Molecular Docking and High-Throughput Screening for Novel Inhibitors of Protein Tyrosine Phosphatase-1B

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High-throughput screening (HTS) of compound libraries is used to discover novel leads for drug development. When a structure is available for the target, computer-based screening using molecular docking may also be considered. The two techniques have rarely been used together on the same target. The opportunity to do so presented itself in a project to discover novel inhibitors for the enzyme protein tyrosine phosphatase-1B (PTP1B), a tyrosine phosphatase that has been implicated as a key target for type II diabetes. A corporate library of approximately 400 000 compounds was screened using high-throughput experimental techniques for compounds that inhibited PTP1B. Concurrently, molecular docking was used to screen approximately 235 000 commercially available compounds against the X-ray crystallographic structure of PTP1B, and 365 high-scoring molecules were tested as inhibitors of the enzyme. Of approximately 400 000 molecules tested in the high-throughput experimental assay, 85 (0.021%) inhibited the enzyme with IC_{50} values less than 100 μM; the most active had an IC_{50} value of 4.2 μM. Of the 365 molecules suggested by molecular docking, 127 (34.8%) inhibited PTP1B with IC_{50} values less than 100 μM; the most active of these had an IC_{50} of 1.7 μM. Structure-based docking therefore enriched the hit rate by 1700-fold over random screening. The hits from both the high-throughput and docking screens were dissimilar from phosphotyrosine, the canonical substrate group for PTP1B; the two hit lists were also very different from each other. Surprisingly, the docking hits were judged to be more druglike than the HTS hits. The diversity of both hit lists and their dissimilarity from each other suggest that docking and HTS may be complementary techniques for lead discovery.

Introduction

High-throughput screening (HTS) of compound libraries is used to discover new lead compounds for drug design.[1−5] When the three-dimensional structure of the target is known or can be modeled, virtual screening using molecular docking can also be used to discover new lead compounds.[6−10] In principle, HTS should discover all of the interesting ligands in a database, whereas the predictions of computer-based docking screens are less reliable and must be tested.

In practice, both docking and high-throughput screens suffer from false positives and false negatives. In HTS, many compounds are tested rapidly and inhibition can be seen or missed due to faults in the assay (false negatives).[11,12] Some classes of compounds appear to inhibit due to interference with properties of the assay or inhibit with poor selectivity, which leads to their overrepresentation in HTS hit lists (false positives).

The problems with molecular docking as a screening method have been widely mooted: the scoring functions are inaccurate, the sampling of conformational states is crude, and many solvent-related terms are typically ignored, among others.[13−20] Nevertheless, molecular docking has some practical advantages. Although it has only a modest ability to distinguish between two compounds that both fit in an active site, it can reliably screen out compounds that do not fit in a binding site or that have grossly wrong electrostatic properties. This allows for careful experimental assays on a relatively small number of database compounds. Also, docking may be used to screen compounds for which there is no actual physical sample at hand. This is particularly useful when deciding on a smaller portion of a collection of commercially available chemistry to acquire. Finally, a docking hit comes with a prediction of a geometry, which allows for inhibitor optimization in the context of a binding site. Such possible advantages are only interesting if molecular docking screens, with all of their drawbacks, can nevertheless discover novel inhibitors at a high enough frequency to be competitive with HTS.

The opportunity to compare the results of a high-throughput screen with a structure-based, molecular docking screen against the same enzyme presented itself in an effort to discover new inhibitors of protein tyrosine phosphatase-1B (PTP1B). This phosphatase hydrolyzes phosphotyrosines on the insulin receptor, deactivating it. Overproduction of this enzyme has been implicated in the onset of type II diabetes, and it is therefore a target for drug discovery.[21,22] For this reason, PTP1B

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Table 1. Hit Rates from High-Throughput and Docking Screens against PTP1B

technique	compds tested	hits with IC_{50} < 100 μM	hits with IC_{50} < 10 μM	hit rate (%)
HTS	400 000	85	6	0.021
docking	365	127	21	34.8[a]

[a] We define hit rate for the docked molecules as 100 times the number of bioactive docked molecules divided by the total number of docked molecules that were bioassayed.

has been the subject of intense study for the past few years.[23−25] A study by Zhang and colleagues has shown that molecular docking could discover several new inhibitors against this target,[26] which made a large scale docking effort appear sensible. In an effort to discover new inhibitors as leads for drug design, we decided to experimentally screen an in-house corporate database of about 400 000 molecules using a high-throughput assay and also to computationally screen a database of 235 000 commercially available compounds using molecular docking followed by experimental testing of 365 top-scoring molecules. A compound from HTS was considered to be worthy of detailed investigation if it inhibited PTP1B at 300 μM. Compounds from molecular docking were considered for further study if they inhibited PTP1B at 200 μM. Subsequently, IC_{50} values were determined for all active compounds, and a compound from either method was considered a "hit" if it had an IC_{50} value less than 100 μM. The large number of compounds tested in both screening methods makes comparison of the hit rates between the two statistically meaningful. It also allows for comparisons of the types of molecules discovered by each technique: where they complement each other, where they overlap, and what sorts of structure−activity relationships (SAR) can be gleaned from the hit lists. To our knowledge, such a comparison has not previously been reported.

Results

HTS. A library of about 400 000 compounds from a corporate collection was screened against human recombinant PTP1B (residues 1−322). Of these, 543 inhibited the enzyme significantly in a single-point assay at 300 μM concentration of compound. In full IC_{50} evaluations, 85 had IC_{50} values between 100 and 1 μM, a hit rate of 0.021% (Table 1).

We make two caveats in comparing the HTS hit rate with the docking hit rate: the conditions for the assays differed in one respect for the two sets of inhibitors, and the criterion for a hit in the initial single-point assays was different. For the HTS experiment, albumin was present at a concentration of 0.33 mg/mL in the assay; for testing the docking-derived molecules, albumin was absent. On the other hand, an initial hit for the HTS assays was a molecule that inhibited significantly at 300 μM whereas only molecules that inhibited significantly at 200 μM were followed up for the docking-derived inhibitors. Our HTS assays preceded the assays on the docking compounds. Between running the HTS assays and testing the docking-derived compounds, we found that we were unable to reproduce literature values for the binding of several known ligands[27] with albumin present. Removing the albumin allowed us to reproduce the literature values, significantly increasing the apparent affinity of the reported inhibitors. We suspect that a higher hit rate would have been found in the HTS assays had albumin not been present.

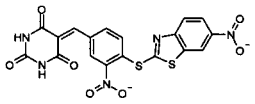
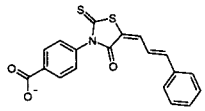
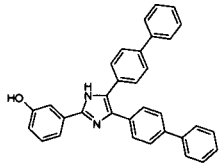
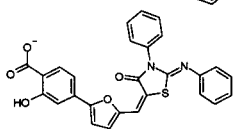
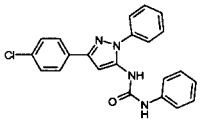
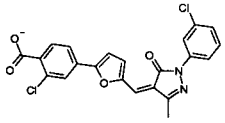
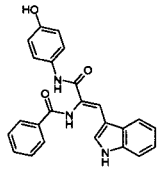
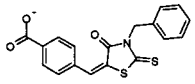
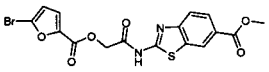
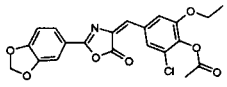
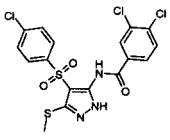
Molecular Docking. The top-scoring 500 molecules from the flexible docking screen of the ACD database and the top-scoring 500 molecules from the flexible docking screen of the combined BioSpecs and Maybridge databases were considered for further evaluation. Of these 1000 compounds, 889 were actually available either commercially or in our own collections. Two major features of the ligands were considered when choosing which of the 889 to select for testing. The first consideration was whether the ligand spanned the two phosphotyrosine sites observed in the crystal structure elucidated by Zhang and co-workers,[28] who found a catalytic phosphotyrosine binding site as well as a lower affinity, noncatalytic phosphotyrosine binding site. They suggested that spanning both sites with a single ligand might provide a highly specific and tight-binding PTP1B inhibitor. In all, 178 "spanners" and 187 "nonspanners" were chosen for testing; 127 of these 365 compounds had IC_{50} values less than 100 μM. Overall, there was some enrichment of spanners among the bioactive hits, with 77 spanners and 50 nonspanners. However, in the list of 21 hits with IC_{50} < 10 μM, there were 10 spanners and 11 nonspanners. The second consideration in selecting ligands for testing was whether the ligand contained a carboxylic acid functionality, which we believed might serve as a reasonable surrogate for phosphate. We found that in the list of 889 top-scoring compounds, 115 contained a carboxylic acid. We tested 47 of these and found that 28 were actually active in the bioassay with IC_{50} values less than or equal to 100 μM.

Of the 500 high-scoring ACD molecules, 118 were tested experimentally; 38 had IC_{50} values of 100 μM or better against PTP1B. Additionally, 15 molecules from a preliminary rigid body docking screen of the ACD were also assayed; eight had IC_{50} values of 100 μM or lower (a combined hit rate of experimentally tested docking molecules of 34.6%). Of the 500 high-scoring BioSpecs/Maybridge molecules, 232 were tested experimentally; 81 had IC_{50} values of 100 μM or better against PTP1B, a hit rate of 34.9%. Overall, 34.8% of the high-scoring docked molecules that were tested (127 of 365) had IC_{50} values of 100 μM or lower (Table 1). The best of these molecules had an IC_{50} of 1.7 μM; 21 had IC_{50} values of 10 μM or better. In a full Lineweaver−Burk analysis, compound **3** (see Table 2) was found to inhibit PTP1B competitively with a K_i value of 10.3 μM (Figure 1).

In an effort to investigate how well-behaved the docking hits were as inhibitors, 25 of the 127 hits were selected for closer kinetic study. Of these, nine were found to be noncompetitive inhibitors with nonclassical kinetics (e.g., very steep IC_{50} curves). Another 12 had well-behaved IC_{50} curves but were either time-dependent or did not fit classical competitive inhibition equations. Four of the 25 appeared to behave as simple, competitive inhibitors (e.g., Figure 1).

The docking hits included a range of functional characteristics, with both polar and apolar molecules selected (see Table 2 for a representative sample). The 127 molecules that inhibited PTP1B had an average molecular weight of 410.34. Twenty-eight of these were aryl carboxylates or salicylates of average molecular weight 356.7, with the carboxylic or salicylic acid group

Table 2. Characteristic Docking Hits

Compound	Structure	Docking Rank	Docking Score (kcal/mol)	IC_{50} vs. PTP1B (μM)
1		406	-33.4	4.1
2		170	-35.0	4.4
3		39	-39.6	8.6
4		116	-36.1	9.0
5		415	-33.3	12.0
6		255	-34.2	19.3
7		440	-33.2	21.5
8		11	-42.01	21.6
9		230	-34.4	34.4
10		284	-34.0	35.0
11		150	-35.7	63.9

docked into the phosphate binding region of the catalytic site and appearing to hydrogen bond with phosphate recognition residues such as Ser216, Gly218, Gly220, and Arg221 (Figure 2A,B). Although the carboxylate groups of the docked compounds were unable to make all of the interactions made by the phosphate moiety in the X-ray structure, any given carboxylate was observed to make some of these interactions; the exact interactions with the phosphate recognition residues differed from compound to compound. For instance, for the compound shown in Figure 2B, nitrogens from the side chain of Arg221 and the backbone of Ser216 appeared to hydrogen bond to the carboxylate, whereas direct interactions with the main chain nitrogen of Gly218 were not observed.

Neutral molecules were also found that scored and inhibited well. These 99 compounds were often larger (average molecular weight 425.51) than the charged molecules, and many spanned both tyrosine binding sites. These larger, neutral molecules appeared to be favored because of overall steric complementarity to the binding site (Figure 2C). For instance, compound **3**

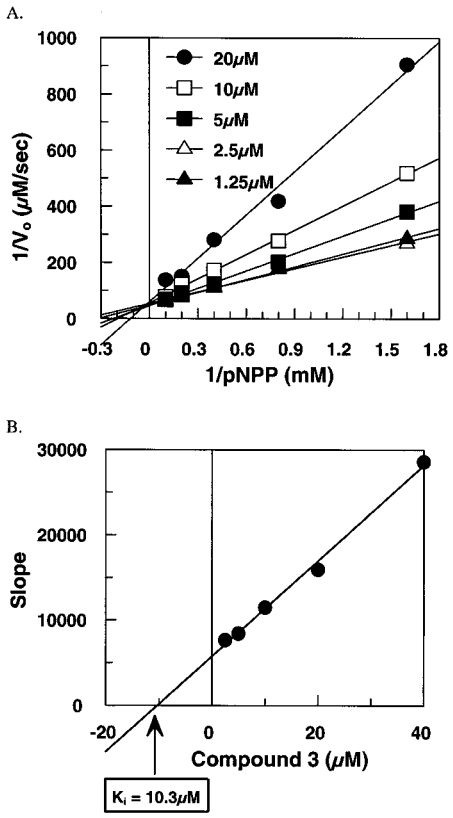


Figure 1. (A) Lineweaver−Burk analysis of the docking hit compound **3**. The concentrations of the inhibitor are shown. The concentrations of the substrate *p*NPP ranged from 0.625 to 10 mM (K_m−16K_m). (B) Replot of slopes from panel A to determine the K_i value.

made only one hydrogen bond to PTP1B (involving its phenolic hydroxyl) but had extensive shape complementarity to the enzyme surface (shown in gray in Figure 2C) and buried much of its surface area. Both the charged and the neutral compounds often sandwiched an aryl ring between Phe182 and Tyr46 of the enzyme (Figure 2B,C). As in the phosphotyrosine crystal structure (Figure 2A), Phe182 often appeared to form a herringbone interaction with the docked ligand whereas Tyr46 often stacked with the ligand aryl ring (Figure 2B). The neutral molecules contained a few general classes of functional groups that were predicted to bind in the catalytic phosphotyrosine site, including, in order of prevalence: unsubstituted heterocycles, nitroaryls, monohalo-phenyls, dihalo-phenyls, methylenedioxybenzenes, and coumarins.

Chemical Informatics. To compare and contrast HTS with docking and to better understand the nature of the hit lists we obtained from each method, we evaluated the diversity and the "druglikeness" of each hit list. Also, we evaluated the structural overlap of the hit lists obtained from HTS and from docking. We characterize the druglikeness of compounds in two ways. One is by applying the Pfizer "Rule-of-5"[29] criteria to each compound. These rules are based on statistical observations of a collection of several thousand known drugs and druglike molecules. According to these rules, a very high proportion of druglike molecules have (i) molecular weight ≤ 500, (ii) calculated log*P* ≤ 5, (iii) number of hydrogen bond donors ≤ 5, and (iv) number of hydrogen bond acceptors ≤ 10.

A compound receives a score of 1 for each rule that it passes; compounds that pass all four Rule-of-5 criteria receive a score of 4 (most druglike) and those that fail all rules receive a score of 0 (least druglike). On average, the HTS hits had Rule-of-5 scores of 2.73 whereas the 365 docking molecules selected for testing had scores of 3.57 and the 127 bioactive docking hits had scores of 3.49 (Table 3).

The second way we assess druglikeness is to determine whether a compound passes a series of property and substructure filters. Three of these are for gross molecular properties: molecular weight (required: 100−600), Daylight Clog*P* (required: −2.00 to +6.00), and number of rotatable bonds (required: ≤15). We also employ a series of 55 substructural filters to remove reactive species, compounds containing heavy metals, and other structural features that make lead optimization very difficult or impossible. These 58 total filters are now used whenever Pharmacia Corporation purchases commercial compounds for general screening purposes. They were not in general use in their final form when the docking molecules were selected. Over 70% of the HTS hits fail our filters (Table 4). In contrast, 70% of the docking hits pass the filters.

To measure the diversity of the respective hit lists, we employed a proprietary clustering algorithm known as "Algorithm5".[30] Algorithm5 is a "fuzzy-clustering" method that clusters without user-supplied parameters; we have found it to be useful in comparing clustering results for a wide variety of small molecule databases. Algorithm5 makes use of the Daylight structural fingerprint, which is based on a two-dimensional structure, and the Tanimoto metric as a similarity measure. For an overall diversity measure, we define the "cluster-based diversity index" (CDI)

$$\text{CDI} = (\text{number of clusters}/\text{number of compounds})$$

that varies from nearly zero (everything in one cluster: CDI = 1/(no. of compounds)) to exactly one (all compounds are singletons; i.e., clusters of one: CDI = (no. of compounds)/(no. of compounds)). Both hit lists were quite diverse by this measure (Table 4). For comparison, the diversity of the Monsanto Company chemical collection of about 250 000 compounds was much lower, with a CDI of 0.234. Most commercial databases we have evaluated for purchase were found to have a CDI of less than 0.5 and sometimes significantly less. The CDI values for the HTS and the docking hits (0.62−0.87) signify diverse collections of chemistry.

As a final experiment, we coclustered the 85 HTS hits with the 889 highest-scoring docking molecules and were surprised to find that there were no mixed clusters whatsoever. This means that the compounds in one hit list have no significant structural similarities to the compounds in the other hit list. As a control, we generated a list of 123 randomly chosen Pharmacia compounds and coclustered it with a randomly chosen selection of 919 ACD compounds (the numbers in each case approximately match the size of the HTS and the top-scoring docking hit lists). Of the fourteen clusters comprised of two or more molecules, three were mixed (i.e., contained molecules from both collections). In a second control experiment, we generated a larger random list of 2051 ACD compounds and 2024 Pharmacia

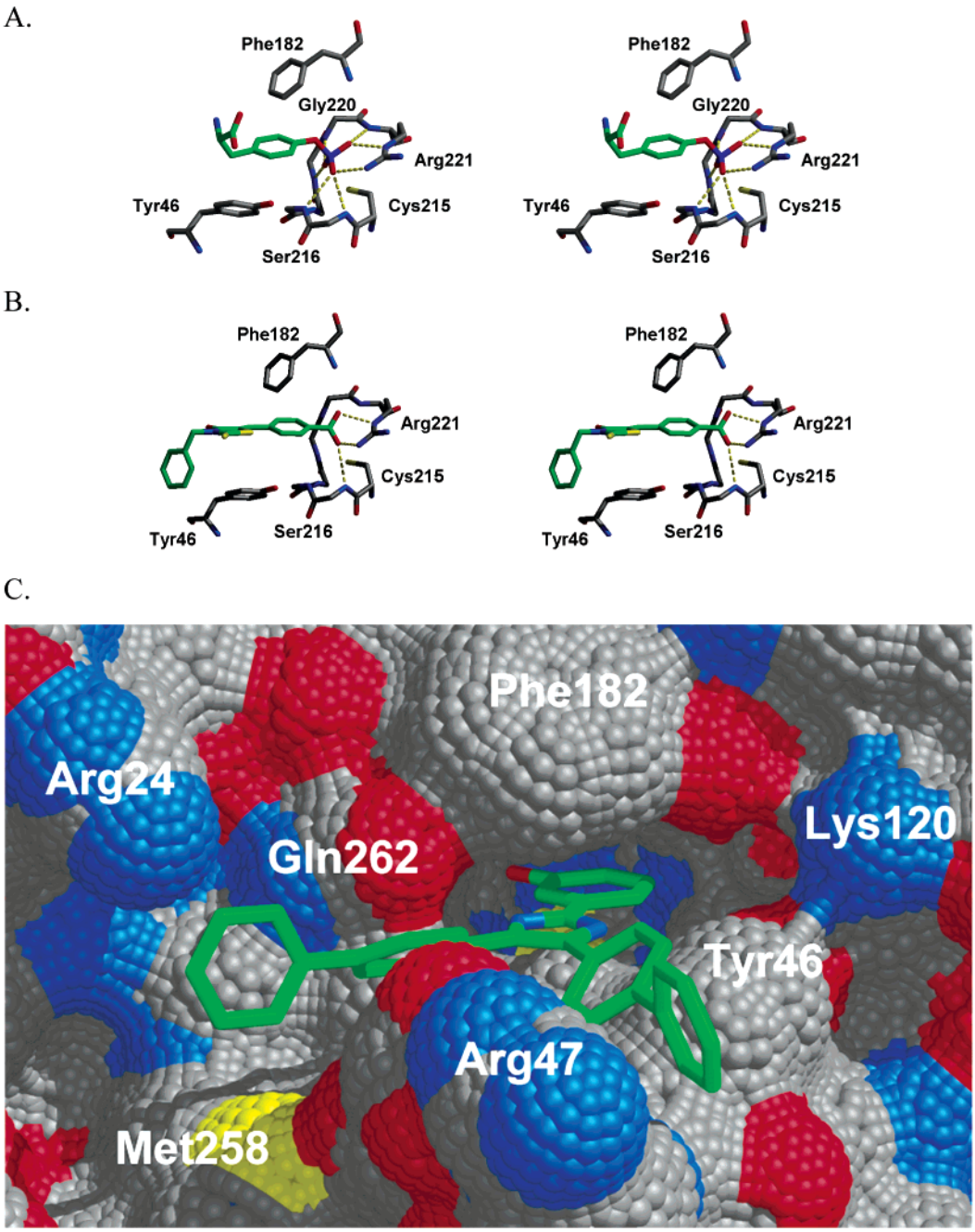


Figure 2. Comparing the docked ligands to phosphotyrosine. (A) Phosphotyrosine in the catalytic site of PTP1B, as determined by X-ray crystallography.[28] Oxygen atoms are in red, nitrogens are in blue, sulfur is in yellow, enzyme carbons are in gray, and ligand carbons are in green. The catalytic cysteine, which was substituted to a serine in the 1pty structure, is represented in the modeled conformation used for docking. Some side chains are not displayed for clarity. (B) Docked orientation of compound **8** in the PTP1B binding site. This compound, a characteristic carboxylic acid-bearing molecule, was a 21.6 μM inhibitor of the phosphatase. (C) The molecular surface of PTP1B showing the docked orientation of compound **3**, which had a K_i of 10.3 μM.

Table 3. Druglike Properties of the HTS and Docking Hits by Rule-of-5 Criteria

list	no. of compds	compds passing at least 3/4 Rules-of-5	compds passing 4/4 Rules-of-5	average Rules-of-5 score
HTS hits	81[a]	49	19	2.73
high-scoring docking molecules	889	773	577	3.47
docking molecules selected for testing	365	332	248	3.57
bioactive docking hits	127	116	73	3.49

[a] Full chemical information was unavailable for four of the 85 HTS hits.

compounds and found that of 119 total clusters, 45 of them were mixed. This was consistent with our initial assumption that the commercial databases and the Pharmacia collection overlap. This overlap arises because we have purchased thousands of compounds from commercial sources over the years, both as discrete

Table 4. Druglike Properties of the HTS and Docking Hits Based on Filters Used at Pharmacia and Diversity Analysis

list	no. of compds	no. filtered out	no. remaining (%)	problem areas/ no. filtered out [a]	CDI[b]
HTS hits	81[c]	63	18 (22.2%)	quat. amines/22 MW/14 rotbonds/10	0.64
high-scoring docking molecules	889	266	623 (70.1%)	Clog*P*/166 $X_3P = X$, X = N, O, S/50 aryl azo/27 ≥2 nitro/23 MW/22	0.62
docking molecules selected for testing	365	82	283 (77.5%)	Clog*P*/70	0.80
bioactive docking hits	127	35	92 (72.44%)	Clog*P*/29	0.87

[a] An itemized list of all rules that were violated by 10 or more compounds, followed by the actual number of violations of the rule. [b] The cluster-based diversity index. The larger the number, the more diverse the group of compounds is found to be. [c] Full chemical information was unavailable for four of the 85 HTS hits.

entities for screening as well as for intermediates in the synthesis of larger molecules. Furthermore, many well-known drug scaffolds are represented both in the Pharmacia collection as well as in commercial databases. Thus, it is surprising that there is no structural overlap between the HTS and the docking hit lists, since a random selection of even small collections of Pharmacia and commercial compounds has some structural overlap. If anything, one would expect the selection done by both HTS and docking to be biased toward compounds with a greater than random probability of structural similarity. To find no overlap whatsoever was completely unexpected.

Discussion

Three interesting results emerge from these studies. The first is the high 34.8% hit rate of the molecules assayed after the docking screen, a 1700-fold enrichment over the random screen hit rate of 0.021% (Table 1). The second is the dissimilarity of the inhibitors in both hit lists to the known ligand, phosphotyrosine. The third is the dissimilarity of the HTS hits from the docking hits. The enrichment over random suggests that docking against the PTP1B crystal structure screened out compounds in the database that were unlikely to inhibit the enzyme and focused on compounds more likely to complement the structure of the binding site. The dissimilarity of the inhibitors to phosphotyrosine suggests that inhibitors lacking a phosphate can be found for PTP1B and other tyrosine phosphatases and kinases, as has been shown by other investigators.[26,31,32] This will be important to the development of sensible drug candidates for this target. The dissimilarity of the HTS and docking hits came as a surprise, particularly in light of control experiments showing that randomly chosen selections of ACD and Pharmacia compounds typically show such similarity. However, the large number of HTS hits that failed the Pharmacia purchase filters suggests that the high-throughput screen identified a significant number of compounds that interact with the protein in undesirable ways, such as via detergent action or covalent interaction due to excessive reactivity of the ligands. A key step in the selection of docking molecules for procurement and testing is to avoid reactive and otherwise undesirable compounds. HTS programs typically involve the testing of all available chemical samples (or a representative subset thereof), and as such, undesirable hits are commonplace. We suspect that the lack of overlap between the two hit lists reflects the widely differing levels of druglikeness of the lists.

It is appropriate to ask if the docking molecules that we have defined as hits are reliably inhibiting, and if so, are they binding at the active site, as predicted by the docking program? Are they druglike? How do they compare as leads with the HTS hits?

We have only counted hit molecules that have IC_{50} values of 100 μM or better. Admittedly, this is a relatively high cutoff for a hit, though certainly not without precedent. Most importantly, the same criterion was used for both HTS and virtual screening hits. If we had used a cutoff of 10 μM, the absolute hit rates for both screening techniques would have decreased, but the enrichment of hits found by virtual screening over HTS would have actually increased to 3800-fold.

To investigate whether they were kinetically well-behaved, 25 of the docking hits were selected for detailed study. Four of the 25 appeared to behave as simple, competitive inhibitors (see Figure 1 for an example); the remaining 21 inhibited by time-dependent or noncompetitive mechanisms. Compounds active in the high-throughput screen probably inhibit by a variety of mechanisms as well, so that if we were to count as hits only those compounds that inhibit via simple, nontime-dependent mechanisms (which are the simplest to understand and to pursue in optimization studies), both the 34.8% docking hit rate and the 0.021% HTS hit rates are probably overestimates. By the same standards, it appears that many of the docking hits are sensible candidates for lead optimization.

To evaluate druglikeness, we considered how many of the docking and HTS hits pass the Pfizer Rule-of-5[29] (Table 3). All but 11 (9%) of the 127 active docking hits passed at least three of the four rules, and 73 (57%) passed all four rules. Conversely, of the 81 HTS hits analyzed, 32 (40%) did not pass at least three of the four rules, and only 19 (23%) passed all four. Consequently, the average Rule-of-5 score for the docking hits was significantly higher than the HTS hits: 3.49 out of a possible 4 for the docking hits and 2.73 out of a possible 4 for the HTS hits. In addition, Table 4 shows that a surprisingly high 78% of the HTS hits actually failed filters that have subsequently come into use at Pharmacia, whereas only 28% of the docking hits failed the

same filters (though we suspect that the use of our filters in advance of HTS or docking would lead to enhanced efficiency and success in both methods.) Judged by these criteria, the docking hits were more druglike than those discovered from screening an in-house pharmaceutical database.

Several interesting features of possible PTP1B inhibitors emerge from the SAR of the docking- and HTS-derived hits. The phosphate moiety can be replaced on the ligands with carboxylate and even neutral functionality that nevertheless appears to bind in the phosphate-binding region of the enzyme. Similar results were observed in an earlier docking study that found seven new inhibitors, including both a carboxylate and a salicylate.[26] In general, the neutral molecules that we found often spanned both phosphotyrosine sites, while the carboxylates and salicylates were frequently docked as nonspanners.

Several caveats should be mentioned. First, the databases that were screened were not the same. The HTS database was an in-house corporate database, whereas the docking database was made up of commercially available compounds. The possibility that the commercially available compounds had more good leads than the in-house database cannot be ruled out. Still, docking databases such as the ACD are dominated by reagents for chemical synthesis and typically would not be considered more druglike overall than a pharmaceutical screening library. Second, the assay conditions were different for the HTS-derived compounds and the docking hits (see Methods and Results); the docking assay conditions were more permissive but also more able to reproduce literature affinity values. We suspect that had albumin been left out of the high-throughput screen, more HTS hits would have been found. Thus, our hit rate enrichment of 1700-fold should be considered an upper estimate. Third, the testing of docking hits was done with a medium-throughput assay, which is probably more accurate and sensitive than the high-throughput assay used for the random screen. This is a feature allowed for by the computational prescreening inherent in docking, which prioritizes a relatively small portion of the database for testing. Finally, we note that the properties of the hits that are discovered by HTS or by docking are highly dependent on the database from which these hits come and on the preprocessing used to filter out unwanted compounds, and these dependencies should be considered whenever comparing HTS with docking.

The correlation between docking scores and IC_{50} values is poor (Table 2). Our docking program remains a screening method. It can discriminate against compounds that are not sensible because of poor steric and electrostatic complementarity, but it cannot reliably distinguish among what we would consider "reasonable-looking" molecules that appear to complement the binding site well. Thus, a molecule such as compound **1** received a docking energy score of −33.4 kcal/mol and was ranked 406th out of the molecules in the database; compound **8** received a docking energy score of −42.01 kcal/mol and was ranked 11th (Table 2). Yet, the IC_{50} values for these compounds are reversed: compound **1** is 5-fold more potent (IC_{50} = 4.1 μM) than compound **8** (IC_{50} = 21.6 μM). The difficulties with ranking compounds can be attributed to an approximate and inaccurate scoring function, failure to consider conformational change in the enzyme, desolvation of the enzyme, and the role of ordered solvent, among others. These contribute to the "scoring problem" in molecular docking, which remains an area of active research.[13−20]

Structure-based methods have been widely mooted as techniques to discover novel leads for drug development, but can they compete with high-throughput random screening—are they worth the trouble? This question cannot be answered by a single study. What can be said is that the absolute hit rate of the docking screen against PTP1B and the 1700-fold enhancement over random screening are encouraging. More inhibitors were discovered by docking than by HTS, even though 1000-fold more compounds were tested by the latter. Unexpectedly, the bioactive docking hits appeared to be more druglike than the HTS hits. More generally, the diversity of both hit lists and their dissimilarity to each other suggest that docking and HTS may be complementary techniques for lead discovery.

Experimental Section

Materials. All chemicals were purchased from commercial suppliers and used without further purification. Human recombinant PTP1B (residues 1−322) was purchased from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA). Phosphotyrosyl dodecapeptide, TRDI(P)YETDYYPRK, corresponding to amino acids 1142−1153 of the insulin receptor kinase regulatory domain, phosphorylated on the tyrosine, was from American Peptide (Sunnyvale, CA). *p*-Nitrophenyl phosphate (*p*NPP) was obtained from Sigma. Selected compounds from the docking calculation were purchased from Specs/BioSpecs, Maybridge, or Sigma-Aldrich Library of Rare Chemicals (SALOR).

HTS. PTP1B activity was assayed using a modification of the technique described by Harder et al.[33] Briefly, the enzyme reactions were carried out in a final volume of 30 μL on Costar 384 well black plates with clear bottoms (part number 3711, Corning Inc., Corning, NY). To each well were added 50 μM of phosphorylated peptide and 0.9 nM (1.6 ng) of PTP1B diluted in 25 mM HEPES (pH 7.0), 0.3 mM EDTA, 3.3 mM DTT, 3.3% glycerol, and 0.03% bovine serum albumin, with or without inhibitors. Following incubation for 60 min at room temperature, the reactions were terminated by addition of malachite green solution (prepared as described in Harder et al.). The plates then were incubated for 15 min at room temperature before measuring absorbance at 620 nm.

Molecular Docking. The site targeted in the docking calculations was defined by the positions of the two phosphotyrosine molecules observed in the complex with PTP1B (PDB code 1pty).[28] The closed, ligand-bound conformation of the active site was used in the docking studies. The atoms of the phosphotyrosine were used as "spheres"[34,35] in the docking calculations; the spheres were labeled[36] according to the expected charge and apparent hydrogen-bonding patterns of the phosphotyrosine atoms in the crystallographic structure. Protons were added to heteroatoms in the PTP1B crystal structure using SYBYL (Tripos, St. Louis, MO). A grid defining the excluded volume of the site was calculated using DISTMAP,[37] part of the DOCK suite. An electrostatic potential for the protein was calculated using the DelPhi program.[38] An AMBER-based[39] van der Waals potential was calculated for the active site using the program CHEMGRID,[39] also part of the DOCK suite. In the DelPhi calculation, the internal dielectric was set to two and the external dielectric was set to 78. To allow for the effect of ligand binding on the dielectric, the binding site was modeled with uncharged, low dielectric atoms inside it, using the positions of the atoms in the two phosphotyrosine molecules. This lowered the dielectric of most of the site to two; these atoms were not used in the calculations

of either the steric or the van der Waals grid and so only affected the electrostatic calculation.[40] In the 1pty structure, the catalytic Cys215 had been mutated to a serine. This residue was "back-mutated" to the wild-type cysteine, using the "swapaa" function in MIDAS.[41] For the modeled Cys215, the χ-1 angle was preserved from that of the serine in the 1pty structure. This catalytic cysteine was modeled as negatively charged, consistent with mechanistic considerations.[42] We presume that this charge state favored less highly charged molecules, which we were more interested in.

Molecules were docked into the active site of PTP1B in multiple conformations, using the Northwestern University version[40,43−45] of DOCK3.5.[39,46] Two databases of molecules were used as follows: about 152 000 compounds from the ACD98.2 database and about 82 000 compounds from the BioSpecs 1999 and the Maybridge 1999 catalog. Only molecules that had at least 17 and no more than 60 nonhydrogen atoms were docked—this amounted to 165 581 molecules docked in total. The atomic properties of these molecules, including van der Waals parameters and partial atomic charges, were calculated as previously described.[39] Conformations were precalculated using SYBYL and stored in a flexibase as described previously.[43]

Up to 500 conformations of each docking molecule were sampled; the average number of conformations for each molecule was 345. For calculating orientations in the site, receptor and ligand "bins" were set to 0.5 Å and "overlap bins" were set to 0.25 Å,[37] with a distance tolerance for matching sphere−atom pairs of 1.25 Å. On average, 341 orientations were sampled for each conformation of each molecule. In total, 19.5 billion docked complexes were calculated. Each was filtered for steric fit[37] and then scored for van der Waals and electrostatic complementarity; these scores were corrected for apolar and polar desolvation energies.[40,47] All ligand configurations were subjected to 10 steps of simplex rigid body minimization.[46] The calculation took 27.7 CPU days on 450 MHz Pentium processors running Linux; the laboratory time was 1 week as four processors were used.

Inhibition. Compounds selected by the docking program were screened for their ability to inhibit the PTP1B dephosphorylation of the insulin receptor peptide. The assay used the malachite green−ammonium molybdate method[33] to detect phosphate liberated from the 1142−1153 insulin receptor phosphotyrosyl dodecapeptide, TRDI(P)YETDYYPRK. Peptide substrate (20 μL) was added to a 96 well plate at a final concentration of 50 μM. Inhibitor dilutions (20 μL) were added to corresponding wells of the plate. All compounds were dissolved in neat dimethyl sulfoxide (DMSO). After dilution, the final concentration of DMSO in all reactions was less than 10%. The assay was initiated with the addition of human recombinant PTP1B (20 μL), at a final concentration of 1 nM in buffer containing 25 mM HEPES (pH 7.4), 125 mM NaCl, 1 mM EDTA, 10% glycerol, and 10 mM DTT. The assay was incubated at room temperature for 10 min and stopped by addition of malachite green−ammonium molybdate reagent (60 μL). The color was allowed to develop at room temperature for 30 min. Sample absorbances were determined at 620 nm using a plate reader (Titertek). Samples and blanks were prepared in triplicate.

Initial rates and inhibitor kinetics were determined by measurement of hydrolysis of the colorimetric substrate *p*NPP. Initial rates at five different fixed inhibitor concentrations were measured at five different *p*NPP concentrations ranging from 0.625 to 10 mM (K_m−16K_m) in buffer containing 25 mM HEPES (pH 7.4), 125 mM NaCl, 10% glycerol, and 1 mM EDTA. The inhibition pattern was evaluated, and K_i was determined using a direct curve-fitting program (GraFit-Erithacus Software).

Chemical Informatics. Similarity clustering was performed using the program Algorithm5.[30] Values for molecular weights, Clog*P* values, number of rotatable bonds, and the number of hydrogen bond acceptors and donors were computed using tools from Daylight Chemical Information Systems (Mission Viejo, CA). Substructure searching was also done using tools from Daylight CIS. The Pfizer Rule-of-5[29] assessment was done as described in the original publication: the number of hydrogen bond donors was taken to be the sum of N−H and O−H bonds; the number of hydrogen bond acceptors was taken to be the sum of N and O atoms. We used Clog*P* as our method of log*P* computation when computing the Rule-of-5 for each compound.

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