Activity of dual SRC-ABL inhibitors highlights the role of BCR/ABL kinase dynamics in drug resistance

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Mutation in the ABL kinase domain is the principal mechanism of imatinib resistance in patients with chronic myelogenous leukemia. Many mutations favor active kinase conformations that preclude imatinib binding. Because the active forms of ABL and SRC resemble one another, we tested two dual SRC-ABL kinase inhibitors, AP23464 and PD166326, against 58 imatinib-resistant (IM*) BCR/ABL kinase variants. Both compounds potently inhibit most IM* variants, and in vitro drug selection demonstrates that active (AP23464) and open (PD166326) conformation-specific compounds are less susceptible to resistance than imatinib. Combinations of inhibitors suppressed essentially all resistance mutations, with the notable exception of T315I. Guided by mutagenesis studies and molecular modeling, we designed a series of AP23464 analogues to target T315I. The analogue AP23846 inhibited both native and T315I variants of BCR/ABL with submicromolar potency but showed nonspecific cellular toxicity. Our data illustrate how conformational dynamics of the ABL kinase accounts for the activity of dual SRC-ABL inhibitors against IM* mutants and provides a rationale for combining conformation specific inhibitors to suppress resistance.

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The small molecule protein kinase inhibitors imatinib (1–5), gefitinib (6), and erlotinib (7, 8) are susceptible to resistance in patients because of amino acid substitutions in the target protein. Imatinib inhibits BCR/ABL by stabilizing the kinase in a catalytically inactive conformation (9). Point mutations in the ABL kinase domain can thwart drug binding by direct steric hindrance or by destabilizing the inactive kinase conformation that is required for imatinib binding (2, 3). Consequently, developing drugs that target the open or active conformation of the kinase may prove effective in rescuing patients who develop imatinib resistance.

Previously, we carried out an in vitro screen for imatinib resistance and identified a large number of mutant amino acid residues outside the active site that did not appear to act by direct steric hindrance of drug binding. Several of these residues were homologous to SRC residues known to play critical roles in maintaining the autoinhibited SRC kinase conformation (10–13), and some previously had been implicated by site-directed mutagenesis in ABL kinase regulation (14, 15). We reasoned that these conformational, or allosteric, mutants exerted effects on drug binding by favoring adoption of the active kinase conformation. Using inferences from the mutagenesis studies, we proposed a model for the assembled ABL kinase that closely resembled the autoinhibited SRC structure (3). Crystallographic and biochemical data published alongside our mutagenesis report confirmed that ABL indeed was regulated in a SRC-like manner (16–18).

The striking similarity between the catalytically active states of the SRC and ABL kinases prompted us to investigate whether SRC kinase inhibitors might be effective against imatinib-resistant (IM*) ABL variants (3, 16). In this report, we have analyzed the activity of AP23464 and PD166326 against 58 IM* variants of BCR/ABL and conducted screens for in vitro resistance to these compounds individually and in combination with imatinib. Our data show that these agents show potent activity against the majority of IM* mutants and are less subject to resistance, with the notable exception of T315I. Based on screening and structural analysis, we chemically modified AP23464 to achieve kinase inhibition of T315I, although the compounds suffered from cellular toxicity. Our results, together with structural modeling, provide important insights into the role of kinase dynamics in mediating drug resistance and suggest that a combination of conformation-specific inhibitors can effectively suppress molecular resistance.

Results

Kinase-Activating IM* BCR/ABL Variants Are Sensitive to AP23464 and PD166326. AP23464 and PD166326 are synthetic small-molecule, ATP-competitive dual-specificity SRC/ABL kinase inhibitors (Fig. 1A). AP23464 and PD166326 inhibited the proliferation of BaF3 cells transformed with BCR/ABL (BaF3-BCR/ABL) with IC50 values of 13.4 and 5.4 nM, respectively. AP23464 and PD166326 are considerably more potent than imatinib and showed no inhibition of untransformed IL-3-dependent BaF3 cells at a concentration of 10 μM. To obtain a comparative inhibition profile, we analyzed the activity of these two compounds against 58 IM* BCR/ABL variants. Both compounds showed greater potency than imatinib against most variants, with the notable exception of T315I (Fig. 1B; see also Fig. 5 and Table 1, which are published as supporting information on the PNAS web site).

AP23464 was superior to PD166326 at inhibiting the P-loop variants L248R, G250E, Q252H, Y253H, and E255K. The variant A269V showed significant resistance against AP23464 (IC50 = 201 nM; 15-fold greater than for native BCR/ABL) but only modest resistance against PD166326 (IC50 = 19.5 nM; 3.5-fold greater; Fig. 1B and Table 1). Interestingly, several variants from the C helix and the C lobe are more sensitive than native BCR/ABL to AP23464 and PD166326 (Fig. 1B and Table 1). We have confirmed the different relative activity profiles of these variants by in vitro autophosphorylation assay (Fig. 5). The higher activity of the AP23464 and PD166326 compounds against the IM* BCR/ABL variants implies more favorable binding to a distinct conformational state promoted by point mutation.

BCR/ABL Mutations Resistant to AP23464. To understand the structure-activity relationships and patterns of resistance for the...
AP23464 compound, we performed a drug selection screen with mutagenized BCR/ABL, as described for imatinib (3). The yield of AP23464-resistant colonies was consistently lower than for imatinib. At the highest concentration of AP23464 tested (500 nM), the yield of resistant colonies dropped to 3 per 10⁶ cells (Fig. 2A). These data suggest that AP23464 is not only more potent than imatinib but is also less susceptible to resistance.

We sequenced the resistant clones. In 200 nM drug, 20 amino acid substitutions were discovered affecting 12 different residues. Four of these residues confer imatinib resistance, whereas eight are unique to AP23464. Nine of 13 clones harbored E255K, H295, T315I, F317V, and C330S as a single mutation, whereas three clones harbored E255K or T315I mutations associated with E373K, D444N, R386S, and L387V (Tables 2 and 3, which are published as supporting information on the PNAS web site). Residue E373 lies at the end of the 7 strand after helix-E, where it appears to modulate the movement of the N and C lobes relative to each other through direct contact with Y320 and T319 of the hinge region; mutation at E373 likely destabilizes the kinase cleft in a manner that is unfavorable for AP23464 binding. Residue H295 lies at the C terminus of helix C and makes contact with S349 of helix E. Mutations at these residues may have a profound effect on the activity of AP23464 and PD166326 alone and in combination with imatinib. (A) Frequency of resistance of BAF3 cells transformed with randomly mutagenized BCR/ABL. For each experiment, 150 × 10⁶ BAF3 cells were transduced with randomly mutagenized BCR/ABL virus. After 16–20 h cells were divided into 15 subgroups and exposed to different drug concentrations, as indicated below each bar. Cells were then plated at a density of 0.25 × 10⁶ per well of a six-well plate. The number of colonies obtained per 10⁶ cells from two independent experiments were averaged and plotted. (B) Frequency of resistance from the BAF3 cells harboring native, nonmutagenized BCR/ABL. For each experiment, 130 × 10⁶ BAF3-BCR/ABL cells were divided into 13 subgroups and exposed to different drug concentrations, as indicated below each bar. Numbers below each bar represent number of resistant colonies from 10⁷ BAF3 cells.
kinetics of opening and closing of the catalytic cleft, which may
contribute significant drug resistance. Clones selected in 500 nM
AP23464 represented a very limited spectrum of mutations at
either T315I or F317V. One clone harbored both mutations T315I
and F317C. Modeling studies with AP23464 revealed that the
mutations T315I and F317V/C cause direct steric blockade to drug
binding and alter the hydrophobic surface of the binding site (data
not shown).

BCR/ABL Mutations Resistant to PD166326. We next selected for
resistance to PD166326. As for AP23464, selection in PD166326
yielded fewer colonies than imatinib (Fig. 2A). Sequence analysis
revealed a range of point mutations identical to IM8 variants, and
several previously undescribed ones (Tables 2 and 3). Like imatinib,
mutation in the P loop residues was a predominant mode of resistance: The mutants G250E and E255K were recovered in 35% and 45% of sequenced clones, respectively. Interestingly, mutations from the Cap, SH3, SH2, CD linker, helices C, E, I, and the activation loop were frequently found in association with G250E or E255K (Table 3). The Y253H mutation was recovered in 13% of
clones, typically associated with secondary mutations: Q30P (cap),
Q252H, A359G, I403L (activation loop), and E281G (C helix).
Y128T was identified as a robust single mutation from the SH2
domain that conferred resistance to 100 nM PD166326. PD166326-
resistant clones that did not have P loop or gatekeeper residue
mutations were found to harbor multiple substitutions from distinct
kinase regulatory motifs. For instance, one clone harbored four
mutations, some of which previously were linked to imatinib
resistance: D233N (CD linker), E292K (C helix), I360L (activation
loop), and V422L (helix F, myristate binding pocket). Four
clones recovered at 500 nM PD166326 each harbor T315I substitu-
tions, illustrating that this mutation remains refractory to even
high concentrations of this potent compound.

Combinations of Inhibitors Select for a Narrow Spectrum of Mutations. Although highly potent, the single inhibitors remain susceptible
to resistance by point mutation in the ABL kinase. PD166326
and AP23464 suppress essentially all resistance mutations at 500
nM, with the notable exception of T315I and F317V/C. However, it
is likely that at this drug concentration, these compounds would man-
ifest undesirable off-target activity against other kinases. An
appealing strategy for suppressing resistance is to combine agents,
on the presumption that their spectrum of resistance will not over-
lap and that lower concentrations of two agents will suppress
resistance. To test this conjecture, we performed in vitro screens for
resistance to combinations of the kinase inhibitors at different
submaximal concentrations (Fig. 2A and Table 2). Combinations of
AP23464 with PD166326 or imatinib reduced the yield of resistant
clones to 3–4 per 106 cells. The resistant clones that survive the combination of AP23464 with PD166326 harbor T315I and F317V
mutations, whereas clones resistant to AP23464 with imatinib
harbor T315I and F317L. The combination of PD166326 with
imatinib was subject to a broader spectrum of resistance mutations:
Three of four clones harbored E255K mutations, and two showed
mutations in the C helix (E281G) or the activation loop (K400Q)
and F-helix (E450K) (Table 3). The triple combination of imatinib,
PD166326, and AP23464 at 5 μM, 50 nM, and 100 nM, respectively,
yielded significantly fewer resistant colonies but failed to suppress
E255K and E279K mutations that are clinically prevalent (Table 4,
which is published as supporting information on the PNAS web site).
Importantly, at higher drug concentrations (200 nM of
AP23464, 100 nM PD166326, and 5 μM of imatinib), resistance was
rare and mediated by the T315I mutation only.

These combination data allow several interesting conclusions: (i)
IM8 mutations are more apt to be cross-resistant to PD166326 than
to AP23464, (ii) combinations of inhibitors reduce the frequency of
resistance in vitro, and (iii) combining even three compounds at
lower drug concentrations will not suppress P loop variants; instead,
high concentrations of each of the drugs is required to be maximally
effective. These in vitro data suggest that combination therapy may
be an appealing front-line strategy for reducing primary resistance,
potentially for the treatment of chronic myelogenous leukemia
patients who have an advanced stage disease at diagnosis and are
often imatinib refractory. Unfortunately, using drugs in combina-
tion may not allow for reduced dosing of individual agents, and
combination therapy may not be a means for reducing side effects
of high-dose regimens.

Selection for Drug Resistance Against Native BCR/ABL. In vitro mu-
tagenesis of BCR/ABL provides a highly sensitive means of
cataloging resistance mutations that might arise during treatment.
To test whether mutagenesis introduces bias into our screen and to
document that mutagenesis enhances screening efficiency, we
performed a screen against cell lines transformed by native, non-
mutagenized BCR/ABL. The pattern of mutations that we recov-
ered was strikingly similar to screens employing mutagenesis,
including the predominance of the P loop mutations Y253H and
E255K and the refractory nature of T315I (Fig. 2B; see Tables 3–7,
which are published as supporting information on the PNAS web
site). Interestingly, many of the clones also harbored multiple
mutations (Tables 6 and 7), implying that multiple mutations are
not an artifact of hypermutation in our system but are detected
because of cooperative effects on drug resistance.

Importantly, the yield of resistant clones from cells trans-
formed with nonmutagenized BCR/ABL was ~10-fold lower than from
cells transformed with a randomly mutagenized library of BCR/ABL plasmids (Fig. 2A and B). Furthermore, sequence analysis revealed that only 70% of colonies isolated in
the absence of mutagenesis harbored relevant point mutations
compared with 100% in screens from mutagenized libraries,
implying that other mechanisms of drug resistance play a larger
role in screens of native BCR/ABL. A previous screen for
resistance against PD166326 by using native, nonmutagenized
BCR/ABL captured only a subset of mutations found in our
screen of mutagenized BCR/ABL and required sequencing of
considerably more clones (19). A recently published screen against dasatinib (BMS-354825) that used mutagenesis showed
efficient identification of resistant clones (20).

AP23464 Variants Designed to Inhibit T315I. A combination of
AP23464 with imatinib suppressed virtually all drug resistance,
except for the vexing T315I variant. Structural modeling reveals that
the phenol ring attached to N-9 of AP23464 targets the deep
hydrophobic pocket of the ABL kinase active site (Fig. 3A and
Supporting Methods, which are published as supporting information on the PNAS web site); access to that site is limited by the
mutant T315I. Therefore, we tested a series of AP23464 analogues with
modifications at the N-9 position for activity against the T315I ABL
kinase (Fig. 3A). AP23848 and AP23980 were only weakly effective
(IC50 = 6.422 nM and 5.055 nM, respectively), whereas AP23846
showed significant potency (IC50 = 297 nM). The key modification in
AP23846 relative to AP23464 (or its exact parent compound
AP23848) is the replacement of the phenol moiety with the ethyl
group at N-9 (Fig. 3A). Replacement of the ethyl group at N-9 with
hydrogen (AP23980) resulted in the loss of inhibitory potency.
AP23464 can dock within the ABL active site and does not clash
with the T315I substitution (Fig. 3B). AP23846 inhibited the
autophosphorylation of both native and T315I-BCR/ABL in a
cellular lysate at submicromolar concentration (Fig. 3C). Cellular
testing showed that AP23846 inhibited proliferation of BaF3 cells
transformed by native BCR/ABL and the T315I variant; unfortu-
nately, it also inhibited proliferation of the parental BaF3 cells,
particularly for the treatment of chronic myelogenous leukemia
patients who have an advanced stage disease at diagnosis and are
often imatinib refractory. Unfortunately, using drugs in combina-
tion may not allow for reduced dosing of individual agents, and
combination therapy may not be a means for reducing side effects
of high-dose regimens.
hydrophobic pocket. However, additional structural features must be engineered into our compound to achieve ABL kinase binding specificity without undesirable toxicity.

Discussion

Mutations in the ABL kinase confer imatinib resistance by either direct steric hindrance to drug binding or by destabilizing the assembled kinase structure that imatinib prefers. The structural resemblance between the catalytically active states of the SRC and ABL kinases prompted us to test the hypothesis that dual SRC-ABL kinase inhibitors would show activity against IMR kinase-activating variants. We tested the activity of two such compounds, AP23464 and PD166326, against a panel of 58 IMR mutants from structurally diverse regulatory and catalytic motifs. Most of the IMR variants are sensitive to one or both of the compounds, with the exception of the P loop variants, T315I, and F317L/H20862V/H20862C. The varying pattern of sensitivity reflects the different affinities of the two compounds for the distinct conformations adopted by the various mutant forms, highlighting the conformational plasticity of the ABL kinase. These data confirm our hypothesis that regulatory mutants that activate the kinase also become susceptible to dual SRC-ABL inhibitors.

The cocrystal structure of PD166326 and ABL shows an open and yet catalytically inactive kinase conformation, with the critical DFG motif rotated away from the catalytic center. In contrast, the cocrystal of AP23464 and SRC (21) demonstrates a DFG conformation that is distinctly more open and catalytically favorable. AP23464 was more effective than PD166326 at suppressing drug resistance, likely because of its association with a more catalytically active kinase conformation. Dasatinib (BMS-354825), which likewise targets the open conformation of the ABL kinase, also has shown reduced frequency of resistance mutations in an in vitro screen for drug resistance (20).

Engineered mutations in the SH2-C-lobe interface and myristate-binding pocket release ABL kinase autoinhibition (17), and mutation of this critical regulatory region is a frequent cause of imatinib resistance (Fig. 4). Interestingly, several of these variants are hypersensitive to both AP23464 and PD166326, again implying that these compounds have higher affinity for the active conformation. Cocrystal structures of ABL and PD173955 show the drug can bind to both the inactive and active conformations, leading to the concept that dual SRC-ABL inhibitors are “conformationally tolerant” (22, 23). Our data suggests significant variation in the apparent binding affinity for specific BCR/ABL variants, thereby...
highlighting the plasticity of the ABL kinase and the role of kinase dynamics in mediating drug resistance.

Selection for resistance against AP23464 or PD166326 alone resulted in the isolation of some previously undescribed mutants not associated with imatinib resistance. Previous screens for drug resistance with the compounds dasatinib (20) and PD166326 (24) likewise discovered previously undescribed mutants at residues G250, E255, T315, and T317, suggesting that each compound will elicit unique variants. Interestingly, identical substitutions of several P loop (L248, G250, Y253, Q252, and E255) and gatekeeper residues (T315 and T317) confer cross-resistance to the structurally distinct compounds imatinib, dasatinib, and PD166326 (20, 24). The common mutants occur chiefly within the active site of the enzyme to which all of the drugs bind. Given the large number of possible amino acid substitutions, this observation highlights the structural constraints imposed on the kinase active site, which must retain the capacity for ATP binding and hydrolysis to maintain transforming function. Mutation at residue F317 appears to be particularly problematic for AP23464 binding because of its abrogation of Van der Waals interactions with the dimethylphosphonophenyl group of the inhibitor. The reduction in surface area provided by the substitution of cysteine or valine residues at F317 destabilizes inhibitor binding. In contrast, the pyridine moiety of imatinib utilizes less surface area from F317 for binding; therefore, substitution at this position causes only modest resistance against imatinib. The reduced frequency of resistant mutations against dasatinib and AP23464 highlights the importance of targeting the active conformation.

Most P loop mutations in our screen carried secondary mutations in the Cap, SH3, SH2, CD linker, C helix, hinge region, SH2-C-lobe interface, activation loop, or myristate-binding pocket. Thus, mutations in the regulatory domains cooperate with P loop variants to reinforce drug resistance. Such multiple mutations were isolated even in screens with nonmutagenized, native BCR/ABL, suggesting that multiple mutations are selected functionally for enhanced drug resistance and do not reflect promiscuous hypermutation during mutagenesis. We predict that similar combinations of mutations will be found in clinical samples. Our data also clearly demonstrate that exposure of cells to a combination of agents with binding preferences for different conformations can suppress most, but importantly not all, drug resistance. Even the combination of PD166326, AP23464, and imatinib at high doses failed to suppress T315I. Combinations at lower doses were not effective and, instead, yielded multiple mutations occurring in concert with E255K and E279K. Neither of these mutations interact directly with the drug, but their association with mutations from regulatory regions (e.g., the SH2-C-lobe interface and CD linker) suggest that they cooperate to disrupt the kinase conformation in a manner that precludes effective binding of all three agents.

ABL Kinase Dynamics Illustrated by Structural Modeling. Mutagenesis followed by molecular modeling of drug-resistant variants provides complementary information to crystallographic data and suggests hypotheses for how mutations might alter the kinase conformation. Mutations affecting the activation loop, helix C, and the SH2-C-lobe interface are very frequent among resistant clones. The PD173955-ABL structure (1M52) indicates that R386 of the activation loop makes a salt bridge with E292 of the C helix and may interact with V289 of the C helix by an induced-dipole moment. Substitution of the polar side group of serine for V289 would predict a stronger contact with the basic side group of R386, which would imply a regulatory communication between the C helix and the activation loop. Such a direct coupling previously has not been described for the tyrosine kinases, but precedent exists for a comparable interaction in the crystal structure of the Jak3 kinase in an active state (24).

The SH2-C-lobe interface is comprised of helix A from the SH2 domain and helices E and I from the C lobe of the kinase. Mutations in this structural motif presumably disrupt the myristate-binding pocket or the docking interactions of the SH2-C-lobe interface, both of which are critical to maintaining the ABL kinase in an assembled state (Fig. 8A, which is published as support information on the PNAS web site). Our modeling analysis suggests that the E507K mutation may disengage D504 from R170 because of the favored interaction of D504 with the oppositely charged K507 (Fig. 8A). Such an interaction would lead to a straightening of helix-I’, a dislodging of the SH2 domain, and a distortion of the myristate pocket. A change in the orientation of helix E might exert a long-range influence on the kinase conformation through disrupted associations between helices E and C, which also are implied by our mutagenesis and modeling (Fig. 8B and C). Residues S349, Y353, and K357 of helix E make direct contact with H295, K294, L298, and E292, respectively, of helix C in the closed conformation, but in the open conformation, the interactions of Y353 and K357 are lost because of the displacement of helices E and C (Fig. 8A and B). Q346 from helix E makes contact with H375 and V377 from 8-8 of the activation loop. Our modeling suggests that mutations of contact residues from helix E might directly influence the helix E on helix C movement, thereby altering the kinase conformation in a manner that would alter the active site. The influence of mutations affecting the SH2-C-lobe interaction on the conformation of the activation loop, and on the movement of helix E and helix C, are hypotheses based on modeling only. Confirmation of any of the above speculations would require the crystallization of individual mutant forms of BCR/ABL or studies of protein dynamics.

A Proof-of-Concept Compound for Inhibiting T315I. T315I serves as a gatekeeper residue that controls access to a hydrophobic region of the enzymatic active site that is not contacted by ATP (1, 9). Mutation at this critical residue confers resistance to almost all ATP-competitive inhibitors of the ABL kinase (22, 25–32). A comparable substitution in platelet-derived growth factor receptor (PDGFR) α and PDGFRβ can be inhibited by PKC412 and SU6668, respectively, compounds that avoid contact with the hydrophobic region (31, 33). AP23846 is a proof-of-concept compound that inhibits the native and T315I kinase and, likewise, avoids the hydrophobic pocket (Fig. 6). Unfortunately, AP23846 is not a drug candidate because of nonspecific cytotoxicity, but this compound provides incentive to explore other structural modifications to identify more specific inhibitors of T315I.

Despite remarkable efficacy, imatinib is not a cure for chronic myelogenous leukemia. Primary refractory disease or relapse with drug-resistance is particularly problematic in advanced phase chronic myelogenous leukemia (34, 35), and some chronic phase patients proceed directly to blast crisis despite apparently effective treatment with imatinib (36, 37). And although virtually all newly diagnosed patients attain complete cytogenetic remission after imatinib, the vast majority retain evidence of residual leukemic clones by PCR (38, 39). The persistence of residual disease raises the specter that dormant leukemic clones eventually might escape drug suppression or provoke disease relapse once imatinib is discontinued. Strategies aimed at greater front-line disease eradication and suppression of resistance are needed, most of which depend on further research into combination chemotherapy and next-generation ABL kinase inhibitors that are active against IM-resistant BCR/ABL variants.

Materials and Methods

Compounds. AP23464 [3-(2-(2-cyclopentyl-6-(4-(dimethylphosphoryl) phenylamino)-9H-purin-9-yl) ethyl)phenol], AP23848 [3-(2-(2-cyclopentyl-6-(4-(dipropylphosphoryl)phenylamino)-
9-H-purin-9-yl)ethyl)phenol], AP23846 [2-cyclopentyl-N-(4-(dimethylphosphoryl)phenyl)-9-ethyl-9-H-purin-6-amine], and AP23980 [2-cyclopentyl-N-(4-dipropethylphosphoryl)phenyl]-9-H-purin-6-amine] were synthesized at Ariad Pharmaceuticals. PD166326 [6-(2, 6-dichlorophenyl)-2-(3-hydroxymethylphenylamino)-8-methyl-8/H/-pyrido [2, 3-d/] pyrimidine-7-one] was synthesized at the Memorial Sloan–Kettering Cancer Center.

**BCR/ABL Mutagenesis and Screening.** BCR/ABL mutagenesis and screening was performed as described in refs. 3 and 40. We chose a graded series of AP23464 concentrations (100, 150, 200, and 500 nM), representing 7.5-, 10-, 15-, and 37-fold multiples, respectively, of the IC50 value determined for growth inhibition of BaF3 cells transformed by native BCR/ABL. The 135 and 200 nM concentrations of AP23464 correspond to 5 and 10 nM concentrations of imatinib in the degree of cell inhibition in this assay. Likewise, we chose PD166326 concentrations for screening at 50, 100, and 500 nM, where 50 and 100 nM concentrations are comparable with 5 and 10 µM imatinib in the inhibitory effect against native BCR/ABL. Genomic DNA was isolated from the resistant clones and a region of BCR/ABL spanning from the BCR fusion to the C terminus of the kinase domain (L528; type Ia numbering) was amplified by PCR with the following primers: BCR/ABLI, 5'-GAGAACAATCGCGGAGCGAC-3'; BCR/ABLII, 5'-CTC-CAGACTGTCACACATTC-3'; BAKR1, 5'-CTGCTTCAACCTGTCAAGGC-3'; and BAKR2, 5'-GACGTCTTCTGGAGGTCCTCG-3'. Amplified PCR products were sequenced. Sequence alignment and analysis was performed by DNASTAR II (DNASTAR, Madison, Wl).

**Cell Viability, Kinase Inhibition, and Western Blotting Assay.** Cell viability, kinase assay, and Western blotting was performed as described in refs. 3 and 40.

**Structural Modeling and Representation.** Structural modeling and docking of the AP23846 on kinase active site were carried out by using INSIGHT II (Accelrys, Inc., San Diego).

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