ELSEVIER



# Chemoproteomics-driven drug discovery: addressing high attrition rates

**Steven E. Hall**

Serenex, 323 Foster Street, Durham, NC 27701, USA

**The advent of multiple high-throughput technologies has brought drug discovery round almost full circle, from pharmacological testing of compounds *in vivo* to engineered molecular target assays and back to integrated phenotypic screens in cells and organisms. In the past, primary screens to identify new pharmacological agents involved administering compounds to an animal and monitoring a pharmacologic endpoint. For example, antihypertensive agents were identified by dosing spontaneously hypertensive rats with compounds and observing whether their blood pressure dropped. In taking this phenomenological approach, scientists were focused on the final goal, in this example lowering of blood pressure, rather than developing an understanding of the target, or targets, the compounds were impacting. With the evolution of rational target-based approaches, scientists were able to study the direct interaction of compounds with their intended targets, expecting that this would lead to more-selective and safer therapeutics. With the industrialization of screening, referred to as HTS, hundreds of thousands of compounds were screened in robot-driven assays against targets of interest (with this goal in mind). However, an unintentional outcome of the migration from *in vivo* primary screens to highly target-specific HTS assays was a reduction in biological context caused by the separation of the target from other cellular proteins and processes that might impact its function. Recognition of the potential consequences of this over-simplification drove the modification of HTS processes and equipment to be compatible with cellular assays.**

High-throughput cell-based assays have enabled scientists to reintroduce some of the biological complexity of a living system. New chemoproteomic approaches share some similarities with the early *in vivo* screens in that the focus is on finding compounds that will elicit the desired effect (in this case at the cellular level rather than the organism level). Following identification of compounds that promote the desired response in a cell-based screen [1,2], further analysis can be initiated to determine the protein target responsible. Despite similarities with their historical counterparts, current cell-based screens differ in three distinct ways. First, the diversity of compounds available has expanded by several orders of magnitude driving the need for increased throughput. Second, highly specific antibodies and other molecular reagents enable the researcher to monitor multiple pathways in phenomenological and highly characterized biochemical screens. Third, new imaging tools allow for temporal processes to be monitored in real time, which is a great advantage in unraveling cause and effect relationships. The ability to monitor discrete pathways within a living cell, in combination with observing the overall cellular response, of an individual compound has enabled the field of chemoproteomics.

Several definitions have been put forward to define chemogenomics and chemoproteomics. Although these terms have been used interchangeably, it would be preferable to use them as distinct terms, given that there are compounds that directly affect gene function (e.g., DNA alkylators) and compounds that directly affect protein function (the majority of drugs). Chemogenomics and chemoproteomics continue to attract a lot of attention with

*Corresponding author:* Hall, S.E. (shall@serenex.com)

1359-6446/06/$ - see front matter  doi:10.1016/j.drudis.2006.04.014

several general reviews [3–6], as well as reviews focused on their application in signal transduction research [7], kinase inhibitor discovery [8,9] and anticancer drug discovery [10,11], published recently.

## The ideal screen

Although screening compound libraries against multiple members of a protein family begins to address the desired scope of target knowledge, the ideal screen should allow for the interrogation of the entire human proteome with each compound library. Armed with this knowledge, scientists can rationally select the most promising hits to pursue into the lead-identification stage. Short of achieving this ideal, discovery approaches that increase our understanding of how individual compounds might interact with protein targets of one or more protein families is an important step forward. These approaches change the discovery process because they focus on methods where the unit process is screening one compound against many targets, rather than screening many compounds against a single target. Technologies that provide the greatest breadth of target affinity information for each compound are desired, they reduce the level of uncertainty.

## Chemoproteomics-driven drug discovery

Chemoproteomic approaches to drug discovery fall into two general categories: focused applications that often involve the detailed study of a single class of compounds with known biological activity, aiming to identify a mechanism of action or the use of a compound series in target validation [12,13]; and broad-based applications that utilize compound libraries to probe protein function, using the binding information to develop an understanding of the links between the targets, chemical structure and biological response. Both applications are dependent on the ability to detect and define accurately the proteins through which compounds exert their biological effects. This review will highlight examples of each approach but will not discuss advances in proteomics that have enabled protein identification on an ever-decreasing scale [14].

## Focused chemoproteomics applications

Given that there are >500 kinases, determining selectivity across the kinase protein family is difficult to assess. This challenge is compounded by the fact that most kinase inhibitors are competitive with ATP and, therefore, can bind to other completely unrelated ATP-utilizing enzymes. Isacchi and colleagues [15] used PHA-539136, one member of a series of pyrrazole-based cyclin-dependent kinase 2 (CDK2) inhibitors, as a ligand to study the selectivity of this inhibitor series. PHA-539136 (Figure 1) was coupled to Actigel-B resin through its terminal amino group and, thus, produced an affinity resin. Following validation that this resin binds CDK2, it was incubated with protein extracts from two different cell lysates. Elution with PHA-539136, and analysis of the eluant by 1D or 2D gel electrophoresis, showed that the two eluants had five proteins in common: heat shock protein (Hsp)90, Hsp70, Hsp27, ATP-synthase α-chain, and β-tubulin. Isothermal titration calorimetry confirmed that all five proteins were binding partners for the inhibitor and three of the proteins, Hsp70, Hsp27 and β-tubulin, bound with submicromolar affinities. Thus, PHA-539136 possessed significant affinity for other ATP-utilizing enzymes (besides kinases). Analogous studies with another CDK inhibitor, olomoucine, showed that this inhibitor displayed significant affinity for another kinase, casein kinase 1 (CK1), [16] and studies of flavopiridol by Schnier and colleagues [17,18] revealed this CDK inhibitor also inhibited two non-kinase targets, glycogen phosphorylase and aldehyde dehydrogenase. Taken together,

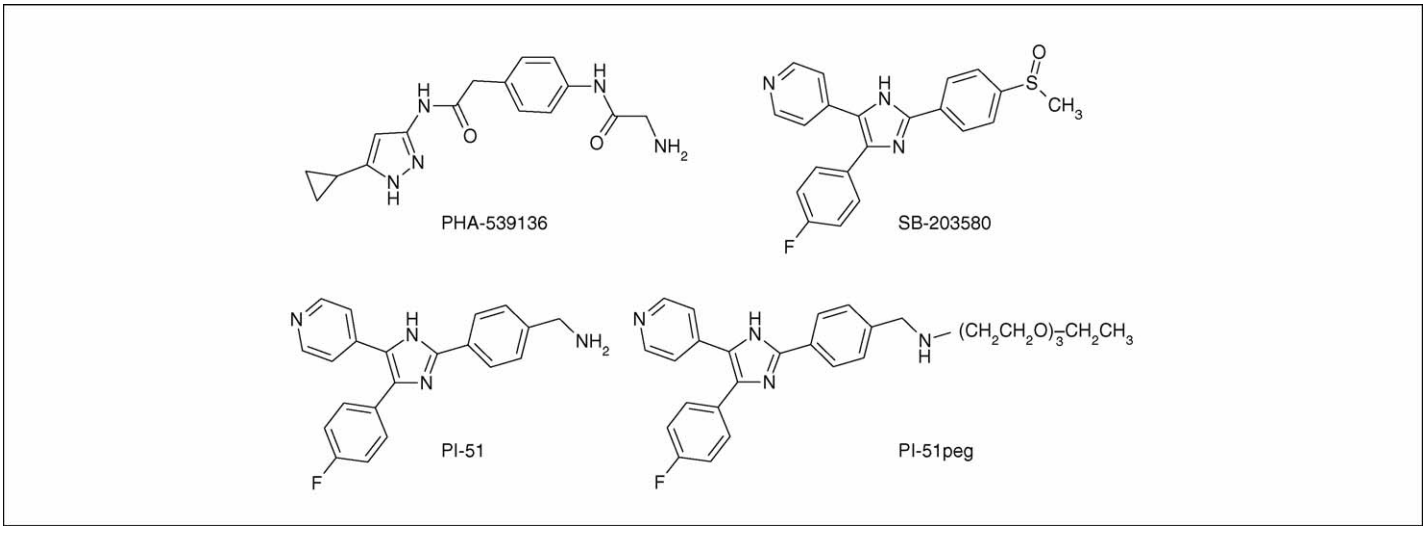


**FIGURE 1**

**Structures of ligands for target pull-down studies.** Determination of secondary targets for the cyclin-dependent kinase 2 (CDK2) inhibitor PHA-539136 was achieved by preparing an affinity matrix with this compound (via coupling of the primary amine group). Incubation with cell extracts and subsequent treatment with PHA-539136 eluted CDK2 and >10 other proteins. High-affinity binding of the inhibitor was determined by isothermal titration calorimetry for the heat shock proteins Hsp70 and Hsp27. Secondary targets for a p38 inhibitor, SB-203580, were determined in an analogous manner, except that a related compound, PI-51, was used as the ligand in the affinity matrix. PI-51peg (PI-51 coupled to polyethylene glycol) was used as a control to assess the effect of the long hydrophilic linker that was used to connect PI-51 to the polymeric support. Incubation of the PI-51 affinity matrix with cellular extracts followed by treatment with SB-203580 eluted the kinases p38, rip-like interacting caspase-like apoptosis-regulatory protein (CLARP) kinase (RICK), cyclin G-associated kinase (GAK), casein kinase 1 (CK1), as well as several other proteins.

these studies underscore the importance of selectivity profiling across multiple protein families.

Covalently attaching ligands to a solid phase has also proved useful in the selectivity studies of other inhibitors. Godl *et al.* [19] explored the profile of the p38 kinase inhibitor SB-203580 (Figure 1) by using the inhibitor as bait to capture protein-binding partners. Because SB-203580 did not have the functionality necessary for coupling to a resin, its close analog PI-51, which had also been shown to be a potent p38 kinase inhibitor, was used as the bait molecule. Both PI-51 and its pegylated derivative (PI-51peg) were shown (by an in-gel kinase assay) to retain potent p38 inhibition. Incubation of the resin with the cell extract resulted in the recovery of known binding partners: p38, multiple c-jun N-terminal kinase (Jnk) isozymes, glycogen synthase kinase β (Gskβ), rip-like interacting caspase-like apoptosis-regulatory protein (CLARP) kinase (RICK), CK1 and cyclin G-associated kinase (GAK). Importantly, the target affinities discovered for PI-51 were replicated for SB-203580 and the latter inhibited RICK at lower concentrations than those required to inhibit p38. In addition, CK1 and GAK were inhibited at concentrations only threefold higher than p38. This led the authors to suggest that some of the biology ascribed to p38 inhibitors should be critically assessed because many studies (>1000 publications) based their conclusions on the assumption that SB-203580 was a highly selective p38 inhibitor. For example, some effects of this compound (e.g. lipopolysaccharide (LPS)-induced cytokine release) could also be the result of RICK inhibition. This study illustrates that great caution should be taken when interpreting data generated using a single inhibitor because, although appearing selective, these data have not been characterized by more comprehensive methods now available.

## Broad-based chemoproteomics applications

### *Protein-based diversity and similarity assessments*

Many diversity methods are based on comparisons between the chemical descriptors for the compounds being evaluated. An alternative approach to define diversity, or similarity, is to compare the pattern of binding affinities of the compounds with a series of unrelated proteins. Kauvar *et al.* [20] described an example of this approach. First, an affinity fingerprint database is generated by measuring the binding of compounds from a small-molecule library against a panel of proteins (reviewed recently by Beroza *et al.* [21,22]). The premise is that the affinity of an individual compound for a variety of different proteins provides a better representation of molecular diversity, or similarity, than pure chemical descriptors. An advantage of this method is the potential to identify chemically novel series of inhibitors that would not have been selected using chemical descriptors alone. Support for this approach comes from the observation that compounds with comparable biological activities (e.g. anti-inflammatory activity) also possess similar protein affinity fingerprints, even when their chemical structures might not be similar.

Hsu *et al.* [23] recently applied this approach, termed target-related affinity profiling (TRAP), to the identification of cyclooxygenase inhibitors. An initial activity model was built using the pattern of protein affinities observed for 19 nonsteroidal anti-inflammatory drugs (NSAIDs) and was then used to select new compounds for screening. Although none of the initial 16 new compounds selected showed significant inhibitory activity, their affinity fingerprints were used to further-refine the activity model. A second round of test compounds produced five actives, three of which displayed potencies that exceeded the standard NSAID ibuprofen. Figure 2 compares the structures of these three compounds with those of the closest known NSAIDs used to build the starting activity model. It is noteworthy that these compounds were identified by assaying a total of only 62 compounds.

## Chemical probes for protein family selectivity

The capability to determine selectivity across large protein families has been expanded through the development of reagents that increase our ability to separate and identify individual family members. Nomanbhoy *et al.* [24] have described an approach to assess selectivity through the use of irreversible chemical probes that are capable of labeling all of the proteins in a single family. These reagents, termed activity-based probes (ABP), label the proteins to which the inhibitor of interest does not bind. These probes enable the identification of uncharacterized protein partners for compounds, without the need to develop specific assays for each target. This approach has been used to characterize additional targets (in the serine hydrolase family) for the dipeptidyl peptidase 4 (DPP4) inhibitor isoleucyl thiazolidine (IT). By using two ABPs (red and green fluorophores), targets of IT were identified through a three-step process. First, the inhibitor and ABP (first color) were co-incubated with the cell lysate of interest. Through reversible binding, the inhibitor protects the enzyme active sites (to which it binds) from the chemically reactive activity probe. Second, dialysis removes both the first probe and the inhibitor. Third, incubation with the second color ABP labels the proteins to which the inhibitor was previously bound. This process identified two DPP4 homologs, DPP8 and DPP9, as potential targets for IT. Other studies using this approach have been published by Adam *et al.* [25] and Patricelli *et al.* [26].

Advances in crystallography have enabled structural chemoproteomic approaches (that utilize the commonalities in structure across proteins from the same family) to define generic scaffolds that can be customized to produce compounds with the desired selectivity. Shin *et al.* [27] analyzed the selectivity of different drugs for the phosphodiesterase (PDE) isozymes PDE4, PDE5 and PDE6. Comparison of the crystal structures of PDE4 and PDE5 revealed a very high degree of similarity, with only two amino acids that would be expected to interact with a chemical inhibitor differing in the region of the active site. In PDE4 replacement of those amino acids, Ala783 and Leu804, with methionines led to the blockade of two subpockets, with which several of the commercially available PDE5 inhibitors interact. Thus, despite the fact that these two proteins were very similar from a structural proteomics perspective, they could be distinguished by a series of inhibitors. Analysis of the structures of PDE5 and PDE6 was used to explain the incidence of a common side-effect of PDE5 therapy, namely cyanopsia or blue vision, which is believed to be caused by inhibition of PDE6. The crystal structure of PDE5 was compared with a homology model of PDE6 to understand the difference in selectivity between tadalafil (700-fold more selective for PDE5, compared with PDE6), sildenafil and vadenafil, which are only 6–15-fold more selective for PDE5 over PDE6. Again, only two amino acids differed between to the two isozymes in the region of

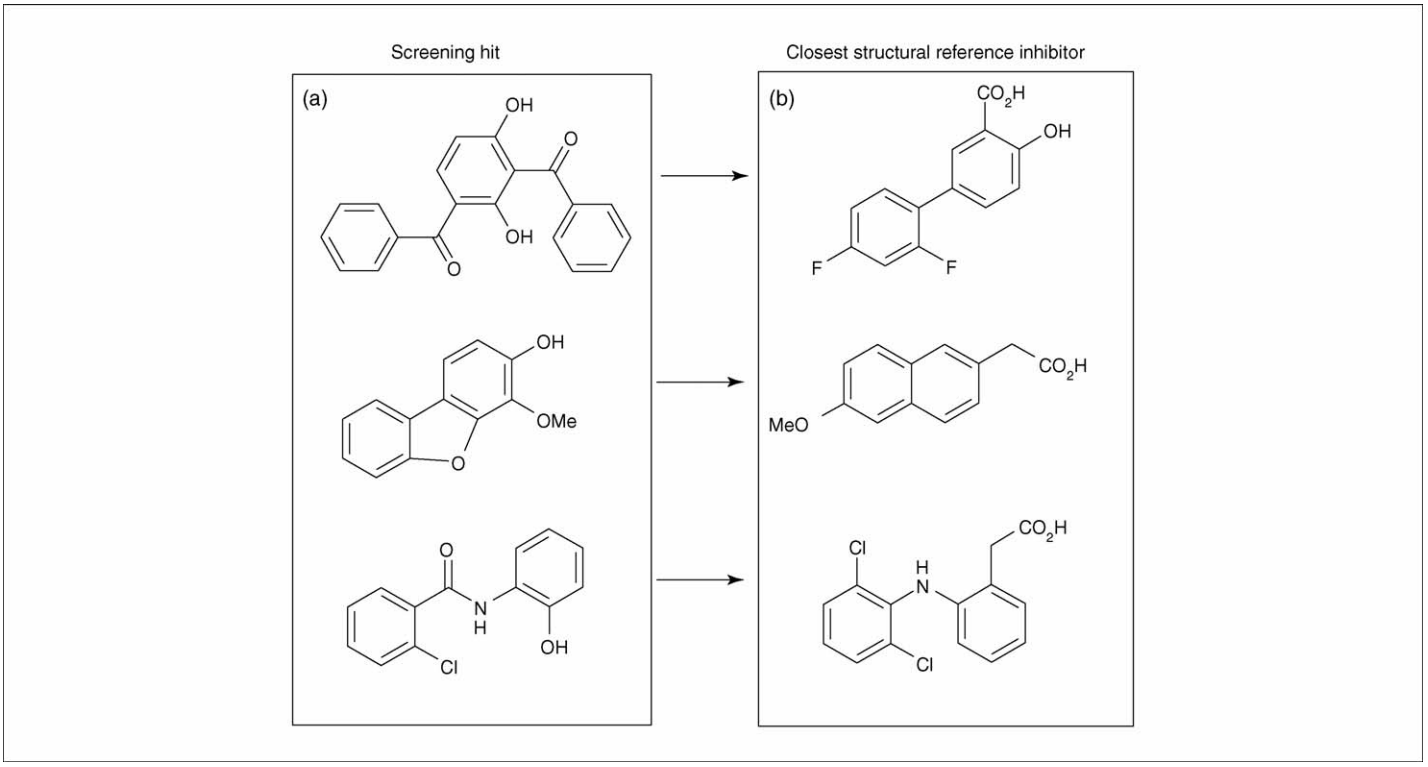


**FIGURE 2**

**Comparing nonsteroidal anti-inflammatory drug (NSAID) actives with reference inhibitors. (a)** The structure of three screening hits that were identified using an affinity fingerprint activity model for cyclooxygenase-1 compared with the structures of the reference NSAIDs **(b)** that had the greatest degree of structural similarity.

inhibitor binding. The authors suggested that the increased selectivity for PDE5 of tadalafil was a consequence of the steric collision between the drug and the terminal methyl group of the leucine side-chain in PDE6, whereas the amino acid that is in this position in PDE5 is less sterically demanding (isoleucine). As a result, tadalafil is only able to bind tightly to PDE5.

## Compound libraries as tools for target discovery

Root *et al.* [28] described the construction of an annotated compound library (ACL) of 2036 biologically active compounds. The aim of the study was to identify new compounds that would kill cancer cells, or subject them to growth arrest. Importantly, the library was populated with compounds with a wide array of different biological activities spanning known antiproliferative compounds, muscle relaxants and antimalarial drugs. Multiple compounds were selected within each biological profile to provide increased structural diversity. These compounds were then used as probes to uncover distinct biological pathways that would either growth-arrest or kill tumor cell lines. From the total of 2036, 85 compounds reduced cell counts by at least 50% (with many unique chemotypes that were not represented in the US National Cancer Institute collection of nearly 250,000 compounds). Comparisons of differential activities in cancer versus normal cells were used to identify compounds with selective antiproliferative activity. Despite the broad range in chemical structure, compounds with a similar mechanism of action displayed a similar degree of selectivity against cancer cells.

Even small compound sets can be valuable as chemical tools for unraveling the relationship of proteins and their physiology. Parsons *et al.* [29] used a 12-member compound set to probe the entire *Saccharomyces cerevisiae* viable deletion set for hypersensitivity. Each of the single-gene deletion mutants were subject to treatment with each compound. With this approach, the authors expected to identify those proteins that are crucial for the survival of the organism under the conditions of a single-gene defect. The chemical–genetic interaction profiles were generated from the study of all 12 compounds against 4700 mutants. Although further work is needed to identify which compound-specific protein enables survival, this approach rapidly narrows the field of candidate proteins that would warrant further study.

## Fragment-based approaches

All of the methods discussed up to this point involve profiling drug-sized molecules, but approaches have been developed that focus on the interaction of low molecular weight compounds, viewed as drug fragments, with individual targets or entire protein families. Several companies have been active in this area including Sunesis, Graffinity and Astex. In one example, Vetter [30] described profiling low molecular weight compounds as arrays of chemical fragments that are constructed on self-assembling monolayers. Binding of a protein to the spatially addressed chemical fragments was detected by surface plasmon resonance. This approach provides comprehensive information on fragments that bind to the protein of interest without any advance knowledge of binding sites. The limitation of this approach is that binding to only a single protein is determined. Dickopf *et al.* [31] recently described the use of this method to discover novel ligands for the Factor VIIa active site.

Several groups have described approaches based on empirical observations in medicinal chemistry. This has led to the privileged structure concept, where the same or similar chemical scaffolds have been used to develop inhibitors to distinct protein targets. Often, a certain chemical structure shows a particular affinity for a target family [e.g. G-protein-coupled receptors (GPCRs) or kinases] but some scaffolds, for example 1,2-diaryl-5-membered aromatic heterocycles, appear in many different classes of agents, including inhibitors of cyclooxygenase, p38 kinase, glucagon receptor, dopamine transporter, and protein tyrosine phosphatase (PTP)1B [32].

## Understanding target selectivity across the entire proteome

The chemoproteomic approaches that have been described in this review were generally developed to either aid the discovery of new targets for compounds (target fishing) or to define the mechanism of action for compounds that possess interesting biological activities [33]. Unfortunately, only a subset of these methods can provide in-depth profiling information for many proteins, knowledge that is needed to improve hit and lead selection. The lack of discriminating information across a series of screening hits is a major contributing factor to the problem that HTS methods have not improved hit-to-candidate conversion rates. This challenge has attracted significant interest [34–37] and several investigators have described empirical-based methods [38–40] and/or computational approaches [41–45] to aid the selection of hits and leads.

Scientists at Serenex were interested in developing an approach to drive the hit-to-candidate process that would be applicable to target discovery, mechanism of action determination and compound profiling. The target space selected for study was the purine-binding proteome. This large family comprises proteins with binding sites for purine cofactors, including ATP, NAD and flavin adenine dinucleotide (FAD). The focus on the cofactor binding site, normally viewed as a site that would lead to nonselective compounds – caused by the conservation of these sites across proteins, was crucial in developing an affinity displacement assay that allowed compounds to be studied against hundreds of targets simultaneously. Central to this approach, which is termed proteome mining (Figure 3), is a purine-affinity media that was optimized to allow binding of diverse protein classes, from kinases to helicases to sulfotransferases – as summarized in Table 1. In the foundation work in this area, Graves and Haystead [46] demonstrated that ATP-based affinity media could be used to capture (reversibly) not only ATP-binding proteins, but also proteins that utilize other purine cofactors.

Haystead and colleagues [47] used this approach to identify two host targets for the antimalarial drug chloroquine, namely quinone reductase 2 (QR2) and aldehyde dehydrogenase 1 (ADH1). Although this method is correctly viewed as a competitive displacement assay, subsequent experiments verified that chloroquine directly inhibited the activity of both QR2 and ADH1 [48,49]. The potential relevance of the latter target is noteworthy because one of the side-effects of long-term chloroquine treatment is the development of retinopathy. ADH1, the enzyme responsible for the production of the visual pigment retinal, represents >95% of retina wet-protein weight. Despite the low affinity of chloroquine for this target, the sheer amount of ADH1 in the retina could explain why the drug accumulates in the eye over extended periods of time. Previous work demonstrated that inhibition of ADH1 leads to retinopathy. Identification of ADH1 as an undesired off-target for chloroquine provides a path forward for the development of safer antimalarial drugs.

Scientists at Serenex were interested in the finding that chloroquine was a potent inhibitor of QR2 because their working

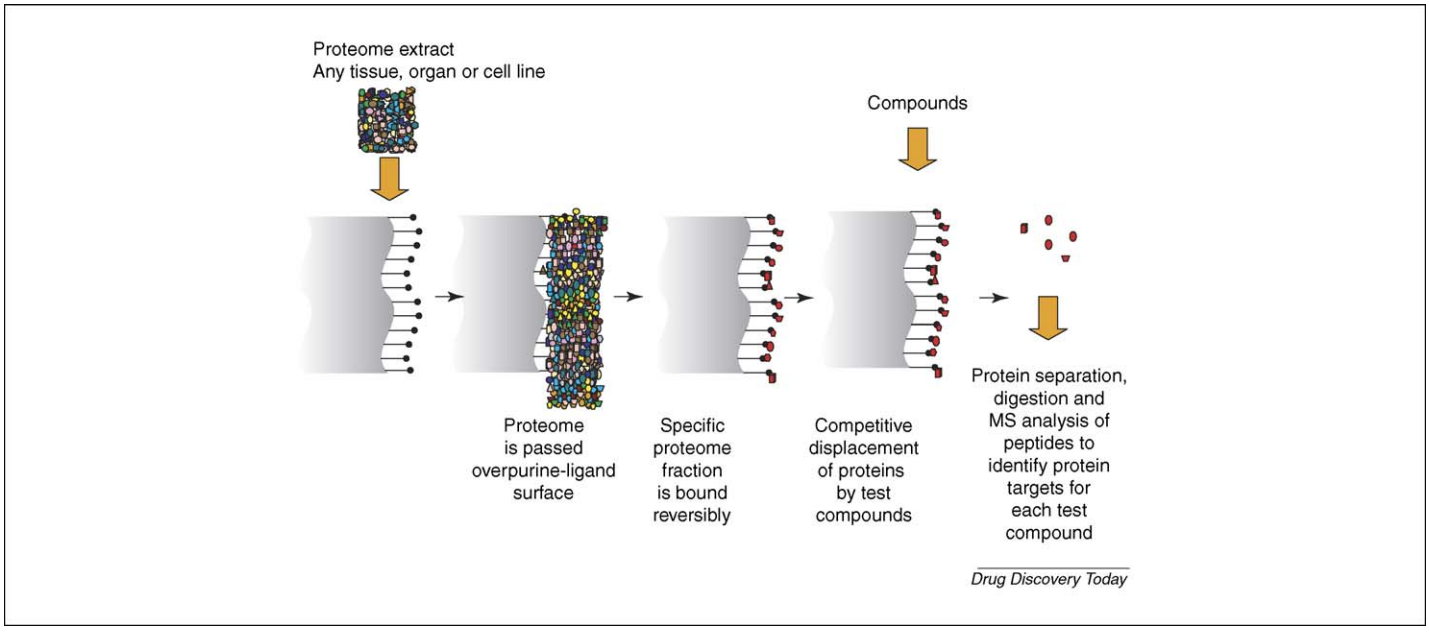


**FIGURE 3**

**Proteome mining process.** Proteome mining consists of a multistep process involving incubation of a proteome extract with purine affinity media, multiple wash steps, competitive elution with members of a compound library and analysis of the eluted proteins by 1D gel electrophoresis and mass spectrometry of the resultant protein bands. Importantly, the protein targets and the compounds being profiled are unmodified and free in solution, thus avoiding artifacts from polymeric supports or the linker functionality.

**TABLE 1**

**Examples of purine-binding protein classes**

| | | | |
|---|---|---|---|
| **Kinases** | **Lipases** | **Helicases** | **Reductases** |
| **Deaminases** | **Transferases** | **Formylases** | **Synthetases** |
| **Phosphorylases** | **Dehydrogenases** | **Carboxylases** | **Sulfotransferases** |

hypothesis was that this target might be responsible for the anti-inflammatory activity of this drug. Proteome mining was used throughout the hit-to-lead process to develop SAR for the identification of selective QR2 inhibitors. Figure 4a illustrates the protein elution profile of chloroquine and Figure 4b illustrates the protein elution profiles of a series of chloroquine-related analogs, each lane represents the elution profile of a unique compound. Some analogs appeared to lose ADH1 activity but, at the same time, eluted a new protein that was identified as alcohol dehydrogenase. Through the use of this profiling approach, selective inhibitors of QR2 were identified, several of which have demonstrated oral activity in mouse models of malaria infection. Further work is in progress to establish a clear link between antimalarial activity, anti-inflammatory activity and QR2 inhibitory potency.

## Proteome mining: an alternative approach to drug discovery screens

To test the applicability of proteome mining to support a screen, a targeted library of ~8000 compounds was designed and screened against the purine-binding proteins from porcine lung (using the methodology illustrated in Figure 3). This custom library was created by similarity methods using known ligands for diverse classes of purine-binding proteins. Examples of compounds, used in generating the library, range from kinase inhibitors (such as flavopyridol, H77 and staurosporin) to dihydrofolate inhibitors (such as methotrexate).

Note that this approach to drug discovery is distinct from the traditional drug discovery process, in which the first step is target selection. In proteome mining target selection does not occur until after the screen is completed because it allows for hundreds of proteins to be screened simultaneously. As described in the following paragraphs, Hsp90 was selected solely on the basis of identifying a selective hit in the proteome mining screen.

## Discovery of Hsp90 inhibitors from a chemoproteomic screen

The screening of an 8000-member focused library generated hits for >50 protein targets spanning several diverse protein families. Of note is the fact that selectivity within a target family (e.g. kinases) did not necessarily correlate with selectivity between target families. The significance of this finding is relevant to kinase drug discovery, where researchers have focused on measuring selectivity against other kinases but have also generally assumed that selective kinase inhibitors would not bind (with any significance) to non-kinase targets.

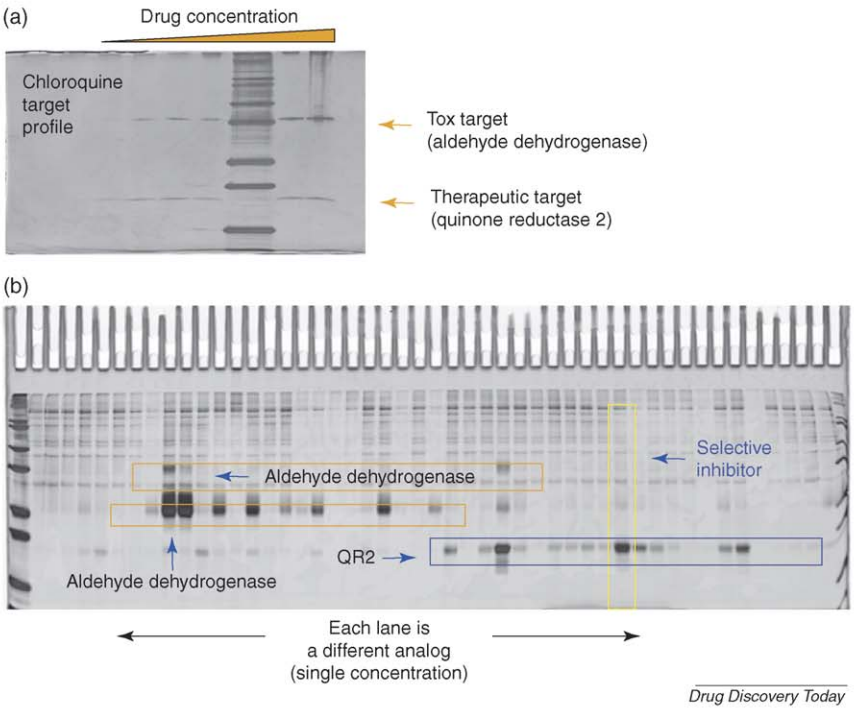


**FIGURE 4**

**SAR development using proteome mining. (a)** Two protein targets for chloroquine were eluted in a concentration-dependent manner, quinone reductase 2 (QR2), the putative target for the anti-inflammatory activity, and aldehyde dehydrogenase, the putative toxicity target responsible for retinopathy that occurs on extended treatment with chloroquine. **(b)** New chloroquine-related analogs were profiled (single concentration) to determine their selectivity in eluting only the desired target, QR2. Note the marked differences in the profile of these analogs, including the unexpected elution of an additional off-target alcohol dehydrogenase.

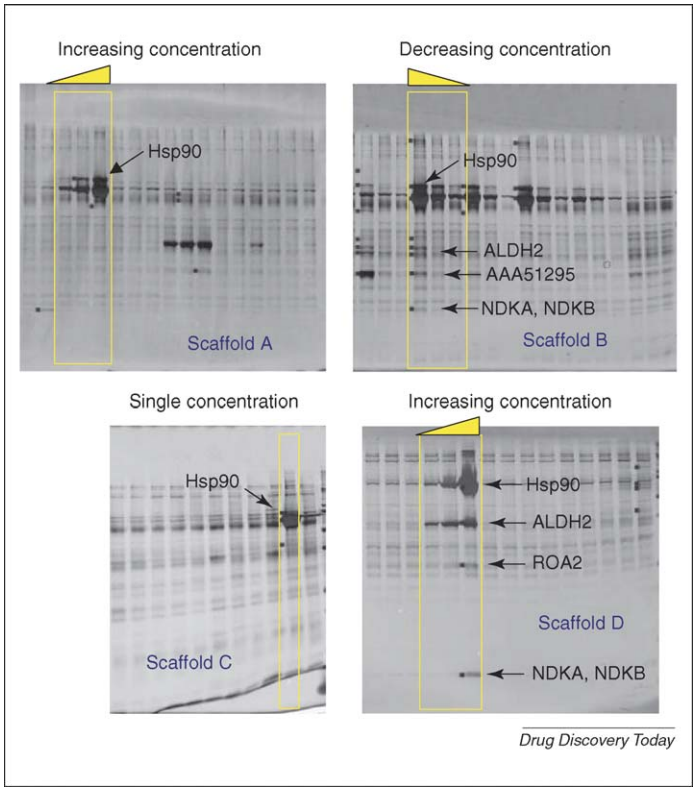


**FIGURE 5**

**Hsp90 screening hits from proteome mining.** Four unique chemical scaffolds were identified having significant affinity for heat shock protein (Hsp)90 – owing to their ability to elute this protein in a concentration-dependent manner. The scaffolds displayed significant differences in selectivity because some of them (e.g. scaffolds B and D) eluted additional targets from multiple protein families. Abbreviations: ALDH2, aldehyde dehydrogenase 2; NDKA, nucleoside diphosphate kinase A; NDKB, nucleoside diphosphate kinase B; ROA2, replication origin activator 2.

One of the proteins eluted in the initial screen was Hsp90, which is currently a target of much interest in oncology research [49–52]. This is because of its role in maintaining, or chaperoning, several oncogenic proteins including several receptor tyrosine kinases such as Her2 and epidermal growth factor receptor (EGFR). Of the 8000 compounds, four unique chemical scaffolds were identified with significant Hsp90 affinity. Figure 5 shows the raw data from that screen. Note that scaffolds B and D were not as selective as scaffolds A and C because they each eluted additional proteins. These proteins included nucleoside diphosphate kinase A (NDKA) and succinyl adenosine homocysteinase (SAHH) in addition to Hsp90. Having this information before initiating the hit-to-lead process enabled medicinal chemists to focus on the most promising scaffold from the outset, resulting in the advancement of the initial hit into a series of highly selective, orally active Hsp90 inhibitors in ~15 months.

## Conclusion

The ability to discern the full complement of proteins to which a drug will bind and potentially exert its effect is central to our ability to develop a clear understanding of the intimate relationship between compound structure, target affinity and biological response. Despite a broad diversity of approaches, this is the ultimate goal of many, if not all, chemoproteomic technologies. To date, insufficiently characterized chemical tools have been used to define the role of proteins but we now know that many compounds do not exert their effects through a single target, instead they have multiple targets. Our challenges remain twofold: to continue the development of robust tools for the screening and characterization of compounds across a broad subset of the proteome; and to be sure that current target annotations are based on carefully characterized chemical probes.



## References


1 Gagna, C. *et al.* (2004) Cell biology, chemogenomics and chemoproteomics. *Cell Biol. Int.* 28, 755–764
2 Croston, G. (2002) Functional cell-based uHTS in chemical genomic drug discovery. *Trends Biotechnol.* 20, 110–115
3 Spring, D. (2005) Chemical genetics to chemical genomics: small molecules offer big insights. *Chem. Soc. Rev.* 34, 472–482
4 Kim, T. (2004) Chemical genomics and medicinal systems biology: chemical control of genomic networks in human system biology for innovative medicine. *J. Biochem. Mol. Biol.* 37, 53–58
5 Rajasethupathy, P. *et al.* (2005) Systems modeling: a pathway to drug discovery. *Curr. Opin. Chem. Biol.* 9, 400–406
6 Stockwell, B. (2004) Exploring biology with small organic molecules. *Nature* 432, 846–854
7 Shokat, K. and Velleca, M. (2002) Novel chemical genetic approaches to the discovery of signal transduction inhibitors. *Drug Discov. Today* 7, 872–879
8 Gallion, S. and Qian, D. (2005) Chemical genetic approaches to kinase drug discovery. *Curr. Opin. Drug Discov. Devel.* 8, 638–645
9 Vieth, M. *et al.* (2004) Kinomics-structural biology and chemogenomics of kinase inhibitors and targets. *Biochim. Biophys. Acta* 1697, 243–257
10 Sehgal, A. (2003) Anticancer drug discovery using chemical genomics. *Curr. Med. Chem.* 10, 749–755
11 Onyango, P. (2004) The role of emerging genomics and proteomics technologies in cancer drug target discovery. *Curr. Cancer Drug Targets* 4, 111–124
12 Zanders, E.D. *et al.* (2002) Probes for chemical genomics by design. *Drug Discov. Today* 7, 711–718
13 Stockwell, B. (2000) Chemical genetics: ligand-based discovery of gene function. *Nat. Rev. Genet.* 1, 116–125
14 Hochstrasser, D. *et al.* (2002) Proteomics and its trends facing nature's complexity. *Proteomics* 2, 807–812
15 Lolli, G. *et al.* (2003) Inhibitor affinity chromatography: Profiling the specific reactivity of the proteome with immobilized molecules. *Proteomics* 3, 1287–1298
16 Knockaert, M. *et al.* (2000) Intracellular targets of cyclin-dependent kinase inhibitors: Identification by affinity chromatography using immobilized inhibitors. *Chem. Biol.* 7, 411–422
17 Kaiser, A. *et al.* (2001) The cyclin-dependent kinase (CDK) inhibitor flavopiridol inhibits glycogen phosphorylase. *Arch. Biochem. Biophys.* 386, 179–187
18 Schnier, J. *et al.* (1999) Identification of cytosolic aldehyde dehydrogenase 1 from non-small cell lung carcinomas as a flavopiridol-binding protein. *FEBS Lett.* 454, 100–104
19 Godl, K. *et al.* (2003) An efficient proteomics method to identify the cellular targets of protein kinase inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* 100, 15434–15439
20 Kauvar, L. *et al.* (1995) Predicting ligand binding to proteins by affinity fingerprinting. *Chem. Biol.* 2, 107–118
21 Beroza, P. *et al.* (2002) Chemoproteomics as a basis for post-genomic drug discovery. *Drug Discov. Today* 7, 807–814
22 Beroza, P. *et al.* (2005) Target-related affinity profiling: Telik's lead discovery technology. *Curr. Top. Med. Chem.* 5, 371–381
23 Hsu, N. *et al.* (2004) Novel cyclooxygenase-1 inhibitors discovered using affinity fingerprints. *J. Med. Chem.* 47, 4875–4880

24 Nomanbhoy, T. *et al.* (2003) Inhibitor focusing: direct selection of drug targets form proteomes using activity-based probes. *Assay Drug Dev Technol.* 1, 137–146

25 Adam, G. *et al.* (2001) Profiling the specific reactivity of the proteome with non-directed activity-based probes. *Chem. Biol.* 8, 81–95

26 Patricelli, M. *et al.* (2001) Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *Proteomics* 1, 1067–1071

27 Shin, D. *et al.* (2005) Structural chemoproteomics and drug discovery. *Biopolymers* 80, 258–263

28 Root, D. *et al.* (2003) Biological mechanism profiling using an annotated compound library. *Chem. Biol.* 10, 881–892

29 Parsons, A. *et al.* (2004) Integration of chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways. *Nat. Biotechnol.* 22, 62–69

30 Vetter, D. (2002) Chemical microarrays, fragment diversity, label-free imaging by plasmon resonance – a chemical genomics approach. *J. Cell. Biochem. Suppl.* 39, 79–84

31 Dickopf, S. *et al.* (2004) Custom chemical microarray production and affinity fingerprinting for the S1 pocket of factor VIIa. *Anal. Biochem.* 335, 50–57

32 Müller, G. (2003) Medicinal chemistry of target family-directed masterkeys. *Drug Discov. Today* 8, 681–691

33 Kley, N. *et al.* (2004) Genomics and proteomics tools for compound mode-of-action studies in drug discovery. *Pharmacogenomics* 5, 395–404

34 Goodnow, R., Jr (2001) Current practices in generation of small molecule new leads. *J. Cell. Biochem. Suppl.* 37, 13–21

35 Ghose, A. *et al.* (1999) A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery 1. A qualitative and quantitative characterization of known drug databases. *J. Comb. Chem.* 1, 55–68

36 Stoughton, R. and Friend, S. (2005) How molecular profiling could revolutionize drug discovery. *Nat. Rev. Drug Discov.* 4, 345–350

37 Swaan, P. and Ekins, S. (2005) Reengineering the pharmaceutical industry by crash-testing molecules. *Drug Discov. Today* 10, 1191–1200

38 Wermuth, C. (2004) Selective optimization of side activities: another way for drug discovery. *J. Med. Chem.* 47, 1303–1314

39 Bemis, G. and Murcko, M. (1996) The properties of known drugs. 1. Molecular frameworks. *J. Med. Chem.* 39, 2887–2893

40 Bemis, G. and Murcko, M. (1999) Properties of known drugs. 2. Side chains. *J. Med. Chem.* 42, 5095–5099

41 Shen, J. (2003) HAD: an automated database tool for analyzing screening hits in drug discovery. *J. Chem. Inf. Comput. Sci.* 43, 1668–1672

42 Roberts, G. *et al.* (2000) LeadScope: software for exploring large sets of screening data. *J. Chem. Inf. Comput. Sci.* 40, 1302–1314

43 Klein, C. *et al.* (2002) Similarity based SAR (SIBAR) as tool for early ADME profiling. *J. Comput. Aided Mol. Des.* 16, 785–793

44 Muegge, I. (2003) Selection criteria for drug-like compounds. *Med. Res. Rev.* 23, 302–321

45 Mestres, J. (2004) Computational chemogenomics approaches to systematic knowledge-based drug discovery. *Curr. Opin. Drug Discov. Devel.* 7, 304–313

46 Graves, P. and Haystead, T. (2003) A functional proteomics approach to signal transduction. *Recent Prog. Horm. Res.* 58, 1–24

47 Graves, P.R. *et al.* (2002) Discovery of novel targets of quinoline drugs in the human purine binding proteome. *Mol. Pharmacol.* 62, 1–9

48 Kwiek, J. *et al.* (2004) Kinetic mechanism of quinone oxidoreductase 2 and its inhibition by the antimalarial quinolines. *Biochemistry* 43, 4538–4547

49 Whitesell, L. and Lindquist, S. (2005) Hsp90 and the chaperoning of cancer. *Nat. Rev. Cancer* 5, 761–772

50 Jackson, S. *et al.* (2004) Hsp90: From structure to phenotype. *Nat. Struct. Mol. Biol.* 11, 1152–1155

51 Dymock, B.W. *et al.* (2004) Inhibitors of Hsp90 and other chaperones for the treatment of cancer. *Expert Opin. Ther. Patents* 14, 837–847

52 Chiosis, G. *et al.* (2004) Hsp90: The vulnerable chaperone. *Drug Discov. Today* 9, 881–888