selectivity by the standards of many of the inhibitors reported on, and there is little to suggest that it has unusually favorable physical/pharmacokinetic properties. Nevertheless, its developers had the wisdom to pick the purest possible test case for the mechanism, where there is a definite medical need but a market size of only 4500 patients a year in the United States. It took real courage to pitch that program to management

and for management to pay for the development, knowing the market size, and we can only hope that the drug fulfils its early clinical promise. Others have now demonstrated that potential utility of kinase inhibitors may go far beyond simply slowing proliferation, provided the understanding of the system is great enough and the imagination of the investigators can match the understanding.

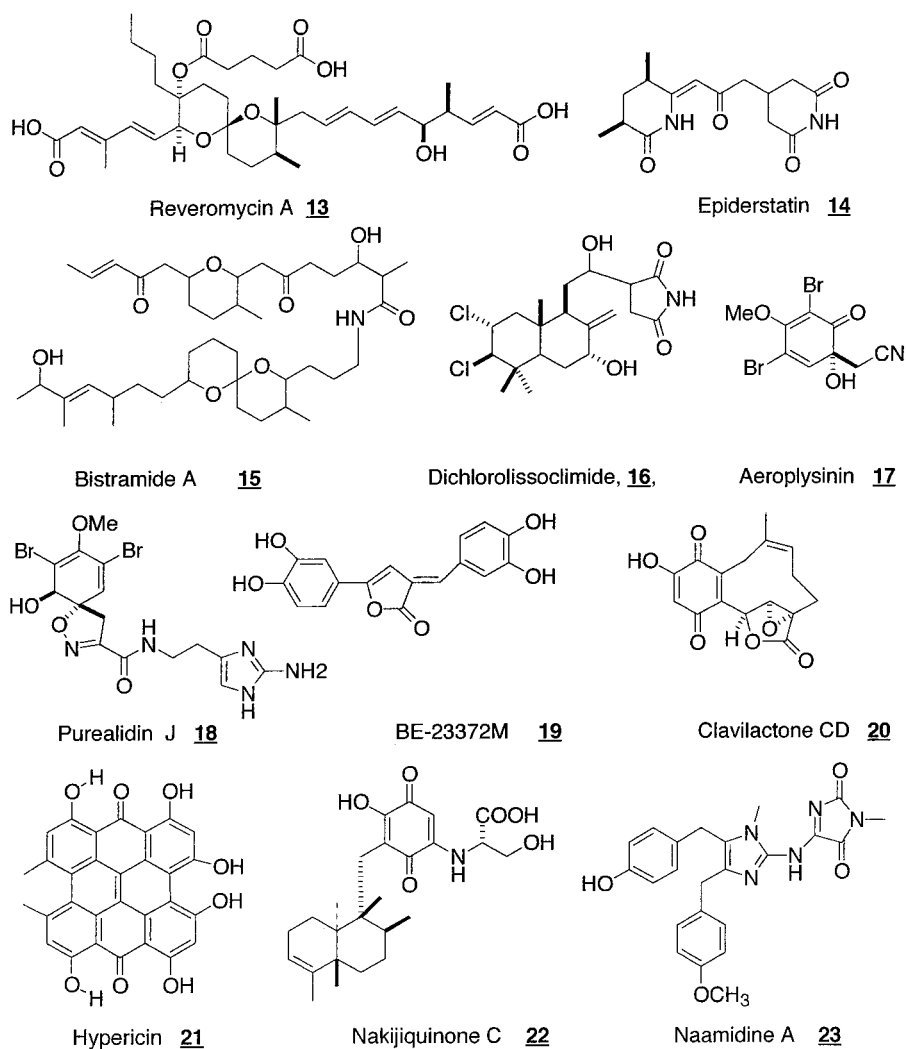
V. Epidermal Growth Factor Receptor TK Inhibitors

A. Introduction

The epidermal growth factor receptor (EGFr or ErbB-1) was identified as a protooncogenic TK by the mid-1980s, making it the first of the TKs to be examined seriously as a drug target. In this case, the science was relatively straightforward from the beginning, despite the family growing to a modest four members, with erbB-2, erbB-3, and erbB4 (HER2-4) being the other members. A close examination of clinical tumors suggests that these receptors and their ligands are associated with a large percentage of all solid tumors, as dysregulation of the system is found in an incredible 60% of them.¹⁰⁸ This dysregulation can occur in several different ways, but all have the effect of leading to an inappropriately strong tyrosine phosphorylation signal passing into the cell. In cellular systems, EGF or other ErbB family ligands often induce robust and sometimes uncontrolled cellular proliferation and can cause cells to change from a normal morphology to typically transformed morphologies.¹⁰⁹ Transfection of excess receptor into cells will also often transform them, and transfection of an intrinsically activated ErbB is usually highly transforming. Ligands dimerize the receptors, activating the tyrosine kinases, and different ligands can lead to homodimerization, as when two EGFr receptors dimerize, or heterodimerization, as when ErbB-3 dimerizes with ErbB-4.¹¹⁰ Therefore, this area was naturally one of the most attractive for the design of kinase inhibitors, and progress has been reviewed extensively.¹¹¹⁻¹¹⁵

B. Early Natural Product Leads

The initial discovery phase of novel EGFr kinase inhibitors (for the ErbBs had not been discovered at the time) did not get off to a good start. The assays used initially to find EGFr inhibitors came up with many hits, some of which do very interesting things biologically but few of which were actually good inhibitors of the target enzyme. Early on, many staurosporins were shown to be very potent EGFr inhibitors, but at the same time they were often being found to inhibit virtually every other kinase that they were assayed against and hence were of little interest for finding EGFr-selective inhibitors and then working out what their true utility is. Cellular assays came up with potent inhibitors, but these tended to have traps for the unwary. Some of the very early hits were natural product inhibitors of the ansamycin class, such as Geldanamycin **9** and Herbimycin A **10** (Figure 5).¹¹⁶ These compounds showed very potent

Chart 3. Various Natural Product Inhibitors of EGFr/ErbB-2

competitive with ATP nor with substrate. This compound also showed 5 μM EGFr potency, some selectivity against S/TKs, and weak in vivo activity against A431 xenografts, but it had an IC_{50} for A431 growth inhibition of 100 nM, strongly suggesting that the observed antitumor activity is, at best, not mediated by EGFr activity alone. Greater selectivity for EGFr over other kinases was reported for Hypericin, **21**,¹³⁷ which has an IC_{50} for EGFr of 370 nM. However, in this case the inhibition was irreversible, and in the presence of light, the IC_{50} improved to 44 nM. Nakijiquinone C, **22**,¹³⁸ is reported as a modestly potent erbB-2 inhibitor, with some selectivity over EGFr inhibition. Once again, its potency in a cellular assay of cytotoxicity is considerably greater than its erbB-2 potency. Another natural product Naamidine A, **23**,¹³⁹ is reported to be an EGFr inhibitor, with good in vivo potency against A431 xenografts (85% inhibition of growth at 25 mg/kg/day). However, in this case the evidence for EGFr inhibition is a cellular mitogenicity assay, along with the statements that **23** does not inhibit EGF binding to EGFr or the kinase activity of c-SRC.

The most obvious thing that the inhibitors discussed immediately above tend to have in common is quite interesting structures and the ability to put both nice chemistry and a therapeutic justification

into an NIH grant. When one starts to analyze these compounds from a drug company point of view, their major features are all unattractive. Few of the compounds appear to be easy to make analogues of, to tighten the SAR. Many of these compounds look as though they are chemically reactive in their own rights or contain groups which are known to be readily metabolized to known reactive, toxic groups. The dominance of quinones or would-be quinones in these compounds would not go unnoticed at any stage in drug development and makes it very difficult to justify programs if these are the only kinds of leads. Another disturbing point is how infrequently an inhibitor is described in a fashion, which is consistent with the described mechanism. When cellular potency is much greater than isolated enzyme potency, the most likely explanations are that the major effect is through another mechanism or through another compound produced by metabolism. Small molecules tend to bind proteins at rates which are close to diffusion controlled, so a prerequisite for 18 h incubations tends to suggest that something else is going on. For some of the inhibitors described above, even the sequence of experiments "proving" the mechanism was not done coherently, again something which does not tend to make one eager to commit resources to follow up on allegedly beguiling results.

Chart 4

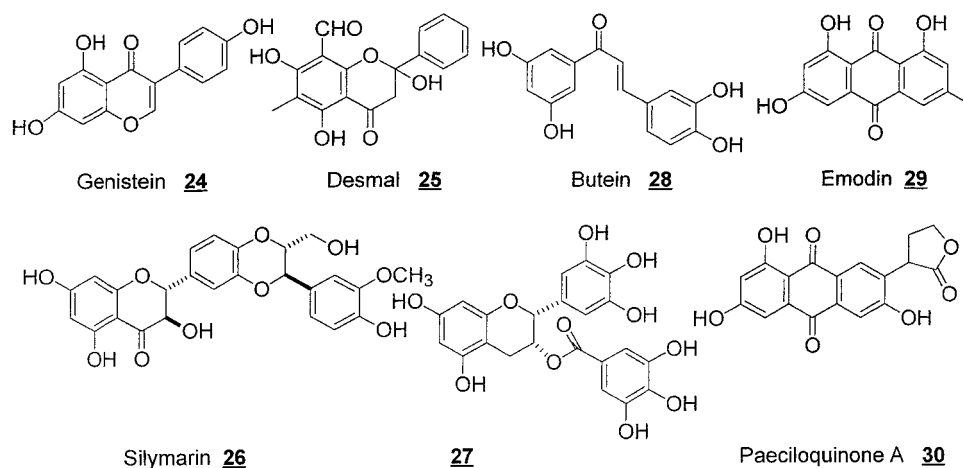
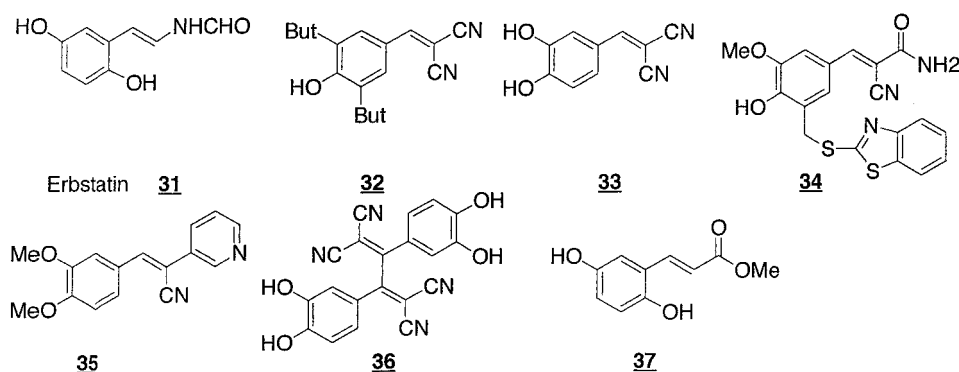


Chart 5



Some of the other natural product hits looked simpler to work on from a chemical point of view but did not offer much else in the way of improvement. Probably the earliest widely used EGFr inhibitor (and still sometimes used today) was the isoflavone genistein, **24** (Chart 4).¹⁴⁰ Genistein inhibits EGFr in the submicromolar range and does so by competing at the ATP-binding site of the enzyme. As described previously, this is one of the most highly conserved domains of the entire kinase, and it came as little surprise that Genistein inhibits many of the kinases it has been tested against subsequently. Recently, **24** has turned out to be a very interesting, selective 50 nM ligand for the estrogen receptor β , so any mechanistic conclusions from breast or ovarian cells are even more questionable than previously thought. Many flavanoids have shown up as EGFr inhibitors, and often the changing substitution patterns can affect selectivity toward different kinases, suggesting that ATP-site competition may not be unusable in designing selective inhibitors.¹⁴¹ Assumptions are often wrong however, and the flavone desmal, **25**,¹⁴² was shown to be a substrate-competitive, not an ATP-competitive, micromolar inhibitor of EGFr. Both the antioxidant flavanoids Silymarin, **26**,¹⁴³ and Epigallocatechin gallate, **27**,¹⁴⁴ are EGFr inhibitors (among many other activities), and both are suggested to be inhibiting the kinase by competition with EGF for binding to the EGF-binding domain of the receptor. In neither case is the evidence very strong to back up this surprising mode of action. An uncyclized flavone analogue, Butein, **28**,¹⁴⁵ is an ATP-competi-

tive inhibitor of EGFr. Two similar looking compounds, the anthracenedione antibiotics Emodin, **29**,¹⁴⁶ and Paecilquinone A, **30**,¹⁴⁷ have been identified and are presumably ATP-competitive, but both have documented activity against other kinases.^{147,148}

C. Erbstatin-Based EGFr Inhibitors

With all of the phenols among the known inhibitors the idea that many of these compounds might be tyrosine mimics was rather attractive, and as we have seen above, a few of the compounds appear to be substrate competitive. One such early natural product hit was Erbstatin, **31** (Chart 5), which appeared to be both ATP and substrate competitive.¹⁴⁹ As the substrate binding domain normally controls the selectivity of kinases, the homology in that domain is much less than that in the ATP-binding or the phosphoryl transfer domains, and it appeared to be most reasonable that substrate-competitive inhibitors would be the easiest to modify to give enzyme selectivity. However, by occupying the ATP binding site as well, one would hope to be able to build inhibitors with the kind of potency seen in the staurosporins. This led to a series of erbstatin-based, potentially substrate-competitive inhibitors called Tyrphostins,¹⁵⁰ e.g., **32** and **33**,¹⁵⁰ **34**,¹⁵¹ and **35**.¹⁵² Furthermore, **34** was stated to be 50-fold selective for erbB-2 inhibition over EGFr, with an erbB-2 IC₅₀ of 350 nM,¹⁵¹ and **35** was reported to slow the growth of MH-85 squamous cell carcinomas in nude mice if dosed intraperitoneally at 10 mg/kg/day from the time that the tumor was implanted.¹⁵² As

our group was actively screening for EGFr and considering the value of erbB-2 inhibitors at the time (see below), these results appeared to be very encouraging but not in accordance with our experience.

The Tyrphostins seem to contain both the readily oxidizable phenol and the potential Michael acceptor described in many of the previous natural product inhibitors, and they did not withstand scrutiny very well. Other kinases turned out to be equally inhibited with many of these compounds, so the early claimed selectivities often appeared to be artifacts of inadequate testing against a kinase panel. More seriously, many unrelated activities were also found. Compound **32** is a much more potent inhibitor of mitochondrial function than it is of EGFr.¹⁵³ Compound **33** inhibits many GTP-utilizing enzymes including transducin¹⁵⁴ as well as topoisomerase I.¹⁵⁵ In addition, it does not appear to be stable in aqueous solution¹⁵⁶ and decomposes to a dimer, tentatively identified as **36**, which is a better TK inhibitor than the parent.¹⁵⁷ Both erbstatin and its ester analogue **37** have been shown to be strong cross-linkers of proteins, presumably via quinone metabolites.^{158,159} Perhaps most discouragingly of all, Workman's group not only could show no *in vivo* activity with **35**, admittedly against the HN5 cell line not the MH-85 cell line, but showed that drug plasma levels were below 1 μM (one tenth of the continuous exposure cellular IC_{50}) within 20 min of dosing, so these compounds have very severe pharmacokinetic problems.¹⁶⁰ In retrospect, trying to develop pharmaceutical agents out of the same chemical family as the riot control agent CS gas, (2-chlorobenzylidene)malononitrile, was probably not the wisest of choices.¹⁶¹

It was against this kind of a developing background that the Parke-Davis group entered the EGFr inhibitor field in 1990. We chose to run a mass screen of the Parke-Davis compound library looking for novel structures. This was a relatively novel approach at the time, as trying to persuade a biochemist to run 100 000 assays by hand on the off-chance that something interesting will turn up was not easy until appropriate robotic assistance became available. What we did not appreciate fully at that time was that we had committed ourselves to climbing two learning curves simultaneously, the kinase inhibitor problem, which is very difficult by itself, and the mass screen learning curve, which itself has plenty of traps for the unwary. We found several of them.

D. Initial Parke-Davis Approach to EGFr Inhibitors

Our first hit was the indolinethione **38** (Figure 6),¹⁶² which showed about 2 μM inhibition of EGFr in a shed membrane assay which we used for the mass screen. The corresponding disulfide dimer **39** turned out to be nearly as active and on standing, especially in DMSO solution, the monomer would turn into the dimer. The SAR of the series was by and large flat,¹⁶³ and the only compound which ever showed much improvement in potency over **38** was the diselenide, **40**,¹⁶⁴ which had an IC_{50} of 150 nM for the enzyme. To rub salt in the wounds, the inhibitors soon showed activity against other kinases,¹⁶⁵ but **40** did show modest efficacy *in vivo*

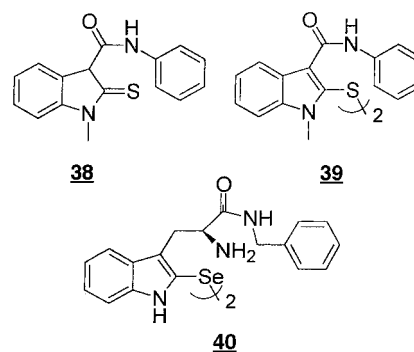


Figure 6.

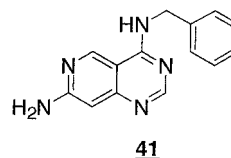


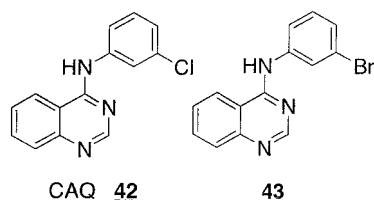
Figure 7.

against A431 tumor xenografts.¹⁶⁴ We spent considerable effort trying to work out the mode of action of these compounds. If they acted as monomers, were they chelators for the manganese put into the assays? If they acted as dimers, were they binding in the ATP pocket as dimers or were they acting as oxidizing agents or thiating agents? If they were acting as thiating agents, was it the dimer itself or its exchange product with glutathione? A recent paper has described the disulfide dimer of glutathione thiating and inactivating creatine kinase,¹⁶⁶ so the inhibition of the enzyme might not even require contact of the inhibitor with the enzyme. As will be seen later, there is some support in our subsequent work for such a mechanism, but the main lesson that we learned from this was that any thiols which turn up in a mass screen are probably best left there.

VI. Anilinopyrimidine Inhibitors

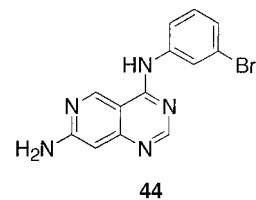
A. Discovery of the Anilinopyrimidine Pharmacophore

Initially, we had discovered that the indolinethiones were as good as the other inhibitors out there, but after a year or so, we realized that such a statement was damning with very faint praise. We had about 20 other hits from our mass screen which looked acceptable, but most of them looked as though they too would react with thiols, and by now we were convinced that we could not build an SAR if the only measured activity was likely to be an unpredictable, and probably irreproducible, reaction with protein thiols. Therefore, we chose to screen out such compounds by a simple chemical technique, running the assay in the presence of excess dithiothreitol to reduce disulfides and react with Michael acceptors. This assay successfully disposes of erbstatin,¹⁶⁷ and it disposed of almost all of our hits, except for a pyridopyrimidine PD 0069896 **41** (Figure 7).¹⁶⁸ Compound **41** was not initially very attractive to us as kinetic analysis showed that it is ATP-competitive and the assay results showed it with quite a wide

**Figure 8.**

range of IC_{50} s (1.5–20 μ M) against the enzyme in the membrane vesicle assay. However, the mechanism was a clean, reversible one and in several other tyrosine kinase assays it showed selectivity for the EGFr receptor. In addition, with the exception of the worrisome “bounce” in the EGFr assay, it behaved exactly as a reversible tyrosine kinase inhibitor ought to behave in cellular assays. When put on A431 cells, it rapidly inhibited EGFr autophosphorylation, with an IC_{50} of 1 μ M, and the inhibitory effect disappeared within a few minutes after the inhibitor was washed off. It was somewhat less potent in inhibiting EGFr autophosphorylation for EGF-stimulated Swiss 3T3 cells, but it had no effect at 100 μ M on PDGFr and FGFr autophosphorylation in the same cells, stimulated, respectively, by PDGF and FGF. In the 3T3 cells, EGF-stimulated proliferation was blocked at 10 μ M but serum-stimulated proliferation was only blocked at 100 μ M and PDGF-stimulated mitogenesis was not blocked at all at that concentration. Buoyed up by this profile, which was the first one we had seen which made sense to us if the mechanism was indeed as proposed, we started SAR studies in this area, and a recent review describes much of the chemistry our effort generated.¹⁶⁹

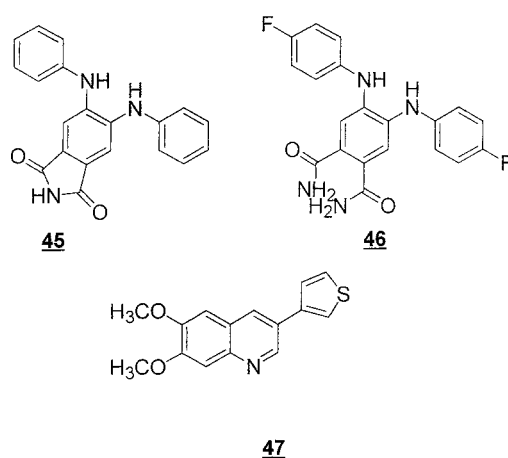
Shortly afterward, selective and potent EGFr inhibitors were revealed in a patent application from Zeneca,¹⁷⁰ which showed some anilinoquinazolines, exemplified by CAQ, **42** (Figure 8),¹⁷¹ as 20–40 nM inhibitors with good selectivity over other kinases. On first seeing this application, we made the bromo analogue **43**, tested it in our assay, and for the first time found that we could repeat a claim made by another research group. This was of considerable importance to us for two reasons. First, when you cannot repeat any literature claims, you (and your management) cannot be sure if it is you or the claims which are irreproducible. Second, even the most casual examination shows that **41** must be related to the anilinoquinazolines, and indeed when **43** was put through the same cellular assays described in the last paragraph, it showed an identical profile to **41**, except it was somewhat more potent (IC_{50} 240 nM). Mechanistically, it was ATP-competitive and highly selective over the other kinases examined. Because of the similarities, we elected to expand our SAR coverage to make highly related pyridopyrimidines and quinazolines as we felt that there really was only one SAR present. This approach had a couple of advantages: it let us see what was important in the bicyclic nucleus by varying it and it also allowed us to optimize substitution patterns in the synthetically easier quinazolines before committing considerably more resources to the same patterns in the synthetically much more challenging pyridopyrimidines. It also had its disadvantages: it committed us to a lot

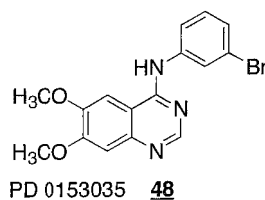
**Figure 9.**

less focused chemistry and it made us more vulnerable to further patent applications from Zeneca. Overall, we reaped both the advantages and the disadvantages quite fully.

Our next facilitating discovery came from an unexpected quarter. None of our initial pyridopyrimidines, most of which had 3-bromoanilino side chains at the 4-position, showed any activity. Most of the quinazolines did show some activity, but the SAR showed no coherence. After a while, suspicion fell on the assay itself.¹⁷² The assay performed as it should with the literature standard inhibitors, but of course they were all compounds of a type we did not want. Furthermore, the assay had its own peculiarities, such as not needing EGF to produce kinase activity. In this case, the detergent present seems to activate the kinase. For kinetic purposes, we had set up a second assay using immunopurified EGFr and a peptide substrate.¹⁷³ This assay required EGF to produce kinase activity but, as it contained no lipid, could not have the hydrophobic transmembrane domains of the RTK embedded in lipid as they normally would be. Therefore, it was difficult to see this assay as any less artifactual than the first one. However, from a practical point of view, the new assay was invaluable. It produced an SAR which was highly robust and reproducible and allowed us to fully appreciate the extraordinary molecular landscape that we were looking at. Compound **41** now had a 580 nM IC_{50} , and the IC_{50} for **43** dropped to 27 nM, as potent as claimed by Zeneca. The replacement of the 4-benzylamino side chain of **41** with the 3-bromoanilino moiety gave us **44** (Figure 9), a 10 nM IC_{50} inhibitor of the enzyme.¹⁶⁸

Shortly afterward, patent applications from Ciba-Geigy revealed the stripped-down staurosporin analogues dianilinophthalic acid derivatives **45** and **46** (Chart 6) as very potent and selective EGFr inhibi-

Chart 6

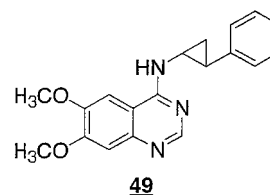
**Figure 10.**

tors, with **46** having an IC_{50} for the enzyme of 1 nM.^{174,175} The selectivity for EGFr was good, and this revealed that allowing the phenyl rings to adopt a different conformation to that seen in staurosporin made the inhibitors very poor ligands for many kinases, where the planar staurosporin binds very well. Another patent application¹⁷⁶ from about the same time showed that the dimethoxyquinoline **47** was a 6 nM inhibitor of the PDGFr tyrosine kinase.¹⁷⁷ Although clearly related to the diarylamine SAR that we were working in, it also is strongly related to some of the Tyrphostins such as **35** and was made as one of a class of “cyclized Tyrphostins”. The electronics are quite different from many of the Tyrphostins, but it is clear that sterically **47** is related to **35** and shows that there must have been a considerable element of noncovalent binding in the affinity of Tyrphostins for their targets. Again, these results were important in our program because they cemented our belief that we were working on a very important kinase inhibitor pharmacophore and was work by outside groups which we could repeat internally. It also meant that if the overall SAR was so large, many more companies might be expected to have found their way into it, so competition would probably become even hotter.

B. Exploration and Development of the Anilinopyrimidine SAR

1. EGFr Enzyme SAR

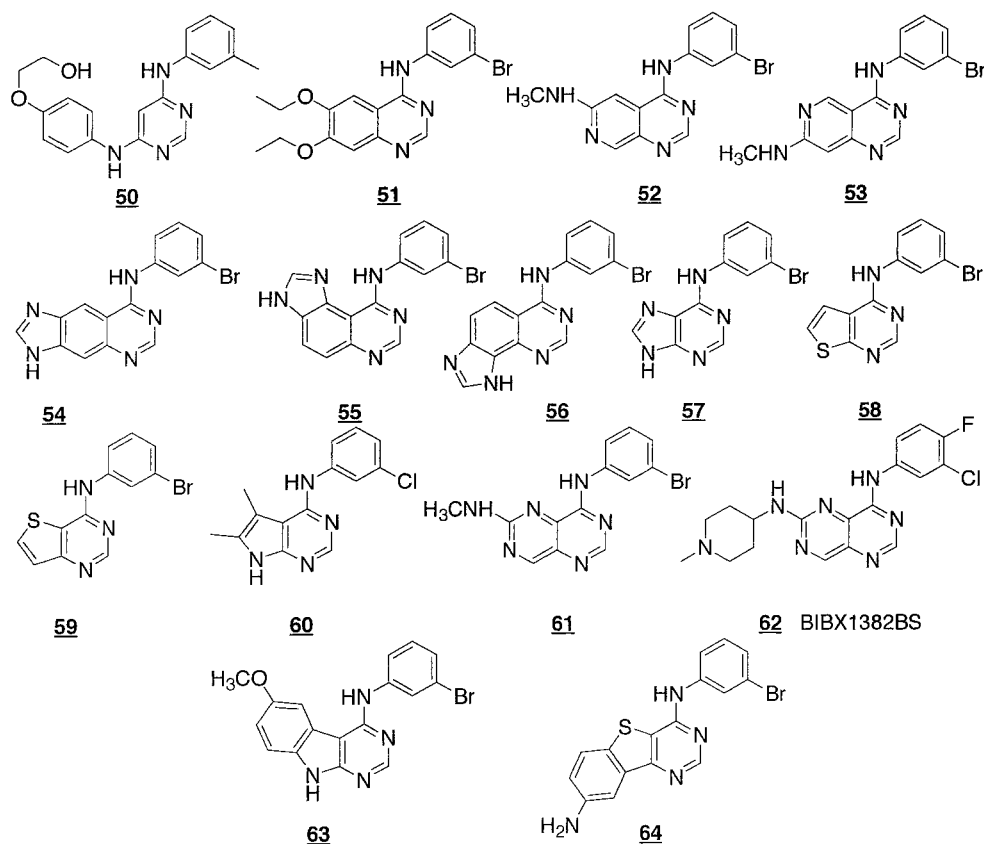
The previously synthesized inhibitors were run through the new assay, and one of them, PD 0153035, **48** (Figure 10), turned out to have an extraordinary potency.¹⁷⁸ After repeating the assay 5 times, our biochemist was ready to state that **48** had an IC_{50} of 29 pM and a K_i of 5.9 pM. Furthermore, the compound was also an ATP-competitive, reversible inhibitor, with no appreciable activity against the dozen or so kinases we counterscreened it against. In cellular assays it showed good selectivity, inhibiting EGFr autophosphorylation with an IC_{50} of 14 and 195 nM for erbB-2. Inhibition was instantaneous, unaffected by DTT, and took about 4 h to go away after wash off, consistent with the very slow unbinding of a tight binding, reversible inhibitor. It also showed the same kinds of results as **41** in other cellular assays, affecting EGFr-induced mitogenesis and morphology changes at around 100 nM but having no effect until above 10 μ M on effects induced by other growth factors, again showing exactly the profile one would expect for such a potent and selective inhibitor. However, the excellent profile of **48** did not protect our cancer program from one of its periodic cancellations but may well have contributed to its resurrection a few months later, luckily before any work had stopped. It also did not protect us from the next

**Figure 11.**

Zeneca patent application^{179,180} which included **48** in its claims, although there was no data to suggest that they had noticed its exceptional potency at that time. Nor could it get us rapid access to in vivo models, physicochemical, pharmacokinetic, or molecular modeling resources, all of which were in very limited supply at that time. As **48** was such an insoluble compound that when first made it had almost not been submitted for testing, this was likely to prove to be problematic down the line. The other very important thing that **48** brought us was credibility in the EGFr inhibitor area. The shortcomings of the previous generation of inhibitors had soured a lot of the cancer research field on TK inhibitors. The publication of **48** followed by our decision to distribute to researchers who asked for it raised our profile considerably and helped to convince many clinicians that the small molecule TK inhibitor field might have something to offer. As a result, we obtained a lot of insight from these researchers as we pushed our program forward and later had access to advice and clinical sites that we probably would not have had an entry to without **48**. One of the big debates in companies is always whether one loses or gains by revealing data, as the obvious downside of prematurely tipping your hand is that it can turn fast followers into rapid overtakers. However, a raised profile and serious interest in your program from experts in the field are themselves very valuable commodities which tend to be undervalued when decisions to publish are taken.

As we ran compounds through the new assay, the SAR became very clear and we explored it quite thoroughly.^{181,182} We had good enzyme and cellular assays running at that time and almost nothing else, so for quite a while we designed those few assays we had. Thus, ironically, the thoroughness of these studies was probably largely driven by our inability to get other types of data useful for drug candidate selection for a considerable period. Both of the nitrogens of the pyrimidine ring were required for good activity as was a secondary N^4 -nitrogen. An aromatic substituent was required either directly on this nitrogen or one carbon removed. The two carbon chain extension showed a dramatic loss of binding potency, but as shown later by Zeneca, if this linker was rigidified by the introduction of a trans cyclopropane, **49** (Figure 11), the compound became very potent with an enzyme IC_{50} of 1 nM.¹⁸³ Although an unsubstituted N^4 -benzyl substituent was as good as an N^4 -phenyl substituent, the benzyl aromatic could not be improved upon by substitution whereas the phenyl was markedly improved by small lipophilic substituents at the 3-position and to a lesser extent at the 4-position, regardless of the parent heterocycle. Inhibitors with monocyclic bicyclic or tricyclic pyrim-

Chart 7



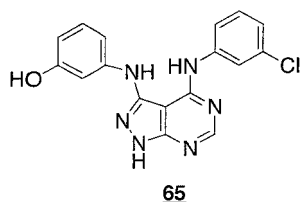
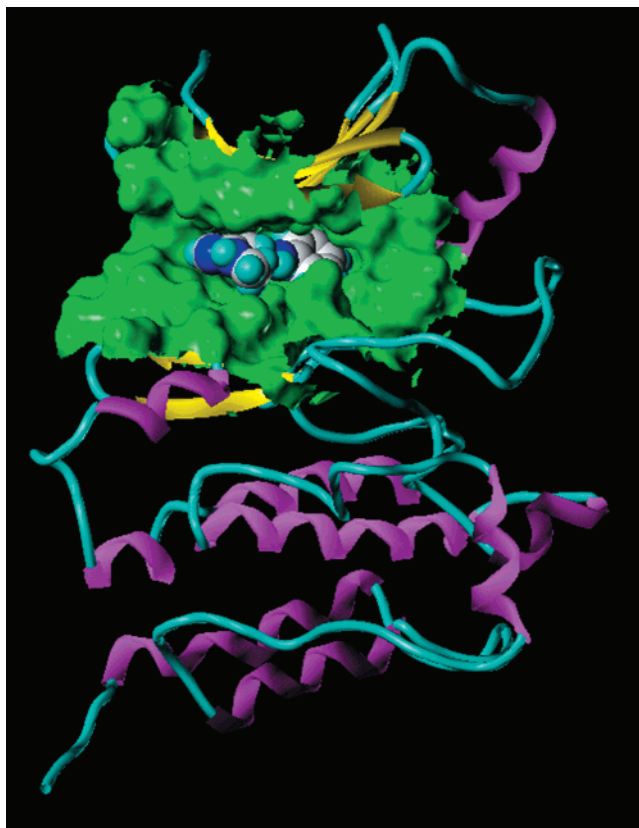
idine cores showed low-nanomolar to low-picomolar potency provided there was no bulk at the C2 position or for polycyclic pyrimidines the 8-quinazolino position. The 5-quinazolino position was somewhat less disfavored, and the 6- and 7-positions were very tolerant of substitution, which were preferably electron-donating amines or ethers. Because of this substitution pattern around the quinazoline ring, linear tricycles were considerably more potent than angular tricycles.

In the area of monocycles, Zeneca published the 4,6-dianilinopyrimidine **50** (Chart 7) as a 1 nM inhibitor,¹⁸⁴ demonstrating that even a bicyclic aromatic was not needed for excellent activity. In the quinazoline area, the most potent inhibitor we found was the diethoxy analogue **51** (IC₅₀ 6 pM).¹⁸² In exploring the pyridopyrimidines, we eventually achieved similar potency with the [3,4-*d*]-ring fusion with PD 0158780 **52** (IC₅₀ 8 pM) and the same methylamino substituent on the original [4,3-*d*]-pyridopyrimidine system gave **53** (IC₅₀ 130 pM), a 70-fold improvement from **44** just by methylating the N⁷-nitrogen.¹⁸⁵ In the tricyclic quinazoline series, the linear imidazo[4,5-*g*]quinazoline **54**¹⁸⁶ showed low-picomolar potency (IC₅₀ 8 pM) and was duly lost to us in another Zeneca patent application.¹⁸⁷ The corresponding angular imidazo[4,5-*f* and *h*]quinazolines **55** and **56** (5- and 8-substituted, respectively) had IC₅₀s of 29 and 270 nM, respectively. Looking at other substituents for the phenyl ring of the quinazoline pharmacophore, we discovered that five-membered rings would still provide potent inhibitors and the adenine **57** and the thienopyrimidines **58** and **59** were all better than 100 nM inhibitors¹⁸⁸ as was the

pyrrolopyrimidine **60** from Ciba-Geigy.¹⁸⁹ Another allowed B-ring was the pyrimidine, and we produced a series of inhibitors exemplified by **61**,¹⁹⁰ just to find that Boehringer Ingelheim had already claimed that series¹⁹¹ and have a member of the series in clinical development, BIBX1382BS **62**,¹⁹² which was stated to have an IC₅₀ of 1 nM against EGF-stimulated cellular proliferation.¹⁹³ In accordance with the SAR, the [6,5,6]-tricycles **63** and **64**¹⁹⁴ would be expected to have geometries intermediate between the linear tricycle **54** and the angular tricycles **55** and **56** and have IC₅₀s in the 1 nM range.

2. Molecular Modeling of the EGFR SAR

The crystal structure of PKA was the first solved for a kinase, and it was also solved with staurosporin in the ATP-binding site.¹⁹⁵ On the basis of this mode of binding, the Ciba-Geigy (now Novartis) team built a homology model of EGFR and proposed a binding mode for the dianilinophthalimide inhibitors **45** and **46**.¹⁹⁶ This had two hinge-region backbone H-bonds to the phthalimide, donor and acceptor, just as are made with the adenine of ATP, with one aromatic ring in the sugar binding domain and the other in a large, normally unused hydrophobic pocket at the rear of the binding pocket. They extrapolated this to the anilinoquinazoline pharmacophore and for pyrrolopyrimidine **60** suggested that the two H-bonds were made to N-1 and N-7H.¹⁹⁷ This puts the aniline into the ribose binding region. On the basis of this model, they developed a series of pyrazolopyrimidine inhibitors, exemplified by the 1 nM inhibitor **65** (Figure 12).¹⁹⁷ With the very interesting SAR data available in our series, molecular modeling also

**Figure 12.****Figure 13.** PD 0158780 bound into EGFr active site.

became an attractive possibility for us and we also built a homology model of the EGFr TK domain. A single docking mode was sought to accommodate all of the different ring systems as they all seemed to belong to the same SAR. However, the Ciba-Geigy model could not accommodate the tricyclic inhibitors such as **54**, and our modeling suggested an alternative binding mode.¹⁹⁸ All of these inhibitors are ATP-competitive, bind the anilino-pyrimidine in the hydrophobic adenine binding pocket, form the hinge-region backbone H-bonds through N-1 and N-3, and place the aniline ring in the hydrophobic “chimney” at the back of the binding site, as shown in Figure 13. This site is normally unoccupied, having no binding function, appears not to be under selection pressure to remain conserved, and is in fact one of the least conserved parts of the kinase core. The 6- and 7- positions of the quinazoline ring point out of the binding site toward solvent, explaining why further rings or large side chains can be accommodated at this site. All of this is very consistent with the known SAR, but it does not fully explain the enormous increase in potency shown by a few compounds such as **48** and **52**. Recently, crystal structures of two analogues of **48** bound to CDK2 and p38, S/TKs, for which **48** has micromolar affinity, have

been published,¹⁹⁹ and they show exactly the predicted binding mode but provide no explanation for the exceptionally potent compounds. Such an explanation may come if EGFr-inhibitor complex crystal structures become available.

3. ErbB Family Selectivity and Potency

Generally the agents described above had excellent enzyme potency for EGFr and enormous selectivity over most of the kinases examined. Selectivity over the other ErbB-family members was not so good, but quinazolines tended to be at least 10–100 fold EGFr selective over ErbB-2 (in cellular assays), exemplified by **48** with cellular IC₅₀s of 14 and 195 nM against EGFr and ErbB-2, respectively. However, the pyridopyrimidines tended to have more ErbB-2 potency, exemplified by **52**, with cellular IC₅₀s of 13 and 52 nM against the two receptors.¹⁶⁸ Early in our program as in most others, selectivity had been a key issue and our initial view was to make the inhibitors as selective as possible for EGFr. However, the flood of literature on ErbB-2, then ErbB-3, and more recently on ErbB-4 changed our view.²⁰⁰ It has become apparent that all four ErbBs are oncogenes in their own right²⁰¹ and that the heterodimers containing two different family members are often far more oncogenic than the homodimers.²⁰² ErbB-3 has active site sequence alterations which either greatly decrease or completely abolish its kinase activity, but its ligand-induced heterodimers with the other ErbBs are all very active TK complexes.²⁰³ Therefore, our strategy became to pursue compounds which showed really good activity in our ErbB-2 assays as well as our EGFr assays. The fact that such compounds tended to be more in the patentable pyridopyrimidine portion of our SAR gave some coherence to the strategy.

When the opportunity arose to test compound **48** in animals against A431 tumor xenografts, we had to deal with its solubility characteristics, which were obviously poor, along with most of the rest of the SAR we had made up to that time. The compound could be made moderately soluble as an isethionate (2-hydroxyethane sulfonate) salt, and this was used for oral and intraperitoneal (IP) dosing. With some solubility and tremendous potency, it appeared to us that we could not fail to get some kind of a proof of concept experiment with **48**. Then, once the idea of EGFr inhibition was validated, we could then go on to fix the problems with our inhibitors and design the actual drug candidate. However, the main activity observed on dosing was precipitation of the drug, with no sign of any effects on tumor growth. After IP dosing, the peritoneum would have visible drug precipitates which showed no signs of disappearing as long as the experiment continued. A pharmacodynamic experiment whereby the drug was dosed IP, tumors were removed after 20 and 180 min, and their EGFr enzymic activity assessed was carried out.²⁰⁴ It showed almost 90% inhibition of the enzyme after 20 min but only 25% after 3 h, suggesting that blood levels after dosing were only transiently high enough and that long term near complete suppression of kinase activity was required for efficacy. At this time

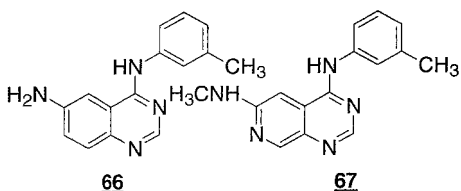


Figure 14.

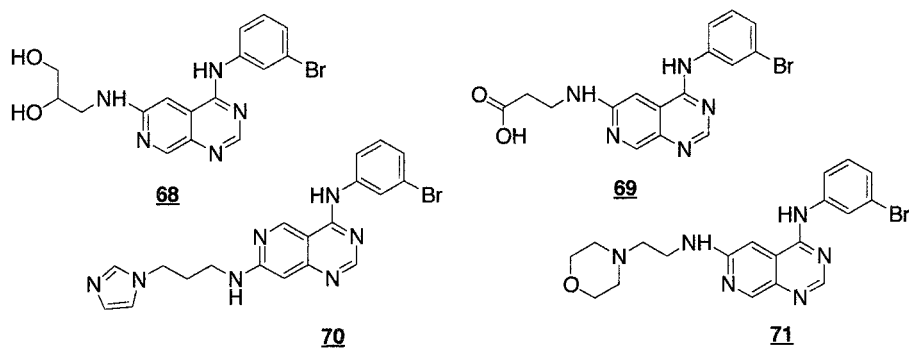
we were only slowly scaling up inhibitors and one of the few compounds available in gram quantities was the tricycle **64**,¹⁹⁴ which showed some possible modest activity when directly injected into tumors. Soon after this particular experiment we got our first pharmacokinetic data, and it showed that blood drug levels and oral bioavailability of compounds such as **48** were very low, but the tricycles, especially **64**, were at least an order of magnitude worse. Its career died immediately, and solubilization now became our major thrust. As the pyridopyrimidines were the least unpromising, patentable, substrates available to work with, they were examined in detail.

Some simple arithmetic makes very tight binding inhibitors such as PD 0158780, **52**, very appealing. With a K_i in the 1 pM range and the definition of K_i as k_{on}/k_{off} , k_{off} must be very large so the inhibitor must be dissociating from the enzyme very slowly, possibly over a matter of hours. If the dissociation is slower than the fall in blood levels after dosing, the enzyme may remain inhibited well after blood levels have fallen below those required by Michaelis–Menten kinetics. In such a situation, the behavior of the inhibitor would be pseudoirreversible, whereas if one has K_i s a thousand times less potent, it is quite reasonable to imagine k_{off} rates on the order of seconds at best and one would expect quite rapid equilibration of bound drug with the plasma. Therefore, our goal at this time was to find a soluble pyridopyrimidine which retained picomolar EGFr inhibition, had good cellular penetration, and also had good ErbB-2 activity. In the cases of ErbB-2, -3, and -4, we did not have the tools available to tell us if our compounds were binding with this kind of affinity so we could only hope for such effects to manifest themselves at a later date.

4. In Vivo Activity of the EGFr Inhibitors

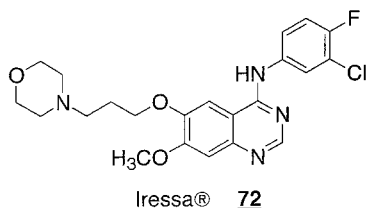
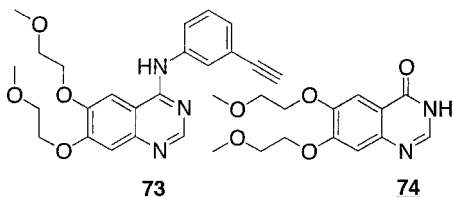
At about this time Zeneca published on a simple quinazoline, **66** (Figure 14), which showed rather good anticancer activity against some EGFr-driven tumors when dosed at 200 mg/kg/day.²⁰⁵ This was

Chart 8



attributed to good pharmacokinetic properties as the compound is quite soluble but not an exceptional enzyme inhibitor. As this was the first credible report of in vivo activity for an EGFr inhibitor, in our opinion, we naturally set out to see if we could repeat it. Compound **66** had an IC_{50} of 6 nM in our EGFr assay, was quite noticeably water soluble, and had appreciable antitumor activity in two of our models with 100–200 mg/kg/day daily dosing. This demonstrated to us for the first time that our in vivo models were capable of reproducing the TK-mediated activity seen elsewhere, which was comforting. The fact that this apparently ordinary inhibitor was as potent as our best compounds in vivo at the time (see below) was less comforting, and the fact that we knew Zeneca had much better compounds under wraps was even less so. We made the pyridopyrimidine equivalent, **67**, but it had an IC_{50} of 420 pM,²⁰⁶ was not much more soluble than **52**, and had no activity in tumors, suggesting that the known lower solubility of the pyridopyrimidines than quinazolines put them beyond such fixes as those that worked for **66**.

The strategy we chose to solubilize the pyridopyrimidines was to place solubilizing moieties on the 6/7-alkylamino side chains, where we felt there would be the least effect on their binding affinities, while retaining the 3-bromoaniline side chain. Three solubilizing entities were looked at: alcohols/polyols, carboxylic acids, and amines.^{206,207} In all cases extending side chains beyond methylamino lost binding affinity for the enzyme with alcohols losing the least, for example, diol **68** (Chart 8) has an IC_{50} of 180 pM. Unfortunately, these compounds were hardly more soluble than **52**. Some carboxylic acid inhibitors, such as **69**, were almost as potent (IC_{50} 270 pM) and were much more soluble than **52**. However, these compounds showed greatly reduced activity against EGFr in cells with an autophosphorylation IC_{50} of 450 nM for **69**, suggesting that the carboxylic acid greatly reduces cellular penetration. The amines generally gave IC_{50} s in the 1–10 nM range, although the best compounds **70** and **71** had IC_{50} s of 510 and 650 pM, respectively. These compounds generally had good solubility characteristics, and moderate plasma levels of the drug were measured with **71**. As an additional bonus, **71** had a better cellular IC_{50} (6.9 nM) than **52**, suggesting that some side chains may help improve cellular penetration. However, **71** only had an IC_{50} of 220 nM against ErbB-2, which made it no better than many quinazolines.

**Figure 15.****Figure 16.**

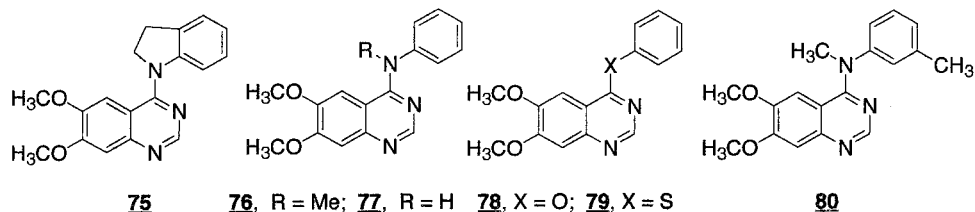
We looked at both **52** and **71** in several antitumor models *in vivo*.²⁰⁶ Unlike the quinazoline **48**, compound **52** showed measurable activity despite its insolubility but the activity was not particularly robust and not very reproducible and overall it was an inferior agent to **66**. As the compound is bright yellow and nude mice are somewhat transparent, it was very obvious on IP dosing that most of the dose simply stayed put. Compound **71** did show somewhat greater activity when dosed IP against the MCF-7 breast cancer line and orally against the HA-125 small cell lung carcinoma line, but the activity was still a lot less than we thought we should be able to obtain. We did start to refine this series, for example, replacing the bromine with methyl, and found that we lost no enzyme potency in doing so. The bromine was really only useful in the exceptionally potent compounds. However, we did not continue on this line of research because one of our other approaches, discussed below, looked much more promising, giving us a chance of completely finessing some of our problems.

Our change in tactics came none too soon, because later disclosures from Zeneca revealed that they had successfully developed a solubilized quinazoline, ZD-1839, Iressa, **72** (Figure 15),²⁰⁸ which is now in Phase II or Phase III clinical trials. This meets most of the criteria that we had set originally for an inhibitor in this class. By its structure, it is obviously a soluble version of **48** and in our hands is about a 1 nM inhibitor of EGFr, with 9 nM potency in the cellular assay. This compound has excellent pharmacokinetic properties, and although it does not have the potency to have a very slow off rate from the enzyme, **72** is retained in cells and *in vivo* seems to act as though it is an irreversible inhibitor.²⁰⁸ In our assay, **72** also shows good potency against ErbB-2 (IC₅₀ 24 nM). In

our *in vivo* evaluation the compound was very potent and with A431 xenografts completely suppressed tumor growth as long as (oral) dosing continued at 10 mg/kg/day. Synergy of **72** with several cytotoxic agents against a variety of tumors, not all of which are high EGFr expressers, has been reported in nude mouse xenograft studies.^{209–211} In clinical trials, Iressa can be dosed up to about 800 mg/day before limiting toxicity (diarrhea, skin rashes, both believed mechanistically related) is reached.^{212–214} At doses of 150 mg/day or above, the target plasma blood levels of >200 nM at all times were easily reached and some form of response, either stopped tumor growth or partial tumor shrinkage, was reported in one-third of the evaluated patients. Activity was seen against non-small cell lung, ovarian, prostate, colorectal, and head and neck cancers. A slightly less potent inhibitor, with excellent pharmacokinetic properties from Pfizer/Oncogene Science is CP 336,774, **73** (Figure 16),^{215–217} another solubilized version of **48**. It is somewhat less potent than Iressa in many models but in the Phase 1 trials produces trough blood levels well above the desired levels (1.25 μM) at 50 mg/day and above.²¹⁸ Skin rashes and diarrhea were also the dose-limiting toxicities (at above 200 mg/day) for **73**, which has advanced into Phase II trials.²¹⁹ The inclusion of the two methoxyethoxy side chains make an astonishing difference to the physical properties of **73**. It is a rather waxy, quite water-soluble compound, nothing like the gritty powders characteristic of most of this series. In fact, the precursor quinazolone **74** could only be precipitated from water with considerable difficulty, whereas most quinazolone/pyridopyrimidones have no aqueous solubility and can be cleaned up after facile aqueous precipitation by washing the residue with any organic solvent one desires. In the original Zeneca patent applications, acetylene had not been claimed as an aniline substituent, possibly because terminal acetylenes are usually rapidly metabolized. Pfizer was able to exploit this gap in Zeneca's patent coverage, and fortunately this acetylene was unusually metabolically stable.

5. Other Inhibitors Based on the Anilinoquinazoline Pharmacophore

With the very interesting activity seen for this pharmacophore, many other companies started to work in the area. This meant that the SAR described above came under intense scrutiny, and a certain amount of correction, as each group looked for areas where the SAR was thinly sketched out and where they might develop a patent position. The Pfizer group showed that 1,*N*-indolines such as **75** (Chart 9) (IC₅₀ 3 nM) could substitute for anilines,^{220,221}

Chart 9

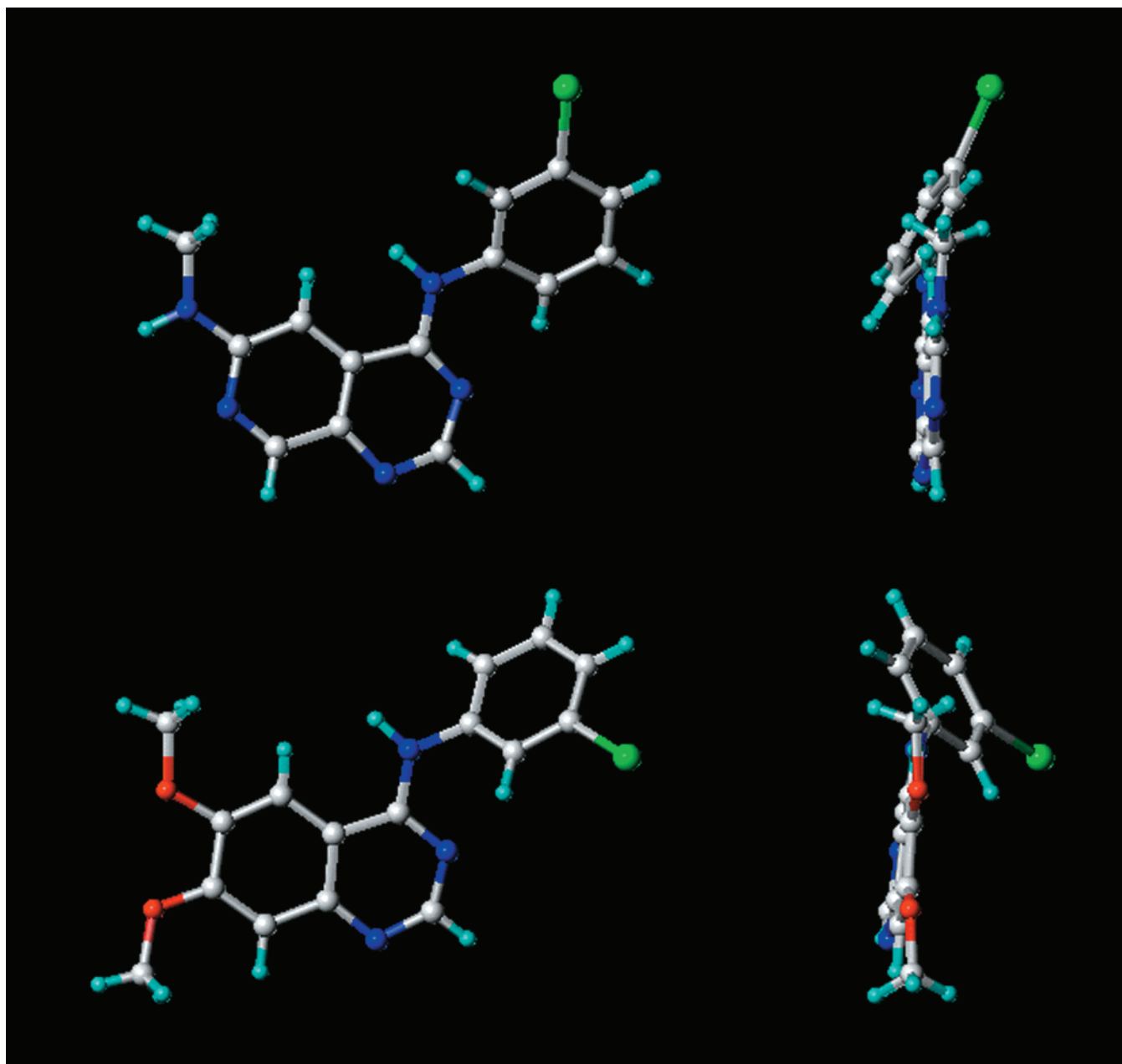


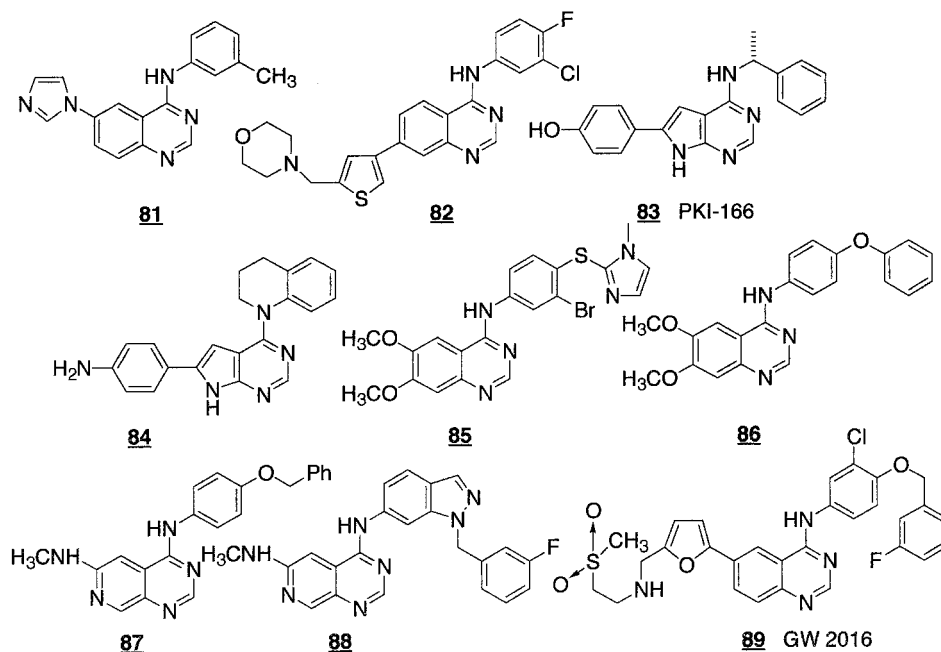
Figure 17. Crystal structures of PD 0153035 and PD 0158780.

despite the fact that simply methylating the nitrogen is very detrimental, with **76** (IC_{50} 4 μ M) showing about a 100-fold loss of potency relative to **77**. In this case²²² the RPR group (now Aventis) showed that both oxygen and sulfur (**78** and **79**, IC_{50} s 20 and 10 nM) were at least equipotent with NH in **77**. **76** was 8-fold selective for the CSF-1R, another RTK, and putting on the 3'-methyl, **80**, (a favorable EGFr substituent), led to a weaker EGFr inhibitor, which has an IC_{50} of 180 nM for CSF-1R and is 66-fold CSF-1R selective. The RPR group did NMR studies and came up with a completely planar, extended conformation for **77**,²²¹ with the N–H bond pointing directly at H-5, which we confirmed by X-ray crystallography. The indoline ring hardly disrupts this conformation, but *N*-methylation causes the C4–N bond to rotate 180° and the anilino ring to move completely out of the plane. Our X-ray studies showed several nanomolar inhibitors to be planar,¹⁹⁰ but interestingly, **48**

and **52** (see Figure 17) had completely planar NH bonds but the bromoanilino rings had about a 40° dihedral (Rubin, J. R., unpublished data) allowing them to enter the hydrophobic chimney of the EGFr ATP-binding domain without much distortion.

Zeneca patent applications revealed that the 6- and 7-positions can tolerate (hetero)aromatic substituents, such as **81** (IC_{50} 26 nM)²²³ and **82** (IC_{50} 42 nM) (Chart 10).²²⁴ A compound from Novartis, which appears to combine this work with both some of our earlier work on enantioselective 4-benzylamino side chains²²⁵ and their earlier work,^{189,226} produced the pyrrolopyrimidine **83** (IC_{50} 13 nM),²²⁷ which has become their clinical EGFr inhibitor PKI-166.^{228,229} Combining further with the indoline work gave **84**²²⁷ (IC_{50} 26 nM), but this compound is also a 2 nM inhibitor of the *v*-abl TK, showing again how selectivity can be lost even in what appears to be the same pharmacophore. The 4-position of the aniline can also

Chart 10



tolerate a lot of bulk, and Zeneca (now AstraZeneca) revealed that the 4-thioimidazole **85** is a 7 nM inhibitor.²³⁰ Burroughs-Wellcome (now GlaxoWellcome or GlaxoSmithKline) put a phenoxy substituent on **86**²³¹ and benzyloxy substituent on **87**,²³² where the pyridopyrimidine nucleus and thienopyrimidine nuclei were utilized, and claimed them to be ErbB-2 selective, with the latter having an IC_{50} of 1.3 nM for ErbB-2. In our hands, **86** was much more potent than claimed on EGFr with an IC_{50} of 11 nM. Glaxo has a series of patent applications where only cellular data (often ~ 1 nM IC_{50} s) is quoted, where use of a bicyclic aniline extends this concept further as exemplified by **88**.²³³ The 6- and 4'-substitution patterns can be put on the same molecule, and Glaxo developed their clinical candidate **89**, GW-2016,²³⁴ in this series.²³⁵ GW-2016 is a balanced EGFr/erbB-2 inhibitor with IC_{50} s of about 10 nM for each of the kinases and an oral bioavailability of 50%,²³⁶ despite having two extra aromatic rings to solubilize, relative to the original pharmacophore.

Compound **90** (Figure 18)²³⁷ represents a more fundamental change in the pharmacophore and claims moderate ErbB-2 activity with little or no EGFr activity. The requirement for a pyrimidine ring has also been circumvented by a group from Wyeth-Ayerst,²³⁸ where the 3-nitrogen is replaced by a C-CN group, giving **91**, which has an IC_{50} of 190 nM

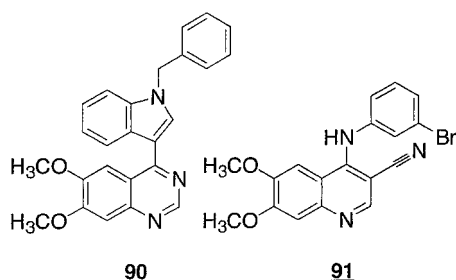


Figure 18.

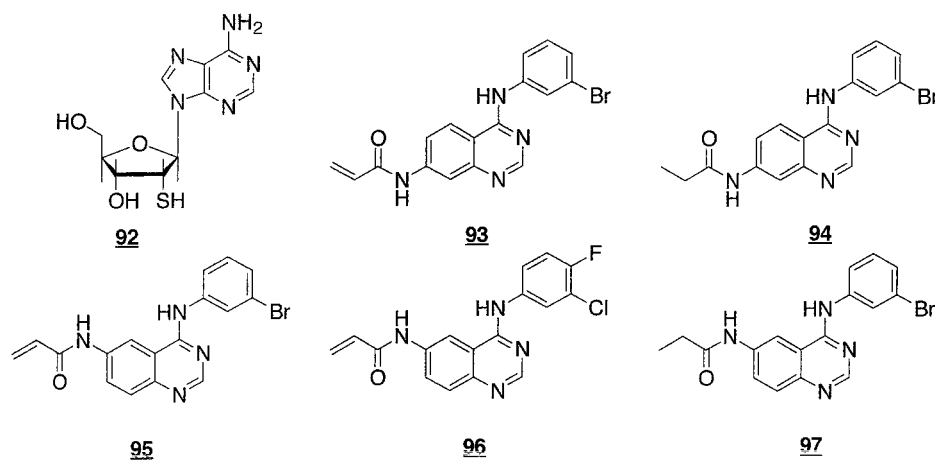
for EGFr, which in their assay system represents only a 3-fold loss in binding from PD 0153035 **48**. The cyano group is postulated to replace a bridging water molecule seen between N-3 and a threonine in the CDK-2 and p38 crystal structures,¹⁹⁹ but whether the huge loss in activity reported for **48** might affect conclusions has to be considered. Additionally, essentially the same compounds, for example, the direct 3-cyanoquinoline analogue of quinazoline **86**, are described by the same authors as MEK inhibitors²³⁹ and claimed as such by Zeneca in several patent applications published late last year,²⁴⁰ suggesting that this is going to be a versatile and potentially promiscuous scaffold.

VII. Making an Irreversible Change in Strategy

A. Reasons for Returning to Covalence

We spent a considerable time trying to build both picomolar potency and solubility into our compounds, for the reasons described above, but never succeeded. It is the personal view of the author that the picomolar inhibitors not only fit deep into the ATP binding cleft, but probably lead to a hydrophobic collapse around the inhibitor, enclosing the inhibitor completely in the cleft. Any long, solubilizing side chain tends to push out into solvent and prevent complete closure of the binding cleft, leading to some loss of binding energy. Thus, it may not be possible to find a low-picomolar inhibitor in this SAR with suitable pharmacokinetic properties. The other way to prevent inhibitors from dissociating from the enzyme is to bond them to it, making them truly irreversible. This would continue the inhibition of the enzyme long after the blood levels of the inhibitors had fallen below inhibitory concentrations. This strategy has several drawbacks, starting with the fact that everyone was probably doing this unwittingly originally and making no useful progress at all. Our first progress had come when we used the DTT screen

Chart 11



to eliminate covalent binders. However, the argument was made that looking for irreversible inhibitors, using a template known to interact specifically with the target enzyme through noncovalent interactions, is quite different from trying to guess blindly if one's compound is in any way targeted to the protein or simply stuck to it. A second point is that irreversible inhibitors have a reputation for being toxic and have a hard time getting any sort of regulatory approval. The counterpoint to this is that the one big exception is cancer, where nondiscriminating DNA alkylators have been used for 40 years and some actually work in some tumors.

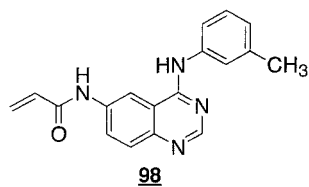
Although no crystal structure of EGFR is available, homology modeling reveals that the members of the EGFR family have a very interesting and unusual cysteine residue (Cys⁷⁷³) which provides a H-bond for the 2'-hydroxyl of the ATP ribose moiety. This residue is usually a serine or glutamine²⁴¹ and provides a near unique, potent nucleophile on the fringe of the inhibitor binding domain of this family. The concept was demonstrated using 2'-thio-2'-deoxyadenosine **92** (Chart 11).²⁴² **92** is a much more potent inhibitor of EGFR than is adenosine but not in the presence of DTT. Mass spectroscopy showed that the protein has thioadenosine covalently attached to it. This demonstrated the principle, but **92** had little potential for development as it was a low-affinity template of unknown selectivity, and it was not felt that disulfide formation would be a very useful mechanism *in vivo*.

B. Development of Irreversible Quinazoline EGFR Inhibitors

As we knew that **48** has very high affinity for the enzyme catalytic site, the 4-anilinoquinazoline nucleus was chosen as the template. At the time we had no binding mode modeled for **48**, so varied alkylating functionalities were attached to the positions of **48**, which are known to tolerate substitution. In the initial set of compounds, only one substituent was found on that template, which did successfully alkylate the enzyme. It was the 7-acrylamide, **93**, which turned out to have IC₅₀s for EGFR of 91 pM and 14 nM in enzyme and cellular assays, respectively.²⁴³ The corresponding propanamide **94** had an enzyme IC₅₀ of 1.9 nM, confirming the good noncovalent

affinity present in the template. Radiolabeling and mass spectral techniques were used to prove the irreversible nature of the interaction for **93** and that the compound had alkylated the target Cys⁷⁷³. However, acrylamide **93** was not a very rapid alkylator of EGFR, with a *t*_{1/2} of 20 min, when put onto A431 cells at 1 μM and then washed off after varying periods, and its *in vivo* activity proved to be no better than similar nonirreversible inhibitors. Placing the acrylamide at the 6-position also led to subnanomolar inhibitors of the enzyme, as exemplified by **95** and **96**, which had enzyme IC₅₀s of 0.84 and 0.75 nM, respectively, with the saturated analogue **97** being of similar potency (0.52 nM).²⁴³ In cellular assays **97** showed rather normal quinazoline IC₅₀s of 15 and 106 nM against EGFR and ErbB-2, respectively, but **95** and **96** showed quite exceptional cellular potencies for both EGFR (2.7 and 3.1 nM, respectively) and ErbB-2 (6.9 and 4.3 nM, respectively). Careful mechanistic analysis of **95** showed that it is also an irreversible alkylator of the EGFR Cys⁷⁷³ residue and that the alkylation is very rapid, being 100% complete at the first time point (2 min) on A431 cells. The selectivity of alkylation was demonstrated in two ways. Incubation of **95** under the same conditions with reduced oxytocin (a peptide dithiol) led to only 1% alkylation of the peptide in 16 h, and there was no appreciable reaction when **95** was incubated with the point-mutated Cys⁷⁷³ → Ser EGFR catalytic domain, despite **95** being a potent inhibitor of what is a catalytically active kinase.

One of the most obvious features of both **95** and **96** was their extreme insolubility. This was not only true in water but in most organic solvents as well. To chromatograph them, the compounds had to be adsorbed onto silica gel by prolonged sonication in acetone and then used as the origin for a dry column. Clearly this did not suggest favorable pharmacokinetic properties. Despite that, both turned out to have excellent antitumor properties even when dosed orally as slurries in Methocel.²⁴⁴ **95** was about one-half as potent as Iressa against A431 xenografts, and **96** was about equipotent to Iressa in that test. We also examined the corresponding 3-methylanilino analogue **98** (Figure 19), which was somewhat more soluble than the other two acrylamides, and this

**Figure 19.**

showed activity against a wide variety of tumors, including the very tough, massively ErbB-2 overexpressing, ovarian line SK-OV-3, MCF-7 breast carcinoma, and A431 by subcutaneous continuous infusion, oral and IP routes.²⁴⁵

C. Discovery of CI-1033: A Soluble, Irreversible, Inhibitor of the ErbB Family with Excellent in Vivo Potency

These results were very encouraging, and the earliest of them had led to the abandonment of the solubilized pyridopyrimidine strategy described earlier. However, despite the excellent in vivo activity, it was clear that the physical properties had to be improved, especially as pharmacokinetics showed very low absorption of both drugs. In the pyridopyrimidines, the morpholinoalkoxy side chains had proved to be about the best at improving both solubility and cellular penetration, and such a side chain was also present in Iressa. Therefore, morpholinopropoxy was among the first solubilizing side chains put onto the 7-position of 6-acrylamides to give the analogues **99** and **100** (Chart 12) (CI-1033).²⁴⁶ These compounds both proved to have first rate activity against isolated enzyme (IC₅₀s 3 and 1.5 nM, respectively), EGFR autophosphorylation (IC₅₀s 5.3 and 1.7 nM, respectively), and ErbB-2 (IC₅₀s 6.4 and 5 nM, respectively). **100** also showed IC₅₀s for inhibition of ErbB-3 and ErbB-4 autophosphorylation of 14 and 10 nM, respectively. **100** has also been shown to be an irreversible inhibitor with very similar mechanistic characteristics to **95**, but its physical properties are much better. CI-1033 is the bis-hydrochloride salt of **100** and has an aqueous solubility of 5 mg/mL and an oral bioavailability of 30% in a rat, with a plasma half-life of 4.8 h. When the pharmacodynamic inhibition of EGFR in A431 tumors was examined in vivo, the effect was very long lasting, with a single 40 mg/kg oral dose retaining 75% inhibition of the enzyme even after 72 h.

Compound **99** showed an exceptional profile in vivo. It was active in 7/10 tumor models it was tested against and in five of those tumors produced either complete cytostasis or actual tumor regressions, as long as dosing was continued. In our benchmark A431 tumor line, regressions were seen even at 5 mg/

kg/day oral dosing. Gratifyingly, **100** proved to be even more potent: in A431 xenografts producing cytostasis at 2.5 mg/kg/day and long-lasting (but not permanent) tumor regressions at 5 mg/kg/day dosed orally. With IV dosing, cytostasis was seen at 1 mg/kg/day. Excellent activity has also been seen in lung, breast, ovarian, glioblastoma, colonic, and pancreatic tumor models. In the A431 tumors, similar efficacy was seen when the compound was dosed orally 5 days a week at 5 mg/kg/dose, twice a week at 12 mg/kg/dose, or once a week at 25 mg/kg. Two recent papers have shown that in cell culture CI-1033 produces good synergy with radiation²⁴⁷ and the topoisomerase I inhibitors SN-38 and Topotecan by inhibiting the induction of a drug efflux pump BCRP.²⁴⁸ The initial profiling of **100** was so impressive and our confidence in our SAR, both enzyme and cellular, and in the rigor and accuracy of our animal models was so strong that **100** was moved forward into development when only 5 g of the material had been made in total. This represented an enormous gamble on behalf of Parke-Davis research management, as the list of unanswered questions about **100** was enormous. For example, there was no demonstration that the chemistry could be scaled up to a feasible manufacturing process, and nothing was known of the toxicity of this deliberately designed alkylating agent.

CI-1033 is a quinazolinone, and we had spent most of our effort in pyridopyrimidines, partially because of the hoped for better profile but mainly because of the patenting difficulties. As the last few paragraphs illustrate, these irreversible quinazolines have an excellent profile and meet all of our initial design goals, including the one area where pyridopyrimidines had a perceived advantage, potency against other ErbBs. We also believed that unsaturated conjugated side chains were a patentable invention in themselves, and we filed patent claims on both quinazolines and pyridopyrimidines. We have also made irreversible pyridopyrimidines, which have excellent profiles, but that story is not yet ready for prime time. Two months before our patent applications were due to publish, a patent application appeared from Wyeth-Ayerst,²⁴⁹ which claimed much of the same subject matter as our quinazolinone claims. This was a real surprise and probably the least pleasant of all the patent surprises we had received as we had no idea that they were even in the kinase inhibitor area let alone the middle of "our" SAR. As their patent has since been granted,²⁴⁹ we have lost inhibitors such as **95**, **96**, and **98** to them. They have since published on the most interesting compound in their original application, the butynamide **101**,²⁵⁰ which has similar potent in vivo activity to **95**. Examination of the Wyeth-Ayerst claims showed that

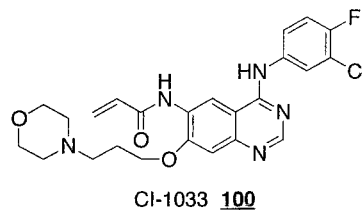
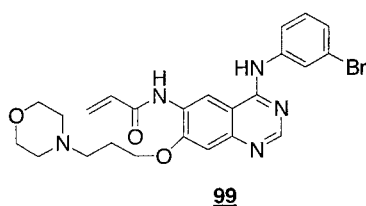
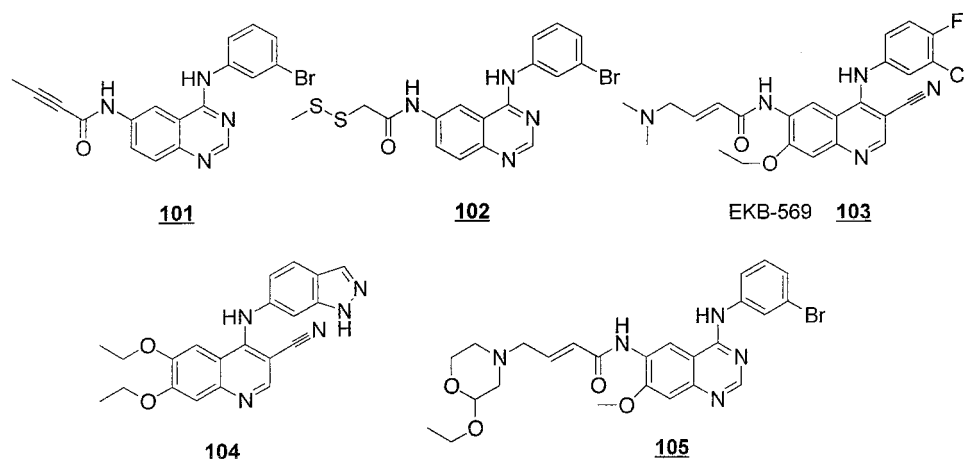
Chart 12

Chart 13



they did not include amine-containing side chains, which were a major focus in our application. Their second application did include such side chains and contained a very interesting twist on the covalent trapping, which probably brings the field full circle back to the indolinethione inhibitors. They used mixed disulfides, such as quinazoline **102**, as a thiol trap. This compound had a 1 pM IC_{50} against the isolated enzyme, presumably because of formation of a disulfide bond with EGFr, but in cells it was enormously weaker ($IC_{50} > 10 \mu\text{M}$),²⁵¹ presumably reflecting the difficulty of using sulfide exchange reactions controllably in the cellular environment. Combining this with their previous cyanoquinoline work,²⁵² the Wyeth-Ayerst team reported on their likely clinical candidate in this area, the irreversible cyanoquinoline EKB-569, **103**.²⁵³ This compound has an IC_{50} for EGFr of 38 nM but is only a 1.25 μM inhibitor of ErbB-2, showing both some Src and MEK/ERK inhibitory activity. It has good bioavailability and a 2 h plasma half-life in mice, and alone and in combination with a COX-2 inhibitor sulindac, it greatly decreased tumors in the *min* mouse, which has a major tumor-suppressor gene, APC, knocked out. These results are in sharp contrast to a study published at the same time using the already proven acrylamidoquinazoline **96**, which showed no effects on tumor formation in the *min* mouse, despite profound inhibition of EGFr in the colonic epithe-

lium.²⁵⁴ However, very similar cyanoquinolines, e.g., **104** (Chart 13) (cf. **88** and **89**) have been claimed by the Wyeth-Ayerst team as reversible, low-nanomolar inhibitors of the MEK dual-function kinase, and other analogues are described in their publications as both c-SRC inhibitors^{255,256} and MEK inhibitors,²⁵⁷ suggesting the experiment may not be as mechanistically clean as it needs to be. Recently, a patent application for 6-acrylamidoquinazolines was published from Boehringer-Ingelheim,²⁵⁸ showing a very similar mixture of Michael acceptors and solubilizing amine functionality to the above-mentioned clinical candidates, as exemplified by **105**, a 1.8 nM inhibitor of EGFr

As indicated above, our initial discovery of how to alkylate EGFr was made by systematically putting alkylators around the template. Some time later, molecular modeling came up with a proposed binding mode for the reversible inhibitors.¹⁹⁸ When the irreversible inhibitors were modeled into the enzyme using this binding mode, the results were quite startling. The C3-vinyl carbon of the 6-acrylamides was placed only about 4 Å from the cysteinyl sulfur atom, essentially within van der Waals radii of one another, with a near perpendicular potential reaction trajectory, suggesting that the placement was nearly ideal to induce a Michael reaction.²⁴³ This is dramatically shown in Figures 20–22. Figure 20 shows the fit of CI-1033 binding in the active site. In Figure

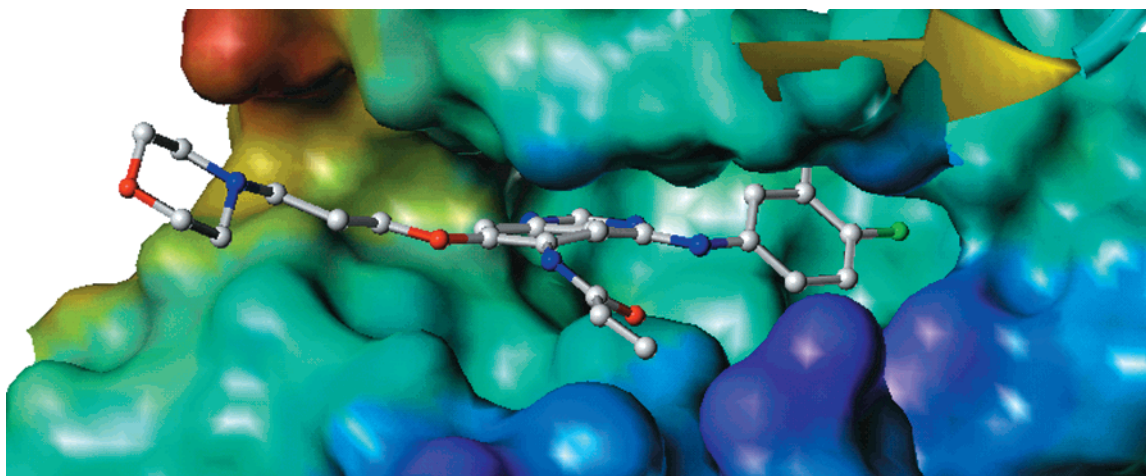


Figure 20. CI-1033 noncovalently bound into EGFr active site.

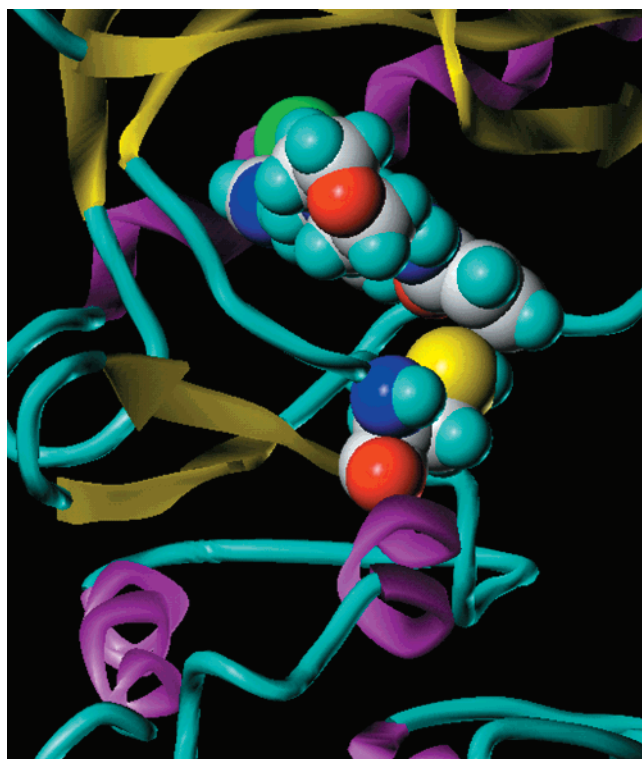


Figure 21. Juxtaposition of CI-1033 and Cys⁷⁷³ of EGFR.

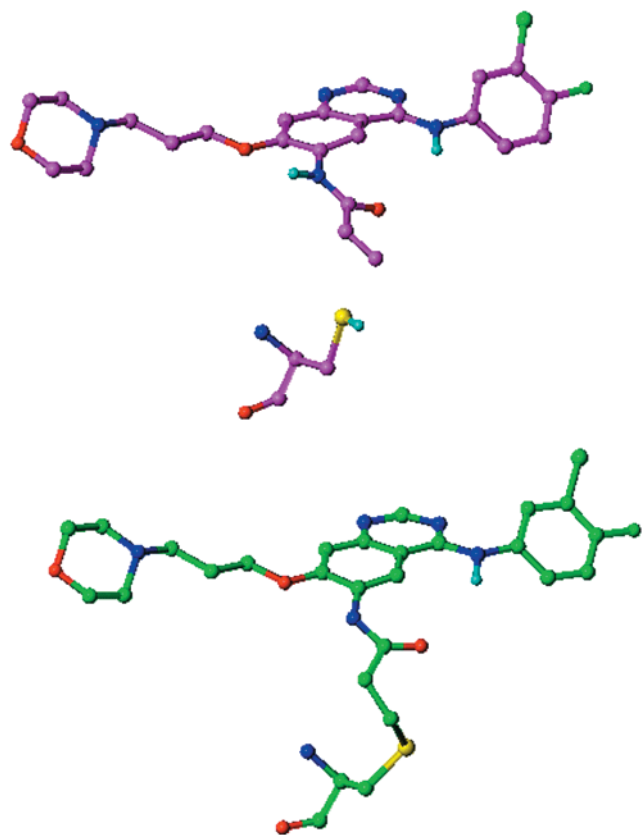


Figure 22. CI-1033 and Cys⁷⁷³: before and after Michael addition.

21, the placement of the CI-1033 side chain with respect to Cys⁷⁷³ is shown, emphasizing that the terminal vinylic carbon is only 3.5 Å from the sulfur atom, and Figure 22 superimposes CI-1033 structures before and after the Michael addition has taken place, emphasizing how little molecular motion is

required for the reaction. In contrast, the corresponding distance for the 7-acrylamides was 8 Å, suggesting that considerable motion would be required before a Michael reaction could take place, explaining the much slower reaction rate.

D. Summary

The development of kinase inhibitors has proved to be very difficult. However, there are now many kinase inhibitors in the clinic, mainly as anticancer strategies. The most dramatic results to date have been obtained with the BCR-ABL inhibitor STI-571, where the background science suggests that a relatively clean shot at the target exists. The most equivocal area remains the PKC inhibitors, where the science started simple but became very complex as people tried to develop agents while the assumptions they were working on changed and fragmented. In the EGFR field, there are at least a half-dozen clinical candidates, and two of these, Iressa and OSI-774, have published data showing clinical efficacy. Several companies have developed very potent and selective inhibitors of the enzyme activity of the EGFR family of RTKs. Both our group and the Wyeth-Ayerst group have achieved very long-lasting enzyme inhibition by designing in a chemically reactive trapping agent into the inhibitors, taking advantage of an unusually placed sulfur atom in the enzyme active site. Selectivity and adequate rates of alkylation have been kept acceptable by using a very poor Michael acceptor but placing it very precisely with respect to the nucleophile. The inhibitors have been given good physicochemical properties by incorporating solubilizing side chains, and this has led to good pharmacokinetic and pharmacodynamic profiles. In our hands, this overall optimization of several properties has led to a compound with exceptional potency in *in vivo* cancer models, and this candidate CI-1033 has been taken forward into clinical development.

E. Epilogue

Although our story comes to a natural end with the description of the discovery and selection of CI-1033, the truth is that it is only the beginning of a much more intense, expensive, and difficult evaluation of the compound. Before CI-1033 could go forward into Phase I trials, a synthesis which produced the compound on the kilogram scale and with a very low, reproducible byproduct profile had to be developed. Formulators had to take this possibly chemically reactive compound and put it into a dose form, which delivered the drug efficiently and prevented it from decomposing appreciably on a time scale of months to years. The compound had to go through toxicology to find out if it had toxicities, which would make it unusable in patients at doses expected to be efficacious, and what toxicity problems would be likely to be manifested in early clinical trials. In cancer biology, experiments continue with new tumors, new dosage forms, and new dosage schedules as efforts are made to work out the most efficient clinical use of the drug. Most importantly but most difficultly, combinations with cytotoxic agents and radiation are

being examined where tumor type, cytotoxic drug, dose of each agent, and schedule for each agent each have to be optimized separately in in vivo models. All of these data have to be documented and sent to the FDA for the various stages of approval and to clinicians who will actually dose patients. Currently, the compound has jumped the hurdles in front of it and has been in Phase I patient trials for some time, and the effort to develop the drug has already exceeded the total effort to find the drug initially. There is no guarantee that the compound will be found efficacious in Phase II/III trials or that the ideal drug cocktails containing it will be identified. Even if the compound works, there is no guarantee that it will prove to be better in the clinic than its rivals, known or unknown, and finally no guarantee that if it makes it to the market, that it will ever sell well enough to recoup its development costs. If this review had been written by anyone else developing kinase inhibitors, they could easily have ended their story on a similar cautionary note, as no kinase inhibitor is yet on the market, helping patients, and generating revenue.

VIII. Acknowledgments

I thank Professor Natalie Ahn for inviting me to contribute to this special edition of *Chemical Reviews*. I also acknowledge some of my key colleagues in developing the work described in this review. In chemistry, I acknowledge Ellen Dobrusin, Hollis Showalter, Denny Macnamara, Hairong Zhou, and Tom Winters. In cancer biology, I acknowledge David Fry, Alan Kraker, Wayne Klohs, Dick Leopold, Bill Elliott, and Pat Vincent. My collaborators in the Cancer Research Laboratory of the University of Auckland in New Zealand, especially Bill Denny, Gordon Rewcastle, Andrew Thompson, and Brian Palmer, also contributed enormously, doing much of the novel chemistry. For molecular modeling, I acknowledge Suzanne Trumpp-Kallmeyer and Jack Bikker, who also kindly provided the illustrations, and Ron Rubin for crystal structures. I also acknowledge the Cancer Research Campaign of New Zealand for partial support of this work.

IX. References

- Boschelli, D. H. *Drugs Future* **1999**, *24*, 515.
- García Echeverría, C.; Traxler, P.; Evans, D. B. *Med. Res. Rev.* **2000**, *20*, 28.
- Lawrence, D. S.; Niu, J. K. *Pharmacol. Ther.* **1998**, *77*, 81.
- Levitt, M. L.; Koty, P. P. *Invest. New Drugs* **1999**, *17*, 213.
- Stover, D. R.; Lydon, N. B.; Nunes, J. J. *Curr. Opin. Drug Discuss. Dev.* **1999**, *2*, 274.
- Traxler, P. M. *Expert Opin. Ther. Pat.* **1997**, *7*, 571.
- Traxler, P. *Exp. Opin. Ther. Pat.* **1998**, *8*, 1599.
- Traxler, P.; Furet, P. *Pharmacol. Ther.* **1999**, *82*, 195.
- Klohs, W. D.; W., F. D.; Kraker, A. J. *Curr. Opin. Oncol.* **1997**, *9*, 562.
- Sedlacek, H. H. *Drugs* **2000**, *59*, 435.
- DeMattia, J. A.; Zhang, W.; Couldwell, W. T. *CNS Drugs* **1999**, *12*, 421.
- Cohen, P. *Curr. Opin. Chem. Biol.* **1999**, *3*, 459.
- Taylor, S. S.; RadzioAndzelm, E. *Curr. Opin. Chem. Biol.* **1997**, *1*, 219.
- Wilkinson, S. E.; Harris, W. *Emerging Drugs* **2000**, *5*, 287.
- Al Obeidi, F. A.; Lam, K. S. *Oncogene* **2000**, *19*, 5690.
- Haluska, P.; Adjei, A. A. *Curr. Opin. Invest. Drugs* **2001**, *2*.
- Morin, M. J. *Oncogene* **2000**, *19*, 6574.
- Dumas, J. *Exp. Opin. Ther. Pat.* **2001**, *11*, 405.
- Buolamwini, J. K. *Curr. Pharm. Des.* **2000**, *6*, 379.
- Coleman, K. G.; Lyssikatos, J. P.; Yang, B. V. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press Inc: San Diego, CA, 1997; Vol. 32.
- Fry, D. W.; Garrett, M. D. *Curr. Opin. Oncol., Endocr. Metab. Invest. Drugs* **2000**, *2*, 40.
- Mani, S.; Wang, C.; Wu, K.; Francis, R.; Pestell, R. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1849.
- Meijer, L.; Kim, S. H. In *Cell Cycle Control*; Dunphy, W. G., Ed.; Academic Press Inc.: San Diego, CA, 1997; Vol. 283.
- Walker, D. H. In *Cyclin Dependent Kinase (Cdk)*; Vogt, P. K., Reed, S. L., Eds.; Springer-Verlag: Berlin, 1998; Vol. 227.
- Senderowicz, A. M.; Sausville, E. A. *J. Natl. Cancer Inst.* **2000**, *92*, 376.
- Rosania, G. R.; Chang, Y. T. *Exp. Opin. Ther. Pat.* **2000**, *10*, 215.
- Adams, J. L.; Lee, D. *Curr. Opin. Drug Discuss. Dev.* **1999**, *2*, 96.
- Salituro, F. G.; Germann, U. A.; Wilson, K. P.; Bemis, G. W.; Fox, T.; Su, M. S. S. *Curr. Med. Chem.* **1999**, *6*, 807.
- Hanson, G. J. *Expert Opin. Ther. Pat.* **1997**, *7*, 729.
- Boehm, J. C.; Adams, J. L. *Exp. Opin. Ther. Pat.* **2000**, *10*, 25.
- Wang, Z. L.; Canagarajah, B. J.; Boehm, J. C.; Kassisa, S.; Cobb, M. H.; Young, P. R.; AbdelMeguid, S.; Adams, J. L.; Goldsmith, E. J. *Structure* **1998**, *6*, 1117.
- Hamby, J. M.; Showalter, H. D. H. *Pharmacol. Ther.* **1999**, *82*, 169.
- Shawver, L. K.; Lipson, K. E.; Fong, T. A. T.; McMahon, G.; Plowman, G. D.; Strawn, L. M. *Drug Discovery Today* **1997**, *2*, 50.
- Showalter, H. D. H.; Kraker, A. J. *Pharmacol. Ther.* **1997**, *76*, 55.
- Ruggeri, B. A.; Miknyoczki, S. J.; Singh, J.; Hudkins, R. L. *Curr. Med. Chem.* **1999**, *6*, 845.
- Myers, M. R.; He, W.; Hulme, C. *Curr. Pharm. Des.* **1997**, *3*, 473.
- Takai, Y.; Kishimoto, M.; Inoue, Y.; Nishizuka, Y. *J. Biol. Chem.* **1977**, *252*, 7603.
- Tamaoki, T.; Nomato, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; F., T. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397.
- Gescher, A. *Crit. Rev. Oncol. Hematol.* **2000**, *34*, 127.
- Caponigro, F.; French, R. C.; Kaye, S. B. *Anti Cancer Drug* **1997**, *8*, 26.
- Harris, W.; Wilkinson, S. E.; Nixon, J. S. *Expert Opin. Ther. Pat.* **1997**, *7*, 63.
- Jarvis, W. D.; Grant, S. *Invest. New Drugs* **1999**, *17*, 227.
- Nishizuka, Y. *Science* **1992**, *258*, 607.
- Dekker: L. V.; Parker, P. J. *Trends Biochem. Sci.* **1994**, *19*, 73.
- Palmer, R. H.; Ridden, J.; Parker, P. J. *FEBS Lett.* **1994**, *356*, 5.
- Hofmann, J. *FASEB J.* **1997**, *11*, 649.
- Nishizuka, Y. *Nature* **1988**, *334*, 661.
- Nishizuka, Y. *FASEB J.* **1995**, *9*, 484.
- Nakanishi, H.; Brewer, K. A.; Exton, J. H. *J. Biol. Chem.* **1993**, *268*, 13.
- Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917.
- Pettit, G. R. *Prog. Org. Chem. Nat. Prod.* **1991**, *57*, 154.
- Lee, H. W.; Smith, L.; Pettit, G. R.; Vinitsky, A.; Smith, J. B. *J. Biol. Chem.* **1996**, *271*, 20973.
- Young, S.; Parker, P. J.; Ullrich, A.; Stabel, S. *Biochem. J.* **1987**, *244*, 711.
- Gescher, A. *Br. J. Cancer* **1992**, *66*, 10.
- Connolly, J. M.; Rose, D. P. *Int. J. Oncol.* **1997**, *10*, 71.
- Lu, Z. M.; Hornia, A.; Jiang, Y. W.; Zang, Q.; Ohno, S.; Foster, D. A. *Mol. Cell Biol.* **1997**, *17*, 3418.
- Doornbos, R. P.; Theelen, M.; van der Hoeven, P. C. J.; van Blitterswijk, W. J.; Verkleij, A. J.; Henegouwen, P. *J. Biol. Chem.* **1999**, *274*, 8589.
- Kieser, A.; Seitz, T.; Adler, H. S.; Coffey, P.; Kremmer, E.; Crespo, P.; Gutkind, J. S.; Henderson, D. W.; Mushinski, J. F.; Kolch, W.; Mischak, H. *Gene Dev.* **1996**, *10*, 1455.
- Cacace, A. M.; Guadagno, S. N.; Krauss, R. S.; Fabbro, D.; Weinstein, I. B. *Oncogene* **1993**, *8*, 2095.
- Murray, N. R.; Fields, A. P. *J. Biol. Chem.* **1997**, *272*, 27521.
- Koya, D.; King, G. L. *Diabetes* **1998**, *47*, 859.
- Tamaoki, T. In *Protein Phosphorylation*; Sefton, B. M., Hunter, T., Eds.; Academic Press Inc.: San Diego, CA, 1998.
- Mehta, A. B.; Virchis, A. E.; Jones, D. T.; Hart, S. M.; Wickremasinghe, R. G.; Prentice, H. G.; Yong, K. L.; Csemak-Renner, A. M. K.; Ganeshaguru, K. *Blood* **1997**, *90*, 3634.
- Kleinschroth, J.; Hartenstein, J.; Rudolph, C.; Schachtele, C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 55.
- Kleinschroth, J.; Hartenstein, J.; Rudolph, C.; Schachtele, C. *Bioorg. Med. Chem. Lett.* **1993**, *5*, 1959.
- Bit, R. A.; Davis, P. D.; Elliott, L. H.; Harris, W.; Hill, C. H.; Hurst, S. A.; Keech, E.; Kumar, H.; Lawton, G.; Maw, A.; Nixon, J. S.; E., W. S. *J. Med. Chem.* **1993**, *36*, 21.

- (67) Jirousek, M. R.; Gillig, J. R.; Gonzalez, C. M.; Heath, W. F.; McDonald, J. H.; Neel, D. A.; Rito, C. J.; Singh, U.; Stramm, I. E.; Melikian-Badalian, A.; Baevsky, M.; Ballas, L. M.; Hall, S. E.; Winneroski, L. L.; Faul, M. M. *J. Med. Chem.* **1996**, *39*, 2664.
- (68) Hu, H. *Drug Discovery Today* **1996**, *1*, 438.
- (69) Cong, F.; Goff, S. P. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13819.
- (70) Sawyers, C. L.; McLaughlin, J.; Goga, A.; Havlik, M.; Witte, O. *Cell* **1994**, *77*, 121.
- (71) Welch, P. J.; Wang, J. Y. *J. Cell* **1993**, *75*, 779.
- (72) Goga, A.; Liu, X.; Hambuch, T. M.; Senechal, K.; Major, E.; Berk, A. J.; Witte, O. N.; Sawyers, C. L. *Oncogene* **1995**, *11*, 791.
- (73) Sionov, R. V.; Moallem, E.; Berger, M.; Kazaz, A.; Gerlitz, O.; Ben Neriah, Y.; Oren, M.; Haupt, Y. *J. Biol. Chem.* **1999**, *274*, 8371.
- (74) Gong, J. G.; Costanzo, A.; Yang, H. Q.; Melino, G.; Kaelin, W. G.; Leverro, M.; Wang, J. Y. *Nature* **1999**, *399*, 806.
- (75) Yuan, Z. M.; Shioya, H.; Ishiko, T.; Sun, X. G.; Gu, J. J.; Huang, Y. Y.; Lu, H.; Kharbanda, S.; Weichselbaum, R.; Kufe, D. *Nature* **1999**, *399*, 814.
- (76) Baskaran, R.; Wood, L. D.; Whitaker, L. L.; Canman, C. E.; Morgan, S. E.; Xu, Y.; Barlow, C.; Baltimore, D.; WynshawBoris, A.; Kastan, M. B.; Wang, J. Y. *Nature* **1997**, *387*, 516.
- (77) Chopra, R.; Pu, Q. Q.; Elefant, A. G. *Blood Rev.* **1999**, *13*, 211.
- (78) Mcwhirter, J. R.; Galasso, D. L.; Wang, J. Y. *J. Mol. Cell Biol.* **1993**, *13*, 7587.
- (79) Voss, J.; Posern, G.; Hannemann, J. R.; Wiedemann, L. M.; Turhan, A. G.; Poirel, H.; Bernard, O. A.; Adermann, K.; Kardinal, C.; Feller, S. M. *Oncogene* **2000**, *19*, 1684.
- (80) Pendergast, A. M.; Quilliam, L. A.; Cripe, L. D.; Bassing, C. H.; Dai, Z. H.; Li, N. X.; Batzer, A.; Rabun, K. M.; Der, C. J.; Schlessinger, J.; Gishizky, M. L. *Cell* **1993**, *75*, 175.
- (81) Goga, A.; McLaughlin, J.; Afar, D. E. H.; Saffran, D. C.; Witte, O. N. *Cell* **1995**, *82*, 981.
- (82) Gishizky, M. L. *Cytokines Mol. Ther.* **1996**, *2*, 251.
- (83) Zou, X. M.; Rudchenko, S.; Wong, K. K.; Calame, K. *Gene Dev.* **1997**, *11*, 654.
- (84) Skorski, T.; Bellacosa, A.; Nieborowska-Skorska, M.; Majewski, M.; Martinez, R.; Choi, J. K.; Trotta, R.; Wlodarski, P.; Perrotti, D.; Chan, T. O.; Wasik, M. A.; Tsichlis, P. N.; Calabretta, B. *EMBO J.* **1997**, *16*, 6151.
- (85) Amarante-Mendes, G. P.; McGahon, A. J.; Nishioka, W. K.; Afar, D. E. H.; Witte, O. N.; Green, D. R. *Oncogene* **1998**, *16*, 1383.
- (86) Lloyd, A. W. *Drug Discovery Today* **1997**, *2*, 162.
- (87) Sawyers, C. L.; Druker, B. *Cancer J. Sci. Am.* **1999**, *5*, 63.
- (88) Carroll, M.; Ohno-Jones, S.; Tamura, S.; Buchdunger, E.; Zimmermann, J.; Lydon, N. B.; Gilliland, D. G.; Druker, B. *J. Blood* **1997**, *90*, 4947.
- (89) Zimmermann, J.; Buchdunger, E.; Mett, H.; Meyer, T.; Lydon, N. B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 187.
- (90) Wang, W.-L.; Healy, M. E.; Sattler, W.; Verma, S.; Lin, J.; Maulik, G.; Stiles, C. D.; Griffin, J. D.; Johnson, B. E.; Salgia, R. *Oncogene* **2000**, *19*, 3521.
- (91) Heinrich, M. C.; Griffith, D. J.; Druker, B. J.; Wait, C. L.; Ott, K. A.; Zigler, A. J. *Blood* **2000**, *96*, 925.
- (92) Schindler, T.; Bornmann, W.; Pellicina, P.; Miller, W. T.; Clarkson, B.; Kuriyan, J. *Science* **2000**, *289*, 1938.
- (93) Alessi, D. R.; Cuenda, A.; Cohen, P.; Dudley, D. R.; Saltiel, A. R. *J. Biol. Chem.* **1995**, *270*, 27489.
- (94) Druker, B. J.; Tamura, S.; Buchdunger, E.; Ohno, S.; Segal, G. M.; Fanning, S.; Zimmermann, J.; Lydon, N. B. *Nat. Med.* **1996**, *2*, 561.
- (95) Marley, S. B.; Deininger, M. W. N.; Davidson, R. J.; Goldman, J. M.; Gordon, M. Y. *Exp. Hematol.* **2000**, *28*, 551.
- (96) Buchdunger, E.; Zimmermann, J.; Mett, H.; Meyer, T.; Muller, M.; Druker, B. J.; Lydon, N. B. *Cancer Res.* **1996**, *56*, 100.
- (97) Horita, M.; Andreu, E. J.; Benito, A.; Arbona, C.; Sanz, C.; Benet, I.; Prosper, F.; Fernandez Luna, J. L. *J. Exp. Med.* **2000**, *191*, 977.
- (98) Druker, B. J.; Lydon, N. B. *J. Clin. Invest.* **2000**, *105*, 3.
- (99) Goldman, J. M. *Lancet* **2000**, *355*, 1031.
- (100) Donato, N. J.; Talpaz, M. *Clin. Cancer Res.* **2000**, *6* (8), 2965.
- (101) Weisberg, E.; Griffin, J. D. *Blood* **2000**, *95*, 3498.
- (102) Hollan, T. *The Scientist* **2000**, *15*.
- (103) Krystal, G. W.; Honsawek, S.; Litz, J.; Buchdunger, E. *Clin. Cancer Res.* **2000**, *6*, 3319.
- (104) Vigneri, P.; Wang, J. Y. *Nat. Med.* **2001**, *7*, 228.
- (105) Wang, J. Y. *J. Oncogene* **2000**, *19*, 5643.
- (106) Newlands, E. S.; Rustin, G. J.; Brampton, M. H. *Br. J. Cancer* **1996**, *74*, 648.
- (107) Fukuda, M.; Asano, S.; Nakamura, T.; Adachi, M.; Yoshida, M.; Yanagida, M.; Nishida, E. *Nature* **1997**, *370*, 308.
- (108) Khazaie, K.; Schirmacher, V.; Lichtner, R. B. *Cancer Metastasis Rev.* **1993**, *12*, 255.
- (109) Raymond, E.; Faivre, S.; Armand, J. P. *Drugs* **2000**, *60*, 15.
- (110) Yarden, Y.; Sliwkowski, M. X. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 127.
- (111) Bridges, A. J. *Curr. Med. Chem.* **1999**, *6*, 825.
- (112) Fry, D. W. *Pharmacol. Ther.* **1999**, *82*, 207.
- (113) Noonberg, S. B.; Benz, C. C. *Drugs* **2000**, *59*, 753.
- (114) Woodburn, J. R. *Pharmacol. Ther.* **1999**, *82*, 241.
- (115) Mendelsohn, J.; Baselga, J. *Oncogene* **2000**, *19*, 6550.
- (116) Uehara, Y.; Murakami, Y.; Mizuno, S.; Kawai, S. *Virology* **1988**, *164*, 294.
- (117) Uehara, Y.; Hori, M.; Takeuchi, T.; Umezuma, H. *Jpn. J. Cancer Res. (Gann)* **1985**, *76*, 672.
- (118) Miller, P.; Schnur, R. C.; Barbacci, E.; Moyer, M. P.; Moyer, J. D. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 1313.
- (119) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. *Cell* **1997**, *89*, 239.
- (120) Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260.
- (121) Murakami, Y.; Mizuno, S.; Uehara, Y. *Biochem. J.* **1994**, *301*, 63.
- (122) Sepplorezino, L.; Ma, Z. P.; Leibold, D. E.; Vinitzky, A.; Rosen, N. *J. Biol. Chem.* **1995**, *270*, 16580.
- (123) Schulte, T. W.; Blagosklonny, M. V.; Romanova, L.; Mushinski, J. F.; Monia, B. P.; Johnston, J. F.; Nguyen, P.; Trepel, J.; Neckers, L. M. *Mol. Cell Biol.* **1996**, *16*, 5839.
- (124) Supino-Rosin, L.; Yoshimura, A.; Yarden, Y.; Elazar, Z.; Neumann, D. *J. Biol. Chem.* **2000**, *275*, 21850.
- (125) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; Moyer, J. D.; Diorio, C. I.; Barbacci, E. G.; Miller, P. E.; O'Brien, A. T.; Morin, M. J.; Foster, B. A.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, M. P. *J. Med. Chem.* **1995**, *38*, 3806.
- (126) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; Diorio, C. I.; Barbacci, E. G.; Miller, P. E.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, J. D.; Moyer, M. P. *J. Med. Chem.* **1995**, *38*, 3813.
- (127) Zheng, F. F.; Kuduk, S. D.; Chiosis, G.; Munster, P. N.; Sepp Lorezino, L.; Danishefsky, S. J.; Rosen, N. *Cancer Res.* **2000**, *60*, 2090.
- (128) Takahashi, H.; Ossada, H.; Koshino, H.; Sasaki, M.; Onose, R.; Nakakoshi, M.; Yoshihama, M.; Isono, K. *J. Antibiot.* **1992**, *45*, 1414.
- (129) Osada, H.; Kikuchi, K.; Makishima, F.; Isono, K. *Oncol. Res.* **1994**, *6*, 11.
- (130) Roussakis, C.; Charrier, J.; Riou, D.; Biard, J. F.; Malochet, C.; Mefflah, K.; Verbist, J. F. *Anti-Cancer Drug Des.* **1994**, *9*, 119.
- (131) Kreuter, M.-H.; Leake, R. E.; Rinaldi, F.; Muller-Klieser, W.; Maidhof, A.; Muller, W. E. G.; Schroeder, H. C. *Comp. Biochem. Physiol.* **1990**, *97B*, 151.
- (132) Hinterding, K.; Knebel, A.; Herrlich, P.; Waldmann, H. *Biorg. Med. Chem.* **1998**, *6*, 1153.
- (133) Kobayashi, J.; Honma, K.; Sasaki, T.; Tsuda, M. *Chem. Pharm. Bull. Tokyo* **1995**, *43*, 403.
- (134) Okabe, T.; Yoshida, E.; Chieda, S.; Endo, K.; Kamiya, S.; Osada, K.; Tanaka, S.; Okura, A.; Suda, H. *J. Antibiot.* **1994**, *47*, 289.
- (135) Tanaka, S.; Okabe, T.; Chieda, S.; Endo, K.; Kanoh, T.; Okura, A.; Yoshida, E. *Jpn. J. Cancer Res.* **1994**, *85*, 253.
- (136) Cassinelli, G.; Lanzi, C.; Pensa, T.; Gambetta, R. A.; Nasini, G.; Cuccuru, G.; Cassinis, M.; Pratesi, G.; Polizzi, D.; Tortoreto, M.; Zunino, F. *Biochem. Pharmacol.* **2000**, *59*, 1539.
- (137) Dewitte, P.; Agostinis, P.; Vanlint, J.; Merlevede, W.; Vandenhede, J. R. *Biochem. Pharmacol.* **1993**, *46*, 1929.
- (138) Kobayashi, J.; Madono, T.; Shigemori, H. *Tetrahedron* **1995**, *51*, 10867.
- (139) Copp, B. R.; Fairchild, C. R.; Cornell, L.; Casazza, A. M.; Robinson, S.; Ireland, C. M. *J. Med. Chem.* **1998**, *41*, 3909.
- (140) Akiyama, T.; Ishida, J.; Nakagawa, S.; Ogawara, H.; Watanabe, S. I.; Itoh, N.; Shibuy, M.; Fukami, Y. *J. Biol. Chem.* **1987**, *262*, 5592.
- (141) Agullo, G.; GametPayrastrre, L.; Manenti, S.; Viala, C.; Remesy, C.; Chap, H.; Payrastrre, B. *Biochem. Pharmacol.* **1997**, *53*, 1649.
- (142) Kakeya, H.; Imoto, M.; Tabata, Y.; Iwami, J.; Matsumoto, H.; Nakamura, K.; Koyano, T.; Tadano, K.; Umezawa, K. *FEBS Lett.* **1993**, *320*, 169.
- (143) Zi, X. L.; Grasso, A. W.; Kung, H. J.; Agarwal, R. *Cancer Res.* **1998**, *58*, 1920.
- (144) Liang, Y. C.; Linshiau, S. Y.; Chen, C. F.; Lin, J. K. *J. Cell Biochem.* **1997**, *67*, 55.
- (145) Yang, E. B.; Zhang, K.; Cheng, L. Y.; Mack, P. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 435.
- (146) Zhang, L. S.; Chang, C. J.; Bacus, S. S.; Hung, M. C. *Cancer Res.* **1995**, *55*, 3890.
- (147) Petersen, F.; Fredenhagen, A.; Mett, H.; Lydon, N. B.; Delmendo, R.; Jenny, H. B.; Peter, H. H. *J. Antibiot.* **1995**, *48*, 191.
- (148) Jayasuriya, H.; Koonchanok, N. M.; Geahlen, R. L.; McLaughlin, J. L.; Chang, C. J. *J. Nat. Prod.-Lloydia* **1992**, *55*, 696.
- (149) Umezawa, K.; Imoto, M. *Methods Enzymol.* **1991**, *201*, 379.
- (150) Gazit, A.; Yaish, P.; Gilon, C.; Levitski, A. *J. Med. Chem.* **1989**, *32*, 2344.
- (151) Osherov, N.; Gazit, A.; Gilon, C.; Levitski, A. *J. Biol. Chem.* **1993**, *268*, 11134.
- (152) Yoneda, T.; Lyall, R. M.; Alsina, M. M.; Person, P. E.; Spada, A. P.; Levitski, A.; Zilberstein, A.; Mundy, G. R. *Cancer Res.* **1991**, *51*, 4430.

- (153) Burger, A. M.; Kaur, G.; Alley, M. C.; Supko, J. G.; Malspeis, L.; Grever, M. R.; Sausville, E. A. *Cancer Res.* **1995**, *55*, 2794.
- (154) Wolbring, G.; Hollenberg, M. D.; Schnetkamp, P. P. M. *J. Biol. Chem.* **1994**, *269*, 22470.
- (155) Aflalo, E.; Iftach, S.; Segal, S.; Gazit, A.; Priel, E. *Cancer Res.* **1994**, *54*, 5038.
- (156) Ramdas, L.; McMurray, J. S.; Budde, R. J. A. *Cancer Res.* **1994**, *54*, 867.
- (157) Ramdas, L.; Obeyesekere, N. U.; McMurray, J. S.; Gallick, G. E.; Seifert, W. E.; Budde, R. J. A. *Arch. Biochem. Biophys.* **1995**, *323*, 237.
- (158) Stanwell, C.; Burke, T. R.; Yuspa, S. H. *Cancer Res.* **1995**, *55*, 4950.
- (159) Stanwell, C.; Ye, B.; Yuspa, S. H.; Burke, T. R. *Biochem. Pharmacol.* **1996**, *52*, 475.
- (160) McLeod, H. L.; Brunton, V. G.; Eckardt, N.; Lear, M. J.; Robins, D. J.; Workman, P.; Graham, M. A. *Br J. Cancer* **1996**, *74*, 1714.
- (161) Jones, G. R. N.; Israel, M. S. *Nature* **1970**, *228*, 1315.
- (162) Thompson, A. M.; Rewcastle, G. W.; Tercel, M.; Dobrusin, E. M.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1993**, *36*, 2459.
- (163) Palmer, B. D.; Rewcastle, G. W.; Thompson, A. M.; Boyd, M.; Showalter, H. D. H.; Sercel, A. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1995**, *38*, 58.
- (164) Showalter, H. D. H.; Sercel, A. D.; Leja, B. M.; Wolfangel, C. D.; Ambroso, L. A.; Elliott, W. L.; Fry, D. W.; Kraker, A. J.; Howard, C. T.; Lu, G. H.; Moore, C. W.; Nelson, J. M.; Roberts, B. J.; Vincent, P. W.; Denny, W. A.; Thompson, A. M. *J. Med. Chem.* **1997**, *40*, 413.
- (165) Fry, D. W.; Nelson, J. M. *Anti-Cancer Drug Des.* **1995**, *10*, 607.
- (166) Reddy, S.; Jones, A. D.; Cross, C. E.; Wong, P. S.-Y.; van der Vliet, A. *Biochem. J.* **2000**, *347*, 821.
- (167) Kraker, A. J.; Wemple, M. J.; Moore, C. W. *Proc. 83rd Annu. Meet. AACR* **1992**, *33*, 85.
- (168) Fry, D. W.; Nelson, J. M.; Slintak, V.; Keller, P. R.; Rewcastle, G. W.; Denny, W. A.; Zhou, H.; Bridges, A. J. *Biochem. Pharmacol.* **1997**, *54*, 877.
- (169) Rewcastle, G. W.; Denny, W. A.; Showalter, H. D. H. *Curr. Org. Chem.* **2000**, *4*, 679.
- (170) Barker, A. J.; Davies, D. H. Eur. Pat. Appl. 0520722 A, 1992.
- (171) Ward, W. H. J.; Cook, P. N.; Slater, A. M.; Davies, D. H.; Holdgate, G. A.; Green, L. R. *Biochem. Pharmacol.* **1994**, *48*, 659.
- (172) Gill, G. N.; Weber, W. *Methods Enzymol.* **1987**, *146*, 82.
- (173) Fry, D. W.; Mcmichael, A.; Singh, J.; Dobrusin, E. M.; Mcnamara, D. J. *Peptides* **1994**, *15*, 951.
- (174) Trinkins, U.; Traxler, P. Eur. Pat. Appl. EP 0 516 588 A, 1992.
- (175) Trinkins, U.; Buchdunger, E.; Furet, P.; Kump, W.; Mett, H.; Meyer, T.; Muler, M.; Regenass, U.; Rihs, G.; Lydon, N.; Traxler, P. *J. Med. Chem.* **1994**, *37*, 1015.
- (176) Spada, A. P.; Maguire, M. P.; Persons, P. E.; Myers, M. R. World Pat. Appl. WO92/20642, 1992.
- (177) Maguire, M. P.; Sheets, K. R.; Mcvety, K.; Spada, A. P.; Zilberstein, A. *J. Med. Chem.* **1994**, *37*, 2129.
- (178) Fry, D. W.; Kraker, A. J.; Mcmichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. *Science* **1994**, *265*, 1093.
- (179) Barker, A. J. Eur. Pat. Appl. EP 0 566 226 A1, 1993.
- (180) Barker, A. J. US Pat. 5,457,105, 1995.
- (181) Rewcastle, G. W.; Denny, W. A.; Bridges, A. J.; Zhou, H. R.; Cody, D. R.; Mcmichael, A.; Fry, D. W. *J. Med. Chem.* **1995**, *38*, 3482.
- (182) 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. *J. Med. Chem.* **1996**, *39*, 267.
- (183) Gibson, K. H.; Grundy, W. J.; Godfrey, A. A.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Barker, A. J.; Brown, D. S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2723.
- (184) Thomas, A. P. World Pat. Appl. WO 95/15952, 1995.
- (185) Rewcastle, G. W.; Palmer, B. D.; Thompson, A. M.; Bridges, A. J.; Cody, D. R.; Zhou, H. R.; Fry, D. W.; Mcmichael, A.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 1823.
- (186) Rewcastle, G. W.; Palmer, B. D.; Bridges, A. J.; Showalter, H. D. H.; Li, S.; Nelson, J.; Mcmichael, A.; Kraker, A. J.; Fry, D. W.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 918.
- (187) Barker, A. J. Eur. Pat. Appl. 0635507, 1995.
- (188) Bridges, A. J.; Denny, W. A.; Fry, D. W.; Kraker, A. J.; Meyer, R. F.; Rewcastle, G. W.; Thompson, A. M. World Pat. Appl. WO 95/19774, 1995.
- (189) Traxler, P. M.; Furet, P.; Mett, H.; Buchdunger, E.; Meyer, T.; Lydon, N. *J. Med. Chem.* **1996**, *39*, 2285.
- (190) Rewcastle, G. W.; Bridges, A. J.; Fry, D. W.; Rubin, J. R.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1820.
- (191) Himmelsbach, F.; von Ruden, T.; Dahmann, G.; Metz, T. US Pat. 5,707,989, 1998.
- (192) Nuijen, B.; Bouma, M.; Henrar, R. E. C.; Brauns, U.; Bette, P.; Bult, A.; Beijnen, J. H. *Int. J. Pharm.* **2000**, *194*, 261.
- (193) Himmelsbach, F.; Dahmann, G.; von Ruden, T.; Metz, T. World Pat. Appl. 97/32880, 1997.
- (194) Showalter, H. D. H.; Bridges, A. J.; Zhou, H. R.; Sercel, A. D.; Mcmichael, A.; Fry, D. W. *J. Med. Chem.* **1999**, *42*, 5464.
- (195) Toledo, L. M.; Lydon, N. B. *Structure* **1997**, *5*, 1551.
- (196) Furet, P.; Caravatti, G.; Lydon, N.; Priestle, J. P.; Sowadski, J. M.; Trinkins, U.; Traxler, P. *J. Comput.-Aided Mol. Des.* **1995**, *9*, 465.
- (197) Traxler, P.; Bold, G.; Frei, J.; Lang, M.; Lydon, N.; Mett, H.; Buchdunger, E.; Meyer, T.; Mueller, M.; Furet, P. *J. Med. Chem.* **1997**, *40*, 3601.
- (198) Palmer, B. D.; TrumppKallmeyer, S.; Fry, D. W.; Nelson, J. M.; Showalter, H. D. H.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1519.
- (199) Shewchuk, L.; Hassell, A.; Wisely, B.; Rocque, W.; Holmes, W.; Veal, J.; Kuyper, L. F. *J. Med. Chem.* **2000**, *43*, 133.
- (200) Klapper, L. N.; Kirschbaum, M. H.; Sela, M.; Yarden, Y. *Adv. Cancer Res.* **2000**, *77*, 25.
- (201) Gullick, W. J.; Srinivasan, R. *Breast Cancer Res. Treat.* **1998**, *52*, 43.
- (202) Tzahar, E.; PinkasKramarski, R.; Moyer, J. D.; Kapper, L. N.; Alroy, I.; Levkowitz, G.; Shelly, M.; Henis, S.; Eisenstein, M.; Ratzkin, B. J.; Sela, M.; Andrews, G. C.; Yarden, Y. *EMBO J.* **1997**, *16*, 4938.
- (203) Waterman, H.; Alroy, I.; Strano, S.; Seger, R.; Yarden, Y. *EMBO J.* **1999**, *18*, 3348.
- (204) Kunkel, M. W.; Hook, K. E.; Howard, C. T.; Przybranowski, S.; Roberts, B. J.; Elliott, W. L.; Leopold, W. R. *Invest. New Drugs* **1995**, *13*, 295.
- (205) Woodburn, J. R.; Barker, A. J.; Wakeling, A. E.; Cartledge, S. A.; Davies, D. H.; Valcaccia, B. E. *Proc. 87th Annu. Meet. AACR* **1996**, *37*, Abstract 2665.
- (206) Rewcastle, G. W.; Murray, D. K.; Elliott, W. L.; Fry, D. W.; Howard, C. T.; Nelson, J. M.; Roberts, B. J.; Vincent, P. W.; Showalter, H. D. H.; Winters, R. T.; Denny, W. A. *J. Med. Chem.* **1998**, *41*, 742.
- (207) Thompson, A. M.; Murray, D. K.; Elliott, W. L.; Fry, D. W.; Nelson, J. A.; Showalter, H. D. H.; Roberts, B. J.; Vincent, P. W.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 3915.
- (208) Woodburn, J. R.; Barker, A. J.; Gibson, K. H.; Ashton, S. E.; Wakeling, A. E.; Curry, B. J.; Scarlett, L.; Henthorn, L. R. *Proc. 88th Annu. Meet. AACR* **1997**, *38*, Abstract 4251.
- (209) Ciardiello, F.; Caputo, R.; Bianco, R.; Damiano, V.; Pomatoc, G.; De Placido, S.; Bianco, A. R.; Tortora, G. *Clin. Cancer Res.* **2000**, *6*, 2053.
- (210) Ciardiello, F.; Caputo, R.; Bianco, R.; Pomatoc, G.; Damiano, V.; De Placido, S.; Bianco, A. R.; Tortora, G. *Proc. AACR-NCI-EORTC Int. Conf.* **1999**, Abstract 28.
- (211) Sirotnak, F. M.; Miller, V. A.; Scher, H. I.; Kris, M. G. *Proc. AACR-NCI-EORTC Int. Conf.* **1999**, Abstract 98.
- (212) Basalga, J.; LoRusso, P.; Herbst, R.; Rischin, D.; Ranson, M.; Maddox, A.-M.; Feyereislova, A.; Averbuch, S. *Proc. AACR-NCI-EORTC Int. Conf.* **1999**, Abstract 29.
- (213) Kris, M.; Ranson, M.; Ferry, D.; Hammond, L.; Averbuch, S.; Ochs, J.; Rowinsky, E. *Proc. AACR-NCI-EORTC Int. Conf.* **1999**, Abstract 99.
- (214) Baselga, J.; Averbuch, S. D. *Drugs* **2000**, *60*, 33.
- (215) Arnold, L. D. World Pat. Appl. WO 95/23141, 1995.
- (216) Moyer, J. D.; Barbacci, E. G.; Iwata, K. K.; Arnold, L.; Boman, B.; Cunningham, A.; DiOrto, C.; Doty, J.; Morin, M. J.; Moyer, M. P.; Neveu, M.; Pollack, V. A.; Pustilnik, L. R.; Reynolds, M. M.; Sloan, D.; Theleman, A.; Miller, P. *Cancer Res.* **1997**, *57*, 4838.
- (217) Pollack, V. A.; Savage, D. M.; Baker, D. A.; Tsaparikos, K. E.; Sloan, D. E.; Moyer, J. D.; Barbacci, E. G.; Pustilnik, L. R.; Smolarek, T. A.; Davis, J. A.; Vaidya, M. P.; Arnold, L. D.; Doty, J. L.; Iwata, K. K.; Morin, M. J. *J. Pharm. Exp. Ther.* **1999**, *291*, 739.
- (218) Siu, L. L.; Hidalgo, M.; Nemunaitis, J.; Rizzo, J.; Moczysgemba, J.; Eckhardt, S. G.; Tolcher, A.; Smith, L.; Hammond, L.; Blackburn, A.; Tensfeldt, T.; Silberman, S.; Von Hoff, D. D.; Rowinsky, E. K. *Proc. 35th Annu. Meet. ASCO* **1999**, *18*, 388a Abstract 1498.
- (219) Karp, D. D.; Silberman, S. L.; Tensfeldt, T.; Csudae, R.; Wirth, F.; Gaynes, L.; Posner, M.; Bubley, G.; Koon, H.; Bergman, M.; Pandya, S.; Schnipper, L. E. *Proc. 35th Annu. Meet. ASCO* **1999**, *18*, 388a Abstract 1499.
- (220) Arnold, L. D. World Pat. Appl. WO 95/23141, 1995.
- (221) Myers, M. R.; Setzer, N. N.; Spada, A. P.; Zulli, A. L.; Hsu, C. Y. J.; Zilberstein, A.; Johnson, S. E.; Hook, L. E.; Jacoski, M. V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 417.
- (222) Myers, M. R.; Setzer, N. N.; Spada, A. P.; Persons, P. E.; Ly, C. Q.; Maguire, M. P.; Zulli, A. L.; Cheney, D. L.; Zilberstein, A.; Johnson, S. E.; Franks, C. F.; Mitchell, K. J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 421.
- (223) Barker, A. J. World Pat. Appl. WO 96/16960, 1996.
- (224) Barker, A. J.; Johnstone, C. World Pat. Appl. WO 97/30044 A1, 1997.
- (225) Bridges, A. J.; Cody, D. R.; Zhou, H.; Mcmichael, A.; Fry, D. W. *Bioorg. Med. Chem.* **1995**, *3*, 1651.
- (226) Lydon, N. B.; Mett, H.; Mueller, M.; Becker, M.; Cozens, R. M.; Stover, D.; Daniels, D.; Traxler, P.; Buchdunger, E. *Int. J. Cancer* **1998**, *76*, 154.

- (227) Traxler, P.; Furet, P.; Brill, W. K. Eur. Pat. Appl. EP 0682027 A, 1997.
- (228) Traxler, P.; Buchdunger, E.; Furet, P.; Gschwind, H.-P.; Ho, P.; Mett, H.; O'Reilly, T.; Pfaar, U.; Thomas, H. *Clin. Cancer Res.* **1995**, *5*, 3750s.
- (229) Bruns, C. J.; Solorzano, C. C.; Harbison, M. T.; Ozawa, S.; Tsan, R.; Fan, D.; Abbruzzese, J.; Traxler, P.; Buchdunger, E.; Radinsky, R.; Fidler, I. J. *Cancer Res.* **2000**, *60*, 2926.
- (230) Brown, D. S.; Morris, J., J.; Thomas, A. P. World Pat. Appl. WO 96/15118 A1, 1996.
- (231) Hudson, A. T.; Vile, S.; Barraclough, P.; Franzmann, K. W.; McKeown, S. C.; Page, M. J. World Pat. Appl. WO 96/09294 A1, 1996.
- (232) Cockerill, G. S.; Guntrip, S. B.; McKeown, S. C.; Page, M. J.; Smith, K. J.; Vile, S.; Hudson, A. T.; Barraclough, P.; Franzmann, K. W. World Pat. Appl. WO 97/13771 A1, 1997.
- (233) Cockerill, G. S.; Carter, M. C.; Guntrip, S. B.; Smith, K. J. World Pat. Appl. WO 98/02438 A1, 1998.
- (234) Glennon, K. C.; Affleck, K.; Cai, Z.; Cockerill, G. S.; Dickerson, S.; Gauthier, C. A.; Griffin, R. J.; Keith, B. R.; Knight, W. B.; Lackey, K. E.; Mullin, R. J.; Rusnak, D. W.; Tadepalli, S. M.; Wissel, P. S.; Wood, E. R.; Zhang, Y. *Proc. 92nd Annu. Meet. AACR* **2001**, *42*, 803. Abstract 4310.
- (235) Cockerill, G. S.; E., L. K. World Pat. Appl. WO 01/04111, 2001.
- (236) Keith, B. R.; Allen, P. P.; Alligood, K. J.; Crosby, R. M.; Lackey, K.; Gilmer, T. M.; Mullin, R. J. *Proc. 92nd Annu. Meet. AACR* **2001**, *42*, 803. Abstract 4308.
- (237) Myers, M. R.; Spada, A. P.; Maguire, M. P.; Persons, P. E. World Pat. Appl. WO 96/39145 A1, 1996.
- (238) Wissner, A.; Berger, D. M.; Boschelli, D. H.; Floyd, M. B.; Greenberger, L. M.; Gruber, B. C.; Johnson, B. D.; Mamuya, N.; Nilakantan, R.; Reich, M. F.; Shen, R.; Tsou, H.-R.; Upešlacis, E.; Wang, Y. F.; Wu, B.; Ye, F.; Zhang, N. *J. Med. Chem.* **2000**, *43*, 3244.
- (239) Zhang, N.; Wu, B. Q.; Powell, D.; Wissner, A.; Floyd, M. B.; Kovacs, E. D.; Toral Barza, L.; Kohler, C. *Biorg. Med. Chem. Lett.* **2000**, *10*, 2825.
- (240) Boyle, F. T.; Gibson, K. H.; Poyser, J. P.; Turner, P. World Pat. Appl. 0068201, 2000.
- (241) Zheng, J.; Knighton, D. R.; Ten Eyck, L. F.; Karlsson, R.; Xuong, N.-H.; Taylor, S. S.; Sowadski, J. M. *Biochemistry* **1993**, *32*, 2154.
- (242) Singh, J.; Dobrusin, E. M.; Fry, D. W.; Haske, T.; Whitty, A.; McNamara, D. J. *J. Med. Chem.* **1997**, *40*, 1130.
- (243) 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.; TrumppKallmeyer, S.; Dobrusin, E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12022.
- (244) Smaill, J. B.; Palmer, B. D.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Bridges, A. J.; Zhou, H. R.; Showalter, H. D. H.; Winters, R. T.; Leopold, W. R.; Fry, D. W.; Nelson, J. M.; Sliantak, V.; Elliot, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. *J. Med. Chem.* **1999**, *42*, 1803.
- (245) Vincent, P. W.; Bridges, A. J.; Dykes, D. J.; Fry, D. W.; Leopold, W. R.; Patmore, S. J.; Roberts, B. J.; Rose, S.; Sherwood, V.; Zhou, H. R.; Elliott, W. L. *Cancer Chemother. Pharmacol.* **2000**, *45*, 231.
- (246) Smaill, J. B.; Rewcastle, G. W.; Loo, J. A.; Greis, K. D.; Chan, O. H.; Reyner, E. L.; Lipka, E.; Showalter, H. D. H.; Vincent, P. W.; Elliott, W. L.; Denny, W. A. *J. Med. Chem.* **2000**, *43*, 1380.
- (247) Rao, G. S.; Murray, S.; Ethier, S. P. *Int. J. Radiat. Oncol., Biol., Phys.* **2000**, *48*, 1519.
- (248) Erlichman, C.; Boerner, S. A.; Hallgren, C. G.; Spieker, R.; Wang, X.-Y.; James, C. D.; Scheffer, G. L.; Maliepaard, M.; Ross, D. D.; Billbe, K. C.; Kaufmann, S. H. *Cancer Res.* **2001**, *61*, 739.
- (249) Wissner, A.; Johnson, B. D.; Floyd, M. B.; Kitchen, D. B. US Pat. 5,760,041, 1998.
- (250) 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.; Greenberger, L. M. *Biochem. Pharm.* **1999**, *57*, 917.
- (251) Wissner, A.; Tsou, H.-R.; Johnson, B. D.; Hamann, P. R.; Zhang, N. World Pat. Appl. 9909016, 1999.
- (252) Wissner, A.; Johnson, B. D.; Reich, M. F.; Floyd, M. B.; Kitchen, D. B.; Tsou, H.-R. US Pat. 6,002,008, 1999.
- (253) Torrance, C. J.; Jackson, P. E.; Montgomery, E.; Kinzler, K. W.; Vogelstein, B.; Wissner, A.; Nunes, M.; Frost, P.; Discafani, C. M. *Nat. Med.* **2000**, *6*, 1024.
- (254) Ritland, S. R.; Gendler, S. J.; Burgart, L. J.; Fry, D. W.; Nelson, J. M.; Bridges, A. J.; Andress, L.; Karnes, W. E. *Cancer Res.* **2000**, *60*, 4678.
- (255) Wang, Y. D.; Miller, K.; Boschelli, D. H.; Ye, F.; Wu, B. Q.; Floyd, M. B.; Powell, D. W.; Wissner, A.; Weber, J. M.; Boschelli, F. *Biorg. Med. Chem. Lett.* **2000**, *10*, 2477.
- (256) Boschelli, D. H.; Yang, Y. D.; Ye, F.; Wu, B.; Zhang, N.; Dutia, M.; Powell, D. W.; Wissner, A.; Arndt, K.; Weber, J. M.; Boschelli, F. *J. Med. Chem.* **2001**, *44*, 822.
- (257) Zhang, N.; Wu, B.; Powell, D.; Wissner, A.; Floyd, M. B.; Kovacs, E. D.; Toral-Barza, L.; Kohler, C. *Biorg. Med. Chem. Lett.* **2000**, *10*, 2825.
- (258) Himmelsbach, F.; Langkopf, E.; Jung, B.; Metz, T.; Solca, F.; Blech, S. World Pat. Appl. WO 0051991, 2000.

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