A Pharmacophore Map of Small Molecule Protein Kinase Inhibitors

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Protein kinases have emerged as one of the major drug target classes that are amenable to the development of small molecule inhibitors. They share a conserved structural similarity in the region of the ATP binding site, where most inhibitors interact. Using a pharmacophore approach, we have explored the features of protein−ligand interactions for a set of 220 kinase crystal structures from the Protein Data Bank (PDB). The resulting "pharmacophore map" shows the interactions made by all ligands with their receptors simultaneously. This gives insight that has been applied in the design of kinase screening sets and combinatorial libraries. An algorithm is described that scores small molecule structures for goodness of fit to the resulting binding site description obtained from the analysis. The algorithm identifies a pose that is close to the crystal structure pose for the majority of ligands, with only the 2D chemical structure as input and no knowledge of the crystal structure from which it was derived. Application of the algorithm to a test screening set gave a useful enrichment of active compounds.

INTRODUCTION

Protein kinases are currently receiving a great amount of attention as drug targets for the development of orally available small molecule inhibitors.[1] They are critical components of cellular signal transducion pathways, the control of which has applications in the treatment of important diseases, particularly in the fields of oncology[2,3] and inflammation. Although of relatively recent origin, several protein kinase inhibitors have been approved for the treatment of cancer and many more are in various stages of clinical development and in the pipelines of major pharmaceutical companies.[4,5]

Protein kinases are a superfamily of enzymes that number over 500 in the human genome,[6] for which there is a great deal of structural information available.[7] They share a common structural fold and catalytic mechanism that have been described in detail.[8,9] The catalytic mechanism involves ATP, which provides a phosphate group for phosphorylation of tyrosine, serine, or threonine residues of a protein substrate. Although kinase domains exist in different molecular and cellular contexts and have different mechanisms of activation,[10] the ATP binding site is highly conserved in both sequence and structure across all members of the superfamily. Most small molecule inhibitors bind to this site and compete with ATP.[11] Many pharmaceutical companies are currently involved in large scale screening of small molecule compounds for activity against kinase targets. The binding site similarities common to all or most members of the superfamily enables the design of kinase-focused screening sets.[12,13] There are third party suppliers that also provide commercially available compound screening sets that attempt to exploit knowledge about kinase binding sites.

We have analyzed a set of kinase ligand:receptor cocrystal structures from the Protein Data Bank (PDB) using a pharmacophore approach, to arrive at a description of interactions made by kinase ligands with their respective targets. This gives insight into the features of ligands that are required for activity against any kinase, i.e., *similarities*, and also the features that confer selectivity between them, i.e., *differences*. The wealth of data now available gives a precise description of the structural features that a small molecules needs to possess to be a high affinity kinase ligand. In the early days of kinase inhibitor design it was thought that target selectivity might be very difficult to achieve. Subsequent experience has shown that some selectivity can be achieved by exploiting the structural differences that exist between receptors;[14−17] however, cross-reactivity remains an issue.

When screening compounds against kinase targets, an important consideration is how to design or put together a screening set of compounds that are (1) compatible with the ATP binding site and (2) contain novel chemotypes with regard to intellectual property. A computational algorithm has been devised that uses the results of the present analysis to select compounds that are compatible with the binding site description given by the pharmacophore map. The algorithm fits the PDB ligands to the pharmacophore map to within a close approximation of the crystal structure pose the majority of the time, without any direct knowledge of the receptor. This method has been used to select compounds and design combinatorial libraries that form the basis of a kinase focused screening set.

MATERIALS AND METHODS

1. Crystal Structure Set for Analysis. We have constructed a database of protein kinase small molecule ligands derived from crystal structures from the PDB,[18] from which a subset of structures was chosen for analysis. In general, the criterion was the presence of a unique drug-like inhibitor in the ATP binding site. Where duplicate ligand structures occur, only the one with the best crystallographic resolution

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was retained. The resulting set consisted of 220 structures. The crystal structure resolution was between 1.3 and 3.5 Å with a median of 2.1 Å. The molecular weight of the ligands was between 149 and 590, with a mean of 379 and a standard deviation of 90.

Structures with ATP and ATP analogues were excluded; although many drug-like ligands mimic the adenine part of ATP, they generally do not mimic the sugar and phosphate regions, so including ATP analogues was considered to skew the analysis. Structures with ligands that do not make the ligand H-bond acceptor (L−HBA) interaction with the hinge region were excluded. This is the most commonly seen interaction in kinase ligands and is described in more detail below. The several ligands that do not make this interaction were not considered interesting from a drug development point of view. Therefore, as a rule for our purposes, all kinase ligands make this interaction. Interestingly, three closely related kinases—Pim1, Pim2, and Pim3—have a proline residue in the receptor in the position of the residue that makes this interaction, and therefore ligands cannot make this H-bond. Therefore these structures were also eliminated from this analysis (should they become targets for drug design, they would be treated separately from a modeling point of view). Finally, the PI3K lipid kinases, although sharing the same overall fold and binding site features of protein kinases, were considered sufficiently divergent from the protein kinases to be excluded.

2. Common Reference Frame. The crystal structure with PDB code "1ATP" was used as a reference structure. This is murine PKA bound to ATP. It was one of the earliest crystal structures to be solved of a protein kinase[19] and has been used as a reference structure in previous analyses.[20,21] An important part of the present analysis involved transforming the coordinates of each structure to a common reference frame. This was done by performing a least-squares superimposition of each structure to the reference structure using the Cα coordinates of a subset of residues. These residues, in 1ATP numbering, were chosen to be 57−59, 70−72, 103−105, 118−124, 126−129, 168−173, and 181−184. These are 30 residues in 7 continuous regions that define the core of the structure around the binding site that is common to all protein kinases, the conformation of which does not change appreciably upon kinase activation (Figure 1). The task is then to identify the equivalent residues in each structure. This is usually clear from a visual inspection on the computer graphics, but to speed the process, a computer program was written that examines the coordinates of each structure and determines which subset of residues has a distance matrix that most closely resembles that of the reference structure and then performs an rms superimposition using this subset of atoms. The selection was then checked manually by visual inspection and all the alignments were observed to be correct. All structures had a root mean square (rms) deviation of <2.0 Å for these atoms and 82% were <1.0 Å. This procedure then gives a set of receptors and ligands aligned in a common reference frame for further analysis. Residues in the receptors can be referred to by the equivalent numbering in the 1ATP structure; for example, the protein H-bond donor residue in the hinge region is 123, and the so-called "gatekeeper" residue is 120.

3. Assigning Pharmacophore Features to Ligands. A common method for analyzing ligand−protein interactions is the assignment of pharmacophore features to ligand structures (and sometimes receptors). The 6 most common pharmacophore types are H-bond acceptor (HBA), H-bond donor (HBD), hydrophobic, negative charge, positive charge, and aromatic. These are the kinds of interactions that are generally observed in crystal structures of ligand−protein complexes of all kinds. Assignment of these types was made to all the superimposed ligands. This was done using the PharmPrint methodology,[22−24] but similar results would be expected using various commercially available modeling packages that incorporate pharmacophore assignment. PharmPrint assigns pharmacophore types to every atom in a small molecule using chemically aware rules based on substructure matching. In addition, the aromatic type is assigned to a pseudo-atom created in the center of an aromatic ring. A default nonspecific "X" type is assigned to atoms that do not have any of the other types assigned.

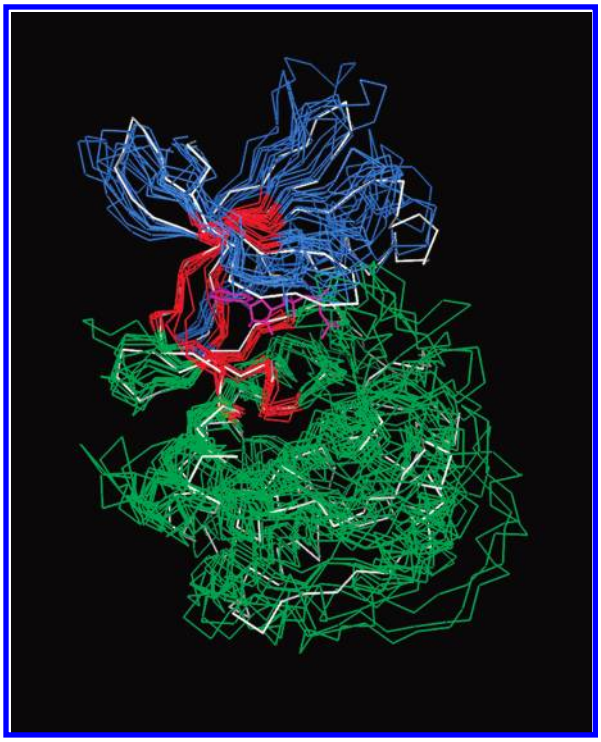

Figure 1. Structural superimposition of 10 different kinases in the reference set. The residues used to perform the rms superimposition, around the ATP binding site, are colored in red. The N-terminal lobe is colored blue, and the C-terminal lobe is colored green.

For the purposes of creating the pharmacophore map, additional constraints were imposed on the assignment of H-bond and hydrophobic atoms. H-bond atoms in the ligand were assigned only if they actually make an H-bond to the receptor, according to the criteria of Baker and Hubbard.[25] In addition hydrophobic atoms were assigned in the ligand only if they make contact with a hydrophobic atom in the receptor, using a cutoff distance of 4 Å.

4. PharmMap Algorithm. Similar to docking algorithms, this consists of two steps. First, there is the generation of a number of combinations of conformations and orientations of the ligand in the binding site—the ligand "pose". Second, a scoring function is applied that calculates a goodness of fit to the binding site.

The scoring function is calculated using the combined ligand atoms in the reference space with their pharmacophore

assignments, plus an excluded volume component calculated from the protein receptor structures in the reference space. Therefore the score gives a generic measure of fit to a kinase target. The scoring function, based on the pharmacophore map, assumes that a favorable pose for a test compound is one where the atom coordinates and pharmacophore types of the test structure correspond closely to those in the map. Therefore a grid of scoring potentials was constructed around the binding site and scores are stored at each point on the grid for each pharmacophore type. At each grid point, the distance to every atom in the map is calculated, and the score for each pharmacophore type at that point is a sum of a function of the distances for that type. Different distance functions were tried; in the end a simple linear function was arrived at that scores a maximum at distance 0.0 Å and declines linearly to a score of 0.0 at 1.0 Å. So, for example, a HBA atom in a ligand scores maximally in the center of a cluster of HBA points in the map.

A conformational database of these ligands was built using the high throughput conformational analysis functionality of MOE, consisting of conformations that retain no memory of the crystal structure. With the default setting this gave an average of 83 conformations per structure. An algorithm was sought to initially place the structures in the pharmacophore map. This consisted of randomly choosing 3 atoms in the ligand and 3 points in the map for a least-squares superimposition, subject to the constraints that the atoms/points have the same pharmacophore types assigned to them and that the distances between them fall into the same distance bin. The distance bins were the 6 previously used in the PharmPrint methodology: 2−4.5, 4.5−7, 7−10, 10−14, 14−19, and 19−24 Å. This then gives a potentially favorable pose suitable for scoring. Many such poses are generated for each conformation, and each pose can be optionally subjected to an optimization involving small rigid rotations and translations of the whole molecule to optimize the scoring function.

5. Compound Test Sets. The 220 PDB ligands were divided into 4 groups for cross-validation. The pharmacophore grid and excluded volume were recalculated for 3 groups, and the ligands in the remaining group were put through the PharmMap program. This procedure was performed 4 times and the results combined.

A database of compounds was put together containing known kinase inhibitors, plus a set of background or "decoy" molecules, to test the ability of the algorithm to discriminate between kinase active and inactive compounds in a screening context. The active set comprised 100 randomly chosen compounds from the Kinase Knowledgebase from Eidogen-Sertanty,[26] with IC50 values better than 1 μM for a kinase target. The inactive set was 1900 randomly chosen compounds from the Comprehensive Medicinal Chemistry (CMC) database from MDL.[27] Both sets were filtered for non-drug-like compounds (metal complexes, etc.) and constrained to be in the molecular weight range of 150−600. Thus, this simulates a screening set of compounds with 5% active molecules.

6. Selectivity Data. Although the focus of the current study is not primarily kinase selectivity, it was considered instructive to explore the issue in the context of the pharmacophore map, at least as a rudimentary first step. Fabian et al.[28] provide screening data for 20 kinase inhibitors, most of which are or have been in clincial development, against 119 kinase assays. The compounds were divided arbitrarily into 3 groups according to the number of assays in which they showed measurable activity: selective (4−23), intermediate (32−42), and nonselective (50−110). For the compounds that have crystal structures available, they were transformed to the common reference frame. Models of the remaining compounds were built by modifying the closest compound in the crystal structure set. The binding poses for all compounds were then examined in the context of the pharmacophore map.

Calculations were executed in the C programming language, and the molecular graphics were displayed using the MOE software from the Chemical Computing Group running on a Silicon Graphics Tezro workstation.

RESULTS

1. Pharmacophore Map. It is instructive to view all the ligands overlapped in the common reference frame, with bond connectivity removed and atoms colored by pharmacophore type. Common interactions are seen to emerge, many of which are well-known and previously described[29] (Figure 2). Most figures are drawn looking "down" from the N-terminal of the protein, with the hinge region on the left, and with the ATP aromatic ring in the plane of the paper/screen. The most relevant parts of the 1ATP protein are shown for context.

General Features. The ligand atoms are densest in the region of the adenine ring of ATP, where the pocket is flat and hydrophobic, with H-bond interactions with the hinge region (described in more detail below). Turning the illustration 90° (Figure 3) shows that atoms in this region occupy a well-defined plane and explains why ligands are predominantly aromatic in this position. The region occupied by the sugar portion of ATP is well-occupied by ligand atoms, but not to as high a density as the purine region. This region is less planar, and clusters of points signifying specific interactions are difficult to discern, the atoms being mainly hydrophobic or X type in character. The region occupied by the ATP phosphate groups is, perhaps surprisingly, very sparsely occupied by ligand atoms. However, there are other places, not occupied by ATP, where ligand atoms are commonly seen. There is a dense cluster of aromatic and hydrophobic groups that extend out toward the solvent from the hinge region, roughly between residues Leu49 in the N-terminal lobe and Gly126 in the C-terminal lobe that flank the opening of the pocket. Further out from this area, exposed to solvent, is a more diffuse group of mainly HBA and HBD groups. There is another clear cluster of aromatic and hydrophobic groups sandwiched between the gatekeeper residue 120 and the conserved lysine 72, at the back of the cleft away from solvent. This is a commonly observed interaction that occurs when the gatekeeper residue is small (e.g., threonine) and is a major determinant of selectivity between kinase targets. The majority of ligands make the above-described interactions; however, there are several examples of ligands that go beyond this region, and two classes of them are notable. One of them, illustrated by, for example, Gleevec[30] (PDB code: 1OPJ), contains ligands which bind to the catalytically inactive conformation of kinases such as ABL, involving a conformational flip of the

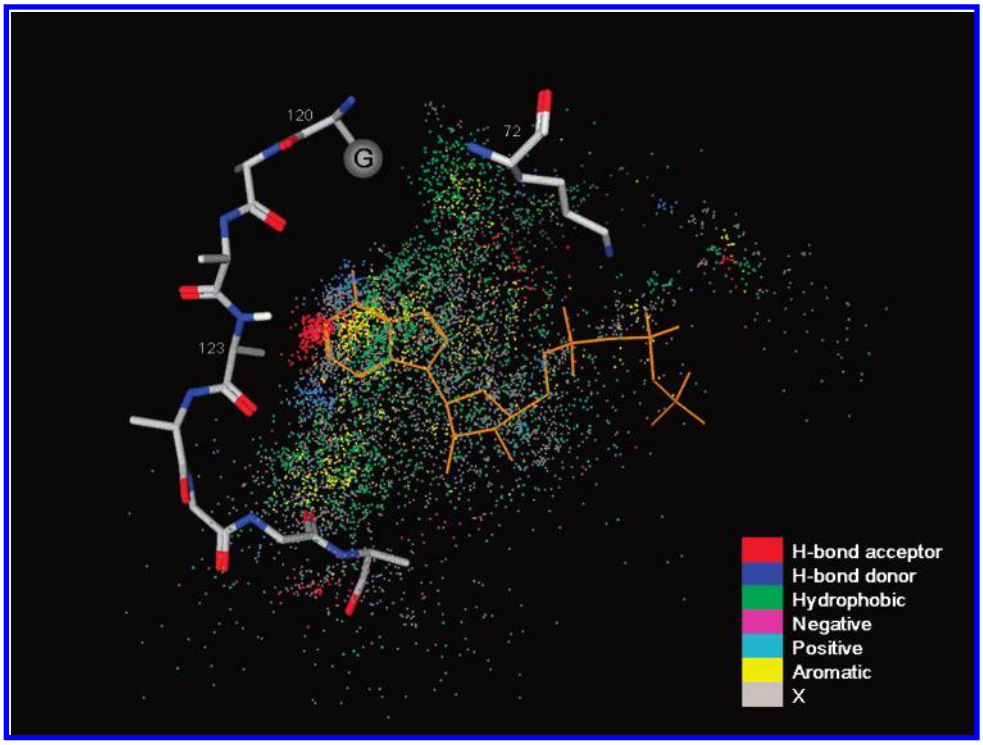

Figure 2. Complete pharmacophore map. All ligand atoms in the reference set of compounds have been transformed to the same reference frame and are displayed colored by pharmacophore type and with bond connectivity removed. The relevant parts of the reference protein (PDB code: 1ATP) are displayed for context. The hinge H-bond donor in the protein is residue 123, the conserved lysine is 72, and the gatekeeper residue is labeled as "G".

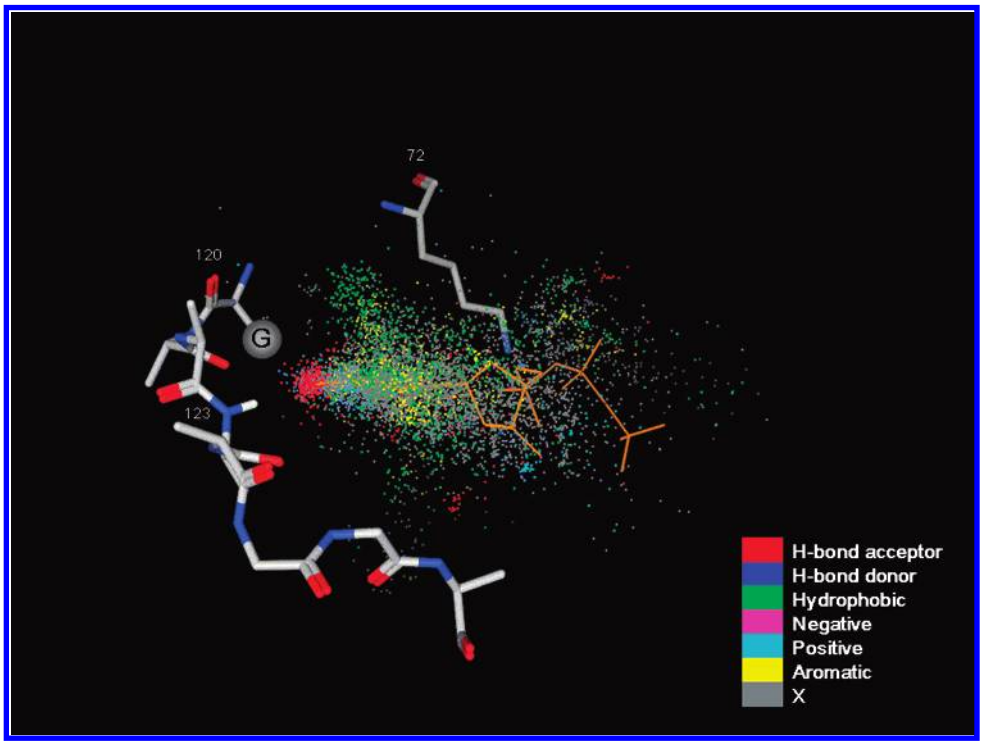

Figure 3. Same data as in Figure 2, but rotated 90°, to show the planar nature of the interactions in the region of the adenine ring of ATP.

DFG motif compared to the active conformation, that opens up a pocket behind the gatekeeper residue, into which part of the ligand protrudes.[31] These ligands are sometimes referred to as type II inhibitors, as opposed to the type I inhibitors that bind to the ATP site of active kinase domains. There are 20 of these so-called DFG-out structures in the data set, involving the kinases ABL, Aurora A, B-RAF, P38, and VEGFR2. Another class is illustrated by the AGC group of kinases, for example, the complexes of PKA with Balanol and H89 (PDB codes: 1BX6 and 1YDT), where part of the ligand extends roughly to the region of the ATP phosphates and interacts with the glycine rich loop.

H-Bond Acceptor (Figure 4). The most common interaction is the ligand−HBA interaction with the hinge region in the receptor. As a result of the selection criteria, this is made by every ligand in the set analyzed and is also made by the

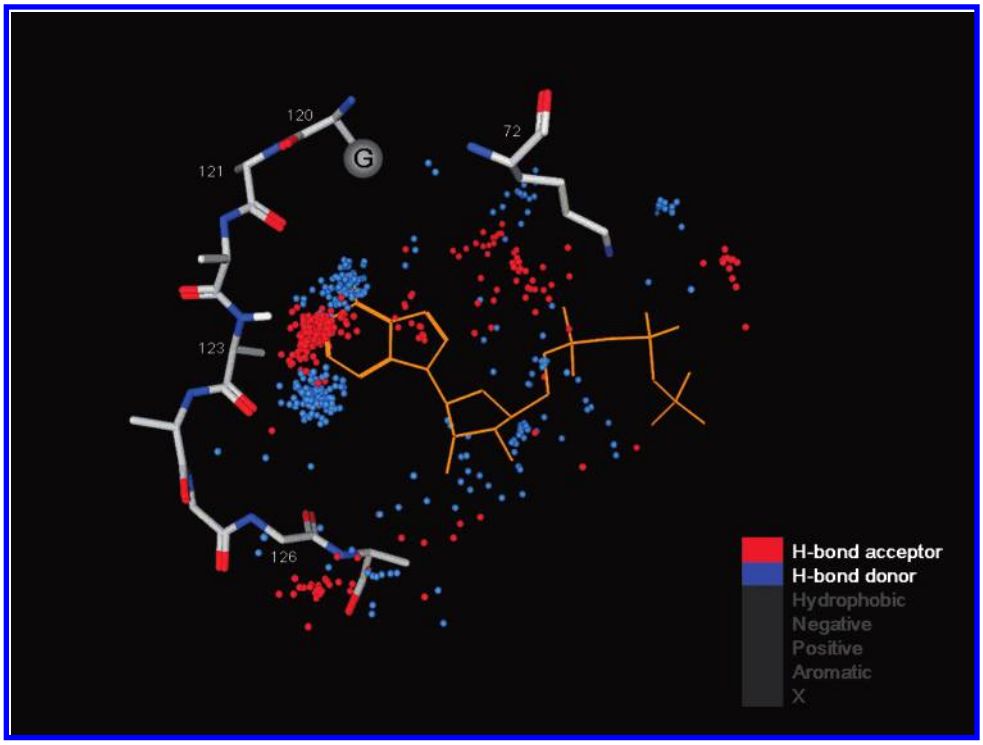

Figure 4. Same data as in Figure 2, but broken down into individual pharmacophore types.

N1 atom in ATP. This is seen clearly as a cluster of ligand−HBA points within the H-bonding distance of the main chain N−H group of residue 123. Other ligand−HBA interactions are less common, but smaller clusters can be seen within the H-bonding distance of the side chain of Lys72, the main chain N−H groups of Asp184 in the floor of the cleft near the gatekeeper, and Glu127 downstream from the hinge region at the opening of the cleft.

H-Bond Donor (Figure 4). To either side of the hinge ligand−HBA interaction, and also interacting with the hinge region, are two clear clusters of ligand−HBD groups. The first of these is equivalent to the N6 atom in ATP and interacts with the main chain carbonyl of residue 121 that points in to the binding site; approximately 40% of ligands make this interaction. The second cluster interacts with the carbonyl of residue 123 and has no equivalent in ATP; approximately 52% of ligands make this interaction. It is observed that 18% of ligands make both of these interactions and 26% make neither. Other smaller clusters are seen to interact with the main chain carbonyl of Glu170 and the side chain oxygen of Asn171 (simultaneously) in the region of the ATP sugar, and with the side chain of Glu91 in the C-helix.

Aromatic Features (Figure 5). The most prominent cluster of aromatic groups corresponds to the six membered aromatic ring of ATP. A second cluster appears roughly between residues Leu49 in the N-terminal lobe and Gly126 in the C-terminal lobe, forming a sandwich type hydrophobic interaction at the periphery of the cleft before the ligand goes out to solvent. Another cluster appears between the gatekeeper residue 120 and the conserved lysine 72; this occurs when the gatekeeper side chain is small and is a major determinant of selectivity in kinase ligands. Aromatic groups also appear throughout the rest of the binding site, but mostly deep inside the hydrophobic cleft.

Hydrophobic Features (Figure 6). Atoms labeled as hydrophobic do not form such well-defined clusters as the other groups above; they are found throughout the binding site but, not surprisingly, are most common in the hydrophobic inner region of the cleft. Highly populated regions are seen around the aromatic regions mentioned above and also in the parts occupied by the adenine and sugar moieties of ATP.

Charged Groups (Figure 7). In protein kinases, unlike some other classes of targets, charge interactions are not a central feature of ligand−receptor interactions. Crystal structures reveal that there are one or two divalent metal cations such as Mg^{2+} that bind along with ATP and neutralize the negative charges on the phosphate groups. Negative charges in synthetic ligands, however, are very uncommon, occurring in only 3% of the compound set and do not form any notable interactions or clusters. On the other hand, positively charged groups such as tertiary amines are quite common, occurring in approximately 37% of compounds in the set analyzed. There is a cluster of positive charges in the region of the O3* sugar atom in ATP and the carbonyl of residue 170 with which it interacts. This is where the amine in staurosporine and related compounds is approximately situated. Other occurrences of a positively charged amine in this region are with the PKA ligands Balanol and H89 (PDB codes: 1BX6 and 1YDT), where in this class of receptors there is a cluster of negatively charged residues flanking this region. Other positively charged groups are present on the periphery of the binding site exposed to solvent, probably the result of the practice of incorporating basic moieties to optimize the drug-like properties of compounds.

X Type (Figure 8). The X pharmacophore type is assigned to any atom that has not been assigned any of the 6 standard types above. As such, these atoms generally fill the space that is explored by the rest of the atom types, while not making specific interactions with the receptor.

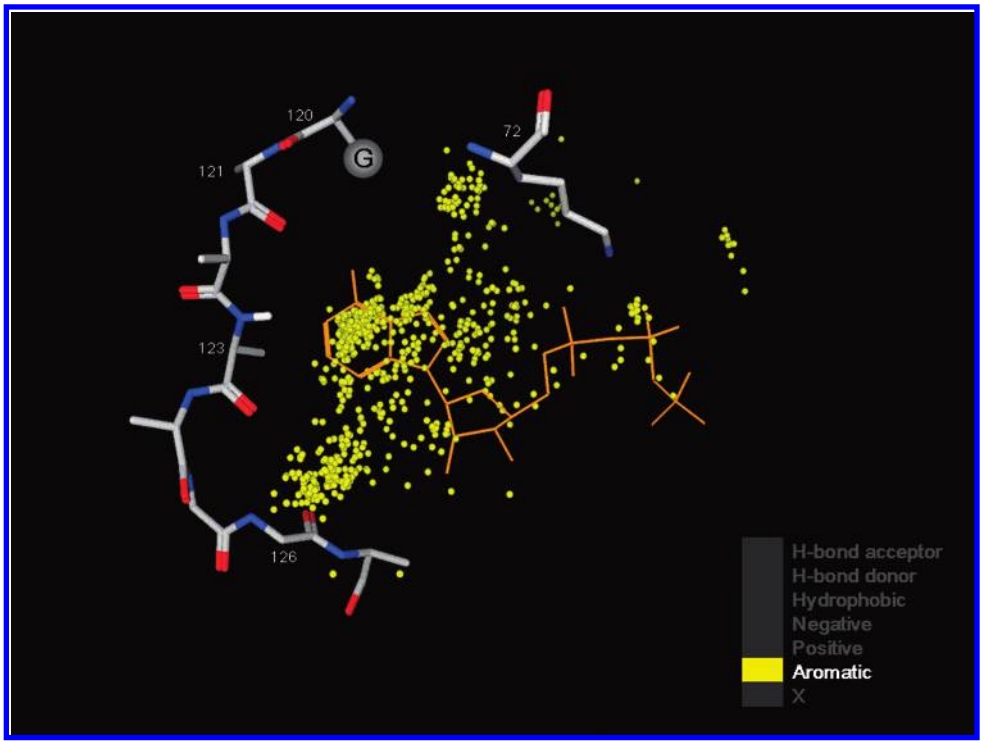

Figure 5. Same data as in Figure 2, but broken down into individual pharmacophore types.

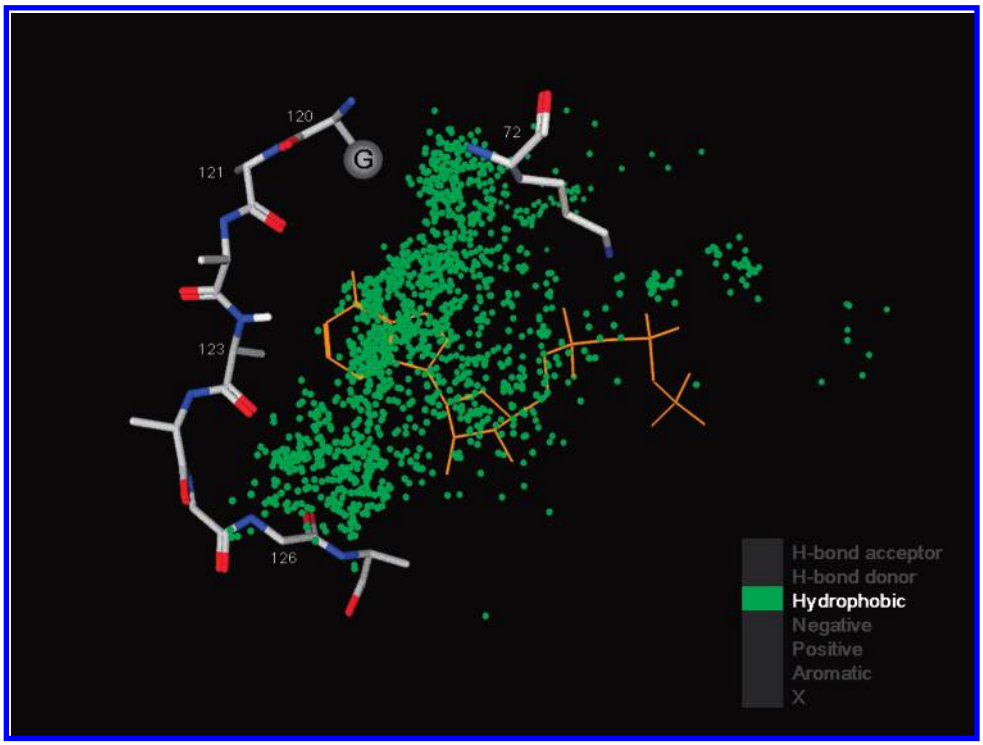

Figure 6. Same data as in Figure 2, but broken down into individual pharmacophore types.

2. PharmMap Algorithm. Although a visualization of the pharmacophore map on the 3D computer graphics, and the interactive positioning of new molecules within the map, is an instructive exercise and a useful tool in itself, we sought to devise an algorithm that automatically orients ligands optimally in the map and calculates a score for goodness of fit, thus giving a measure of kinase-ligand-likeness for the structure. The 220 PDB ligands were put through the algorithm using the cross-validation procedure described in Materials and Methods. Thus, each ligand is fitted to a pharmacophore map that was not generated from its own crystal structure. Analysis of the final poses showed that over half (53%) of the poses were within 2.5 Å rms of the crystal structure. From visual inspection, it was determined that, within this cutoff, ligands are generally in the correct pose and make the correct interactions, and differences are mostly in the periphery of the molecule, whereas outside the cutoff molecules can be flipped over, not making the correct interactions. If the crystal structure conformation is used as input for each ligand and the algorithm only needs to orient

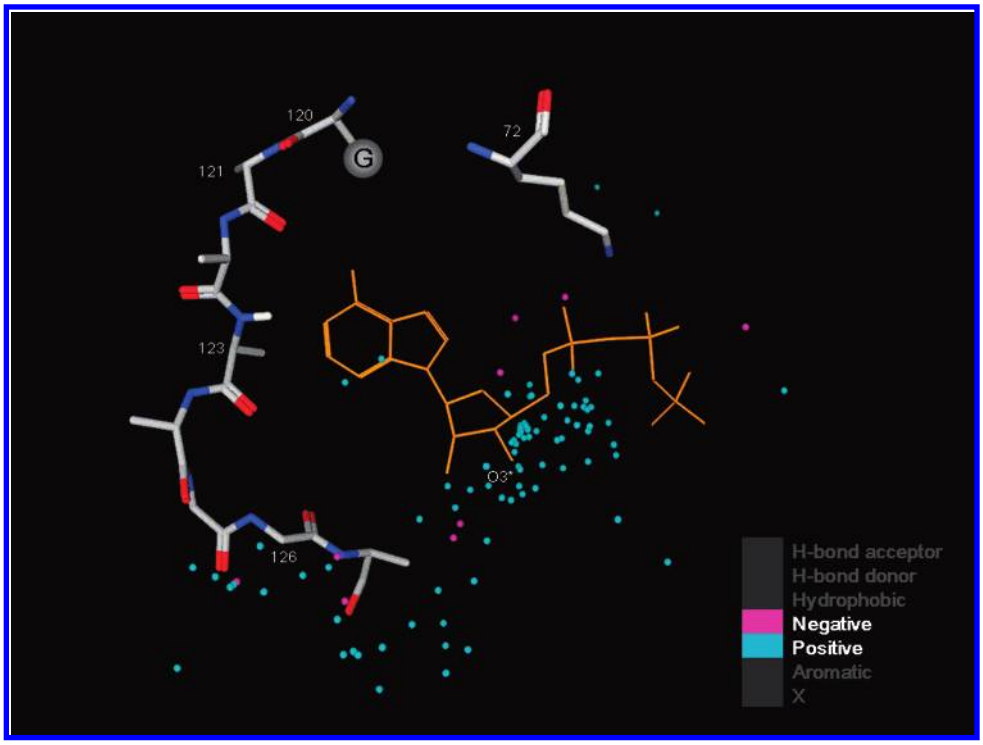

Figure 7. Same data as in Figure 2, but broken down into individual pharmacophore types.

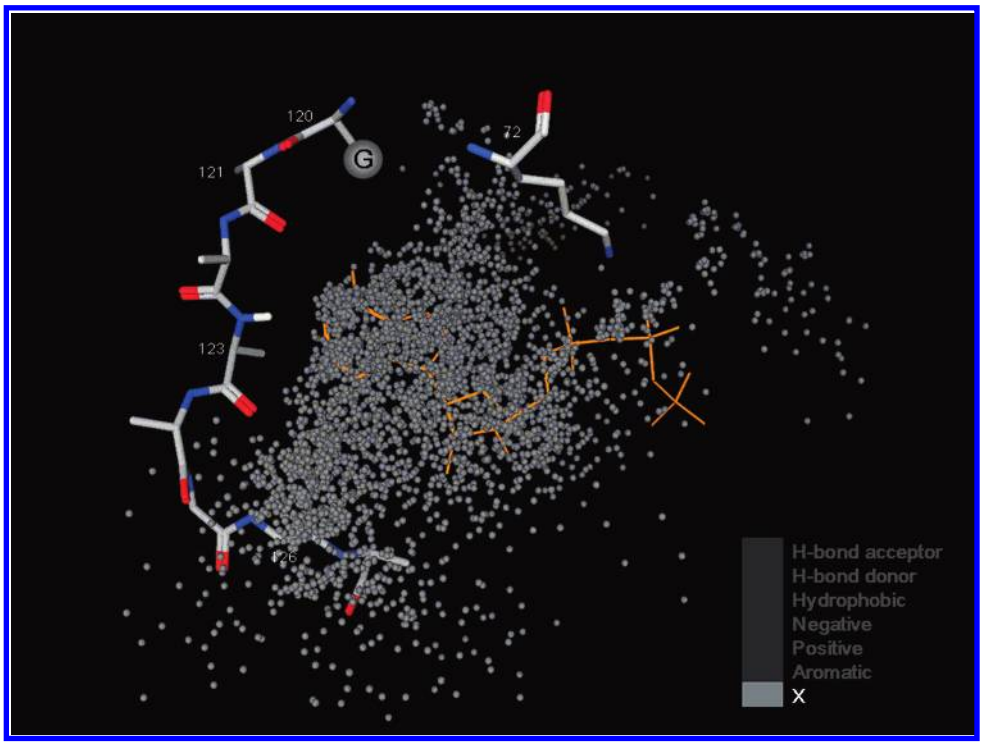

Figure 8. Same data as in Figure 2, but broken down into individual pharmacophore types.

the ligand in the map, this figure rises to 80%, suggesting that a more thorough conformational analysis might improve the score significantly. A difficulty is that it is sometimes seen that ligands have different binding modes with different receptors.[32]

The algorithm was applied to the virtual screening test set described in Materials and Methods, designed to simulate the case where a set of drug-like compounds is screened that contains 5% active compounds. The compounds were rank ordered by score. Figure 9 shows the enrichment plot when the top scoring compounds are selected from the set. For example, the top scoring 5% of the set includes 39% of the active compounds, an enrichment factor of nearly 8-fold, and the top scoring 10% of the compounds contain 51% of the actives, an enrichment factor of over 5-fold. This demonstrates the ability of the algorithm to discriminate between active and inactive compounds in a screen against multiple kinase targets.

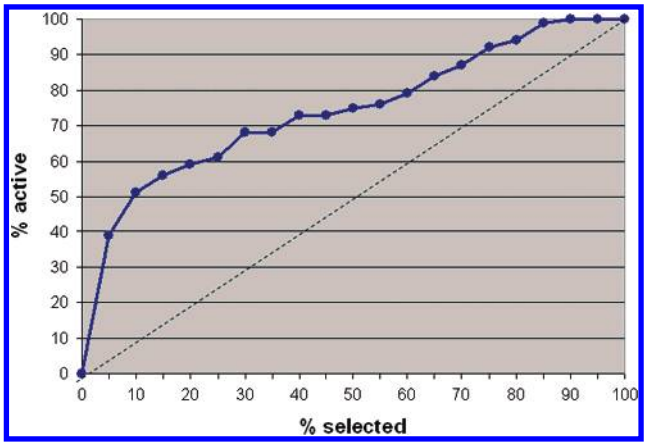

Figure 9. Enrichment plot for the test screening set (see text).

DISCUSSION AND CONCLUSION

We have used a pharmacophore description of ligand atoms in crystal structure complexes of protein kinases with their inhibitors in order to identify common or necessary structural requirements for ligand binding. The resulting pharmacophore map allows the visualization of the interactions of all ligands simultaneously in 3D on the computer graphics. Common interactions are seen to emerge that agree with previous observations, though to our knowledge have not been illustrated in this way before.

The PharmMap algorithm generates poses and scores for newly presented small molecule structures in an automated fashion. An enrichment has been demonstrated in a test set. This is with a view to selecting compounds for screening and designing combinatorial libraries, when the compound sets are to be screened across many or unanticipated kinase targets. In recent years it has become more common for pharmaceutical companies to set up multiple assays for a target class and to screen them in parallel.

Thus, the goal here has been to identify common features and interactions that are necessary for screening molecules to contain. This is derived from the similarities between the structures analyzed. Another major task is then to address the question of selectivity between kinase targets, i.e., to explore the differences between them. A complete treatment of selectivity needs to consider the structural details of each ligand−receptor complex individually. However, the current results give some general pointers. Figure 10 shows the compounds from the Fabian et al.[28] set displayed in the reference binding site in a similar rendering as the pharmacophore map. The nonselective compounds are colored red and appear to be confined to the region around the hinge and out to solvent. The intermediate (yellow) and selective (green) compounds explore regions further away, in this case the area of the gatekeeper residue and beyond it. Another possible area for exploration might be the region around the ATP phosphates and glycine rich loop; however, this is not illustrated well by this particular set of compounds. This result is in agreement with other studies, e.g., Aronov and Murcko,[29] who found that frequent hitters in their kinase assays map to a five point pharmacophore centered around the hinge region (although not all of the nonselective compounds in the Fabian et al. set satisfy all the points in this pharmacophore). To some extent, selectivity can be achieved by the elaboration of combinatorial scaffolds or by analogue development that is part of the hits to lead process.

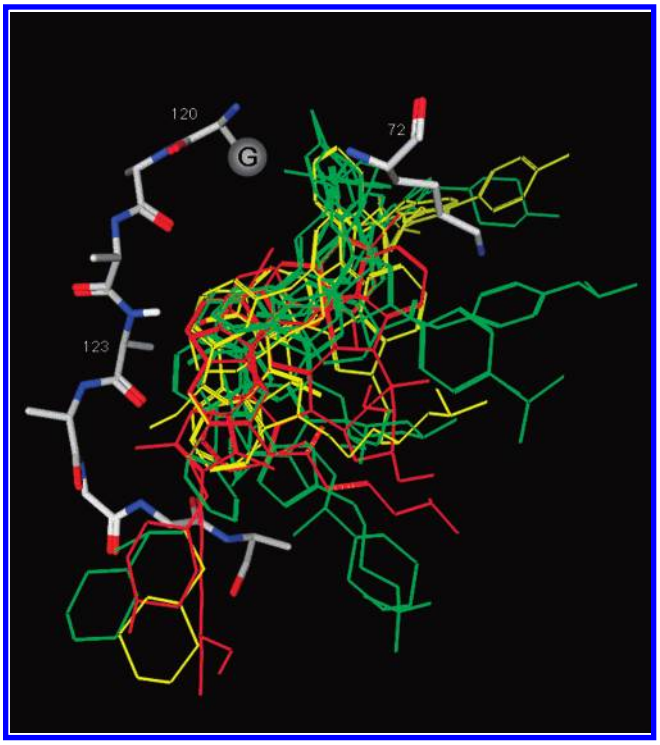

Figure 10. Compound set of Fabian et al., illustrated in the same orientation as the pharmacophore map and colored by selectivity (green = selective; yellow = intermediate; red = nonselective).

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REFERENCES AND NOTES

(1) Cohen, P. Protein Kinases—The Major Drug Targets of the Twenty-First Century? *Nat. Rev. Drug Discovery* **2002**, *1*, 309−315.
(2) Collins, I.; Workman, P. Design and Development of Signal Transduction Inhibitors for Cancer Treatment: Experience and Challenges with Kinase Targets. *Curr. Signal Transduction Ther.* **2006**, *1*, 13−23.
(3) Arora, A.; Scholar, E. M. Role of Tyrosine Kinase Inhibitors in Cancer Therapy. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 971−979.
(4) Krause, D. S.; Van Etten, R. A. Tyrosine Kinases as Targets for Cancer Therapy. *N. Engl. J. Med.* **2005**, *353*, 172−187.
(5) Dancey, J.; Sausville, E. A. Issues and Progress with Protein Kinase Inhibitors for Cancer Treatment. *Nat. Rev. Drug Discovery* **2003**, *2*, 296−313.
(6) Manning, G.; Whyte, D. B.; Martinez, R.; Hunter, T.; Sudarsanam, S. The Protein Kinase Complement of the Human Genome. *Science* **2002**, *298*, 1912−1934.
(7) Noble, M. E. M.; Endicott, J. A.; Johnson, L. N. Protein Kinase Inhibitors: Insights into Drug Design from Structure. *Science* **2004**, *303*, 1800−1805.
(8) Hanks, S. K.; Hunter, T. The Eukaryotic Protein Kinase Superfamily: Kinase (Catalytic) Domain Structure and Classification. *FASEB J.* **1995**, *9*, 576−596.
(9) Hanks, S. K.; Quin, A. M.; Hunter, T. The Protein Kinase Family: Conserved Features and Deduced Phylogeny of the Catalytic Domains. *Science* **1988**, *241*, 42−52.
(10) Nolen, B.; Taylor, S.; Ghosh, G. Regulation of Protein Kinases: Controlling Activity through Activation Segment Conformation. *Mol. Cell* **2004**, *15*, 661−675.
(11) Liao, J. J. Molecular Recognition of Protein Kinase Binding Pockets for Design of Potent and Selective Kinase Inhibitors. *J. Med. Chem.* **2007**, *50*, 409−424.
(12) Schurer, S. C.; Tyagi, P.; Muskal, S. M. Prospective Exploration of Synthetically Feasible, Medicinally Relevant Chemical Space. *J. Chem. Inf. Comput. Sci.* **2005**, *45*, 239−248.

(13) Muegge, I.; Enyedy, I. J. Virtual screening for kinase targets. *Curr. Med. Chem.* **2004**, *11*, 693−707.
(14) Fischer, P. M. The Design of Drug Candidate Molecules as Selective Inhibitors of Therapeutically Relevant Protein Kinases. *Curr. Med. Chem.* **2004**, *11*, 1563−1583.
(15) Cherry, M.; Williams, D. H. Recent Kinase and Kinase Inhibitor X-ray Structures: Mechanisms of Inhibition and Selectivity Insights. *Curr. Med. Chem.* **2004**, *11*, 663−673.
(16) Li, B.; Liu, Y.; Uno, T.; Gray, N. Creating Chemical Diversity to Target Protein Kinases. *Comb. Chem. High Throughput Screening* **2004**, *7*, 453−472.
(17) Knight, Z. A.; Shokat, K. M. Features of Selective Kinase Inhibitors. *Chem. Biol.* **2005**, *12*, 621−637.
(18) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(19) Knighton, D. R.; Zheng, J. H.; Ten Eyck, L. F.; Ashford, V. A.; Xuong, N. H.; Taylor, S. S.; Sowadski, J. M. Crystal Structure of the Catalytic Subunit of Cyclic Adenosine Monophosphate-Dependent Protein Kinase. *Science* **2006**, *253*, 407−414.
(20) Deng, Z.; Chuaqui, C.; Singh, J. Structural Interaction Fingerprint (SIFt): A Novel Method for Analyzing Three-Dimensional Protein−Ligand Binding Interactions. *J. Med. Chem.* **2004**, *47*, 337−344.
(21) Chuaqui, C.; Deng, Z.; Singh, J. Interaction Profiles of Protein Kinase-Inhibitor Complexes and Their Application to Virtual Screening. *J. Med. Chem.* **2005**, *48*, 121−133.
(22) McGregor, M. J.; Muskal, S. M. Pharmacophore Fingerprinting. 2. Application to Primary Library Design. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 117−125.
(23) McGregor, M. J.; Muskal, S. M. Pharmacophore Fingerprinting. 1. Application to QSAR and Focused Library Design. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 569−574.
(24) Deanda, F.; Stewart, E. L. Application of the PharmPrint Methodology to Two Protein Kinases. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 1803−1809.
(25) Baker, E. N.; Hubbard, R. E. Hydrogen Bonding in Globular Proteins. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97−179.
(26) Eidogen-Sertanty Inc., San Diego, CA.
(27) Elsevier MDL, San Ramon, CA.
(28) Fabian, M. A.; William, H. B. I.; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J.-M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. A Small Molecule-Kinase Interaction Map for Clinical Kinase Inhibitors. *Nat. Biotechnol.* **2005**, *23*, 329−336.
(29) Aronov, A. M.; Murcko, M. A. Toward a Pharmacophore for Kinase Frequent Hitters. *J. Med. Chem.* **2004**, *47*, 5616−5619.
(30) Capdeville, R.; Buchdunger, E.; Zimmermann, J.; Matter, A. Glivec, (STI571, Imatinib), a Rationally Developed, Targeted Anticancer Drug. *Nat. Rev. Drug Discovery* **2002**, *1*, 493−502.
(31) Liu, Y.; Gray, N. S. Rational Design of Inhibitors That Bind to Inactive Kinase Conformations. *Nat. Chem. Biol.* **2006**, *2*, 358−364.
(32) Aronov, A. M.; Baker, C.; Bemis, G. W.; Cao, J.; Chen, G.; Ford, P. J.; Germann, U. A.; Green, J.; Hale, M. R.; Jacobs, M.; Janetka, J. W.; Maltais, F.; Martinez-Botella, G.; Namchuk, M. N.; Straub, J.; Tang, Q.; Xie, X. Flipped Out: Structure-Guided Design of Selective Pyrazolylpyrrole ERK Inhibitors. *J. Med. Chem.* **2007**, *50*, 1280−1287.

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