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Acquisition of Inhibitor-Sensitive Protein Kinases through Protein Design

Anthony C. Bishop[†] *and Kevan M. Shokat**[†‡]

DEPARTMENTS OF [†]CHEMISTRY AND [‡]MOLECULAR BIOLOGY, PRINCETON UNIVERSITY, PRINCETON, NJ 08544, USA

ABSTRACT. Protein phosphorylation is the major post-translational modification used by eukaryotic cells to control cellular signaling. Protein kinases have emerged as attractive drug targets because heightened protein kinase activity has been associated with several proliferative diseases, most notably cancer and restenosis. Until now, it has been very difficult to confirm the utility of protein kinases as inhibitor targets because very few small molecules that selectively inhibit one particular kinase are known. Discovery of highly specific kinase inhibitors has been slow because the protein family contains approximately 2000 members, all of which share a conserved active site fold. Recent work in several laboratories has sought to circumvent the problem of kinase structural degeneracy by engineering drug sensitivity into Src family tyrosine kinases and mitogen-activated protein kinases through site-directed mutagenesis. By introducing a unique non-naturally occurring amino acid into a conserved region of the enzyme's binding site, a target protein kinase can be rapidly sensitized to a small molecule. Introduction of the engineered kinase into a cell line or animal model should greatly expedite the investigation of protein kinase inhibition as a viable drug treatment. The purpose of this review is to summarize these recent advances in protein kinase drug sensitization. PHARMACOL. THER. 82(2–3):337–346, 1999.

KEY WORDS. Protein kinase inhibitors, Src family tyrosine kinases, mitogen-activated protein kinases (MAPKs), protein engineering, PP1, pyridinylimidazoles.

CONTENTS

ABBREVIATIONS. EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; PKC, protein kinase C; SAPK, stress-activated protein kinase.

1. INTRODUCTION

Protein phosphorylation is used by all cells as an essential control element of many different cell signaling events (Shokat, 1995). The body of cellular processes regulated by protein phosphorylation is impossible to list. In fact, there may be no cellular signal transduction pathway that is not phospho-regulated in one way or another. Even higher order processes, such as sensory perception and memory, can be analyzed at the level of individual protein phosphorylation events (Philpot *et al.*, 1997).

The first discovery of the role of protein phosphorylation was made in the context of phosphorylase regulation by cyclic AMP in glucose metabolism. Since these seminal studies, hundreds of protein kinases have been discovered using a variety of strategies. The sheer number of protein kinases (roughly 2% of the proteins in the human genome) has made their study a challenge to contemporary biochemical and genetic methods (Hunter, 1987). The interconnectivity of many cell signaling pathways complicates the problem further.

Our laboratory seeks to develop new chemical approaches aimed at deciphering the precise components of the interconnected web of kinase-mediated signal transduction. In recent years, the term "chemical genetics" has been coined to encapsulate the idea that small molecule ligands that are specific for a given gene product would be invaluable tools in dissecting complex biochemical networks

*Corresponding author.

(Mitchison, 1994). Traditionally, medicinal chemists have pursued these same small molecule probes (as drugs) by generating chemical diversity (synthesizing many compounds), which are focused on a particular protein target, one target at a time. The stunning size and conserved active site fold of the protein kinase super-family presents two serious problems for this approach. First, the simple generation of a small molecule inhibitor that is unique for a single family member out of a group of highly homologous proteins that may number as many as 2000 is a daunting chemical problem. When this problem is multiplied by the presence of 2000 target protein kinases, the goal of the chemical genetics vision appears unreachable. In addition, even when it appears that a uniquely specific inhibitor of a given protein kinase has been discovered, there is currently no assay that can confirm the selectivity across the breadth of the kinase super-family. Taken to its extreme, this problem points out the need for up to 2000 *in vitro* assays to unambiguously confirm the selectivity of each putative specific kinase inhibitor. Despite the size of the problem, traditional inhibitor screens have produced a number of highly selective kinase inhibitors. The bulk of the success has come in the identification of inhibitors for the receptor tyrosine kinases, particularly the epidermal growth factor receptor (EGFR) (Fry *et al.*, 1994; Traxler *et al.*, 1997b) and the platelet-derived growth factor receptor (Buchdunger *et al.*, 1995). This work has been reviewed previously (Showalter and Kraker, 1997).

Recently, our laboratory has described a novel combination of synthetic chemistry and genetic manipulation that approaches the chemical genetics problem of protein kinase inhibitor design from an angle opposite that of traditional medicinal chemistry (Bishop *et al.*, 1998). The crux of this strategy is to overcome the problem of genetic redundancy in Src family tyrosine kinase active sites by introducing structural diversity in the target kinase through site-directed mutagenesis. The design of small molecules that specifically bind and inhibit the engineered target is thus greatly facilitated. In addition, recent work in multiple laboratories has shown that point mutations in the ATP-binding sites of multiple mitogen-activated protein kinase (MAPK) family members are sufficient to confer sensitivity to pyridinylimidazole inhibitors, molecules that previously were known to inhibit p38 MAPK selectively (Wilson *et al.*, 1997; Eyers *et al.*, 1998; Gum *et al.*, 1998). The purpose of this review is to summarize these recent advances in the use of combined chemical and genetic approaches for protein kinase inhibitor design, as well as the utility of these advances in studying the cellular roles of individual protein kinases.

2. CHEMICAL GENETIC DESIGN OF Src FAMILY TYROSINE KINASE INHIBITORS

2.1. *Inhibitors of Wild-Type Src Family Tyrosine Kinases*

Members of the Src family of protein tyrosine kinases are vital cytosolic components of the signal transduction machinery in cellular processes as diverse as lymphocyte activation, cell adhesion, and oncogenesis (Bolen *et al.*, 1992). The prototypical Src family members, v-Src and c-Src, have been widely studied as inhibitor targets (Levitzki, 1996). Because of its putative role in human cancer, c-Src may prove to be a potential drug target. Elevated c-Src activity is found in tissue samples taken from human breast, lung, and colon tumors (Levitzki, 1996). In addition, the inhibition of Lck has been investigated extensively because of its importance in the early steps of T-cell receptor-mediated immune response (Li *et al.*, 1991; Faltynek *et al.*, 1995; Hanke *et al.*, 1996). Very few data are available concerning *in vitro* or cellular inhibition of the remaining known Src family kinases (Fyn, Lyn, Hck, Yes, Fgr, Blk, Yrk).

While the field of Src family kinase inhibitors has been reviewed previously (Levitzki, 1996; Showalter and Kraker, 1997), it will be instructive for this review of new inhibitor design approaches to mention a few of the most potent and selective Src family blockers identified to date (see Fig. 1 for structures). Historically, the benzoquinone ansamycins, herbimycin A (**1**) and geldanamycin (**2**), as well as another macrocycle, radicicol (**3**), have received much attention due to their ability to reverse the transformed morphology of fibroblasts that express activated forms of the Src tyrosine kinase (Uehara *et al.*, 1986; Murakami *et al.*, 1988; Kwon *et al.*, 1992). While these natural products do inhibit Src *in vitro*, little has been reported concerning their selectivity between different tyrosine kinases. In addition, the reversion of transformed phenotype due to ansamycin or radicicol treatment recently has been shown to proceed through a mechanism independent of kinase inhibition. From the emerging data, it appears that these molecules bind to heat-shock protein-90 in transformed cell lines and subsequently target the heat-shock protein-90-associated oncogenic tyrosine kinases for proteasomic degradation (Whitesell *et al.*, 1992, 1994; Sepp-Lorenzino *et al.*, 1995; Stebbins *et al.*, 1997; Schulte *et al.*, 1998; Sharma *et al.*, 1998).

Several molecules have appeared in the literature that potently inhibit Src family kinases, but are not able to discriminate between the Src members (or no Src family selectivity is reported). Most notably, Hanke *et al.* (1996) reported that the pyrazolo[3,4-*d*]pyrimidine PP1 (**4**) inhibits both Fyn and Lck at an IC_{50} of ~5 nM. While this molecule is selective with respect to the non-Src family kinases (EGFR, ZAP-70, JAK-2, protein kinase A [PKA]), it is only 4-fold selective with respect to Hck and 30-fold with respect to c-Src. In addition, work in our laboratory has shown that PP1 also effectively inhibits the non-Src family member tyrosine kinase, c-Abl ($IC_{50} \approx 30$ nM) (Y. Liu, A. C. Bishop and K. M. Shokat, unpublished results). In an attempt to discover more potent and selective EGFR blockers, Traxler *et al.* (1997a) have discovered the most potent c-Src inhibitor published to date (**5**, IC_{50} 2 nM). This molecule shows moderate to strong selectivity with respect to EGFR, v-Abl, protein kinase C (PKC)α, and cyclin-dependent kinase-1, but no data for Src family members other than c-Src are reported.

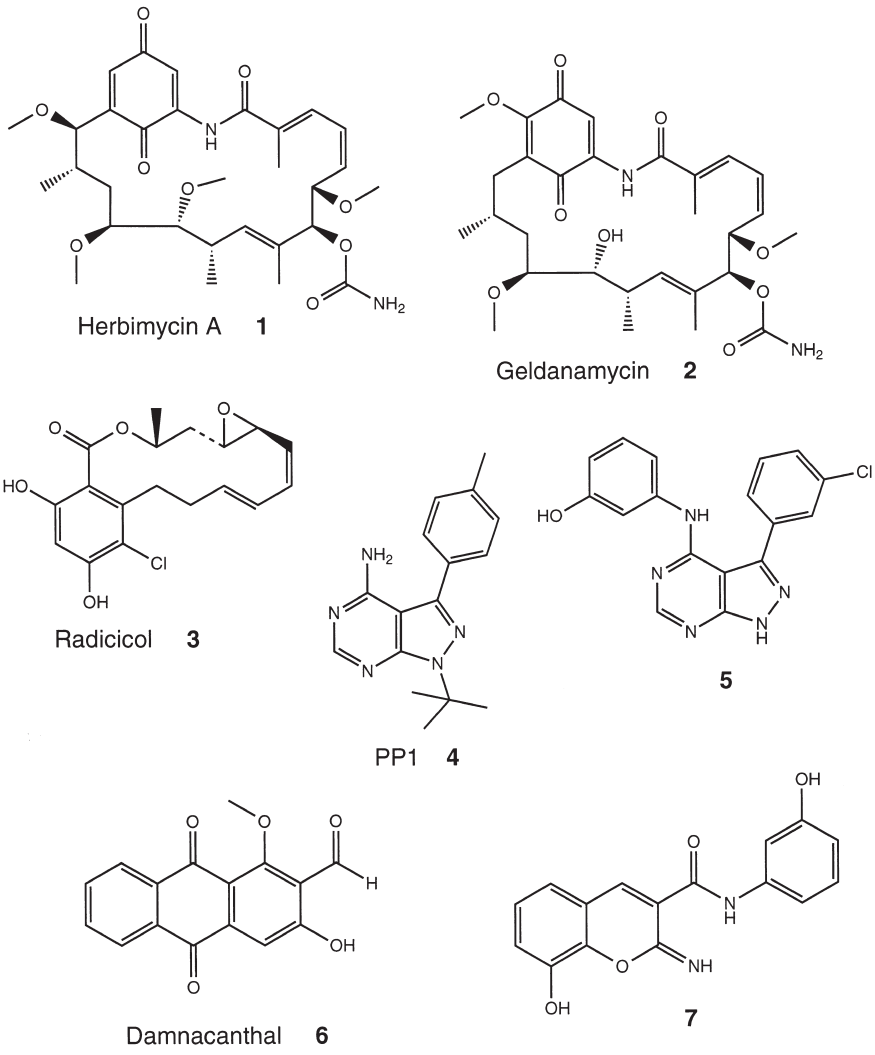

FIGURE 1. Inhibitors of wild-type Src family tyrosine kinases (see text for references).

Through traditional inhibitor screens, no molecules that demonstrate high selectivity between different Src family kinases have been identified. The most effective to date are the natural product damnacanthal (**6**) (Faltynek *et al.*, 1995) and a 3-(*N*-phenyl)carbamoyl-2-iminochromene (**7**) synthesized by Huang *et al.* (1995). Damnacanthal (IC_{50} for Lck, 17 nM) demonstrated 7- and 20-fold selectivity for Lck over Src and Fyn, respectively. The most selective iminochromene strongly inhibited c-Src (IC_{50} 120 nM), with approximately 18-fold selectivity with respect to Lck, <285-fold selectivity over Lyn, and 570-fold selectivity over Fyn. Unfortunately, the authors of this report did not describe any *in vitro* inhibition of non-Src family kinases or demonstrate any cellular kinase inhibition.

2.2. *Engineering a Unique Pocket in Src Family Tyrosine Kinases*

It has been shown in several systems that protein engineering can confer unique small molecule specificity on a rationally designed protein (Clackson, 1998). A recent series of papers from our laboratory has shown that Src family kinases can be re-engineered in a systematic fashion to contain a unique pocket in the ATP-binding site (Shah *et al.*, 1997; Bishop *et al.*, 1998; Liu *et al.*, 1998a,b).

By examining protein kinase crystal structures, Shah *et al.* (1997) recognized that there is a structurally conserved amino acid residue at the base of the ATP binding pocket that is in close contact with the exocyclic amine (N^6) of ATP's adenine ring (Ile338 in v-Src, from this point on referred to as position 338) (DeBondt *et al.*, 1993; Zheng *et al.*, 1993). Analysis of protein kinase sequence alignments confirmed that residue 338 contains a bulky side chain (usually Thr, Ile, Leu, Met, or Phe) in all known eukaryotic protein kinases (Hanks and Quinn, 1991). Thus, it was inferred that mutation of residue 338 to a smaller amino acid (e.g., glycine) would create a novel binding pocket that could uniquely bind small molecules that would be excluded by every wild-type kinase in the genome. Initial work showed that residue 338 could be mutated to glycine without altering the phosphoacceptor specificity (Shah *et al.*, 1997) or biological function of the v-Src kinase (K. Shah and K. M. Shokat, unpublished results). The space-creating mutation caused only a modest drop in k_{cat} and a modest increase in the K_m for ATP. It was also shown that the mutation strategy could be readily extended to the Src

family kinase Fyn, implying that the mutation of position 338 to glycine could be used as a general tool for systematically expanding the binding pocket of a given target protein kinase (Liu *et al.*, 1998a).

Subsequent modeling studies by Liu *et al.* (1998b) predicted that the 338 position of protein kinases acts as a "molecular gate" to a natural binding pocket surrounding N^7 of ATP, a pocket that is not utilized in ATP binding. Thus, removal of the gate (mutation of 338 to a small amino acid) would be expected to give access to a significantly larger unique binding pocket than would be expected from most single-point mutations. All of these data suggested that genetic introduction of a single-point mutation should confer unique drug sensitivity to the engineered Src family kinase, if a suitable inhibitor could be designed and synthesized.

2.3. *Design and Synthesis of Inhibitors Targeted to the Engineered Kinase*

To identify a cell-permeable small molecule that uniquely inhibits Ile338Gly v-Src, Bishop and colleagues tethered bulky chemical groups to the previously described Src family inhibitor PP1 (Hanke *et al.*, 1996; Bishop *et al.*, 1998). Through modeling of PP1 in the active site of the Src family kinase Hck, it was predicted that PP1 would bind in an orientation analogous to that of ATP, therefore presenting its exocyclic amine (N^4) to the space surrounding residue 338 (Sicheri *et al.*, 1997). This amine was used as a chemical hook to which they could attach hydrophobic moieties that would prevent binding of the derivatized molecules to wild-type protein kinases. The same hook would concurrently generate novel van der Waals interactions with the engineered v-Src. From a panel of 10 PP1 analogues, the prediction of binding orientation was confirmed by the finding that all of the molecules were preferentially bound by the engineered v-Src with respect to the wild type (see Fig. 2). The analogue that was over 20-fold more potent for Ile338Gly [4-(*p-tert*-butyl)benzamido-1-*tert*-butyl-3-phenylpyrazolo[3,4-*d*]pyrimidine, IC_{50} 430 nM, **17**] than any other in the panel contains a surprising large 11-carbon substituent, providing further support for the validity of the 338 "molecular gate" hypothesis. The selectivity of **17** was confirmed against wild-type v-Src, Fyn, Abl, PKA, and PKCδ, all of which were inhibited at least 700-fold less effectively than the target kinase. Bishop *et al.* (1998) also showed that the equivalent mutation in Fyn also made it sensitive to inhibition by **17** (IC_{50} 830 nM), showing that a chemical genetic approach to protein/small molecule recognition can rapidly circumvent the selectivity problem inherent in the structural degeneracy of homologous protein families.

To identify more potent inhibitors of the engineered Src family kinases, we have subsequently generated an expanded panel of PP1 analogues, consisting of over 25 molecules with increased chemical diversity at the N^4 and N(1) positions of the pyrazolo[3,4-*d*] pyrimidine ring. Screening of these putative inhibitors against Ile338Gly v-Src yielded

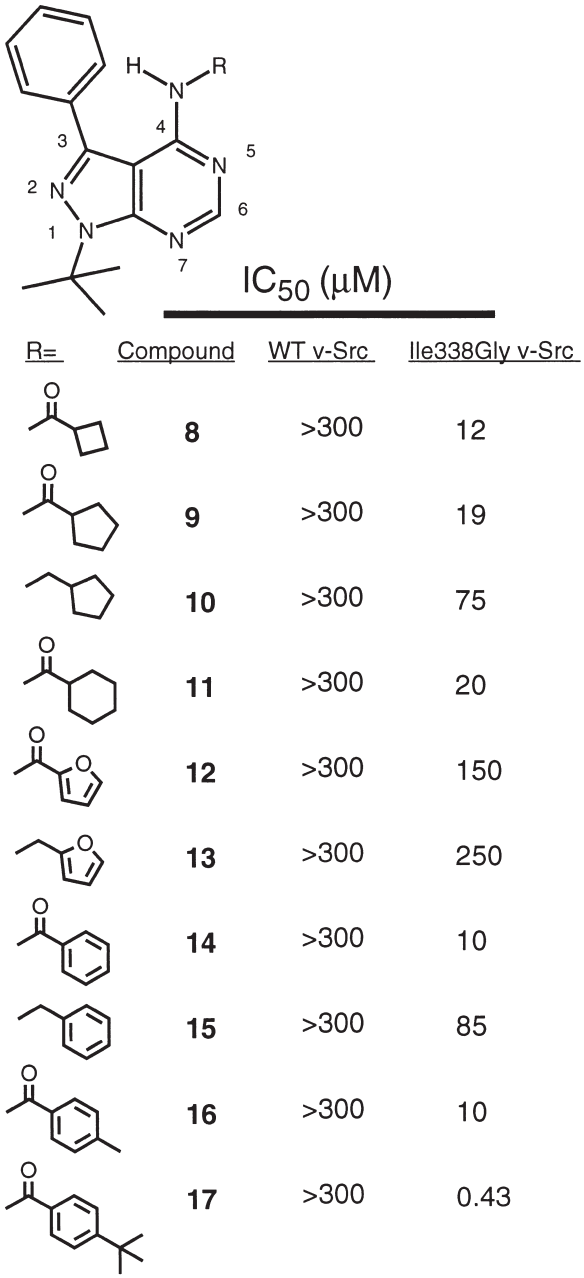

FIGURE 2. Selective inhibition of engineered v-Src by N^4 derivatized PP1 analogues. Modified from Bishop *et al.* (1998).

no potent inhibitors. All of the molecules in Fig. 3 were found to have an IC_{50} for the target kinase of greater than 10 μM (A. C. Bishop and K. M. Shokat, unpublished results). Examination of the cumulative data from both inhibitor panels strongly suggests that any alkyl substitution at N^4 greatly reduces binding of the PP1 analogues for both wild-type and engineered Src kinases. However, acyl substitutions at N^4 are very well tolerated. The basis for this dichotomy is not well understood and currently is under investigation.

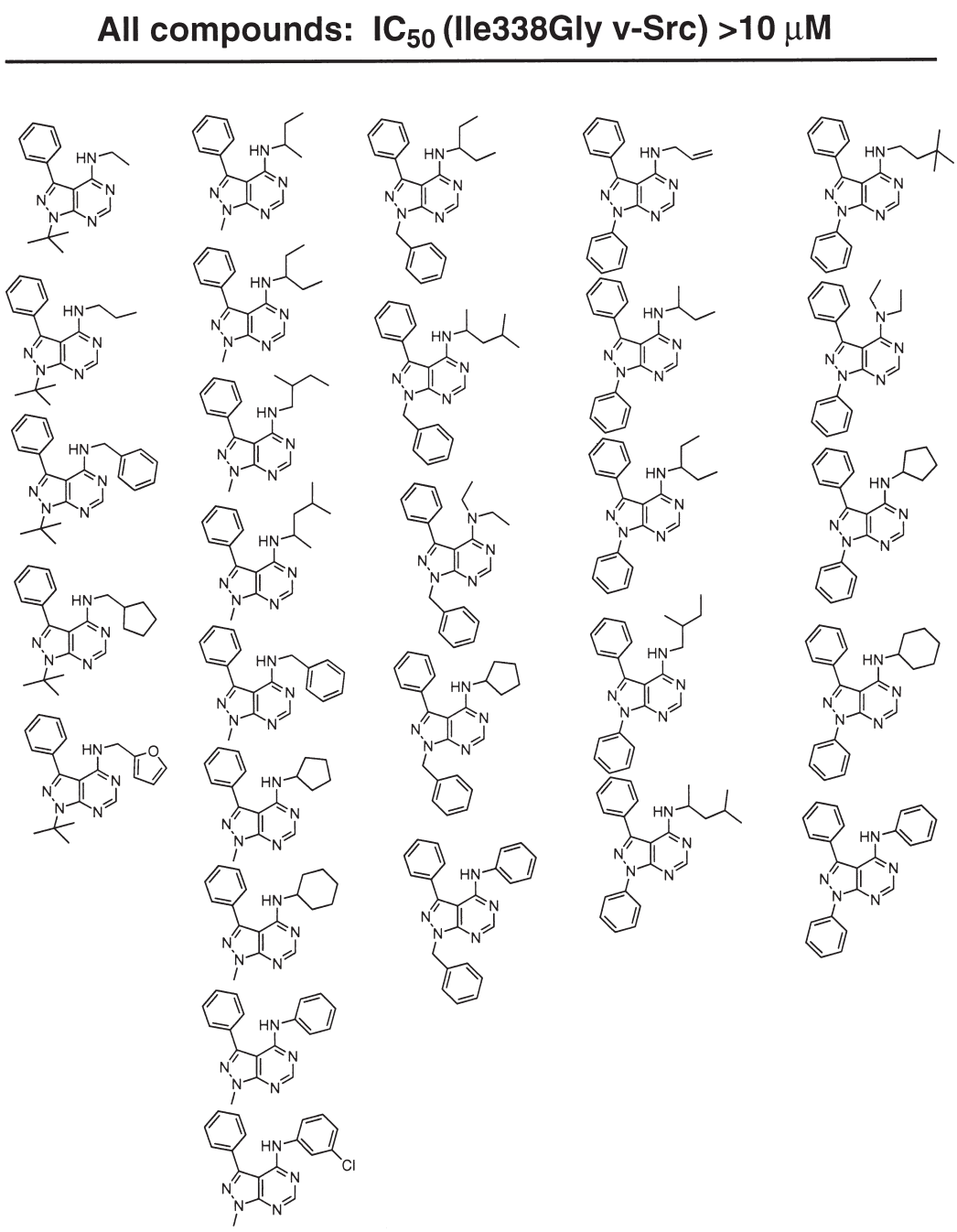

FIGURE 3. Second-generation panel of N(1)- and N^4-diversified PP1 analogues.

2.4. *Unique Cellular Target Inhibition through Chemical Genetic Design*

Identification of a selective enzyme inhibitor through chemical genetic design is only useful if the inhibitor can be used in a relevant cellular context to probe the target's function inside the cell. To demonstrate cellular inhibition of the target kinase by **17,** Bishop *et al.* (1998) treated NIH 3T3 fibroblasts, which expressed either wild-type or Ile338Gly v-Src, with the mutant-specific inhibitor. The authors found that at 100 μM, **17** had no effect on the phosphotyrosine level of cells expressing wild-type v-Src, whereas the phosphotyrosine level of cells that expressed the target kinase was moderately reduced. More strikingly, under prolonged drug treatment, it was found that the Ile338Gly expressing cells selectively reverted to the flattened morphology of non-transformed fibroblasts. This phenotype was confirmed by staining the actin stress fibers that are characteristic of normal fibroblasts (see Fig. 4). No change in the morphology or actin organization was observed for the wild-type v-Src-expressing cells. Thus, it was demonstrated that a small molecule inhibitor, which is uniquely selective for a tyrosine kinase oncogene product, can revert

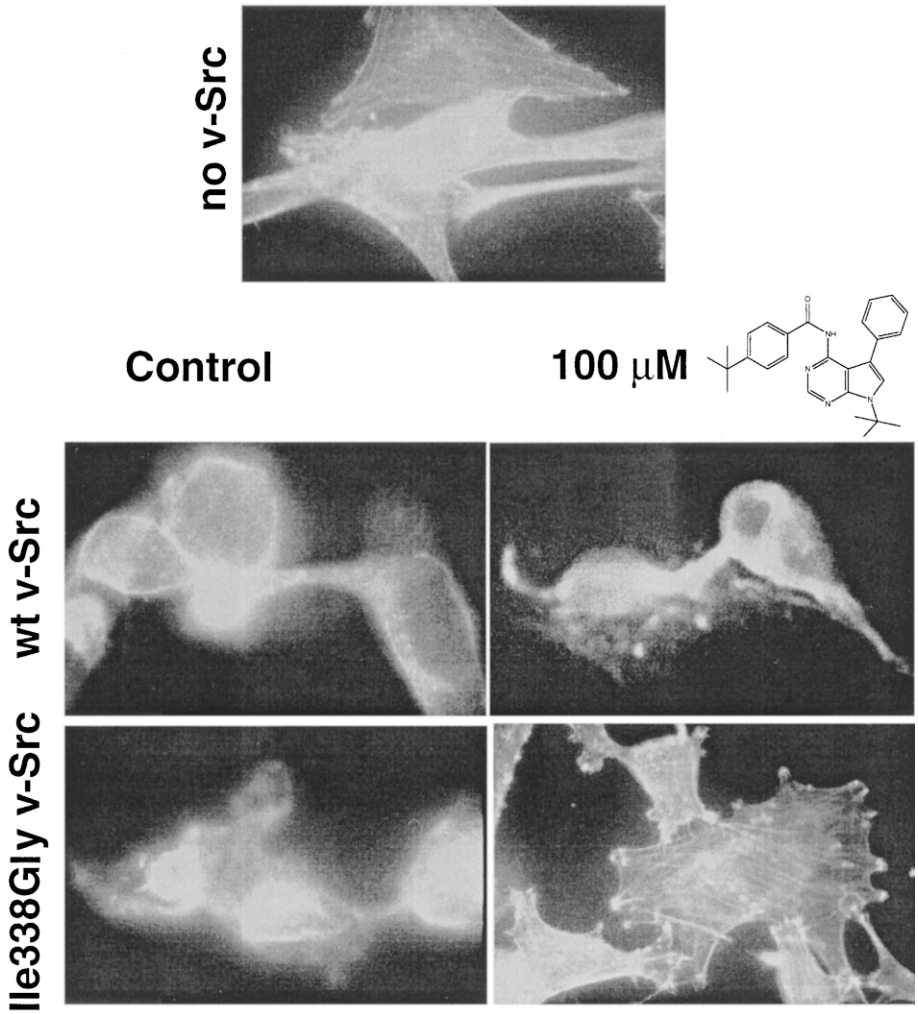

FIGURE 4. Selective reversion of transformed morphology in fibroblasts expressing Ile338Gly v-Src. Cells were stained with phalloidin-FITC and visualized by fluorescence microscopy. Modified from Bishop *et al.* (1998).

the morphological changes associated with cellular transformation. Only through chemical genetic design coupled with the built-in control of the wild-type target homologue could such a determination of cellular specificity be made.

3. GENETIC MANIPULATION OF MITOGEN-ACTIVATED PROTEIN KINASE HOMOLOGUES TO GENERATE NOVEL INHIBITOR SENSITIVITY

3.1. *Pyridinylimidazoles*

Lee *et al.* (1994) reported a remarkable finding. In one of the more striking demonstrations of the power of chemical genetics, the authors used a radiolabeled 2,4-diaryl-5-pyridin-4-yl-imidazole (SB 206718, **18**; see Fig. 5) to directly photoaffinity label this small molecule's direct cellular targets in the human cell line THP.1. It was known that this class of pyridinylimidazoles potently inhibits the production of cytokines (interleukin-1 and tumor necrosis factor), which are associated with inflammatory response. Surprisingly, the authors were able to isolate a single radiolabeled protein target from the cellular milieu and subsequently clone two cDNAs that were identical to one another outside of a 75-nucleotide region. The cloned genes encode MAPK homologues cytokine-suppressive anti-inflammatory drug-binding protein-1 and -2 (also named Stress-Activated Protein Kinase [SAPK] 2a or p38).

The stunning selectivity of the pyridinylimidazole family for p38 led to further investigation of the structure-activity relationships of these molecules (Boehm *et al.*, 1996; Gallagher *et al.*, 1997). It was found by Gallagher *et al.* that both the 4-phenyl and 5-pyrid-4-yl groups are required for maximum inhibition of p38. Halogens at the *meta* or *para* position of the 4-phenyl ring yield the most potent inhibitors of p38, with the most widely used pyridinylimidazole analogue containing a *para*-fluoro moiety (SB 203580, **19**). As can be seen from the structures in Fig. 5, potent pyridinylimidazole p38 inhibitors can contain substitutions at either C(2) or N(1). The IC_{50} (p38) for SB 203580 is 42 nM, but it does not inhibit the MAPK extracellular signal-regulated kinase (ERK)2 ($IC_{50} > 10$ μM) and does not inhibit PKA ($IC_{50} > 100$ μM) or PKCα ($IC_{50} > 100$ μM).

3.2. *Structural Studies on p38/Pyridinylimidazole Complexes*

Predictably, the high specificity of the pyridinylimidazole compounds led to a series of structural studies aimed at de-

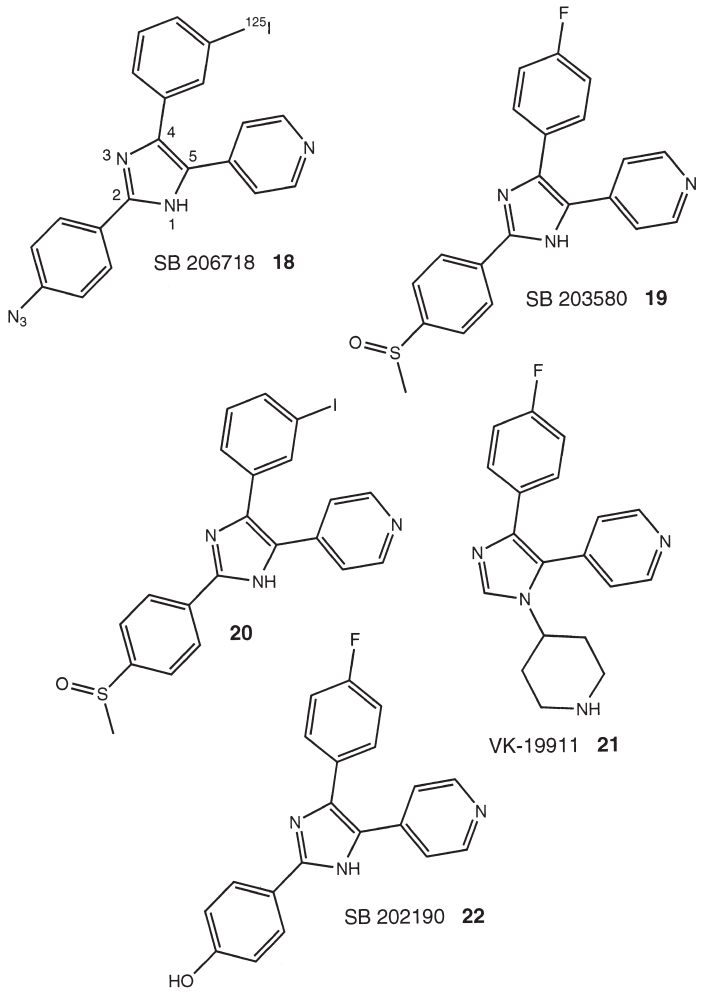

FIGURE 5. Chemical structures of pyridinylimidazole p38 inhibitors.

termining the basis for the p38 selectivity. Within 2 months of each other, 2 different groups published high-resolution crystal structures of pyridinylimidazoles bound to p38 (Tong *et al.*, 1997; Wilson *et al.*, 1997). These structures, along with concurrent biochemical work, confirmed that pyridinylimidazole inhibitors bind in the ATP-binding site of p38 (Young *et al.*, 1997). In the first published structure, Tong *et al.* co-crystallized p38 with a 4-*meta*-iodo phenyl analogue of SB 203580 (**20**). Wilson *et al.* crystallized VK-19911 (**21**), an N(1) piperidino analogue, with p38. The binding orientations of the inhibitors in the two co-crystal structures are exactly analogous. In both structures, N(4) of the pyridine ring makes a hydrogen bond with the backbone amide of Met109. This hydrogen bond mimics a similar interaction made by N(1) of ATP (Zheng *et al.*, 1993). In addition, the C(4) phenyl rings of both inhibitors occupy equivalent binding pockets, comprised of backbone atoms from residues 51–53 and 104–106, along with the side chains of Val38, Ala51, Leu75, Leu86, and Leu104. Interestingly, in the structure reported by Tong *et al.*, a second molecule of the SB 203580 analogue was also bound at a site 30 Å from the ATP-binding site. However, the significance of this finding with regard to the specificity of pyridinylimidazole inhibitors is unclear, as this second inhibitor binding pocket was not utilized in the co-crystal structure of p38 with the parent molecule SB 203580 or VK-19911.

	106													
p38	F	N	D	V	Y	L	V	T	H	L	M	G	A	D	L
p38β2	F	S	E	V	Y	L	V	T	T	L	M	G	A	D	L
SAPK3	F	T	D	F	Y	L	V	M	P	F	M	G	T	D	L
SAPK4	F	Y	D	F	Y	L	V	M	P	F	M	Q	T	D	L
JNK1	F	Q	D	V	Y	I	V	M	E	L	M	D	A	N	L
JNK2β	F	Q	D	V	Y	L	V	M	E	L	M	D	A	N	L
ERK2	M	K	D	V	Y	I	V	Q	D	L	M	E	T	D	L
								338							
v-Src	E	P	I	Y	I	V	I	E	Y	M	S	K			

FIGURE 6. Sequence alignment of MAPK homologues at the positions surrounding 106. For reference, the alignment of v-Src is also shown.

3.3. *Pyridinylimidazole Sensitivity through Site-Directed Mutagenesis*

From their structural studies, along with analysis of the sequence alignments of the MAPK family, both Tong *et al.* (1997) and Wilson *et al.* (1997) proposed that Thr106 (equivalent to 338 of Src) may be an important residue for the determination of the observed specificity of pyridinylimidazole inhibitors for p38. The side chain of this residue helps make up the *para*-fluoro phenyl ring binding pocket. The sequence alignment of MAPKs shown in Fig. 6 shows that only p38 and p38β2 have threonine at position 106. These enzymes are uniquely sensitive to pyridinylimidazoles. All of the other MAPK homologues possess a larger side chain (Met in SAPK3, SAPK4, c-Jun N-terminal kinase [JNK]1, and JNK2β or Gln in ERK1 and ERK2) at this position. To test the hypothesis that this inhibitor sensitivity was mediated by the side chain of residue 106, Wilson *et al.* (1997) mutated Thr106 of p38 to both methionine and alanine. As predicted, Thr106Met showed resistance to pyridinylimidazoles that was equivalent to SAPK3 (IC_{50} >> 10 μM for both VK-19911 and SB 202190), while Thr106Ala p38 retained its sensitivity to inhibition.

Two recent papers have expanded the scope of the inquiry into MAPK acquisition of SB 203580 sensitivity through active site mutations (Eyers *et al.*, 1998; Gum *et al.*, 1998). Eyers *et al.* carried out a systematic investigation of Thr106 mutations in five MAPKs. The authors found that mutation of the wild-type methionines of SAPK3 and SAPK4 to threonine conferred inhibition sensitivity that was directly comparable with that of wild-type SAPK2b ($IC_{50} \approx 300$ nM), but still 10-fold lower than SAPK2a. Even smaller side chains (Ala, Gly) were then introduced in all four of these enzymes. For each of these SAPKs, the SB 203580 sensitivity for 106 mutations lies in the pattern Ala > Gly > Thr >> Met, suggesting that the methyl side chain of alanine is the ideal size to pack with the para-fluoro-phenyl ring efficiently. Not surprisingly, mutation of the corresponding methionine of the less closely related JNK1 to threonine and alanine also increased its SB 203580 sensitivity, but to a substantially lower level than was demonstrated with SAPK3 and SAPK4. Through investigation of the primary sequences of these kinases, the authors realized that all of the SAPKs and JNKs contained a Leu residue at the position 2 residues N-terminal to Thr106 of p38, with the exception of JNK1 (Ile). Thus, it was demonstrated that while the single mutation of this isoleucine to leucine changed the inhibitor sensitivity of JNK1 only very slightly, the double mutant (Met106Ala, Ile104Leu) possessed SB 203580 sensitivity (IC_{50} 30 nM) that was comparable with that of the SAPK3 alanine mutant.

A report from Gum *et al.* (1998) took a somewhat different approach to the problem. These authors mutated 21 residues in the ATP-binding site of p38, and assayed both the kinase activity and radiolabeled SB206718 crosslinking sensitivity of these mutants. It was discovered that kinase activity can be decoupled from pyridinylimidazole binding capability, as two mutants were discovered that were dead enzymes, but were still efficiently photoaffinity labeled by SB 206718 (K152A, A157L). In a more directed mutagenesis strategy that strongly parallels the work by Eyers *et al.* (1998), the authors investigated the effect of exchanging not only the 106 position in the various SAPKs, but also triple mutations of residues 106–108. In these positions, p38 contains the amino acids T-H-L, SAPKs 3 and 4 contain M-P-F, and JNK1 contains M-E-L. When the 106–108 region of SAPK3 and SAPK4 were replaced with T-H-L, the resulting mutants each demonstrated SB 203580 sensitivity that was equivalent to each other and to wild-type p38 ($IC_{50} \sim 0.5$–1 μM). This represented an ~10-fold gain over the single Met106Thr mutations of SAPK3 and SAPK4. (Note: due to different assays, the inhibition data from Eyers *et al.* and Gum *et al.* should not be compared directly.) The more distant relative JNK1 fit the paradigm. Mutations of the M-E motif of JNK1 to T-H of p38 led to SB 203580 sensitivity that was comparable with that of wild-type p38 and roughly an order of magnitude greater than the single mutant.

4. CONCLUSIONS AND PERSPECTIVES

In a span of a little over a year, two functionally important and structurally distinct classes of kinases, the Src family tyrosine kinases and the MAP serine/threonine kinases, have been shown to be amenable to a systematic and generalizable drug-sensitization strategy. Amazingly, analogous mutations of an equivalently positioned residue are sufficient in both families to confer potent small molecule sensitivity on the target kinase. This consistency in the mutagenesis data implies that the limiting factor in using engineered kinase/inhibitor systems in biological research will lie in the identification of the uniquely specific small molecules. This becomes particularly apparent when it is realized that in the MAPK sensitization studies, all of the experiments started with an incredibly specific small molecule, and through protein design, it was made less specific. By contrast, the Src family studies started with a weakly selective inhibitor and combined chemical and protein design to generate a unique kinase/inhibitor pair that was functional in a cellular context.

These first steps in the identification and characterization of "chemical sensitive mutants" of protein kinases have demonstrated that the field holds great promise. Since transfected cell lines, and even "knock-in" mice, can now be generated rapidly, this ability to genetically "program" the kinase of interest for unique inhibition by a small molecule should greatly expedite the process of testing the effects of selective inhibition of a given kinase in a whole cell or in an animal model. Furthermore, the unique pairing of a designed target/small molecule pair will allow for the unambiguous assignment of the activity of a specific kinase to an induced phenotype.

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