DESIGNING SCREENS: HOW TO MAKE YOUR HITS A HIT

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The basic goal of small-molecule screening is the identification of chemically 'interesting' starting points for elaboration towards a drug. A number of innovative approaches for pursuing this goal have evolved, and the right approach is dictated by the target class being pursued and the capabilities of the organization involved. A recent trend in high-throughput screening has been to place less emphasis on the number of data points that can be produced, and to focus instead on the quality of the data obtained. Several computational and technological advances have aided in the selection of compounds for screening and widened the variety of assay formats available for screening. The effect on the efficiency of the screening process is discussed.

A GUIDE TO DRUG DISCOVERY

The science of high-throughput screening (HTS) has undergone a series of significant periods of evolution. Through the early and mid-1990s, increasing screening capacity was clearly identified as the challenge for HTS. This was fuelled by an expanded number of targets divined by molecular biology and an increased number of compounds generated by combinatorial chemistry. Indeed, industry conferences largely focused on the technological innovations required to reach ultraHTS (that is, 100,000 compounds screened per day). A series of advances in the areas of automation and miniaturization have largely accomplished this goal, and attention has more recently focused on understanding which targets should be screened and what other roles HTS can play aside from lead identification. Approaches such as higher-content screening[1], the use of compounds to aid in target validation (for example, chemogenomics and chemical genomics)[2,3], or high-throughput properties assessment (for example, cytochrome P450 (CYP) inhibition, hERG channel inhibition, *in vitro* metabolism and so on) are beginning to show that capabilities initially established for compound screening can be expanded for use in other key aspects of the drug-discovery process. However, the basic goal of small-molecule screening remains the identification of reasonable starting points for prosecution with medicinal chemistry.

The 'right' approach to screening depends heavily on the strengths of the organization involved and the targets being pursued. This is particularly true for smaller companies, which might not have the resources to institute multiple screening platforms and approaches. In the case of enzymatic screening, the expression and purification of protein is often a limiting step for the execution of high-throughput screens and can limit the targets pursued or the methods used for screening. Chemistry resources are also a key consideration. An organization rich in chemistry resources and talent might focus on weaker-binding, structurally novel hits, with the confidence that the starting point can be appropriately optimized. Conversely, companies stronger in biology might choose to profile hit compounds more extensively before committing chemistry resources to a project.

Given the relatively limited scale of this review, we have focused on a subset of issues that are crucial for successful screening, namely, assay design, compound selection and tailoring the screening enterprise towards the existing strengths of the organization. These approaches are largely discussed in the context of biochemical screening, but many of the concepts apply equally to cell-based approaches.

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Box 1 | **Screening metrics**

The goal of screens is normally to identify inhibitors (and, in some cases, activators) of the target of interest (that is, the hits). So, it is important to differentiate *bona fide* inhibitors from false positives in the assay. This is largely related to the quality of the assay used. Assay quality has traditionally been judged by factors such as signal/noise or signal/background ratio. However, both of these metrics define assays mainly on the basis of assay window, that is, the difference between the assay background and the maximal signal. The size of the signal window and the precision of the data from the assay are two key factors in assigning statistically significant inhibition to an assay. Z′ is now the most commonly used metric to define HTS assay quality and is defined in the following EQN 1:

$$Z' = 1-(3\sigma_{c+} + 3\sigma_{c-})/(\mu_{c+} - \mu_{c-}) \quad (1)$$

where σ_{c+} is the standard deviation of the positive control for the assay, σ_{c-} is the standard deviation for the negative control and $\mu_{c+} - \mu_{c-}$ define the mean value for the for the positive and negative control[4].

The advantage of this metric is that the numerator accounts for the scatter in the positive and negative data, so a smaller change in the signal for a precise assay is afforded more significance. In practice, enzymatic assays with values Z′ >0.5 have acceptable characteristics for high-throughput screening (HTS).

'Hits' in HTS can be assigned in several ways. The first is to simply set a cut off at a percentage inhibition above which a compound is deemed a hit. This implies that compounds with an IC_{50} less than a certain threshold are of interest. The second approach is to examine the distribution of percentage inhibition obtained from the screen, and then define a hit on the basis of the point at which this inhibition becomes statistically significant. This is a more statistically rigorous approach, but does not triage the hits obtained on the basis of potency. A third approach is to define the number of hits that will be put through follow-up. This has the practical advantage that the screen output is tailored to the capacity of the organization to perform follow-up assays, and is helpful for targets with very high hit rates. A variant of this strategy is often employed when screening natural product mixtures, in which the screening concentration of the mixture is varied to obtain a predefined hit rate.

What makes a good assay?

Several metrics exist to quantify the quality of screening assays with respect to reproducibility and data scatter (Z/Z′, the signal-to-noise ratio[4]) (BOX 1). These metrics can greatly aid in defining at what point a result from a screen becomes significant (the statistically significant amount of inhibition in an assay, for example) and are also useful for maintaining the same standard for assay data over multiple days of screening, or for comparison of data quality across screens.

A number of factors dictate the components of a screening assay. The first consideration should be the relevant target biology and the goal of the screen. For example, functional cell-based approaches can offer significant advantages for receptor screening. Receptor-binding assays cannot differentiate between agonists and antagonists. Furthermore, it has been demonstrated that receptor ligands need not compete with the endogenous ligand to affect receptor activity[5]. So, a cell-based approach is likely to be more effective in identifying receptor agonists, as receptor function can be directly assessed. A similar case can be made when screening for compounds that interact with ion channels, particularly if one is interested in activity-state modulators.

Biochemical screening can provide significant advantages over cell-based approaches, particularly for intracellular targets. A well-optimized biochemical assay will, generally, have less data scatter than a cell-based approach. Furthermore, the presence of a single target in the assay simplifies follow-up after the screen. Biochemical screening can also provide a larger series of chemical starting points for intracellular targets, as the hits obtained are not initially required to possess significant potency and cell-permeability. Biochemical screens can also be performed at higher compound concentrations, which aids in the identification of novel chemical classes (FIG. 1). This added structural diversity can be particularly important when working with competitive target classes for which the relevant chemical matter is heavily patented.

Types of screens and readouts

The selection of an assay for screening needs to be guided by the goals of the screen and the practical constraints for the target of interest (FIG. 2). These constraints include amount of protein available, substrate requirements for the enzyme and capacity for follow-up chemistry and biology after the screen is completed. Biochemical assays can generally be divided into separation-based assays (in which the reaction product is detected after its separation from the starting material) and homogeneous assays (in which the detection of the product does not require a separation step). Most homogeneous assays rely on some form of proximity detection. For example, fluorescence resonance energy transfer (FRET) is the basic principle behind a number of biochemical and cell-based assays that are widely used in HTS[6]. In FRET-based assays, a fluorescent molecule is excited by energy at a certain wavelength, and an acceptor molecule then captures the emission energy from the donor fluorophore (FIG. 2). The efficiency of the capture process is dependent on the distance between the donor and acceptor. A simple example is a protease assay in which the donor and acceptor are on opposite ends of the same peptide. Cleavage of the peptide by a protease increases the distance between the donor and acceptor, disrupting FRET and thereby increasing the signal from the donor fluorophore. A number of biochemical assay types are listed in TABLE 1.

Most of the innovation in HTS assay design has been targeted to improve throughput and to remove assay artefacts arising from the presence of the compounds being screened. Separation-based assays (FIG. 2) have the advantage that the compound has usually been separated from the reaction product at the time of detection, which minimizes compound interference effects. Furthermore, these assay types tend to have larger signal windows than homogeneous formats, as the reaction product is the only source of signal in the assay. Indeed, it is often the case that the background in a homogeneous assay format will increase as the concentration of the substrate in the assay is increased, limiting the concentration of substrate that can be used in the assay. However, homogeneous formats require fewer addition or reagent-transfer steps, making them easier to automate and/or miniaturize. The decreased assay complexity can also help reduce scatter in the assay data, as there are fewer opportunities to accumulate errors in the measurement.

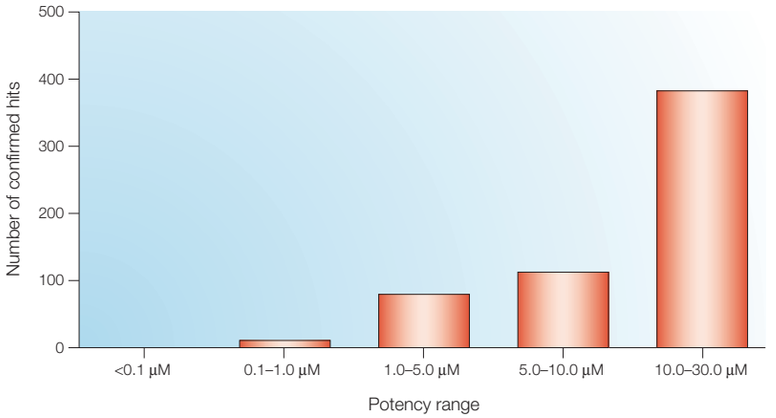


Figure 1 | **Screening at higher concentration leads to greater structural diversity.** It is always the case that more hits are obtained in a screen when higher screening concentrations of compound are assessed. As a natural consequence, more chemical scaffold types (that is, more structural diversity) are typically observed when a screen is run at higher compound concentrations. FIGURE 1 exemplifies the distribution of hits obtained from an unbiased chemical library (a library not containing large clusters of compounds derived from a common scaffold) screened against a kinase. Compounds profiled in this screen were initially obtained by screening in duplicate at 30 μM, then 'confirmed' by titration of the same compound from a fresh aliquot of a dimethyl sulphoxide (DMSO) dissolved stock of the compound.

Compound selection

Although assay technology and appropriate biochemistry contribute to a successful screen, no factor has a larger role than the compounds used for the screen. Compound selection dictates the size of the screening collection required, the quality of positive and negative data and, most importantly, the value of the hits obtained to medicinal chemistry. Factors such as physical properties, target class and 'drug-likeness' are all important considerations, and computational approaches can be valuable in addressing several of these issues. It is important to consider features that make molecules desirable as leads — that is, focusing the screen — as well as identifying molecules that should be avoided — that is, filtering the screen. During the past ten years, a great deal of effort has gone into the development of computational methods for filtering and focusing screening libraries. Filtering methods range from simple counting schemes to more sophisticated methods that attempt to 'learn' from examples of drugs and non-drugs. A variety of methods have also been used for focusing screening libraries towards specific targets. Groups have achieved success by using techniques such as protein–ligand docking, similarity searching, pharmacophore searches and property profiling.

Filtering screening collections

As mentioned above, the objective of computational filtering methods is to identify and remove potentially problematic compounds. Removing these compounds streamlines the screening process by decreasing potential false positives. Perhaps the best-known example of the filtering approach is the 'rule-of-5' (REFS 7–9), which was published in 1997 by Lipinski and co-workers[7]. The authors analysed 2,245 compounds from the World Drug Index, and found that in a high percentage of compounds, the following rules were true: hydrogen-bond donors ≤ 5, hydrogen-bond acceptors ≤ 10, molecular mass ≤ 500 daltons and logP ≤ 5. Subsequent analyses of additional drug databases by other groups have reached

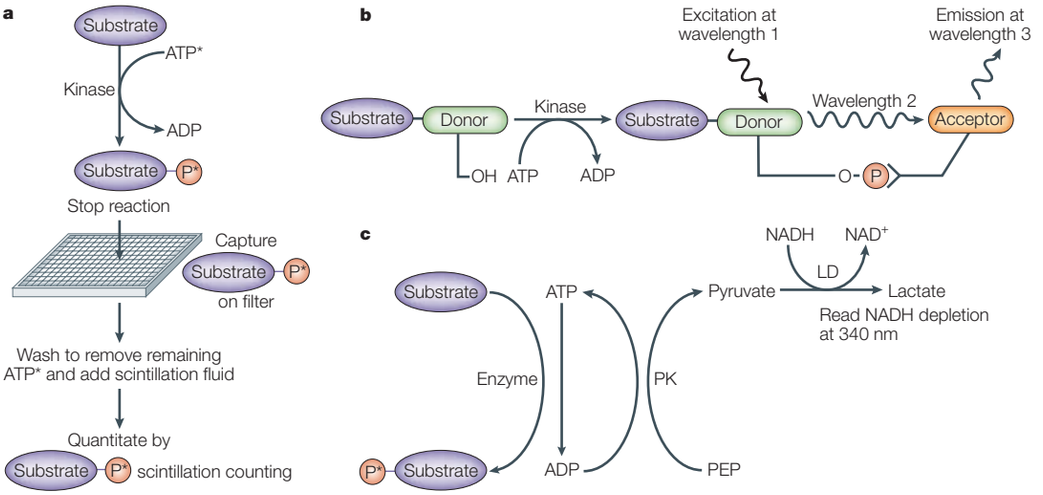


Figure 2 | **Examples of screening assays.** This figure outlines the principles behind three assay types used for screening. The examples illustrate their application to kinase assays, but in all cases the methods are applicable to other target types (TABLE 1). A standard separation-based assay is depicted in **a**, in which radiolabelled ATP transfers a labelled phosphate moiety from ATP to substrate. The substrate is subsequently captured on a membrane, and separated from the ATP substrate by filtration. The signal is measured by addition of scintillate and quantitated by counting. **b** | Outlines the general principles of FRET, a homogeneous assay format. In this case, a labelled substrate is phosphorylated allowing it to bind to a second molecule labelled with an acceptor group. The proximity of the two groups allows energy transfer between the donor and acceptor. This causes a shift in wavelength for the signal emitted from the assay and detection of the product without separation from the substrate. **c** | Another homogeneous assay format in which the conversion of ATP to ADP is coupled to the conversion of NADH to NAD using two enzymes, pyruvate kinase (PK) and lactate dehydrogenase (LD). This decreases the A340 of the assay, which is used to monitor the progress of the reaction.

similar conclusions. Lipinski noted that the majority of drugs that violate the rule-of-5 are antibiotics, antifungals, vitamins and cardiac glycosides. The authors suggest that despite the violations, these classes of compounds are orally bioavailable because they possess groups that act as substrates for transporters.

Another highly effective method of filtering large collections of compounds is to eliminate compounds containing moieties that might be toxic or reactive, or which might interfere with biological assays. One example of such a filtering approach is the REOS (Rapid Elimination Of Swill) program developed at Vertex[10]. The primary function of the program is to analyse potential screening compounds and 'filter out' molecules which might be problematic (Swill) (BOX 2).

During the past five years, there has been a tremendous growth in the number of new computational methods being developed for the prediction of absorption, distribution, metabolism, elimination and toxicity (ADMET) parameters[11,12]. There are differing philosophies as to where these methods should be introduced in the drug-discovery process. One school of thought suggests that as compounds undergo significant changes during the lead-optimization process, it might be premature to use predictive ADMET in compound selection at the screening stage. However, analyses of the progress of a number of drug-discovery programmes have pointed out that drug candidates are typically larger and more lipophilic than the initial lead[13,14]. On the basis of these observations, it might be beneficial to select screening compounds that are predicted to be soluble and orally bioavailable. Our approach has been to apply simpler, heuristic ADMET prediction methods during compound selection; more sophisticated ADMET methods are then applied during later optimization stages.

Table 1 | **Common assay methods for high-throughput screening**

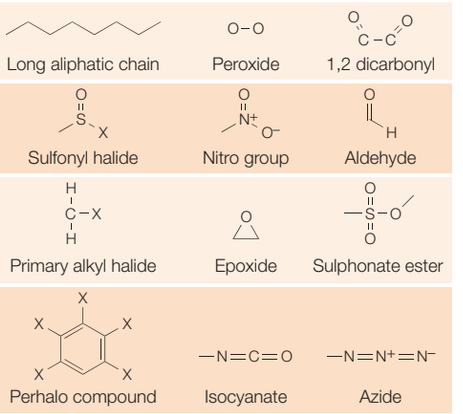
Assay name	Key aspects	Common applications	Method to remove compound interference*
Homogeneous assays			
Fluorescence polarization	Product is detected by binding to a partner of higher molecular mass. This decreases the tumbling of the complex, thereby maintaining polarization of the emitted light	Kinase, receptor and protease assays	Polarization of light is independent of signal intensity, and therefore is not affected by compound quenching or emission
HTRF/LANCE	Time-resolved fluorescent donor (lanthanide), acceptor labelled with APC	Kinase, receptor, protease and helicase assays	Emission lifetime is longer than typical compound emission. Large emission/excitation wavelength shift moves detected signal away from most compound-derived signals
Conventional FRET	Number of donor/acceptor pairs in common use, such as Dabsyl/Edans, coumarin/fluoroscein and others	Kinase, protease and phosphatase assays	Can be run in kinetic format; so, the rate of reaction is independent of initial fluorescence or absorbance
SPA/Flashplate	Detects radioisotopes in close proximity to a solid scintillant, encased in bead or plates	Kinase, ligand binding and helicase assays	Emitted signal can be corrected for compound effects at scintillation counting
Alpha Screen	Luminescent homogeneous proximity assay. Donor bead excited at 370 nm, which generates singlet oxygen, diffuses to acceptor bead and emits at 520–620 nm	GPCR and kinase assays	Large emission/excitation wavelength shift separates assay signal from short wavelength compound emissions
Coupled enzyme assays	Couples conversion of ATP to ADP with decrease in A340 by conversion of NADH to NAD	Any ATP-dependent assay (for example, kinase and helicase assays)	Kinetic measurements are independent of initial absorbance of assay, and are therefore not affected by compound absorbance
Separation-based assays			
Filter-binding assays	A radioactive substrate (typically γ^{32}P-ATP) transfers a label to a charged peptide, which is bound to a filter with the opposite charge	Kinase, polymerase assays	Compound is removed by filtration of the sample
Precipitation/filtration assay	A radioactive substrate is precipitated after completion of the assay, then captured on glass fibre of a similar filter	Kinase, receptor binding, polymerase, GTP exchange (GPCR) assays	Compound is removed by filtration of the sample
ELISA and similar formats	Products from a reaction are captured in a plate using a specific antibody to the reaction product. A similar approach is to use a biotinylated substrate molecule, which is captured on a strepavidin-coated plate after assay is complete	Most common biochemical targets	Compound is removed by filtration of the sample

*Theoretical basis for removal of compound interference effects. In practice compound interference is often observed, particularly with highly fluorescent or coloured compounds. APC, allophycocyanin; ELISA, enzyme-linked immunosorbent assay; FRET, fluorescence resonance energy transfer; GPCR, G-protein-coupled receptor; HTRF/LANCE, homogeneous time-resolve fluoroscence/lanthanide chelate excitation; NAD, nicotinamide adenine dinucleotide.

Box 2 | **Rapid Elimination Of Swill**

Rapid Elimination Of Swill (REOS) is a hybrid method that combines a set of functional group filters with some simple counting schemes similar to those in the rule-of-5. The initial filtering is based on a set of set property filters. The default values for these filters are shown in the TABLE . In addition to the property filters, REOS also employs a set of more than 200 rules based on the presence of chemical functionality known to be problematic. These rules have been developed over the past seven years and are the result of contributions by scientists throughout the Vertex organization. Rather than providing a simple 'accept/reject' facility, REOS allows the user to specify a maximum allowed quantity for each functional group rule. Importantly, rules can be custom tuned to suit the needs of specific targets or assay formats. Examples of the functional group filters employed by REOS are illustrated in the figure.

Property	Minimum	Maximum
Molecular weight	200	500
LogP	–5	5
Hydrogen-bond donors	0	5
Hydrogen-bond acceptors	0	10
Formal charge	–2	2
Number of rotatable bonds	0	8
Number of heavy atoms	15	50



The filtering techniques described above provide a convenient and efficient means for identifying compounds that an experienced medicinal chemist would tend to avoid. However, these methods do not necessarily identify molecules that a medicinal chemist would consider 'interesting' as leads. In addition to eliminating molecules containing undesirable fragments, it is also important to ensure that molecules in a screening collection contain functionality known to impart biological activity. One example of this approach is the work of Muegge and co-workers[15]. In this work, each molecule was assigned a score on the basis of the presence of structural fragments typically found in drugs; the fragments used in this study are shown in FIG. 3. A molecule is given one point for each non-overlapping fragment. Molecules with a score between two and seven are classified as drugs, otherwise they are classified as non-drugs.

Approaches such as REOS are more effective when experienced chemists and biologists are available to contribute rules to the knowledge base. In some organizations, particularly those focused on a single technology, an effort such as REOS might not be practical. An alternative approach is to design a computer program capable of 'learning' to distinguish drugs from non-drugs. The development of computer programs that can learn classification rules forms the basis of a branch of computer science known as machine learning[16]. Machine learning programs operate by examining a set of training examples, each of which belongs to a particular class. A learning program then derives a rule (or set of rules) that assigns new examples to these classes.

Neural networks are examples of one such automated classification method[17]. This technique uses a highly connected network, modelled on a biological nervous system, to create an output classification in response to a set of input values. The neural network is initially presented with a set of labelled examples and a set of attributes describing these examples. The program then 'learns' the relationship between these variables and the desired output by creating a nonlinear relationship between the attributes. A neural network designed to identify drug-like compounds is typically presented with a set of molecules labelled as drug (1) or non-drug (0), as well as a set of descriptors for each molecule. The program then learns relationships between the descriptors and uses these relationships to assign a score between 0 and 1 to each molecule. Those molecules with scores closer to 1 are classified as drugs, whereas those with scores closer to 0 are classified as non-drugs. Neural networks have been shown to be able to distinguish drugs from non-drugs with a 75–85% success rate[18,19]. One potential advantage of neural networks is that they are not subject to

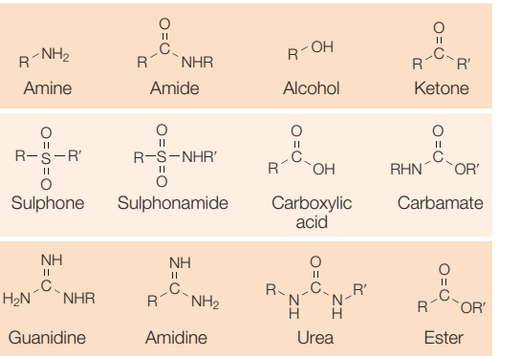


Figure 3 | **Functional groups used in the scoring scheme developed by Muegge and colleagues[15].**

the inherent biases of human experts. However, as with any computational analysis, the output of a neural network should be carefully scrutinized for agreement with scientific intuition.

Focusing screening collections

The techniques described above provide a means of identifying compounds that should not be included in a screening collection. However, in order to design an effective screening library, it is also necessary to identify compounds with a high probability of being hits in a given assay. One way of improving hit rates is to design screening libraries that are targeted to a particular gene family. There are a number of approaches to designing targeted screening libraries. When protein crystal structures or homology models are available, a screening library can be designed from a structure-based perspective[20,21]. An alternative and often complementary approach is to design a screening library on the basis of information gleaned from known active compounds. For gene families such as kinases[22] and G-protein-coupled receptors[22,23], there are hundreds of known active compounds that can be used as starting points for a ligand-based design.

Structure-based library design is typically carried out by computationally docking sets of compounds into a rigid model of the protein active site[24]. In the past, docking methods were primarily used to identify small sets of molecules (10–100) which would be active against a single target. More recently, docking methods have been used to prioritize combinatorial libraries and create screening libraries focused on specific gene families. Although docking is a relatively well-developed technique, it still suffers from several limitations. One limitation is that docking studies are typically carried out using a rigid model of the receptor. Although a few docking methods allow a certain degree of side-chain flexibility, these programs are currently unable to model loop movements or induced-fit effects, which are found in many systems. The most prominent limitation of docking programs results from the scoring functions that they use to predict binding affinity. Although a great deal of progress has been made in this field, an accurate, generally applicable scoring function is still not available[24–26]. The lack of quality in scoring function can end up being less crucial when the objective is to enrich a screening library, rather than to identify single compounds. When designing a screening library targeted to an entire gene family, it can be more important to identify molecules with a certain shape or a set of key features.

Ligand-based approaches typically begin with a collection of molecules known to bind to a set of related targets. This collection of ligands is then used to perform similarity searches against one or more databases of commercially available compounds. Searches can be performed by using either single molecules as a search query or a representation created by combining multiple known ligands[27]. A key factor in performing these types of searches is novelty. A similarity metric that is too literal will tend to identify only those molecules that are close analogues of the known ligands used to perform the search. It is highly likely that these analogues will already be covered by existing patents, and will therefore be of less value as starting points in a drug-discovery effort. The ideal similarity metric will identify molecules that are functionally equivalent to known ligands, but patentably distinct. There are a plethora of methods for determining the similarity of two molecules[28]. Recent studies have shown that it is difficult to predict, *a priori*, which methods will perform best for a particular target. In our experience, extensive empirical validation is required to identify and tune similarity metrics to a particular problem.

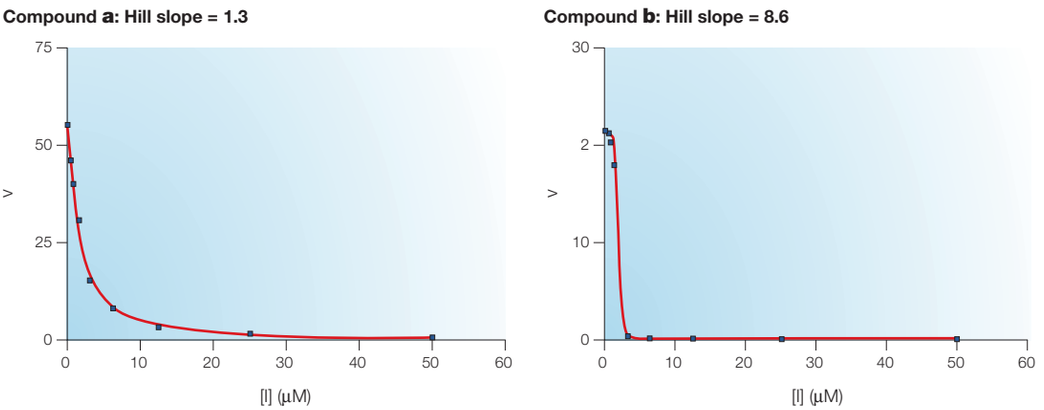


Figure 4 | **Examples of titration curves for compounds obtained from screening.** Compounds **a** and **b** have similar IC_{50} values, but very different slope factors (also referred to as HILL SLOPES[33]). A Hill slope can be useful in describing the binding or kinetic behaviour of a target with ligand-binding stoichiometry greater than one. If the target being screened has a relatively simple kinetic mechanism, and, as such, the expectation of a single inhibitor-binding site, the Hill slope for the titration of a compound should be approximately one. When Hill slopes with values much greater than one are obtained, a general interpretation is that more inhibition of the target is being observed than would be expected if the compound had a simple binding mechanism. At the molecular level, there are a number of possible explanations for such behaviour; however, in a practical sense they are compounds that are less likely to be easily optimized in a subsequent medicinal chemistry effort. In the Vertex triage, compound **a** would be accepted, and compound **b** would be eliminated from further consideration. For a rigorous kinetic analysis of inhibition of enzymes with multiple binding sites, see REF. 32.

HILL SLOPE
In the program *Prism*, the curves are fitted to the equation:

$$y = \text{bottom} + \frac{(\text{top-bottom})}{1+10^{(\text{Log IC50}-x)\,-\,\text{Hill slope}}}$$

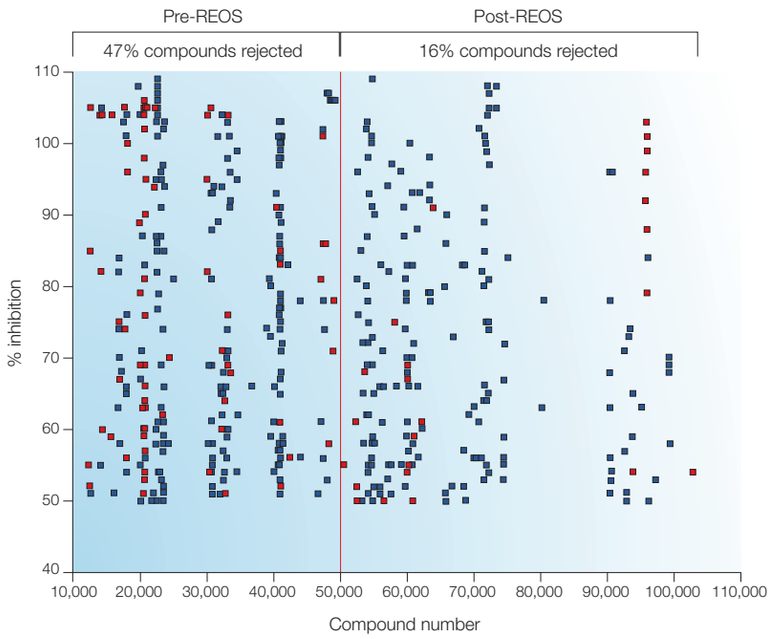


Figure 5 | **The effect of REOS (Rapid Elimination Of Swill) on screening efficiency.** The hits obtained from a kinase screen were inspected by a group of medicinal chemists. The compounds of interest are represented in blue, and the rejected compounds in red. The selections were unbiased by initial potency. In this example, compounds with compound numbers less than 50,000 were not pre-filtered using REOS before inclusion in the screening deck. As such, one of the main positive aspects of REOS filtering for screening is an increase in the proportion of the hits obtained from screening that are of interest to medicinal chemistry. Most of the undesirable compounds identified in this example were removed from the screening collection in a subsequent re-plating effort.

An integrated screen approach for protein kinases

A number of the concepts discussed above were applied in the design of the Vertex kinase-screening program. The approach was tailored to take advantage of the strength of the organization in protein expression, enzymology, chemistry and computational chemistry. Vertex made the strategic decision to organize its early drug-discovery effort around gene families. A key goal of this effort is to efficiently use information obtained from screening the first target in a gene family to aid the prosecution of subsequent targets in the same family. There are two important facets of this approach: enabling the re-screening of compounds from ongoing medicinal chemistry efforts to look for starting points on new targets, and increasing the efficiency in transitioning from the initial screening result to follow-up in medicinal chemistry by providing both structure–activity relationship (SAR) and selectivity information about the hits obtained directly from the HTS.

The main aspects of the compound selection strategy are outlined in the sections above. All of the compounds selected for screening are subjected to REOS analysis. After plating, screening compounds with poor dimethyl sulphoxide (DMSO) solubility are identified by visual inspection and routinely culled from the main screening collection. The compounds are screened at known molar concentrations, simplifying the relationship between the observed percentage inhibition and IC_{50}. To improve the quality of the initial screening data and decrease the false-positive rate, compounds are routinely screened in duplicate. For the kinase-directed libraries, compounds are screened at two concentrations, again to account for compound solubility concerns. Finally, the compounds synthesized for the programme were >85% pure.

Assay selection is largely guided by quality considerations, rather than throughput. Two extremely robust assay formats are a kinetic-coupled assay[29] and conventional radioactive filter binding. A useful estimate for the overall quality of the screening approach is the follow-up rate, which in this case is defined as the frequency with which a compound initially observed as a hit is found to replicate with a reasonable dose response when a fresh aliquot of the compound is assayed. The kinetic-coupled assay routinely provides follow-up rates of 70% or higher, and data provided by a fully automated filter-binding platform are of similar quality (C. Memmot, M. Namchuk, presented at the International Symposium on Laboratory Automation and Robotics, Boston Massachussetts, USA (2002)).

The ready comparison of data obtained from screening different kinases (that is, selectivity — the difference in compound potency against different targets) requires some standardization of the enzymatic parameters for the screening assay. For example, all of the ATP concentrations are fixed at K_m for the individual kinases. Furthermore, every effort is made to screen with full-length kinase constructs, as minor changes in the protein structure can occasionally affect the binding of small molecules to the protein. In cases where a less robust screening assay is selected owing to throughput or protein-consumption issues, the re-testing of the hits obtained from screening is executed with one of these two methods. This again helps to improve the quality of the counter-screening data, as minor variations in titration data obtained from different assays are relatively common[30].

The early examination of the mechanism of action of the hits obtained from screening is also extremely useful. At a basic level, such data can be obtained from examining the slope factor (Hill slope values[33]) from the titration of screening hits (FIG. 4). For enzyme targets with simple kinetic mechanisms (no complex allosteric regulation, for example), a Hill slope value of much greater than one is a reasonable means of removing compounds with low potential as leads. More thorough mechanistic analysis of the hits obtained can help further define the most promising hits from the screen and aid in defining the SAR. Indeed, any computational model will usually assume that the compound maintains a single mode of binding and is helped by segregation of compounds by mechanism of action.

The application of this focused screening approach has produced several tangible benefits. Lower false-positive rates, coupled with better initial data quality, has helped streamline the hit follow-up process, decreasing the amount of follow-up screening required to validate the primary screening result. The

focused re-screening approach has also improved our ability to obtain more hits and compound classes from screening[3]. Finally, the use of REOS filtering in compound selection has dramatically improved the odds of obtaining chemically 'interesting' hits in the screening process (FIG. 5).

Conclusions

In the post-genomic era, target-based HTS has become a staple for project initiation in the pharmaceutical industry. A number of factors play a role in dictating the success of the approach. Improvements in assay methods and automation have provided the tools to better align the screen being conducted with the biology of the target being examined. Matching these assay methods with more judicious compound selection for screening is likely to increase both the frequency of obtaining chemically tractable starting points for medicinal chemistry and the quality of the data obtained from HTS.

The improvements in modern screening technology and combinatorial chemistry have created the opportunity for scientific inquiry on an unprecedented scale. High-throughput properties assessment and cell-based functional assays aid in annotating the initial results from screening and guiding how lead molecules should be optimized. However, the explosion in the data available is yet to create a commensurate increase in the efficiency of the drug-discovery process[31]. Indeed, it can be argued that obtaining data is no longer the key issue facing early drug discovery, and that the challenge has now become selecting the right experiments to run and the right point in the compound optimization process to apply the information.

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