# Generation of Monospecific Nanomolar Tyrosine Kinase Inhibitors via a Chemical Genetic Approach

**Anthony C. Bishop,[†] Chi-yun Kung,[†] Kavita Shah,[†] Laurie Witucki,[†] Kevan M. Shokat,*[,†,‡] and Yi Liu[†]**

*Contribution from the Department of Chemistry and Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544*

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**Abstract:** Selective protein kinase inhibitors are highly sought after as tools for studying cellular signal transduction cascades, yet few have been discovered due to the highly conserved fold of kinase catalytic domains. Through a combination of small molecule synthesis and protein mutagenesis, a highly potent ($IC_{50}$ = 1.5 nM) and uniquely specific inhibitor (4-amino-1-*tert*-butyl-3-(1′-naphthyl)pyrazolo[3,4-*d*]pyrimidine) of a rationally engineered v-Src tyrosine kinase (Ile338Gly v-Src) has been identified. Both the potency and specificity of this compound surpass those of any known Src family tyrosine kinase inhibitors. The molecule strongly inhibits the engineered v-Src in whole cells but does not inhibit tyrosine phosphorylation in cells that express only wild-type tyrosine kinases. In addition, the inhibitor selectively disrupts transformation in cells that express the target v-Src. The structural degeneracy of kinase active sites should allow the same complementary inhibitor/protein design strategy to be widely applicable across this entire enzyme superfamily.

## Introduction

The current explosion in the number of newly discovered genes underscores the need for small molecule ligands that can be used to elucidate and control gene function. Convergent engineering of protein/small molecule interfaces has emerged in recent years as a powerful method for generating novel ligand/receptor pairs with high specificity.[1−3] By introducing chemical diversity into the target protein as well as the small molecule, unique binding interactions can be designed and exploited more efficiently than through traditional medicinal chemistry. Such approaches have been used to chemically explore a number of biological systems. FK506-binding protein has been engineered to preferentially bind nonnatural FK506 analogues by Schreiber and co-workers,[4,5] as well as Clackson and co-workers.[6,7] This system has been used extensively to selectively dimerize receptors and control gene expression in a cellular context.[8] Nuclear hormone receptors have also been shown to be amenable to chemical genetic design. Corey and co-workers demonstrated that mutations at two amino acid residues in the retinoid X receptor are sufficient to create two new classes of receptors with novel ligand specificities.[9] In a more medicinally applicable system, Smith and co-workers engineered the protease, carboxypeptidase A1, to hydrolyze a prodrug of methotrexate that is resistant to hydrolysis by wild-type proteases.[10]

Work in our laboratory has focused on engineering protein kinases to uniquely recognize rationally designed small molecule substrates and inhibitors.[3,11−13] Protein kinase catalyzed phosphorylation of the hydroxyl moiety of serine, threonine, or tyrosine is the central posttranslational control element in eukaryotic signal transduction.[14] The phosphorylation state of a given protein can govern its enzyme activity, protein−protein binding interactions, and cellular distribution. Phosphorylation and dephosphorylation is thus a "chemical switch" that allows the cell to transmit signals from the plasma membrane to the nucleus to ultimately control gene expression in a highly regulated manner.[15] Highly selective, cell-permeable inhibitors of individual kinases would allow for the systematic investigation of the cellular function of a kinase in real time and, thus would provide invaluable tools for the deconvolution of phosphorylation-dependent processes in signal transduction cascades.[16−21]

We recently described a combined chemical and genetic approach which enables the rapid generation of highly selective

* To whom correspondence should be addressed. E-mail: shokat@princeton.edu.

† Department of Chemistry.

‡ Department of Molecular Biology.

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small molecule inhibitors for one engineered protein tyrosine kinase in vitro and in whole cells.[3] The strategy involves using a point mutation to create a unique pocket in the ATP binding site of the kinase of interest that does not occur in any other protein kinase in the genome. A specific inhibitor of the engineered kinase is then synthesized by derivatizing a known kinase inhibitor with a bulky group designed to fit the novel active site pocket. By using genetic manipulation to introduce a unique structural difference into the conserved kinase active site, highly selective inhibitors can be identified from very small panels ($\leq$10 compounds) of putative inhibitors.[3]

We have selected the Src family of tyrosine kinases as our initial inhibitor design target. The Src family is composed of 10 highly homologous cytosolic kinases, which are critical components in an array of cell signaling pathways ranging from lymphocyte activation to cell growth and proliferation.[22] Constitutive activation of these enzymes can lead to oncogenic cell transformation, making them putative drug targets for cancer therapies.[23] Because of their importance in the regulation of these fundamental cellular processes, many studies have focused on developing small inhibitors for the Src family kinases.[23,24] However, the potent inhibitors that have been discovered lack the high selectivity that would be required for probing the cellular function of a target kinase. Conventional inhibitor screens have produced no molecules that can discriminate between the active sites of the various Src family kinases. The functional importance of the Src family kinases coupled with the dearth of effective inhibitors for these enzymes makes them ideal targets for our chemical genetic design strategy.

## Results and Discussion

**Inhibitor Design and Modeling.** Initially, we utilized the $N^4$ exocyclic amine of the Src family kinase inhibitor 4-amino-1-*tert*-butyl-3-phenylpyrazolo[3,4-*d*]pyrimidine (**1**, Figure 1a) as a chemical hook to which we could tether bulky groups to destroy the molecule's affinity for wild-type kinases (**1** is a *des*-methyl modified analogue of PP1, reported by Hanke et al.[25]). While this approach did yield a very selective inhibitor (**2**) of the engineered v-Src kinase (I338G v-Src), it was not totally satisfactory because we lost more than 1 order of magnitude in binding energy from the starting affinity of the parent molecule for wild-type Src family kinases (see Figure 1a)[3].

To increase the potency of our inhibitors, we modeled the binding of **1** in the active site of the Src family kinase, Hck.[13,26] Using the molecular graphics program GRASP,[27] we compared the protein surface map of the ATP binding pocket of Hck (Figure 1b,c.) with the corresponding predicted map of the expanded pocket in the engineered protein kinase, T338G Hck (Figure 1d). From this model, we deduced that derivatization of $N^4$ was not the only means of generating complementary van der Waals interactions with the unique binding pocket of I338G v-Src. It could be seen from the surface map that derivatization of the $C^3$ phenyl ring of **1** (for example, phenyl replaced with naphthyl, compound **6a**) with a bulky group leads

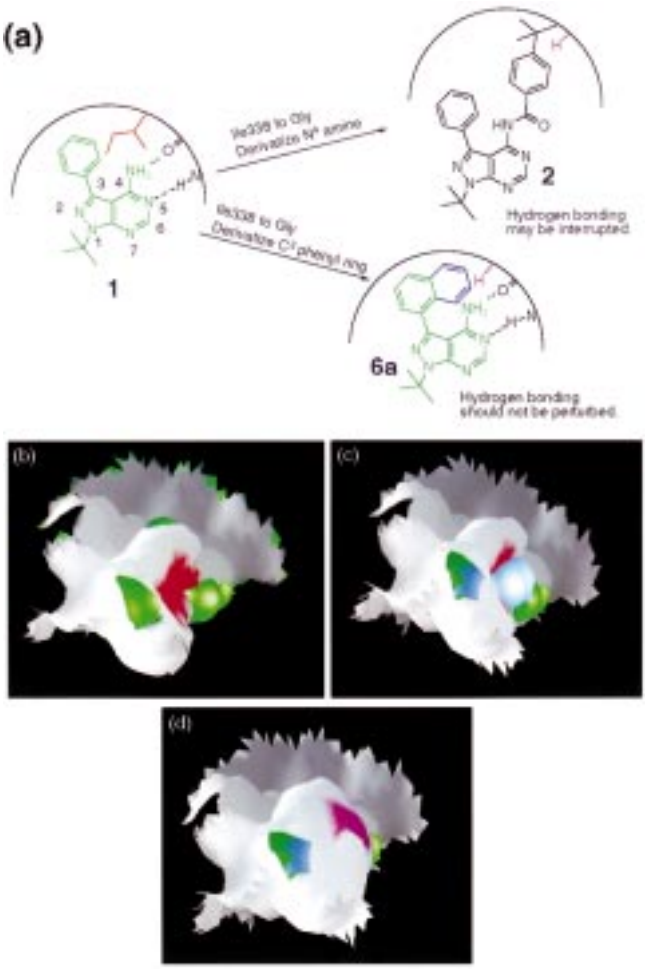


**Figure 1.** (a) Schematic representation of the predicted binding orientation of two classes of derivatized pyrazolo[3,4-*d*]pyrimidines. Analogues that were derivatized at $N^4$ may have lost potency due to an interruption of the ATP-like hydrogen-bonding network. This network is presumably intact in the $C^3$-derivatized inhibitors. The color scheme is the same as in b−d. (b−d) Proposed structural basis for the $C^3$-derivatization strategy as shown by a surface representation of the ATP binding pocket of the Src family tyrosine kinase, Hck,[26] with **1** bound, and the proposed effect of enlarging the ATP binding pocket to accommodate derivatized analogues of **1**. (b) Wild-type Hck + **1**. The solvent accessible surface of Hck within 5 Å of **1** is shown in gray; the surface of **1** is shown in green. The portion of Hck's surface formed by Thr338 is shown in red. The binding orientation of **1** was predicted as described previously.[3] (c) Wild-type Hck + **6a**. Model of the unfavorable packing of **6a** into the wild-type Hck ATP binding site, showing the steric clash between the added phenyl ring (shown in blue) of the naphthyl moiety and the side chain of Thr338. (d) Thr338Gly Hck + **6a**. Model of the improved fit of **6a** into the enlarged ATP binding pocket of the engineered kinase. The portion of Hck's surface formed by Gly338 is shown in purple. The conformation of **6a** was chosen by manually checking multiple rotamers of the bond between $C^3$ of the pyrazolo[3,4-*d*]pyrimidine and the naphthyl ring for steric clash with the residues in the Hck ATP binding site using the program InsightII. The rotamer with the least steric clash determined in this manner was then imported into the program GRASP to produce the surface maps. These figures were created using GRASP.[27]

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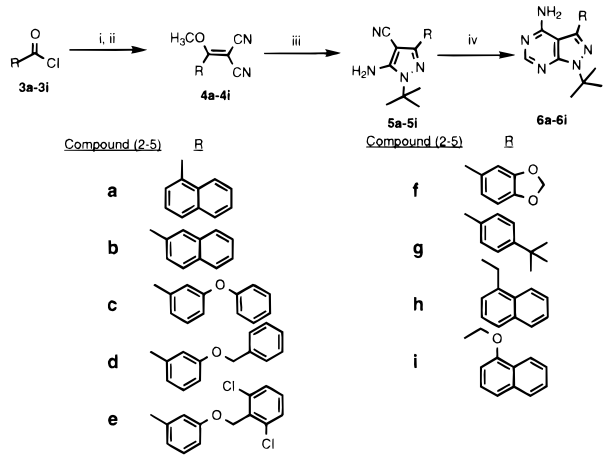
**Scheme 1.** Synthesis of $C^3$-Derivatized PP1 Analogues[a]




[a] Conditions: (i) 2 equiv of NaH, 1 equiv of malonitrile, THF, rt, 0.5 h; (ii) 5 equiv of $NaHCO_3$, 5 equiv of dimethyl sulfate, dioxane/$H_2O$ (6/1), 80 °C, 1 h; (iii) 1 equiv of triethylamine, 1 equiv of *tert*-butylhydrazine hydrochloride, EtOH, reflux, 1 h; (iv) formamide, 180 °C, 12 h.

**Table 1.** 50% Inhibitory Concentrations ($IC_{50}$'s) of $C^3$-Derivatized PP1 Analogues for Engineered and Wild-Type Src Family Tyrosine Kinases

| compd | I338G v-Src ($\mu$M) | v-Src ($\mu$M) | Fyn ($\mu$M) |
|---|---|---|---|
| **6a** | 0.0015 | 1.0 | 0.60 |
| **6b** | 0.0015 | 1.0 | 0.13 |
| **6c** | 0.14 | 40 | 0.80 |
| **6d** | 0.0070 | 26 | 6.0 |
| **6e** | 0.13 | 300 | 84 |
| **6f** | 0.025 | 3.1 | 0.040 |
| **6g** | 0.099 | 65 | 9.2 |
| **6h** | 0.0042 | 28 | 1.1 |
| **6i** | 0.045 | >300 | >300 |

to steric clash between the derivatized inhibitor and the molecular surface created by Thr338 (Figure 1c). Mutation of residue 338 to glycine generates a unique binding pocket that is predicted to be large enough to accept the naphthyl analogue of **1** (Figure 1d). We reasoned, therefore, that derivatization of this phenyl group with hydrophobic substituents should afford compounds that complement the corresponding I338G v-Src active site, without disrupting any potential hydrogen-bonding interactions at $N^4$. In addition, this added bulk at the $C^3$ moiety should cause these molecules to be sterically incompatible with the active sites of wild type tyrosine kinases, affording high specificity for the suitably engineered v-Src.

**Inhibitor Synthesis and Screening.** A small panel of $C^3$-derivatized PP1 analogues (**6a**−**i**) was synthesized as shown in Scheme 1.[28] The group of modified inhibitors was screened against the catalytic domain of the target kinase, I338G v-Src, which was expressed in bacteria and purified as a glutathione-S-transferase (GST) fusion protein.[12] All of the $C^3$-derivatized analogues are more potent inhibitors of I338G v-Src than the most potent molecule (**2**, $IC_{50}$ = 430 nM) identified from the first generation panel of $N^4$-derivatized compounds[3] (see Table 1). Four of the molecules (**6a**,**b**,**d**,**h**) inhibit the target kinase at low nanomolar concentrations with the two naphthyl isomers (**6a**,**b**) exhibiting the greatest potency ($IC_{50}$ = 1.5 nM).[29] Under the conditions of our assay, the parent molecule, PP1, inhibited its optimal target, Fyn, at only $IC_{50}$ = 30 nM. These data show that an inhibitor design strategy combining enzyme engineering with directed small molecule synthesis cannot only achieve the potency of molecules identified through screening of large libraries but can lead to a significant increase (20-fold in the case of **6a**,**b**) in affinity over previously optimized inhibitors of wild-type kinases. To our knowledge, **6a** and **6b** are the most potent inhibitors of any Src family tyrosine kinase that have been reported to date[30].

Importantly, all nine molecules show striking selectivity for I338G v-Src with respect to the wild-type enzyme. The in vitro selectivities range from ≈120 for the piperonyl compound (**6f**) to as high as >6500 for the naphthoxy methyl derivative (**6i**). This range is similar to the selectivities generated by derivatization of $N^4$, further validating both our prediction of binding orientation for the parent inhibitor as well as our modeling of the expanded binding site of I338G v-Src[3]. These selectivities compare favorably to those from other strategies, which combine protein engineering with small molecule recognition,[4−6] as well as strategies that utilize selection techniques to identify tight binding proteins from large pools of mutants.[31]

To further confirm the selectivity for the target kinase, the panel was screened against wild-type Fyn (Table 1). This is presumably a more stringent control than inhibition of wild-type v-Src because Fyn is more potently inhibited by PP1. Three of the four most potent inhibitors (**6a**,**d**,**h**) showed sufficient selectivity for the target kinase (≥100-fold) with respect to wild-type Fyn. The most potent inhibitor of I338G v-Src, **6a**, binds wild-type Fyn at 600nM representing 400-fold selectivity for the designed target.

**Cellular Inhibition of the Target Kinase.** Two cell culture systems were employed to investigate the utility of compound **6a** as a specific kinase inhibitor in the context of a whole cell. First, NIH3T3 fibroblast cell lines that express either wild type or I338G v-Src were generated by retroviral infection.[3] Because v-Src is a highly activated, oncogenic tyrosine kinase the majority of the tyrosine phosphorylation in these cells is a result of v-Src expression.[32] To investigate the selective inhibition of the engineered v-Src, both wild-type and I338G v-Src expressing cells were incubated with varying concentrations of **6a**. Antiphosphotyrosine immunoblots of lysates derived from these cells demonstrate that **6a** strongly diminishes tyrosine phosphorylation in a concentration-dependent manner within minutes (Figure 2, lanes 3−8). In agreement with the in vitro potencies, the ablation of phosphotyrosine signal from **6a** is much more rapid and complete than that caused by the most potent $N^4$-derivatized analogue in the same cell line.[3] Wild-type v-Src expressing cells in the presence of 500 nM **6a** show no loss of phosphotyrosine signal (lanes 1 and 2) and can be grown in the presence of the compound for days with no loss of tyrosine phosphorylation or apparent cytotoxicity (not shown).

To further confirm the selectivity of **6a**, Jurkat cells (a human-derived T cell line with no engineered kinases) were treated with the general protein tyrosine phosphatase inhibitor, per-

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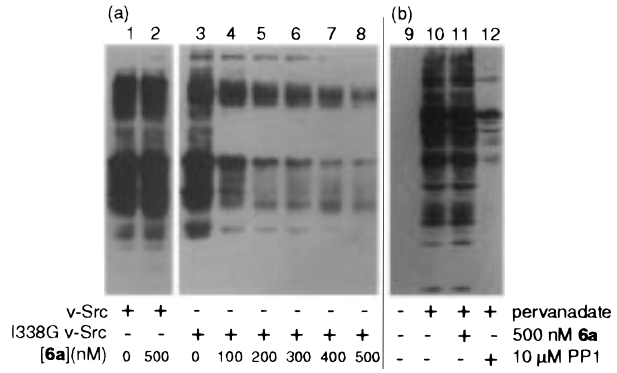


**Figure 2.** (a) Effect of **6a** on tyrosine phosphorylation in NIH3T3 fibroblasts expressing either wild-type v-Src (lanes 1, and 2) or I338G v-Src (lanes 3−8). Cells were treated with the indicated amount of **6a** in 0.5% DMSO for 30 min and immediately lysed. Cellular proteins were separated by polyacrylamide gel electrophoresis (10%) and transferred to nitrocellulose. Tyrosine phosphorylated proteins were visualized by immunoblotting with a monoclonal anti-phosphotyrosine antibody (4G10). (b) Effect of **6a** on tyrosine phosphorylation in wild-type Jurkat cells. $10^6$ Jurkat cells were incubated at 37 °C for 30 min in the presence of 0.5% DMSO (lanes 9, and 10), 500 nM **6a** (lane 11), or 10 $\mu$M PP1 (lane 12). Cells in lanes 10−12 were subsequently treated with 0.5 mM pervanadate for 10 min before lysis. Tyrosine phosphorylated proteins were visualized as in (a).

vanadate, which covalently binds to a catalytically essential cysteine in the active site of phosphotyrosine phosphatases.[33] The presence of pervanadate effectively shifts the cellular equilibrium between phosphotyrosine and tyrosine, giving rise to a large increase in tyrosine phosphorylation (see Figure 2, compare lane 10 to lane 9). When these cells are treated with 500 nM **6a** (lane 11), there is no detectable decrease in phosphorylation, indicating that none of the wild-type tyrosine kinases in Jurkat cells are appreciably inhibited by the designed inhibitor. PP1 was used as a positive control for inhibition of wild-type kinases at 10 $\mu$M (lane 12), the concentration at which it has previously been shown to strongly suppress T cell receptor-mediated tyrosine phosphorylation.[25]

**Selective Disruption of Cellular Transformation.** The rapid generation of highly selective kinase inhibitors should have far-reaching applications in the pharmacological validation of protein kinases as viable drug targets. To test this idea, we investigated whether compound **6a** could selectively disrupt oncogenic transformation in cells that expressed the target kinase. Normal NIH3T3 fibroblasts have long fibers of polymerized actin across the cells that can be visualized by staining the cells with phalloidin conjugated to rhodamine (Figure 3a).[34] Cells that express an oncogenic protein (either wild type or I338G v-Src) are rounded and therefore have a diffuse pattern of actin (Figure 3b). Wild-type v-Src expressing cells that are treated with **6a** appear indistinguishable from untreated wild-type cells, suggesting that **6a** has no effect on this nonmutant cell line. However, cells expressing the target kinase have clear actin fibers and appear indistinguishable from normal NIH3T3 fibroblasts when incubated with 250 nM **6a** for 16 h. From these data it is clear that small molecule inhibition of v-Src's catalytic activity is sufficient to block its role in oncogenesis.

## Conclusion

Directed structure-based design of kinase/inhibitor pairs has yielded monospecific, cell-permeable inhibitors of engineered

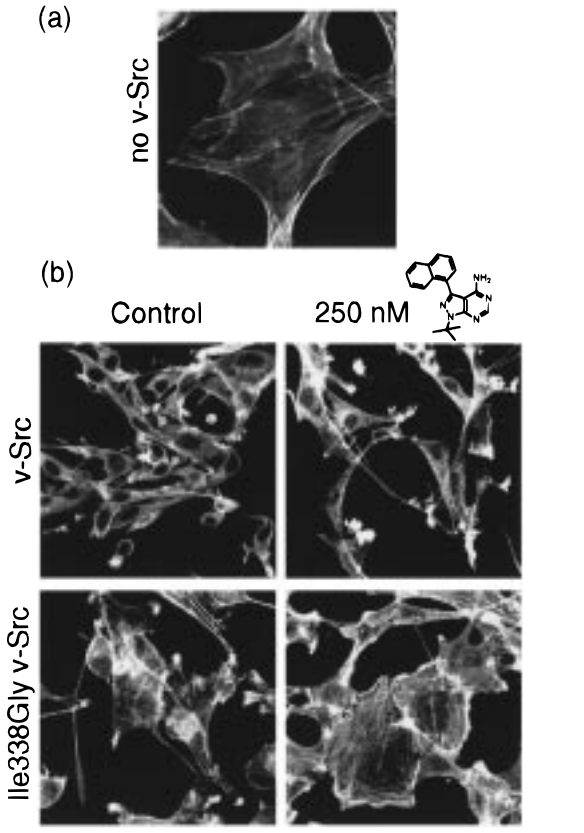


**Figure 3.** I338G v-Src transformed fibroblasts selectively acquire a flattened morphology and selectively regain actin stress fibers upon incubation with **6a**. (a) Nontransformed NIH3T3 cells. (b) Cells transformed by either wild-type v-Src or I338G v-Src were treated with 0.5% DMSO or 250 nM **6a** in 0.5% DMSO for 16 h. All cells were fixed, stained with phalloidin−rhodamine, and visualized by confocal microscopy.

Src family kinases with potencies that have not been attainable with conventional inhibitor screening methods. By mutating the active site of v-Src, we have not only differentiated one protein kinase from all others but simultaneously created a newly accessible binding site to use in designing more potent inhibitors. Thus, we can increase both potency and selectivity compared to traditional inhibitor design strategies. Our design is highly generalizable, owing to the conservation of kinases at the site corresponding to Ile338 in v-Src.[12] In fact, recent work has shown that the sensitivity of mitogen-activated protein kinases (MAPKs) to pyridinylimidazole inhibitors is in large part controlled by the side chain of residue 106, which corresponds to 338 of Src.[35−37] The primary advantage of our approach to the design of selective kinase inhibitors for the study of protein kinase function is the ability to genetically "program" the kinase of interest for unique inhibition by a small molecule. This allows for the unambiguous assignment of the activity of a specific kinase to the induced phenotype. The combination of genetic manipulation and small molecule control of enzyme activity should have far-reaching applications in the pharmacological validation of protein kinases as viable drug targets in cells as well as whole organisms.

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## Experimental Section

**Chemical Synthesis.** All starting materials and synthetic reagents were purchased from commercial suppliers unless otherwise noted. Acid chlorides that were not readily commercially available (**3c**,**d**,**e**,**h**,**i**) were synthesized by treating the corresponding carboxylic acids with excess oxalyl chloride and catalytic DMF in diethyl ether.[38] All PP1 analogues were synthesized according to Hanefeld et al.[28]

**4-Amino-1-*tert*-butyl-3-(1′-naphthyl)pyrazolo[3,4-*d*]pyrimidine (6a):** white powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.92 (s, 9H), 5.04 (m, 2H), 7.43−7.73 (m, 4H), 7.92−8.02 (m, 3H), 8.34 (s, 1H); HRMS (EI) molecular ion calcd for $C_{19}H_{19}N_5$ 317.16427, found 317.16247.

**4-Amino-1-*tert*-butyl-3-(2′-naphthyl)pyrazolo[3,4-*d*]pyrimidine (6b):** white powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.88 (s, 9H), 5.55 (m, 2H), 7.56−8.00 (m, 6H), 8.16 (s, 1H), 8.39 (s, 1H); HRMS (EI) molecular ion calcd for $C_{19}H_{19}N_5$ 317.16427, found 317.16359.

**4-Amino-1-*tert*-butyl-3-(*m*-phenoxyphenyl)pyrazolo[3,4-*d*]pyrimidine (6c):** white powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.83 (s, 9H), 5.61 (s, 2H), 7.08−7.49 (m, 9H), 8.35 (s, 1H); HRMS (EI) molecular ion calcd for $C_{21}H_{21}N_5O$ 359.17483, found 359.17325.

**4-Amino-1-*tert*-butyl-3-(*m*-benzyloxyphenyl)pyrazolo[3,4-*d*]pyrimidine (6d):** white powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.85 (s, 9H), 5.17 (s, 2H), 5.55 (s, 2H), 7.10 (d, $J$ = 8 Hz, 1H), 7.27−7.48 (m, 8H), 8.34 (s, 1H); HRMS (EI) molecular ion calcd for $C_{22}H_{23}N_5O$ 373.19049, found 373.18833.

**4-Amino-1-*tert*-butyl-3-(*m*-(2′,6′-dichloro)benzyloxyphenyl)pyrazolo [3,4-*d*]pyrimidine (6e):** white powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.85 (s, 9H), 5.36 (s, 2H), 5.74 (s, 2H), 7.11−7.51 (m, 7H), 8.36 (s, 1H); HRMS (EI) molecular ion calcd for $C_{22}H_{21}Cl_2N_5O$ 441.11263, found 441.11050.

**4-Amino-1-*tert*-butyl-3-piperonylpyrazolo[3,4-*d*]pyrimidine (6f):** white powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.83 (s, 9H), 5.70 (s, 2H), 6.05 (s, 2H), 6.96 (d, $J$ = 8 Hz, 1H), 7.13−7.27 (m, 2H), 8.34 (s, 1H); HRMS (EI) molecular ion calcd for $C_{16}H_{17}N_5O_2$ 311.13841, found 311.13777.

**4-Amino-1-*tert*-butyl-3-(*p*-*tert*-butylphenyl)pyrazolo[3,4-*d*]pyrimidine (6g):** white powder; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.38 (s, 9H), 1.84 (s, 9H), 5.83 (s, 2H), 7.58 (dd, $J$ = 8 Hz, 12 Hz, 4H), 8.33 (s, 1H); HRMS (EI) molecular ion calcd for $C_{19}H_{25}N_5$ 323.21125, found 323.21024.

**4-Amino-1-*tert*-butyl-3-(1′-naphthylmethyl)pyrazolo[3,4-*d*]pyrimidine (6h):** white powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.85 (s, 9H), 4.76 (s, 2H), 5.04 (s, 2H), 7.19 (d, $J$ = 6 Hz, 1H), 7.39 (t, $J$ = 8 Hz, 1H), 7.55 (t, $J$ = 4 Hz, 2H), 7.79−7.92 (m, 2H), 8.20 (d, $J$ = 8 Hz, 1H), 8.24 (s, 1H); HRMS (EI) molecular ion calcd for $C_{20}H_{21}N_5$ 331.17993, found 331.17951.

**4-Amino-1-*tert*-butyl-3-(1′-naphthoxymethyl)pyrazolo[3,4-*d*]pyrimidine (6i):** beige powder; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.83 (s, 9H), 5.57 (s, 2H), 6.12 (s, 2H), 7.07 (d, $J$ = 7 Hz, 1H), 7.39−7.54 (m, 4H), 7.84 (d, $J$ = 8 Hz, 1H), 8.25 (d, $J$ = 8 Hz, 1H), 8.35 (s, 1H); HRMS (EI) molecular ion calcd for $C_{20}H_{21}N_5O$ 347.17483, found 347.17408.

**Protein Expression and Purification** Site-directed mutagenesis and cloning of the genes for the glutathione-S-transferase fusion proteins of wild-type v-Src catalytic domain, I338G v-Src SH1, and WT Fyn into the pGEX-KT plasmid was carried out as described previously.[11,12] These kinases were expressed in DH5α *E. coli* and purified on immobilized glutathione beads (Sigma).

***In Vitro* Kinase Inhibition Assay.** $IC_{50}$'s for putative kinase inhibitors were determined by measuring the counts per minute (cpm) of $^{32}$P transferred to an optimized peptide substrate for src family kinases (IYGEFKKK). Various concentrations of inhibitor were incubated with 50 mM Tris (pH 8.0), 10 mM $MgCl_2$, 1.6 mM glutathione, 1 mg/mL of BSA, 100 $\mu$M IYGEFKKK, 3.3% DMSO, the appropriate kinase, and 11 nM (2 $\mu$Ci) [$\gamma$-$^{32}$P]ATP (6000 Ci/mmol, NEN) in a total volume of 30 $\mu$L for 30 min. Reaction mixtures (25 $\mu$L)were spotted onto a phosphocellulose disk, immersed in 10% HOAc, and washed with 0.5% $H_3PO_4$. The transfer of $^{32}$P was measured by standard scintillation counting. $IC_{50}$ was defined to be the concentration of inhibitor at which the cpm was 50% of the control disk. When the $IC_{50}$ fell between two measured concentrations, it was calculated based on the assumption of an inversely proportional relationship between inhibitor concentration and cpm between the two data points.

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**Supporting Information Available:** Experimental procedures (PDF). See any current masthead page for Web access instructions.

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