Crystal Structures of the Lyn Protein Tyrosine Kinase Domain in Its Apo- and Inhibitor-bound State*

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The Src-family protein-tyrosine kinase (PTK) Lyn is the most important Src-family kinase in B cells, having both inhibitory and stimulatory activity that is dependent on the receptor, ligand, and developmental context of the B cell. An important role for Lyn has been reported in acute myeloid leukemia and chronic myeloid leukemia, as well as certain solid tumors. Although several Src-family inhibitors are available, the development of Lyn-specific inhibitors, or inhibitors with reduced off-target activity to Lyn, has been hampered by the lack of structural data on the Lyn kinase. Here we report the crystal structure of the non-ligated form of Lyn kinase domain, as well as in complex with three different inhibitors: the ATP analogue AMP-PNP; the pan Src kinase inhibitor PP2; and the BCR-Abl/ Src-family inhibitor Dasatinib. The Lyn kinase domain was determined in its “active” conformation, but in the unphosphorylated state. All three inhibitors are bound at the ATP-binding site, with PP2 and Dasatinib extending into a hydrophobic pocket deep in the substrate cleft, thereby providing a basis for the Src-specific inhibition. Analysis of sequence and structural differences around the active site region of the Src-family PTKs were evident. Accordingly, our data provide valuable information for the further development of therapeutics targeting Lyn and the important Src-family of kinases.

Phosphorylation of the C-terminal tail (pTyr508 in Lyn) inhibits activity through promoting its association with the kinases own SH2 domain. In contrast, phosphorylation of a residue within the activation loop (pTyr397) results in activation of the enzyme. Enzymic inhibition is also enhanced by the binding of the kinases SH3 domain to a left-handed polyproline type II helix situated between the SH2 and kinase domains (2). The SH2 and SH3 domains are also utilized by the enzyme for its activation, as well as inactivation, through specific interactions with proteins containing polyproline and/or phosphotyrosine motifs, thus also allowing specific targeting of the kinases to specific substrates/subcellular compartments (3).

The involvement of Src family kinases in various signaling cascades including cytokine receptor pathways (4, 5) is gradually being elucidated (6). Lyn is expressed in hemopoietic cells of erythroid/myeloid and B lymphoid origin (7–9), neuronal cells, prostate cells (10), colon cells (11), and is involved in the transmission of signals from a number of receptors such as Epo (7, 12, 13), c-Kit (14), B cell antigen, and c-Mpl (15) receptors (4). Lyn phosphorylates a number of signaling molecules, including PI 3-kinase, PLCγ2, H51 (16), Cbp (17), STAT5 (18), and MAP kinase, and Lyn−/− mice display severe defects in the immune system (19) and the erythroid compartment (13, 20).

An important role for Lyn in leukemia has been suggested by several studies (21–25). Primary acute myeloid leukemia (AML) cells display elevated Lyn kinase activity (21) and Lyn is critical for maintaining AML cell proliferation and anti-apoptotic pathways (26). While the BCR-Abl fusion protein is the initiating molecule for chromic myeloid leukemia (CML), there is a crucial downstream role for Lyn in BCR-Abl-induced leukemogenesis (23–25). There is a direct link between Lyn and BCR-Abl signaling pathways as Lyn phosphorylates Tyr177 of BCR-Abl (27, 28), thus recruiting the adaptor Gab2, both of which are essential for BCR-Abl oncogenesis (29). Significantly, Imatinib-resistant CML cells have elevated Lyn levels and kinase activity (22). Further, ablation of Lyn from Imatinib-resistant CML cells resulted in the induction of apoptosis (30).

A significant role for Lyn in the development of certain solid tumors has also come to light. Colon carcinoma cells utilize Lyn in the activation of the Akt anti-apoptotic pathway, and drug-resistant cells show elevated Lyn kinase activity (11). Lyn is also involved in the signaling mechanisms regulating prostate cancer cells (31). Significantly, inhibition of Lyn in prostate cancer cell lines resulted in reduced proliferation in vitro and in prostatic xenograft models (10). Thus several lines of evi-
dence point to a significant involvement of Lyn in both leukemia and solid tumor development.

The Src-family as well as other cytoplasmic (e.g. Abl) and receptor (e.g. EGF-R) tyrosine kinases are important targets or therapeutic intervention (32, 33). Several specific (e.g. Imatinib) and some less specific (e.g. Dasatinib) small molecules have been generated that mostly act as ATP competitive inhibitors and have been successfully employed for leukemia/cancer treatment in the clinic (32, 33). Crystal structures of Src (1, 34), Hck (35, 36), and Lck (37, 38) have enabled a detailed investigation of how the Src family of kinases are regulated, and the way in which small molecule inhibitors can inactivate these enzymes. To extend our understanding, and provide a structural framework for the design of specific inhibitors to the Src family of kinases, we have determined the crystal structures of the kinase domain of Lyn in complex with 3 different inhibitors, AMP-PNP,2 PP2, and Dasatinib, as well as the non-ligated form of the enzyme, at 2.5–2.7-Å resolution.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—All plasmid constructs were generated by site-directed mutagenesis using oligonucleotides and subcloned in-frame into the appropriate vector and confirmed by sequencing. The murine Lyn kinase domain (amino acids 239–512) was subcloned into the baculovirus expression vector pFastBacHTA (Invitrogen) generating a His₆-tagged fusion resulting in a 5-amino acid N-terminal extension (GAMDP) to the Lyn kinase domain after tobacco etch virus (TEV) cleavage. The murine Csk-binding protein (Cbp, amino acids 74–474) was subcloned into pET44a (Merck, Darmstadt, Germany) to be expressed as a NusA-His₆-tagged fusion in bacteria (Rosetta2, Merck), with replacement of the thombin with a TEV cleave site using site-directed mutagenesis, for expression and purification using the Profinia system (Bio-Rad) for use as a biologically significant substrate for Lyn kinase assays.

**Protein Expression and Purification of Lyn Kinase Domain**—Recombinant bacmid DNA was isolated and used to transfect *Spodoptera frugiperda* (Sf9) insect cells. Baculovirus obtained from the transfection culture was used to infect Sf9 cells grown in suspension to a density of 2 × 10⁶ cells per ml, at a multiplicity of infection greater than 10, and harvested 48 h after infection. Cells were resuspended in a buffer consisting of 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5% glycerol, 3 mM β-mercaptoethanol, 0.1% thesis, supplemented with complete protease inhibitors mixture (Roche Applied Sciences), lysed by sonication and centrifuged at 45,000 × g for 1 h at 4 °C. The supernatant was filtered and loaded onto ProBond nickel-chelating resin (Invitrogen). After extensive washing, the recombinant protein was eluted with buffer plus 100–300 mM imidazole and Lyn-containing fractions were pooled. The His₆ tag was removed by treatment with TEV protease during overnight dialysis against 20 mM Tris HCl, pH 8.0, 25 mM NaCl, 5% glycerol, 2 mM DTT, 0.5 mM EDTA, at 4 °C. The digested protein was bound to a HiTrap Q column (GE Healthcare) equilibrated in the same buffer and eluted with a NaCl gradient. Lyn-containing fractions were pooled, concentrated to 2.5 ml and loaded onto a Superdex 75 gel filtration column (HiLoad 16/60; GE Healthcare) equilibrated in 20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 2 mM DTT, 0.5 mM EDTA. Purified Lyn kinase domain was concentrated to 8 mg/ml for crystallization.

**Protein Analysis and Western Blotting**—Mass spectrometric analysis of purified Lyn protein before and after auto-kinase assays were performed by Proteomics International (East Perth, WA, Australia) on protein gel plugs that were destained, trypsin-digested, and peptides extracted according to standard techniques (39). Peptides were analyzed by electrospray ionization mass spectrometry (LC/MS) using the Ultimate 3000 HPLC system (Dionex, Sunnyvale, CA) coupled to a Q TRAP 4000 mass spectrometer (Applied Biosystems, Foster City, CA). Tryptic peptides were loaded onto a C18 PepMap100 reversed phase column (Dionex) and separated with a linear gradient of water/acetonitrile/0.1% formic acid (v/v) at a flow rate of 300 nl/min. Spectra were analyzed to identify proteins of interest using Mascot sequence matching software (Matrix Science, London, UK) with the data base and taxonomy set as Ludwig NR and All Taxonomy, respectively. The phosphorylation detection was achieved with precursor ion scanning for a loss of 79 mass units in negative mode (PO₃⁻) and subsequent MSMS fragmentation of those selected peptides to determine their identity. Protein concentration was estimated using the Bio-Rad D₅₀ protein assay according to the manufacturer’s instructions, using bovine serum albumin as a standard.

Exo-kinase kinase assays were performed essentially as previously described (7), with minor modifications, using NusA-Cbp (5 mg/ml) as substrate in 50 mM Tris-HCl, pH 7.4, 1 mM DTT, 10 mM MgCl₂, 50 μM ATP, at 30 °C. Kinase inhibitors Dasatinib (Bristol-Myers-Squibb, New York, NY), PP2 (Merck) and SU6656 (Merck) were added to reaction mixtures 10 min prior to the addition of ATP. Auto-kinase reactions were undertaken under identical conditions to those described for the exo-kinase assays, only no NusA-Cbp substrate was added. Reactions contained 0.1 μg/10 μl Lyn kinase and were stopped by the addition of SDS loading buffer. Reactions were then analyzed by Western blotting for phosphotyrosine incorporation into the Cbp substrate.

Western blotting was performed essentially as described previously (7, 40). Antibodies used included anti-phosphotyrosine 4G10-HRP (Millipore, Billerica, MA) and anti-phospho-Src (pY416) (Cell Signaling Technology, Danvers, MA). Secondary antibodies were coupled to horseradish peroxidase (Amer- sham, Buckinghamshire, UK) and detected by enhanced chemiluminescence (Amerham Biosciences). Western blots were quantitated using a ChemDoc XRS (Bio-Rad) and Quantity One (v4.5.2, Bio-Rad).

**Crystalization of Lyn Kinase Domain and Formation of Inhibitor Complexes**—Crystals were grown at 4 °C using the hanging-drop vapor-diffusion method. Purified Lyn kinase domain was mixed with an equal volume of a reservoir solution containing 23% polyethylene glycol 3350, 0.1 M NaCl, and 0.1 M Na.Hepes, pH 7.5. Crystals formed after 1–3 days. Inhibitors PP2 (1-tet-butyl-3-(4-chlorophenyl)-2H-pyrazolo[4, 5-e]pyrimidin-1-ium-4-amine) and Dasatinib (BMS-354825; N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-

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2 The abbreviations used are: AMP-PNP, adenosine 5′-[(β,γ-imino)triphosphate; PTK, protein-tyrosine kinase domain; VDW, van der Waals; DTT, dithiothreitol; r.m.s.d., root mean square deviation.
aryl)-2-methylpyrimidin-4-ylamino(thiazole-5-carboxamide) in DMSO at 10 mM, or diluted 10-fold in reservoir solution, were added to drops containing Lyn crystals, and incubated at 4 °C for 1–2 h. AMP-PNP and MgCl₂ at 50 mM each were added to crystal-containing drops and incubated for up to 3 weeks at 4 °C.

X-Ray Data Collection, Structure Determination, and Refinement—Crystals were flash-frozen using the reservoir solution plus 10% glycerol as a cryoprotectant. Data sets at 2.5–2.76-Å resolution were merged and processed using MOSFLM and SCALA in the CCP4i suite. The crystals, with unit cell dimensions 

TABLE 1
Data collection and refinement statistics

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<tr>
<th></th>
<th>Apo-Lyn</th>
<th>Lyn-PP2</th>
<th>Lyn-Dasatinib</th>
<th>Lyn-AMP-PNP</th>
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<td>48.7-2.76 (2.91-2.76)</td>
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<td>23.1</td>
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a The value in parentheses is for the highest resolution bin (approximate interval, 0.1 Å).

b Rmerge = Σ[Ihkl–<Ihkl>]/ΣIhkl

c R-factor = Σ[Ihkl–|Fhkl|]/|Σ[Ihkl]| for all data except 5%, which was used for the Rfree calculation.

FIGURE 1. Biochemical analysis of the purified Lyn kinase domain. A, Lyn auto-phosphorylates its activation loop. Purified Lyn kinase domain was analyzed for phosphorylation of Tyr⁹⁰⁷ using the Src pY⁴¹⁶-specific antibody. Phosphorylation of Tyr⁹⁰⁷ detected by Western blotting was quantitated relative to total protein using Quantity One (Bio-Rad). B, exo-kinase activity of Lyn. Analysis of the Lyn kinase domains ability to phosphorylate Cbp. Western blots using anti-pY-HRP coupled antibodies were quantitated as in A above. C, determination of IC₅₀ values for Dasatinib, PP2, and SU6656 for the Lyn kinase domain. Kinase assays were performed using Cbp as a substrate with various concentrations of inhibitors (Dasatinib, PP2, SU6656) and Western blots of phospho-Cbp analyzed as in A above.

and SCALA in the CCP4i suite. The crystals, with unit cell dimensions 

RESULTS AND DISCUSSION

Biochemical Analysis of Purified Lyn Kinase Domain—The purified kinase domain of Lyn was assessed for post-translational modifications by Western blot and mass spectrometry analysis. Using an antibody specific for the common activation loop phosphorylation site found in Src family kinases (pTyr⁴¹⁶
in Src, pTyr<sup>397</sup> in Lyn), only after auto-activation was significant reactivity observed (Fig. 1A). This was confirmed by mass spectrometric analysis, showing the purified kinase domain was essentially unphosphorylated at this site, but upon incubation with Mg<sup>2+</sup>-ATP it became rapidly phosphorylated. The enzyme was also able to rapidly phosphorylate the substrate Cbp in exo-kinase assays showing the capacity of the purified protein to display functional activity (Fig. 1B). Further, the ability of small molecules to inhibit the enzymes ability to phosphorylate Cbp were analyzed, showing Dasatinib (IC<sub>50</sub> 11 nM), PP2 (IC<sub>50</sub> 9 nM), and SU6656 (IC<sub>50</sub> 35 nM) could all strongly inhibit the activity of Lyn (Fig. 1C). The PP2 and Dasatinib inhibitors were then used to examine the structural basis of binding and specificity by x-ray crystallography.

Structure of the Lyn Kinase Domain—To determine the overall atomic architecture of the Lyn PTK, and provide a baseline for structural comparison of inhibitor binding to Lyn, the kinase domain of murine Lyn was crystallized in the absence of inhibitor and its structure determined at 2.5-Å resolution and refined to an R<sub>fact</sub> = 20.5% and R<sub>free</sub> = 23.8%. The refined model, comprising residues 239–501, is typical of the bi-lobal protein-tyrosine kinase fold and closely resembles the structures of Src, Lck, and Hck (Fig. 2A). Briefly, the N-terminal lobe of the PTK consists of a 5-stranded anti-parallel twisted β-sheet and a single large α-helix, termed the αC helix. The C-terminal lobe is larger and predominantly helical, but includes a 2-stranded anti-parallel β-sheet. Between the two lobes is a deep cleft that forms the ATP-binding pocket, and connecting them is a loop that borders the cleft and forms a hinge that confers flexibility to the overall structure, allowing for relative movement of the lobes.

The catalytic activity of protein tyrosine kinase can be regulated by the phosphorylation state of the activation loop Tyr residue (Tyr<sup>397</sup> in Lyn) and is correlated with movements in the loop and αC. The region of the activation loop spanning this residue is thus highly flexible, and in the apo Lyn structure residues 393–399, inclusive, are disordered and not included in the model, thus not revealing the phosphorylation state of Tyr<sup>397</sup>, although Western blot and mass spectrometric analyses clearly indicated that the Lyn PTK was unphosphorylated. Structural comparison with unphosphorylated, inactive Src and Hck, and active, phosphorylated Lck (Fig. 2B) shows that despite the lack of phosphorylation in Lyn PTK, the activation loop and αC are in the active conformations.

AMP-PNP Binding to Lyn—To provide a model for the substrate-bound state of the enzyme, Lyn crystals were soaked with the non-hydrolyzable ATP analogue adenyl imidodiphosphate (AMP-PNP) in the presence of Mg<sup>2+</sup> ions and the resulting complex was solved at 2.7 Å resolution and refined to an R<sub>fact</sub> = 19.9% and R<sub>free</sub> = 24.5%. As expected, AMP-PNP binds in the cleft between the two lobes of the kinase domain (Fig. 3A) forming extensive van der Waals (VDW) and hydrogen-bonding interactions. The planar adenine ring is sandwiched between the hydrophobic residues of the N-terminal lobe (Leu<sup>253</sup>, Val<sup>261</sup>, and Ala<sup>275</sup>), the hinge region (Phe<sup>321</sup> and Met<sup>322</sup>) and the C-terminal lobe (Leu<sup>374</sup>). In addition, two hydrogen bonds link the adenine group through N<sup>6</sup> and N<sup>1</sup> to the hinge residues Glu<sup>220</sup> and Met<sup>222</sup>, respectively, as seen with AMP-PNP-binding to other Src family kinases. In the published complexes of AMP-PNP with Src (1) and Lck (38), the ribose group is hydrogen-bonded to the Ser residue conserved in all but one Src-family PTKs (326 in Lyn) either through a water-mediated hydrogen bond in Src, or directly in the case of Lck where the side chain points toward the ligand. In the Lyn structure, as in Src, Ser<sup>326</sup> is pointing away from the ribose group, but at the resolution of this Lyn structure, no hydrogen-bonded water molecule is detected. Asp<sup>385</sup> of the conserved DFG motif forms hydrogen bonds with the α- and β-phosphates and a water-mediated hydrogen bond to the imido-nitrogen. The β-phosphate group interacts with the glycine loop residues Ala<sup>255</sup> and Gly<sup>259</sup> via a water molecule that is accommodated by a small shift in the loop location with respect to the apo structure. A third water molecule links Arg<sup>369</sup> to the γ-phosphate. Thus, extensive contacts and hydrogen bonds anchor the substrate analogue and poise the tri-phosphate group for catalysis.
PP2 Binding to Lyn—For insight into the structural basis of small molecule inhibition of Lyn PTK by the pan-Src kinase inhibitor PP2, the PP2-Lyn complex was formed by soaking apo Lyn crystals with PP2, and the structure was solved at 2.76Å resolution and refined to an $R_{	ext{free}}$ of 19.5% and $R_{	ext{free}}$ of 23.1% (Fig. 3B). PP2 binds to Lyn kinase in a very similar position to AMP-PNP, with the respective purine rings almost co-planar. The two adenine group hydrogen bonds to residues Glu$^{320}$ and Met$^{322}$ are present, with a third, absent in the AMP-PNP structure, between N25 of the inhibitor and Thr$^{319}$OG. This hydrogen bond, seen in PP2 binding to Lck (38) and the closely related inhibitor PP1 binding to Hck (36), is made possible by the...
purine group of PP2 inserting slightly more deeply into the nucleotide-binding cleft than in AMP-PNP, and a concomitant movement of Thr319 to accommodate the chlorophenyl moiety of the inhibitor. The chlorophenyl ring is rotated some 67° with respect to the plane of the purine ring system and buried in a hydrophobic pocket not occupied by ATP, having VDW interactions with Lys275, Glu290, Met294, Ile317, Thr319, and Ala384. The ter-butyl substituent extends toward the ribose-binding pocket and makes VDW contacts with Val261, Ser326, and Leu374.

**Dasatinib Binding to Lyn**—Dasatinib is a multi-target kinase inhibitor recently approved for treatment of chronic myeloid leukemia, and showing promise in the treatment of several solid tumors. For further insight into the structural basis of specific inhibitor binding, Lyn crystals were soaked with Dasatinib and the resulting complex structure solved to 2.6-Å resolution and refined to an R_{eq} = 19.8% and R_{free} = 23.1% (Fig. 3C). The deep hydrophobic pocket that accommodates the chlorophenyl moiety of PP2 here is occupied by the 2-chloro-6-methyl phenyl group of Dasatinib, which makes extensive VDW contacts with Lys275, Glu290, Met294, Val303, Ile317, Thr319, and Ala384. The aminothiazole ring of Dasatinib is located approximately where the purine ring of PP2 is situated, participating in a hydrogen bond to Met322N through the ring nitrogen, and having VDW interactions with Lys275, Glu290, Met294, Ile317, Thr319, and Ala384. The main chain atoms of these residues are superposable in the Lyn-PP2 structure with related PTKs similarly reveals a high degree of structural conservation. Given the high degree of sequence identity among the Src-family as well as the closely related Ab1 kinase.

**Comparison of Inhibitor Binding to Lyn and Related Structures**—The binding of the three structurally distinct inhibitors to Lyn caused only minor changes in the overall structure from the unliganded state, with root mean square (r.m.s.) deviations upon binding of 0.27 Å over 244 Ca atoms for AMP-PNP, 0.24 Å over 235 Ca atoms for PP2, and 0.39 Å over 230 Ca atoms for Dasatinib (Fig. 4). Comparison of these Lyn structures with related PTKs similarly reveals a high degree of structural conservation. Given the high degree of sequence identity among the Src-family of PTKs (Fig. 5A) this structural conservation is not surprising and highlights the significant challenge faced in designing inhibitors specific to individual members.

AMP-PNP binding is expected to closely mimic that of substrate ATP. The Lyn-AMP-PNP structure reveals 2 hydrogen bonds to main chain atoms of highly conserved residues in PTKs and phosphate interactions with catalytic residues as well as conserved regions of the glycine loop. By contrast, PP2 exerts Src-family selectivity through a unique hydrogen bond to Thr319, a residue conserved among this group of kinases (Fig. 5A). Furthermore, the chlorophenyl moiety is positioned within a hydrophobic pocket, access to which is made possible by Thr319 substituting the gate-keeper Met residue conserved in many PTKs (38). Superimposing the Lyn-PP2 complex with the published Lck-PP2 structure (Fig. 5B) reveals a small r.m.s. deviation of 0.68 Å over 230 Ca atoms. Only 2 amino acid substitutions between Lyn and Lck exist around the active site. The hinge residues Ala323 and Lys324 of Lyn correspond to Lck Glu320 and Asn321. Indeed, these sequence positions are the most variable around the active site of the Src-family kinases. The main chain atoms of these residues are superposable in the two structures, and the small size of PP2 means that the side chains of neither residue are contacted by the molecule, consistent with PP2 being a pan-Src family inhibitor. Nonetheless, the amino acid substitutions at these positions may present exploitable differences in the future design of more selective inhibitors.

Dasatinib was originally designed as a selective inhibitor to the Src kinases (42) and subsequently identified as a useful BCR-Ab1 inhibitor (43). Dasatinib is able to inhibit forms of BCR-Ab1 harboring point mutations that confer resistance to Gleevec (Imatinib) and arise during drug treatment. This results from Dasatinib binding to the active state of the kinase rather than being limited to the inactive state in the case of Gleevec (44). The Lyn-Dasatinib complex determined here is the first reported structure of this inhibitor with an Src-family PTK. The specificity of Dasatinib is exerted through similar active site interactions to those of PP2. Structural alignment of the Lyn-Dasatinib complex with the Ab1 complex (41) gives an
r.m.s. deviation of 0.870 Å over 236 Ca atoms. The structural features highlighted above are also present in Abl, accounting for the cross-reactivity of the compound. However, a major difference is observed in the glycine loop between Abl and Lyn, with an 8.2-Å movement of the Co of Lyn Phe²⁵⁸ to the corresponding Abl Tyr²⁵³ (Fig. 5C). Abl Tyr²⁵³ makes several VDW contacts with the aminothiazole and amide moieties of Dasatinib that are absent in the Lyn complex. The repositioning of the glycine loop also enables the carbonyl oxygen of Abl Gly²⁴⁹ to contact the pyrimidine methyl group, which is not seen between the inhibitor and Lyn Gly²⁵⁴. Although the Glycine loop of PTKs is quite flexible, and thus would be expected to accommodate some variety of conformations, it is notable that the Abl glycine loop is in a similar position in the
recently reported structure of the Abl-Nilotinib inhibitor complex (45), and in both cases quite distinct to the location in all 4 Lyn structures reported here (Fig. 4), as well as in other published Src-family PTK structures. Such a distinct structural difference between the Lyn and Abl complexes likely accounts for much of the >10-fold higher affinity of Abl (IC_{50} < 1 nM) (42) than Lyn (11 nM, this work) for Dasatinib. The variable hinge residue Ala^{233} (Thr^{319} in Abl) makes VDW contact with the hydroxethyl-piperazine moiety of Dasatinib through the main-chain carbonyl oxygen. In the Abl complex, Tyr^{220} is within VDW contact distance, which is not seen with Lys^{324} of Lyn. These structural features not only are likely to contribute to different binding affinities for Dasatinib, but, importantly, point the way to further exploitation in the development of future, more specific compounds.

The current study reports the structure of Lyn PTK. Analysis of the binding mode of a series of structurally distinct inhibitors reveals features accounting for their varying specificity to Lyn and other PTKs. Kinase inhibitors are becoming an increasingly important class of chemotherapeutic agents. With the growing recognition of Lyn as a key player in several leukemias as well as important class of chemotherapeutic agents. With the growing

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REFERENCES

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