Docking Study Yields Four Novel Inhibitors of the Protooncogene Pim-1 Kinase†

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To supplement the hits from a high throughput screen, docking was performed against Pim-1 kinase. Glide docking was augmented with a filter to require traditional or aromatic CH··O hydrogen bonds to the kinase hinge. Four diverse actives, of 96 molecules assayed, had K_i values between 0.091 and 4.5 uM. This gives a 14-fold enrichment over the earlier HTS run, and the two crystal structures solved confirmed the binding modes predicted by docking.

Introduction

Human Pim-1 kinase is a highly conserved serine-threonine kinase named for the genomic site where it was discovered, Proviral Integration site for MuLV (murine leukemia virus).[1] Pim-1 is expressed predominately in hematopoietic cells, though during embryonic development it is expressed in the neural retina, olfactory epithelium, and forebrain.[2] In hematopoietic cells, at least, Pim-1 appears to play a role in cell survival/apoptosis, differentiation, and proliferation. To control these crucial functions, Pim-1 is highly regulated at the transcriptional, post-transcriptional, translational, and post-translational levels.[3] As might be expected from its expression profile and its roles in survival, differentiation, and proliferation, Pim-1 has been shown to be overexpressed in a variety of human leukemias.[4] Additional evidence for its oncogenic role comes from studies in transgenic mice, which show that overexpression of Pim-1 leads to a dose-dependent susceptibility to spontaneous tumor formation and to increased susceptibility to chemical- and radiation-induced tumorigenesis.[3] These findings suggest that Pim-1 may be a valuable anticancer drug target. The fact that Pim-1 knockout mice showed no obvious phenotype suggests that side effects for such a drug should be minimal. Presumably this is due to redundancy with two other Pim-1 homologues, Pim2 and Pim3/kid-1.[5]

Given this promising target profile, we began a drug discovery program targeting Pim-1. A high throughput screen was performed to identify inhibitors of Pim-1 kinase activity. Due to the low 0.3% hit rate from this screen and a lack of leads with the desired combination of potency, selectivity, and properties profile, a virtual screen was conducted in an attempt to find additional lead series.

Since several structures of Pim-1 have been published,[6,7] we have attempted to use the available structural information to improve the potency and selectivity of the inhibitors from the docking experiment. To increase the docking hit rate, we applied filters requiring all hits to form two fairly conserved kinase inhibitor interactions. The docked pose of each ligand had to accept a hydrogen bond from the side chain of Lys67 and donate a hydrogen bond to the backbone carbonyl of hinge residue Glu121. Not all known Pim-1 inhibitors make these two hydrogen bonds, but all make at least one of the two, and we reasoned that ligands forming both interactions in their docked pose would have better hit rates and potencies. Further, we did not restrict hydrogen bonding donors to traditional OH and NH groups, but also allowed aromatic CH groups as donors[8,9] to the Glu121 carbonyl oxygen. In the crystal structure of the triazolopyridazine compound, **1**, shown in Figure 1, a bifurcated hydrogen bond is observed in which two aromatic CH groups interact with a common main chain carbonyl. The inhibition constant (K_i) for this compound is 11 nM and its crystal structure was used as the docking target for virtual screening.

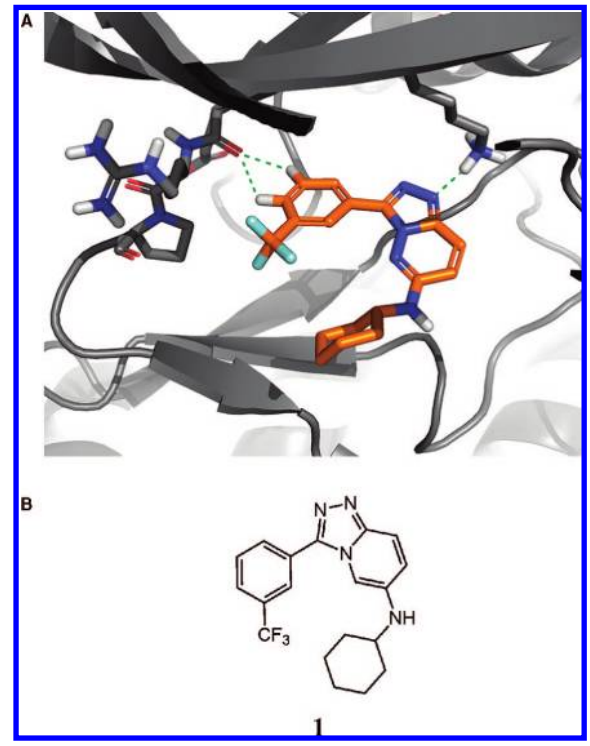

Figure 1. (A) Triazolopyridazine ligand 1 bound to PIM-1 in the crystal structure used in docking studies. Key interactions with Glu121 and Lys67 are highlighted in green. (B) 2-D structure of ligand 1.

The one concern that could be raised with requiring all docking hits to form the two hydrogen bonds mentioned above is that those two interactions are conserved across all kinases and this requirement might normally lead to nonselective inhibitors. Fortunately, Pim-1 has a unique structural feature which we believed would allow the targeting of Pim-1 selective inhibitors in the screening process. In almost all protein kinases, the backbone NH of the hinge residue equivalent to residue 123 of Pim-1 donates a hydrogen bond to an acceptor in the bound

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ligand. In a recent survey of kinase inhibitor crystal structures, at least 96.5% of kinase ligands formed this hydrogen bond.[10] However, in Pim-1 this residue is a proline, so no such hydrogen bond can be formed. The absence of this key hydrogen bond in Pim-1 inhibitors offers the potential for excellent selectivity in our docking hits despite the requirement for the two other conserved interactions.

Methods

Methods used for protein expression, purification, and crystallization are identical to those described previously, as are the methods used in the determination of inhibition constants.[6]

Docking into the active site of Pim-1 was carried out for approximately 700000 commercially available molecules. These molecules were compiled from a large set of vendor catalogs, filtered with REOS,[11] and enumerated for tautomers and stereoisomers at undefined stereocenters using Corina.[12] Because charge–charge interactions are not a key factor in kinase inhibitor binding and the uncharged forms of amines and carboxylic acids are capable of the appropriate hydrogen binding interactions, molecules were docked in their neutral form with inappropriate interactions manually filtered as described below. Compounds were docked into the crystal structure of compound **1** (PDB code 3BGQ) with all structural waters removed. Docking was performed with Glide 3.5[13] in standard precision mode without further minimization, using Glidescore for ligand ranking. The hydrogen bond to Lys 67 was enforced within Glide, and several other filters were applied in postprocessing steps. First, to eliminate molecules that adopt excessively strained conformations in their docked pose, all hits with internal energy greater than 1.5 kcal/mol per rotatable bond were eliminated. An automated filter was then applied to remove all hits unless they contained a hydrogen bond donor (OH, NH, or aromatic CH) within 3.6 Å of the backbone carbonyl oxygen of Glu 121. Third, to counteract the tendency of empirical scoring functions to produce better scores for larger molecules,[10,14,15] a fixed number of hits was taken from each 50 Dalton molecular weight range (i.e., the top 200 molecules with molecular weight between 200 and 249 were selected, the top 200 between 250 and 299 molecular weight, etc.) This was applied in place of analogous corrections based on solvation, entropy, and/or molecular size.[14,15] Finally, the poses of each of these 1200 molecules were visually inspected. A number of hits were eliminated due to poor hydrogen bond geometry with Glu 121 (donor−hydrogen−acceptor angle <90°). Others were removed because an unrealistic tautomer or charge state was involved in a key interaction with the protein (e.g., a carboxylic acid donating a hydrogen bond). A final filter was imposed by the timing and expedience of the ongoing research project. Only those compounds that had previously been purchased for our corporate compound collection (but not included in the original screening set) were put into the new Pim-1 screen. This was an essentially random subset of 96 of the compounds that passed all the filters described above.

Results and Discussion

In the experimental assessment of the inhibition constants for the 96 hits from virtual screening, four compounds showed K_i values of less than 10 μM. The four hits are shown in Figure 2 with K_i values that range from 0.091 to 4.5 μM. These four hits represent four distinctly different scaffolds (the highest 2-D similarity among these molecules is 0.42), so the Pim-1 project benefited significantly from this small additional screening effort. To objectively measure the success of a virtual screening exercise, the true hit rate from a virtual screen should be compared to the true hit rate from a high throughput screen of a large set of random molecules. In actuality, true enrichment rates can rarely be calculated; the enrichment rates published in virtual screening studies are typically based on retrospective screens with the set of

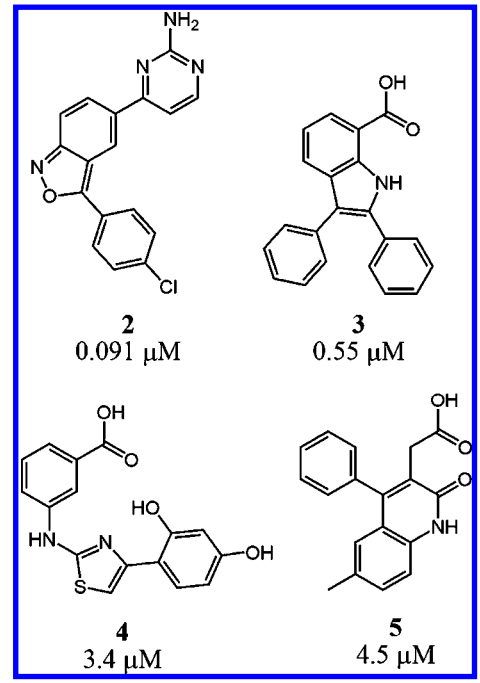

Figure 2. Structures and PIM-1 K_i values of the docking hits that showed activity in the PIM-1 inhibition assay.

"noninhibitors" made up of random drug-like molecules, some of which would actually inhibit the target if they were screened. Less frequently, a prospective screen has been done to give a hit rate from the virtual screen, but there is no screen of random molecules to give comparison for the enrichment calculation. In this study our initial hit rate of 0.3% can be compared to the virtual screening hit rate of 4.2% to give a 14-fold enrichment. This enrichment rate is quite high compared to traditional docking in kinases (0–15-fold),[16,17] and moderate compared to constrained docking results (5–26-fold)[10] achieved in similar, recently published studies. A number of factors may have kept this study from matching the highest enrichment levels achieved in the earlier constrained docking study. It could be argued that our attempt to counteract the tendency of scoring functions to overscore large ligands and our decision to dock all molecules in their neutral form were detrimental to the results. However, we believe the largest factor is likely to be that the absence of the central kinase hinge hydrogen bond donor in Pim-1 makes it a more difficult case for constrained virtual screening. Nevertheless, a 14-fold enrichment over traditional random screening is an unqualified success.

We would like to address one potential concern with these enrichment results: that of bias among the virtual screening compound set leading to an artificially elevated hit rate. We do not believe this to be an issue. The initial Pim-1 HTS screen included 14% kinase-associated molecules (those purchased or synthesized in-house for screening against a particular kinase or kinases in general). The nonkinase molecules were purchased or synthesized in-house for other programs (e.g., IMPDH) or for general screening. The Pim-1 HTS compounds were deliberately enriched in kinase-associated compounds with the hope that this would lead to an improved hit rate from the HTS. The virtual screen considered only commercially available compounds in the corporate collection, and it selected a significantly lower percentage (5/96, 5.2%) of kinase-associated compounds. As this was the case, the enrichment rate seems very unlikely to have resulted from bias in the compound sets.

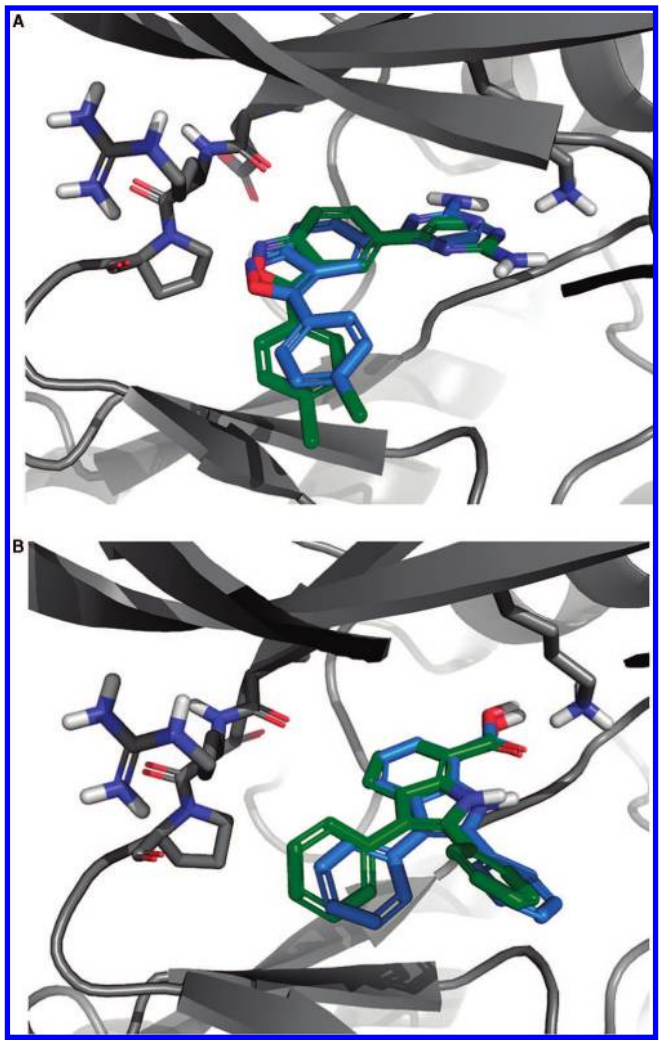

Figure 3. (A) Crystal structure (green) of 2 bound to PIM-1 compared to the Glide-predicted binding mode (blue). (B) Crystal structure (green) and Glide-predicted binding mode (blue) of 3 in PIM-1.

Another test of the success of a virtual screening effort is the accuracy of the binding mode predictions. We were able to obtain Pim-1 crystal structures for compounds **2** and **3**, and these are depicted in Figure 3. The heavy atom RMS deviation between the docked and crystal structures measures 1.74 and 0.87 Å for molecules **2** and **3**, respectively. These low RMS deviations are impressive considering that the Pim-1 crystal structure used for docking had a very different ligand in its active site. The larger RMSD for molecule **2** is due to a change in conformation of Gly 89 of Pim-1. With this change in conformation, an aminopyrimidine-Gly 89 hydrogen bond formed in the docked structure is no longer possible, leading to a change in aminopyrimidine conformation. This result could not have been predicted without accounting for receptor flexibility in the docking calculations, but correcting the aminopyrimidine conformation reduces the RMSD to 0.96 Å. Attempts to determine crystal structures for ligands **4** and **5** were unsuccessful.

The two crystal structures that were determined share an interesting feature. Both ligands donate an aromatic CH hydrogen bond to the backbone carbonyl of Glu 121. This is also true of the docked poses of compounds **4** and **5**, though these interactions have not been confirmed crystallographically. It is somewhat surprising that all four hits donate this hydrogen bond through an aromatic CH group since the hydrogen bonds formed with OH and NH donors would be expected to be significantly stronger than those formed by aromatic CH donors. However, in the Pim-1 ATP site the polar NH of the central hinge residue is replaced by the more hydrophobic proline side chain, creating a hinge region significantly less polar than that of most kinases. In this environment the more hydrophobic CH donor may be preferable to the more polar heteroatom donors.

Conclusions

This study describes a relatively rare example of a full prospective docking-based virtual screening with experimental follow-up. Because a random screen had been carried out on Pim-1 prior to this work, a true enrichment rate could be calculated. The virtual screen produced four hits among 96 compounds tested experimentally. The enrichment rate of 14-fold represented a significant improvement over the random screen and provided four distinct, novel scaffolds for medicinal chemistry optimization. Also, crystallographic studies were carried out to determine the crystallographic binding modes of the confirmed hits from virtual screening. In the two crystal structures determined, the docked structures were within (on average) 1.3 Å of the true structures. This level of accuracy confirms the value of the virtual screen not only for lead generation, but also for structural guidance in lead optimization.

Supporting Information Available: X-ray crystallography data collection information and refinement statistics are available free of charge via the Internet at http://pubs.acs.org.

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